

CHANGES IN THE PROTEIN VALUE OF BREADS, PREPARED FROM WHEAT FLOURS OF DIFFERENT EXTRACTION DEGREE, IN THE COURSE OF THE BAKING PROCESS

By

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Introductory considerations, objects of the research

One of the important tasks of modern technological development work is the as far as possible complete preservation of food proteins of dietetic value during the various food processing operations. The largest part of cereals, and particularly of wheat, playing an important role in the food stock of most countries, is consumed in form of bakery products. In the course of the baking operation, characteristic of baking industry, the dough is exposed to high temperatures. One part of the reactions, proceeding at higher temperatures, may affect the free and bound amino acids of the dough and may influence thereby the dietetic values.

The change in quality of flour proteins occurring during baking has been investigated by KENNEDY and SABISTON, [6] STROMNAES and KENNEDY, [12] KROHN, CLARKE and KENNEDY, [7] GATES and KENNEDY, [4] MCGARR, GOTTHOLD and KENNEDY, [9] BLOCK et al., [2]. In most of the cases, the change in PER (Protein Efficiency Ratio) value was experimentally measured in rats. The PER index is based on the gain in weight of the animals. The gain in weight in grams, is referred to one gram of protein consumed. In the baking technologies investigated, protein quality decreased by an average of 10 to 40%. MORGAN (1931) established already long ago that the protein value of the crust of white bread was lower than that of the soft part. HUTCHINSON et al. [5] found that the decrease in utilizable lysine quantity was higher in the crust of the bread than in the soft part. The lysine-damaging effect of the baking process has been discussed also by others, thus by MAURON et al. [8] ROSENBERG and ROHDENBURG [11], CARPENTER and MARCH [3].

BAUM [1] investigated the change in protein quality during baking by microbiological method, with the test organism *Tetrahyman pyriformis* W, and found that the baking technology used by them impaired the protein

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quality. After baking for 90 minutes, the RNV value of the crust decreased by 64 to 65%, and the RNV value of the full bread by 43 to 48%. The breads investigated were prepared from a 60/40% mixture of rye flour type 1150 and wheat flour type 812. The composition and mode of preparation of bread corresponded to the standard valid in the GDR. BAUM remarks that the values he found can be compared with data published in the literature only after due circumspection, because most of the data are relevant to bread made of pure wheat flour, and both manufacturing technology and baking temperature may be different.

BAUM determined also the lysine content of the bread samples (by microbiological method), and found that the lysine content of the crust decreased by 72 to 75%, that of the full bread by 28 to 33% during baking.

Research work summarized in this study was undertaken on the one hand with the aim to compare the biological value of breads prepared from wheat flour of different extraction degree, and on the other hand to clear the rate of possible changes occurring during baking. In addition to this double aim, the investigations have been used also to compare the different methods, serving for the determination of the biological value of proteins, on wheat protein as an actual model. For this purpose, using the data on amino-acid composition, the MITSHELL, KORPÁCZY, FAO/WHO indexes, further the limiting amino acids were calculated on the one hand, and the relative dietetic value has been determined on the other hand by a biological method (*Tetrahymena pyriformis* W).

The biological value of proteins and methods of determination

The concept of the biological value of nutriment proteins indicate to what degree proteins of various origin yield the amino acids necessary for human or animal organism, i.e. to what extent they are suitable to cover the amino-acid requirement of the organism. The biological value of nutriment proteins, which is characterized on the basis of different tests by different indexes, depends primarily on the amino-acid composition of the protein, but it is influenced also by various factors, which are independent of the amino-acid composition of the proteins, and characteristic of fodder, or in human relation of dietetics.

A difference must be made between the concepts "protein quality" and "protein utilization efficiency". The first is the property of the protein proper, and is determined primarily by the amino-acid composition of the protein; the latter depends, besides the quality of the protein also on the quantity of protein contained in the food, on the essential constituents and energy content of food and on the physiological circumstances.

