CHEMICAL CHANGES
DURING FOOD PROCESSING

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DEVELOPMENTS IN FOOD SCIENCE 21

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Chemical Changes during Food Processing

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PREFACE

It is no easy task to introduce a book whose aim is to describe in detail the manifold phenomena and processes which take place during the processing and storage of food.

Food processing is as old as mankind. As human society has evolved and knowledge has increased, so also has food processing developed in parallel. With the relatively recent expansion in the understanding of nutritional physiology, the requirements imposed on the processing of food have changed. At first sight it seems strange that food science as a whole has not assembled a volume of knowledge to match its long history, for it must be admitted that the development of knowledge in this sphere has lagged a long way behind that in other areas of science. A description of the changes occurring in food processing and storage was acquired only slowly. This can be put down to the complex chemical heterogeneity of foods and the correspondingly complex reactions and processes which take place, so that until fairly recently advances were concerned more with description than with understanding. It is only in the last few decades that powerful new instrumental methods of analysis and particularly of separation have revealed new aspects of food science and have thus hastened its development.

The majority of the older textbooks on food chemistry therefore confined themselves to a description of the reactions of food commodities and attempts at their explanation. This was naturally so, since the hitherto assembled corpus of knowledge did not permit a more generalized and perceptive approach. When we prepared the manuscript for the Polish edition of our book on food chemistry in 1975, we took the alternative and more informative course of dealing with the changes occurring in foodstuffs from the aspect of the different constituents in the foods regardless of the particular food item *per se*. The response showed that it had achieved its purpose, and based on this experience we published a further book, in Czech, in 1980.

We now submit to the wider scientific public a monograph on Chemical Changes during Food Processing. In common with our previous books, its basic pattern is concerned with the description of changes occurring in the food constituents. A substantial difference, however, is that we assume some previous knowledge of food chemistry. This allows us to concern ourselves only with those changes in different food constituents which have an important bearing on the nutritional, sensory, hygiene and technological aspects of the food industry. Only natural food constituents are considered; the entire sphere of food additives and contaminants has been omitted intentionally.

This, then, is a food chemistry approached in a new way with a minimum of descriptive matter, and focused on the changes which occur during processing and storage. Our objective has been to generalize these complex reactions which take place in different foods and thus to promote the understanding and development of this scientific discipline. Our readers will judge to what extent we have succeeded in this task.

The Authors

CONTENTS

Chapter 1

PROTEINS, PEPTIDES AND AMINO ACIDS

1.1 INTRODUCTION

This chapter is intended to provide basic information on protein changes during food processing and storage. The main stress is laid on the changes connected with the quality of produced foods, and an underlying knowledge of protein chemistry is hence required.

First of all the physico-chemical changes are described. Denaturation of proteins from the theoretical point of view is discussed, using practical examples from food processing (meat and meat products, eggs, milk, cereals, fruits and vegetables). As to chemical changes, attention is paid mainly to enzymatic and non-enzymatic hydrolysis, redox changes of proteins, interaction with phenolic substances and formation of protein salts. Each reaction is demonstrated on the most important examples from food technology: ripening and autolysis of meat, ripening of cheese, enzymatic changes of proteins during dough making and during malting and brewing, production of yeast autolysates, enzymatic protein hydrolysates, non-enzymatic protein hydrolysates, effect of redox reactions on dough proteins; meat proteins, effect of tannins on proteins and their influence on haze formation, formation of protein salts in canning technology and the interaction of casein with calcium.

The second part of this chapter deals with peptides and amino acids. Conformation changes of amino acids during the technological production of protein isolates are discussed. The redox activity of some amino acids, especially the sulphur-containing ones is demonstrated on their changes during the processing of vegetables and during bread baking. Other reactions of great importance are: enzymatic and non-enzymatic decarboxylation and deamination of amino acids, cyclization of amino acids, formation of N-nitroso compounds, the enzymatic browning reaction and the Strecker degradation of amino acids. All these reactions are described on practical examples from food technology. In most of the existing monographs on food chemistry the changes of proteins, peptides and amino acids are dealt with in terms of the overall technological procedure without paying attention to the classification of each reaction. In this chapter a theoretical explanation of each type of reaction is given and this is then followed by examples taken from different branches of food technology.

This approach makes it easy to recognize the most important reactions occurring during the overall procedure, to identify their mechanisms, and thus to control these reactions in the directions which yield products of highest quality.

