Use of Micro-organisms in Food Processing

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Chapter 1.
LACTIC ACID BACTERIA AND LACTIC FERMENTATIONS

Food-borne disease and food spoilage are topics in which we view microbes as pests, sometimes very dangerous pests. Now we are going to look at them in a very different light, specifically as useful tools of food production. The basic principles are the same. We will still be talking about the source of the microbes in the food, the conditions which select for their proliferation, and what happens as they proliferate. The difference is that the focus is on how to facilitate the process rather than how to minimize it. The topic of food fermentation also differs from topics of microbial problems in food in that there are fewer types of microbes under discussion. Traditionally food fermentation has meant the growth of specific lactic acid bacteria, yeast, molds, or, more rarely, endospore-forming bacteria in food. Because the fermented food industry in this country is concerned mostly with lactic fermentations, we will begin this section with this group of bacteria.

The field of food fermentation is rapidly changing into a field more appropriately termed industrial fermentation or fermentation biotechnology. With traditional fermentations, the end product has been the altered food, and the research has been directed at maximizing quantity and quality by manipulating the fermentation conditions and selecting for the best microbial workhorses. The modern extension of food fermentation technology consists of processes designed to obtain particular compounds using microbial metabolism as the chemical machinery. Food fermentation research is now focused on the custom designing of the microbial agents to optimize clearly defined aspects of the fermentation process. In either situation, the food microbiologist needs a good understanding of the microbial agents. Thus this section includes material not only about fermentation processes, but also about the metabolism of the primary agents of food fermentation in this country.

I. Defining “Food Fermentation”

A. “Fermentation” has been defined in several ways:

1. **Common (non-microbiologist) usage:** Fermentation means making alcoholic beverages.

2. **Basic microbiologist usage:** Fermentation refers to energy metabolism (as covered in our section on the effect of atmosphere on microbial growth) that does not involve an electron acceptor, thus is anaerobic.

3. **Industrial usage:** Fermentation refers to the growth of microbes on a large scale to obtain specific products.

4. **Food scientist usage in context of fermented foods:** Fermentation is the process of making a food in which the characteristic properties are the result of extensive microbial growth.
These definitions are similar in that they all refer to the growth of microbes. They represent extensions of a pre-industrial concept of fermentation associated with the making of food or beverages using naturally occurring processes that require no aeration.

B. Basic food fermentation patterns

Fermented foods can be grouped on the basis of the primary substrate used by the microbial agent. There are actually relatively few themes, although small variations in the details can result in very different tasting products.

<table>
<thead>
<tr>
<th>MAINSTREAM ENERGY METABOLISMS HARNESSED IN FOOD FERMENTATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugars → Lactic acid, acetaldehyde, acetic acid, CO₂, diacetyl, ethanol</td>
</tr>
<tr>
<td>Yeast → ethanol, CO₂</td>
</tr>
<tr>
<td>Proteins → amino acids, peptides, amino acids, other amines</td>
</tr>
<tr>
<td>Molds, Bacillus (Clostridium) → NH₃, NH₃</td>
</tr>
<tr>
<td>Yeast → Smaller sugars, Esters</td>
</tr>
<tr>
<td>Starch → Sugar - not usually released, Sugar - polymers, Oligosaccharides</td>
</tr>
<tr>
<td>Molds, Bacillus → YEAST</td>
</tr>
<tr>
<td>Fat → (Glycerol - not usually released), Fatty acids</td>
</tr>
<tr>
<td>Molds → Ketones</td>
</tr>
</tbody>
</table>
II. THE LACTIC ACID BACTERIA

Special focus is given to this group because they are the basis of most fermented food produced commercially in this country. As a group, they can be described as Gram positive bacteria which form lactic acid as their primary metabolic end-product.

A. Important characteristics of all lactic acid bacteria

We have already looked at these characteristics in the context of spoilage potential. The difference here is that the type of spoilage they cause, namely souring, is a desirable process in the context of food fermentation.

