

Effect of Baking on the Nutritive Value of Pakistani Bread

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(Manuscript received 10 April 1978)

The nutritive value of Pakistani breads prepared from wheat, maize, rice, barley, millet, triticale and sorghum were measured chemically, by proximate, fatty acid and amino acid analyses and biologically in N-balance experiments with growing rats. Yeast was not used in the preparation of doughs. The levels of lysine, threonine and tryptophan were in several cases negatively affected by the baking procedure. According to FAO 1973 scoring pattern, lysine and threonine were found to be the first and second limiting amino acids in most of the breads. Methionine does not appear to be the third limiting amino acid. Net protein utilisation was negatively affected by baking only in the case of barley (6%) and maize (9%) whereas the biological value was improved in triticale (8%) and sorghum (6%) compared to the corresponding unprocessed samples. The baking procedure did not affect the digestible energy in triticale. Net dietary protein calories percentage values indicate that the cereal breads, except sorghum, had values higher than 5%.

1. Introduction

In Pakistan it has been reported that cereals provide over 72 and 76%, respectively, of the daily calories and protein intakes of an average person.¹ In certain areas, cereals constitute as much as 85% of the total caloric intake.² Wheat, in the form of bread, is by far the most common cereal product consumed and forms 88% of the total cereal intake.³ Breads are also cooked from other cereals and consumed in different seasons of the year.

Cereal protein is known to be of poor quality because of its low content of certain essential amino acids, particularly lysine.⁴ Lysine and threonine were found to be the first and second limiting amino acids respectively for wheat,⁴ barley,⁵ rice⁶ and triticale.⁷ The sulphur containing amino acids have been shown to be the third limiting for wheat,⁴ corn⁸ and triticale.⁷ Contrary to these reports, it has been claimed⁹ that the order of limiting amino acids in wheat protein is lysine, tryptophan, methionine, isoleucine and threonine.

Although there is a vast literature on the protein quality of flour and of yeast bread, there is still controversy regarding the influence of baking on protein quality. Eggum¹⁰ reported that baking caused partial reduction of lysine, tryptophan, methionine and cystine in European rye and wheat breads, whereas Clegg and Davis¹¹ and Maleki and Djazayeri¹² observed no change in the available lysine content of flour due to baking of European and Arabic wheat breads, respectively. Recently, Eggum and Duggal¹³ reported a 5% reduction in the net protein utilisation of Indian wheat chapati as compared to wheat flour. On the other hand, Shyamala and Kennedy¹⁴ found the protein efficiency ratio of Indian chapati to be considerably higher than that of unprocessed wheat. Thus, there are conflicting reports with regard to second and third limiting amino acids in bread and the effect of baking on the protein quality of Indian, Arabic and European breads.

Since there is no published work on the protein quality of Pakistani bread made from cereals such as wheat, maize, rice, barley, millet, triticale and sorghum, the present work was initiated to

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study the effect of baking without yeast on the nutritive value of Pakistani bread made from different cereals. Yeast is not commonly used in bread making in Pakistan.

2. Experimental

2.1. Experimental animal and diets

The experimental procedure has been described by Eggum.¹⁵ Groups of five Wistar male rats, each weighing approximately 75 g, were used. The preliminary period lasted for 4 days and the balance period for 5 days. The rats were weighed at the beginning of the experiments and divided into groups of five such that the average weights of the groups differed by no more than ± 0.5 g. Weighing was repeated at the end of the preliminary and balance periods: access to feed and water was prevented 3 h before weighing. Each animal received 150 mg N and 10 g dry matter daily throughout the preliminary and balance periods. The N content of the diet was adjusted by using a basal diet consisting of a N-free mixture (Table 1).