If the metabolizable energy content of food is low, the efficiency of protein utilization decreases, but it decreases also if protein consumption is higher than needed. Therefore, the biological value of food proteins of various origin must be tested under standard conditions, under which the efficiency of utilization is maximal.

Since there is no possibility in general for the determination of the nutritive value of proteins by direct feeding experiments, we have to fall back on laboratory methods of chemical, microbiological or biological character, the results of which show a significant correlation with the results of direct feeding experiments. Various indices determined by laboratory methods indicate mostly well the biological value of food proteins, but they always make only cautious extrapolation possible.

The elaboration of the first chemical index, calculated on the basis of amino-acid composition, is linked with the name of MITSCHHELL and BLOCK. Briefly, their method consists in comparing the essential amino-acid composition of the protein tested with the amino-acid composition of a protein considered as ideal (ovalbumin). Expressing the quantity of the single amino acids of the protein to be evaluated as percentage of the corresponding amino-acid quantities of ovalbumin, the essential amino acid occurring in the relatively lowest quantity, or by another name, the limiting amino acid is obtained. As an example, the proteins of soya-bean and wheat are compared in Table 1 with ovalbumin.

It is seen from Table 1 that if the protein investigated contains more than ovalbumin from one of the essential amino acids, this is considered as 100%.

Table 1

The essential amino-acid content of egg, wheat and soya-been proteins
(1 — percentage of the protein content, 2 — relative percentage referred to ovalbumin)

Amino acid	Egg		Soya-bean		Wheat	
	1	2	1	2	1	2
Tre	4.9	100	3.8	68	2.8	57
Val	7.1	100	5.6	79	4.7	66
Met	3.2	100	1.3	41	1.8	56
Met + Cys	5.3	100	2.3	43	3.0	57
Ile	5.9	100	5.6	95	4.6	78
Leu	8.8	100	8.1	92	7.8	88
Tyr + Phe	9.3	100	7.7	83	7.1	76
Lys	7.8	100	6.3	81	2.2	28
His	2.6	100	2.6	100	1.9	70
Trp	1.4	100	1.5	100	1.0	71

The table contains the data tyrosine + phenylalanine. Animal experiments proved namely that in the case of considerable tyrosine content the phenylalanine demand decreases. Essentially, the Mitschell index takes the relative quantity of the limiting amino acid into consideration, presuming that the incorporation of food protein is only controlled by the limiting essential amino acid.

The FAO/WHO index takes a standard of comparison calculated on the basis of the demand of human organism into consideration. In the calculation of the FAO/WHO index, the quantities of the single essential amino acids of the protein to be evaluated are referred to the total quantity of essential amino acids. This series of data obtained for the protein to be tested is compared with a similar series of values, calculated on the basis of the FAO/WHO standard. The limiting amino acid, giving the new index value, can be found also in this case.

The FAO/WHO standard and the corresponding data of ovalbumin are shown in Table 2.

OSER introduced in 1951 the calculation of the essential amino-acid index, named after him. The basic concept is also here a comparison with some basic protein, however, its mode of calculation differs in several features. OSER takes into consideration the combined quantity of methionine and cysteine, ignores the effect of tyrosine, and takes as essential arginine. The index is calculated by averaging the relative essential amino-acid quantities, referred to ovalbumin. As a modification of the OSER index, MITSCHELL calculated in a similar way, but omits arginine in his index.

It is a generally accepted opinion that the results obtained by the method of calculation used by OSER and MITSCHELL approach best the biological value of proteins, determined on the basis of human and animal experiments. KORPÁCZY, LINDNER and VARGA (1961) point out two shortcomings in the calculation of the EAA index, and try to eliminate them by an improvement of the method.

