Food and Beverage Mycology

Second Edition

Larry R. Beuchat

FOOD AND BEVERAGE MYCOLOGY

Second Edition

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Cover: Ascospores of *Neosartorya fischeri*.
Frontispiece: Ascospores of *Neosartorya fischeri* (top), and *Talaromyces flavus* (bottom).

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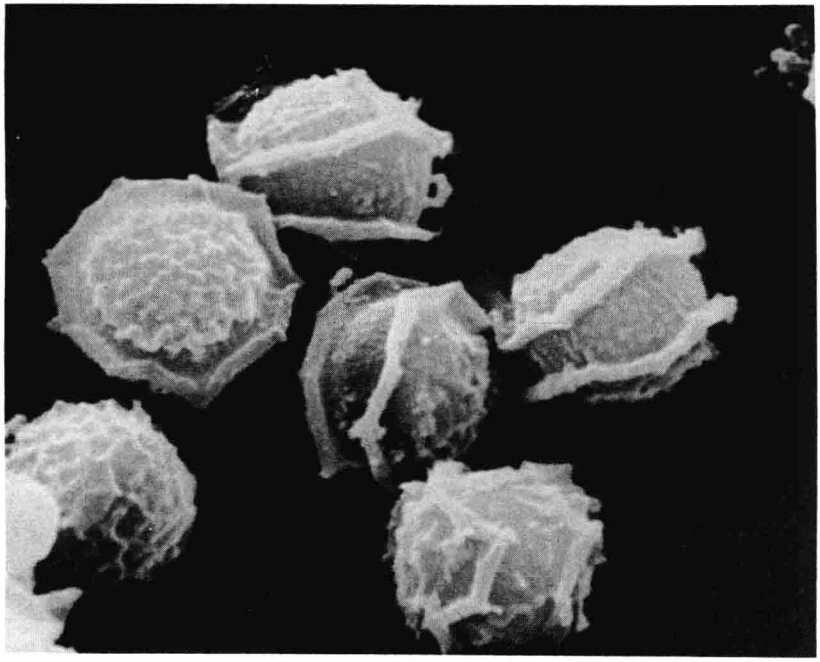

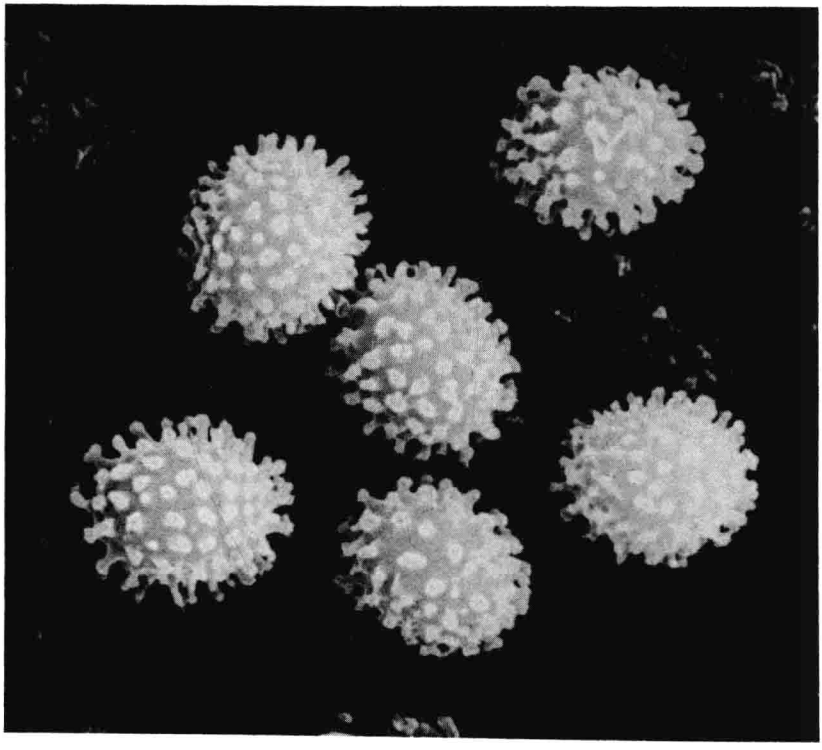

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Foreword

Fungi have contributed to the shaping of man's welfare from the beginning of civilization. However, the acquisition of knowledge regarding this group of microorganisms is too often neglected by students, professionals, and the general public.

Microbiology is too frequently equated with bacteriology, which deals with only one group of those microbes encompassed by the term, and thus the mycological aspects are generally minimized in classes and textbooks. Yet it has been the fungi which have provided much of our information in genetics, biochemistry, and nutrition. In fact the catalysts for biological transformation, enzymes, derive their name from the original isolation source—yeasts.