Table 1. Composition (parts by weight) of the nitrogen-free mixture

| | |
|--|-----|
| Potato starch (autoclaved) | 767 |
| Sucrose | 90 |
| Cellulose powder | 52 |
| Soy bean oil | 52 |
| Mineral mixture ^a | 40 |
| Vitamin mixture ^b (mixed with autoclaved potato starch) | 20 |

^a To provide per kg diet: CaCO₃, 2.74 g; calcium citrate, Ca₃C₁₂H₁₀O₁₄·4H₂O, 12.33 g; CaHPO₄·2H₂O, 4.51 g; K₂HPO₄, 8.75 g; KCl, 4.99 g; NaCl, 3.08 g; MgSO₄, 1.53 g; MgCO₃, 1.41 g; ammonium ferric citrate (20.5–22.5% Fe), 0.61 g; MnSO₄·H₂O, 8.0 mg; CuSO₄·5H₂O, 3.1 mg; KI, 1.6 mg; NaF, 20.3 mg; AlNH₄(SO₄)₂·12H₂O, 3.6 mg.

^b To provide per kg diet: retinol equivalent, 1.2 mg; cholecalciferol, 7.5 μ g; thiamin, 0.8 mg; riboflavin, 2 mg; nicotinamide, 8 mg; pantothenic acid, 2 mg; α -tocopherol, 0.4 mg; pyridoxine, 0.2 mg.

The experimental diets were weighed out into plastic boxes with tightly fitting lids for each of the preliminary and balance periods. The diet was weighed each day from these boxes in four daily allowances in the preliminary period and five daily allowances during the balance period. Any remaining diet was weighed and taken into consideration in the calculation of the experimental results.

Wheat, maize, rice, barley, millet (Bajra), triticale and sorghum grains were ground to whole meal flours and breads were baked as follows: the doughs were first prepared by mixing the flour with water (70% of wheat, barley and triticale flours; 75–80% of rice, millet, maize and sorghum flours) and kneaded. Yeast was not added to any of the doughs. A portion of the dough was rolled on a wooden board until it was flat and circular. It was then placed on a heated flat iron plate at 220–230°C temperature. Wheat, barley and triticale breads were baked for 1.5–2 min while rice, millet, maize and sorghum breads were cooked for 7–10 min. All the bread samples were freeze-dried, ground and incorporated into the test diets (Table 1), at the expense of autoclaved potato starch.

2.2. Analytical methods

The chemical composition of the diets was determined according to standard methods.¹⁶ Acid hydrolysis followed by ether extraction was used for the estimation of fat according to the method of Stoldt.¹⁷ The caloric value of the diets and faeces was determined in an IKA-calorimeter (Adiabatic). Metabolisable energy (ME) of the diets was calculated according to Miller and Payne.¹⁸

The fatty acids were methylated according to Metcalfe *et al.*¹⁹ and analysed by g.l.c. The liquid phase was 15% ethyleneglycol succinate supported on Chromosorb W (AW, DMCS) 80–100 mesh. Amino acid analyses were carried out according to Weidner and Eggum²⁰ and Eggum.²¹ Tannin was estimated as described by Eggum and Christensen²² and statistical analyses were based on methods described by Snedecor.²³ All the assays were performed in duplicate.

3. Results

3.1. Chemical composition

The values for the chemical composition of different flours and the corresponding breads are shown in Tables 2 and 3. As recommended by Tkachuk,²⁴ a factor of 5.7 was used for the conversion of nitrogen to protein.

Table 2 reveals that the protein content of various breads ranged from 6.9 to 14.5%. Fat and ME values were highest in maize and millet breads. Fibre contents were higher in barley and sorghum breads than in all other breads. Sorghum bread contained highest contents of ash (2.9%). Baking of bread in general, increased the values of calcium and sulphur whereas the phosphorus and

