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Development of Chemical Investigations on Artificial and Synthetic Foods.

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I. INTRODUCTION

The idea that it was opportune for the start of practical studies on the production of food by industrial methods, avoiding agriculture, was suggested by A.N.Nesmeyanov at the beginning of 1961. This idea was based on the one hand on the work of the great chemists of the past—D.I. Mendeleev and M. Berthelot—and on the other, on an understanding of the contemporary state of the science of the physiology of nutrition and the achievements of the chemical industry[1]. Actually, at this time it appeared that man could satisfy all his physiological requirements by living exclusively on rations consisting of substances made synthetically. This gave a confidence in the knowledge of the main ingredients of food.

The great output of organic synthesis, which reaches millions of tonnes per annum in the production of polymeric products, made the question of the creation of a food products industry a real subject. Thus were all the proteins needed for feeding a country with a population of 250 million to be produced industrially it would be necessary to make about 6 million tonnes per annum.

Moreover, at this time the problem of the shortage of foodstuffs, especially proteins, became very acute. According to United Nations Organization data, about two-thirds of the population of the world are now in a state of chronic malnutrition, and often complete starvation[2].

No less alarm is aroused by the progressive destruction of the Earth's exposphere and the associated depletion of natural resources[3].

Hence in contrast to the XIXth century there were the need and real reasons for a practical solution of the problem of the production of synthetic food. There still remained one difficulty without overcoming which it would be impossible to think of the propaganda of new ways of obtaining food. This difficulty consists in the high cost of synthetic foodstuffs used for making synthetic rations. These substances—amino-acids, fatty acids, some vitamins—sold as reagents would be excessively dear. But economics should in the final analysis decide the choice between various methods for obtaining food. Furthermore it would be necessary to give the synthetic mixtures taste in the widest meaning of this word, including odour, taste itself, form, texture, etc.

To solve all these problems laboratory investigations were made under the guidance of A.N.Nesmeyanov in the Order of Lenin Institute of Organoelemental Compounds of the Academy of Sciences of the USSR into the production of edible amino-acids, proteins, and other foodstuffs, the study and reproduction of pleasant natural odours of prepared food, and the physical and chemical processes of structure formation. The need arose for the development of a series of theoretical and practical problems of biochemistry, organic, physical, and analytical chemistry and the foundations of a new branch of science—that of synthetic food—were really laid.

On comparing the various paths for the industrial production of foodstuffs that now most promising is microbiological synthesis. Nesmeyanov first understood the great potential possibilities of this method and under his initiative complex studies on the growing of yeasts on petroleum hydrocarbons were undertaken in the Soviet Union in 1963. As a result, by 1967 several experimental industrial plants for yeast bio-mass had been commissioned.

This bio-mass contains ~40% protein rich in amino-acids, as well as fats and vitamins. It is now being used as an animal food product but it is much more profitable to use these yeasts directly in human nutrition[3]. Naturally for this the beneficial constituents should be separated, freed from ingredients having doubtful nutritive value, and converted into an edible form with the necessary odour and taste.

The present review deals with the studies, mainly under the leadership of A.N.Nesmeyanov, directed to the solution of this problem.

2. PROTEINS AND NUTRITION

Most of the amino-acids needed for active life are obtained by man in the form of various proteins present in the products of plant and animal origin. The great protein deficiency makes the problem of increasing the effectiveness of the use of food proteins very acute and also that of acquiring new unusual sources of proteins which can be used for solving the problem of nutrition directly or through animal husbandry.

The first path—the use of proteins of unusual origin directly in human food—is the more promising, since shortening the nutritive chain increases the effectiveness of use of the proteins by a factor greater than five, that is, to bring about the same results much less protein must be made than in the production of protein food products by traditional methods via animal husbandry[1].

In addition, the separation from various sources of proteins with their simultaneous purification from extraneous substances, sometimes also harmful impurities, enables not only the biological value of the proteins when

used in nutrition to be increased but also food products to be obtained with the required amino-acid composition and with many other nutritive components of the necessary composition, taken in optimal amounts, that is, to convert the foodstuffs into a sufficiently powerful means of regulating the biochemical processes of the human organism.

Table 1. Principal oil-bearing crops.

Name	Mean protein content, %	Lipid content, %	World production, tonnes	Chief producing countries	Factors preventing use for food
Soya bean	42	20	35 000	U.S.A., China	inhibitors of trypsin, chemotrypsin, haemoglutinin, saponins
Groundnut	27	48	14 800	India, Senegal, Nigeria	aflotoxins, saponins
Cotton seed	30	30	20 600	U.S.A., India, U.S.S.R., Mexico, U.A.R., Brazil, Pakistan	gossypol, aflotoxins
Eastern sesame	25	50	1500	China, India, Sudan Mexico	oxalates, fibres
Sunflower seed	30	40	6840	U.S.S.R., Argentina, Uruguay, South Africa, Turkey	fibres

Apart from the usual animal and plant sources of proteins already recommended in nutrition, there is a large number of sources of sufficiently rich proteins, especially plant proteins, which are not widely used in nutrition owing to accompanying impurities. Table 1 gives information about the world production of oil-bearing crops containing large amounts of proteins, sufficiently rich in nutritive respects [4]. However, cotton seeds, which are rich in protein, contain the toxic substance gossypol, soya beans contain a series of growth inhibitors and inhibitors of digestive enzymes, castor plant seeds contain ricin and several allergins, and certain fruits contain glucosides which liberate cyanides and various inhibitors of biological processes [5]. Naturally, such protein products can be widely used in nutrition only after isolating the proteins and freeing them from the harmful impurities.

Table 2.

Components	*Torula*	*Candida tropicalis*	Components	*Torula*	*Candida tropicalis*
Dry substances, %	93.0	92.7	Vitamins, mg per kg		
Protein (total), %	34.0	43.6	thiamin (B_1)	80	3.16
Carbohydrates, %	34.0	21.0	riboflavin (B_2)	120	75
Fats, %	7.8	18.5	nicotinic acid (P-P)	800	180—200
Inorganic substances (ash), %	7.5	4.43	panthothenic acid (B_3)	47	150—192
			pyridoxin (B_6)	26	23

There are still richer sources of valuable proteins in the micro-world among the various algae, yeasts, and non-pathogenic micro-organisms [6-9]. Thus the widely distributed yeasts of the *Candida tropicalis* and *Torula* cultures, the basis for the growth of which are wastes from the alcohol industry and hydrocarbons, are rich sources of valuable proteins [10] (Table 2).

The exceptionally high rate of synthesis of proteins by micro-organisms and also the possibility of the organization of their production on a large scale by industrial methods offers great possibilities for rapidly increasing protein resources by microbiological means. The results given in Table 3 on the rate of accumulation of bio-masses by various organisms show that the rate of synthesis of proteins by micro-organisms is more than 1000 times the rate of synthesis of proteins by higher organisms.

Table 3. Time for a twofold increase in the biomass of growing organisms in the phase of maximal growth [11].

Organism	Time, h
Bacteria and yeasts	0.3—2
Fungi and chlorella	2—6
Grass and plants	144—288
Chicks	288—576
Piglets	576—864
Beef cattle	720—1500

It is noteworthy that unlike most plant proteins, which are often deficient in lysine, tryptophan, and methionine, the proteins which are synthesised by micro-organisms are considerably closer in composition to animal proteins and can be readily assimilated in an isolated state.

Despite repeated attempts to use various yeasts in human nutrition without separating the proteins from them, especially under unusual conditions (war, drought, etc.), microbiological products have not yet found wide application in nutrition without additional chemical treatment owing to several of their properties (flavour, odour, appearance).

One of the possible ways of using valuable foodstuffs which are present in microbiological raw material, and above all in various yeasts consists in the isolation from the hydrolysis products of the microbiological raw material of all the amino-acids suitable for use in human nutrition.

No less promising is the isolation from the micro-biological raw material of all the proteins it contains, their purification, and use directly in foods. In this instance all the factors (colour, odours, and extraneous flavour) which hinder the wide use of new sources of proteins in nutrition can be removed, which simultaneously increases the biological value of such proteins. For example, Mitsuda and coworkers [12] showed that the proteins isolated from *Micrococcus glutamicus* does not differ in amino-acid content from the protein of hen's eggs.

The amino-acid composition of the microbiological proteins isolated (Table 4) shows that they are much more balanced than plant proteins.

