

Microbial influences on fermented beans

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Harvested soybeans, ready for fermenting. Credit: Wikimedia Commons

Fermented foods are deeply seated in global food culture. Many recipes are highly guarded, passed through generations and shrouded in mystery. The microbial communities that make up fermented foods are often diverse, but typically only a few species contribute significantly to the final product.

Additionally, fermented foods and drinks (termed ferments in this article) with similar flavor profiles are often fermented by similar microbes, or at least microbes that carry out similar metabolic processes. By exploring the variety of fermentations from essentially the same starting material we can observe the impact microbes have on the final product.

Fermentation's role in the field of microbiology is hard to overstate. Significant advances in early microbiology history stemmed from trying to understand why some ferments go wrong. For example, in the mid 1800s Louis Pasteur noticed that [wine was souring](#) because it was colonized by an organism smaller than yeast, which turned out to be lactic acid bacteria.

Metabolically, fermentation simply describes processes where organic molecules act as donors and acceptors of electrons. The microbial community that drives fermentation can either be the microbes that are natively on the [food](#) (termed spontaneous fermentation), or a part of a previous ferment known as a starter, mother or backslop.

Starter cultures typically help expedite the first stages of fermentation. This is because the starter will seed the ferment with a larger population of organisms than are natively present on the food starting material. Starter cultures can also ensure more fermentation consistency and quality by maintaining similar organisms. Larger scale production tends to rely on starter cultures, while many traditional fermented foods utilize the native communities of microorganisms.

People maintain starters for long periods to make sure they can continue making the fermented foods they enjoy. For example, in popular history, [sourdough starters were kept warm by Alaskan miners by keeping them on their person](#).

The microbiological processes underlying food fermentation are highly dynamic, with many ferments characterized by multiple stages as the microbes change their environments with the addition of each metabolic waste product. Furthermore, different ferments are characterized by different microbes that each contribute to the unique flavor profiles.

How fermentation shapes food

Fermentation influences five primary aspects of food:

1. Preservation.
2. Digestibility.
3. Toxin reduction.
4. Probiotics.
5. Taste.

Preservation

Fermentation is often associated with the idea of food preservation. This is because the fermenting microbes influence their environment in ways that exclude many of the organisms that cause rancidity or make people sick. For example, in acidic ferments, lactic acid bacteria produce acids that exclude dangerous microbes, such as *Clostridium*.

Digestibility and Toxin Reduction

Fermentation can facilitate healthy food consumption by [improving digestibility](#) and [performing toxin reduction](#). This is especially true in the case of soybeans, as well as many other plant-based foods.

Plant matter is often composed of molecules that reduce the bioavailability of nutrients, inhibit digestive enzymes or even inhibit

overall cell function. During fermentation, microbial enzymes can break down many of these toxins, including cyanogenic glycosides, which can inhibit cellular respiration. Additionally, some toxins are removed during preparation steps, such as removing the outer hull of seed pods or through cooking.

Probiotics

The health benefits of fermented foods are thought to extend beyond making foods easier to digest and less toxic. In particular, scientists are very interested in the influence of the microbial constituents of the ferment as potential probiotics.

There is mixed evidence regarding the effects of fermented foods on gut health. Many of these food-associated microbes are considered transient and have limited ability to colonize the gut, meaning that they simply pass through the digestive tract (or something to this effect). Yet, one study indicates that some [situations of dysbiosis can be mitigated, with the help of lactic acid bacteria](#) from a kefir-like fermented milk product.

The authors explored the ability of microorganisms (a consortia of Bifidobacteria, Lactococcus, Lactobacillus and Streptococcus) from the probiotic drink to colonize rat guts with different initial microbiomes. They observed that individuals that did not maintain the Lactococcus lactis had a more resilient microbiome that was characterized by a higher relative abundance of Lachnospiraceae. This suggests that microbiomes going through flux are more likely to be impacted by probiotics in fermented foods.

Taste

And of course, taste! Secondary metabolites, such as lactic acids, esters

and free amino acids that are produced during fermentation can significantly alter the flavor profile of the food. For example, in sourdough cultures, the balance of lactic acid bacteria and yeasts will determine just how sour the bread gets. A higher proportion of [lactic acid bacteria](#) results in more sour bread.

Further, by adjusting the environmental conditions one can influence what metabolic pathways are favored, thereby encouraging production of different acids or esters, which also contribute to the flavor profile of the food.

