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The amino acid composition and protein quality of high-protein gluten bread

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With 3 tables

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In recent years intensive processing techniques have attracted a great deal of public attention. With the rapid increase in our understanding of the biochemical lesions underlying many disease states, it has become possible more recently to formulate a whole range of new products that can be applied in the dietary management of specific disorders.

Bread tailored for example for slimming and for diabetics is made by adding protein to flour, this protein may be gluten, skim milk or soya flour (1, 4). Processes have been worked out to separate the gluten, major wheat protein from the starch and then dry it. Its good taste and economic price make it a valuable companion in food fortification (5). The general quality of the bread produced is superior in its protein content (12). Gluten-rich bread is very suitable as a diabetic food, where carbohydrate intakes must be restricted. Junior forms of diabetics are now increasing; for children both the quality and quantity of dietary proteins are of importance for their growth.

It is the aim of the present work to determine the amino acid composition of gluten-rich dietetic bread. Its protein quality was also assessed in rat feeding experiment.

Materials and methods

The wheat sample used in the course of this work was a soft winter variety (Triticum vulgaris, Gizza 155). Low grade (second-class) flour was obtained at long extraction of 87.5%, to which wheat gluten protein was added. This composite flour was used for the preparation of high protein-gluten bread (dietetic bread). After baking, the bread was sliced by the manufacturer and wrapped in ½-kg packages, 3 kgs of dry dietetic bread was used in this study.

Sampling

The bread was ground and samples were analyzed for moisture, nitrogen, fat, crude fibres, and ash using the usual standard methods (2).

For amino acid determination, the ground sample was hydrolyzed with 6 N HCl for 24 h at 110 °C in a stream of nitrogen (2). The acid hydrolysate was evaporated in vacuo, redissolved in citrate buffer pH 2.2 and an aliquot was applied to the amino acid analyzer (6).

The tryptophan content was also determined colorimetrically according to the method of *Spies* (11).

Rat feeding experiment

Male Sprague-Dawley rats aged 21±2 days were used for the assessment of the protein quality of the dietetic bread. The experiment corresponded nearly with the experimental procedure described by the German group on the Protein Evaluation (9). For the first three days, the rats were fed a mixture of equal parts of a practical rat diet and a mixture of the experimental diets. After three days the food was removed for 6 hours, and the rats were weighed and distributed over 7 blocks of rats of equal weights. Within each block the rats were randomized for diet and cage. In the experiment one group received a protein-free diet which consisted of the following in (%): cottonseed, 5; salt mix (9), 6; vitamin mix, (9), 2; cellulose, 4; cornstarch, 83. Three groups of six animals each received diets which contained three levels of casein + dl-methionine. At the lower dietary protein levels (3.5, 7.0%), the casein diets were supplemented with 0.3% dl-methionine; whereby at 10% dietary protein level, the casein diets were supplemented with 0.5% dl-methionine.

Three groups of six animals each received diets containing 3.5, 7.0, 10.0% protein derived from the dietetic bread. The diet was prepared by substituting dietetic bread for the starch in the protein-free diet, cellulose was also omitted in this case.

The animals were killed with chloroform after 21-day-feeding. They were weighed and the abdomen was opened. The carcasses were placed in tared beakers, weighed and dried at 95 °C until constant weight. For larger animals this required approximately 3 days.

A group of animals was analyzed at the start of the experiment for body water content. These values were used to calculate the original body water of each animal, and then the gain in body water of each animal during the experiment was calculated.

For each group standard error of the means was calculated for data on weight gain, PERs, and NPUs.

The Net protein Utilization (NPU) was calculated according to the method of *Bender* and *Miller* (3) from the values of body water.

$$NPU = \frac{(BW_T) - (BW_F)}{I} \times 100,$$

whereby: BW_T = the body water of the rats on the protein diet at the end of 21-day-feeding experiment;

BW_F = the body water of the rat on N-free diet;

I = total protein intake during the whole feeding experiment.

The slope-ratio analysis described by *Snedecor* (10) includes the calculation of the linear regression line, $y = a + bx$; for dietetic bread and for casein; y is the response and x is the amount of protein consumed by each rat.

The ratio of the slope for dietetic protein to that of the reference casein + methionine diet is the measure of potency.

The Relative Nutritive Value (RNV) is defined as the slope of the regression line between weight gain and protein intake for the dietetic bread divided by the slope of the close response line obtained with the standard protein, casein + dl-methionine.

Results and discussion

Chemical analysis of whole wheat and dietetic bread are presented in table 1. The results of protein analysis indicate that dietetic bread is higher in protein (38.1% in the dry matter) as compared to whole wheat (12.55% in the dry matter) which is due to addition of gluten, gluten accounts 80% of the protein of whole wheat flour (5). The amino acid composition of gluten protein was given by *Williams* (12) with a lysine content of 2.1 g/16 g nitrogen. *Hepburn* et al. (8) tested the availability of eight essential amino

Table 1. Chemical analysis of whole wheat and dietetic bread.

Material	Protein %	Ash %	Fat %	Total carbohydrate %	Fiber by difference %	Calories
Whole wheat	12.55	1.52	1.93	82.10	1.90	403.57
Dietetic bread	38.10	2.82	1.43	56.87	0.78	395.87

acids in gluten, wheat and bread. They concluded that, in general, amino acids availability tended to be highest in gluten; the authors found that the methionine in the gluten was 96% available, whereas valine showed the lowest availability.

The amino acid composition of whole wheat and dietetic bread is presented in table 2. Lysine is the first limiting amino acid in the whole wheat and also in dietetic bread (13). The chemical score for dietetic bread was found to be 43% which is quite similar to the chemical score (44%) found in the whole wheat kernel.

