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Methods for the regulation of the functional properties of isolated myofibrillar proteins in order to expand their use as foodstuffs

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ABSTRACT. Data characterising the specific features of the chemical, enzymic, mechanical, and combined modifications of myofibrillar proteins are described systematically with the aim of expanding their possible use in food systems. Attention is concentrated on the mechanisms of the enzymic and chemical reactions with food proteins of myofibrillar origin, carried out in order to regulate specific functional properties of the protein systems.

The problem of toxicity, biological value, bioavailability, and metabolic pathways of the modified food proteins is discussed.

The prospects and limitations of enzymic and chemical modification of myofibrillar proteins are indicated.

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I. Introduction

For a successful solution of the problem of food supply, it is timely to take into account and assess the possibility of using new sources of food protein, including those which had been previously neglected.[1,2] Apart from the non-traditional sources of proteins of plant origin, to which a fair amount of attention has been devoted,[3,4] rich resources of proteins with a high food value are provided by various objects in the Pacific Ocean: the Antarctic krill,[5–10] certain species of fish, in particular myctophid fish,[11–13] as well as fish with a low commercial value.[14–18]

Isolated fish proteins, free from sarcoplasmic proteins and lipids, have no specific taste or smell and can be used in any food system ranging from delicatessen fish products and meat substitutes to dessert dishes.[5,6,19,20] The isolation of proteins from non-traditional sources requires additional stages involving the removal of toxic, ballast, or antinutritive components (specific pigments, proteolytic enzymes, lipids which are difficult to remove, etc.). Myofibrillar proteins are more sensitive to chemical and physical effects than plant proteins. The proteins isolated from muscle tissue do not therefore meet the current requirements from the standpoint of functional properties. On the other hand, the possibility of the wide scale application of proteins of this kind in food products does in fact depend on the level of their functional properties.

Functional properties (FP) is the term given to a set of physicochemical characteristics of an individual protein, which determines its behaviour in aqueous media (swellability, solubility, gel formation, etc.), and in the heterophase systems of the type water–oil, oil–water (emulsifying capacity), or water–gas (capacity for foam formation), as well as in multicomponent mixtures. The functional properties of the protein are manifested as a function of its nature and the character of its interaction with other components of the given food system.

Studies in which the relation between the structure of the protein and its functional properties is determined are believed to play virtually the main role in modern food protein chemistry.[21–25] Chemical modification of proteins makes a considerable contribution to the solution of this problem making it possible to trace the correlation between certain physicochemical parameters of the protein and the manifestation of a particular functional property.

In selecting a method for the regulation of the functional properties of a food protein, one must take into account all the characteristic features of its structure and the character of the changes entailed by a particular type of modification.

The functional properties of the protein are determined by many factors:

(a) They are determined mainly by its surface characteristics, which are in turn determined by the quaternary structure of the protein (the size and shape of the protein molecule, its overall surface charge, and its surface hydrophobicity). The stability of the protein depends on the type of bond between the subunits and the ease of their dissociation.

(b) The relation between the intermolecular and intramolecular bonds of various types (hydrogen, ionic, hydrophobic, electrostatic, van der Waals) characterises the flexibility of the molecule, i.e. its capacity for conformational changes without the disruption of the native structure of the protein.

(c) The aminoacid sequence dictates the charge distribution in the molecule and the type of packing of the peptide chains, i.e. the geometrical disposition of the hydrophobic sections.

(d) The secondary and tertiary structures of the protein determine ultimately the steric accessbility and the reactivity of the functional groups of the protein.

The set of the above factors does in the main determine the individual features of the protein.[26]

Any kind of modification alters a whole set of physicochemical properties of the protein and these processes must be examined in detail, since they open a pathway to the deliberate regulation of the functional properties of food protein systems.

The aim of the present review is to give a systematic account of the available information about the ways in which food proteins isolated from muscle tissue are treated in order to regulate their functional properties. The narrow selection of the subjects for discussion makes it possible to trace the trend in the changes of particular properties of the protein as a function of the type of influence on the latter.

It is possible to differentiate three main aspects of the regulation of the functional properties of food proteins: enzymic treatment, chemical modification, and physicochemical methods of influencing the protein. These processes are not mutually exclusive and can be successfully used in a combined procedure.

II. Enzymic treatment of proteins

The processes involving the regulation of the functional properties of myofibrillar food proteins with the aid of enzymes (usually proteolytic) have been widely discussed in the literature.[27–36]

By means of the partial hydrolysis of the protein, it is possible to achieve an increase in its solubility, emulsifying activity, foam-forming capacity, and ability to stabilise emulsions and foams.

In the treatment of muscle tissue by proteases, micro-organisms were used initially: *Thamnidium sp.* in order to soften meat and *Aspergillus glaucus* in order to alter the consistency and taste of fish.[37] The proteases produced by micro-organisms apparently caused the breakdown in the first place of the structure of the sarcomers, increasing thereby the softness of the tissues but at the same time increasing also the viscosity and stickiness of the product. This procedure has not been found extensive application because of the considerable losses of the product.

The most effective method of increasing the solubility of a protein is enzyme hydrolysis. Proteolytic enzymes cleave specific peptide bonds causing the breakdown of the protein molecule into definite fragments. In contrast to acid or alkaline hydrolysis, this process is not accompanied by any side reactions. Myosin, as the main component of myofibrils and having the most complex structure, is most frequently used for a detailed investigation of the behaviour of muscle proteins. The mechanism of the hydrolysis of myosin by food digesting enzymes of the series of peptidases has been studied in fair detail.[38]

For example, trypsin initially splits the myosin molecule into two large fragments: the heavy meromyosin (MW = 200 000) and the light meromyosin (MW = 150 000). Globular fragments or myosin "heads" (MW = 120 000) are then split off from the heavy meromyosin leaving the C-2 subfragment (MW = 100 000).

Papain abstracts in the first place the myosin heads, acting subsequently in a similar manner.

The high degree of hydrolysis of the protein achieved on treatment by active enzymes such as pepsin, papain, and pronase[39–41] is undesirable since it frequently entails the loss of the emulsifying and foam-forming capacities[42] and leads to the appearance of an appreciable bitter taste due to hydrophobic peptides.[41,43] It is very difficult to optimise this process.[44]

In many cases, enzymic treatment was resorted to in order to stabilise the muscle tissue homogenates before extracting the protein.[45,46] This method is especially convenient in isolating proteins from small oceanic fish. The fish are wholly ground up and are then subjected to enzymolysis, whereupon the partially hydrolysed protein can be extracted to a large extent. Amylosubtilin and protosubtilin were used on fish objects,[45] while papain and ficin were employed for the analogous treatment of the skeletal muscle tissue of cattle.[46]

A successful version of the enzymic modification of isolated *Rockfish* fish proteins has been achieved using the "Rhozyme P-11" preparation.[47,48] After 15 min hydrolysis at pH 6.5–6.7 and 30 °C (enzyme : substrate = 1 : 75), an emulsifying activity exceeding the control parameter for the initial isolated protein by a factor of almost 1.5 was obtained. For higher degrees of hydrolysis the emulsifying activity fell, while the stability of the emulsions increased (to a maximum after hydrolysis for 30–60 min) but subsequently it too decreased. The solubility in water of the fish hydrolysate obtained reached 20%. The authors do not mention a bitter taste.[47]

It is of interest to note that weak hydrolytic treatment of the *Rockfish* isolated protein with bromelin (10 min at pH 7; temperature 25 °C) only slightly diminished its emulsifying and foam-forming capacities, whereas the above properties of the same isolated proteins subjected to preliminary acylation were increased sharply[49–51] (for details, see Section IV).