Table 2. Chemical composition (dry basis) of flour and bread samples

| | g per 100 g | | | | | | | | | | | |
|-----------------|----------------------|------------------------|---------|-------------|--------------------|-------|-----------------|--------------|-------|------|----------------|------|
| | Protein (N × 5.7) | Available carbohydrate | | | Calories per 100 g | | | mg per 100 g | | | g per 100 g | |
| | | Fat | hydrate | Crude fibre | Ash | Total | ME ^a | Ca | P | S | | Fe |
| Wheat flour | 11.2 | 2.2 | 81.1 | 1.2 | 1.6 | 436 | 414 | 31.4 | 395.4 | 38.4 | 5.1 | 0.50 |
| Wheat bread | 11.4 | 2.1 | 78.1 | 1.4 | 1.4 | 433 | 411 | 33.0 | 175.5 | 40.3 | 5.5 | 0.37 |
| Maize flour | 10.4 | 5.7 | 74.0 | 2.3 | 1.6 | 461 | 438 | 16.8 | 330.3 | 29.6 | 4.2 | 0.48 |
| Maize bread | 10.5 | 5.2 | 72.5 | 2.6 | 1.5 | 455 | 432 | 27.1 | 325.0 | 31.4 | 4.5 | 0.35 |
| Rice flour | 8.1 | 1.4 | 90.1 | 0.7 | 0.6 | 432 | 410 | 12.5 | 125.5 | 31.9 | 2.9 | 0.11 |
| Rice bread | 8.1 | 1.4 | 89.4 | 0.7 | 0.6 | 429 | 407 | 17.5 | 120.5 | 36.3 | 2.5 | 0.09 |
| Barley flour | 14.4 | 3.9 | 64.9 | 4.3 | 2.2 | 454 | 431 | 62.4 | 374.3 | 29.5 | 7.0 | 0.81 |
| Barley bread | 14.5 | 3.8 | 63.4 | 4.4 | 2.2 | 451 | 428 | 80.4 | 369.3 | 35.8 | 8.3 | 0.72 |
| Millet flour | 12.2 | 5.5 | 73.7 | 1.8 | 1.8 | 459 | 436 | 22.5 | 338.2 | 33.8 | 8.1 | 0.72 |
| Millet bread | 12.2 | 5.4 | 71.1 | 1.8 | 1.7 | 455 | 432 | 30.3 | 335.6 | 43.3 | 6.2 | 0.62 |
| Triticale flour | 14.1 | 2.6 | 67.4 | 2.7 | 2.0 | 442 | 420 | 44.4 | 399.6 | 31.1 | 2.9 | 0.70 |
| Triticale bread | 14.0 | 2.6 | 64.4 | 2.7 | 2.1 | 444 | 422 | 64.8 | 399.7 | 33.5 | 3.1 | 0.48 |
| Sorghum flour | 6.9 | 4.5 | 67.4 | 4.8 | 3.0 | 447 | 425 | 44.8 | 313.9 | 25.9 | 10.1 | 1.90 |
| Sorghum bread | 6.9 | 4.9 | 64.8 | 4.8 | 2.9 | 446 | 424 | 53.9 | 323.9 | 35.6 | 10.7 | 1.67 |

^a ME, Metabolisable energy.

Table 3. Fatty acid composition (g per 100 g measured f.a.) of flour and bread

| Fatty acids | Wheat | | Maize | | Rice | | Barley | | Millet | | Triticale | | Sorghum | |
|-------------|-------|-------|-------|-------|-------|-------|--------|-------|--------|-------|-----------|-------|---------|-------|
| | Flour | Bread | Flour | Bread | Flour | Bread | Flour | Bread | Flour | Bread | Flour | Bread | Flour | Bread |
| 12:0 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.4 |
| 14:0 | 0.2 | 0.2 | 0.1 | 0.1 | 1.9 | 1.8 | 0.8 | 0.7 | 0.1 | 0.1 | 0.2 | 0.2 | 0.1 | 0.3 |
| 16:0 | 23.6 | 24.3 | 15.8 | 15.7 | 39.9 | 37.4 | 25.7 | 26.3 | 20.1 | 20.4 | 18.6 | 18.7 | 17.4 | 18.0 |
| 16:1 | 0 | 0 | 0.4 | 0 | 0 | 0 | 0 | 0 | 0.6 | 0.6 | 0 | 0.6 | 0.9 | 0.9 |
| 18:0 | 1.0 | 1.0 | 2.4 | 2.1 | 3.5 | 3.4 | 1.1 | 1.2 | 2.9 | 2.9 | 0.9 | 1.0 | 1.4 | 2.0 |
| 18:1 | 9.9 | 10.0 | 33.0 | 32.7 | 22.4 | 24.6 | 12.1 | 12.6 | 25.5 | 26.3 | 15.0 | 15.8 | 35.4 | 35.7 |
| 18:2 | 60.7 | 60.2 | 45.9 | 47.2 | 30.1 | 30.8 | 54.3 | 52.9 | 45.8 | 44.7 | 59.3 | 58.8 | 43.3 | 40.9 |
| 18:3 | 4.7 | 4.3 | 2.3 | 2.2 | 2.2 | 1.9 | 6.0 | 6.2 | 5.0 | 4.9 | 6.0 | 5.0 | 1.5 | 1.8 |

tannin contents were slightly reduced. Iron contents and fatty composition (Table 3) were not affected by baking of breads.