In determining the food value of the total proteins isolated by extraction with urea from a *Torula* strain it was found that the product isolated is assimilated and converted much better than entire yeast cells [13]. When chlorella with unbroken cells was used as a source of proteins similar results were obtained. Here it was found that > 40% of the nitrogen was not absorbed in the gastrointestinal tract [14].

The zooplankton of the Pacific Ocean can be a vast source of proteins [15], and their role for increasing the protein resources becomes real after the development of

accessible and economically favourable methods of separation and purification of proteins present in marine microorganisms.

The need for the thorough purification of microbiological proteins destined for food purposes is also confirmed by the investigation of the biological value and harmlessness of the protein–vitamin concentrate obtainable from yeasts cultivated on hydrocarbons and at present usable only as animal food [16,17].

Table 4. Amino-acid composition of proteins in % of the sum of the ten most important amino-acids.

Amino-acid	Products								
	meat (pork)	dried milk (fat-free)	whole egg	protein of *Micrococcus glutamicus*	protein of the yeast *Torula*	white bread	oatmeal	ground-nuts	corn-flakes
Arginine	11.8	6.0	10.3	14.4	—	9.5	12.3	30.1	4.4
Valine	10.7	13.3	12.1	14.1	8.8	15.9	13.1	9.7	12.2
Isoleucine	9.9	10.5	9.9	11.1	13.4	12.0	9.7	8.3	9.3
Leucine	18.4	19.5	15.9	17.3	18.2	20.4	19.0	14.7	39.2
Lysine	16.8	16.3	13.1	12.3	21.0	7.3	8.1	8.7	1.8
Methionine	5.5	4.9	6.1	3.8	1.2	3.8	4.6	2.1	4.9
Thionine	8.9	8.8	8.6	9.2	12.8	8.9	8.7	5.7	8.5
Tryptophan	2.8	3.1	2.9	2.8	2.4	3.7	2.8	2.6	1.5
Tyrosine	6.9	8.3	7.2	5.4	10.0	5.7	7.7	6.5	4.6
Phenylalanine	8.3	9.4	9.4	9.3	11.9	14.1	14.2	11.4	13.5

The extraction and purification of proteins from microbiological raw materials are also necessary for the subsequent creation from them of edible forms suitable for human beings. Most of the products for feeding human beings have a complex structure consisting of three main elements—fibres, films, and gels, which can be obtained and even now are being made for technical purposes in industry [18] from proteins which are polymers of α-amino-acids.

Proteins, like mixtures of amino-acids, are white powders, almost devoid of odour and taste, of little use in this form for food. However, the complex of physicochemical properties of proteins allows any structural elements of the usual nutritive forms to be obtained from them and new nutritive forms based on them to be created, as shown below.

As is well known, after fermentation and separation from the culture medium the bio-mass contains many substances of nutritive value: up to 40% (on the dry weight) of proteins, a certain amount of free amino-acids, fats, polysaccharides, vitamins, nucleic acids, and various other compounds. From the chemist's viewpoint a single-cell organism is a complex system consisting of polymers of three types and a large number of low-molecular-weight compounds entering both into the composition of cell walls, membranes, and other structural elements of cells and also found in protoplasm.

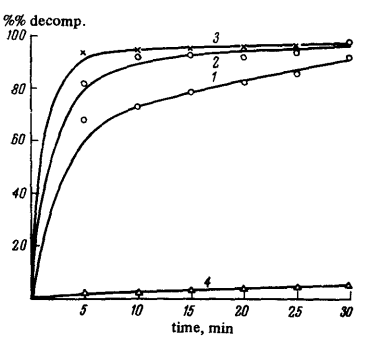
Owing to the semipermeable nature of the cell membranes and other structural formations of the cells, part of the low-molecular-weight compounds—inorganic salts, vitamins, free amino-acids, and part of the lipids—can be separated from the cell material without destroying the cell and its structural elements.

In accordance with their chemical nature, proteins usually dissolve comparatively readily in water or aqueous salt solutions at various pH values, depending on the nature of the protein, and the resulting solutions can be isolated pure. However, owing to the insolubility of the outer sheath of unicellular organisms and the polymeric nature of the proteins their isolation in a pure form without destruction of the cell membranes is almost impossible.

In the literature there are several descriptions of methods of extracting native proteins from microbiological material in which the foremost aim pursued was to retain the enzymic activity of the proteins, their supermolecular structure, and also to obtain narrow protein fractions and, if possible, individual proteins [19-21]. These methods are usually distinguished by low reproducibility, complexity of the technical procedure, and low suitability for obtaining proteins in large amounts.

By using experience on the production of finely disperse aluminium for the synthesis of Ziegler catalysts for the polymerisation of ethylene, success was achieved in creating a method of breaking down the cell material in sufficiently large amounts (1 kg of dry material per operation). As a result of a large number of experiments it was found that at 10–15°C there is almost complete destruction of the cell walls in 15–20 min.

It was found that the effectiveness of the destruction of the cell walls depends on the nature of the medium in which the destruction is carried out; thus the Figure shows the change in the degree of destruction of the cell walls, calculated from the results of electron microscopy in different media depending on the time and temperature.



1) Hexane +7°C; *2*) hexanes −70°C; *3*) methylene chloride −70°C; *4*) water, +2°C.

As the Figure shows, water is quite unusable for these purposes. The most suitable medium for the destruction of the cell walls was methylene dichloride, widely employed in the production of acetate silk. The destruction is just as effective in other low-boiling dehydrated organic solvents: diethyl ether, acetone, alcohol, etc. The great merit of the method is its universality which allows proteins to be obtained from various microbiological and plant sources.

The destruction of cell membranes in methylene dichloride, in which alkanes and lipids dissolve readily, is accompanied by effective extraction of fats and hydrocarbons from the microbiological material and also of fat-soluble vitamins, which can then be isolated from the extractant and used. Complete extraction of the lipids greatly facilitates the further separation of the proteins and ensures a higher degree of purity of the final product.

After thorough removal of the organic solvent the broken-down cell material can be used for the isolation of nucleic acids and proteins.

Nucleic acids, which as a result of enzymolysis are converted into nucleotides [22] from which sodium inosinate and guanylate—the best taste-developing additives—are removed in the form of nucleoprotides by extraction with 10% NaCl. The proteins are transferred into the solution after extraction of the nucleic acids by treatment of the disrupted cell material with dilute alkali solutions (0.2M NaOH) and are purified by reprecipitation with hydrochloric or acetic acid or by electrolysis. The residues of the cell walls and other structural fragments of the cells in the isolation of the nucleic acids and proteins are separated from the solutions by centrifuging or filtration.

Table 5. Amino-acid composition of microbiological proteins and proteins of animal origin.

Amino-acid	Mouse actin	Casein	Protein of the yeast *Candida tropicalis*	Protein from *Saccharomyces cerevisiae*	Protein from *Micobacterium rubrum*	Protein from chlorella
Alanine	5.7	3.4	5.7	6.2	8.2	7.0
Arginine	5.9	3.9	3.9	4.2	8.1	4.1
Aspartic acid	9.8	7.6	10.6	10.0	7.4	7.8
Valine	4.4	5.7	5.4	5.5	7.0	3.4
Histidine	2.3	2.6	2.2	2.1	1.6	1.2
Glutamic acid	13.3	20.3	11.5	12.6	12.2	9.6
Glycine	4.7	2.1	4.2	4.4	4.0	4.7
Isoleucine	6.8	5.8	5.7	5.4	3.9	2.3
Leucine	7.4	7.1	8.8	6.9	7.3	5.3
Lysine	6.8	8.1	7.2	9.2	3.2	5.7
Methionine	4.0	2.3	2.2	1.2	1.6	1.8
Proline	4.6	6.9	3.6	4.0	2.2	3.6
Serine	5.3	5.7	3.8	5.2	2.5	3.5
Tyrosine	5.2	7.3	4.4	3.6	1.9	2.9
Threonine	6.3	4.4	4.1	5.4	4.1	4.1
Tryptophan	1.8	1.5	1.5	1.3	1.2	1.3
Phenylalanine	4.3	4.1	5.4	4.7	3.5	3.0

Note: The data for mouse action and casein are given in the Ajinomoto catalogue, Part 4, 1965. The amino-acid analysis of the microbiological proteins was obtained on an automatic analyser of Czechoslovak manufacture. The results for the last two products are rather low, owing to their containing carbohydrate impurities.