The traditional employment of meat proteins in food systems imposes certain requirements on the latter. Solubility is not as important for them as a high emulsifying capacity, gel formation, and ability to bind high- and low-molecular-weight components of the system.

By treating the myofibrillar proteins of bovine skeletal muscle with papain, it was possible to increase their emulsifying capacity by 40% with formation of very stable emulsions.[46]

Owing to their low level of functional properties, proteins isolated from bovine cardiac muscle tissue have not found extensive applications despite their high biological value. Modification using ficin made it possible to employ such isolated proteins as a valuable additive for the replacement of beef in meat systems (up to 30%) without impairing their nutrient and organoleptic properties.[52]

In order to improve the emulsifying and gel forming capacities of myofibrillar proteins from the skeletal muscles of domestic birds, treatment with the preparation "Milezyme AFP-2000" was tried. It was noted that, with increase in the degree of hydrolysis, the solubility of the proteins rises but the capacity for gel formation falls rapidly at the same time.[53] It is possible to obtain a modified product, which has been recommended as an active emulsifying additive to meat systems.[54]

Enzyming processes used for the modification of myofibrillar proteins hardly extend beyond the framework of proteolysis. There have been several communications on the use of transferases which can serve as activators for the covalent attachment of aminoacids or aminoacid esters to proteins[55,56] (see Section IV).

It can be inferred from the data presented that the spectrum of functional properties which can be improved by treating myofibrillar proteins with enzymes is so far relatively narrow. The principal factor determining the level of the required functional properties in the modified object is the degree of hydrolysis of the protein. On the other hand, the main difficulty in carrying out proteolytic processes is in monitoring the degree of decomposition of protein molecules.

The application of immobilised enzymes was predicted a fairly long time ago,[57] but this technique has not so far found an application in practice.

Despite the difficulty of controlling enzymic processes on an industrial scale, some workers believe that this aspect of the modification is the most promising on the grounds that such treatment does not entail the loss of nutrient properties and in addition increases the assimilability of the isolated proteins.[3,5,58]

In our view, the use of enzymic processes on isolated proteins subjected to a preliminary chemical modification is effective (for further details, see Section IV).

III. Chemical modification

Chemical modification is of great interest for the regulation of the functional properties of food proteins.[21,58–67] It involves many more possibilities for the transformation of proteins than enzymic modification. Apart from the simplest operations associated with the degradation of peptide chains and the hydrolysis of amide groups of asparagine and glutamine, it makes it possible to alter the structure of the protein by creating new covalent or ionic bonds.

With the aid of chemical reagents, it is possible to introduce hydrophilic and hydrophobic groups into the protein molecule, to enrich the protein in aminoacids present in insufficient amounts, and to attach polyaminoacid chains. By taking into account the nature of the groups introduced and by regulating the degree of modification, it is possible to alter specifically the overall charge and hydrophobicity of the protein system, which in fact determines its functional properties.

1. Alkaline and acid hydrolysis of proteins

One of the simplest methods of solubilising proteins is alkaline and acid hydrolysis. Since 1970, alkaline hydrolysis has been used widely for the improvement of the functional properties of fish protein concentrates.[68–70] Such treatment usually increases the solubility and the emulsifying and foam-forming capacities of the protein.

Treatment of myofibrillar proteins with alkalies and acids in order to cleave the peptide bonds induces profound irreversible denaturation changes and is accompanied by the appearance of a number of side reactions. Alkaline hydrolysis is also accompanied by the degradation of alkali-labile aminoacids, the partial racemisation of aminoacids, and the β-elimination process with participation of hydroxy- and mercapto-aminoacids leading to the formation of dehydroalanine, which readily interacts with lysine, cysteine, ornithine, and ammonia.[71–74] The products of these reactions are respectively lysinoalanine, lanthioline, ornithinoalanine, and β-aminoalanine. This diminishes the food value of the protein by depleting it in essential aminoacids, by the partial loss of assimilability as a result of the formation of intermolecular cross-links, and by the accumulation of antinutrient substances. The question of the toxicity or harmlessness to man of dehydroalanine derivatives still remains open.[3,72]

Comparatively recently it has been demonstrated experimentally (on skeletal muscles in fish) that lysinoalanine is formed only under very severe alkaline conditions at pH 12 and above and at temperatures not below 90 °C.[75] Thus the possibility of using relatively mild hydrolysis for food proteins has not been rejected at the present time.[3,36] Acid hydrolysis of myofibrillar proteins has not come to be widely employed although according to some specialists[5] it has advantages compared with alkaline hydrolysis mainly as a result of the lower degree of racemisation of aminoacids.

One of the principal disadvantages of hydrolytic processes is the formation of bitter peptides (even for low degrees of hydrolysis of the protein), which is especially characteristic of isolated fish proteins.[20,42]

2. Solubilisation of proteins by salt formation

Proteins, which are polymeric ampholytes, have the characteristic ability to interact with cations and anions. Two types of interaction are possible: the formation of salt "bridges" and the specific sorption of ions on the surface of the protein.

In terms of their positive influence on the solubility of proteins, cations and anions can be arranged in the following sequence:[76]

$$Ca^{2+} > Mg^{2+} > Li^{+} > Cs^{+} > Na^{+} > K^{+} > NH_4^{+}$$

$$SCN^{-} > ClO_4^{-} > I^{-} > NO_3^{-} > Br^{-} > Cl^{-} > CH_3COO^{-} > F^{-} > HPO_4^{2-} > SO_4^{2-} >$$

$$> {}^{-}OOC{-}CHOH{-}CHOH{-}COO^{-} > {}^{-}OOC{-}CH_2{-}\underset{\displaystyle COOH}{\overset{\displaystyle |}{C}}(OH){-}CH_2{-}COO^{-}.$$

Proteinates have a much higher solubility than desalinated proteins. The change in the solubility of proteins is attributed to the specific interaction of ions (not only electrostatic interaction), which entails changes in the surface charge of the protein and in its conformation, ensuring a higher degree of hydration. A change in the ionic strength of the solution also promotes an increase in the solubility of the protein.[3,76,77]

