Protein Nutritional Value of a Biscuit Processed by Extrusion Cooking: Effects on Available Lysine

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The aim of this study was to measure the effect of extrusion cooking on available lysine. The product studied was a protein-enriched biscuit. Available lysine was analyzed chemically as fluorodinitrobenzene (FDNB)-reactive lysine and biologically with growing rats. A comparison was also made with total lysine assayed after conventional acid hydrolysis. The decrease in lysine during extrusion cooking, calculated as percentage of the content in the raw material, was similar with all methods under mild process conditions, about 11%. However, at more severe process conditions the biological assay showed a more pronounced decrease than the chemical methods. Lysine retention was negatively influenced by increased process temperature and positively by increased moisture content of feed. In addition to the lysine loss, there was also a decrease in sulfur-containing amino acids, arginine, and tryptophan. Extrusion cooking under conditions giving a realistic product appears to affect available lysine similarly to, e.g., baking.

From a technological point of view, extrusion cooking of food can be classified as a high-temperature, short-time (HTST) treatment. The process is mainly used for precooking and/or texturization. A variety of products, e.g., crisp bread, biscuits, breakfast cereals, weaning foods, full fat soy flour, and snacks, are processed in extruders, and new applications are made currently.

As the process is under development regarding applications and equipment, and as the nutritional value is of great importance in some of the products, it is essential to study the effect of different processing parameters on the nutritional value.

In the extruder the product is subjected to intense mechanical shear through the action of one or two rotating screws. The cooking takes place at high temperature, high pressure, and a comparatively low water content.

The Maillard reaction is known to be enhanced by high temperatures and intermediate water contents, such as occur during extrusion cooking. Lysine is particularly interesting as it is essential to man and the most reactive protein-bound amino acid, due to its free ϵ-amino group.

The effect of extrusion processing (Brabender-Plasticorder, PL-V500) of lysine-fortified rice was studied by Tsao (1976). The available lysine content, analyzed by the 2,4,6-trinitrobenzenesulfonic acid (TNBS) method (Ousterhout and Wood, 1970), was 89–97% of that in the unprocessed material. In a study by Noguchi et al. (1982) available lysine was analyzed as 1-fluoro-2,4-dinitrobenzene (FDNB)-reactive lysine according to Booth (1971). The product, a protein-enriched biscuit, was processed in a Creusot-Loire BC 45 extruder at different process conditions, and the retention of lysine ranged from 60 to 100%. Pongor and Mâtrai (1976) processed soybeans in a Brady 400 type extruder. The available lysine content did not change up to a process temperature of 144 °C but decreased 20–30% at temperatures around 150 °C. Available lysine was meausured as the difference between total lysine and residual lysine after treatment with FDNB. Beaufrand et al. (1978) studied the effect of extrusion cooking (Creusot-Loire BC 45) on lysine in relation to the composition of the raw material. The availability of lysine measured with the FDNB method by difference decreased considerably when sucrose was replaced with fructose, a reducing carbohydrate.

Depending on the kind of Maillard products formed, the different chemical methods (FDNB, TNBS, total lysine) can be expected to correlate more or less well with biological assays. It has been shown that the TNBS method grossly overestimates biologically available lysine in the presence of early Maillard products. Thus, formylfructosyllysine that is biologically unavailable was 80% "available" with this method. The FDNB method, on the other hand, showed only 15% availability for this compound, when determined as reactive lysine, while the FDNB method by difference showed 53% availability (Hurrell and Carpenter, 1981). Erbersdobler et al. (1982) compared the reliability of different methods for determination of available lysine in heated protein/sugar mixtures. The FDNB method gave the best correlation with in vivo determinations.

The main purpose of the present investigation was to compare the FDNB method for determination of available lysine with a biological assay in extruded products. A Creusot-Loire BC 45 twin screw extruder was used. This equipment has a great versatility regarding applications.

MATERIALS AND METHODS

Chemical Analysis. Nitrogen was determined by the method of Kjeldahl. Amino acids were analyzed after acid hydrolysis (6 N HCl, 110 °C, 24 h) by ion-exchange chromatography. Sulfur-containing amino acids were determined after performic acid oxidation. The equipment used was a Durrum D-500. Tryptophan was analyzed spectrofluorometrically after incubation with papain in urea (Öste et al., 1976). When the chemical score was calculated, the FAO provisional amino acid scoring pattern was used (FAO/WHO, 1973). Available lysine was analyzed as FDNB-reactive lysine as described by Booth (1971) with a slight modification according to Noguchi et al. (1982).

