

Practical
**Food Microbiology and
Technology**
Second Edition

Practical
Food Microbiology
And Technology
Second Edition

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WESTPORT, CONNECTICUT
THE AVI PUBLISHING COMPANY, INC.

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Preface to Second Edition

An attempt has been made in the Second Edition to bring the original manuscript up-to-date and to reorganize segments of the First Edition of the book. New tables and the latest laboratory methods for isolating and culturing microorganisms of importance to the food industry also have been added.

The authors wish to thank the following for reviewing portions of the revised book and for making many helpful suggestions: Dr. A. W. Anderson, Oregon State University; Dr. Nancy Bigley, Ohio State University; Dr. Gene Geisman, Ohio State University; Dr. Herbert O. Hultin, University of Massachusetts; Dr. Maynard A. Joslyn, University of California, Berkeley; Dr. Lloyd L. Kempe, University of Michigan; Dr. James J. Jezeski, University of Minnesota; Dr. John T. R. Nickerson, Massachusetts Institute of Technology; Dr. Herbert Ockerman, Ohio State University; Dr. Z. John Ordal, University of Illinois; Dr. Carl S. Pederson, New York State Agricultural Experiment Station; Dr. Andrew Peng, Ohio State University; and Dr. Fred Stephens, Ohio State University.

The assistance of Mrs. Carol Day, Miss Marie Francis, and Miss Becky Gould is also acknowledged with thanks.

July 1970

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Preface to the First Edition

The interrelationships between food microbiology and food technology are very intricate. Micro-organisms associated with foods may be classified as desirable and undesirable. In fact micro-organisms are excellent biochemists and many of their reactions are beneficial and even indispensable in certain foods.

It is assumed that students in food microbiology and food technology have had basic courses in microbiology and chemistry. Therefore, they should be familiar with morphology, biochemical behavior and control measures that may govern the activity of desirable and undesirable micro-organisms associated with foods.

The purpose of this book is to emphasize a few basic concepts that are related to some of the biochemical changes induced by micro-organisms from a practical viewpoint in processing certain foods.

Obviously the composition of foods will influence the kind and nature of biochemical changes caused by micro-organisms in food.

Food spoilage is discussed from a food technologist's viewpoint, with emphasis on appropriate control measures. Food additives, including antibiotics are stressed because they are legally used in some cases while in other instances they may be detrimental. The role of radiation in food technology is explained and discussed.

Food poisoning is emphasized because it is on the increase in spite of the sanitary measures taken at the present time.

A discussion on sources and treatment of water supplies is stressed. The food industry is a large consumer of water which must be potable in order to produce a good food product and eliminate public health hazards resulting from a contaminated water supply.

New advances in methods and research activities will no doubt be published before this book is printed. Nevertheless the basic general principles discussed here will still apply to the field of food microbiology and technology processes.

No attempt has been made to list microbial standards for raw and processed foods. This information is readily available from various sources. Moreover, microbial standards are constantly changing due to new technological developments in the food industry. Also there are many differences in viewpoints among food technologists, biochemists, food microbiologists and sanitarians as to specific standards for various food products.

The author is grateful for the advice and encouragement so generously given by his associates in the preparation of the subject matter, especially Doctors A. R. Winter, Poultry Science; Wilbur Gould, Horticulture Products; F. E. Deatherage, Agricultural Biochemistry; W. F. Gray, Botany and Plant Pathology; J. M. Birkeland and G. W. Malaney, Microbiology; and Robert Angelotti, Robert A. Taft, Environmental Center, Food Division, Cincinnati, Ohio. The aid of Dr. G. M. Dack, Director, Food Research Institute, University of Chicago, Chicago, Illinois, is also acknowledged.

The author is particularly indebted to Dr. George Mountney for his assistance in reading proof and making many helpful suggestions.

March, 1962

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Introduction: Food and Man

GENERAL ASPECT OF FOOD

Man is the only species in the animal kingdom that seeks to adjust his environment to his own personal needs. It has been a constant struggle through the ages for man to keep up with the constant changes in the environment in which he lives. Technological development has raised him from a cave man existence to a high standard of living in which he has more leisure, works less and enjoys life according to his own dictates.

