Synthetic Amino Acids and Their Use in Fortifying Foods

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This paper gives a general review of the synthesis of the amino acids methionine, lysine, threonine, and tryptophane, and their use in fortifying foods. The latter point is discussed from the nutritional and economic–technological points of view.

The chemical industry has up to now been highly successful in efforts to raise the quantity of foodstuffs available to mankind. To illustrate this point we need only refer to the manufacture of fertilizers—which in 1965 was responsible for the production of about 17.5 million tons of bound nitrogen alone—and to the preparation of herbicides and insecticides, which have made contributions that cannot easily be overrated. It may rightly be said that these branches of chemical industry, which are concerned with raising the output of natural products important to man and with protecting them from deterioration, will show further extensive development also in the future.

On the other hand, efforts at improving the quality of foodstuffs have remained limited to supplementation with vitamins, mineral salts, and flavors. Methods for improving the biological value of protein by supplementation with essential amino acids have not been developed to any large extent, in spite of the fact that such supplementation offers great possibilities for the upgrading of cereal proteins. Among the essential amino acids, L-lysine and methionine rank first in order of importance, followed by threonine and tryptophane.

SYNTHESIS OF AMINO ACIDS

In spite of the fact that the production capacity for L-glutamic acid is 10 to 20 times greater than that for all other amino acids together, further details about the preparation of this acid will not be given, since it is used for flavoring and not for fortifying foods; in other words, it is a nonessential amino acid.

The ratio of the production capacities for the two important essential amino acids, lysine and methionine, is about 1 to 20. One of the reasons is the fact that lysine is primarily deficient in human foods, and methionine is deficient in feeds for animals, above all in broiler rations. The advantage of amino acid supplementation in animal nutrition is much easier to evaluate, since it can be expressed in dollar cents per pound of weight gain. In human feeding, taste aspects are rated above optimum biological effects, such as improved health and growth, and greater resistance to diseases. Such effects do not lend themselves to direct measurement.

Methionine. The synthesis of methionine has long been known and has not changed essentially in the last 25 yr. The starting material used in nearly all technical processes for the production of methionine is acrolein, which can be made by a vapor-phase oxidation of propylene with the aid of a catalyst. Addition of methyl mercaptan to the double bond of acrolein effects an almost quantitative yield of β-methyl mercaptopropionaldehyde, which can subsequently be converted in three ways (Figure 1): (a) with HCN, in the so-called cyanohydrin synthesis producing the corresponding hydroxynitrile; (b) with HCN + NH_3 to the corresponding aminonitrile; and (c) with HCN, NH_3, and CO_2 to the corresponding hydantoin.

Upon saponification of the nitrile group, these products yield either DL-hydroxymethionine or DL-methionine. Route (a) is preferred in the U.S. and routes (b) and (c) are preferred in other countries.

Unlike most other essential amino acids, methionine has the same biological value in the DL-modification and in the form of the α-hydroxy acid as in the L-modification (which latter form occurs in natural products).

This is due to the fact also that higher organisms can convert α-hydroxy- and D-methionine to L-methionine. Of most of the other amino acids, including lysine, only the L-modification is biologically active.

Lysine. Literature indicates many ways of preparing lysine. Sixty-five years ago the first successful lysine synthesis was carried out by the German chemist Fischer (1902). It would take too long to mention all the synthesis methods described in literature; we shall give instead an idea of the various principles on which such methods may be based. On account of the presence of the asymmetric α-carbon atom, two optical isomers can occur, the D-isomer and the L-isomer. As mentioned above, only the latter is biologically active. All chemical synthesis processes yield a mixture of D- and L-lysine, so that a resolution of the optical antipodes is necessary.

The principles of the synthesis of lysine fall into a number of categories, depending upon the way in which the C_6 chain is formed. The synthesis method can be based on the combination of two C_3 fragments, of a C_4 with a C_2 fragment, of a C_5 with a C_1 fragment, or the use of a C_6 product as starting material, in which case a further distinction may be made into open and cyclic products.

So far, only one of all these possibilities has reached the stage of technical realization. This process was developed by our company; work on it was started in 1959. The basic material we use, caprolactam, has many attractive aspects. In the first place it is very cheap and available in large amounts. Furthermore, the molecule already comprises three of the four structural elements of lysine: 6 carbon atoms and, potentially, the ϵ-amino group and the carboxylic acid function.