3.2. Protein quality

Table 4 shows the total amino acid composition of flours and breads. The amino acid composition of flour differed from that of bread due to baking. Lysine, the most heat-sensitive amino acid was reduced slightly in the case of wheat and maize bread, 6% in barley bread and 7% in sorghum bread. The content of threonine in various breads were also lowered slightly in millet, triticale and sorghum, 6–7% in rice and wheat, 18% in barley bread. The content of tryptophan seemed to be most affected and was lower by 8–10% in the case of millet, barley triticale and sorghum, 17% in maize and 27% in rice breads than those of respective flour values.

Table 4. Amino acid composition (g per 16 g N) of flour and bread

| Amino acids | Wheat | | Maize | | Rice | | Barley | | Millet | | Triticale | | Sorghum | |
|---------------|-------|-------|-------|-------|-------|-------|--------|-------|--------|-------|-----------|-------|---------|-------|
| | Flour | Bread | Flour | Bread | Flour | Bread | Flour | Bread | Flour | Bread | Flour | Bread | Flour | Bread |
| Aspartic acid | 4.6 | 4.5 | 5.5 | 5.8 | 8.3 | 8.1 | 5.1 | 4.4 | 6.7 | 6.6 | 5.9 | 5.9 | 6.5 | 6.5 |
| Threonine | 2.8 | 2.6 | 3.2 | 3.2 | 3.4 | 3.2 | 2.9 | 2.3 | 3.2 | 3.1 | 2.9 | 2.8 | 3.3 | 3.2 |
| Serine | 4.8 | 4.5 | 4.4 | 4.4 | 4.6 | 4.4 | 3.7 | 3.1 | 4.4 | 4.3 | 4.0 | 4.0 | 4.3 | 4.3 |
| Glutamic acid | 34.9 | 33.5 | 19.0 | 20.2 | 18.1 | 18.1 | 26.2 | 25.6 | 23.5 | 23.0 | 27.3 | 27.2 | 18.9 | 19.6 |
| Proline | 10.7 | 10.5 | 8.5 | 8.9 | 4.3 | 4.2 | 10.7 | 10.6 | 7.0 | 6.9 | 8.6 | 8.6 | 7.5 | 7.9 |
| Glycine | 3.7 | 3.6 | 3.4 | 3.4 | 4.2 | 4.1 | 3.6 | 3.7 | 3.3 | 3.2 | 4.1 | 4.1 | 3.8 | 3.7 |
| Alanine | 3.2 | 3.1 | 7.2 | 7.2 | 5.3 | 5.3 | 3.7 | 3.7 | 6.2 | 6.2 | 3.7 | 3.8 | 8.0 | 7.8 |
| Valine | 3.8 | 3.9 | 4.5 | 4.5 | 5.4 | 5.4 | 4.6 | 4.6 | 4.8 | 4.7 | 4.2 | 4.2 | 4.7 | 4.7 |
| Isoleucine | 3.4 | 3.6 | 3.4 | 3.5 | 4.0 | 3.9 | 3.5 | 3.4 | 3.8 | 3.8 | 3.4 | 3.5 | 3.6 | 3.6 |
| Leucine | 6.9 | 6.8 | 12.7 | 12.8 | 7.7 | 7.6 | 6.7 | 6.7 | 8.9 | 8.8 | 6.4 | 6.4 | 11.2 | 11.1 |
| Tyrosine | 3.0 | 3.1 | 4.0 | 4.1 | 4.6 | 4.6 | 3.0 | 3.0 | 3.0 | 3.0 | 2.8 | 2.9 | 3.6 | 3.5 |
| Phenylalanine | 4.7 | 4.7 | 4.5 | 4.6 | 4.8 | 4.7 | 4.9 | 4.9 | 4.5 | 4.4 | 4.2 | 4.2 | 4.4 | 4.4 |
| Lysine | 2.3 | 2.2 | 2.5 | 2.4 | 3.4 | 3.4 | 3.2 | 3.1 | 2.7 | 2.5 | 2.9 | 2.9 | 2.7 | 2.5 |
| Histidine | 2.1 | 2.1 | 2.7 | 2.7 | 2.1 | 2.1 | 2.0 | 2.0 | 2.1 | 2.0 | 2.2 | 2.2 | 2.2 | 2.0 |
| Arginine | 4.2 | 4.1 | 4.4 | 4.3 | 7.6 | 7.5 | 4.7 | 4.7 | 4.5 | 4.3 | 5.4 | 5.3 | 4.6 | 4.0 |
| Methionine | 1.6 | 1.6 | 2.1 | 2.1 | 2.9 | 3.1 | 1.7 | 1.6 | 2.2 | 2.2 | 1.7 | 1.7 | 2.3 | 2.3 |
| Cystine | 2.0 | 2.0 | 2.0 | 1.9 | 2.0 | 2.0 | 2.0 | 1.8 | 1.9 | 1.7 | 2.0 | 2.0 | 2.2 | 2.3 |
| Tryptophan | 1.0 | 1.0 | 0.6 | 0.5 | 1.1 | 0.8 | 1.1 | 1.0 | 1.3 | 1.2 | 1.1 | 1.0 | 1.0 | 1.0 |