The use of salt components in food protein systems is limited by their toxicity and cost. The most widely used additives are sodium chloride and inorganic phosphates.[78–85]

The moisture retaining capacity of meat systems is frequently regulated with the aid of salts. Sodium chloride, pyrophosphate, or triphosphate is usually introduced to increase such capacity.[78,86] The affinity of myosin for various inorganic phosphates is not unambiguous. It has been demonstrated for the interaction of myosin and with sodium pyrophosphate, triphosphate, and hexametaphosphate.[83,84] Among them, hexametaphosphate shows the greatest affinity for myosin, the character of its binding to myosin differing from that of the other phosphates. In the presence of Na^+ and especially Mg^{2+} or Ca^{2+} cations, the affinity of triphosphate and pyrophosphate for myosin increases sharply. More precisely, the affinity of myosin for these metal phosphate complexes is greater than for the phosphates themselves. This rule does not extend to hexametaphosphate. On the contrary, the presence of NaCl inhibits the interaction of myosin with hexametaphosphate.[83,84] These data indicate a high degree of specificity of the ionic interactions of myofibrillar proteins.

The modern concept concerning the influence of phosphate and chloride anions on the solubility of myofibrillar proteins reduces mainly to electrostatic effects. As a result of the interaction of anions with myosin filaments, electrostatic repulsion forces arise and weaken the binding of the filaments. It has been suggested that at the critical salt concentration in solution, certain types of bonding of the filaments (as a rule in the regions of the *M*- and *Z*-lines) can break down. Furthermore, the bonding of the myosin heads to actin weakens until complete dissociation. Thus the introduction of anions promotes the depolarisation of thick filaments with liberation of myosin molecules.[76] In general, it has been found that inorganic phosphates are universal polyfunctional additives to isolated muscle tissue proteins. For example, sodium hexametaphosphate is introduced to ensure the complete precipitation of fish protein in the form of the phosphate complex,[47] while the addition of sodium triphosphate to the isolated fish protein is recommended in order to optimise the drying process.[87] Phosphates can be used conveniently to modify myofibrillar proteins, because they possess also antidenaturing,[88–90] antiseptic,[82,91–93] and antioxidant activities.[82,87,94] Phosphates have proved useful as active synergistic agents in relation to phenolic antioxidants.[94] On the other hand, the presence of polyphosphates increases the affinity binding of carbonyl compounds

formed on oxidative decomposition of lipids; actomyosin phosphate should not therefore be added to isolated proteins which need to be defatted.[95]

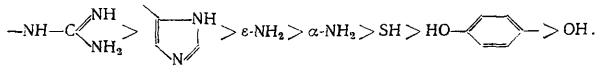
Mention should also be made of the ability of certain anions to protect actomyosin against cryodenaturation. For this purpose, glutamate, tartrate, and maleate are used together with phosphates at low temperatures.[96–98]

3. Acylation of myofibrillar proteins

One of the most widely used procedures in the chemical modification of food proteins is acylation.[21,59,61,63,64,99,100] Among acylating agents, acetic and succinic anhydrides are the most widely used. They are cheap, convenient to employ, and relatively harmless, they do not form toxic derivatives with proteins, and are distinguished by a high activity. The acylation reaction is usually carried out at a reduced or room temperature at alkaline pH.

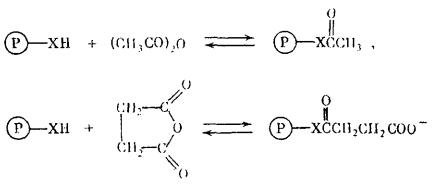
The degree of involvement of the different nucleophilic groups of the protein in the reaction varies as a function of the conditions (mainly the pH), the character of the anhydride, and the influence of inhibiting additives.

In the series based on decreasing nucleophilicity, the functional groups of the side chains in the aminoacid residues are arranged in accordance with their dissociation constants:



However, their reactivity can be influenced by adjoining groups in the peptide chain with which donor–acceptor or electrostatic interaction is possible or hydrogen bonds may be formed.[77]

The reaction of the protein with acetic and succinic anhydrides can be represented in a general form by the following scheme:



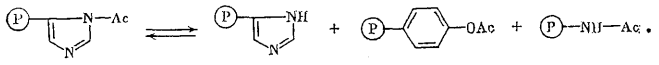
X = NH, O, S; Ⓟ = protein.

The fundamental difference between these processes is that the groups formed as a result of the interaction of the protein with acetic anhydride are compact and electrically neutral. On the other hand, the reaction with succinic anhydride introduces into the protein molecule more bulky negatively charged substituents. The latter reaction induces a sharper shift of the isoelectric point of the modified protein to the region of acid pH and more profound conformational changes than in the case of acetylation. This is in fact the cause of the greater possibilities, even when the degrees of modification are low, for the improvement of the solubility and the emulsifying and foam-forming capacities by succinylation rather than by acetylation.

In principle, all types of basic groups of the protein can interact with carboxylic acid anhydrides. In practice, however, the principal reaction centres are the ε- and α-amino-groups, mercapto-groups, and the most active hydroxy-groups of the protein.

The highly basic guanidine groups of the arginine residue are not acylated because under the reaction conditions (at a pH usually not exceeding 9.5) they exist in the protonated state.[77]

The *N*-acylimidazole derivatives of the histidine residue are so active that they themselves function as acylating agents, i.e. the *N*-acyl group migrates from the imidazole ring to the neighbouring sterically accessible amino- or phenoxy-groups of the protein:[63,64]



The half-life of *N*-succinylimidazole at pH 8.5 is 1 min.[101]

The terminal amino-groups of the protein, the ε-amino-groups of lysine, the SH groups of cysteine, and the OH groups of tyrosine are readily acylated, while the hydroxy-groups of serine and threonine are less reactive.[100] The amide bonds formed are stable, while the *O*- and *S*-acyl bonds, which are relatively stable in acid media, are rapidly hydrolysed in weakly alkaline media (already at pH 8.0). Tyrosine derivatives are especially labile. For example, the *N*-hydroxysuccinimide ester of tyrosine (*O*-Suc-tyrosine) is spontaneously deacylated at pH 8.0–8.5.[100,102]

It has been established with the aid of the reaction with salicylaldehyde that the accessible and active ε-amino-groups of lysine in native myosin constitute only 2.5% of the total number of lysine ε-amino-groups, while moderately active residues constitute about 30%. Other residues are sterically inaccessible and can react with the modifying agent only after the denaturation of the protein.[103]

The effect of acylation on the conformation of the myofibrillar proteins has been studied in detail on myosin and actin.[104–111] When only the surface ε-amino-groups of lysine have been modified, the conformation of myosin does not change.[104,105] Succinylation under conditions ruling out the denaturation of myosin (pH 7.0; 0 °C) showed that such chemical action induces only the dissociation of the light chains of myosin, while in other respects the structure of the protein remains intact.[106] These observations were confirmed in the analogous acetylation of myosin. Fractions with molecular weights of 17 000, 19 000, and 20 000, representing the light chains of myosin bound non-covalently to the C-1 subfragment, have been isolated and identified.[107,108]