1. They are ubiquitous flora of animals and plants.

Implication: They are already on the food that we want to be fermented.

2. They are strictly fermentative in terms of energy metabolism and rely on free sugars as the substrate.

Implication: They do not attack the large molecular weight structural components of a food and do not produce “bad” flavors associated with the metabolism of amino acids and fatty acids. They produce acids as the primary end-product, and thus lower the pH.

3. They are microaerophils.

Implication: They can grow throughout the bulk of the food (except for the surface, most raw foods present a microaerophilic environment).

B. Important genera

1. Basis of genus differentiation;

a) Morphology:

RODS vs COCCI vs tetrads
b) Fermentation products from glucose in the absence of air:

HOMOFERMENTATIVE vs HETEROFERMENTATIVE

- Lactic acid only
- Lactic acid, ethanol, and CO\(_2\) in equal amounts

The basis of this difference is that the heterofermentative genera cannot phosphorylate both ends of the hexose, which is necessary to produce two ATP from each glucose. All other enzymes for sugar metabolism are found in both groups. The pathways for glucose breakdown are shown on the next page.

2. Primary genera:

a) Lactobacillus - rods (homofermentative or heterofermentative)

b) Streptococcus - cocci, form pairs and chains homofermentative

c) Leuconostoc - cocci, form pairs and chains heterofermentative

d) Pediococcus - cocci, form tetrads homofermentative

FERMENTATIONS OF GLUCOSE IN THE ABSENCE OF OXYGEN

<table>
<thead>
<tr>
<th>Homolactic Fermentation</th>
<th>Heterolactic Fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUCOSE</td>
<td>GLUCOSE</td>
</tr>
<tr>
<td>2 ATP, 2 ADP</td>
<td>6-Phospho gluconate</td>
</tr>
<tr>
<td>1.6 bis-phospho fructose</td>
<td>NAD, NAD-2H</td>
</tr>
<tr>
<td>2 Glyceraldehyde 3-phosphate</td>
<td>CO(_2)</td>
</tr>
<tr>
<td>2 NAD, 2NAD-2H, 4 ATP</td>
<td>Pentose phosphate</td>
</tr>
<tr>
<td>2 Pyruvate</td>
<td>Acetyl CoA</td>
</tr>
<tr>
<td>2 LACTATE</td>
<td>Glyceraldehyde 3-phosphate</td>
</tr>
<tr>
<td></td>
<td>Acetaldehyde</td>
</tr>
<tr>
<td></td>
<td>CO(_2)</td>
</tr>
<tr>
<td></td>
<td>Pyruvate</td>
</tr>
<tr>
<td></td>
<td>ETHANOL</td>
</tr>
</tbody>
</table>
C. Important fermentation products

1. Lactic acid is always a major product

Lactic acid production is the basis of the lowering of pH. It is the final low pH (typically 4.5 to 3.5) that makes the fermented food resistant to spoilage.

2. Other end products depend on which metabolites are available. Important variables include:

   a) Which sugars are present.
   
      e.g. Pentose utilization results in less CO₂.

   Fructose can be reduced to mannitol in a reaction that helps to regenerate NAD.

   b) Presence or absence of air.

   In the absence of air, pyruvate and acetyl-CoA must be used to restore NAD, as shown in the above pathways. This results in maximal lactate and ethanol production. If air is present, NAD can be restored by means of NADH OXIDASE:

   ![NADH Oxidase Reaction](image)

   Thus pyruvate can be used to make other compounds most notably DIACETYL, and acetyl-CoA can be used to generate more ATP, yielding ACETIC ACID as an additional end product, and also ACETALDEHYDE need not all be further reduced to ethanol:
c) Presence of other sources of pyruvate, most notably CITRATE.

