

# Mixed Cultures in Vegetable Fermentations

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## Introduction

Many vegetables have been preserved by salting, with various degrees of fermentation, depending upon the salt concentration. Cucumbers, cabbage (for sauerkraut) and olives account for the largest volume of fermented vegetables in the western hemisphere, but smaller quantities of peppers, carrots, cauliflower, and okra are preserved by brining.<sup>2</sup> The fermentation of vegetables involves a complexity of physical, chemical, and microbiological factors that have been well characterized over the past several decades. The reader is encouraged to consider previous reviews on this subject<sup>3</sup> in addition to the original research cited.

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<sup>3</sup>Andersson et al. 1988; Etchells et al. 1975; Fernandez Diez 1983; Fleming 1982; Pederson and Albury 1969; Stamer 1983, 1988; Vaughn 1954, 1982.

Microbial activities during the natural fermentation and storage of vegetables have been divided into four stages: initiation of fermentation, primary fermentation, secondary fermentation, and postfermentation (Table 4.1). This classification was based upon characterizations of the fermentations of cucumbers, cabbage, and olives by various researchers during this century. This review is organized according to this classification to emphasize similarities and dissimilarities in the fermentation of these commodities. Although some overlapping of activities among stages occurs, criteria for distinguishing the stages are sufficiently distinct to serve as a guide in developing controlled fermentation methods.

During the past 30 years, efforts have been made to develop pure-culture and controlled fermentation methods, many of which are discussed herein. Some novel control procedures have been employed commercially, such as the purging of CO<sub>2</sub> from fermenting cucumbers to prevent bloater formation. However, comprehensive controlled fermentation methods such as those used in the production of alcoholic beverages and fermented dairy products have not been used commercially on a large scale. Economic and technical reasons are responsible for this state of affairs. It is conceivable that technological advancements will mandate commercial acceptance of improved methods for fermentation control. One such advancement could be the development of microbial cultures with novel and valuable properties. The likelihood of developing such cultures during the next decade seems reasonable, considering the recent progress in genetic technology of microorganisms. To take advantage of such microbial technology, however, other technological advances also must occur (e.g., improved

TABLE 4.1 Stages of Microbial Activities During the Natural Fermentation of Vegetables

Stage	Prevalent microorganisms (conditions)
Initiation of fermentation	Various gram-positive and -negative bacteria
Primary fermentation	Lactic acid bacteria, yeasts (sufficient acid has been produced to inhibit most bacteria)
Secondary fermentation	Fermentative yeasts (when residual sugars remain and LAB have been inhibited by low pH) Spoilage bacteria (degradation of lactic acid when pH is too high and/or salt/acid concentration is too low; e.g., propionic acid bacteria, clostridia)
Postfermentation	Open tanks: surface growth of oxidative yeasts, molds, and bacteria Anaerobic tanks: none (provided the pH is sufficiently low and salt or acid concentrations are sufficiently high)

methods for sanitization and containment of the vegetables). It is the intent of this review to provide a better understanding of the principles that govern successful fermentation of vegetables, with the hope of encouraging the development of improved controlled fermentation methods that may eventually become commercially acceptable.

### Initiation of Fermentation

Many vegetables, in the presence of appropriate concentrations of salt (NaCl) and under suitable environmental conditions, will undergo fermentation by lactic acid bacteria (LAB). Although the number of LAB is usually quite low compared to the total number of microorganisms (Table 4.2), the LAB eventually predominate due to the production of acids and other products which restrict growth and survival of other groups of microorganisms. The rate and consistency of LAB gaining predominance also is a reflection of many factors, including initial populations of all microorganisms, physical and chemical properties of the vegetables, and the environment (physical and chemical) in which the vegetables are held. Each vegetable may reflect unique responses during initiation of fermentation as exemplified with cucumbers, cabbage, and olives. Salt is known to exert two important effects in its preservative role: It directs the course of microbial activities and it prevents softening of the vegetable tissue. Although salt is commonly added in vegetable fermentations, the concentration used (Table 4.3) and its effects on the fermentation and product quality vary widely.

### Cucumbers

Pickling varieties of cucumbers are used in various methods for commercial processing, including brine fermentation. Fermentation accounts for about 40 percent of commercially processed cucumbers, with pasteurization (40 percent) and refrigeration (20 percent) ac-

TABLE 4.2 Microorganisms on Raw Vegetables Used for Fermentation

Microorganism	Number/g fresh weight		
	Cucumber <sup>a</sup>		
	Fruit	Flower	Cabbage <sup>b</sup>
Total aerobes	$1.6 \times 10^4$	$1.8 \times 10^7$	$1.3 \times 10^5$
Enterobacteriaceae	$3.9 \times 10^3$	$6.4 \times 10^6$	$3.9 \times 10^3$
Lactic acid bacteria	$5 \times 10^0$	$2.6 \times 10^4$	$4.2 \times 10^1$
Yeasts	$1.6 \times 10^0$	$3 \times 10^3$	<10

<sup>a</sup>From Etchells et al. 1975.

<sup>b</sup>After trimming outer leaves. From Fleming et al. 1988a.