Biological Assay of Available Lysine. Biologically available lysine was estimated from nitrogen balance experiments with growing rats in the following way: Each diet was tested on five male rats (Sprague-Dawley, 75 g). Each rat received 150 mg of nitrogen/day, corresponding to 9.4% protein in the diet. After a 4-day equilibrium period a nitrogen balance was performed for 5 days. Urine and feces were collected separately and analyzed for nitrogen. Fecal "metabolic" nitrogen excretion and urinary

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Table I. Conditions of Extrusion Cooking

sample	moisture content of feed, %	feed rate, kg wet wt/h	screw speed, rpm	mass[a] temp, °C	pressure,[a] bars	minimum residence time, s
I	13	40	80	170	195 ~ 200	42
II	13	40	80	193	150 ~ 160	42
III	13	40	80	210	95 ~ 105	44
IV	18	40	80	210	75 ~ 85	44

[a] Mass temperature and pressure measured just before the die.

"endogenous" nitrogen were determined with a diet containing 4.5% protein from diethyl ether extracted hen's egg. Calculations of true digestibility (TD), biological value (BV), and net protein utilization (NPU) were performed with the Thomas-Mitchell equations as described by Eggum (1973).

The diets were supplemented with minerals, vitamins, chloine chloride, and corn oil in optimal amounts for growth as described earlier (Burvall et al., 1977). Cellulose powder, 5%, was added as bulking agent and sucrose, 10%, to improve the edibility of the diet. Corn starch was used to adjust the dry matter content.

Lysine was made limiting in the diets by mixing the sample under investigation with wheat gluten (BDH Chemicals, Ltd.) in the proportions 68:32 regarding nitrogen from the sample and wheat gluten, respectively.

Extrusion Processing. A mixture containing 22% protein was processed in a Creusot-Loire BC 45 twin screw extruder. The protein fraction consisted of 26% wheat protein, 25% casein, and 49% soy protein. The composition of the raw material was as follows: 42% wheat flour (5.5% ash content); 20% corn starch (Fleurine, Corn Products, USA); 20% sucrose (finely ground); 11% soy protein isolate (Promine D, Central Soya); 6% sodium caseinate (low viscosity, Francexpa, 75 Paris); 1% NaCl. The blend was thoroughly mixed for 30 min to get an even distribution of ingredients before processing (M 20-G Lödige mixer, Paderborn, West Germany). The product was a protein-enriched, cereal-based biscuit with the shape of an expanded ribbon.

Product temperature and pressure were measured in the compression chamber just before the die, with a TPT 463 E Dynisco probe (Westwood, MA). The product was processed under four different conditions as shown in Table I. Mass temperature was 170–210 °C just before the die. The moisture content of feed was generally 13% or 18% in one experiment. When the water content was increased from 13 to 18%, it was necessary to increase the temperature of external heaters in order to keep the same product temperature just before the die.

Minimum residence time was determined by using erythrosin and was found to be close to 42–44 s. The screw diameter was 44.2 mm and the screws consisted of five segments (FCCCRCD) (feed CCCRC die; C = compression, R = reverse flighted). The die was rectangular and had an height of 1.5 mm, a width of 22.25 mm, and a length of 47 mm.

The product processed under the mildest conditions was slightly yellowish and can be considered a realistic biscuit. The biscuit processed at 210 °C was brown and must be considered overprocessed. All samples were packed in aluminum–polyethylene bags under vacuum and stored at −20 °C until analysis. Before chemical analysis and biological evaluation, the samples were ground to pass a 0.5-mm screen.