Introduction of the α-amino group is the only major problem. In the position where the substitution has to be made in the caprolactam ring there is insufficient reactivity. Therefore, the caprolactam must be modified in order to establish the right reactivity in the right place. This is done quantitatively (Figure 2) by reacting 1 mole of caprolactam with 2 moles of phosgene, with formation of 2-chloroazacycloheptene-*N*-carbochloride. This intermediate was originally nitrated in concentrated sulfuric acid. Although the yields were extremely high, the reaction mixture had some draw-

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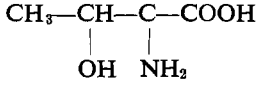
backs, such as high viscosity, thermal instability, and production of waste acid. These were successfully overcome by introduction of liquid sulfur dioxide as a solvent. The nitration product is subsequently hydrolyzed to DL-nitrolactam, which, in its turn, is hydrogenated to DL-α-aminocaprolactam. The simple hydrolysis yielding lysine is postponed until after the optical resolution step.

Optical Resolution. All synthesis routes result in a mixture of the optical antipodes. This racemic mixture must be resolved into the pure L-modification and the D-modification. The latter is racemized into the DL-mixture, which is recirculated to the main stream. So far, optical resolution has been realized on a large industrial scale only with lysine and glutamic acid. Two nonbiological procedures for optical resolution may be used, *viz.* crystallization of diastereoisomeric salts formed from the DL-mixture and an optically active acid, and selective crystallization from a salt of the DL-mixture and an optically inactive acid, through seeding with the pure L-salt.

In the case of lysine production, both lines are open to us. In the present lysine plant, diastereoisomeric salts are formed by reacting DL-aminolactam with L-pyrrolidonecarboxylic acid (a derivative of L-glutamic acid). Whereas in the classical procedure, equivalent amounts of DL-aminolactam and the L-acid have to be used, only half the amount of L-acid is needed here. Complete precipitation of the "L,L"-salt is effected, and, no DL-salt being formed, D-aminolactam remains in solution, ready for the racemization step. After isolation the "L,L"-salt is split up, by ion exchange, into L-aminolactam and L-pyrrolidonecarboxylic acid, the latter of which is recirculated. Finally, hydrolysis of L-aminolactam produces L-lysine, which is sold as the HCl-salt. The principal advantages of this resolution procedure are high selectivity and low consumption of raw materials. Of course, alternative synthetic routes to lysine have our attention. Therefore, other resolution procedures, based on the principle of selective crystallization of lysine salts, have been developed. Because free lysine itself shows poor crystallization characteristics, lysine salts formed with optically inactive acids are used. In the procedures meant here, a supersaturated solution of DL-lysine salt is seeded with crystals of the pure L-lysine salt, causing selective crystallization of L-lysine salt from the solution of the racemic form.

Threonine. α-Amino-β-hydroxybutyric acid has two asym-



metric C-atoms, and can therefore occur in four diastereomeric modifications, of which only the L-threo modification shows biological activity.

Among the many possible synthesis methods, only the one which offers the best prospects (for technical realization), *viz.* the condensation of acetaldehyde by means of the Cu-complex of glycine, will be discussed. This condensation was first described by Sato *et al.* (1957) (Figure 3).

With the aid of Na_2CO_3, the reaction can be effected in water at a temperature of 60° C. In an alkaline medium the DL-threonine complex is stabler than the DL-allothreonine (threo to allo ratio = 3 to 1). Under certain conditions, the difference in solubility between the threo- and allo-complexes offers the possibility of separating the two complexes immediately after the synthesis; at the same time it is possible to convert the DL-allothreonine to DL-threonine, *e.g.*, via the Cu-acetaldehyde complex. The resolution of DL-threonine can be done, *e.g.*, by selective crystallization.