The yield of proteins in laboratory experiments is 65–75% (25–29% of the dry weight) at a purity of 80–95%. The protein products obtained do not contain mineral impurities (ash < 0.5%) or nucleic acids (traces of phosphorus) or organic impurities of a lipid or hydrocarbon nature. The only impurity in the product is 10–15% oligosaccharides entering into the composition of the cell walls, which can be removed by additional purification if necessary.

The amino-acid composition of the total proteins obtained (see Table 5), especially from yeast cultures, shows the exceptionally high value as a future source of rich nutritive proteins, since they are very similar in composition to the best proteins of animal origin (proteins of milk, meat, etc.).

The determination of the concentration in the initial material and in the liberated proteins of the three limiting amino-acids—tryptophan, methionine, and lysine—and also the determination in them of the available lysine convincingly confirms that in the separation and purification the biological value of the microbiological proteins is greatly increased (Table 6).

Table 6. Amino-acid contents of microbiological raw material and of the proteins isolated, obtained on its basis.

Name of initial material	Tryptophan, % calculated on proteins		Methionine, % calculated on proteins		Total lysine, %, calculated on proteins		Available lysine %, calculated on proteins		Ratio of available to total lysine	
	initial material	total proteins	initial material	total proteins	initial material	total proteins	initial material	total proteins	initial material	total proteins
Candida tropicalis	0.88	1.52	1.33	2.10	6.60	7.40	5.47	6.82	0.82	0.92
Saccharomyces cerevisiae	0.93	1.27	1.37	1.75	8.78	9.20	6.25	7.92	0.71	0.86
Micobacterium rubrum	0.83	1.20	1.42	1.82	4.51	5.18	3.16	3.59	0.70	0.69
Chlorella	1.51	1.53	1.98	2.10	7.53	5.30	4.52	4.55	0.60	0.85

It is quite natural that the possibility of using such isolated proteins directly for human nutrition will be determined after detailed biomedical investigations both of proteins themselves and of new food products based on their use. In their physicochemical properties the isolated proteins from yeast cultures are very similar to casein and completely suitable for obtaining granulated food products of the artificial-albumen caviar type, the method for making which was developed in the Institute of Organoelemental Compounds. The specimens of black granular roe obtained by using yeast proteins did not differ in either physico-mechanical or organoleptic properties from the roe obtained from milk casein.

3. AMINO-ACIDS

In recent years important results have been obtained in studying human nutrition with mixtures of amino-acids, although much still remains to be done. It seems definite that in maintaining the nitrogen balance of the human organism it is immaterial whether the person is fed with proteins or the mixtures of the constituent amino-acids.

Table 7. Amino-acid composition of a diet.

Amino-acid	Quantity, g day^{-1}	Amino-acid	Quantity, g day^{-1}
Isoleucine	1.78	Phenylalanine	2.42
Leucine	2.60	Tyrosine	0.66
Lysine	3.60	Threonine	1.21
Methionine	1.88	Tryptophan	0.62
Cysteine	0.31	Valine	1.78

Many investigations on the prolonged feeding of people with synthetic diets have been published recently [23-28]. As an example there is the amino-acid composition of the diet [23], used for feeding 22–25 year old men (Table 7). The remaining (replaceable) nitrogen was given in the form of a 1 : 1 (by weight) mixture of glycine and diammonium citrate. Evidently this mixture is the best source of exchangeable nitrogen [29-31].

The use of such diets no longer in medicine but for food purposes can be limited on the one hand, by the need for their texturing and culinary treatment, and on the other by the possible high cost of individual natural amino-acids. As regards the texturing, the studies which will

be described below have shown that this obstacle can be overcome. As regards cost, progress in the synthesis of amino-acids achieved in recent years gives a basis for hoping that there will be no significant difficulties here.

A very promising method for obtaining mixtures of edible amino-acids is by growing unicellular micro-organisms (usually yeasts on carbohydrate or hydrocarbon raw material), hydrolysis of the proteins thus obtained to amino-acids, and their isolation in pure form.

The growing of yeasts has now been developed very well and is carried out on an industrial scale. The bio-mass thus obtained contains ~40% proteins. The action of proteolytic enzymes on this bio-mass leads to the hydrolysis of the protein molecules [32].

The sum of chromatographically pure amino-acids can be isolated from the product thus obtained. To do this displacement ion-exchange chromatography is used [33]. The solution is passed successively through cation-exchange and anion-exchange resins and the amino-acids, as amphoteric compounds, are separated from all the other impurities which are not amphoteric. The product is a white powder completely soluble in water. The amino-acid composition corresponds to the amino-acid composition of the initial enzyme hydrolysate. The change of the amino-acid composition in the desired direction can be brought about by addition of a little of the pure amino-acids. In this form the product can be used as a basis for synthetic meat and other edible products, for constituents of synthetic diets, and also in various branches of medicine.

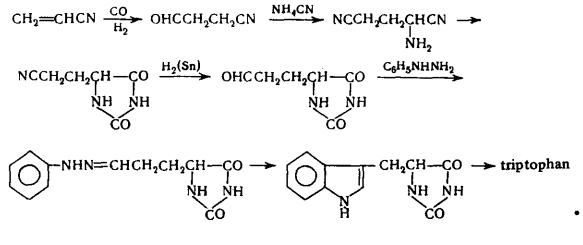
Many amino-acids are obtained on an industrial scale by microbiological means, by growing microbes which produce certain amino-acids on culture medium. The best results are obtained for *L*-glutamic acid and *L*-lysine [34]. The raw material usually employed is glucose or the cheaper molasses with the addition of ammonium salts as a source of nitrogen and of other salts required by the micro-organisms. However, there are results showing that alkanes can also serve as a source of carbon for the micro-organisms which produce amino-acids [35]. The most important precursor of glutamic acid—α-ketoglutaric acid—is isolated in the medium on growing certain strains of yeasts on a mixture of normal hydrocarbons of composition C_{14}–C_{26}. More than 800 g ketoglutaric acid are obtained from 1 kg alkanes [36].

In several instances a purely chemical synthesis is also economically suitable. An example is the production of *L*-lysine from caprolactam, effected by the Dutch firm of Stamicarbon on an industrial scale [37].

The industrial production of methionine in the USSR is based on the following scheme [38]:

$$CH_2{=}CHCHO + CH_3SH \rightarrow CH_3SCH_2CH_2CHO \xrightarrow{NH_3,KCN} CH_3SCH_2CH_2CHCN \rightarrow$$
$$\rightarrow CH_3SCH_2CH_2CHCOOH \quad (NH_2)$$

In Japan there is a combined method for obtaining glutamic acid and tryptophan from acrylonitrile [39]. The part relating to tryptophan includes the following stage:



As regards threonine, the best method for making it can be considered to be the Akabori synthesis carried out on an industrial scale in Japan. The reaction consists in the interaction of copper glycinate with acetaldehyde in alkaline medium [40]. As our last investigations showed [41], a necessary condition for the occurrence of this reaction is the formation of a Schiff base, since if *L*-alanine is introduced into the reaction instead of glycine the rate of racemisation of its copper complex is very greatly increased in the presence of acetaldehyde and the methyl-threonine obtained is always racemised.

On the other hand, *NN*-dimethyl-*L*-alanine not only does not racemise in the presence of acetaldehyde but does not even give a hydroxyamino-acid.

In 1957 Nesmeyanov and Freidlin developed a new method of synthesis of ω- and α-halogeno-acids on the base of telomerisation. The amination of these compounds gives the most varied aliphatic, aromatic, and heterocyclic amino-acids [42].

Several new syntheses of the most important unsubstituted amino-acids have been described recently [43]. The most promising for industry of the synthesis is based on aliphatic nitro-compounds. The key substance in all these syntheses is nitromethane—a cheap and accessible substance. Nitromethane can be readily converted [44] into methyl nitroacetate, $O_2NCH_2CO_2CH_3$, a very reactive compound having a carboxyl group and a potential amino-group.