In the field of food microbiology, the role of the fungi is also often underemphasized. Text and reference books concerning themselves with the beneficial and detrimental aspects of fungi as they affect food and drink vary in emphasis and inclusion of current information. "Food and Beverage Mycology" is filling a long-existing hiatus in books available to students, professionals, and lay persons interested in the role fungi play in food availability in both unprocessed and processed forms.

The book, with contributions by many recognized authorities in food microbiology, concentrates on fungi involved with food and beverage products. Information is provided on what fungi are, what they do, their involvement in all classes of food products, and methodologies used in detecting and quantifying fungal populations and mycotoxins. For the student, professional, and lay person, this book will bring together in one place the information they desire on fungi influencing the foods and beverages that are available to the world's population.

Martin W. Miller

Preface

In the course of studying molds and yeasts and their relationships to food and beverage spoilage and processing, I have observed a great need for the compilation of such information into one book. Too often the student of food microbiology, the quality assurance technician, and the sanitarian must search through several "microbiology" sources to obtain specific information on food and beverage mycology. Our increased knowledge of the significant role fungi play with respect to food and beverage deterioration, mycotoxin production, fermentation, and potential sources of industrial ingredients and dietary protein further emphasizes the need for special consideration of food mycology separate from the food bacteriology.

As was the plan of the first edition of this book, after a chapter on classification and descriptions of fungi associated with food and beverages, basic information on one of the most important environmental factors affecting fungal growth, namely water activity, is covered followed by discussions on specific commodity areas. In response from many readers of the first edition, a chapter on poisonous mushrooms has been added to this edition to provide a more complete story on the macrofungi. This is followed by chapters on the use of fungi and fungal metabolites as components of the human diet. Public health hazards implicated by the presence of mycotoxin producers in foods are treated in a separate chapter followed by chapters describing the detection of mycotoxins and fungal contaminants in foods and beverages.

Again, as in the first edition, it has been necessary to choose what to omit from the various topics presented in the book, since discussions of all aspects in so short a space are not possible. The practical and applied apsects of mycology as it relates to foods and beverages are stressed. Special efforts have been made by the authors to summarize in the form of tables information from a large number of authoritative sources. Also, valuable references are provided for each subject area.

My sincere gratitude is expressed to the authors who have contributed to this book. Their expert knowledge in particular areas of food and beverage mycology has made my job as editor enjoyable.

Larry R. Beuchat

Contents

1

Classification of Food and Beverage Fungi

E. S. Beneke
K. E. Stevenson

Fungi comprise a large group of microorganisms which affect our food supply as a result of their infection of living plant tissue and fruit, their ability to invade and decompose harvested and processed foods and beverages, and their capacity to produce toxic secondary metabolites. They are responsible for a major portion of food deterioration in developing countries. On the other hand, the preservative effects brought about by fungal fermentation of foods and beverages are well-known benefits. In addition, various types of mushrooms, referred to as macrofungi, are an important part of human diets in many countries.

Fungi of importance in food and beverage industries can be classified in the Eumycota or true fungi. Traditionally the true fungi have been divided into three classes—Phycomycetes, Ascomycetes, and Basidiomycetes—based on the mode of sexual spore production, with the Deuteromycetes or Fungi Imperfecti reserved for organisms for which no sexual stage was known. However, some taxonomists (e.g., Ainsworth, 1973; Ainsworth and Sussman 1965) prefer to divide the fungi into phyla (Whitaker 1969) and divisions which are further divided into classes. The divisions of fungi can be classified as follows:

1a	Motile cells (zoospores) present	*Chytridiomycota*
1b	Motile cells absent	2
2a	Perfect state absent	*Deuteromycota*
2b	Perfect state present	3
3a	Perfect state spores zygospores	*Zygomycota*
3b	Zygospores absent	4
4a	Perfect state spores ascospores	*Ascomycota*
4b	Perfect state spores basidiospores	*Basidiomycota*

Since the class Phycomycetes was a miscellaneous assemblage, two of the divisions, Chytridiomycota and Zygomycota, now comprise representatives of this former class.

In most fresh, moist foods, fungi do not grow well due to competition from bacteria. However, in foods which have conditions such as lowered water activity, pH less than 4.5, or refrigerated temperatures, fungi may proliferate. In other words, fungi usually become predominant under conditions which tend to markedly retard bacterial growth. This characteristic will be illustrated in detail in several of the following chapters. Indeed, conditions adverse to bacterial growth are often employed in laboratory media used to detect and identify fungi in foods and beverages (Chapter 17). In the laboratory, microbiologists commonly separate cultures of true fungi into two main groups—yeasts and molds—based on colonial and microscopic appearance. The molds are typically filamentous and are primarily classified according to the morphology of hyphae, sexual spores, and asexual spores. Yeasts, on the other hand, exist mainly as single cells, i.e., they lack many morphological criteria used to classify filamentous fungi, and are normally classified separately with an emphasis on physiological characteristics. Due to the divergent methods used for classification of yeasts and molds, the two groups are discussed separately in the remainder of this chapter.