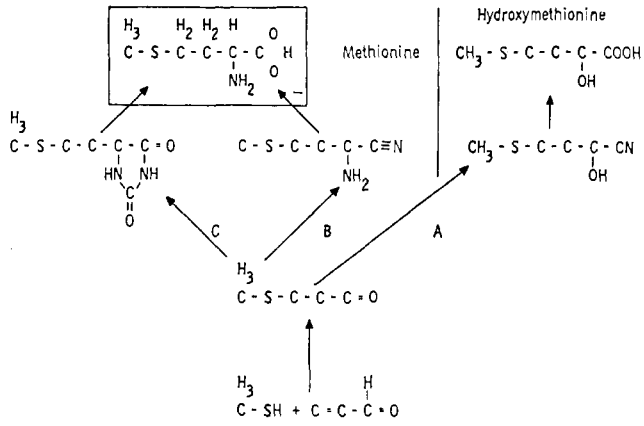


Figure 1. Commercial synthesis of methionine

Tryptophane. Also for the preparation of tryptophane, numerous methods have been described in literature. Many of them start from indole or indole derivatives. Some are based on the so-called Fischer indole synthesis and an ester condensation. However, none of these processes is suitable for technical realization. The Japanese firm of Ajinomoto uses β-cyanopropionaldehyde, which is obtained by an oxo-reaction from acrylonitrile, and is also used, for instance, in the synthesis of glutamic acid (Figure 4). This β-cyanopropionaldehyde is reacted to form cyanoethylhydantoin, which, upon selective hydrogenation in an acid medium, gives the corresponding aldehyde. Reaction of this aldehyde with phenylhydrazine produces phenylhydrazone. Ring closure in an acid medium, followed by alkaline hydrolysis, yields DL-tryptophane. The resolution of the racemate can be effected either through crystallization of salts of the diastereo acid, or through selective crystallization.

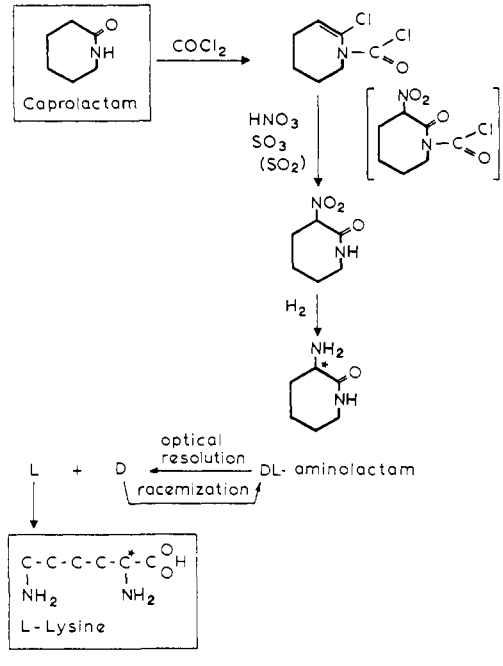


Figure 2. DSM lysine synthesis

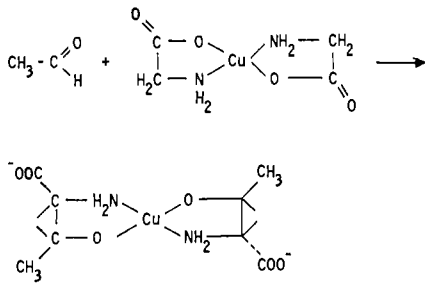


Figure 3. Synthesis of an active threonine Cu complex

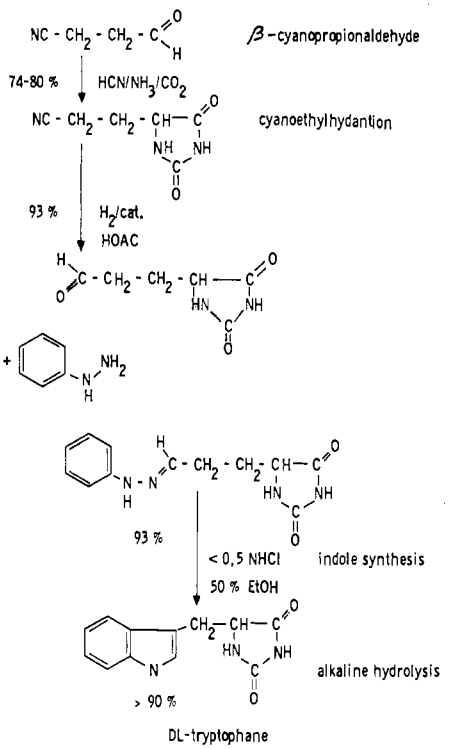


Figure 4. Preparation of tryptophane

PROSPECTS OF AMINO ACIDS IN FORTIFYING FOODS

Let us now turn to the second part of our paper, dealing with the prospects of pure amino acids as ingredients in fortifying foods. In doing so, we shall focus our attention on the problem of providing a rapidly expanding world population with enough and adequate food, and not consider the extent of the world food problem. The latter subject has been repeatedly dealt with in rather thorough studies. An excellent survey with recommendations was published recently by a Panel and some Subpanels of the President's Science Advisory Committee (Report, 1967) in the United States. An adequate solution of the food problem, of course, depends on a close interplay between agriculture—providing the basic foods—and the chemical industry producing the fortifying additives needed by man and animal. The use of these fortifiers will now be discussed from two points of view: nutritional and economic-technological.