Pyruvate is a major source of carbon for building cell structures. If it is plentiful, then more of it can be diverted into side reactions producing flavor compounds such as diacetyl. Citrate is a source of pyruvate in milk (milk contains about 0.2%).

\[
\text{CITRATE outside} \xrightarrow{\text{citrate permease}} \text{CITRATE inside} \xrightarrow{\text{Pyruvate}} \text{+ Acetate} + \text{CO}_2
\]

\[\text{CITRATE} \xrightarrow{\text{citrate permease}} \text{CITRATE inside} \]

Pyruvate + Acetate + CO₂

\[\text{CITRATE} \xrightarrow{\text{citrate permease}} \text{CITRATE inside} \]

Pyruvate + Acetate + CO₂

d) Presence of other compounds that feed into carbon (rather than energy) metabolism.

Like most microbes that are described as lacking extracellular hydrolases, lactic acid bacteria do digest protein to the limited extent that the amino acids can be made available for cell building reactions. Depending on which proteins are present, there may be by-products of this activity that contribute flavor to a fermented food. For example:
PROTEIN → Amino acids, including cysteine

CYSTEINE → METHANETHIOL

\[ \begin{align*}
\text{H}_2\text{CSH} & \quad \text{flavor component of cheddar cheese} \\
\text{HCNH}_2 & \\
\text{COOH} & 
\end{align*} \]

3. Product balance depends on other conditions of the fermentation.

a) pH

Example: the permease for citrate requires a pH of around 5 for full activity. At lower or higher pH, then, less diacetyl will be produced.

b) Temperature

Different species and different reactions within a given microbe exhibit different temperature dependencies. Raising temperature above the optimal for a given fermentation tends to reduce the range of end products, resulting in more lactic acid and fewer subtle flavors.

III. SIMPLE LACTIC DAIRY FERMENTATIONS

Many of the commonly sold fermented dairy products are the result of the growth of lactic acid bacteria. A basic fermentation theme characterizes all of them. The variations in this theme result in different products.

A. The common theme

1. Milk is inoculated. This occurs naturally (fresh raw milk contains up to 100,000 lactics/ml), but almost all commercial fermentation involve inoculation of pasteurized milk with pure cultures of lactic bacteria.

2. Inoculated milk is incubated. The lactics grow, metabolize the lactose, and produce lactic acid and other flavor compounds.

3. Acid production results in coagulation of the casein (the "curds" separate from the whey).

4. If cheese is the object, the casein is removed from the whey and incubation continues.
B. The variations

The diagram on the next page shows many of the common dairy products that are simple lactic fermentations. Note that variation in incubation temperature (32°C vs 40°C) results in very different products. Also note that different parts of the milk become different products even when the same bacteria are involved.

**COMMON DAIRY FERMENTATIONS**

[Note: Aging refers to incubation at warm temperatures which permit the proliferation of bacteria in the milk (almost all lactic acid bacteria)]

Commonly used cultures:
*Streptococcus cremoris, S. lactis subsp. diacetylactis, Leuconostoc remorisor*
**S. thermophilus, Lactobacillus bulgaricus, L. acidophilus**

Unless otherwise noted, incubations at 32°C
IV. DAIRY FERMENTATIONS WITH LACTICS AND OTHER MICROBES

A number of fermented dairy products involve other types of microbes in addition to the lactic acid bacteria:

A. Bacteria other than lactics

Example: Swiss cheese

1. Inoculated milk is heated to 50°C which selects for certain thermophilic lactics, typically *S. thermophilus* and *L. helveticus*. Like the yogurt bacteria, they produce acetaldehyde as a major end product.

2. Coagulation is achieved by adding a calf rumen extract (contains rennin) which also contains bacteria of the genus *Propionibacterium*. (Actually pure cultures are generally now used in place of the natural source).

