



BC Centre for Disease Control  
An agency of the Provincial Health Services Authority

Environmental Health Services

# Food Issue

## Notes from the Field

### Tempe (tempeh) fermentation

Request received from:	Vancouver Coastal Health Authority
Date of request:	October 12, 2016
Issue ( <i>brief description</i> ):	Evaluate food safety of tempe fermentation recipe

*Disclaimer: The information provided in this document is based on the judgement of BCCDC's Environmental Health Services Food Safety Specialists and represents our knowledge at the time of the request. It has not been peer-reviewed and is not comprehensive.*

#### Summary of search information:

Internet sources: PubMed, Google Scholar-linked citation sources, MMWR

#### Background information:

Tempe is classified as a compact cake-form product which is prepared from dehulled soybeans through fermentation with *Rhizopus* spp. by Codex.<sup>1</sup> This product originated in Indonesia and the earliest reference to this traditional ethnic food dates back to the 1600's.<sup>2</sup> It is an affordable protein staple in Indonesia, also common in the Netherlands, where there is a large Indonesian sub-population. The alternate spelling, tempeh, is derived from the Dutch.<sup>2</sup> Tempe has some interesting nutritional characteristics; fermentation renders the soybeans more digestible (via reduction of flatulence inducing oligosaccharides),<sup>3</sup> inhibits phytic acids commonly known as anti-nutrients thereby increasing mineral bioavailability, increases the vitamin B<sub>12</sub> and folate content, and tempe researchers have shown this food has antibacterial affects, such as reducing adhesion of enterotoxigenic *E. coli*.<sup>2,4</sup>



Fresh tempe (fermented soybean cake)  
Surono, 2016<sup>2</sup>



Assorted tempe tasting: bean and pea varieties  
Guixer, 2015<sup>5</sup>

The process of tempe manufacture includes two types of fermentations. First, the soybeans are soaked in water and undergo lactic acid bacteria (LAB) fermentation at room temperature for less than one day. This is a naturally occurring fermentation without the addition of a starter culture. This step should lower the acidity of the food to below 4.6. The beans are dehulled, drained, and boiled. The mashed beans are placed into perforated plastic bags and inoculated with the *Rhizopus* mold inoculum, then allowed to ferment for approximately one to two days at warmer temperatures, generally between 22 and 38°C.<sup>4,6</sup> During this second fermentation period, the mold releases ammonia, which is essentially an alkaline fermentation that raises the pH of the tempe to between 6.8 and 8.0, with optimal pH at 7.2.<sup>2,4</sup> The product at this stage is ready to eat, fresh, but has a very short shelf-life. Estimates provided in tempe production guides suggest that tempe must be eaten within one to four days, and may be refrigerated for up to one week.<sup>6</sup> However, the longer the tempe is held, the more likely ammonia will continue to be elaborated by the *Rhizopus* culture, rendering the product “smelly” and with black spores. See food flow-chart figure below.

Tempe products have also been made with combinations of wheat, millet, other cereal grains, rice and coconut.<sup>4,7</sup>

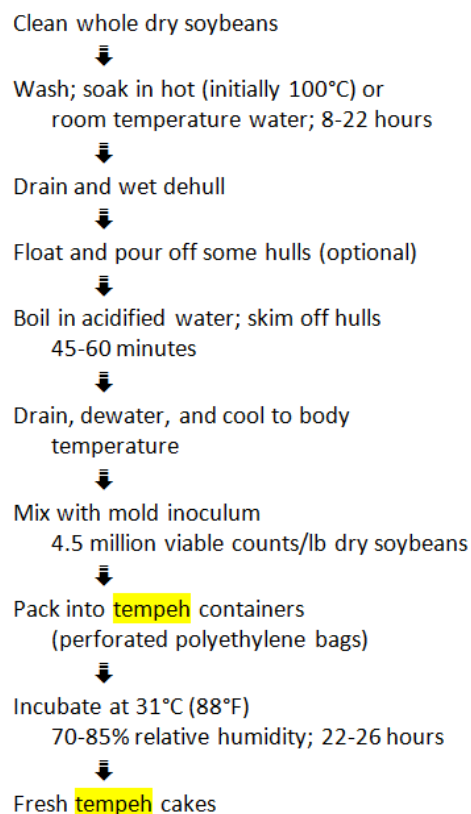


Figure adapted from Shurtleff and Aoyagi, 1979.<sup>6</sup>

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### *What are the risks associated with tempe fermentation*

Biological and chemical (toxin) hazards are associated with tempe products. Microbiological quality of tempe sold in the Netherlands revealed bacterial counts exceeding 10,000 CFU/g in 13% of samples for *S. aureus*, 11% for *B. cereus* and 3% for generic *E. coli*.<sup>8</sup> In a review of *B. cereus* food poisonings in China, fermented bean curds, black soybeans, and soy paste were implicated.<sup>9</sup> One outbreak in Yunan, China that affected 139 persons implicated fermented black beans, from which two heat stable emetic (cereulide) strains of *B. cereus* were isolated.<sup>9</sup> *Staphylococcus* spp. were also isolated from fermented products. A review of sofu, another fermented soy-bean product from China, also found samples where *B. cereus* levels exceeded 10,000 CFU/g.<sup>10</sup>

**To control these hazards, acidification of the first fermentation step is required.** This will prevent *B. cereus* and *S. aureus* from growing during the stage beans are soaked. Although there is a cooking step later on, heat stable toxins formed and elaborated during the soaking period would not be destroyed by a cook step.