Nutritional Aspects. The level of dietary protein required is lower as this protein is better adapted to the individual's needs, *i.e.*, as it is better balanced. An example in point is given in Figure 5, which is based on data given by Hegsted and Chang (1965) on protein utilization in rats, with various levels of protein intake from different protein sources. It is also possible to improve the nutritional value of the dietary protein by addition of the amino acid most deficient in comparison to the individual's need. Results on lysine supplementation of wheat protein found in our studies on rats are given in Figure 6, which shows that protein waste can be largely prevented by supplementing the diet with an adequate, even though rather small, quantity of the first limiting amino acid.

It is clear, then, that fortification of cereals with pure amino acids results in an improvement of protein quality, and hence in an increase of the amount of utilizable protein. While fortification of cereal proteins does not increase the total amount of protein consumed, more of this protein is made utilizable, and this has more or less the same effect as consuming more protein of poorer (biological) quality. Moreover, the fortification method offers the definite advantage of being an immediately effective procedure in covering the actual need for additional protein without requiring simultaneous changes in food habits or having adverse effects on the organoleptic properties of cereal foods. However, an effective attack on hunger and malnutrition should first of all prevent the occurrence of famine by correcting shortages in the total food supply, and next aim at preventing malnutrition. The question arises how the target areas can make optimal use of their agricultural potential to formulate adequate foods in terms of quantity and

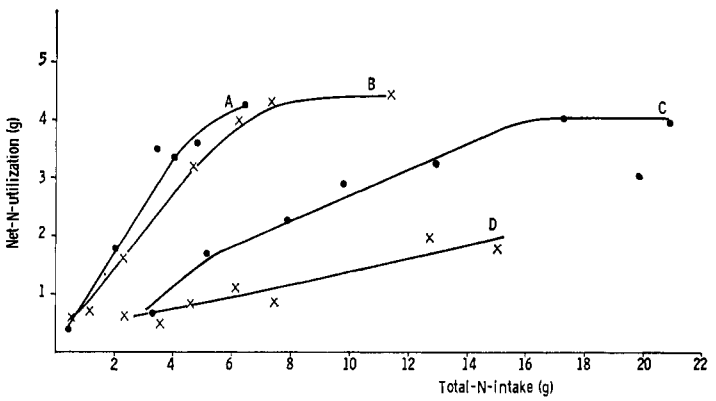


Figure 5. *N*-intake and net-*N*-utilization in rats on various levels and sources of dietary protein (Hegsted and Chang, 1965)

A = lactalbumine, B = casein (70), C = soy protein (34), D = wheat-gluten (18.7)

Table I. Efficiency in Animal Production

	Pigs	Broilers	Eggs/Hen/Year
Weight gain	80 kg	1.2 kg	...
Edible protein	10 kg	0.15 kg	1.5 kg
Protein intake	40 kg	0.5 kg	7.5 kg
Protein conversion	25%	30%	20%
Energy in edible product	125 Mcal	2.0 Mcal	20.0 Mcal
Energy intake	875 Mcal	8.0 Mcal	135.0 Mcal
Energy conversion	15%	25%	15%

Table II. 1966 World Production of Some Cereals and Vegetable Protein Sources[b]

	Area (10^6 ha)[a]		Production (10^6 t)[a]		Crop (t/ha)	
Wheat	217		308		1.42	
Corn	102	389	239	663	2.33	1.80
Barley	70		116		1.65	
Soybeans	31.3		39.0		1.24	
Sunflowerseed	7.8	70.6	9.1	67.8	1.16	1.01
Cottonseed	31.5		19.7		0.63	

[a] 1 ha = 2.471 acres; 1 ton = 1000 kgs. [b] FAO (1967).