3. After the lactic acid concentration is high, the *Propionibacterium* begins to ferment it, producing:
   a) Propionic acid (nutty taste)
   b) Acetic acid (sharp bite)
   c) CO₂ (holes in the cheese)

B. Yeasts

Example: Kefir - a Balkan drink quite different from the product sold as kefir in this country [American kefir is actually liquid yogurt].

1. Milk is inoculated with “beads” derived from previous batch which contain lactic acid bacteria (*L. acidophilus* and probably others) and a lactose-fermenting yeast: *Saccharomyces kefir*.

2. The two types of microbes metabolize the lactose simultaneously, the yeast producing ethanol and CO₂, and the lactics producing lactic acid.

3. During the fermentation, the beads swell up and rise to the top. They consist of a polysaccharide (formed by the bacteria) in which the microbes are embedded. They are removed, dried, and saved for the next batch.
C. Molds

Examples: Camembert and Roquefort cheeses
1. Fermentation begins just as in simple lactic case. Several different kinds of lactics are possible.

2. After the initial lactic fermentation, the cheese is surface inoculated with a mold, usually a species of Penicillius, e.g. _camembertii_ or _roquefortii_.

3. The molds attack the higher molecular weight milk compounds and also metabolize some of the lactic acid. Result:

\[
\begin{align*}
\text{Protein} & \rightarrow \text{peptides, NH}_3 \text{ [bitter taste, pH goes up]} \\
\text{Fats} & \rightarrow \text{fatty acids (peppery taste)} \\
\text{Fatty acids} & \rightarrow \text{methyl ketones (unique flavor)}
\end{align*}
\]

Breakdown of fats and protein results in crumbly texture and a product less resistant to the growth of other (possibly pathogenic) microbes.

V. NON-DAIRY LACTIC FERMENTATIONS

A. Sausage fermentation

There are many kinds of fermented sausages, the most common in this country being salami and pepperoni. The process is very similar to the simple lactic dairy fermentations.

1. The meat is chopped, salted, spiced, and packed.

This procedure selects for the growth of naturally occurring lactic acid bacteria, which are often added in the form of a sample from the last completed batch. The chief lactic agent is usually _Lactobacillus plantarum_ and/or _Pediococcus acidilactici_. Most modern commercial sausage preparation employs pure cultures of these organisms maintained in sausage-like media.

Chopping and packing makes a microaerophilic environment. Meat sugar (glucose) is the primary substrate. Salting inhibits the growth of potential competitors. Spices may contain manganese which aids the survival of lactic acid bacteria in the air.

2. A source of nitrite is typically added.

This can be done by adding nitrite per se or by adding nitrate along with a microbe that readily reduces it to nitrite in this environment (usually a _Micrococcus_ species.)
Nitrite contributes to the taste, color, and, most importantly, to protection against *C. botulinum*.

3. **Considerations**

It is essential that the growth of the lactics begins immediately because the acid that they produce is what makes the sausage resistant to the growth of other salt tolerant microbes such as *S. aureus*.

B. **Vegetable fermentations, e.g. Pickles and Sauerkraut**

The traditional fermentation of vegetables is very similar to fermentations, although most of the commercial processes involve shortcuts that significantly stretch our definition of fermentation. The sauerkraut fermentation is described below.

1. **The cabbage is chopped, salted, and packed.**

As with sausages, this process selects for the growth of naturally occurring lactic acid bacteria. The salt serves an additional function of leaching out nutrients from the plant cells. Without the salt, the lactics cannot obtain the necessary substrates for growth. Sugars (primarily glucose) are the energy source for the lactics. Sucrose is often added to the fermentation to make sure there is an adequate amount.

2. **The fermentation involves a progression from heterofermentative to homofermentative lactics.**

The heterofermenters, principally *Leuconostoc mesenteroides* initiate the process because they are the air-tolerant. The homofermenters, principally *Lactobacillus plantarum*, take over when the acid concentration becomes too high for the heterofermenters. Note the progression in the diagram below.
3. Variations

a) The vegetables need not be chopped. This makes it a little bit trickier to ensure growth of lactics.

b) Acetic acid may be added at the start to make sure that non-lactics are inhibited from the onset.

c) Acetic acid and lactic acid may be used as a substitute for lactic acid bacterial growth. Some pickles sold are actually just acidified vegetables.