Food is one of the governing factors which forces man to adjust to his surroundings. His ability to obtain food is closely related to fertility of the soil, climate, and ultimate sources of food supply.

Insects, micro-organisms, and other animals compete with him for the available food supply. For example, many organisms are undesirable from the public health standpoint and for the role they play in bringing about the spoilage of many foods, thus rendering them inedible for human consumption. Thus far, man, through technological developments, has been able to increase or at least hold his own food supplies stable in competition with other organisms for food. The development of insecticides, fungicides, germicides, and other chemicals has enabled him to maintain a balance between himself and other living organisms.

Many kinds of living organisms are highly desirable for the part they play in creating, preserving, and processing certain kinds of food, for example, the honey bee not only produces honey but also pollinates the flowers of many fruit trees.

Man has been able to utilize many animals as a source of food for himself; therefore, a balance between man and food animals has been carefully controlled. Many other animals are not used for human food, and, naturally, a problem is presented in their control. Fortunately, through scientific developments, the animal called man has been successful in reducing the populations of these animals in the competition for survival.

Food is the ever dominant concern of the human race; it is one of the first things demanded after birth, and throughout life it is essential for health and happiness.

Lin Yutang in his book, "The Importance of Living," gives us food

for thought: "That revolutions, peace, war, patriotism, international understanding, our daily life and the whole human social structure are influenced by food." Perhaps this is why men refuse to work, soldiers refuse to fight, prima donnas refuse to sing and senators refuse to debate without food.

Historical Food Supply

Prehistoric man and other animals consumed their food in the raw state. Later in the evolution of man he began to cook his food. Wandering tribes and animals usually found food in abundance in the natural state; when it was less abundant they simply moved to new sources. When the natural supply of food was plentiful, there was very little trouble in the animal kingdom. However, when the potential supply was less abundant, then competition and warfare resulted. Our forefathers practiced this art and we see parallels to this struggle among wild animals that live in thick woodlands. In the course of man's development, he and animals learned to store foods for future use. For instance, squirrels store nuts in season for winter use. Man learned to preserve certain foods very early by drying, souring, and later by cooking food. Moreover, nature has endowed many kinds of birds with an instinct to migrate during certain seasons, primarily for mating and also in search of an abundant food supply. Snakes and bears undergo a process of annual hibernation. During this period, animal metabolic activity is low. No food is consumed but life is sustained by utilizing a reserve deposit of fat in the body.

Sources of Our Food Supply

It has been estimated that there are two billion acres of farm land in the world today devoted to the production of food for human consumption. This does not include the acreage used for growing sugar, potatoes, and cereals. However, one billion acres of tropical, subtropical and arid lands now untilled could be used if irrigation and fertilizers were available. There are over 300 million acres in the temperate zone in the same category.

The population of the world is increasing at the rate of about 40-50 million a year and more food must be obtained. Soil erosion and improper farming practices have done much to deplete the soil of its fertility. However, steps are being taken to reduce these practices to a minimum. High fertility levels of the soil can be maintained while at the same time increasing the crop yield. Nitrogen-fixing bacteria are playing an important role in keeping soil fertility up to a high

level. The use of commercial fertilizer and good farm practices will go a long way in supplying an ever-increasing food supply.

Practically all of our food is derived from animal and vegetable sources from land and sea. Processes are being developed for making waste products usable for food, for example, the conversion of wood cellulose to digestible carbohydrates. For animals, German farm chemists have been pioneers in the field of animal feeds for many years. It has been stated that 60 per cent of forest products are wasted in the form of sawdust and branches after the logs suitable for lumber have been removed from the tree. It has been estimated that 600 lbs. of sugar can be obtained from one ton of wood.

Moreover, man is not able to make edible food out of air and water as the plant does in the processes of photosynthesis. Our food supply in the future may be substantially increased if the proteins of grass can be transformed into beefsteak more efficiently than by comparable processes used by the beef or dairy cow today.

A variety of algae called *Chlorella* has been investigated as a source of edible protein. Something like 45 tons of algae protein can be produced annually on one acre of soil. With such a yield of food, one can see great possibilities of an increased food supply grown on a few acres of land. One pound of algae protein is comparable to two large pork chops and to one-half pound of butter in nutritive value.