One of these deficiencies is that the OSER index covers only the essential amino acids, but neglects the quantity of non-essential amino acids. However, the experiments of ROSE and co-workers called the attention to the fact that the biological value is influenced also by the non-essential amino acids. In human experiments, only essential amino acids were supplied as nitrogen

Table 2

Amino acid	Thr	Met+Cys	Val	Ile	Leu	Phe+Tyr	Lys	TRy	Total
Ovalbumin	47	57	66	54	76	93	70	17	490
FAO/WHO standard	40	35	50	40	70	60	55	10	360

source over a longer period, which resulted in disturbances in nitrogen metabolism. The nitrogen equilibrium became balanced again only when the essential amino acid diet has been changed, and at least 25% of non-essential amino acids has also been provided for the organism. ROSE established on the basis of these experiments that the ideal ratio in the equilibrated protein is 75% essential amino acid to 25% non-essential amino acid.

KORPÁCZY and co-workers showed also the other deficiency of the OSER index. In proteins, containing more of one of the essential amino acids than does egg protein, OSER ignores the quantity above hundred, and calculates consequently with hundred. LINDNER established that this excess of essential amino acid imbalanced the equilibrium of the amino acids, and hereby, the protein was not of full value anymore. All this is solved by taking the reciprocal of the original value, and using this value in the calculations.

Microbiological methods are particularly promising for the characterization of the biological value of food proteins, because they are less costly and laboursome than animal experiments, and moreover, are biologically specific and suitable for series tests. Several attempts are reported in the international literature for the development of a microbiological method, which gives similar results as animal experiments. The basis for this is that the essential amino-acid demand of certain microorganisms with proteolytic activity is similar to that of the higher animals, and their reproduction under adequate conditions is proportional to the biological value of the protein in the nutrient medium, i.e. with its utilizable essential amino-acid content.

Among the possible test organisms, *Tetrahymena pyriformis* W holitrich ciliated protozoon occupies a special place, because it requests the same essential amino acids as the rat in growth (lysine, tryptophane, methionine, leucine, isoleucine, valine, histidine, arginine, phenylalanine and threonine). It produces extracellular proteinases, and can utilize therefore proteins also in intact state. The nutrient medium is formed by the test nutrient solution and the protein to be investigated. The test nutrient solution contains all those nutrient ingredients, which are necessary for *Tetrahymena pyriformis* W, in a quantity and ratio such that the propagation of the organism shall not be influenced by the accompanying substances inevitably present in protein samples (e.g. vitamins, nucleotides, etc.), and the growth in cell number shall be exclusively proportional to the quality of the protein, or more exactly, to its utilizable amino-acid content.

Test substances and methods

Flours BL 55 and BL 80 were used in our experiments, the baking tests were made in accordance with the Hungarian standard specifications, with a starting flour quantity of 30 g and a quantity of water corresponding to

the Valorigraph (Farinograph) test. Baking time ranged from 30 to 60 minutes.

Investigation of the amino acid composition of the bread samples

The bread samples prepared by the prescribed method have been dried at room temperature. From this product a grist (of flour fineness) has been prepared, serving to the determination of the amino-acid composition of the proteins of bread. Before the amino-acid determination, the following hydrolysis method was applied.

Protein hydrolysis

200 mg of the bread-flour was weighed into a hydrolyzing ampoule, and 5 ml of 6 n hydrochloric acid was added. Prehydrolysis was carried out on a water bath for 15 minutes, while 0.2 ml of (melted) phenol was added. After pre-hydrolysis, further 5 ml of 6 n hydrochloric acid were added to the hydrolysate, and the hydrolyzing ampoule was sealed. The hydrochloric acid hydrolysate was evaporated on a rotary vacuum film evaporator to an oily product. The evaporation residue was dissolved in trisodium citrate buffer of pH 2.2, transferred into a 50 ml normal flask, and the volume was made up to the mark. 10 ml of this stock solution was filtered, and 1 ml of this solution was introduced with the sample dispenser of the automatic amino-acid analyser into the ion-exchanger resin column.

Amino-acid analysis procedure

An automatic analyser Model AAA 881 was used for the analysis. Separations were performed on a strongly acid cation exchange resin Aminex A6 (Bio-Rad).