When the degree of modification is above 3%, the myosin molecules acquire a more open-chain conformation and the degree of coiling into a helix diminishes.[107] On succinylation up to 50% and above, the conformation of proteins is seriously damaged.[112]

As regards biological activity, on succinylation (even for low degrees of the latter), myosin loses the ATPase activity and the ability to bind to F-actin.[107]

Acetylated myosin is distinguished by a high thermal stability, i.e. resistance to thermocoagulation.[107] It is believed[105,108] that the lysine ε-amino-groups located on the surface (the most accessible) are responsible for the thermal stability of myosin. On modification with a reagent specific to amino-groups (β-naphthoquinone-4-sulphonic acid), the thermal stability of myosin increased linearly with increase in the degree of substitution of amino-groups from 0 to 2.5%. The blocking of the surface lysine ε-amino-groups probably reduces the capacity of the heavy myosin chains for aggregation during their thermocoagulation.[76,109,110]

Studies of the influence of chemical modification on the properties of actin have been shown that up to 30% of the amino-groups and up to 40% of the SH groups are capable of entering into the succinylation reaction, as a result of which actin becomes biologically inert, losing the capacity for polymerisation and for binding to myosin. It is of interest that the selective blocking of the mercapto-groups of F- and G-actin does not affect their biological activity.[111]

Apparently the ε-amino-groups of lysine play the main role in actin, as in myosin.

Modelling of biochemical reactions has shown how specific and complex are the processes involving the changes in the conformation of the muscle tissue proteins. For example, enzymic phosphorylation of the light chains of myosin (LC-2) alters only the steric orientation of the heads of the C-1 myosin subfragment.[113,114]

Proteins isolated from animal sources, containing many different components of structural proteins, were subjected to chemical modification in the studies discussed below. Sarcoplasmic proteins and lipids are as a rule removed in order to avoid the oxidative spoliation of the isolated proteins during storage and to eliminate their specific smell and taste. Thus the principal components of the isolated proteins are myosin, actin, and tropomyosin, while the minor components are troponin and actinin.[76] According to the literature,[76] myosin contains only 17% of the basic aminoacid residues and 38% of the polar functional groups, actin contains 13% of the basic residues and 33% of the polar residues, while tropomyosin contains 19% of the basic residues and 50% of the polar residues. The cysteine residues are concentrated predominantly in myosin (65% of all the SH groups) and actin (29% of the SH groups). The accessibility of the groups enumerated above to chemical reagents is various, depending on the degree of denaturation of the modified protein system and on the nature of the selected agent.

The composition of the isolated proteins depends on the object, the kind of muscle tissues, the method of isolation and treatment, and the degree of denaturation of the proteins — on the conditions used in carrying out each stage of the process. The available literature data on the modification of isolated proteins can therefore be compared only fairly arbitrarily.

The influence of the conditions in the acylation reaction on the properties of isolated fish proteins has been investigated[100] with quantitative determination of the functional groups of the protein involved in the reaction. It was established that the rate of acylation depends mainly on the pH of the medium. For example, at 0 °C and pH 9.5, 90% of all the ε-amino-groups of lysin are acetylated, the corresponding figures at other pH values being 85% at pH from 8 to 8.5,[115] 75% at pH 7.5, and 65% at pH 6.5. Nevertheless, in the presence of a large excess of acetic anhydride and for longer reaction times, complete acylation is attained even at pH 6.5.[100]

The rate of the reaction between acetic anhydride and the SH and OH polar groups of the protein falls appreciably as the pH is reduced. Whereas 35% of all the polar groups (SH and OH) are acetylated at pH 9.5 and 25% at pH 7.5, not more than 20% are acetylated at pH 6.5. Under these conditions, the mercapto-groups of the cysteine residue were modified even at low concentrations of acetic anhydride, while the rate of acylation of tyrosine increased only at high concentrations of the anhydride. More than 50% of the SH groups of cysteine, 30–40% of the OH groups of tyrosine, and of the order of 20% of the hydroxy-groups of serine and threonine were acetylated in the pH range 7.5–9.5. On succinylation, the same proportions were observed, but the degree of involvement of all the groups was somewhat lower because of the lower reactivity of succinic anhydride.

The introduction of an inhibiting additive (sodium sulphite) alters the pattern in the acetylation of fish protein: the involvement of the ε-amino-groups is reduced by 20%, that of the SH groups is reduced by 60%, and that of the OH groups of serine and threonine is reduced by a factor of two. Tyrosine is not then acetylated at all.[100] In the succinylation process, sodium sulphate inhibits most strongly the SH groups without affecting the reactivity of the OH groups of serine and threonine; *O*-Suc-tyrosine was not detected.[100] One should recall that it is readily deacylated.[102]

It is seen that, regardless of the acylation conditions, the main reaction centres (apart from the terminal α-amino-groups) are the ε-amino-groups of lysine and the degree of modification of the protein by acylation has therefore come to be estimated in terms of the percentage of the substituted ε-NH_2 group (the number of the accessible ε-NH_2 groups of lysine in the given protein system is adopted as 100%).

There exist biological, microbiological, and chemical methods for the determination of the accessible lysine in proteins.[116–119] One of the most reliable and trustworthy chemical methods is based on the reaction of the primary amino-groups with trinitrobenzene-sulphonic acid.[119]

A number of studies have been devoted to the investigation of the functional properties of acylated isolated fish proteins.[50,51,87,120–125] The emulsifying activity of the succinylated *Rockfish* isolated protein increased in proportion to the degree of modification. For example, it amounted to 1.3 g per mg of the protein for 30% modification and increased by a factor of two for 77% modification.[120] The isolated hake proteins acquired emulsifying properties as a result of succinylation which were superior to those of gelatine. The stability of the emulsions obtained was specially emphasised.[121] It is important to note that the changes in the emulsifying capacity of the succinylated isolated proteins at different pH and after the introduction of monovalent and divalent cations (Na^+, Ca^{2+}) were of a fundamentally different character than for the unmodified proteins.[121]

Acetylation and especially succinylation appreciably increased the foam-forming capacity of fish proteins, but the stability of the foams diminished compared with the control.[51]

By subjecting the homogenates of plant and animal tissues to acetylation simultaneously with succinylation, protein products with effective emulsifying and foam-forming properties are obtained together with a high solubility and water-absorption capacities. It has been recommended that such products be used as food surfactants.[122–124] Acylated myofibrillar proteins have a characteristic solubility profile: they are almost insoluble in an acid medium below the isoelectric point of the protein. The isoelectric point is displaced towards acid pH in proportion to the degree of modification of the protein. This is associated with the decrease in the number of free ε-amino-groups of lysine. The solubility increases sharply at neutral and weakly alkaline pH precisely because of the displacement of the isoelectric point.[121]

The absorption of water by the *Rockfish* isolated protein increases by a factor of two on acetylation and by a factor of five on succinylation.[51] Succinylation increases the capacity of the isolated protein for rehydration and its thermal stability (it did not coagulate even at 100 °C).[120]

It has been noted that acylation lowers somewhat the capacity for gel formation but does not destroy it: 3% solutions of the acetylated protein and 7% solutions of the succinylated protein formed gels of the same kind as a 2% solution of gelatine.[51]

A method has been proposed for the preparation of viscoelastic gels from fish proteins succinylated to a low extent (up to 10%), the stability of the gels being inversely correlated with the degree of modification.[125]

It has become evident that the acylation of isolated fish proteins makes it possible to use them as powerful emulsifying and foam-forming additives to food systems (Table 1).