Protein score, based on the FAO scoring pattern (Table 5) indicates that lysine is the first limiting amino acid in all the flours and breads, threonine is the second limiting amino acid in wheat, rice, barley, millet, triticale and sorghum breads, whereas tryptophan was found to be the second limiting amino acid in maize bread. Isoleucine is the third limiting amino acid in barley, millet and sorghum breads, valine in wheat and triticale breads, threonine and tryptophan in maize and rice breads respectively.

Results obtained on the effect of baking on true protein digestibility (TD), biological value (BV) and net protein utilisation (NPU) are summarised in Table 6. The TD of wheat, maize, rice and millet breads was not affected by baking, but was significantly lower in barley, triticale and sorghum breads compared with the corresponding flour values. The BV of maize and barley breads was significantly lower, while the BV of triticale and sorghum breads was significantly higher. The BV of wheat, rice and millet breads was not different from the unprocessed samples. NPU of triticale bread was significantly increased and was significantly lowered in case of maize and barley breads from processing. The NPU values of wheat, rice, millet and sorghum breads were similar to their unprocessed flours. The effect of baking on the digestible energy of cereals was tested by feeding two groups of rats on triticale flour and bread. No significant difference between the digestible energy of flour (87%) and bread (86%) was observed.

Table 5. Protein score and the limiting amino acids of flour and bread samples

| Sample | Protein score ^a | Limiting amino acids | | |
|-----------------|----------------------------|----------------------|------------|------------|
| | | First | Second | Third |
| Wheat flour | 49 | Lysine | Threonine | Valine |
| Wheat bread | 47 | Lysine | Threonine | Valine |
| Maize flour | 41 | Lysine | Tryptophan | Threonine |
| Maize bread | 39 | Lysine | Tryptophan | Threonine |
| Rice flour | 58 | Lysine | Threonine | Isoleucine |
| Rice bread | 58 | Lysine | Threonine | Tryptophan |
| Barley flour | 63 | Lysine | Threonine | Isoleucine |
| Barley bread | 62 | Lysine | Threonine | Isoleucine |
| Millet flour | 49 | Lysine | Threonine | Isoleucine |
| Millet bread | 46 | Lysine | Threonine | Isoleucine |
| Triticale flour | 61 | Lysine | Threonine | Valine |
| Triticale bread | 60 | Lysine | Threonine | Valine |
| Sorghum flour | 46 | Lysine | Threonine | Isoleucine |
| Sorghum bread | 43 | Lysine | Threonine | Isoleucine |

^a Based on FAO/WHO 1973 scoring pattern.

