PHYSICAL CHEMISTRY OF FOOD PROCESSES
VOLUME 2

Advanced Techniques, Structures, and Applications

Edited by

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To those who introduced us to the love of books,
the notions of scholarship and research,
and the nurturing of a body of knowledge.

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This reference, Volume 2 of a two-volume set, offers unique coverage of state-of-the-art techniques and their applications for food science, food technology, biotechnology, and biopolymer studies.

An invaluable synthesis of information previously found only in numerous texts and research articles, this volume offers detailed, up-to-date coverage of both scattering (light, X-ray, neutron) and spectroscopic techniques (NMR, ESR, FT-IR, vibrational, circular dichroism) along with numerous applications. The limitations and advantages of each technique are discussed in the context of specific applications.

Containing contributions by a distinguished international group of researchers, Volume 2 addresses topics of special interest to the food technologist, including:
• starch structure and gelatinization • structure and hydration of pectins • structures of gels and gelling mechanisms • physico-chemical aspects of food proteins (milk, cereals, and soy proteins) and enzymes

In-depth chapters also examine
• the structures of proteins, with emphasis on food proteins and enzymes
• secondary structures of proteins, enzymes, food proteins, polysaccharides, and nucleic acids
• NMR theory, techniques, and applications to biopolymers and starch
• neutron diffraction of hydrated protein crystals
• quantitative composition analyses of foods by supercritical fluid chromatography (SFC)
• genetic engineering of bacteria, along with novel techniques and applications

Enhanced with over 300 illustrations, many of them presenting original results, *Physical Chemistry of Food Processes: Volume 2* is an important working tool for food scientists and food technologists, as well as researchers in biochemistry, physical chemistry, biotechnology, and other biomedical areas. It is also an excellent textbook for graduate and advanced undergraduate food science students.

About the Editors

Ioni C. Baianu is Associate Professor of Food Chemistry, University of Illinois at Urbana, and he also edited Volume 1 in this two-volume set.

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Physical Chemistry of Food Processes

Volume 2

ADVANCED TECHNIQUES, STRUCTURES, AND APPLICATIONS

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Preface

The second volume in this series is concerned with those techniques and methodologies that are widely used to study biopolymers, in general, and food biopolymers, in particular as well as their specific applications. Whereas in Volume 1, which is a textbook intended for both undergraduate and graduate students, such techniques are only briefly outlined (Chapter 5), in this volume the topics are covered *extensively* and with a view to future applications. Therefore, Volume 2 is designed as a *reference* book and provides details that are hard to find in original articles, reviews, or even in recent books assembled by many contributors.

Because *molecular structure* determination is an essential part of *Molecular Biology* and the latter is concerned with explaining functions at the molecular level, this volume will be useful as a reference book not only to food chemists/food scientists, but also to molecular biologists, physical biochemists, physical chemists, biophysicists, and biotechnologists.

Although the emphasis is placed on biopolymers in solution, hydrated protein crystals are also discussed in detail (Chap. 4) and other studies of solid food materials/biopolymers are presented (Chap. 9).

Both scattering (light, X-ray, and neutron) and spectroscopic techniques (NMR, ESR, FT-IR, and Vibrational Circular Dichroism (VCD)) are presented in detail with numerous applications and suggestive illustrations.

Furthermore, genetic engineering and novel biotechnology methodologies are presented, together with quantitative composition analysis of foods by Supercritical Fluid Chromatography (SFC). The editors' introduction (Chap. 1) provides a detailed guide to all chapters in the book, thus helping the reader to find the chapter(s) most suited to reader's interests.

Much of the information presented in Volume 2 is hard to find in existing textbooks or review articles, and represents state of the art and beyond. The assembly of this *unique* reference book would not have been possible without the hard work and strong determination of both contributors and editors who worked harmoniously as a team.

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PHYSICAL CHEMISTRY OF FOOD PROCESSES

VOLUME 2

Advanced Techniques, Structures, and Applications

Contents

1

Application of Physical and Chemical Principles and Techniques to the Study of Biopolymers, Foods, and Food Processes: The Approach

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Today, researchers in the field of food science and technology look toward the future with a great deal of enthusiasm. In particular, they expect that *biotechnology* holds the promise of developing new designer-type products for food and nonfood uses, for example, medical, with tailor-made functionalities. Here, it is hoped that the modification of food proteins via enzyme and chemical modification, and more importantly, genetic engineering, will provide the vehicle for success. Ultimately, genetic engineering may even allow the possibility of creation of transgenic animals and plants whose by-products will need only a small amount of processing to achieve the desired product. Chapter 12 suggests several genetic engineering developments on the basis of current successes. Related food processing applications and immobilized bacterial cell applications are indicated in Chapter 13. However, the historic problem of developing quantitative measures for food protein *structure-functionality* relationships is that the researcher or developer is limited to costly hit-or-miss experiments that have a minimal success rate.