Table III. Requirements for Energy and Protein

Age groups	Energy kcal/kg/day	Protein g/kg/day	Protein Energy g/100 kcal
½–1 yr	100	3.0	3.0
5 yr	80	2.5	3.0
15 yrs	65	2.0	3.0
Adult	40	1.0	2.5
	Present in Cereals		
Wheat	3275	120	3.7
Corn	3325	90	2.7
Rice	3500	75	2.1
	kcal/kg	g/kg	g/100 kcal

quality. This means that yield is evidently the primary aim at which agricultural production should be directed; if quality has to be sacrificed to quantity, compensating methods for upgrading the quality must be employed. From this point of view the conventional methods for producing foods from animal and plant origin will be discussed.

Animal Production. Although the nutritional quality of animal foods (*i.e.*, eggs, milk, meat, fish) must be rated high, animal production is intrinsically inefficient (Table I). Even with modern production methods, the conversion of feeds to animal products involves a waste of about 75% of the calories and proteins supplied. Therefore, foods of animal origin cannot provide a general solution to the world food problem. However, the unique ability of animals to convert products such as forages, byproducts, wastes, and other materials unfit for human consumption—even chemicals like urea—to high-quality food should be recognized and maximized.

Plant Production. Table II summarizes data on the 1966 world output of some cereals and vegetable protein sources. Among other things it appears that the average harvest of cereals from arable land is larger by approximately a factor of two than the average harvest of oil seeds from the same area. This striking difference may be attributed largely to differences in the amount of sunlight-energy required for the photosynthesis of carbohydrates on the one hand and proteins on the other. Therefore, even if all the environmental agents like water, fertilizers, and pesticides should be supplied in

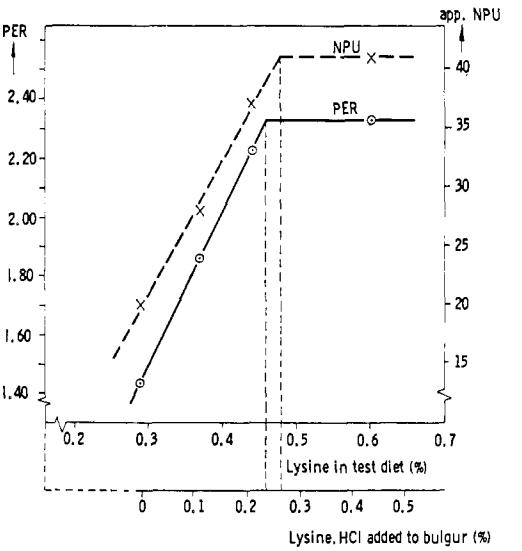


Figure 6. Lysine supplementation of bulgur (Jenneskens, 1969)

the most favorable quantities, and adequate efforts at genetic change should be used, cereal crops will be significantly larger than the yield of vegetable foods rich in protein (De Wit, 1968).

Although there are a few factors which may to some degree disturb the foregoing conclusions, one may tentatively state that cereal crop production should be strongly recommended as an effective approach to increasing the world food production.

However, cereals (particularly rice) have a low protein content. In Table III a comparison is made between the energy and protein contents of some cereals and the corresponding quantities required in human nutrition. It appears that notwithstanding their low protein content, cereals could almost completely eliminate the current protein shortage if their amino-acid composition were made to fit human requirements in an optimum way. The latter condition is not ful-

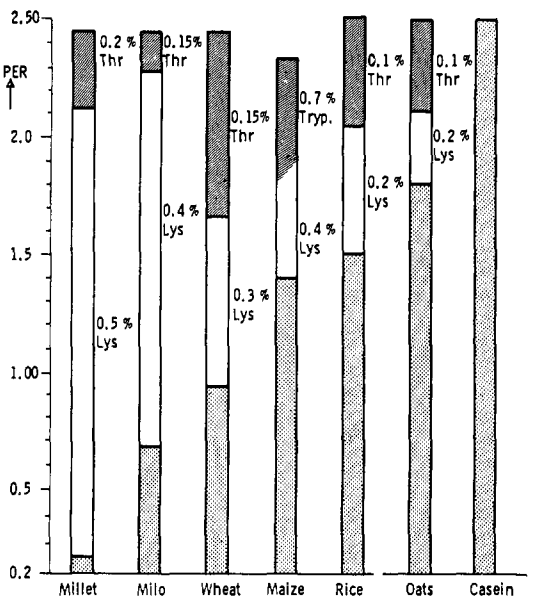


Figure 7. Nutritional effects in amino acid enrichment of cereal proteins

▨ = unsupplemental, ▨ = supplemental, □ = lysine supplemented (Howe *et al.*, 1965)