Table II. Amino Acid Composition of Raw Material and Corresponding Extruded Samples (I-IV)

	g/16 g of N[a]				
sample	raw material	I	II	III	IV
isoleucine	4.7	4.6	4.7	4.5	4.5
leucine	8.4	8.1	8.2	8.2	8.2
total lysine	6.3	5.4	4.9	3.9	4.5
FDNB-lysine	5.8	5.4	4.9	3.7	3.9
methionine }[b]	2.1	1.8	1.6	1.5	1.5
cystine }	1.4	1.3	1.2	1.2	1.2
phenylalanine	5.2	4.7	4.6	5.0	4.9
tyrosine	4.1	3.7	3.6	3.8	3.7
threonine	4.1	3.8	3.9	3.9	4.0
tryptophan[c]	1.7	1.7	1.6	1.5	1.5
valine	5.3	5.0	5.1	5.1	5.0
aspartic acid	9.3	9.1	9.2	8.9	9.0
serine	6.3	5.7	5.7	6.0	6.0
glutamic acid	24.0	24.0	24.0	23.1	23.4
proline	8.4	8.1	8.1	8.7	8.4
glycine	3.7	3.5	3.3	3.6	3.5
alanine	3.9	3.6	3.7	3.9	3.8
histidine	2.7	2.6	2.5	2.5	2.5
arginine	6.1	6.1	5.0	4.8	5.1

[a] The figures are mean values from duplicates. [b] Performic acid oxidation. [c] Spectrofluorometrical assay.

The biscuits extruded under the four different conditions will be referred to as samples I–IV. The mixture of ingredients before extrusion will be refered to as raw material.

RESULTS

Amino Acids. The amino acid contents are shown in Table II. There was a substantial decrease in total lysine with increasing process temperature. The loss was 13% in the mildest processed sample (I) and 37% in the most severely processed one (III). An increased water content had a clearly beneficial effect on the lysine retention. There was also a decrease in sulfur-containing amino acids, in arginine, and in tryptophan. After lysine the loss in methionine was most pronounced, amounting to 26–28% in the samples processed under the most severe conditions. Cystine decreased about 17% and the loss in arginine was up to 20%. The tryptophan content did not change at the lower temperature, but at 210 °C the loss was 10%. The other analyzed amino acids did not change significantly.

FDNB-Reactive Lysine. The decrease in FDNB-reactive lysine was moderate in the sample processed at 170 °C, about 7%. At 210 °C the decrease was substantial, about 37%. At this temperature, an increase in water content from 13 to 18% H_2O gave a higher retention of FDNB-lysine (Table II).

Biological Assay of Available Lysine. In the raw material calculation of the chemical score (c.s.) showed that the sulfur-containing amino acids were limiting. In order to make lysine limiting in all the test diets, wheat gluten was added. On the basis of the amino acid analysis, a diet was chosen with 68% of its nitrogen coming from the extruded samples or from the raw material and 32% from gluten. As there was also a decrease in the sulfur-containing amino acids during extrusion, all diets were supplemented with L-methionine (0.4 g/16 g of N). For the raw material and sample (I) extruded at the lowest temperature, the score for threonine and valine was very close to that of lysine after mixing with gluten. Those amino acids were therefore also added to all diets, 0.4 and 0.3 g/16 g of N, respectively.

Chemical scores for the diets are given in Table III. Lysine was limiting and the range of c.s. was 59–88%. As a control c.s. was calculated also from data of the re-

Table III. Chemical Score of the Sample/Gluten Mixtures

chemical score	raw material/gluten[d]	sample I/gluten[d]	sample II/gluten[d]	sample III/gluten[d]	sample IV/gluten[d]
isoleucine	109	108	108	106	106
leucine	113	111	111	111	112
lysine	88	78	71	59	67
methionine + cystine	116[a]	107[a]	103[a]	101[a]	101[a]
phenylalanine + tyrosine	149	138	135	143	141
threonine	101[b]	97[b]	98[b]	99[b]	100[b]
tryptophan	139	144	136	128	127
valine	104[c]	100[c]	100[c]	101[c]	100[c]

[a] 0.36 g of L-methionine/16 g of N added. [b] 0.38 g of L-threonine/16 g of N added. [c] 0.29 g of L-valine/16 g of N added. [d] N of sample/N of gluten = 68/32.

Table IV. True Digestibility (TD), Biological Value (BV), and Net Protein Utilization (NPU) of the Sample/Gluten Mixtures

	TD ± SD	BV ± SD	NPU ± SD
raw material/gluten[a]	96.9 ± 0.9	73.4 ± 1.8	71.1 ± 2.0
sample I/gluten[a]	96.8 ± 1.2	69.2 ± 2.0	67.0 ± 2.0
sample II/gluten[a]	95.5 ± 0.9	62.8 ± 3.0	59.9 ± 3.3
sample III/gluten[a]	92.7 ± 1.1	55.0 ± 2.2	51.0 ± 2.4
sample IV/gluten[a]	94.6 ± 1.3	61.1 ± 1.7	57.7 ± 1.9

[a] N of sample/N of gluten = 68/32.

quirements of the growing rat given by NAS/NRC (1972). Those data also include histidine and arginine. Lysine was found to be clearly limiting in all diets also according to these calculations.