General Biological Characteristics for Food

Food is a perishable product and is subject to attack by micro-organisms. If it were not subject to biochemical changes, it would not be suitable for energy production in man. These changes may be desirable or undesirable from an economical, nutritional, and public health standpoint depending upon the kind of micro-organisms present which are in turn determined by environmental factors.

These biochemical activities are brought about in many complicated yet interrelated processes by enzymes. All living tissues possess enzyme systems which are endogenous and are just as much a part of the tissue as other constituents that make up the cell. These enzymes are, as a rule, not destroyed after the product reaches maturity but may continue to function long after or even during the storage of the food product. For example, everyone is familiar with what happens when an apple is peeled. The removal of the outer skin exposes the meat of the apple to atmospheric oxygen and, within a short time, the apple will turn brown indicating that a biochemical change has taken place. The change is brought about by enzymes.

Then too, micro-organisms also possess complex enzyme systems.

This is important because as organisms grow in various foods, enzymes are liberated in the food and they in turn can bring about profound changes in the product very much in the same way as the inherent enzymes. In other words, enzymes, whether they are inherent or secreted by micro-organisms, produce significant changes in food. Many examples will be discussed in succeeding pages to emphasize the importance of these biochemical changes in different foods.

Functions of Food for the Living Cell

Perhaps we are beginning to get some concept of food. It might be in order to ask this question: what is the purpose of food? The physiologist says we eat food because: (1) it gives energy to sustain life, (2) it promotes growth, (3) it repairs damaged and worn out tissues, (4) it makes reproduction possible, and (5) the consumption of food provides a unique psychological or esthetic effect. In other words, there is a great deal of pleasure and satisfaction in tasting and consuming well prepared food.

FOOD AND MICRO-ORGANISMS

The activity of inherent enzymes present in foods may be stimulated by man in his efforts to process or preserve food. In frozen and dehydrated foods, enzymes, unless inactivated, will ultimately bring about off-flavors, off-odors, and off-color. One basic principle in food processing and preservation is to inactivate or destroy the inherent enzymes present. This can be done by heating, which not only destroys the enzymes but eliminates undesirable micro-organisms. These organisms, like any living cell, produce enzymes and they in turn can play an important role in the quality of the food product.

During processing and preserving food, changes take place in concentrations of the constituents, also a change in pH may occur. These variables may be conducive to enzyme activity. Moreover, the addition of certain ingredients to the food during the processing may be important. For example, salt may inhibit many micro-organisms, but at the same time permit halophilic bacteria to grow and produce undesirable changes in the food. Another illustration is a case in which butterscotch flavored starch puddings failed to set when cooled. It was found that one ingredient of the flavor was made from Mexican materials. This product showed a positive diastase action which partially liquefied the pudding starch during cooking.

Nature can be very uncooperative at times as evidenced by the way she has placed amino acids and aldehydes in contact with each other in food products. This condition may be desirable in some products

and undesirable in others. On the favorable side, a variety of breakfast foods owe their satisfying flavors to a chemical rearrangement of these constituents. Many times a new breakfast food can be developed by controlling the time, temperature, and moisture relationship. Then if sugar is added reactions occur which may destroy the moisture relationship.

Sugars in general parallel the reactivity of amino acids in foods. Carbohydrates having a free aldehyde or ketone group naturally react more rapidly than those which first have to be hydrolyzed or broken down to release these groups.

In processing certain foods, if the temperature is increased the number of molecules of water in proportion to the number of molecules of amino acids increases. This is because an amino acid has served as a catalyst which brings about a reaction causing dehydration of carbohydrates in the foodstuff.

Unfortunately objectionable changes sometimes occur due to this complex combination of factors. If these variables are not carefully controlled, certain obvious changes occur in the food product such as: (1) darkening of the product, (2) development of a bitter flavor, and (3) unpleasant odor.

To suppress undesirable flavors or induce desirable flavors in food products, the food technologist recognizes the basic constituents in the product and by altering their natural relationships he can determine to a large extent the quality of the food product.