The amino acids were eluted by the stepwise gradient method of Dévényi. The buffer system consisted of trisodium-citrate solution in the following order of concentration:

- Buffer A pH 3.18 Na⁺ concentration 0.2 n
- Buffer B pH 4.16 Na⁺ concentration 0.8 n
- Buffer C pH 6.00 Na⁺ concentration 1.5 n.

With the single-column method and changing of the three buffers, one complete analysis required 230 minutes. The amino acids were separated under isothermal conditions at 52°C.

The order of elution of the amino acids was as follows: Asp., Thr., Ser., Glu., Pro., Gly., Ala., Cys/2., Val., Met., He., Leu., Try., Phe., NH₃, Sys., His., Arg. (abbreviations according to IUPAC nomenclature).

Calculation of the protein value

From the amino-acid composition data three kinds of indices were calculated: the Mitschell index, the FAO/WHO (1972) index and Korpáczy's index. Computation was performed according to WÖLLER et al. [13] with the aid of a special computer program.

Investigation of the relative nutritive value with *Tetrahymena pyriformis* W test organism

For the numerical characterization of the quality of protein, the cell count value obtained at the end of four days of incubation was taken as basis expressed as percentage of the cell count values obtained for full egg. The index obtained is the so-called Relative Nutritive Value (RNV).

Methods

Maintenance of the strain. The strain *Tetrahymena pyriformis* W, known also under the names *Glaucoma pyriformis* and *Tetrahymena geleii*, was grown on peptone-based liquid culture medium in test tubes, under sterile conditions, at 25°C. The organism does not require light. Composition of the nutrient solution for the maintenance of the strain:

Peptone (DIFCO)	20 g/lit.
yeast extract (DIFCO)	1 g/lit
glucose	5 g/lit
NaCl	1 g/lit
pH	7.1 g/lit

Transoculation is done weekly with a Pasteur pipette, under sterile conditions.

Composition of the nutrient medium. The nutritent medium consists of the chemically defined test nutrient solution and of the sample to be tested. Composition of the nutrient medium (10 ml):

- Solution A: 2 ml of nucleotide solution (fivefold concentration)
 2 ml mineral salt solution (fivefold concentration)
 4 ml protein suspension (equivalent to 3 mg N)
- Solution B: 1 ml of vitamine solution (tenfold concentration)
- Solution C: 1 ml of glucose solution (tenfold concentration)

Stock solutions A, B and C are each separately thermally sterilized (at 121°C for 10 minutes), and the nutrient medium is compounded under sterile conditions. The pH of solution A is adjusted to 8.2 before sterilization.

Preparation of the stock solutions needed for the compounding of the nutrient medium:

Nucleotide solution of fivefold concentration: 75.0 mg of guanylic acid (Fluka) and 62.5 mg of cytidilic acid (Fluka) are dissolved in a conical flask under gentle heating on a water bath in about 30 ml of water. In another flask, 50.0 mg of adenylic acid (Fluka) is dissolved in the presence of 1 or 2 drops of conc. HCl in about 30 ml of water on a water bath. In a third flask, 25.0 mg of uracil (Fluka) is dissolved after addition of 1 or 2 drops of conc. NH_4OH in about 30 ml of water on a water bath. After complete dissolution, the solutions are admixed in a 100 ml flask, and filled up with distilled water to the mark.

Mineral salt solution of fivefold concentration:

(a)	$\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$	2.8 g
	$\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6 \text{H}_2\text{O}$	1.25
	$\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$	0.025
	ZnCl_2	0.0025
		g g g g

The above compounds are dissolved in a 200-ml conical flask in about 100 ml of distilled water, washed into a 200-ml normal flask and filled up with distilled water to the mark. The concentration of this solution is hundredfold.

(b)	$\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$	600 mg
	$\text{CuCl}_2 \cdot 2 \text{H}_2\text{O}$	60 mg
	$\text{FeCl}_2 \cdot 6 \text{H}_2\text{O}$	15 mg

The above compounds are dissolved in a 200-ml conical flask in about 100 ml of distilled water, washed into a 200-ml normal flask and filled up with distilled water to the mark. The concentration of this solution is hundredfold.