C. The sourdough fermentation

Sourdough bread differs from ordinary bread in that the yeast growth is accompanied by the growth of lactic acid bacteria. Yeast provides the leavening, and the lactics provide the sour flavor. This kind of bread is not really as unique as you might think. Before clean pure cultures of baker's yeast (Saccharomyces cerevisiae) became available, the yeast used was probably a mixture of yeast and lactics. In addition, flour contains lactic acid bacteria, which are part of the normal flora of plants.

A very famous type of sourdough bread originated in San Francisco. It is famous because it is particularly sour and consistently so. According to local legend, it is not possible to make it anywhere else; you can take the starter to another place, but it will
not produce the same bread. About 20 years ago, a team of food microbiologists decided to figure out what made this bread unique. Below is a summary of what they found over a period of several years.

1. **The sourness of SF sourdough was due to acetic acid.**

Most bread contains some acetic acid, probably due to the growth of small numbers of lactic acid bacteria. The SF sourdough was found to contain 10 times more than the average, which suggested extensive growth of heterofermentative lactic acid bacteria.

2. **The formulation involved few ingredients and cooler rising time:** the bread was made only with flour, water, salt and starter, and was proofed at $30^\circ$C.

This suggested that the fermentation microbes were different from those used in typical bread.

3. **Analysis of starters revealed two unique microbes:**

   a) Yeast: *Torulopsis holmii* (more recently named *Candida milleri*), which differs from baker's yeast in that it:

   - Uses glucose, but not maltose. (Maltose is the major soluble sugar released from starch by amylases present in the flour)
   - Grows at $30^\circ$C, but not at $37^\circ$C.

   b) Lactic acid bacterium: *Lactobacillus sanfrancisco*, so-named because it did not resemble any known lactic species. It is unique in that it:

   - Uses maltose, but not glucose! (Apparently due to the lack of a glucose permease).
   - Requires a special growth factor, originally termed “Fresh Yeast Extract” because that was the only substance that worked, but now known to be a small peptide that happens to be released by growing yeast.

   c) The two microbes grow as symbionts, as shown in the flour chromatograms on the next page.

4. **Why can't the bread be made elsewhere?**

   Although the starter can be taken to another place, it is gradually overtaken by microbes indigenous to the new site. Apparently the bay area climate is crucial to the symbiotic growth of these particular microbes. Like all fermentations, SF sourdough bread is its own ecosystem, the survival of which depends on maintenance of all the factors contributing to the microbial growth.
CHROMATOGRAMS OF SOLUBLE SUGARS IN FLOUR AFTER 6 HOURS INCUBATION WITH WATER AND SALT AND INDICATED INOCULA

A. No additions

B. With S.D. yeast

C. With S.D. bacteria

D. With S.D. yeast & S.D. bacteria
Chapter 2.
FERMENTATIONS INVOLVING EXTRACELLULAR 
HYDROLASES AND OTHER COMPLEX FERMENTATIONS

Food fermentations involving the lactic acid bacteria as the sole or initial fermentation agents are based on the conversion of soluble sugars to acids as the major products and are characterized by only minor alterations in the macromolecular structure of the food. A relatively high concentration of soluble simple sugars and microaerophilic conditions, are the key features which pre-determine this sequence of events. The acids produced prevent the growth of other bacteria which could digest the macromolecular components, and the lack of oxygen prevents significant mold growth.