Since the foam-forming and foam-stabilising properties of the succinylated fish protein are not inferior even to those of egg albumin, it is believed[87] that it can replace egg protein up to 50% in confectionery, for example in sweets or in whipped dessert dishes, and can be used in fruit desserts instead of gelatine[51] and in ice cream instead of caseine.[50]

Table 1. Functional properties of acetylated and succinylated fish proteins (the degrees of modification are equivalent and amount to 50–60%).[87]

Properties	Ac	Suc
Emulsifying activity at a protein concentration of 5 mg × ml^{-1}, % emulsion	61	97
Emulsifying capacity, g of oil/100 mg of protein	53	132
Capacity for gel formation, minimum concentration of protein solution (%) for the formation of a stable gel	4	3
Foam-forming capacity, volume of foam in ml	550	600
Stability of foam, syneresis volume in ml	71	32
Water absorption, ml of water/1 g of protein	15	20

The addition of succinylated fish protein to meat products in an amount of 10–20% does not impair their organoleptic properties.[87]

Similar results have been obtained in studies on the modification of structural meat proteins by acylation.[58,126] Acetylated and succinylated heart muscle proteins from cattle proved to be satisfactorily soluble in aqueous media at a low ionic strength and exhibited a higher emulsifying activity even at pH 6.0 than that of the native protein at pH 7.4, which is sufficient for meat systems.[126] Furthermore, anhydrides with a more complex structure were used for modification, such as benzene-1,2,4-tricarboxylic anhydride (BTCA) and benzene-2,3,4,5-tetracarboxylic dianhydride (BTCDA). In the reactivity series, they occupy an intermediate position between acetic anhydride (AA) and succinic anhydride (SA):

$$AA > BTCDA > BTCA > SA.$$

All the anhydrides quoted, with the exception of AA, reacted much more slowly with the SH and OH group of the protein than with the amino-groups. In the presence of the greatest excess of the anhydride, not more than 1/4 of all the SH groups of cysteine reacted and the products of the reaction of SA with serine and threonine were not detected. A sharp increase in the solubility of the derivatives was noted in all cases even for low degrees of modification.[126] Unfortunately no data have been quoted on the assimilability of the new modified proteins.

Summarising the data on the acylation of myofibrillar proteins, one must emphasise that acylation alters to different extents (depending on the nature of the acylating agent and the reaction conditions) the overall charge on the macromolecule, the isoelectric point being displaced to the acid region. As a rule the structure of the protein becomes less helical, which entails an enhancement in its hydrophobic properties. The tendency towards dissociation into subunits increases and the tendency towards specific polymerisation diminishes. Simplifying the pattern of the real changes in the modified protein system, the increase in solubility, in the moisture retaining capacity, and in swellability are usually attributed to the shift of the hydrophilic–hydrophobic balance towards an increased hydrophilicity; the increase in the emulsifying and foam-forming capacities is frequently explained by an enhancement of hydrophobic properties.

The set of chemical reagents used for the modification of myofibrillar food proteins is unfortunately extremely small. There is no information in the literature concerning isolated muscle proteins about promising methods such as, for example, phosphorylation, deamidation, and esterification.

When the chemical agents for the regulation of the functional properties are compared with enzymes, the following features may be noted. Chemical modification does not lead to the loss of the binding and gel-forming properties of proteins, of the kind observed in enzymic reactions. The degree of proteolytic decomposition has a stronger influence on the functional properties of the protein system than the degree of chemical modification. On the other hand, the specified level of chemical modification can be more readily ensured than a rigorously defined degree of proteolysis. From the standpoint of the preparation of highly functional isolated proteins, which remain stable on storage and during treatment in a specific food system, chemical modification has advantages compared with enzymic modification (see Section VI). However, certain types of chemical modification lower to different extents the nutrient value of food proteins and their assimilability, which does not occur in the enzymic treatment.

IV. Combined chemical and enzymic modification of proteins

In a number of instances, combined chemical and enzymic treatment of food muscle proteins has been applied extremely effectively.[50,51] The weak hydrolytic effect of bromeline on the succinylated *Rockfish* protein makes it possible to obtain excellent foams, as regards bulk and stability, at protein concentrations of 3–4% over a wide pH range. The stability of these foams was greater than that of the foams based on egg and soya proteins.[50] The maximum positive effect in the improvement of the foam-forming properties was obtained by mild proteolysis of 40–50% succinylated fish proteins.[51,127] However, under all the conditions tested, this procedure reduced the emulsifying activity, gel formation, and swellability.[51,127]

It has been reported[20] that, regardless of the sequence in which the acylation and proteolytic processes are carried out on fish proteins, products with similar functional properties are obtained, although, in principle the results should not be the same. If chemical modification is carried out in the first stage and the acylated proteins are then subjected to proteolysis, then in this instance proteolysis proceeds more slowly and certain peptide bonds are not split at all. Larger fragments of the protein molecules, chemically modified to a lesser extent, should be obtained in this way. If, other conditions being equal, the above processes are interchanged, then proteolysis proceeds without obstacles and the resulting protein fragments, having a larger number of accessible reaction groups, react chemically. This procedure should lead to more profound chemical transformations of smaller protein fragments.

Using these hypotheses, one may recommend the first method for the activation of the surface properties of a protein and the second

for increasing its solubility. From the standpoint of the retention of the nutrient value of the protein, the first method is preferable.

Another aspect of the successful use of the combined chemical and enzymic modification of the protein involves the covalent attachment of aminoacids or their esters with the aid of enzymes.[55,56,127,128] Using papain, the covalent attachment of the alkyl esters of leucine to succinylated fish protein has been achieved.[127] By varying the length of the hydrocarbon chain of the alkyl ester of leucine, the authors obtained superactive emulsifying and foam-forming agents: the C_4-C_8 alkyl groups correspond to the maximum foam-forming activity and the $C_{10}-C_{12}$ groups correspond to the maximum stability of the foams and emulsifying activity.