Table IV shows the results of the nitrogen balance experiments. Since the wheat gluten used is completely digestible, the digestibility changes demonstrated correspond to a decrease from about 95% in the raw material and the mildest extruded sample (I) to about 89% in the most severely extruded one (III). In sample IV, processed with higher water content, the digestibility change was less pronounced. The biological value of the sample/gluten mixture decreased from 73% in the mixture with the raw material to 55% in the mixture with sample III, extruded at 210 °C. Again the sample processed at the same temperature but at a higher water content (IV) was less affected.

In order to get a quantitative measure of biologically available lysine, a second experiment was performed, in which free amino acids were added to wheat gluten in amounts equal to the amino acid content in the raw material. The ratio of "sample" nitrogen to gluten nitrogen was the same as in the first experiment. The lysine content was varied at the expense of alanine to keep total nitrogen constant in all mixtures. The chemical score for lysine was varied in the interval 50–88% to make a standard curve that would fit the samples. The full composition of amino acids in the standard mixtures is given in Table V.

The results from the nitrogen balance study performed on these standard mixtures are given in Table VI. The BV and NPU (almost identical in this experiment because of the TD close to 100%) can be expressed as a function of "added" lysine as shown in Figure 1. A linear correlation was obtained with the correlation coefficient of 0.99.

Comparison of FDNB-Reactive Lysine, Total Lysine, and Biologically Available Lysine. Available lysine measured biologically, BV = f (available lysine), was consistently lower then FDNB-lysine (Table VII). In the raw material and in the sample extruded at higher water content (18%), the results were in good agreement, however, the methods differing only by 5 and 3%, respectively. For the other extruded samples the difference was 10–20% (Figure 2). When the calculation of biologically available lysine is based on NPU, taking into account also the digestibility, the overestimation of available lysine with the FDNB method is more pronounced (Table VII).

Table V. Amino Acid Composition of the Standard Diets

	g/16 g of N		
	gluten	free amino acids	c.s. of mixture[a]
isoleucine	1.2	3.2	108
leucine	2.3	5.7	
lysine	0.6	2.2-4.2	50-88
methionine + cystine[b]	0.6 + 0.8	1.8 + 1.0	116
phenylalanine	1.6	3.5	147
tyrosine	1.1	2.8	
threonine	0.9	3.2	101
tryptophan[c]	0.2	1.1	139
valine	1.3	3.9	104
aspartic acid	1.1	6.3	
serine	1.8	4.3	
glutamic acid	11.1	16.2	
proline	3.8	5.7	
glycine	1.0	2.5	
alanine	0.9	4.7-2.7	
histidine	0.7	1.8	
arginine	1.2	4.2	

[a] N of sample/N of gluten = 68/32. [b] Performic acid oxidation. [c] Spectrofluorometrical assay.

Table VI. True Digestibility (TD), Biological Value (BV), and Net Protein Utilization (NPU) of the Standard Diets

added lysine, g/16 g of N	TD ± SD	BV ± SD	NPU ± SD
2.18	100.9 ± 0.8	54.6 ± 1.7	55.1 ± 2.0
2.73	100.6 ± 0.3	61.9 ± 1.2	62.2 ± 1.3
3.28	100.3 ± 0.6	69.2 ± 0.7	69.5 ± 0.9
3.82	100.3 ± 0.7	76.4 ± 1.2	76.6 ± 0.8
4.23	100.7 ± 0.5	77.7 ± 2.2	78.2 ± 2.5