(c)	KH_2PO_4	3.5 g
	K_2HPO_4	3.5 g

The above compounds are dissolved as described above in distilled water and their volume is made up to 200 ml. The concentration of this solution is hundredfold.

From each of the solutions (a), (b) and (c), portions of 5 ml are transferred with a pipette into a 100-ml normal flask, and the flask is filled up with distilled water to the mark. This solution is of fivefold concentration.

Preparation of vitamin solution of tenfold concentration

(1) Vitamin mixture (dry)	
Ca-panthotenate	25 mg
Nicotinic acid amide	25 mg
Pyridoxine HCl	250 mg
Pyridoxal HCl	25 mg
Pyridoxamine HCl	25 mg
Riboflavine HCl	25 mg
Thiamine HCl	250 mg
Meso-inosite	25 mg
p-Aminobenzoic acid	25 mg

The above compounds are triturated in a mortar, homogenized, placed into an airtight closed drying vessel covered with black paper, and stored in a refrigerator. The mixture can be stored for a year.

- (2) Choline chloride solution: 625.0 mg of choline chloride solution is weighed into a 100-ml normal flask, dissolved in distilled water, and the flask is filled up with distilled water to the mark.
- (3) Biotin solution: 31.3 mg of biotin is dissolved in a 500-ml normal flask in distilled water, and the flask is filled up with distilled water, and the flask is filled up with distilled water to the mark.
- (4) Folic acid solution: 31.3 mg of folic acid is dissolved in a 500-ml normal flask in 10 ml of 0.1 n NaOH, and the flask is filled up with distilled water to the mark.
- (5) DL-alpha-liponic acid solution: 10 mg of DL-alpha-liponic acid is dissolved in a 500-ml normal flask in a few drops of 96% ethanol, and the flask is filled up with distilled water to the mark.

After the preparation of the stock solutions, the vitamin solution of tenfold concentration is composed in the following way: 33.8 mg of vitamin dry mixture (1) is dissolved in a 200-ml normal flask in about 50 ml of distilled water (under heating on a water bath), and after cooling, 2 ml each of choline chloride solution (2) biotin solution (3), folic acid solution (4) and DL-alpha-liponic acid solution (5) are added. The flask is filled up to the mark, and sterilized in a conical flask, stoppered with paper wadding, for 10 minutes at 121°C.

Preparation of glucose solution of tenfold concentration: a 15% glucose solution is prepared, and sterilized in the same way as the vitamin solution.

Preparation of iso-osmotic salt solution: 0.9 g of NaCl is dissolved in 100 ml of distilled water.

Preparation of formaldehyde solution: to 90 ml of 40% formaldehyde solution, 50 ml of the salt solution marked (c) is added, followed by 460 ml of distilled water. This solution is used for the killing of the cells.

Preparation of the sample: The air-dry bread samples were comminuted in an electric milling apparatus, and were then defatted in a Soxhlet apparatus by extracting twice for 8 hours with diethylether. The sample was then comminuted until the whole quantity passed a 0.2-mm sieve. The nitrogen content of the sifted samples was determined by Kjeldahl's method.

Inoculation, incubation and evaluation: The experiments were carried out in 100-ml conical flasks at a final volume of 10 ml. From each sample four parallels were weighed in. The sterile mixture of the sample and the nutrient solution was inoculated with 0.2 ml of the three-day *Tetrahymena* culture, which represents a starting cell count of 1000 cells/ml as an average. The samples were incubated at 25°C for 4 days, then the parallel samples were combined, and cell count values were twice evaluated from these stock solutions in three different dilutions. Tenfold, fivefold and twofold dilutions were used. The cell population was photographed in a counting chamber of Fuchs-Rosenthal type through a microscope. From each sample a total of twelve pictures were made. After the evaluation of the films the cell count values were plotted on log-log millimeter paper as a function of dilution, and extrapolated graphically to zero dilution.