Transglutaminase is frequently used for the covalent "attachment" of aminoacids to the protein.[55,56,128,129] The efficiency of the reaction involving the attachment of the aminoacid residue increases if the amino-groups in the attacked protein were acylated beforehand. Intermolecular cross-links, which reduce the digestibility of the protein, are not then formed. After the enzymic treatment, the amino-groups can be regenerated. Maleic, 2,3-dimethylmaleic, and citraconic anhydrides, whose *N*-acyl residues are cleaved in an acid medium, were used for the reversible acylation. This procedure has been recommended for increasing the solubility and emulsifying capacity of isolated proteins from plant and animal raw materials.[55,56]

It has been suggested that fish proteins, to which hydrophobic aminoacid (leucine, norleucine, and alanine) have been "attached" enzymically, be used as food antifreeze agents. The alkyl residue of the ester group should also be hydrophobic ($R = C_8-C_{10}$). An antifreeze of this kind is non-toxic and is very effective in the storage of food products in a fresh form at low temperatures.[129]

V. Physicochemical modification methods

The physicochemical methods for the treatment of isolated food proteins include the following procedures: complex formation with natural biopolymers (proteins, polysaccharides, etc.) and also with monomeric compounds (carbohydrates, lipids); mechanical treatment of different kinds, heat treatment, etc.

Extensive studies on these lines have been carried out by Tolstoguzov and co-workers on proteins of plant origin.[3,130]

Changes in the functional properties of food proteins can be achieved by varying the hydrophilic–hydrophobic balance in the protein system as a result of specific protein–protein interactions. Studies of the conjugates of myosin with plant proteins have aroused considerable interest.[131–138] It has been noted that, when mixed solutions of myosin and 11S soya globulins are heated, hybrid aggregates are formed.[131] Basic subunits, formed on partial dissociation of the 11S globulins, and the heavy chains of myosin are capable of interacting. The degree of binding increases on raising the temperature in the range from 85 to 100 °C. As a result of thermal denaturation, the subunits undergo a conformational rearrangement accompanied by aggregation with formation of qualitatively new energetically more favourable protein complexes. Hydrophobic interactions play a significant role in this process.[132]

Myosin is able to aggregate with β-conglycinin in a similar way at temperatures above 85 °C.[133] The formation of mixed conjugates can be detected by gel electrophoresis. The aggregates obtained are distinguished by a high solubility and thermal stability. The latter property permitted the pasteurisation of meat systems with added soya protein.[134,135]

When solutions of actomyosin were heated with the "CIF" fraction of soya protein, the formation of stable gel structures of a new type was observed.[136,138] The denatured soya protein was associated in a definite manner with the actomyosin network, and, according to the scanning electron microscope data, the resulting structure differed appreciably from that of the thermogels formed by the individual components. According to the authors' hypothesis, the formation of disulphide linkages between the two protein partners is possible in this instance.[136]

Hybrid protein complexes can also be obtained by intermolecular cross-linking. The possibility of the covalent binding of myosin to globular proteins such as soya protein, casein, and gluten as a result of the formation of an amide bond between the γ-carboxy-groups of glutamic acid and the ε-amino-groups of lysine, both in the presence and absence of an activator enzyme (transglutaminase), was investigated.[55,56,128,137] The optimum binding conditions depend on the nature of both components. The percentage binding of soya protein and fibrin to myosin increased on raising the pH of the medium and temperature, while the binding of caseine was favoured by an increase in temperature and a decrease in pH. With participation of the enzyme, the maximum amounts of proteins bound to 1 g of myosin are 0.58 g of caseine, 0.30 g of gluten, and 0.15 g of soya protein.[128]

Complex formation of the type of protein–protein interaction found a practical application before its theoretical interpretation. It was noted in an early study[87] that, when proteins of different nature are dried together, not only are highly nutritious protein mixtures obtained but their functional properties are also retained at a high level. For example, instead of adding sodium chloride and phosphates to fish proteins in order to optimise their drying, it is more convenient to dry mixtures of fish and grain proteins which, together with a gain in the functional properties of the protein concentrate, has a positive economic effect. The grain additives (from 10 to 30 wt.%) can be wheat, rice, and virtually any kind of flour.[87,139]

The addition of plant proteins to semi-finished meat products ensures, by virtue of complex formation, the minimum losses of the moisture-retaining capacity of the system on heat treatment.[140]

Conjugates formed by proteins with carbohydrates possess as a rule highly effective functional properties. The ability of carbohydrates to protect muscle proteins against denaturation under both low-temperature conditions and on heat treatment has been known for a long time.[19] The antidenaturing capacity of saccharides in relation to fish proteins decreases in the sequence maltose > sorbital > sucrose ≃ maltitol > glucose > lactitol. At negative temperatures, carbohydrates exhibit under these conditions a highly protecting effect at lower concentrations.[141–143] When fish proteins are dried together with monosaccharides, highly hydrophilic complexes are produced. The solubility of such complexes is proportional to the percentage content of the saccharide and depends on the nature of the latter. In terms of their influence on the solubility of the adduct with fish protein, the monosaccharides tested can be arranged in the following sequence: glucose ⩾ sucrose > fructose.[19] Glycerol and partly hydrolysed starch have similar properties.[19] The solubility of conjugates of fish protein with starch is 25% of the solubility of the freshly isolated protein.

By drying fish protein with 10% of added glucose, sorbital, or sucrose, complexes with a high emulsifying activity and an enhanced thermal stability are obtained. However, they partly lose their nutrient value as a consequence of the Mayar reaction.[19]

With the aid of complex formation (for example with carbohydrates), it is possible to improve the capacity of acylated myofibrillar proteins for gel formation. For example, the aggregation of succinylated fish proteins with glucono-delta-lactone leads to the formation of gels distinguished by a high stability and formed even in oil-containing systems.[121]

The addition of glucono-delta-lactone and citric acid promotes the retention of functional properties by ground beef.[86]

Together with nucleotides, lipids bound to myofibrillar proteins protect them against denaturation. The mechanism of the protecting action requires further study.[62,144,145]

Certain functional properties of meat systems can be altered by simple procedures such as heat treatment. By selecting the optimum conditions (temperature and pressure), the moisture retaining capacity, tenderness, succulence, and other properties of meat specimens of different quality can be regulated.[146–148] The changes occurring in the properties can be explained by the partial or complete decomposition of individual components of the myofilaments: connectin and nebulin.[148]

Simple mechanical treatment of proteins, both animal and plant, for example, grinding in a vibromill, are known to be capable of improving their solubility and moisture-retaining capacity as a consequence of the mechanical degradation of sarcomers.[148,149]

Combined mechanochemical modification is much more effective. Under different conditions in the treatment of fish homogenates with acetic or succinic anhydride in a vibromill, it is possible to increase greatly their moisture-retaining and foam-forming capacities and their ability to stabilise foams.[149,150]

Presumably, among the physicochemical methods of treatment, the complex formation procedure is the most promising: it has no pronounced disadvantages or limitations of the kind charactersitic of chemical or enzymic modification (from the standpoint of toxicity or the loss of nutrient value). In general, practice has shown that the best results in the transformation of the functional properties of food proteins can be obtained by an appropriate combination of various modification procedures, for example, chemical and enzymic methods or chemical methods and complex formation.