Micro-organisms in Foods

Without a basic knowledge of microbiology man cannot comprehend the significance and importance of micro-organisms in the biological world. Micro-organisms are widely distributed in nature wherever favorable environmental factors are present for their growth. Usually food supply, temperature, and moisture are the main factors that determine the growth of micro-organisms. No food product after it reaches maturity, commonly referred to as "ripeness," is free of organisms. However, there is a marked difference in the microbial content of foods. Hickory nuts, pecans, and other shell products are protected by a semi-impermeable shell which offers the maximum protection to the nut meats against biological activity. But in the course of time these products will undergo slow biochemical changes which alter their natural flavor. In contrast, fresh raw milk is a highly perishable product and sours in a short time in a warm temperature.

Bacteria, molds, and yeasts constitute a large group of micro-

organisms. Obviously their presence in food is widespread. Their competition with man and other animals can be quite significant in a food supply. Micro-organisms derive their energy and other metabolic activities from food exactly as man does. They may cause significant biochemical changes in food. Fortunately a few of these changes are desirable, such as the transformation of fluid raw milk into cheese, or the production of sauerkraut from raw cabbage. These examples are but a few used in the food industry. Industrial uses of the products from various types of fermentations are well known.

Recently micro-organisms have been used to assay for certain vitamins and amino acids in food products. The microbiologist does not understand all of the complex biochemical changes that may take place in food during the natural fermentation process. However, he does know that the chemical and physical composition of the food is markedly changed as compared to the raw product. Food microbiologists and biochemists have found many challenging research problems in the field of food processing and food microbiology. The production of biological acidity is a very complex problem since many intermediate products are formed, and they in turn may determine very largely the type of end products that may be formed during the fermentation process. The organic acids thus formed may serve as a food preservative. Actually these acids may inhibit or destroy many kinds of organisms, which may render the food product inedible. Hence, biological acidity is one of nature's ways of preserving food from further microbiological activity.

Sauerkraut is an excellent example of self-preservation by the production of acids by micro-organisms, and in addition the formation of various "esters" which contribute to its characteristic flavor.

Again bacteria, molds, and yeasts may be highly undesirable in foods by causing food spoilage. A more complete discussion will be given elsewhere. There is the ever present group of organisms that may be dangerous to public health. Certain food-borne diseases have been recognized in which man, other animals, and insects may contaminate the food and make it potentially dangerous for human consumption. Moreover, there are other organisms which, when present in certain foods, may grow and liberate an exotoxin which causes food poisoning in man.

Classification of Micro-organisms Found in or on Foods

Perhaps a broad classification of organisms in foods may be based on their ability to attack the various constituents in foods. All micro-organisms found in food may be classified into aerobes and anaerobes.

Nearly all foods contain carbohydrates which vary from simple monosaccharides to complex polysaccharides such as starch. Not all organisms can attack the various carbohydrates. Starch is very difficult to break down by organisms, although a few micro-organisms called "starch splitters" are capable of attacking it. Monosaccharides and disaccharides are readily attacked by a large group of organisms. Most fruits are rich in monosaccharides, hence a spontaneous fermentation takes place with the production of acids and gases. Milk contains lactose, a disaccharide, which undergoes lactic acid fermentation very quickly. The production of acids by acid forming organisms is very common in foods rich in carbohydrates. As a rule molds and yeasts are not active in the production of biological acidity in foods.

Foods rich in proteins also undergo biochemical changes. As a rule the protein molecule is more complex than the carbohydrate, hence is much more difficult to break down. Certain bacteria and molds which are capable of attacking the protein molecule are called "protein digesters." Protein digesting organisms, like any living cell, possess a complex enzyme system and through specific enzymes the protein is broken down to amino acids. This form of protein is simple enough for the living cell to utilize it as a source of energy. Several end products such as acids, gases, and protein degradation products are formed during the protein digestion process. Some of these products are desirable as in cheese ripening.

Many foods contain fats although the composition of the fat will vary markedly with different foods. Fats are composed of glycerol and fatty acids. Vegetable fats as a rule contain members of the higher fatty acid series such as stearic and others. Such fats do not undergo decomposition as readily as the animal fatty acid series. Butter, for example, becomes rancid very quickly due to the formation of large amounts of butyric acid, a member of the lower fatty acid series.

In general fats are not attacked by micro-organisms as readily as the carbohydrates and proteins in foods. In cheese ripening the breakdown of the milk fat is a part of the desirable changes involved in the ripening.