One *Salmonella* Paratyphi outbreak in the United States associated with unpasteurized tempe was linked to contaminated starter *Rhizopus* culture.<sup>11</sup> Mycotoxins are also a potential hazard from cultures of *Rhizopus microspores*<sup>9</sup> and are known to elaborate rhizonins and rhizoxins. Other toxic metabolites may be produced from *Aspergillus* and other molds that may also be growing on the fermenting product.<sup>9</sup>

While Steinkraus (2002) generally reports that tempe is a safe product, one type of tempe has been associated with serious illness – “tempe bongkrek” made from coconut. *Burkholderia (Pseudomonas) cocovenenans*, a bacteria that can grow in this product may elaborate a very toxic (bongkrek) acid that is lethal.<sup>7</sup> Since 1951, at least 1000 people are known to have died, and in 1988 Indonesian banned the production of tempe bongkrek.<sup>3</sup>

There are three types of ways to inoculate fermentations: backslopping, where a portion of the previous ferment is used as starter culture, wild-type fermentation, and pure starter culture.<sup>12</sup> **To control these hazards, it is necessary to have *Rhizopus* starter culture that is purchased from a commercial supplier that can provide a certificate of analysis (COA) that the culture is pure. It must be verified free of *Salmonella*, and should additionally be verified free of *Staphylococcus aureus* and *Bacillus cereus*.** The re-use of starter culture is not recommended. Commercially purchased *Rhizopus* starter culture should be stored refrigerated to preserve quality.

Vacuum-packaging sales of tempe need to be assessed for the potential risk of botulism. Although the soy-beans are boiled, there is a potential for spores of botulism to survive this process. As the beans are boiled for longer than 10 minutes, a shelf-life of more than 10 days is allowable.<sup>13,14</sup> Studies of soy and tempe products have shown that botulism toxin can be produced in tempe both during the mold fermentation period and in the final product (based on studies where *C. botulinum* bacteria is inoculated into the final product); botulism toxin was detected when temperature abused tempe was held in

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vacuum packaging (within 5 days in vacuum-packaged tempe held at 25°C and within 4 weeks in vacuum-packaged tempe held at 15°C).<sup>15</sup> Home-fermented tofu has been known to cause botulism when it is temperature abused.<sup>16</sup>

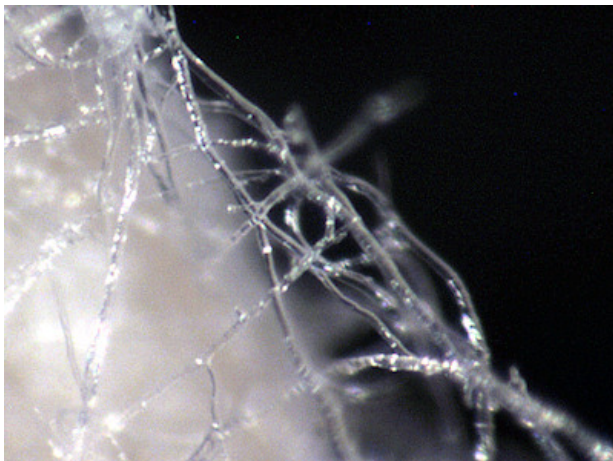
A list of concerns stemming from a review of illnesses reported with fermentation processes include<sup>9</sup>

- Contaminated raw ingredients
- Lack of pasteurization
- Poorly controlled natural fermentation
- Inadequate storage and maturation conditions
- Consumption without prior cooking

**It is critical, therefore, that (1) sanitary handling and conditions are present during fermentation and during handling and packaging, (2) environments are properly controlled for temperature and moisture to allow optimal maturation and fermentation, (3) products are properly pasteurized before packaging, and (4) cold-chain of this product is properly maintained. Further, consumer packaging should indicate that (5) the product must be refrigerated, and (6) the product must not be eaten raw, particularly if sold in a raw state.** Botulinum toxin is heat-labile, and can be inactivated by cooking.