In the Institute of Organoelemental Compounds of the Academy of Sciences of the USSR and the Leningrad Pedagogic Institute syntheses of amino-acids from the nitroacetate have been developed, based on condensation with aldehydes, addition to an activated double bond, and alkylation of alkyl halides. Thus in the condensation of nitroacetic ester with acetaldehyde in the presence of catalytic amounts of sodium acetate α-nitro-β-hydroxybutyric ester is formed. Quantitative yield of the reaction product is observed when it is carried out in the absence of a solvent and at 10–12°C.

$$CH_3CHO + O_2N{-}CH_2COOCH_3 \xrightarrow{CH_3COONa} CH_3{-}CH(OH){-}CH(NO_2){-}COOCH_3 \xrightarrow[CH_3COOH]{H_2(Pd/C)}$$
$$\rightarrow CH_3{-}CH(OH){-}CH(NH_2){-}COOCH_3 \xrightarrow{H^+} DL\text{-threonine.}$$

The reduction of α-nitro- β-hydroxybutyric ester is effected over Pd catalyst in an autoclave [45].

Other α-amino-acids (leucine [46], phenylalanine [47], etc.) are also obtained when almost the same sequence of reactions is used:

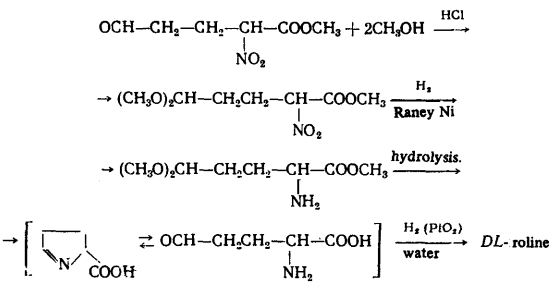
$$R{-}CHO + O_2N{-}CH_2{-}COOCH_3 \rightarrow R{-}CH(OH){-}CH(NO_2){-}COOCH_3 \xrightarrow{-H_2O}$$
$$\rightarrow R{-}CH{=}C(NO_2){-}COOCH_3 \xrightarrow[2)\ \text{hydrolysis}]{1)\ H_2\ (\text{Raney Ni})} R{-}CH_2{-}CH(NH_2){-}COOH\,.$$

The addition of methyl nitroacetate to an activated double bond in the presence of bases was proposed as the basis of the synthesis of *DL*-proline [48]:

$$O_2N{-}CH_2COOCH_3 + CH_2{=}CH{-}CHO \rightarrow OCH{-}CH_2CH_2{-}CH(NO_2){-}COOCH_3\,.$$

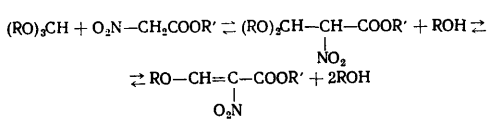
It appeared that when the addition product is hydrogenated over Raney nickel catalyst *DL*-proline is formed, but in low yield and badly contaminated with impurities. This made necessary the preliminary protection of the aldehyde group, which under conditions of reducing alkylation can take part in side-reactions.

The final scheme for making *DL*-proline free from impurities is:

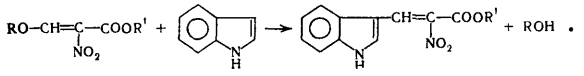


An intermediate in the synthesis of *DL*-proline is Δ^1-pyrrolidonecarboxylic acid which is known to participate in the biosynthesis of proline.

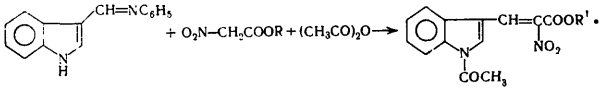
An original method for the synthesis of *DL*-tryptophan was also recently developed[49]. It is based on the condensation of indole with esters of β-alkoxy-substituted α-nitroacrylic acids. The latter is now available and readily obtained by the reaction of a nitroacetic ester with esters of orthoformic acid:



The reaction of indole with α-nitro-β-alkoxyacrylic esters takes place very smoothly and in the absence of catalysts and solvents it leads to an ester of α-nitro-β-(3-indolyl)acrylic acid:

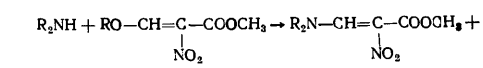


N-Acyl derivative of the same ester was obtained in the reaction of the anil of indolaldehyde with a nitroacetic ester in acetic anhydride[50]:



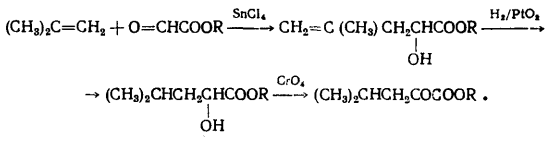
The reduction of both esters and the hydrolysis led to *DL*-tryptophan.

The reaction of α-nitro-β-alkoxyacrylic esters with amines takes place similarly to the reaction with indol[51]. Various substituted esters of β-amino-α-nitroacrylic acid—precursors of αβ-diamino-acids—are formed thereby:



One of the most important diamino-acids—lysine—was obtained by the addition of nitroacetic ester to nitrobutadiene followed by hydrolysis and saponification[52]. The synthesis of *DL*-leucine[53] and *DL*-glutamic acid[54-56] from nitroacetic ester was also described. The path to leucine goes through the alkylation with an alkyl halide, and glutamic acid is obtained after addition to an acrylic ester.

The problem of how much α-amino-acids can be replaced in the diet by the correspondingly constructed α-hydroxy- and α-keto-acids is very interesting. In the last example there is additional interest in that α-keto-acids do not have an asymmetric α-carbon atom. In this connection mention must be made of the interesting synthesis of a keto-analogue of leucine[58,59], by the scheme:



Naturally racemic amino-acids are obtained in the chemical synthesis. The examples of asymmetric synthesis described so far have gone no further than laboratory investigations[57].

The food value of the racemates of amino-acids is naturally lower than that of the natural optically active forms.

The most promising way of resolving the racemates of α-amino-acids is the use of optically active ion-exchange resins, which have become available as the result of investigations conducted in the Institute of Organoelemental Compounds of the Academy of Sciences of the USSR[60]. On the basis of copolymers of styrene with divinylbenzene having a three-dimensional lattice structure of varying density according to the divinylbenzene content, dissymmetric resins containing various optically active compounds (*L*- and *D*- forms of α-amino-acids, their esters and amides, optically active amines, etc.) as ionogenic groupings with an exchange capacity of 3–3.5 mg-equiv. litre^{-1} were synthesised.

On using such exchangers for the resolution of α-amino-acid racemates there is strong enrichment of the eluates by one of the atipodes and in some instances their complete separation[61]. Continuation of the investigations in this direction undoubtedly provides a suitable method of isolating both the *L*- and *D*-isomers of α-amino-acids which gives this method great advantages over others. The *D*-isomers of the *L*-amino-acids thus obtained should find application for carrying out biomedical investigations; after racemisation they can be returned to the separation which allows the total quantity of synthetic α-amino-acids to be used for food purposes.

As regards the analysis of the optical purity of mixtures of amino-acids polarimetric methods are suitable for solving the problems posed by the specific features of the method itself (additivity of total rotation, low sensitivity, and accuracy). It has been shown[62-64] that several compounds which contain asymmetric carbon atoms (amino-acids, hydroxy-acids, alcohols) can be converted into diastereoisomeric esters as a result of the treatment by a corresponding optically active reagent.

The diastereoisomers obtained are chromatographed in the gas phase. This gives a satisfactory separation, both between the stereoisomers and from other substances, including optically active ones. The possibility of a quantitative analysis is determined by the optical purity of the second component used for obtaining the diastereoisomers. Hence it is easy to reach an accuracy exceeding 0.5 rel.% at a sensitivity (in the sense of the possibility of discovering an opposite enantiomer) of 0.2 rel.%[65].

These principles were used in the analysis of protein enzymolysates and hydrolysates. It was shown that the main mass of amino-acids which are present in the enzyme hydrolysate of yeasts are the natural *L*-isomers. The gas chromatographic determination of the ratio of the optical isomers was first used in studying the Akabori reaction. It was found that the copper complex of *L*-alanine reacts with an excess of acetaldehyde and racemic *threo*- and *erythro*-methylthreonines are formed.

The rate of racemisation of the initial substance was less than the rate of formation of the final racemic products. The total experimental results suggested that the reaction proceeds through the copper complex of the imine (Schiff base) which is rapidly racemised by the formation of a carbanion, which reacts with acetaldehyde [41].

4. CHEMISTRY OF ODOUR AND TASTE

The successful progress of the work on the synthesis of food products made it necessary to start investigations of the chemistry of odour and taste in 1963.