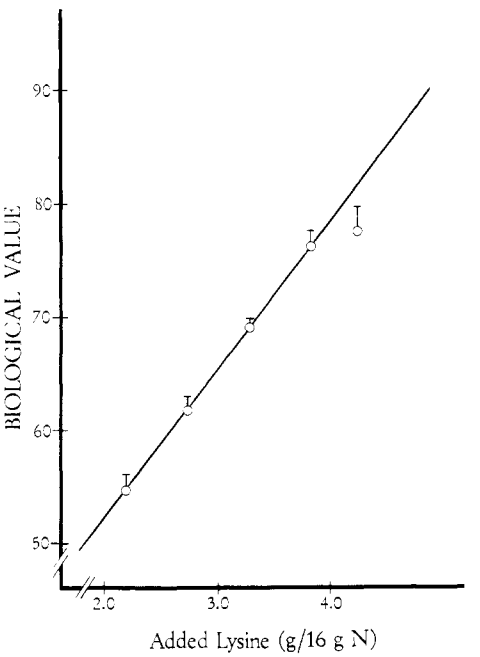

Figure 1. Biological value as a function of lysine added to the standard diets.

Table VII. Effects of Extrusion Cooking on Lysine: Comparison between Total Lysine, FDNB-Reactive Lysine, and a Biological Determination Performed on Rats

	lysine, g/16 g of N			
			biologically available	
	total	FDNB reactive	BV	NPU
raw material	6.3 (100)[a]	5.8 (100)	5.5 (100)	5.1 (100)
extruded samples				
I (170 °C, 13% H_2O, 80 rpm)	5.4 (87)	5.4 (93)	4.8 (88)	4.5 (87)
II (193 °C, 13% H_2O, 80 rpm)	4.9 (77)	4.9 (85)	4.0 (73)	3.6 (70)
III (210 °C, 13% H_2O, 80 rpm)	3.9 (63)	3.7 (63)	3.1 (56)	2.6 (50)
IV (210 °C, 18% H_2O, 80 rpm)	4.5 (72)	3.9 (68)	3.8 (70)	3.4 (65)

[a] Values in parentheses are percent.

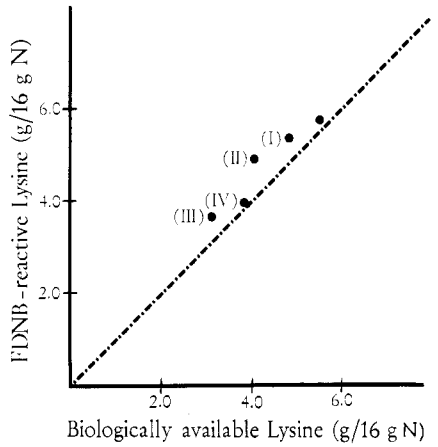

Figure 2. FDNB-reactive lysine as a function of biologically available lysine calculated from the biological value. The extruded samples are indicated (I–IV).

Total lysine after acid hydrolysis was in general higher than both FDNB-reactive lysine and biologically available lysine, as shown in Table VII.

The decrease in lysine during extrusion cooking calculated as a percentage of the lysine content in the raw material was similar for total lysine and for available lysine calculated from the BV. FDNB-reactive lysine showed a slightly lower relative decrease for samples extruded under the milder conditions (I and II). The decrease in available lysine calculated from NPU was more pronounced under the more severe process conditions, due to the decreased digestibility.

DISCUSSION

Determination of FDNB-reactive lysine or other in vitro methods does not reflect changes in the protein digestibility. Lysine with a free ϵ-amino group may be physiologically unavailable if adjacent peptide bonds are not split in the digestive tract. This may, therefore, cause an error in the chemical determination of available lysine in samples with a decreased protein digestibility.

An objection to the biological assay is that the standard curve was based on free amino acids. The rate of digestion and absorption can be expected to differ from that of the test protein (Mathews and Adibi, 1976). Nitrogen balance experiments in which mixtures of free amino acids have been compared with the corresponding proteins show divergent results, however. Amino acid mixtures corresponding to casein and egg albumin gave BV and NPU similar to the proteins. For soy protein the NPU and BV for the corresponding amino acid mixtures were significantly higher than for the protein, whereas in the case of gluten NPU was higher and the BV lower for the amino acid mixture (Forsum and Hambraeus, 1977). In our study the test protein was a mixture of casein, soy, and wheat protein. Thus, it is not possible to predict whether our biological assay would give a negative or a positive error, if any. In severely processed samples toxic derivatives, if produced, could influence the biological respons. This is, however, an objection to all biological measurements.

The standard curve BV = f (available lysine) is not valid for BV < 40. At such low levels of lysine the curve is not linear (Bender, 1961).