Man's Control of Micro-organisms

Man, through his understanding of food chemistry and food microbiology, is now able in a considerable measure to control the micro-organisms present in foods for the encouragement and improvement of desirable changes and for the suppression of undesirable and deteriorative effects caused by micro-organisms. Table 1 shows the organisms which cause spoilage in some important foods.

TABLE 1
SPOILAGE ORGANISMS OF IMPORTANT FOODS

Class of Food Products	Genera Dominating When Spoilage Occurs During Standard Conditions of Storage
Milk and milk products	<i>Streptococcus</i> , <i>Lactobacillus</i> , <i>Microbacterium</i> , Gram-negative rods, ¹ <i>Bacillus</i>
Fresh meat	Gram-negative rods, ¹ <i>Micrococcus</i> , <i>Cladosporium</i> , <i>Thamnidium</i>
Poultry	Gram-negative rods, ¹ <i>Micrococcus</i>
Sausage, bacon ham, etc.	<i>Micrococcus</i> , <i>Lactobacillus</i> , <i>Streptococcus</i> , <i>Debaromyces</i> , <i>Penicillium</i>
Whale meat	<i>Streptococcus</i> , <i>Clostridium</i> , Gram-negative rods ¹
Fish, shrimp	Gram-negative rods, ¹ <i>Micrococcus</i>
Shellfish	Gram-negative rods, ¹ <i>Micrococcus</i>
Eggs	<i>Pseudomonas</i> , <i>Cladosporium</i> , <i>Penicillium</i> , <i>Sporotrichum</i>
Vegetables	Gram-negative rods, ¹ <i>Lactobacillus</i> , <i>Bacillus</i>
Fruits and Juices	<i>Acetobacter</i> , <i>Lactobacillus</i> , <i>Saccharomyces</i> , <i>Torulopsis</i>
Cereal grains	<i>Botrytis</i> , <i>Penicillium</i> , <i>Rhizopus</i> , <i>Aspergillus</i> , <i>Fusarium</i> , <i>Monilia</i> , <i>Penicillium</i> , <i>Rhizopus</i>
Bread	<i>Bacillus</i> , <i>Aspergillus</i> , <i>Endomyces</i> , <i>Neurospora</i> , <i>Rhizopus</i>

¹This includes, particularly, strains of *Achromobacter*, *Pseudomonas* and *Flavobacterium*, with exclusion of the *coli-aerogenes* group.

Composition of Foods

The microbiology of foods is a comprehensive subject and is concerned not only with spoilage but also with beneficial changes in food brought about by micro-organisms. Spoilage may be injurious to health when such food is eaten. Changes in physical appearance, flavor, and odor in spoiled foods compared with normal food are very difficult to evaluate. One group of people selects certain kinds of cheese because of their peculiar fine flavors and odors, while others would consider such products spoiled. Individuals have not learned to perceive the changes that affect the nutritive values in foods that may be important to health. Food may contain traces of arsenical sprays or salts of other heavy metals which are detrimental but which cannot be detected by taste.

Our discussion of food spoilage will be confined largely to changes in the food due to micro-organisms and to certain enzymes. Let us consider some of the factors associated with spoilage and also with some desirable biochemical changes. Obviously these changes will depend very largely on the composition of the foods.

Foods are made up of carbohydrates, proteins, fats, minerals, water, vitamins, hormones, nucleotides,¹ and other unnamed constituents often called "growth factors," as well as a complex endogenous enzyme system. There is evidence to indicate that the fertility of the soil affects the composition and nutritive value of the food. However, more study and confirmation is necessary before any definite conclusions can be drawn regarding soil fertility and human nutrition.

In considering the general composition of foods, a few examples will serve to show how the constituents may vary in different foods by altering the composition by processing and handling. In addition, the stage of maturity, climatic conditions, and other factors such as inherent acidity may vary widely in different foods. Moreover, a food product such as butter is mainly fat; meat, on the other hand, has a high protein content and fruits of various kinds have appreciable amounts of carbohydrates. Obviously, one would not expect the

¹ Nucleotides are components of the bacterial cell which absorb ultraviolet light and by means of this property help to distinguish between Gram-positive and Gram-negative organisms.