However, many fermentable foods do not contain high concentrations of simple sugars. Some have very few soluble nutrients of any kind. In this case, fermentation must be initiated by organisms capable of attacking the large molecular weight structural components. Other foods have very modest concentrations of soluble sugars, and the result is a mixed fermentation in which lactic growth is accompanied by the growth of other organisms. Finally, some foods contain sugar derivatives which may be soluble, but which require special enzymatic action before the useful part of the molecule becomes available. The fermentation of foods in these three categories does not follow the pattern described in the last chapter. In many cases, the process is often quite complex and still poorly understood. Here we will look at some examples.

I. ONE-STEP FERMENTATIONS BY EXTRACELLULAR HYDROLASE PRODUCERS

(a) The Basic Picture
Molds and Bacillus species are common fermentation agents of foods composed primarily of protein or starch. Unlike the lactic fermentation foods, the product here is more susceptible to spoilage than the starting food because it contains more soluble nutrients. The advantage of the product is increased digestibility rather than stability.

The basic process is outlined below. Typically it begins with a soaking or cooking step which serves to inactivate destructive enzymes in the food, reduce the microbial load, and increase the partial digestion of the materials so that the inoculum will thrive.

Inoculate & 
Beans, 
Wheat, 
or rice
Softened materials 
with reduced flora 
and inactivated native enzymes
Fermented Food

Incubate
Partial digestion involving proteases, amylases, and some lipase action
(b) TEMPEH - Soybeans fermented by *Rhizopus*

1. Soaked and heated beans are packed in trays and inoculated (traditionally the inoculum was on the leaves in which the beans were wrapped)
2. Inoculated beans are incubated about 24 hours at a warm temperature, around 30°C.
3. Result: a cake of beans held together by the mold mycelium, with features as follows:
   a) The soy protein no longer contains pancreatic inhibitor (a problem in unfermented soy products), and soluble nitrogen is increased about 4-fold.
   b) A nutty taste; some bitterness and NH$_3$, depending on extent of incubation.

(e) NATTO - Soybeans fermented by *Bacillus subtilis*

1. Soaked and heated beans are spread in a thin layer and inoculated. (traditionally the inoculum was on the rice straw in which the beans were wrapped)
2. Inoculated beans are incubated about 20 hours at a very warm temperature, around 40°C.
3. Result: a mass of beans held together by a sticky polymer synthesized by the bacteria.
   • Increased digestibility is a result, probably to about the same extent as determined for tempeh.
   • Flavor contains some NH$_3$, but is otherwise quite different from tempeh because different hydrolases are synthesized by *Bacillus*.

II. COMPLEX FERMENTATIONS INITIATED BY MOLDS - SOY SAUCE

Once the extracellular hydrolases have liberated soluble nutrients, many other organisms can grow in the food. Soy sauce is an example of the fermentation involving the sequential growth of hydrolase-producers followed by microbes that rely on the soluble nutrients. The starting material can be soybeans or wheat or a combination thereof. There are many variations on the soy sauce fermentation. A typical process is described below.

A. Initial step: the KOJI stage

   • Microbes: *Aspergillus oryzae* or *A. soyaee*

   • Incubation: about 3 days at about 30°C

   • Result: a mash with greatly increased concentrations of soluble nutrients - sugars, peptides, and free amino acids (including glutamic acid).
B. Koji is soaked in salt, concentration about 18% to produce the MOROMI

C. Moromi is very slowly fermented

1. Microbes: both yeast *Saccharomyces rouxii* and lactic acid bacteria *Lactobacillus delbrueckii* and/or *Pediococcus soyae*.

2. Incubation: 3-6 months, starting at about 15°C, then gradually warming to about 25°C.

   During this time *Aspergillus* growth continues to some extent, but gradually the yeast dominate, and finally the lactic acid bacteria finish the fermentation.

3. Result: Product is high in peptides and amino acids (including MSG) and is acidic (final pH around 4.7)

III. SPECIAL LACTIC FERMENTATIONS FOR PRODUCTION OF FLAVORS

Some flavorings that we use are fermented products. Often the key volatile components are released from complex saccharides by bacteria, and usually we are otherwise very poorly informed about the other microbial events associated with the fermentation.