Composition of the test nutrient solution (tenfold concentration)

Ca-panthotenate	5.25 $\mu\text{g/ml}$
Nicotinic acid amide	6.25 $\mu\text{g/ml}$
Pyridoxine HCl	62.50 $\mu\text{g/ml}$
Pyridoxal HCl	6.25 $\mu\text{g/ml}$
Pyridoxamine HCl	6.25 $\mu\text{g/ml}$
Riboflavine HCl	6.26 $\mu\text{g/ml}$
Thiamine HCl	62.50 $\mu\text{g/ml}$
Meso-inosite	6.25 $\mu\text{g/ml}$
p-Aminobenzoic acid	6.52 $\mu\text{g/ml}$
Choline chloride	62.50 $\mu\text{g/ml}$
Folic acid	0.625 $\mu\text{g/ml}$
Biotin	0.625 $\mu\text{g/ml}$
DL-alpha-liponic acid	0.20 $\mu\text{g/ml}$
MgSO ₄ · 7 H ₂ O	1400.00 $\mu\text{g/ml}$
Fe(NH ₄) ₂ (SO ₄) ₂ · 6 H ₂ O	625.00 $\mu\text{g/ml}$
MnCl ₂ · 4 H ₂ O	12.50 $\mu\text{g/ml}$
ZnCl ₂	1.25 $\mu\text{g/ml}$
CaCl ₂ · 2 H ₂ O	300.00 $\mu\text{g/ml}$
CuCl ₂ · 2 H ₂ O	30.00 $\mu\text{g/ml}$
FeCl ₃ · 6 H ₂ O	7.50 $\mu\text{g/ml}$
K ₂ HPO ₄	1750.00 $\mu\text{g/ml}$
KH ₂ PO ₄	1750.00 $\mu\text{g/ml}$
Uracil	500.00 $\mu\text{g/ml}$
Adenylic acid	1000.00 $\mu\text{g/ml}$
Guanylic acid	1500.00 $\mu\text{g/ml}$
Cytidylic acid	1250.00 $\mu\text{g/ml}$
Glucose	150 000.00 $\mu\text{g/ml}$

Table 3

Amino acid	Nr of sample									
	5	10	8	11	10	5	4	8	11	4
ASP	4.3	5.3	5.7	5.1	4.9	4.7	4.8	5.0	7.3	4.5
THR	2.2	2.0	2.1	2.5	2.3	2.3	2.5	2.5	1.2	2.9
SER	3.9	5.7	5.2	5.6	5.4	5.6	5.9	5.5	6.3	4.2
GLU	32.4	34.6	31.8	28.4	28.4	28.9	28.3	30.4	26.9	35.4
PRO	12.3	15.0	10.7	13.1	14.7	13.6	14.1	12.7	14.2	11.2
GLY	3.8	3.3	3.2	3.9	4.0	5.2	4.1	4.0	4.3	3.5
ALA	3.9	3.4	3.8	3.7	3.9	3.1	3.9	3.8	3.5	3.0
1/2 CYS	0.6	0.6	0.9	0.6	0.6	0.6	0.5	0.5	0.5	0.9
VAL	3.4	3.9	5.0	4.9	4.8	5.7	4.9	5.0	5.7	4.6
MET	0.9	0.4	0.6	0.2	0.3	0.4	0.3	1.1	1.2	0.4
ILE	4.1	3.4	3.8	3.9	3.8	3.5	3.6	3.3	3.1	3.8
LEU	7.3	6.7	6.4	6.8	6.8	6.5	6.9	7.8	7.5	6.5
TYR	3.7	3.3	3.2	3.3	3.9	3.4	3.7	3.7	3.8	1.7
PHE	5.3	4.5	5.3	5.1	6.1	5.5	5.2	5.4	5.6	4.2
LYS	2.6	1.9	2.8	2.8	2.6	3.0	3.1	2.4	2.2	1.6
HIS	2.4	2.0	2.9	2.3	2.4	2.4	2.6	2.2	2.4	2.1
TRP	0.7	0.8	0.5	0.2	0.2	0.3	0.2	0.6	0.9	0.5
ARG	5.7	3.9	5.0	6.3	4.1	4.7	4.8	5.3	4.9	6.2
Ammonia content	0.6	0.5	1.1	0.9	0.8	0.8	0.7	0.7	0.7	1.6
Degree of amidation	15.1	11.5	24.2	21.9	21.4	19.3	19.2	15.9	16.7	34.2
Mitchel index	44.753	47.099	44.574	37.377	38.435	40.697	39.188	51.641	48.524	44.618
Korpáczy index	41.802	52.795	39.968	33.818	35.077	37.556	35.779	60.973	56.322	41.948
FAO/WHO-1972]	49.351	50.000	50.214	19.216	23.195	32.804	25.807	52.300	49.700	36.673
Limiting AA	MET + CYS	TRY	MET + CYS	TRY	TRY	MET + CYS	TRY	TRY	TRY	LYS