Like every purified polymer, the protein constituting the basis of the artificial food has hardly any odour or taste. To make it appetising, it must be given the texture, appearance, flavour, and odour of some particular food product.

The taste of most natural products can be imitated by combinations of four dominating taste sensations, acid, salt, hot, and sweet.† The necessary taste sensations can be matched by combinations of chemicals. The use of natural flavour intensifiers such as sodium *L*-glutamate [69], disodium inosine-5'-monophosphate and disodium guanosine-5'-monophosphate [70] and various others greatly improves the flavour of the product.

The imitation of odour is one of the most complex tasks in the problem of creating synthetic food. Unfortunately it is still impossible to synthesise aromatic compositions with given properties. Attempts to create a theory of odour [71-85] have been made repeatedly [71-85]. That which is best known is the stereochemical theory of Amoore, in which a geometric correspondence of the form and dimensions of the molecules with definite vacancies on the surface of the receptor was postulated. A classification of odours into seven types was proposed: flowery, ethereal, camphor, musky, minty, acrid, and putrid [76,77]. This theory was subjected to thorough criticism [86,87] which was admitted to be correct by Amoore himself, and is now abandoned.

Dyson and Wright's theory [78-82] had many supporters. Wright thought that the sensation of odour arises as a result of vibrational movements proper to the molecules of the odorant and sought to find a correlation between the infrared spectrum of a molecule in the 500–50 cm^{-1} range and its odour. However, under the pressure of the experimental facts Wright admitted that the specificity of the odour could not be connected with the characteristic frequencies of the molecule in the far infrared range [88].

The most promising theory at present is the "membrane puncture" theory developed by Davies and Taylor [83-85] by analogy with the theory of flavour proposed earlier by Beidler [66,89]. According to this theory the corresponding impulse of an olfactory receptor can be the result only of an energetically rich process therefore it is improbable that the primary source of the impulse would be the energy of adsorption or dissolution. The mechanism of the penetration of the odorant to the receptor surface includes the adsorption of the odorant molecule from the mucus surface layer which covers the olfactory surface, then diffusion through the water-mucus layer and adsorption on the surface of the membrane of the olfactory cells, its phosphate-lipid protein layer. The adsorption on the surface of the cell membranes leads to the breakdown of the ordering of its surface layer. This brings about a change in potential existing between the wall of the neurone and the external medium. An ionic current arises because of the transfer of K^+ and Na^+ ions and consequently an electrical impulse, that is, a response to the odorant.

However, this theory is still insufficiently developed for the prediction of odour on the basis of the physicochemical properties of molecules. In connection with this, the starting point in the creation of aromatic compositions is the interpretation of the odour of natural food products [90].

The concentration of odour in a food product is negligible; it usually amounts to a few parts per million, and the number of components often exceeds 200. Therefore an investigation of the nature of odour is unthinkable without specially fine methods of analysis.

In the Institute of Organoelemental Compounds of the Academy of Sciences of the USSR the task was attempted of developing standard free gas chromatographic methods of identifying various classes of organic substances. For this purpose it was necessary to find a relation between the gas-chromatographic behaviour and the structure of the compound. Such a task could be solved only if well reproducible gas-chromatographic parameters existed. The Kovats retention index [91] was first used for the identification of the components of odour; it has good reproducibility [92] and can be used successfully in work on polar stationary phases, even such as ββ′-hydroxypropionitrile [93].

The investigations made showed that there is a marked relation between the index and the structure of the compound investigated, namely the number of carbon atoms, the number of functional groups, and the boiling point. The existence of such regularities gives the possibility of predicting gas chromatographic parameters and consequently of identifying a substance in the absence of a standard.

As an example there is the standard-free method of identifying n-aliphatic amines [94-96], which is now unique in studying the amines in an odour. An analysis of a mixture of primary, secondary, and tertiary aliphatic amines is among the most difficult of gas chromatographic tasks owing to their adsorption on solid carriers and frequent instances of the superposition of chromatographic zones.

This problem was solved by using the known ability of primary and secondary amines to form a hydrogen bond with solid alkali carried on a sorbent [97,98]. The heat of formation of a hydrogen bond found from the chromatographic results and equal for primary amines to $\Delta H = -6.3$ kcal $mole^{-1}$ and the secondary to $\Delta H = -3.6$ kcal $mole^{-1}$ includes a considerable contribution to the value of the index. The retention indices of the tertiary amines on various columns changes slightly.

Further study of the gas chromatographic behaviour of amines allowed a series of equations to be found (Tables 8 and 9) connecting the index with the physicochemical properties of the amines.

Table 9 gives equations for the variation of the index with the boiling point for the primary aliphatic amines. For secondary and tertiary n-aliphatic amines such a relation is observed only on a column with liquid paraffin +2% KOH. The equation has the form $I^{lp}_{100} = 4.0\ (t_k + 85)$ for secondary and $I^{lp}_{100} = 4.0\ (t_k + 79)$ for tertiary amines.

† Apart from the taste sensations listed there are various others, for example, cold, pungent, caustic, metallic, soapy, etc. The existing idea of the presence of only four taste sensations and of four corresponding receptors is inaccurate. In reality each taste receptor has a specific spectrum of responses to tasty substances [66-68].

Optimal conditions were found similarly for the gas chromatographic analysis of mixtures of n-aldehydes, methyl ketones, and α-unsaturated aldehydes[99]. The data needed for the identification are given in Tables 10 and 11.

Table 8. Equations connecting the retention indices with the number of carbon atoms in the molecule of aliphatic amines of normal structure*.

Primary amines, $n \geqslant 2$	Secondary amines, $n \geqslant 4$	Tertiary amines, $n \geqslant 9$
$I^{ts}_{100}=100n+283$	$I^{ts}_{100}=100n+176$	$I^{ts}_{100}=85n+162$
$I^{lp}_{100}=100n+386$	$I^{lp}_{100}=100n+176$	$I^{lp}_{100}=86n+146$
$I^{tw}_{100}=100n+461$	$I^{tw}_{100}=100n+266$	$I^{tw}_{100}=85n+183$
$I^{peg}_{100}=100n+568$	$I^{peg}_{100}=100n+330$	$I^{peg}_{100}=85n+200$

*n is the number of carbon atoms in the amine; I^{ts}_{100}, I^{tw}_{100}, I^{peg}_{100} and I^{lp}_{100} are the indices of the amines at a temperature of 100° in columns with tristearin (ts), Tween 80 (tw), polyethylene glycol-1000 (peg), and 5% liquid paraffin +2%KOH (lp) on "Novator" detergent.

In the general form the scheme of analysis leads to the following: after the concentration of the volatile substances from the food product a definite class of organic compounds is extracted with the aid of chemical reactions. For the gas chromatographic analysis of the mixture of substances of each class a system of three or four columns of different polarity was selected to rule out errors due to the superposition of peaks on the chromatograms. The subsequent identification was made on the basis of the values of the indices and their relation with the number of carbon atoms and boiling point of the test substances.

Table 9. Variation of the retention indices with the boiling point of the amine (t_b).

Equation	Amine	t_b, °C, calculated from I^{ts}_{100}	I^{tw}_{100}	I^{peg}_{100}	t_b, °C (published data)
$I^{ts}_{100}=4.0\ (t_b+91)$	propylamine	54	55	55	45—50
	butylamine	80	80	80	78—79
	pentylamine	104	105	105	104
$I^{peg}_{100}=4.0\ (t_b+116)$	hexylamine	131	130	130	130
	heptylamine	155	155	155	155
$I^{tw}_{100}=4.0\ (t_b+135)$	isopropylamine	35			33—34
	isobutylamine	69			68—69
$I^{peg}_{100}=4.0\ (t+164)$	isopentylamine	96			95
	vinylamine	56			56
	allylamine	54			53

The extract of the odour usually contains many organic substances belonging to different homologous series. In this instance the study of the relation between the index and the structure of the compound assists the identification. The additivity of the contributions reflecting the specific interaction of each functional group with the substrate of the column was established. It appeared that if a substance has several functional groups the total contribution to the value of the index will be equal to the sum of the contributions of all the functional groups. Table 12 gives the equations found for the calculation of the indices of the substances with several functional groups.

Table 10. Equations connecting the retention indices with the number of hydrocarbon atoms in the molecule of monocarboxylic acids*.