Table 6. Effect of baking on the protein quality of Pakistani breads

| | True digestibility (%) | Biological value (%) | Net protein utilisation (%) | Net dietary protein calorie (%) |
|-----------------|------------------------|----------------------|-----------------------------|---------------------------------|
| Wheat flour | 96.0 | 55.0 | 53.0 | 5.7 |
| Wheat bread | 95.0 | 56.0 | 53.0 | 5.9 |
| Maize flour | 95.0 | 61.0 | 58.0 | 5.5 |
| Maize bread | 94.0 | 57.0 ^c | 53.0 ^c | 5.2 |
| Rice flour | 100.0 | 71.0 | 71.0 | 5.6 |
| Rice bread | 100.0 | 71.0 | 72.0 | 5.7 |
| Barley flour | 88.0 | 70.0 | 62.0 | 8.3 |
| Barley bread | 85.0 ^b | 68.0 ^a | 58.0 ^b | 7.9 |
| Millet flour | 93.0 | 60.0 | 56.0 | 6.3 |
| Millet bread | 92.0 | 62.0 | 57.0 | 6.4 |
| Triticale flour | 93.0 | 66.0 | 61.0 | 8.2 |
| Triticale bread | 91.0 ^a | 71.0 ^c | 65.0 ^c | 8.6 |
| Sorghum flour | 56.0 | 91.0 | 51.0 | 3.3 |
| Sorghum bread | 52.0 ^c | 96.0 ^c | 50.0 | 3.3 |

^a $P < 0.05$ significantly different from corresponding value of flour.

^b $P < 0.01$ significantly different from corresponding value of flour.

^c $P < 0.001$ significantly different from corresponding value of flour.

Net dietary protein calorie percentage (NDp cal %) of the different flour and bread samples were calculated according to Miller and Payne²⁵ (Table 6). The NDp cal % of the breads lie between 3.3 and 8.6. The values of all the breads, except sorghum bread, were greater than 5 NDp cal %.

4. Discussion

The most important factors determining the loss in nutritive value are the duration and temperature level of heat treatment and the levels of moisture and reducing substances.²⁶

The processing conditions employed in the present work for preparing the different breads did not decrease the nutritive value to a large extent, even in the samples (rice, millet, maize, sorghum)

exposed to 220–230°C for 7–10 min. However, making breads from barley and maize resulted in 6 and 9% decrease in NPU respectively, due to losses in the most limiting amino acids lysine, threonine and tryptophan. The results of the present studies, although the effect of processing was not so severe, agree with those found earlier.^{13,27}

However, BV for triticale and sorghum breads were 8 and 6% higher respectively than those of unprocessed samples. The moderate heat treatment employed apparently had a positive influence on the nutritive value due to destruction of antimetabolites. On the other hand the very high BV of sorghum is quite unrealistic. The sorghum used in this experiment was a local Pakistani variety having a dark brown colour with a bitter taste, and one would have expected a high tannin content. Tannin estimated by the present method gave only 1.9% in the sorghum flour. This tannin value cannot explain the very low digestibility values. However a specific analytical procedure has to be undertaken to be able to analyse for all polyphenols in sorghum (Arora, S. K., personal communication). The present tannin values for sorghum are apparently far too low. If the tannin content is higher than determined in the present work this might not only explain the low TD values but also the high BV values. As discussed previously,²² tannin has a highly negative effect on protein digestibility. However, tannin reacts primarily with the non-essential amino acids proline, glutamic acid, glycine and alanine. Consequently the BV of the absorbed protein must increase.

The results also indicate that in the flours and breads of wheat and triticale, threonine is the second limiting amino acid and that valine is the third. Similar results have been reported²⁸ for whole wheat and wheat flour. Methionine does not appear to be the third limiting amino acid in the flour and bread samples.

According to FAO²⁹ the protein allowances for different age groups in terms of NDp cal % are 8.0, 7.8, 5.9, 8.4, 4.6 and 9.5 for infants, toddler, child, adolescent, adult and lactating mothers, respectively. All evaluated cereal breads, except sorghum, give NDp cal values higher than 5% and should theoretically meet the protein requirement if consumed in adequate amounts.