TABLE 4.3 Salt Concentrations Used for the Fermentation and Storage of Vegetables

Method of salting	Salt concentration, % <sup>a</sup>		Vegetable
	Fermentation	Storage	
Dry salting	1.5-2.5	1.5-2.5	Cabbage
Brining	5-8	5-16	Cucumbers
Brining	4-8	4-8	Green olives

<sup>a</sup>The concentrations of salt indicated generally are used by commercial firms in the United States. Modified from Fleming, 1982.

counting for the remainder (Fleming and Moore, 1983). The primary purpose of fermentation is to serve as a means for temporary preservation of the cucumbers in bulk tanks until they are needed for processing into various types of sweet and sour pickles. The fermented cucumber products may or may not be pasteurized, depending upon the type of product and its intended use.

The cucumbers may be harvested by hand or mechanically, and usually are graded by size before being placed in bulk tanks containing a salt solution where they are allowed to ferment. The cucumbers usually are not intentionally washed before brining, so the initial microbial load reflects the soil type and weather conditions to which the fruit were exposed, as well as harvesting and handling methods. The fruit may be rinsed if they are received in a water-cushioned hopper or pit or conveyed in liquid. Most companies do not brush-wash the fruit before brining, although washing has been suggested for use in controlled fermentation procedures (Etchells et al. 1973, Fleming et al. 1988a). The microbial load on the raw fruit may vary widely, particularly if the flower remains attached, as is more common with smaller fruit (Fig. 4.1).

The flower is of particular significance also because it may harbor pectinolytic enzymes from mold growth which may result in softening of the cucumbers. In the southeastern United States where softening was a severe problem with small cucumbers for many years, pectinolytic enzymes resulting from mold growth within the flowers were found to be the causative agents of softening (Bell 1951, Etchells et al. 1958). Previously, aerobic, spore-forming bacilli had been thought to be responsible for softening (Vaughn et al. 1954). The softening problem with small fruit was greatly reduced by removal of the flowers before brining or by draining of the cover brines from the cucumbers after about 37 h (Etchells et al. 1955). In recent years the practice of draining brine from cucumbers to prevent softening has been curtailed due to environmental problems associated with salt disposal. Attempts now are made to remove the flowers from fruit be-

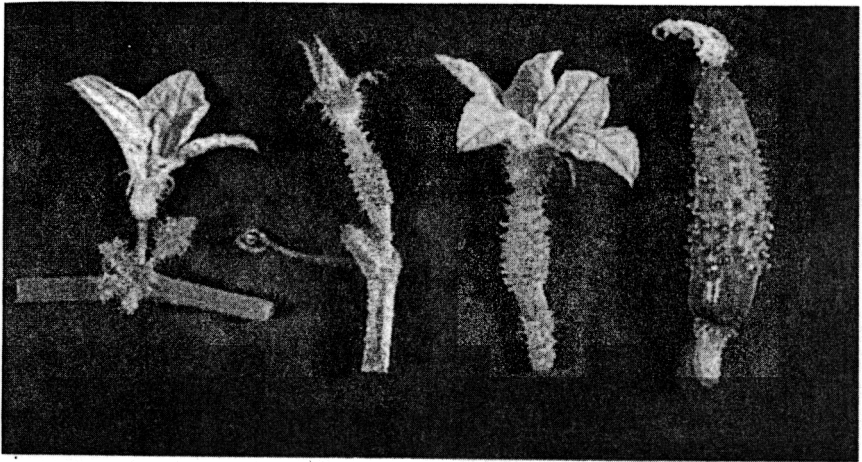
STAMINATE FLOWER  
AND BUDSPISTILLATE FLOWER  
UNOPENEDPISTILLATE FLOWER  
OPENEDIMMATURE FRUIT WITH  
DRIED FLOWER

Figure 4.1 Flowers and immature fruit of the cucumber plant. (From Etchells et al., 1953.)

fore brining. Also, the addition of calcium salts to the cover brine has been shown to reduce softening of brined cucumbers (Fleming et al. 1978, 1987), even in the presence of mold polygalacturonase (Buescher et al. 1979, 1981).

The microflora are located mostly on or in the outer surface of raw cucumbers (Samish et al. 1963). For many years some scientists in the field considered the interior of healthy, fresh cucumbers to be sterile. However, it has been reported that the interior of fresh cucumbers may contain a latent population of bacteria (Samish and Dimant 1959, Meneley and Stanghellini 1974), probably of the family Enterobacteriaceae. Furthermore, it has been shown that bacteria may grow within the cucumbers during fermentation (Samish et al. 1963), and that the relative amount of fermentation by lactic acid bacteria within the cucumbers and in the brine surrounding the cucumbers can be manipulated by modifying the internal gas atmosphere of the fresh fruit before brining (Daeschel and Fleming 1981). Oxygen within the fresh cucumbers is quickly metabolized after the fruit are brined, and  $\text{CO}_2$  is formed. The  $\text{CO}_2$  is much more soluble than oxygen, thus a vacuum is formed within the fruit (Corey et al. 1983, Fleming et al. 1980). Bacteria may enter the fruit through stomata located in the skin (Fig. 4.2). Yeasts do not enter the fruit because of their larger size (Daeschel et al. 1985).

Cucumbers, graded by size, are conveyed into bulk tanks of 7500- to 75,000-L capacity containing brine (6 to 12% NaCl). The tanks are