Already, researchers in the pharmaceutical area have been attempting to use genetic engineering for developing new drugs with differing biological functionality. To date, they have been successful in shortening the time necessary for expression of a new gene in *Escherichia coli* and even tissue cultures. However, they are still subjected to a hit-or-miss type of procedure for obtaining the desired biological functionality. In recent years, their efforts have changed to include developing structure-biological function relationships. Chapter 16 documents the use of liposomes in cell culture research which is one of such recent developments that hold promise for pharmaceutical and biomedical applications.

The functionality required by food science and technology is more difficult. Not only is biological functionality of proteins often needed, but other less-defined functionalities such as colloidal stability, dispersibility, gel formation abilities, emulsifications, whippability, etc., are mandated under a variety of environmental conditions (temperature, pH, protein concentration, salt addition, etc.). Furthermore, three-dimensional structures determined by X-ray crystallography of the enzyme-substrate complex and a variety of inhibitors are generally available to the pharmaceutical researcher. The study of such systems in solution by Small-Angle X-ray Scattering techniques will be discussed in some detail in Chapter 2. The majority of proteins important to the food industry cannot be crystallized. Therefore, physical and chemical methodologies for solutions are the only ones routinely available to the food researcher. Thus, a multidisciplinary approach must be used, and the appropriate methodologies must be employed to develop, quantitate, and predict protein **structure-food functionality** relationships. To obtain such a quantitation in complex food systems, it is often necessary to employ digital computers with specialized programs.

In any broad, applied area it is generally true that such integrating approaches are called for to match the variety and complexity of the systems under investigation. This is certainly true in food science. An attack on a basic problem will be most effective when it can summon a number of relevant techniques to be used in concert. Each technique separately may give a view of a slice of the problem, so to speak, while a full perspective is obtained only from the multidimensional view afforded by the integration of more than just a single technique. Basic concepts and techniques available for such purposes were introduced in Volume 1 of this book. The following chapters present state-of-the-art techniques that are being applied to both biopolymers and food systems.

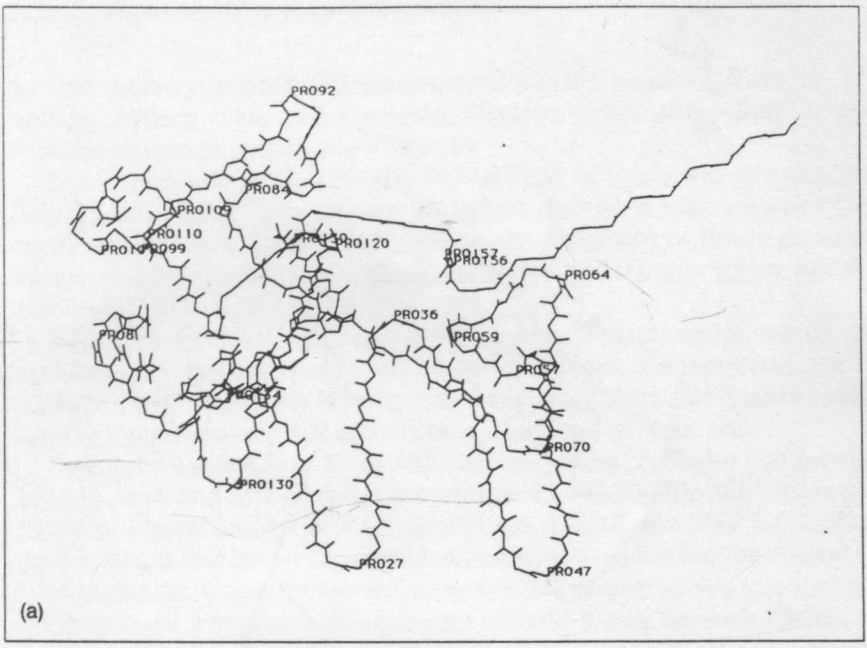
A case in point is the recent development of a predicted three-dimensional structure for κ-casein and α_s-casein. In this case, a research group has recently employed molecular modeling techniques in conjunction with secondary structure sequence-based prediction algorithms, and com-