Alkanals	2-Alkenals	Methyl ketones
$I^{apm}_{125}=100n+188$	$I^{apm}_{125}=100n+251$	$I^{apm}_{125}=100n+171$
$I^{pega}_{125}=100n+555$	$I^{pega}_{125}=97n+732$	$I^{pega}_{125}=100n+558$
$I^{apm}_{50}=100n+179$	$I^{apm}_{50}=100n+243$	$I^{apm}_{50}=100n+167$
$I^{\beta\beta'}_{50}=82n+820$	$I^{\beta\beta'}_{50}=81n+993$	$I^{\beta\beta'}_{50}=81n+856$

*n = number of carbon atoms in the compound; I^{y}_{x}, where x is the temperature of the analysis and y the liquid phase: Apiezon-M (apm), polyethylene glycol adipate (pega), and hydroxydipropionitrile ($\beta\beta'$) on celite.

In an analysis on a polar column the contribution to the index which characterises a functional group rises sharply whereas the contribution introduced by the peculiarities of the structure of the carbon skeleton does not change appreciably. In this connection a very useful quantity for the identification is ΔI, the difference between the indices of a single substance on polar and non-polar columns. ΔI estimates the contribution of a functional group to the value of the index.

Table 11. Variation of the retention indices with the boiling point of monocarboxylic compounds.

Equation	Aldehydes and methyl ketones	Calculated t_b, °C from I^{apm}_{125}	I^{dif*}_{125}	I^{pega}_{125}	I^{apm}_{50}	$I^{\beta\beta'}_{50}$	t_b, °C (published data)
$I^{apm}_{125}=3.8\ (t_b+78)$	C_2-al	24	15	29	24	25	20
$I^{dif}_{125}=3.8\ (t_b+104)$	C_3-al	50	50	49	50.5	51	49.5
	C_4-al	76	75	74	76	75	75.7
$I^{pega}_{125}=3.8\ (t_b+175)$	C_5-al	103	103	103	103	101	103
	C_6-al	129	130	129	130	126	129
$I^{\beta\beta'}_{50}=3.4\ (t_b+260)$	C_7-al	156	157	156	156	151	155
$I^{apm}_{125}=4.2\ (t_b+58)$	C_3-one	57	56	63	52	61	56
$I^{dif}_{125}=4.2\ (t_b+84)$	C_4-one	80	80	83	78	82	79.6
	C_5-one	101	101	102	101.5	101	101
$I^{pega}_{125}=4.2\ (t_b+154)$	C_6-one	127	126	125	128	127	127.5
$I^{apm}_{50}=3.8\ (t_b+74)$	C_7-one	150	150	149	154	152	151.5
$I^{\beta\beta'}_{50}=3.25(t_b+285)$	C_8-one	173	175	173		176	173

*dnp = dinonyl phthalate.

From Table 13, clearly despite the difference in the value of the indices of the substances of normal and isostructure the value of ΔI for a single functional group is almost the same.

A comparison of the results of an analysis on two phases—polar and non-polar immediately gives the possibility of finding a substance with the same functional group.

A knowledge of the functional group in turn allows the number of carbon atoms (n) and boiling point of the compound being determined to be found, by using the appropriate equation.

Table 12. Relation between the retention index and the structure of the substance being analysed.

Equation for variation of the index with the number of carbon atoms $n \geqslant 2$ and the nature of the functional group	Functional group	dI*
For tertiary amines $I_{100}^{peg} = 100\,n + 586$	$-NH_2$	586
For diamines $I_{100}^{peg} = 100\,n + 586.2$		
For secondary amines $I_{100}^{peg} = 100\,n + 330$	>NH	330
For tertiary amines $I_{100}^{peg} = 85\,n + 200$	>N—	200
For alkenes-1 $I_{125}^{apm} = 100\,n - 17$	$>C{=}CH_2$	−17
For n-alkanals $I_{125}^{apm} = 100\,n + 188$	$-C(=O)H$	188
For ω-alkenals $I_{125}^{apm} = 100\,n - 17 + 188$	$H_2C{=}C$ … $-C(=O)H$	
For α-alkenals $I_{125}^{apm} = 100\,n + 251$	$>C{=}C-C(=O)H$	251
For methyl ketones $I_{125}^{apm} = 100\,n + 171$	>C=O	171
For n-mercaptans $I_{60}^{se**} = 101\,n + 297$	—SH	297
For n-sulphides $I_{60}^{se} = 91\,n + 330$	—S—	330
For n-disulphides $I_{130}^{se} = 93\,n + 546$	—S—S—	546

*∂I is a quantity characterising the interaction of the functional group with the substrate of the column.
** = silicone elastomer SE-30.

Table 13. Quantities ΔI characteristic for n-aliphatic amines.

Amine	$\Delta I^{peg\text{-}tw}$*	Amine	$\Delta I^{peg\text{-}tw}$
Methylamine	173	Dimethylamine	90
Ethylamine	111	Diethylamine	63
Propylamine	107	Dipropylamine	60
Butylamine	109	Dibutylamine	62
Pentylamine	105	Dipentylamine	66
Hexylamine	106	Triethylamine	34
Heptylamine	106	Tripropylamine	15
Isopropylamine	100	Tributylamine	16
Isobutylamine	103	Tripentylamine	17

*$\Delta I^{peg\text{-}tw}$ is the difference in the indices of the amine on columns with 10% polyethylene glycol-1000 and Tween-80.

The methods of identification considered above relate to isothermal conditions of analysis. Evidently, the analysis of the components of an odour may sometimes be more convenient by use of gas chromatography with programmed temperature. Therefore attempts were repeatedly made to use isothermal data for identification under conditions of programmed gas chromatographic analysis. A series of empirical formulae was found [100,101].

Recently success was achieved in relating theoretically the isothermal index with parameters which can be determined experimentally [102,103]. A formula was obtained:

$$I_{t_0/\beta} = I_{t_0} + \frac{\beta\tau_x}{2}\frac{\partial I}{\partial t} \qquad (1)$$

in which $I_{t_0/\beta}$ is the index of the test substance with linear programming of the column temperature as a rate β, deg min^{-1}, from an initial temperature of t_0; I_{t_0} the isothermal index at temperature t_0, τ_x the time for the loss of the test substance under the programming conditions, and dI/dt the temperature increment of the index per 1 deg.

Table 14. Composition of monocarboxylic compounds and of volatile components of 5-month Russian cheese.

Aldehyde	Relative %	Aldehyde	Relative %	Ketone	Relative %
Ethanal	5.4	Isopentanal	1.9	Acetone	42.0
Propanal	0.15	Acrylaldehyde	3.3	Butanone	13.7
Butanal	2.0	Pentenal	0.1	Pentanone	5.6
Pentanal	0.7	Butenal	0.17	Hexanone	0.3
Hexanal	0.95	Octenal	0.01	Heptanone	4.6
Heptanal	0.2	Nonenal	0.13	Octanone	0.03
Octanal	0.9	Decenal	0.05	Nonanone	1.6
Nonanal	0.6	2,4-Heptadienal	0.01	Decanone	0.06
Decanal	0.7	2,4-Octadienal	0.04	Undecanone	0.5
Undecanal	0.12	2,4-Nonadienal	0.04		
Isobutanal	0.16				

*A total of 86%; 11 peaks were not identified.

Table 15. Change in the quantitative ratio of volatile amines in Russian cheese on storage, relative %.

Amine	4-months cheese	10-months cheese	Amines	4-months cheese	10-months cheese
Primary			*Tertiary*		
Methylamine	7.4	0.2	Trimethylamine	14.3	3.0
Ethylamine	—	0.3	Triethylamine	4.8	2.8
Propylamine	0.6	—	Tripropylamine	1.2	0.1
Isopropylamine	0.2	0.1	*Heterocyclic*		
Isobutylamine	12.8	0.5	Pyridine	29.6	4.9
Isopentylamine	0.6	0.1	2-Picoline	7.5	52.4
Secondary			3-Picoline	—	0.3
Dimethylamine	2.5	2.5	Piperidine	0.4	15.4
Diethylamine	2.5	1.1	Pyrrolidine	—	0.2
Dipropylamine	2.9	0.5	Hexamethyleneimine	—	0.3
Di-isopropylamine	9.0	8.8			
Dibutylamine	1.2	0.4			
Di-isobutylamine	0.3	0.1			
Unidentified secondary amines	2.3	6.4			

Formula (1) was verified on a large volume of experimental material and allows a substance to be identified in a programmed analysis from the values of isothermal indices.