In general, processing of cereals into Pakistani bread (without yeast) affects the nutritive quality only to a minor extent.

Acknowledgements

The authors are grateful to Miss I. Jacobsen, Mrs A. Tommerup and Miss M. Jensen for co-operation and assistance during the course of research work.

References

1. Ministry of Health, Government of Pakistan *Nutrition Survey of Pakistan* Directorate of Nutrition Survey Research, Islamabad, 1970.
2. Ali, S. M.; Khan, M. A. *Nutrition Survey of Northern Areas of Pakistan* University of Agriculture Press, Lyallpur, Pakistan, 1976.
3. Khan, M. A.; Almas, K.; Abid, A. R.; Yaqoob, M. *Pakistan J. Agric. Sci.* 1976, **13**, 167.
4. Bender, A. E. *Science N.Y.* 1968, **127**, 874.
5. Howe, E. E.; Jansen, G. R.; Gilfillan, E. W. *Am. J. Clin. Nutr.* 1965, **16**, 315.
6. Rosenberg, H. R.; Culik, R.; Eckert, R. E. *J. Nutr.* 1959, **69**, 217.
7. Shimada, A.; Cline, T. R. *J. Anim. Sci.* 1974, **38**, 941.
8. Oestermer, G. A.; Meade, R. J.; Stockland, W. L.; Hanson, L. E. *J. Anim. Sci.* 1970, **31**, 1133.
9. King, K. W.; Sebrell, W. H. Jr.; Severinghans, E. L.; Slovick, W. O. *Am. J. Clin. Nutr.* 1963, **12**, 36.
10. Eggum, B. O. *Aminosyrekoncentration og proteinkvalitet* Stougaards Forlag, Copenhagen, 1968.
11. Clegg, K. M.; Davis, N. *Proc. Nutr. Soc.* 1958, 17 abstr. X.
12. Maleki, M.; Djazayeri, A. *J. Sci. Fd Agric.* 1968, **19**, 449.
13. Eggum, B. O.; Duggal, S. K. *J. Sci. Fd Agric.* 1977, **28**, 1052.
14. Shymala, G.; Kennedy, B. M. *J. Am. Diet Ass.* 1962, **41**, 115.
15. Eggum, B. O. *406. Beretn.* Forsøgslab, Copenhagen, 1973.
16. Association of Official Agricultural Chemists *Official Methods of Analysis* Washington, DC, 1970, 10th edn.
17. Stoldt, W. *Fette, Seifen, Anstrichmittel.* 1952, **54**, 206.
18. Miller, D. S.; Payne, P. R. *Br. J. Nutr.* 1959, **13**, 501.
19. Metcalfe, L. C.; Schmitz, A. A.; Pelka, J. R. *Analyt. Chem.* 1966, **38**, 514.
20. Weidner, K.; Eggum, B. O. *Acta Agric. scand.* 1966, **16**, 115.
21. Eggum, B. O. *Acta Agric. scand.* 1968, **18**, 122.

22. Eggum, B. O.; Christensen, K. D. *Breeding for Seed Protein Improvement Using Nuclear Techniques* International Atomic Energy Agency, Vienna, 1975.
23. Snedecor, G. W. *Statistical Methods* Iowa State University Press, Ames, Iowa, 1956, 5th edn.
24. Tkachuk, R. In *Nutritional Standards and Methods of Evaluations for Legume Breeders* (Hulse, J. H.; Rachie, K. O.; Billingsley, L. W., Eds.) International Development Research Centre, Ottawa, Canada, 1977.
25. Miller, D. S.; Payne, P. R. *J. Nutr.* 1961, **74**, 413.
26. Bender, A. E. *J. Fd Technol.* 1972, **7**, 239.
27. Milner, C. K.; Carpenter, K. J. *Cereal Chem.* 1969, **46**, 426.
28. Sure, B. J. *J. Nutr.* 1953, **50**, 235.
29. F.A.O. Committee on Protein Requirements *Nutrition Studies No. 37* Rome, Italy, 1965.