bined these with global secondary structure analysis via Fourier Transform Infrared (IR) and Raman spectroscopy. The vibrational spectroscopy technique was chosen over the traditional circular dichroism approach for obtaining experimental information on these milk proteins, which allowed them to accurately obtain the fraction and type of β-turns as well as the usual β-sheet, α-helix, and loop or unordered structure fractions. In this case, ordinary circular dichroism (CD) would only allow an estimate of the sheet and the possible turn fractions.

Figure 1-1a shows a backbone structure, while Figure 1-1b shows a stereo-chain time view of this recently proposed κ-casein structure. Here, two "doglegs" resulting from two antiparallel stranded sheets are observed. These side chains (not shown) on these sheets are hydrophobic, indicating interaction sites for hydrophobically controlled self-association or complexing with other caseins.

Figures 1-2a and 1-2b show, respectively, a backbone and a stereo-chain trace-view of this recent α_s-casein B model. Here, two domains are observed that are connected via extended and α-helical structures. The right domain is predominantly hydrophilic, whereas the left hydrophobic domain also contains two sets of antiparallel sheet structure (doglegs), just as earlier observed with the κ-casein structure. The importance of the model is that, now, a hydrophobic sheet-sheet interaction site between κ-casein and α_s-casein, as well as between proteins of the same kind, can be employed as a mechanism for the complexing ability of these two important food proteins. This structure adds more rigidity and specificity to the interaction that was, until now, thought to be the result of random coil interactions. The model can now be tested via genetic engineering or chemical and enzymatic modification to obtain a desired increase or decrease in complexing ability for κ- and α_s-casein. An alternative approach by vibrational CD will be presented in Chapter 8, as well as representative examples for food proteins, polysaccharides, and nucleic acids.

Another, more general, example is provided by the study of water associated with food systems. Controversies on this topic have raged so intensively and for so long that even expressions like "hydration" and "bound" water have threatened to become loaded terms. The practical impact of the topic is, of course, beyond dispute, because hydration properties of foods influence, and even determine, consumer acceptance, storage conditions, shelf life, functionality, quality control, food formulation, and product development (food engineering). All of this underlies the necessity for a rational treatment and one would hope for at least some general agreement on the broad outlines of a comprehensive theory. Solutions to these problems are now provided by powerful new techniques and methodologies for elucidating the structure, conformations and hydration of food systems. Such