The methods of identification developed allowed for the first time extremely valuable information to be obtained about the composition of amines and carbonyl compounds in the odours of various natural food products [104-108]. Tables 14–17 give the results.

It is interesting to note that the composition of the volatile components of cheeses have been intensively studied abroad but so far in every instance no more than 11 monocarboxylic compounds were found by gas chromatography [109-111], whereas 30 were identified with the aid of the standard-free method [112].

The use of analytical methods developed in the Institute of Organoelemental Compounds made it possible to establish for the first time that in the volatile components of food products which contain proteins there is a large number of organic bases. Table 15‡ gives the composition of the amines identified in the aroma of 4- and 10-month Russian cheese. During the storage of cheese there is an accumulation of heterocyclic bases. The proportions of some of them were determined by the absolute calibration method (Table 16).

Table 16. Accumulation of amines during storage of Russian cheese.

Substance	Concn. mg per kg cheese	
	4-months	10-months
Piperidine	1.5	50.0
2-Picoline	13.0	80.0
Pyridine	36.0	3.3
Triethylamine	6.0	3.0

The composition of the amines in the volatile components of soft caviar and salmon roes were determined similarly with the aid of standard-free gas chromatography [104,105].

The amines identified are shown in Table 17, which indicates that the relative proportions of the amines is rather specific for each foodstuff. Thus in the volatile components of soft caviar the pyridine content is 21 times that in salmon roe, in which trimethylamine predominates. This shows its organoleptic properties. On the basis of these results an imitation odour was created for synthetic soft caviar. On comparing the composition of the amines in the odour of such varied foodstuffs as caviar and cheese (Tables 15 and 17) the great similarity in the qualitative composition of the amines is seen [106]. This may be because both products contain proteins of animal origin which serve as the precursors of the amines.

The composition of the nitrogenous bases in the volatile components of the foodstuffs is extremely diverse. In addition to the primary amines, secondary and tertiary aliphatic amines are present. The presence of secondary and tertiary amines is somewhat unexpected. It is generally accepted that aliphatic amines are formed in the decarboxylation of amino-acids. However, only primary amines can arise in this manner; secondary and tertiary amines can be formed only as a result of alkylations, which, with the exception of methylation, are unknown. It was interesting to find whether these amines are products of the activity, or arise as a result of decomposition of the organism after the death. For this purpose the composition and kinetics of accumulation of the amines in the volatile components of rapidly growing organisms was studied. The lactic acid bacterium *Streptococcus lactis* was selected as the subject of the investigation.

Table 18 gives the composition of the amines identified in the volatile components of *Streptococcus lactis* at the stage of the logarithmic phase of growth. The organic bases were identified with the aid of the gas chromatographic method of analysis described above.

Table 17. Amines and volatile substances of soft caviar and salmon roes.

Amine	Sturgeon roe, rel. %	Salmon roe, rel. %
Primary		
Methyl-	2.2	3.2
Ethyl-	2.5	—
Propyl-	12.0	—
Isopropyl-	2.0	0.1
Butyl-	3.1	0.3
Isobutyl-	—	1.8
Pentyl-	2.8	0.1
Secondary		
Dimethyl-	2.8	1.0
Diethyl-	2.8	0.4
Dipropyl-	2.6	0.3
Di-isopropyl-	8.6	2.3
Dibutyl-	2.4	0.3
Di-isobutyl	1.8	0.1
Tertiary		
Trimethyl-	3.4	87.5
Triethyl-	9.0	0.3
Tripropyl-	—	0.1
Heterocyclic		
Pyridine	40.4	0.3
2-Picoline	1.4	0.6
Piperidine	—	0.5
Pyrrolidine	—	0.1
	99.8%	99.3%

Table 18. Kinetics of the change in concentration of amines in the volatile substances of lactic acid bacteria.

Amine	Growth of culture, hours		
	6	24	30
Primary			
Methylamine	5.2	0.7	0.1
Ethylamine	0.3	0.1	0.1
Isopropylamine	traces	0.2	0.2
Butylamine	1.5	0.2	0.1
Isobutylamine	0.8	0.7	0.7
Pentylamine	—	0.4	0.4
Isopentylamine	0.7	0.7	0.1
t-Butylamine	1.9	1.3	0.2
Secondary			
Dimethylamine	0.1	1.2	0.1
Diethylamine	2.5	0.5	3.6
Dipropylamine	4.0	1.2	0.7
Di-isopropylamine	9.4	2.8	1.3
Di-isobutylamine	—	1.2	0.6
Butylisopropylamine	15.9	50.6	56.1
Pyrrolidine	2.9	0.4	0.1
Piperidine	5.6	traces	0.1
Unidentified	0.2	0.5	traces
Tertiary			
Trimethylamine	25.7	7.2	2.8
Triethylamine	18.3	2.0	1.7
Tripropylamine	0.7	1.3	0.3
t-Aliphatic amines	4.1	27.0	30.9

As Table 18 shows, the total concentration of amines in the growth of lactic acid bacteria increases, their ratio in the mixture changes sharply, and the concentration of butylisopropylamine and tertiary amines with ten carbon atoms and b.p. 166°C increases [113]. This shows that in the exchange process the bacterial cell secondary and tertiary amines are synthesised, which suggests the existence of specific enzyme systems carrying out the process of synthesis of the amines. Hence a phenomenon

‡ Under the conditions of the analysis amines appearing on the chromatogram up to n-dodecane were identified.

new in principle in biochemistry has been discovered—the synthesis of secondary and tertiary amines by living organisms.

The results of the analysis of the components of the vapour made at the Institute of Organoelemental Compounds of the Academy of Sciences of the USSR shows that under exceptionally thorough work with the aid of gas chromatography it was possible not just to obtain interesting results about the composition of complex aromas of foodstuffs but also to arrive at an understanding of the processes of formation of the food odours. The results obtained show that the problem of odour and taste can be solved successfully. Its solution will enable synthetic foodstuffs to be successful and accepted by people.

5. STRUCTURAL-PHYSICAL PROBLEMS OF ARTIFICIAL FOODS

The production of artificial food requires the solution of yet another great problem—the giving of the usable foodstuffs (of natural, artificial, or synthetic origin) of taste, odour and an attractive appearance. It must be emphasised that each of these factors plays an extremely important part and an underestimate of any of these can lead to the impossibility of admitting and assimilating the food even when its composition is ideal. It is quite evident that an unpleasant or even simply unfamiliar taste of a food provokes in any person an apprehension or even a refusal to use it. The same can naturally be said also about the odour of food.

However, the colour of food and its form are no less serious factors; their being unusual can bring about extremely unpleasant physiological sensations. For example, it was found that artificial soft caviar, the preparation of which was developed in the Institute of Organoelemental Compounds of the Academy of Sciences of the USSR [114-118], having the same composition, structure, and physical properties, exhibiting the same odour and flavour, but differing in colour, aroused greatly differing reactions in a meal. Whereas caviar with the natural colour (greenish grey, dark grey) was regarded as excellent, that coloured bright green was impossible to eat.

Therefore the giving to artificial food of taste and odour and also an attractive appearance was an important problem. It will be examined in greater detail.

It is usually thought that taste is determined by a combination of four sensations, namely, acid, sweet, salt, and hot. However, this is true only in relation to the chemical aspect of taste, since the taste receptors found in the cavity of the mouth actually perceive just these principal taste sensations. Hence this part of the problem of the creation of flavour is readily solved. It is sufficient just to select correctly the ratio of four carriers of these sensations, for example, salt, sugar, any food acid, and coffee.

It is much more difficult to solve another part of the problem of taste having a physical or physicochemical character, which includes above all the creation of a physical structure and definite complex of physical properties for the artificial food.

Actually, the taste sensations in eating are determined not only by the chemical action of the food on the taste receptors but also by the distribution of the sensations of acid, sweet, salt, and hot in relation to individual receptors and also by the mechanical action of the food on the tongue, palate, and teeth, the degree of wetting of the food by the saliva, and by the change in the physical properties of the food–saliva system during its interaction, by bringing about swelling and other physicochemical processes. All this requires a thorough physicochemical investigation of the substances used for preparing artificial foods. Here an investigation of structural and mechanical characteristics is of most value.