VI. The nutrient value and the bioavailability of the modified food proteins

Among all the procedures for the modification of food proteins enumerated above, chemical treatment gives rise to special risks from the standpoint of the toxicity and bioavailability of the protein. The principal disadvantage of the chemical modification is believed to be the decrease in the nutrient value of the food proteins. Indeed, the action of chemical reagents leads to a definite loss of the bioavailability of the protein (different for each kind of chemical modification and for each type of modified objects). At the same time, the blocking of the most reactive groups of the protein leads to the formation of more stable, albeit less nutritious, non-toxic products.

The loss of nutrient value and the decrease in the assimilability of the protein products are the negative phenomena which accompany any traditional treatment of the protein (drying, canning, cooking, and even the storage process).[36,151] They are due to the involvement of particular chemical reactions of the active functional side groups in essential aminoacids. The ε-amino-groups of lysine and the SH groups of cysteine are believed to belong to the class of the most reactive groups. For example, during the storage of both frozen fish and of dry isolated fish proteins, undesirable side products are formed, one of which is formaldehyde. The latter reacts readily with the amino-, mercapto-, and hydroxy-groups of the protein and to a lesser extent with the amide groups, reducing the assimilability of the protein.[152,153] Myosin interacts with certain free aminoacids even at –20 °C, the reactions of the protein with taurine, lysine, and histidine accelerating the denaturation aggregation of the protein.[97]

In the oxidative degradation of lipids, the percentage of the ultilised protein (fish or meat) can be reduced by a factor of two, the maximum loss involving under these conditions tryptophan, lysine, histidine, methionine, and cysteine.[36,154,155]

During the heat treatment of fish at temperatures starting from 50 °C, the mercapto-groups of cysteine undergo vigorous oxidation with formation of disulphide bonds. A greater decrease in assimilability was observed for lean fish compared with fat fish. The SH groups responsible for the binding to lipids are apparently protected against oxidation.[156] When proteins are heated to temperatures ranging from 80 °C upwards, there is also the possibility of the interaction of the lysine ε-amino-groups with the accessible carboxy-groups of glutamic and aspartic acids.[157,158] The same reaction can be initiated by enzyme action rather than heat,[159] which naturally leads to a decrease in the amount of the accessible lysine.

A shift of the isoelectric point of the protein in fish concentrates from pH 5.0 to 4.5 as a result of lyophilic drying has been noted, which is incontrovertible evidence for the involvement of the ε-amino-groups in the intramolecular and intermolecular cross-links of the protein.[19]

When fish is canned, not only is it not possible to avoid a decrease in the assimilability of the protein by approximately 15% [160] but it is also necessary to achieve constant monitoring of the accumulation of the potentially toxic compounds formed as a consequence of the occurrence of the Mayar reaction during sterilisation as has been shown for salmon and plaice.[161]

Among aminoacids, lysine is the most active participant in the reaction with sugars.[162] A linear decrease in assimilability as a consequence of the loss of accessible lysine, in fact as a result of the Mayar reaction, has been noted for a whole series of food proteins.[74] It is therefore recommended that serious attention should be paid to the analysis of food products with a relatively high carbohydrate content if they are heat treated.[74,163] For example, it has been shown that 19 days after the addition of 10% glucose to a system based on fish protein with an 18% moisture content at 35 °C half the accessible lysine is lost.[163] Sodium sulphite (or SO_2) is added to inhibit the Mayar reaction but this measure is not universal.[153]

Acylation of proteins appreciably reduces the possibility of the occurrence of this undesirable reaction and also of other side reactions undergone by the unmodified protein under the conditions of alkaline and heat treatment, on storage, and on drying.[36,153,157,158,164,165]

Individual types of modification of food proteins, especially chemical modification, lead to profound changes in the protein structure. Studies on bioavailability, assimilability, and metabolic pathways for the modified systems are therefore of primary importance together with other complex medicobiological problems as regards toxicity and carginogenic and mutagenic properties of new products.[166–169]

A definite trend has arisen in recent years towards reducing the content of common salt in food products for man.[58] When food proteins are used in the form of proteinates, an optimum limit to the content of inorganic ions, which does not affect the health of man,

must also be found. In those cases where alkaline hydrolysis is used to solubilise proteins, it is essential to desalinate the product.[87,170–172]

The nutrient properties of protein isolated from chicken muscle tissue have been compared in detail[173] with those of the muscle tissue separated mechanically in the form of a homogenate. It was shown that alkaline extraction followed by acid precipitation does not entail any appreciable loss of nutrient value of the protein, but its functional properties are then appreciably impaired.[173]

As regards chemically modified food proteins, most information concerning medicobiological tests refers to acylated proteins. Since the available literature data are fairly contradictory, it is apparently desirable to compare data referring to acylated proteins of different kinds.

The content of accessible lysine is known to diminish during the acylation of protein. The role of lysine in human and animal nutrition is extremely important. The deficiency of this essential aminoacid induces serious disturbances in metabolic processes, in the first place in the lipid metabolism.[174] Despite the fact that the set of methods for the determination of the available lysin *in vivo* is entirely satisfactory,[2] the interpretation of the experimental data is frequently complicated by side effects.[172] The prospects for the chemical modification of food proteins depend on the validity of biological tests. The question of the assimilability of lysine from its ε-amide derivatives is mainly disputed. The deacylase activity has been investigated on animals in fair detail. As early as 1943, data were obtained on the partial assimilation of ε-Ac-lysine by young rats.[175] The rate of growth of experimental animals receiving ε-Ac-lysine perorally was lower than for the controls. However, on doubling the dose of ε-Ac-lysine, the difference in the growth of rats was indistinguishable. When ε-Ac-lysine was administered intra-abdominally, partial assimilation of lysine by the animals was also noted.[175] These data agree with the conclusions reached in a whole series of studies on these lines namely that rats assimilate approximately 50% of lysine and mice assimilate about 30% from a diet containing ε-Ac-lysine.[157,158,176–178]

Using di-, tri-, and tetra-peptides containing the ε-Ac- and ε-Suc-lysine residues as models of the fragments of the acylated protein, their proteolysis was investigated *in vitro* under the influence of enzymes from the gastrointestinal tract.[179,180] All the α-peptide bonds adjoining the modified lysine were cleaved, (in contrast to the ε-amide bond) in conformity with the nature of the enzyme tested. However, the initial rates of proteolysis were lower in attack on ε-Ac- and especially ε-Suc-peptides.[179]

One of the little investigated aspects of the assimilation of acylated proteins is the stage where their hydrolysis products are absorbed. In experiments on the isolated small intestine of the chicken, it has been shown that ε-Ac-lysine is absorbed by the cells of the mucous membrane almost just as readily as free lysine, while ε-Suc-lysine is absorbed much less effectively. Lysine modified by aliphatic acid or glutamic acid derivatives is hardly transported through the small intestine of chicks.[180]

The results of Leclerc and Benoiton[181] demonstrate the selective activity of animal kidney acylases: only the ε-formyl and ε-acetyl derivatives of lysine deacylated. The deacetylation of the ε-Ac-lysine by the chick kidney tissue homogenate takes place fairly vigorously (30% of the ε-Ac groups are split off in 4 h). The data presented show that, although the ε-acylated proteins are biodegradable, from the standpoint of their true utilisation acetylated derivatives have undoubted advantages over all other acylated proteins.