#### *Previous guidance on tempe fermentation from British Columbia*

Environmental Health has been contacted about tempe on one other occasion, when a customer complaint about black mold formation on commercial tempe was brought forward to an inspector in Northern Health. Our response to that question was that tempe is a mold-ripened product, although it was possible that environmental contamination could cause black mold to appear on the product. Black spores are colouration are common on aged tempe as *Rhizopus*, although the mycelium (mold strands or hyphae) are white, once this organism sporulates, the spores are black.<sup>4</sup>



Mycelium of *Rhizopus oryzae* on fresh tempeh



Sporangia of *Rhizopus oryzae* on overripe tempeh

Source: TopCultures<sup>17</sup> ([www.tempeh.info](http://www.tempeh.info))

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BCCDC further issues general fermentation advice in a food issue note titled, “Raw food handling to limit existing hazards”.<sup>18</sup> The fermentation specific food safety advice in this document, however, does not address the specific hazards associated with an alkaline fermented food that has a more neutral pH.

### *Previous guidance on tempe from elsewhere*

According to Dr. Brian Nummer, culture manufacturers MUST be an inspected facility, and must be able to provide a COA for microbial analysis.<sup>19</sup> This is absolutely recommended to ensure culture safety. Further, he recommends manufacturers perform a test batch with new cultures. Excellent sanitation principles are also recommended to control and minimize environmental and cross contamination issues.<sup>19</sup>

The incubation time, temperature and length of fermentation vary depending on the recipe used, but time is inversely proportion to temperature increase (higher temperatures give shorter fermentation periods).<sup>4</sup> **Operators should control the temperature and humidity to optimize fermentation, and prevent spoilage and growth of spoilage organisms.**

The issue of storage dates for tempe vary depending on whether the product, after fermentation, has been pasteurized. Some manufactures blanch pasteurize tempe to allow for longer refrigerated storage of two to three weeks.<sup>6</sup> For this product, if it is refrigerated at retail, a pull-date on the product is advisable for quality and safety.<sup>6</sup> Prolonged refrigerated storage of tempe can result in undesirable odour (from increasing ammonia), black spots for sporulating *Rhizopus*, or growth of harmful pathogenic bacteria. Growth of undesirable bacteria and molds occur following increases in ammonia production that ultimately inhibit *Rhizopus* culture.<sup>4</sup> However, even when tempe is made incorrectly, it is more likely the *Rhizopus* culture will be overgrown by another spoilage organism.<sup>6</sup>

Tempe culture should be stored in the refrigerator to keep its quality and be protected from cross-contamination.<sup>17</sup>

According to one site (photo right)<sup>20</sup> tempe (presumably pasteurized) can be kept refrigerated for up to two weeks, and frozen for six months.

Longer refrigerated storage has been reported after longer pasteurization cooking periods. The length of storage time will depend on how the product is cooked following fermentation, and on the packaging and handling.

Lalibela Farms manufactures unpasteurized organic tempe that is distributed frozen, they advise to thaw and use within three days. They also include a consumer advisory to not eat the product raw, and to cook.<sup>21</sup> Storage of tempe can be improved if the tempe is dried, fried, dehydrated, frozen, blanched, steamed or canned.<sup>4</sup>



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One tempe culture manufacturer advises that raw tempe should be cooked to an internal temperature of 80°C for at least 60 seconds.<sup>17</sup>

**Operators should determine the optimal shelf-life of their product through quality testing. Tests should include checks for indicator organisms (coliforms, total plate counts, yeasts) and pathogens (*E.coli*, *Listeria*, *Staphylococcus*, *Bacillus*). Shelf-life tests should be repeated at least annually to verify product performance. The length of tempe storage should include a safety margin that includes potential temperature abuse by consumers. A use-by date and/or pull-by date at retail is recommended.**

#### Recommendations from BCCDC:

1. *Rhizopus* mold starter cultures must be purchased from a commercial supplier, and come with a certificate of analysis that verifies the culture is free from *Salmonella* and *Staphylococcus aureus* / *Bacillus cereus*.
2. Do not re-use mold product as a starter.
3. The soaking and boiling of the soy-beans must occur in potable acidified water, and the pH of the soaking and boiling water must be at or below pH 4.6.
4. Environments for fermentation must be properly controlled for temperature and humidity (moisture). Excess moisture and overheating can cause quality issues and growth of spoilage organisms. Operators are advised to discard batches that do not conform to normal appearance of quality of tempe.
5. Raw tempe should be cooked before packaging in reduced oxygen atmosphere. Tempe should be cooked to an internal temperature of 80°C
6. Operators should determine the optimal shelf-life for their product through quality testing as suggested above.
7. Proper labelling on the packaging is essential for consumers. If the tempe is sold unpasteurized there should be an instruction that consumer are not advised to eat the product raw, and to cook before consumption.
8. A pull-by or use-by date should be clearly stamped on the packaging. The operator is responsible to establish the shelf-life through quality testing of the product.

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