A. VANILLA production

Vanilla beans from certain tropical orchids are "cured" or "conditioned" for several months, during which time lactic acid bacteria hydrolyze several glucosides, most notably *glucovanillin*. They metabolize the glucose part of the molecule, leaving behind the aromatic ring. "Curing" takes 1-2 weeks (depending on the procedure). The bean pH drops from about 5.5 to 3.5, and bacterial counts reach 10⁹/gram.

B. TEA

There are 3 major kinds of tea according to the general processing methods:

1. GREEN TEA: Processing of Green Tea is minimal. The harvested tea goes through 3 stages—steaming/panfrying, rolling, and drying. This kind of tea is also called “UNFERMENTED TEA”.

2. OOLONG TEA: The so-called “SEMI-FERMENTED TEA”. Some fermentation, basically the tea after withering is left indoor and incubated at ambient temperature for a number of hours. When the tea has reached a low level of color changes, it is roasted at 250–260°C for about 15 minutes. This stops the enzymatic process. The tea is then rolled and dried to produce the final product.
3. **BLACK TEA**: The black tea is the “FERMENTED TEA”. Its processing has a more lengthy and extensive fermentation step. Fermentation starts at rolling of the tea leaves. The rolling process breaks the tea leaves into smaller pieces and allows the enzymes, in particular, polyphenol oxidase, to mix with the polyphenols in the tea leaves. The tea leaves after rolling are incubated at a specified or controlled condition for a number of hours until the tea is dark brown. It is then roasted and dried to make the final product.

The drying of harvested tea leaves and subsequent “fermentation” in the Oolong and Black Tea production are accompanied by significant microbial growth, including molds, yeasts, and bacteria. It has been suggested that they are an important factor in flavor development, but little is known about the details of their growth. Alternatively some scientists believe that the important changes are due to enzymes in the tea leaves, producing groups of compounds called theaflavins and thearubigins which are themselves flavors and also react with amino acids and sugars to form more complex flavors. They think that microbial growth is of no consequence in flavor development.

**IV. MIXED FERMENTATIONS INVOLVING MANY ORGANISMS**

Some natural products appear to be the result of the simultaneous growth of many different microbes, up to a dozen or more. In fact, different batches may contain different organisms. The two fermentations described below fall into this category. However, in each case there are certain essential events that occur reproducibly, and are attributed to microbial types that are always present during the fermentation.

**A. COFFEE**

Before the actual coffee bean (the cotyledon) can be released, pectinolytic bacteria such as *Erwinia* (or, more rarely, pectino-lytic molds) digest the pectin. Free sugars released typically support the growth of lactic acid bacteria, and the final product is acidic.

**B. COCOA**

Beans removed from the pod are dried in heaps that are inoculated by fruit flies. Fermentation takes about a week.

Probably *Bacillus* species or molds initiate the process by breaking down the pulp structure, thereby making the soluble sugars and organic acids (malic and citric) available to other microbes, mostly yeast and lactic acid bacteria.

The lactic acid bacteria produce lactic and acetic acids from the sugars and also additional lactic acid from malic and citric acids. Sugar metabolism lowers pH, but malic/citric acid metabolism raises it. Yeast (or other unidentified microbes) uses the organic acids, thereby raising pH. Yeast also produces ethanol from the sugars (little pH change), which is subsequently converted to acetic acid by acetic acid bacteria (lowering pH). Different air concentrations favor some reactions over others, but the net pH
reaction in the pulp is one of increases over the initial pH (3.6 - 4.0). The pulp eventually disappears, but the beans (cotyledons) absorb acids from it. Thus the final bean pH is significantly lower than the initial pH of about 6.5. Some chocolate reaches a pH of 4.5, but "better" chocolate is not so acidic. (pH around 5.4)