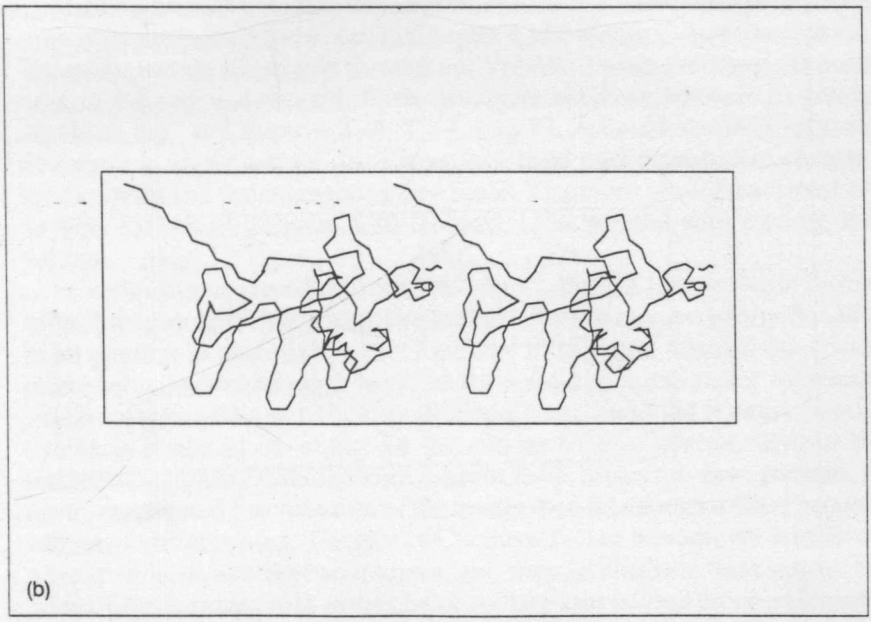
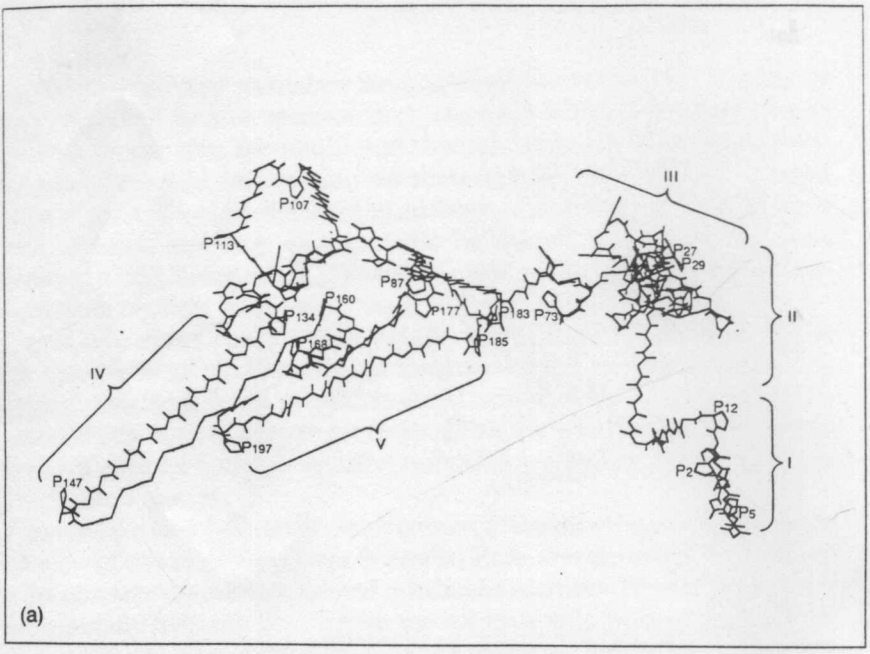


FIGURE 1-1. (*a*) Chain trace of κ-casein; prolines are indicated. (*b*) Stereo view of the 3-D model of κ-casein, showing the backbone without the side chains.



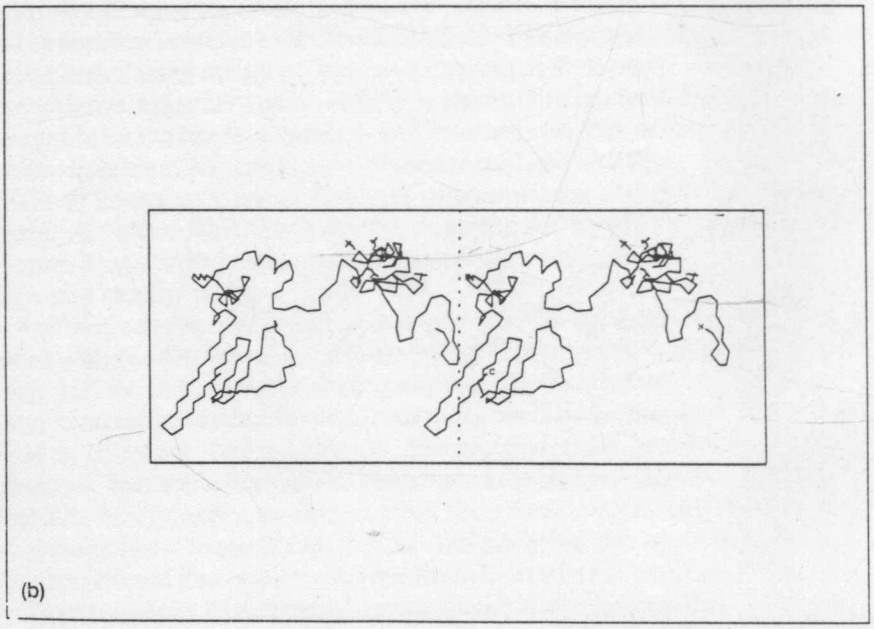


FIGURE 1-2. (*a*) Chain trace of α_{s1}-casein; prolines are indicated. (*b*) Stereo view of the 3-D molecular model of α_{s1}-casein, showing the backbone without side chains.