Table 2. Amino acid composition of dietetic bread in relation to wheat proteins and FAO/WHO Reference Pattern (1973).

Amino acid	Whole wheat	Dietetic bread	FAO/WHO
Essentials			
Lysine	168.5	163.9	344.0
Methionine	92.8	81.1	
Cystine	150.1	133.7	
Total sulfur			219.0
Isoleucine	200.7	210.6	250.0
Leucine	412.5	425.2	437.0
Total aromatic	263.4	269.8	375.0
Tryptophan	70.2	68.0	62.0
Threonine	171.6	161.2	250.0
Valine	262.0	249.9	312.0
Total	1791.8	1763.4	2249.0
Nonessentials			
Aspartic	337.0	258.6	
Glutamic	1763.0	2097.1	
Alanine	217.6	195.5	
Proline	286.6	288.0	
Serine	606.8	702.1	
Glycine	249.9	225.5	
Arginine	281.5	221.6	
Histidine			
NH_3	242.1	289.6	
Total	3984.5	4278.0	
Chemical score =	44.0	43.0	

It was found that baking had no effect on destruction of the amino acids of dietetic bread, it has nearly the same values as whole wheat. Threonine is the next limiting amino acid in whole wheat proteins and also in dietetic bread as compared to FAO/WHO reference amino acid pattern of 1973 (13).

The data obtained in the feeding experiment are presented in table 3. These data show the mean weight gain and gain in body water. The PER values reveal that dietetic bread had a value of 1.34 at 10% dietary level compared with a value of 3.0 for reference casein diets. Rats fed dietetic bread at 10% protein level showed an increase of 10.3 ± 0.4 g body water during the three week feeding experiment, compared with a figure of 32.1 ± 2.0 g in the casein group. Measurement of body water was further used for the calculation of the net protein utilization in dietetic bread, giving a figure of 39.2 compared with a figure of 67.2 in the casein group.

The estimated potency obtained from the slope ratios by the method described by *Hegsted* et al. (7) has been expressed as percentage of the reference casein diet + dl-methionine. The results of the feeding experiment were evaluated utilizing body water and weight gain as the measure of response as shown in table 3.

Generally speaking, similar results were obtained regardless of the measure of response, the use of body water resulted in a nutritive value estimates of 24% of the reference casein diet, compared to relative nutritive value of 20% when weight gain was the measure of response.

Combination of the relative nutritive value and protein content in one index, the utilizable protein, gave value of 7.50 ± 0.08%. This figure is still lower than the level recommended by *Hegsted* (10%), as the minimum level for food proteins having good biological value.

Ingestion of such food proteins may prove to be very useful for diabetic children, who should be maintained on a restricted carbohydrate diet, and still need dietary protein for growth. In 7-year-old school children the FAO/WHO estimate is 0.77 g of reference protein/kg/day. Thus one would estimate that they would require $(0.77 \times \frac{100}{22}) = 3.5$ g/kg/day of gluten-rich dietetic bread. Children in this age group have an estimated caloric requirement of 78 cal/kg. For a child to receive 3.5 g/kg gluten-rich protein he would need around 12 g bread/kg body weight. It would appear from this calculation that gluten-rich materials would be useful for school-age children. In considering cost, gluten-rich foods are still more economic compared to foods such as skim milk, fish or soya flour.

Summary

Dietetic bread is mainly produced for diabetics. Gluten accounts 80% of the protein of whole wheat flour. Forty per cent wheat gluten are incorporated in the dough to increase the protein content and decrease the starch in the final product. Gluten-rich foods are still of low cost as compared to others such as skim milk, soya and fish flour.

Zusammenfassung

Diätbrot wurde in Ägypten hauptsächlich für Diabetiker hergestellt. Zur Herstellung dieses Brotes wird ein Gemisch aus 60 Teilen Weizenmehl und 40 Teilen

Table 3. Mean change in body weight, body water, Protein Effiency Ratio (PER), Net Protein Utilization (NPU), and Relative Nutritive Value (RNV) of weanling rats fed dietetic bread at different protein levels.

Protein in diet	Intake				Change in body				PER		RNV	NPU		RNV
%	Food		Nitrogen		Weight		Water							
	$\bar{x}_{(g)}$	$S_{\bar{x}}$	$\bar{x}_{(mg)}$	$S_{\bar{x}}$	$\bar{x}_{(g)}$	$S_{\bar{x}}$	$\bar{x}_{(g)}$	$S_{\bar{x}}$	$\bar{x}$	$S_{\bar{x}}$		$\bar{x}$	$S_{\bar{x}}$	
Reference casein											100			100
3.5	152.0	4.9	900	30	4.8	1.3	4.0	0.9	0.84	0.18		55.2	1.7	
7.0	221.0	5.4	2870	120	44.0	3.1	22.0	1.8	2.39	0.12		43.2	3.0	
10.0	244.0	11.9	4140	230	79.2	5.4	51.7	2.4	3.00	0.14		59.6	4.0	
Dietetic bread											20			24
3.5	88.4	11.01	480	50	3.0	0.8	44.4	0.5	1.08	0.17		76.5	10.1	
7.0	103.0	15.2	1340	200	6.70	1.3	9.3	0.6	1.02	0.23		44.0	10.7	
10.0	97.0	11.0	1480	160	10.90	0.8	10.3	0.4	1.34	0.14		39.2	3.9	

Gluten eingesetzt. Der Proteingehalt des Produktes beträgt 38% in der Trockensubstanz. Der Proteinwert des Brotes hat einen PER-Wert von 1,34 in Rattenverfütterungsversuchen.

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