YEAST CLASSIFICATION

Yeasts as a group are difficult to define. They are fungi which at some stage in their life cycle exist primarily as single cells which reproduce by budding or fission. Typical structural characteristics of a yeast cell are illustrated in Fig. 1.1. By general agreement fungi which are black-pigmented or pluri-nucleate are not considered to be yeasts, although *Aureobasidium (Pullularia) pullulans* has often been referred to as the "black yeast." This is a subjective system and representative yeasts are classified in the Ascomycota, Basidiomycota, and Deuteromycota.

A monumental work, "The Yeasts: A Taxonomic Study" (Kreger-van Rij 1984) provides the most comprehensive basis for yeast taxonomy at the present time. Several changes in yeast taxa have occurred since 1970 when the previous edition (Lodder 1970) was published. At the genus level, there have been two important changes involving yeasts associated with foods and beverages. First, imperfect yeasts formerly in the genus *Torulopsis* have been transferred to the genus *Candida,*

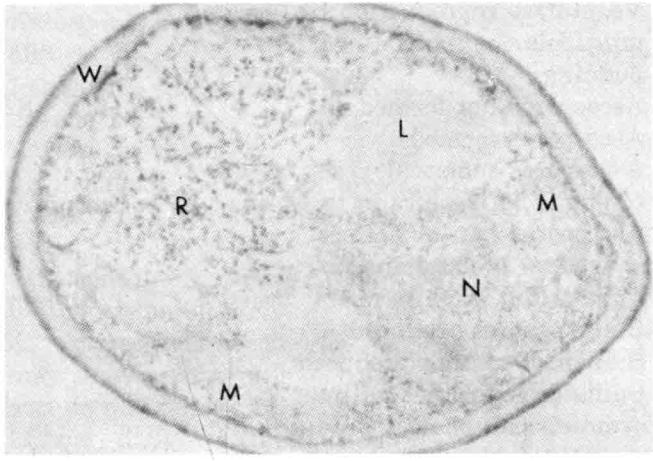

Fig. 1.1 Electron photomicrograph of a vegetative cell of *Candida steatolytica*. Several features common to yeast cells are visible, including cell wall (W), nucleus (N), mitochondria (M), lipid droplets (L), and ribosomes (R). (22,500×)

and the name *Torulopsis* is recognized for human mycoses. Second, some of the ascosporogenous yeasts formerly classified as *Saccharomyces* have been reassigned to two reinstated genera, *Torulaspora* and *Zygosaccharomyces*. At the species level there has been a slight trend in "lumping" or merging species. This is due primarily to questions concerning the validity of recognizing separate species based upon the fermentation or assimilation of a single sugar. The book by Barnett *et al.* (1983), "Yeasts: Characteristics and Identification," also provides an excellent description of yeasts associated with food and beverage spoilage and manufacture.

The following key is designed for generic classification of yeasts which are commonly isolated from foods and beverages. In order to facilitate identification of unknown isolates, yeast genera of the Ascomycota and Deuteromycota are included in the key:

1a	Vegetative reproduction exclusively by cross wall formation, i.e., fission	*Schizosaccharomyces*
1b	Vegetative reproduction by budding	2
2a	Vegetative reproduction by bipolar budding, cells usually apiculate or lemon-shaped	3

2b	Vegetative reproduction by multipolar or multilateral budding	5
3a	Ascospores not formed	*Kloeckera*
3b	Ascospores formed	4
4a	Ascospores spherical, conjugating in pairs in the ascus	*Saccharomycodes*
4b	Ascospores hat- or helmet-shaped, or globose and not conjugating in pairs in the ascus	*Hanseniaspora*
5a	Ballistospores produced	6
5b	Ballistospores not produced	7
6a	Ballistospores bilaterally symmetrical; usually produce carotenoid pigments	*Sporobolomyces*
6b	Ballistospores rotationally symmetrical; carotenoid pigments not produced	*Bullera*
7a	Cells ogival; strong acetic acid aroma produced; cells short-lived on malt agar	8
7b	Cells differ from above description (7a) by one or more characteristics	9
8a	Ascospores formed	*Dekkera*
8b	Ascospores not formed	*Brettanomyces*
9a	Ascospores formed	10
9b	Ascospores not formed	21
10a	Nitrate assimilated	11
10b	Nitrate not assimilated	12
11a	Ascospores spherical with warty walls	*Citeromyces*
11b	Ascospores hat- or saturn-shaped or hemispherical without warty walls	*Hansenula*
12a	Glucose fermentation weak, slow, or absent	13
12b	Glucose vigorously fermented	17
13a	Abundant development of true mycelium	*Saccharomycopsis*
13b	True mycelium absent or limited if present	14