Test results and their evaluation

The amino-acid compositions of the samples tested and the indices calculated are summarized in Table 3. Data show primarily that an important part of the differences to be observed is not significant. In summary, certain difference can be established to the advantage of the flour of higher extraction degree, due primarily to the difference in lysine content. This difference asserts itself particularly where lysine is the limiting amino acid. Changes brought about by the baking time are also predominantly non-significant, since in the single cases the changes of the various indexes are not of the same direction. A more decisive decrease in biological value was found for breads made from flour BL-50.

The RNV values of breads investigated by us are summarized in Table 4. It can be established that neither the RNV value of breads made of flour BL-50, nor that made of flour BL-90 change significantly as a function of baking time. This is seen also from Figs 1 and 2.

On the other hand, the RNV values of bread samples made of flour BL-90 were in general significantly higher than those for flour BL-50. These results are shown in Table 5.

Table 4
Test results on the protein quality of bread samples prepared from flours BL-50 and BL-90

Sample mark	Baking time (min)	Nitrogen* (%)	Cell count $\times 10^{-4}$ ml	RNV value (%)
BL-50: 10	30	2.1	34.0 \pm 6.0	17.0 \pm 3.0
11	30	2.2	34.0 \pm 6.0	17.0 \pm 3.0
2	40	1.9	36.0 \pm 4.0	18.0 \pm 2.0
	40	2.1	32.0 \pm 6.0	16.0 \pm 3.0
7	50	2.1	36.0 \pm 8.0	18.0 \pm 4.0
8	50	2.2	36.0 \pm 2.0	18.0 \pm 1.0
4	60	2.1	28.0 \pm 7.0	14.0 \pm 3.5
5	60	2.2	33.0 \pm 6.0	16.5 \pm 3.0
BL-90: 10	30	2.2	40.0 \pm 10.0	20.0 \pm 5.0
11	30	2.2	40.0 \pm 8.0	20.0 \pm 4.0
2	40	2.2	38.0 \pm 4.0	19.0 \pm 2.0
3	40	2.2	42.0 \pm 4.0	21.0 \pm 2.0
7	50	2.2	38.0 \pm 5.0	19.0 \pm 2.5
8	50	2.3	44.0 \pm 8.0	22.0 \pm 4.0
4	60	2.2	44.0 \pm 6.0	22.0 \pm 3.0
5	60	2.3	40.0 \pm 2.0	20.0 \pm 1.0

*The values of nitrogen content are referred to samples extracted with ether.

