Traditional mixed cultures have several other defie advantages. First, they can be produced quite easily by local craftsmen under ordinary nonsterile workshop conditions, using only local materials. Second, local production virtually guarantees that they are well adapted to the local environment. And third, in developing countries they generally cost only a fraction as much as pure cultures purchased from a central laboratory source.

It is important to remember that the presence of unwanted microorganisms (such as spoilage bacteria) in a mixed culture can lead to poor-quality tempeh. Steinkraus et al. (1961), for example, reported that unwanted bacteria that developed when the pH was too high contributed undesirable odors to the tempeh. Moreover, there is a definite danger of food poisoning when the content of an inoculum is unknown, as in the case of coconut-presscake tempeh, where a *Pseudomonas* contaminant can even cause fatalities. Fortunately no such problems have been reported in the case of other tempeh types. Foods such as miso and shoyu having a high salt content protect themselves, since food-poisoning bacteria cannot tolerate the salt.

Commercial tempeh makers in the West (especially those using low technology) report having difficulty with unwanted organisms (especially spoilage bacteria) that ruin their tempeh unless highly sanitary conditions are maintained. Several reasons have been given to explain why this occurs, but does not take place even under rel-

1. In Indonesia the shop environment is so heavily saturated with tempeh microorganisms that, even if deliberate inoculation were omitted, an inoculation would take place automatically, and good tempeh would generally result, although with a longer fermentation time.

2. The pure-culture starters used in the West, like agricultural monocultures, often lack stability and are vulnerable to contaminants once they start to propagate. Studies show that beneficial microorganisms in Indonesian mixed-culture starters are antagonistic to many alien strains that try to enter – they dominant or crowd them out.

 In new Western shops using simple technology, the main problems may well be due to improper temperature and humidity control.

4. The tempeh organisms used in Indonesia are "natives" which have adapted over the centuries to local temperatures, predators, and humidity conditions. In North America, where the conditions are completely different, these "transplants" or "imports" cannot be expected to be as hardy or as resistant to local competition. Thus it would not be surprising if it becomes increasingly easy to make fine American tempeh as we begin to use local *Rhizopus* strains, develop hardy mixed cultures, and/or give imported Indonesian strains time to adapt to their new environment. We may even have to adapt the basic tempeh-making process to suit our clite.

Preparing Soybeans for Fermentation

Having looked from various points of view at the microorganisms which activate the tempeh fermentation, let us now study the process step by step, starting at the beginning. Since soy tempeh is by far the most popular variety and since most of the scientific studies on tempeh have been done using samples made with soybeans, the word tempeh in the following sections will be used to refer to soy tempeh, keeping in mind that many of the basic principles would also apply more or less to grain, soy & grain, other legume, or (nonlegume) seed tempehs.

Precooking, Soaking, and Prefermentation: To make tempeh (as described in Chapters 6 and 8) whole dry soybeans are washed, precooked for 15 to 30 minutes (or placed in a vat with boiling-hot water), and allowed to soak (hydrate), in tropical countries, for an average of 22 hours (although Boorsma in 1900 described a soak of 2 to 3 days). Cooking the beans prior to soaking loosens the hulls, making the beans easier to dehull later, and ensures the proper hydration of even the hardest beans. Precooking will kill all the molds and most of the bacteria present on the beans; the shorter the precook, the more bacteria will survive to later activate the prefermentation. The soak in hot water softens the beans, thus reducing the total cooking time and fuel required, stimulates the prefermentation, and removes mold inhibitors and flatus factors (see below). Moreover, soaking in just enough water to cover the beans extracts most of the so-called soy "off-flavors" without leaching out too much of the carbohydrates. During the soak, bacteria that adhere to the soybean hulls and survive the precook cause a rather active prefermentation. The soak water becomes turbid, with a slightly acid flavor, not unlike that of sour milk. A head of foam may rise owing to carbon dioxide formation. Most important, the prefermentation lowers the pH of the beans to within the range of 3.5 to 5.0. After inoculation this low pH inhibits the growth of a great many undesirable bacterial contaminants (which may be acquired during soaking) without hindering the growth of the Rhizopus mycelium. If this low pH is not attained, it becomes necessary to use more starter, which is expensive. In Indonesia, where the soybeans are generally soaked in wooden vats, bacteria that activate the prefermentation live in the cracks of the vats and continually, automatically inoculate successive batches. In nontropical climates where cold weather prevents a natural prefermentation, researchers have found that the pH can be lowered by the addition of lactic acid. However, as described in the previous section, the other benefits of prefermentation do not result. Indonesian tempeh scholars are not yet certain which bacteria are essential to the prefermentation: Bacillus polymyxa, Pediococcus, Streptococcus, and Lactobacillus have each been named as possibilities. Steinkraus et al. (1961, 1965) have done preliminary tests inoculating the soak water with the bacteria Lactobacillus plantarum or L. brevis; after a 15-hour prefermentation at 32° C. (89.6° F.) the pH had dropped to 5.5. Using bacteria isolated from

APPENDIX E

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