1.2 PROTEINS

1.2.1 Denaturation and hydration reactions

Native proteins represent a highly ordered structure possessing a relatively stable conformation which is optimal as regards protein function.

Denaturation of proteins is defined as fundamental conformation changes in all parts of their molecule leading to the complete loss of biological activity and natural functionality. The arrangement of the polypeptide chains becomes more random and similar to the amorphous state. The course of the conformational changes and the corresponding biological response is often gradual and reversible (p. 14).

Denaturation does not involve any changes in the primary structure. On the contrary, all other higher structures (secondary, tertiary and quarternary) are altered. Many proteins are formed from just one single polypeptide chain, and therefore the tertiary structure represents their highest degree of spatial organization. On the other hand, some proteins are composed of more than one polypeptide chain. The mutual spatial arrangement of the individual polypeptide chains, called sub-units, represents the quarternary structure which is the necessary condition for full biological activity. The individual sub-units are mutually bound mainly by hydrophobic and electrostatic bonds or by other types of non-covalent interactions, such as hydrogen bonding, dipole-dipole interactions, and van der Waals attractive forces [1]. In general, disulphide bonds are not involved in maintaining the quarternary structure.

The quarternary structure represents a rather labile formation which may be dissociated to individual sub-units by treating the protein with salts, detergents, various chemical compounds, and by changes of pH.

Electrostatic bonds develop between two opposite charges in juxtaposition. Aspartyl, glutamyl and terminal α-carboxyl residues interact with lysyl, guanidyl, arginyl and terminal α-amino groups. Hydrogen bonds are formed between atoms which have unshared pairs of electrons, mainly oxygen and nitrogen. Dipole-dipole interactions are known, for instance, between the hydroxyl groups of two serine residues. Where there is an imbalance in charge distribution within the molecules, the dipoles may interact with one another. Hydrophobic interactions of non-polar side chains of amino acid bonds in the polypeptide chain are caused by mutual repulsion of water. Van der Waals interactions represent complex interactions between non-polar groups and molecules [2].

The most stable bonds are disulphide bridges. They can be disrupted by reducing agents which convert the disulphide bonds to sulphydryl groups. Proteins in which the tertiary structure is fixed by disulphide bridges are, in general, more resistant to denaturation. Many of extracellular enzymes contain disulphide bonds and, therefore, they are more stable than the intracellular enzymes which contain disulphide bonds only rarely.

The stability of proteins against denaturation is affected by some other phenomena. Metallic ions may help to maintain the tertiary structure of proteins and enhance in this way their stability against denaturation. This is well known for many enzymes, such as Ca^{2+} for α-amylase (EC 3.2.1.1) and trypsin (EC 3.4.4.4), Mg^{2+} and Co^{2+} for glucose isomerase (EC 5.3.1.9).

Protein stability is also enhanced by its solvation. It is known from the theory of globular proteins that the hydrophobic amino acid residues are located inside the globule, while the hydrophilic (polar) groups are situated outside the globule and exposed to the solvent (water). Due to this arrangement, a few layers of water molecules are bound to the surface of the protein. The first layer of water molecules is bound tightly and the strength of the binding decreases for the succeeding layers. It is difficult to determine exactly how much water is really bound to the protein surface in solution, but it is supposed that globular proteins bind about 0.2—0.5 g of water per gram of protein [3]. Random coiled proteins, such as gelatin, can bind as much as 99 times their weight of water (at 4° C) due to the entrapment of water inside the lattice-like structure [1]. Water binding is not limited only to globular proteins. Insoluble proteins organized into fibres, membranes and other structures (e.g. contractile and connective tissue fibres of muscle) adsorb water, and the degree of hydration is related to their alternating physical and chemical properties [4].

In general, a high degree of protein hydration increases its stability against denaturation and succeeding precipitation. For instance, casein with a low degree of hydration is readily precipitated by a change of pH to its isoelectric point, while ovoalbumin forms a stable dispersion (p. 10).