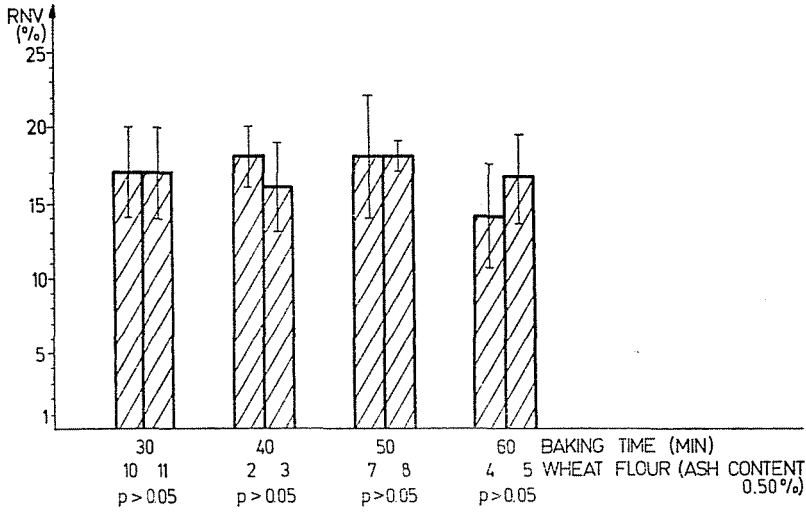


Fig. 1

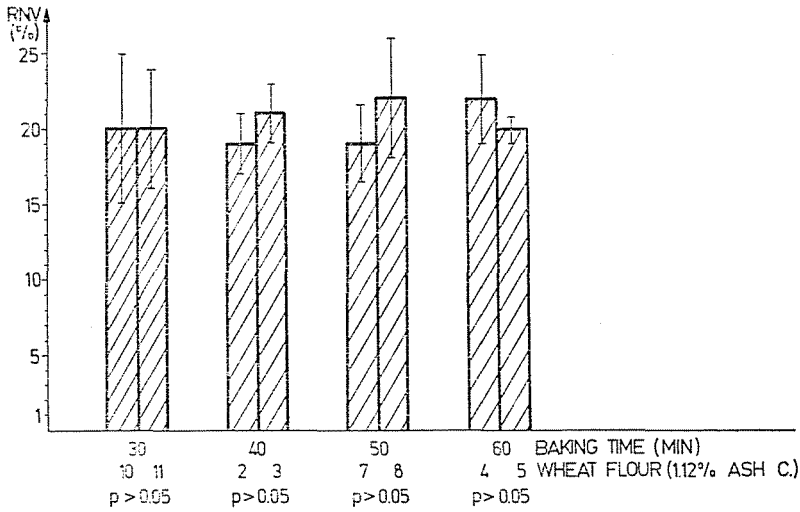


Fig. 2

Table 5

Comparison of the relative nutrient value (RNV) of bread samples prepared from flours BL-50 and BL-90

Baking time (min)	Relative nutrient value (%)		
	BL-50	BL-90	Difference
30	17.0 ± 3.0	20.0 ± 4.0	3.0 (p < 0.05)
40	17.0 ± 2.5	20.0 ± 2.0	3.0 (p < 0.02)
50	18.0 ± 2.5	20.5 ± 3.3	2.5 (p < 0.10)
60	15.3 ± 3.3	21.0 ± 2.0	5.7 (p < 0.01)

The protein values of the flours of cereals and the breads made of them are determined generally by the utilizable quantity of lysine, or more exactly: in such cases, there is a strict correlation between the biological value of the protein and the utilizable quantity of lysine. The RNV values of the samples investigated permit to conclude that the utilizable lysine content of the full bread does not change as much as to reduce the RNV value. The protein-degenerating role of the Maillard reaction was more manifested in the crust of the breads. Thus, the causal influence of baking conditions can be investigated more sensitively, by following the change in RNV value of the crust alone.

Summary

The amino-acid composition of two wheat flours of different extraction degrees (ash content 0.50 and 1.12%) and its changes during baking of breads made from wheat flours were investigated. The biological value was also determined by biological test (*Tetrahymena pyriformis* W) and calculation of different indices.

The differences observed are in many cases small and not significant. In final account a certain difference can be established to the advantage of the flour of higher extraction degree, due primarily to the difference in lysin content. A more decisive decrease in biological value was found for breads made of flour of lower extraction during baking.

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