Back to the basics: Alkaline fermentation

Typically, we think of fermentation as being an anaerobic process, but that is not always the case in "fermented" foods. In Western cuisine, fermented foods are typically acidic; however, fermented foods can also be produced through alkaline processes or have phases at different pHs. Acidic fermentation, fermentation that results in a decreased pH, is a highly studied process. However less literature exists on alkaline fermentation, where amino acids are metabolized to ammonia, which raises the pH of the culture.

Foods that are alkaline fermented are typically more protein rich and commonly include legumes (such as soybeans) and seeds. For example, [natto](#), dawadawa and [kinema](#) are all alkaline fermented foods, made using legumes. Most alkaline [fermented foods](#) use native [microbial communities](#), from which *Bacillus* species tend to be highly abundant after the cooking process enriches for them.

Natto, a fermented soybean product characterized by stringy polymers that bind the beans together, is influenced by microbial processes in multiple ways. The production of natto from soybeans occurs when *Bacillus subtilis* subspecies *natto* secretes proteases that break down the

proteins of the soaked and cooked soybeans. Then, a secondary fermentation driven by [glutamate dehydrogenases and ureases](#) leads to natto's iconic ammonia smell.

Strings of natto are primarily made up of poly-gamma-glutamate (γ -PGA), an extracellular polymeric substance (EPS) of *Bacillus subtilis* (natto). The EPS derived from the production of natto is also of interest as a [polymer for manufactured products including biodegradable fibers, heavy metal absorber](#), among other uses.

Another *Bacillus*-driven alkaline ferment is the seed-based condiment from Western Africa, which goes by the names Dawadawa, ogiri, soubala, iru and netetu among others. To produce Dawadawa, locust bean seeds (or other large seeds) are boiled and dehulled, then fermented for a few days as flavor develops. [Bacillus subtilis is often a primary organism](#) involved in this fermentation; other involved organisms include members of the genera *Leuconostoc*, *Staphylococcus* and *Micrococcus*.

There is limited information about the role of fungi in this fermentation process. It is thought that the cooking process encourages the dominance of *Bacilli* because they can form heat-resistant spores, and inoculating the seeds with *Bacillus subtilis* spores improves the reproducibility of the fermentation process.

Kinema is produced in a similar manner as dawadawa, but with soybeans and in a different region—the Eastern Himalayas. Like natto, kinema fermentation forms stringy polymers, but to a slightly lesser extent than natto. The fermentation is also carried out by [native microorganisms](#), primarily *Bacillus* species and other Firmicutes

In terms of fungi, the main species are *Wallemia canadensis* and *Pichia sporocuriosa*, but their functional role is not well defined. As with other alkaline ferments, amino acids are released by the microbes providing

human consumers a ready source of nutrients, as well as an umami or meat-like flavor.

Mold-driven fermentation of soybeans

Like alkaline ferments, many other foods with a higher protein content, such as beans and seeds, are fermented with molds. Mold-driven ferments are generally shaped by the action of just a few molds; *Rhizopus*, *Mucor* and *Aspergillus*.

Compared to many bacterial ferments, mold-based ferments are often fermented at somewhat higher temperatures. Propagation methods are also somewhat different than for bacterial cultures because it is often useful to wait for parts of the culture to sporulate in order to transfer to the next ferment.

Tempeh and [Koji](#) are two soybean ferments [mediated by molds](#). Tempeh production involves soaking, cooking and drying the beans then inoculating with a starter culture of *Rhizopus*. Then, this mixture is formed into a shape (typically blocks) and covered with an air permeable wrapper.

This process must be watched and mixed fairly carefully, as the interior of the fermenting tempeh can become too warm through the metabolism of the molds. As the mold grows, it forms a mycelium that binds the tempeh block together. This fermentation process usually takes between 1.5 and three days, depending on temperature and starting inoculum.

Rhizopus improves the soybean nutrient quality by breaking down proteins, making iron more bioavailable and removing toxic compounds. *Koji*, on the other hand, is typically made using *Aspergillus* species, but it is made from a variety of starting materials including rice and barley, in addition to soybeans. *Koji* is the starting culture for other products

including miso, soy sauce and saké.

Interestingly some foods, like amazaké, use koji as a source of enzymes, rather than relying on growth of the mold itself. Amazaké relies on high temperatures to encourage enzyme activity to release sugars from rice to make a pudding or drink.

Fermentation broadens the foods that we are able to eat and the amount of time we have to eat them before they spoil. Different fermentation processes and organisms are used to generate wildly different flavors, which we highlight by exploring the fermentation processes used to make natto, dawadawa, kinema as well as tempeh and koji.

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