Table IV. Economic Evaluation of Cereal Protein Enrichment with Lysine and with Protein-Rich Foods

	1	2	3	4	5
Wheat flour[a] (gm)	542.00	542.00	507.00	506.00	535.00
Lysine.HCl[a] (gm)	...	1.09	...	...	...
Soybean flour[a] (gm)	...	...	29.00	...	...
Skim milkpowder[a] (gm)	...	...	...	36.1	...
Fish protein concentrate[a] (gm)	...	...	...	...	12.5
Total protein (gm)	49.9	51.0	61.1	59.6	58.3
Chemical score	26	48	41	42	42
Utilizable protein (gm)	**13.0**	**24.5**	**24.5**	**25.0**	**24.5**
Energy (kcal)	2000	2000	2000	2000	2000
Costs of protein enrichment (2 = 100)	...	100	145	245	172
Cost of protein vitamin mineral } Enrichment (2 = 100)	...	100	116	140	120

[a] Prices in U.S. $/100 kg: wheat flour, 7.30; lysine.HCl, 250.00; soybean flour, 22.50; skim milkpowder, 25.74; fish protein concentrate, 41.66.

Table V. Distribution of Lysine in Whole Wheat Containing 8.0% of Lysine after Impregnation

	Weight-fraction (in % of whole wheat)	Free-lysine-content (%)	Free lysine as percentage of lysine input
Bran	0.5	5.27	0.3
Groats	2.7	8.42	2.9
Middlings	4.5	6.50	3.7
Flour	92.3	7.99	93.1
	100.0		100.0

filled, however. It has been demonstrated in many studies that fortification with pure amino acids offers an effective procedure to raise the poor nutritional quality of cereal protein. This is illustrated in Figure 7, based on data given by Howe *et al.* (1965), which indicate that the high-rated score of animal protein can be equalized by property supplemented cereal protein.

Economic and Technological Aspects. An alternative approach to cereal fortification with pure amino acids familiar to all nutritionists is the combination of several protein sources in a complementary way. Since none of the methods developed for this purpose have been tested for their efficiency in large populations, a comparison can be made only on the basis of cost and technological feasibility.

Table IV compares the cost of cereal fortification with lysine *vs.* supplementation with protein concentrates. In all of the rations here compared, the caloric supply was kept constant at 2000 kcal. For this purpose the quantity of cereals in the diets was reduced in correspondence to the energy contribution of the protein sources. Within a cereal group approximately equal amounts of utilizable proteins are available. The results of these calculations demonstrate obviously lower costs for fortification with lysine. However, in addition a correction has to be made for the differences in vitamins and minerals, to ward off errors in our approach to equality of nutritional value. A more realistic comparison, including enrichment with vitamins and minerals up to the NAS/NRC (1964) recommendations, is also illustrated in Table III.

It may be concluded that lysine supplementation of cereals is still preferable to the alternative fortification methods. Moreover, with the present state of technology, fortification by mixing native protein sources can, on the whole, be done only for flours and textured foods. However, enrichment of cereals with amino acids (and whether or not with additional vitamins and minerals) can be done in various ways such as: dry mixing, impregnation of whole cereal grains, and solution in processing water (*e.g.*, with bakery products).

Although impregnation techniques for whole grains are more expensive than the other more simple processes, this method is not complicated. Results, as obtained by us with impregnation of whole wheat with an aqueous solution of lysine, are given in Table V. The lysine content of whole wheat can be increased up to 10%, while most of the lysine is recovered in the flour and not in the gluten fraction. Moreover, the appearance of the whole wheat is unchanged, and milling gives no problems.

SUMMARY

The present state of amino-acid production by chemical synthesis and fermentation permits enlargement of output volumes. In particular DL-methionine and L-lysine can be supplied on a commercial scale at competitive prices. Against the background of the world food problem, maximization of cereal production is the most important key towards eliminating current and future food shortages. Cereals supplied in amounts adequate to cover caloric needs can be substantially upgraded with pure amino acids (and vitamins/minerals). Lysine supplementation of cereals ranks first as an attractive way of improving cereal protein quality from both an economic and a technological point of view.

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