Often, the improvement of protein stability against denaturation is achieved by its interaction or conjunction with various substances, mainly polymeric ones. The complex proteins, such as glycoproteins, lipoproteins and nucleoproteins etc. are as a rule more resistant to denaturating factors than the simple proteins, a point which is of great importance for food technologists. For instance, a lot of enzymes contain a carbohydrate moiety and all of them are found to be relatively stable (e.g. glucose oxidase, EC 1.1.3.4, invertase, EC 3.2.1.26, cellulase EC 3.2.1.4). The effect of the carbohydrate moiety is ascribed to its ability to prevent dehydration, which is considered to be the first step in the course of protein denaturation.

A wide variety of treatments used in food processing may cause denaturation of proteins. These include heat, surface changes, changes of pH and salt concentrations, dehydration (even at low temperatures), different additives etc. Usually the final denaturating effect is caused by a combination of various factors which disrupt the original protein conformation.

Different denaturating agents may lead to different conformational changes, so that any given protein may be subjected to several different denaturation reactions. Various broken bonds during the unfolding of the polypeptide chain may be renewed by recombination with the other parts of the chain or with other proteins in the locality or even with some other non-protein substances. Such interactions may diminish the rate of protein denaturation. Other substances present in the system affect the denaturation rate, for example sugars (sucrose, glucose) protect proteins against heat denaturation [5].

The definition of protein denaturation (characterized mainly by the loss of biological activity) used by biochemists is not sufficiently broad for the food technologist, who is interested not only in inactivation of enzymes (in blanching, drying etc.) but also in the technological properties of the denatured proteins.

Denaturation alters many properties of proteins which are important from the technological point of view and for food quality. Denatured protein is

usually less soluble or even insoluble, it increases the viscosity of the food and enhances different association reactions. Denaturation also increases the reactivity of side chain groups, mainly those which were originally oriented towards the interior of the original molecule. This is especially true of the sulphydryl/disulphide groups and their interchange.

Denatured proteins are more susceptible to hydrolysis by proteolytic enzymes and, therefore, in many cases their digestibility and utilization are enhanced. The process of protein denaturation taking place during different technological treatments (blanching, pasteurization etc.) leads to enzyme inactivation and the elimination of the toxic effect of various proteins (microbial toxins, natural enzyme inhibitors etc.).

Changes occurring in protein structures on heating may be briefly summarized as shown in Fig. 1.1. These changes include first the so-called pre-denaturational transitions, i.e. minor conformational changes occurring prior to denaturation and, as the reaction proceeds, changes designated as denaturation (resulting predominantly in random-coil configuration of protein structure). Following the pre-denatured state, the heated proteins very often react, either with themselves and/or with other food constituents forming higher-molecular weight aggregates (precipitates, gels etc.). These post-denaturation reactions are virtually irreversible [6—8].

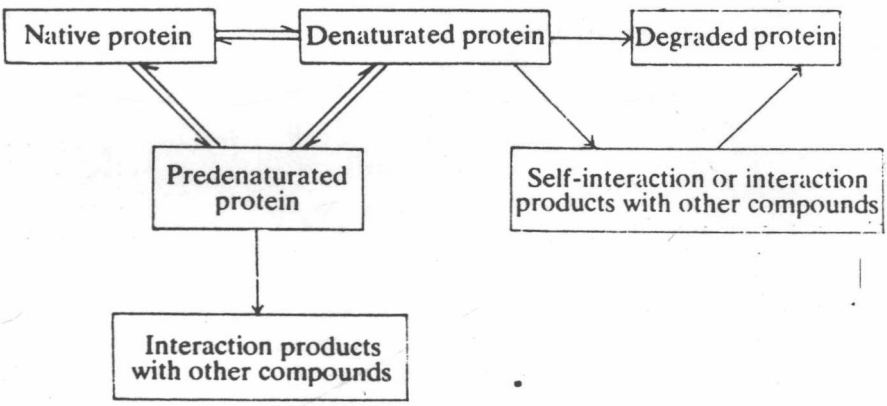

Fig. 1.1. Protein changes on heating.

Depending on such factors as time, temperature, moisture content, and the presence or absence of reducing substances, heating may have either a beneficial influence (inactivation of protein inhibitors and other enzymes, improved digestibility of proteins, availability of amino acids and improved flavour) or a detrimental influence (alteration of some of the linkages between amino acids, formation of new amino acid linkages and undesirable products).

One of the first noticeable changes of proteins on heating (even at temperatures around 100° C) is the loss of labile amino acids such as cystine/cysteine and lysine and the formation of gases such as ammonia and hydrogen sulphide (p. 5).