Under the process conditions used in the present study, lysine was damaged at the fastest rate. The lysine retention was highly dependent on the process temperature and on the water content of the feed. Other amino acids—cystine, arginine, and tryptophan—known to participate in the Maillard reaction (Hurrell and Carpenter, 1977) were also affected. In addition to losses in lysine, Beaufrand et al. (1978) reported significant losses of arginine and histidine during extrusion cooking in a Creusot-Loire BC 45 extruder.

The decrease in *total* methionine in our study is more difficult to interpret as the thioether group does not normally combine with reducing sugars (Cuq et al., 1978).

The reason for reduced availability of protein-bound methionine during heat treatment of proteins is not fully known, although a decreased protein digestibility and oxidation of methionine residues are believed to be important. During heat treatment methionine may be oxidized to methionine sulfoxide and methionine sulfone. Methionine sulfone is unavailable whereas methionine sulfoxide is partly available at least in the rat (Cheftel, 1979). Available methionine can decrease considerable during heat treatment (Rao and Mc Laughlan, 1967).

Pongor and Mâtrai (1976) studied the changes in methionine during extrusion cooking of soybean in a single screw extruder. Unaltered methionine, that is, free methionine recovered after acid hydrolysis, was used as a measure of availability. Corrections were made to compensate for destruction of methionine during hydrolysis. At a process temperature of 149 °C, the decrease in methionine was 10%. Total methionine measured after oxidation to sulfone did not change, however.

As total methionine normally is determined as methionine sulfone, it is not likely that oxidation during processing has any great impact on the result. To account for the prominent loss in the samples processed at the most severe conditions in our study, methionine must have been altered in such a way as to make oxidation to sulfone impossible. However, losses in methionine could not be found in the most severely extruded sample (sample III) when analyzed directly without prior oxidation to methionine sulfone. This discrepancy deserves further investigation.

Losses in total methionine has been reported in the literature (Miller et al., 1965a). In a study by Donoso et al. (1962) heat treatment of pork resulted in a 16% loss in total methionine. Miller et al. (1965b) studied different methods for determination of total methionine and con-

cluded that analysis of methionine sulfone could give divergent results in heated materials. That is, a decrease in total methionine after heat treatment could be due to analytical artifacts.

The raw material used in our study contained only small amounts of reducing sugars initially. The reason for the considerable loss in lysine at the higher temperatures is probably production of reducing monosaccarides through hydrolysis of sucrose. This has been reported to take place during extrusion cooking by Noguchi et al. (1982), and these authors suggested that the Maillard reaction might be limited by the formation of reducing sugars through sucrose hydrolysis. Dextrinization of starch has also been reported (Tsao, 1976; Mercier, 1977; Sahagun and Harper, 1980), which could further aggrevate lysine loss.

Under the mildest conditions chosen in our study (170 °C, 13% H_2O, 80 rpm) the lysine loss was about 11% regardless of analytical method. This loss is close to that reported during bread baking (Rosenburg and Rohdenburg, 1951). The similar decrease in total and available lysine at mild conditions in our study indicates that the mechanism of lysine damage is destruction rather than reduced availability. This is in agreement with results found during high-temperature, short-time baking of pizza crust (Tsen et al., 1982a) and balady bread (Tsen et al., 1982b). However, under more severe extrusion conditions (210 °C, 13%, H_2O, 80 rpm) there is a discrepancy between the chemical methods and the biological assay (37 vs. 50% lysine loss). This discrepancy was mainly due to a reduction in the protein digestibility. The product processed at this high temperature was dark brown in color and *cannot* be considered a realistic product. Losses of this magnitude (50%), however, have been reported when baking protein-enriched biscuits containing skim milk powder (Carpenter and March, 1961). When conventional baking, microwave baking, and steaming were compared, conventional baking significantly reduced the availability of lysine (Tsen et al., 1977). However, only minor differences were seen in total lysine between the three methods of baking. Thus, depending on the food process and/or processing temperature, it seems as if the nature of lysine damage may vary.

For practical purposes determination of total lysine after acid hydrolysis or of FDNB-reactive lysine seems useful to predict deterioration of protein nutritional value during extrusion cooking, when expressed relative to the content in the raw material.

Registry No. Lysine, 56-87-1; arginine, 74-79-3; tryptophan, 73-22-3.

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