With the aid of a microbiological test, it has been demonstrated convincingly that the acylation of the hydroxy- and mercapto-groups of caseine does not lower its nutrient value. In those cases where the ε-amino-groups of lysine were acylated, the biological value of the protein fell with increase in the size of the acyl group. The biological value of caseine fell from 100 to 48% on acetylation (degree of modification 35%) and to 12% on succinylation (degree of modification 74%).[182] The low and moderate degrees of succinylation of caseine and lactalbumin hardly lower their nutrient value, as shown enzymically *in vitro* and by growth tests on rats.[183,184] It has been reported that mice fed on highly acetylated caseine grew, developed, and multiplied.[178] However, they were smaller and leaner than the control animals and had fewer offspring. Chicks receiving acetylated egg albumin (degree of modification 67%) and succinylated egg albumin (degree of modification 55%) showed a weight increment during growth which was greater in the first instance and smaller in the second. However, both groups grew more slowly than the control animals. In those cases where lysine had been added to the acetylated egg albumin, the growth of the chicks was normalised.[172]

The bioavailability of the succinylated protein of unicellular organisms fell by a factor of 10. The estimate was carried out *in vitro* using the pepsin–pancreatin enzyme system.[185]

When proteins deficient in lysine were acetylated, the decrease in biological value was inappreciable. After the complete acetylation of the protein isolated from *Vicia-faba*, a decrease in nutrient value was not noted.[186] We shall compare these data with the available information concerning the nutrient value of acylated muscle proteins.

Groninger and Miller[115] investigated *in vitro* the susceptibility to attack by pepsin and trypsin of fish protein 50% acetylated and 40% succinylated. They reported that the rate of proteolysis by pepsin did not change compared with the unmodified protein. On the other hand, trypsin hydrolysed less effectively the succinylated protein and still less so the acetylated protein, in agreement with other data.[187]

The bioavailability of 90% succinylated fish protein has been estimated[121] from the percentage of the lysine liberated on treatment with pancreatin *in vitro*. The bioavailability of the modified protein fell by a factor of 10.

It has been demonstrated on several generations of rats and mice that the consumption of acetylated and succinylated fish proteins[87] as well as acetylcaseine is not harmful.[188] Histological studies on sections of the liver, the lungs, the kidneys, and the adrenal glands of the experimental animals did not reveal pathological effects and no changes in the internal organs themselves were noted.[187,188]

The metabolism of the acetylated and succinylated fish proteins was investigated on rats by introducing the ^{14}C radioactive label into the ε-amide groups of the protein.[115] The degree of utilisation of the protein was estimated from the distribution of ^{14}C in the carbon dioxide and urine from the animals. After the intravenous administration of a solution of ε-Suc-lysine or the hydrolysate of the Suc-protein, 87 and 93% of ε-Suc-lysine was eliminated with urine respectively, while after the above substances were administered perorally 75 and 72% of ε-Suc-lysine was eliminated from the organism. According to the final data, after intravenous administration, ε-Suc-lysine is not assimilated at all, while ε-Ac-lysine is assimilated to an extent of approximately 25%. After the peroral administration to rats, about 14% of the lysine from ε-Ac-lysine and approximately 50% of lysine from the acetylated protein are assimilated.[115] It has been noted that the metabolism of the succinylated derivatives is much slower than that of the acetylated derivatives.[115]

It has been suggested that the partial utilisation of lysine after the peroral consumption of acyllysine derivatives or acylated proteins can be achieved as a result of the activity of their intestinal microflora.[180,189,190]

The assimilation of lysine on biodegradation of acylated proteins takes place with a higher coefficient of utilisation than the assimilation of lysine from its ε-amide derivatives.[166,190,191] The difference between the mechanisms of these processes has been confirmed experimentally.[180] When the membrane food digestion process was modelled on the isolated chick small intestine, it was noted that the ε-Ac-lysine-containing peptides are partly deacylated, although the elimination of the ε-Ac-group from ε-Ac-lysine was not observed under these conditions[180] (Table 2).

Table 2. Biological value of acylated food proteins.

Protein	Degree of modification, %	Coefficient of effectiveness of protein, %	Refs.
Caseine	0	100	—
Ac-casein	35	48	[182]
Suc-casein	74	12	[182]
Ac-casein	100	64	[188]
Suc-casein	100	12	[188]
FPC*	0	110	[120]
Suc-FPC	30—40	79	[120]
FPC	0	101	[115]
Ac-FPC	50	103	[115]
Ac-FPC	75	85	[115]
Suc-FPC	25	96	[115]
Suc-FPC	40	73	[115]
Suc-FPC	75	35	[87]
FPC	0	41	[121]
Suc-FPC	90	4	[121]
Caseine	—	2,5**	[58]
Myofibrillar protein from ox heart muscle (MPOHM)	—	2,83**	[58]
Acetylated MPOHM	—	2,55**	[58]
Succinylated MPOHM	—	2,36**	[58]

*FPC = fish protein concentrate.
**In grammes per gramme of increment.

The utilisation of proteins with covalently attached aminoacids requires additional study. The metabolism of such proteins is undoubtedly difficult,[117] because the isopeptide bond formed is not cleaved by digesting enzymes and the isopeptides themselves are not absorbed into the blood, as shown in relation to glutamylisopeptides.[180] From the standpoint of Feeney and others,[64–66] the bioavailability of proteins with isopeptide fragments is determined by the nature of the grafted aminoacids and their steric accessibility to the enzyme.

In our view, instead of the covalent binding of the aminoacids, it is more rational to enrich the isolated proteins by adding polyaminoacids. The excellent assimilation of polylysine is the basis for this recommendation.[66,192]

There are doubts concerning the bioavailability of hybrid protein conjugates obtained by grafting glutamic acid and lysine residues.[128,137] However, it is believed[137,193,194] that glutamyllysine-cross-linked proteins are fully assimilated in the intestine, although as a rule the rate of metabolism is extremely low.

Objective assessment and timely introduction of promising methods for the modification of isolated proteins makes it possible to intensify the ultilisation of food proteins from traditional and non-traditional sources in order to obtain new products for mass consumption.

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