Since two of the main ingredients of food—proteins and polysaccharides—are high-molecular-weight substances, the structure-physical task of creating specific forms of artificial food seems [119] part of the general physicochemical problem of creating a desired structure and complex of physical properties for polymeric articles, that is, part of the problem which has occupied investigators working in the field of the physical chemistry of polymers for many years [120].

It must be particularly emphasised that processes of dissolution or dispersion of proteins or polysaccharides, their mixing with one another and with other foodstuffs, odour carriers, dyestuffs, etc. and also the formation of gels from such systems is hindered by a series of problems relating to the physicochemical theory of true and colloidal solutions of polymers. Among such problems there are, for example, the swelling and solubility of food polymers in water, fats and other solvents, the mutual solubility of proteins and polysaccharides, the surface properties of solutions and gels of these polymers, the formation of a structure in solutions of the polymers, the relation between various mechanical properties of the solutions and gels, and their composition and supermolecular structure.

Therefore the whole approach to the modern physicochemistry of polymers can be used for the development of procedures for the production of various forms of artificial food.

The preparation of a specific form of artificial food, for example, caviar, groats, meat, fish, etc. requires, on the one hand, the determination of the complex of structural and physical properties characteristic for the natural product, and on the other hand the development of procedures for the formation of artificial food exhibiting these characteristics. Whereas the first aspect of the problem requires the use of methods of polymer physical chemistry the second aspect seems closely connected with many technological procedures developed in the production of plastics, resins, polymer films, and polymer fibres, adhesives, and lacquers. Actually among foodstuffs there are a series of corresponding forms, namely, homogeneous and heterogeneous, liquid and solid systems (broths, milk, jelly, cheese), layer forms (confectionary), fibrous forms (meat, fish), granular forms (roe, berries, groats), etc. Here the structure can be fine or coarse (porous and frothy foods, emulsions, suspensions, stuffed foods, etc.) and mechanical or other physical properties are extremely varied.

Hence there is a very far-reaching analogy between food and commercial polymer goods allowing the enormous existing experience from the development of various polymers in a variety of articles to be widely used in the creation of one or another form of artificial food. In the development of procedures for the structuring and forming of artificial foodstuffs specific sanitary and hygiene requirements must be borne in mind.

The solution of the problems of artificial food required the development of a large complex of physicochemical investigations of proteins, polysaccharides, and compositions based on them. This is also of general interest for the physical chemistry of polymers. These include, for

example, the investigation of the properties of aqueous and oily solutions and the dispersion of individual food polymers and their mixtures, the study of the effect on these of liquid and gel-forming systems of substances which differ in nature (fats, vitamins, enzymes, sodium chloride, etc.) and also in conditions (temperature and pressure).

To create artificial macaroni and grain articles [121] it is necessary to give them a definite stability of form and volume since on boiling groats or macaroni swelling should occur, but not breakdown of the particles.

Corresponding investigations [122-126] led to the creation of articles of artificial food containing all the necessary nutritive substances (proteins, starch, etc.) with a special space lattice structure. This lattice, completely harmless for the organism, constitutes a negligible part of the mass of the food product but nevertheless gives it elasticity and strength, ensuring the retention of the entity on swelling in boiling water. This naturally gives to the product a certain quality of taste depending on the mechanical properties of the food.

From the physicochemical aspect these investigations led to interesting new results on the physicochemical properties of three-dimensional polymer structures, in particular the properties of the double lattice structures, for example, systems which contain two lattices simultaneously—gelatin and calcium alginate [127].

At present the most far-reaching physicochemical investigations relate to the production of artificial soft caviar. Taking this into account and also the special value of such a delicatessen product this subject will be discussed in greater detail.

In general the preparation of granular forms of artificial food products, for example, artificial groats, berries, roe, etc. leads to the preparation of granules of edible jelly of definite dimensions, chemical composition, and structure, and with the necessary physicochemical properties [128], the creation on these granules of a covering and their colouring the desired shade [123].

In accordance with the process developed for the preparation of artificial caviar various foodstuffs of animal, plant, and microbiological origin and also the products of chemical synthesis can be used. However, at present casein is used as the food raw material; it is a high-quality protein and the problem of its use for feeding is extremely urgent. The essence of the process is as follows. To prepare edible granulated gels, a hot solution of gelatin containing casein or other food substances is supplied dropwise into previously chilled vegetable oil or liquid paraffin.

The resulting granules are kept in the oil at 5–10°C until a gel with a modulus of elasticity of 10^4 dyn cm^{-2} is formed, after which they are separated from the oil and washed with cold water to free the surface from traces of the latter. The formation of gelatin gels is complicated here by the presence of a considerable proportion of casein and other foodstuffs, and also by sorption of oils by the protein components of the gel. Thus, sorption of hydrocarbons by the liquid paraffin leads to a significant change in the melting point, rate of swelling and mechanical properties of the granulated gels. The phase state of the initial mixture of foodstuffs, the conditions of formation, structure, and the corresponding complex of physicochemical properties of granulated gels is determined by the possibility of preparation of a high grade product. To study the effect of these factors on the properties of granulated gels simple and precise methods of determining the temperature range for their fusion, the rates of swelling, and pliability were developed [128] and also a method of investigating the mechanical properties of gels under conditions of uniaxial compression [129,130] and a method of determining the degree of swelling [131] were developed. The possibility of removing oil from the surface of granulated gels when these are washed with water depends significantly on the surface properties of the gel. Gelatin gels are very poorly wetted by water [132]. The introduction of globular proteins into them leads to a distinct change in surface properties, depending on the nature of the protein introduced [133]. The addition of egg albumen is accompanied by some increase in the hydrophobic nature of the gelatin gel, whereas the introduction of even small proportions of casein makes them hydrophilic.

Therefore unlike granules of pure gelatin gel, those of gelatin containing casein are readily washed by water from oil on remixing. The latter treatment of granular gels with aqueous extractants of vegetable tanning agents allows sufficiently definite dense and elastic walls of tanning products to be obtained. The homogeneity of the covering here is determined by the degree of removal of oil from the surface of the granules and by the surface properties of the gel. Aqueous extracts of green tea or the wastes from tea production are used for tanning the granules. [114,123] The colouring of their walls grey-black for the imitating of sturgeon roe is done by treating the granules with solutions of iron(III) salts of bile acids [114,115], that is, by using the well-known reaction of phenolic compounds with iron(III) ions. The properties of the granules are greatly influenced by reagents and media with different osmotic properties applicable for the development of granulated gels (washing, tanning, colouring), and also the conditions of cooking and subsequent storage of the product. The volume properties of the granulated gels do not change appreciably during their tanning. The intensity of the swelling of the gel is depressed in this as a result of the resistance of the granule sheath to extension. The diffusion into the gel of iron(III) ions being used for colouring the sheath is accompanied by the tanning of the proteins through the entire volume of the gel, as a result of which the pliability and intensity of swelling of the gel decrease and its melting point rises.

To make caviar and granules of a gel coated with coloured elastic sheaths, sodium chloride, vegetable oil, fish oil and various other tasty and aromatic substances are added. The combination of casein, vegetable oils, fish oil, and also the additional mineral salt and vitamins introduced weakens the high food values of the product, for the preparation of which high-grade but nevertheless expensive edible raw material was used. In connection with the fact that tea extracts are used for tanning granulated gels their effect has been investigated on the enzyme activity of pepsin, trypsin, and chromotrypsin in vitro [134]. It was shown that the complex of tanning agents of green tea not only does not have an inhibiting action on enzymes under these conditions, but even raises the activity of pepsin and chemotrypsin. Whereas the comparative investigation of the vulnerability of proteins to enzymes of the gastrodigestive tract in vitro and of the same proteins in the composition of various sorts of roe investigated showed that the biological value of these proteins increases on their inclusion in the composition of the product [135]. The investigations of the physicochemical and mechanical properties and also the biological value of the granulated gels allowed the conditions for the preparation of granulated gels to be determined and the influence of various factors on the composition and properties of the prepared product to be characterised, which was a

necessary stage in the development of a method of making artificial soft caviar.

As a result of the studies made in the Institute of Organoelemental Compounds of the Academy of Sciences of the USSR, under the direction of A. N. Nesmeyanov, the first results on the creation of a high quality artificial caviar have been obtained. These studies created the necessary chemical, physicochemical, and technological basis for subsequent development of the investigations in this new and interesting field of science and industry.

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