87

Production of Tempe, an Indonesian Fermented Food

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INTRODUCTION

Tempe (or tempeh) is a solid fermented soya bean product that is consumed widely in Indonesia. In recent years, there has been considerable interest in the West, especially the USA, in developing tempe as an alternative protein source. Hesseltine was one of the first to make a detailed description of the fermentation in his authoritative review of oriental fermented foods (Hesseltine, 1965). Only one micro-organism, *Rhizopus oligosporus* (Fungi: Zygomycotina) is required for the process and the preparation is extremely rapid, taking only two days, at most, for completion. In this it differs from other soya fermentations, such as miso and shoyu, which involve fungi, yeasts and bacteria in a multi-stage fermentation, which may take months or years to completion. Unlike these fermentations tempe is a solid "cake", which is perishable and is consumed as a meat substitute, rather than as a condiment.

Rapidity and simplicity make tempe preparation ideal for demonstrating the principle of food fermentations, and, with reasonable care, the product can be guaranteed to be free of contaminating moulds and mycotoxins.

EXPERIMENTAL

Technical requirements for the production of tempe are modest, but successful preparation depends on adherence to a number of practical points, which are outlined in the flow diagram and

experimental notes. Ultimate disposal of the product depends on the gastronomic predilections of student and tutor, as well as interpretation of the Health and Safety at Work Act. Tempe is delicious thinly sliced and fried in soya oil until light brown. Garlic and tamarind make useful spices and the tempe can be treated as a meat substitute in the menu.

Day 1 *(1 h)*

Preparation of beans

Weigh out approximately 500 g of soya beans (previously soaked overnight in tap-water and allowed to drain) (*see* note 1).

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Suspend the beans in fresh tap-water and dehull by hand (*see* note 2).

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Discard water, drain the beans and place in a saucepan or glass beaker. Add an excess of water *(see* note 3).

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Bring to the boil and boil for 5 min.

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Immediately drain the beans **thoroughly** through a sieve (into a sink) and spread them over a clean tray (surface sterilized with alcohol).

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Allow to cool, stirring continuously with a spatula or spoon until a temperature of 35–40 °C is reached *(see* note 4).

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Add inoculum and mix the whole mass thoroughly by hand. Do not allow to cool excessively (*see* note 5).

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Place the inoculated beans in fermentation containers, either plastic bags or Petri dishes *(see* note 6).

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Incubate in the dark between 25 and 38 °C *(see* note 7).

Preparation of inoculum

Sporulating culture of *R. oligosporus* on 3 % malt agar (*see* note 11).

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Add 10.0 ml sterile distilled water + "Tween 80" (*see* note 12).

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Dislodge spores with a sterile paintbrush or strong loop.

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Add to a sterile centrifuge tube, centrifuge for 5 min, discard supernatant.

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Resuspend spores in 0.5 ml sterile distilled water.

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Add to small quantity of beans in a clean beaker (alcohol sterilized) and mix thoroughly. Use as inoculum for main bean mass.

Day 2 *(15 min)*

Examine tempe and assess the state of fermentation after 24 h in incubator *(see* note 8). If in plastic bags, reverse the incubation position. Check the temperature of the incubator. If necessary, remove the tempe to a cooler incubator *(see* note 9).

Day 3 *(15 min)*

The tempe should by now be ready and can be stored if necessary at 10 ˚C for up to 1 week or can be deep frozen immediately. The product should be a firm white cake *(see* note 10).

Notes and Points to Watch

1. Soaking is necessary to remove inhibitors from the soya beans and to loosen the hulls. There should be at least 5 cm depth of soak-water over the beans. If necessary, larger quantities of beans can be soaked together for class use.
2. Dehulling is a laborious process. It can be done simply by squeezing beans between thumb and forefinger. Loose hulls can be floated off with a jet of water. In fact, 100% dehulling is unnecessary but a fairly high percentage is required for a successful fermentation, as the presence of the hulls inhibits penetration of the beans by the *Rhizopus* mycelium. A little ingenuity will soon provide more efficient ways of dehulling.
3. There should be about 5 cm depth of water over the beans.
4. This is an important stage. The beans should be vigorously stirred to promote evaporation of excess water. If possible a perforated tray should be used to promote draining of water. An excess of water will promote bacterial growth (especially *Bacillus subtilis)* and inhibit the mould, resulting in bad tempe. The surface of the beans should be almost dry without a very obvious film of water. If necessary, use a fan to promote drying. Do not inoculate until the temperature falls below 40 ˚C.
5. Below 35 ˚C, the lower the temperature of the bean mass when placed in the fermentation containers, the slower will be the fermentation, because of the insertion of a "lag phase" required for temperature build-up. In general, fermentation is most successful if the operations are carried out in a warm room.
6. The choice of fermentation container is important. In Indonesia the traditional method requires wrapping the beans in banana leaves, but fortunately plastic bags are even better.

   Place the soya beans in a new (and therefore partially sterile) plastic bag and flatten the contents out to a "cake" about 2.0 cm thick; 250 g wet weight of beans will give a cake about 24 x 12 cm. The area of the cake is not important, but the thickness should always be about 2.0 cm. Plastic bags can either be heat-sealed (with a hot needle) or the opening can be folded over tightly up to the edge of the cake, moulded straight with a ruler, and secured with metal or plastic clips. The plastic bag should then be pierced at 1–2 cm intervals all over its surface. Such holes should not be larger than the needle and a hot, but **not** red hot, needle gives excellent results. Plastic bags

can then be laid in the incubator but should never be placed on top of each other as this restricts aeration. They should be turned after the first 16–20 h of incubation.

Petri dishes provide an alternative container but produce tempe of slightly poorer quality. The new square type plastic dish is the best and used dishes, that have been thoroughly washed and sterilized with alcohol, are perfectly good. The beans should be packed down into the dishes by leaving them about "half a bean" proud of the edge and then forcing the lid down. Square dish lids can be secured with a rubber band, round dishes by a strip of *Sellotape.* Aeration by holes is, in this case, unnecessary, though the square dishes may benefit from a small hole in the centre top and bottom.

7. The speed of fermentation is determined by the incubation temperature. Incubation temperatures above 40 °C and below 25 °C will not produce good tempe. A temperature of 37-38 °C will produce tempe within 22 h; a temperature of 28–30 °C will take up to 48 h to produce tempe.
8. Within 16–20 h there should be a covering of mycelium over the beans but the beans themselves will still be visible. Wet patches without mycelium mean that bacterial contamination has occurred and the tempe should be discarded.
9. After about 12–16 h the fermentation begins to generate heat. If a small closed incubator is used with large quantities of tempe, temperatures within the tempe cake may reach 50 °C and the growth of the *Rhizopus* is checked. If the tempe is excessively hot at the 16–24 h stage, it can be transferred to a cooler incubator or the door of the incubator can be left ajar. Ventilation appears to be important, in any case, for successful fermentation.
10. The final stage of the fermentation is reached when the soya beans are completely covered in a dense mass of white mycelium. A section through the cake reveals that the spaces between the beans are completely filled with mycelium. The cake itself should be firm and should only bend a little when held by one corner. Soft cake which tends to break up and has the interstices only partly filled by mycelium, indicates either bacterial contamination or too high a temperature in the late stages of fermentation. The tempe should have a pleasant, slightly sweet smell and nutty flavour; off odours and excessively sweet taste indicate bacterial contamination; ammonia production indicates that the fermentation has gone too far. Other problems which may occur include a black colouration around the sites of the aeration holes; this is due to sporulation by the fungus, but does not affect the quality. Heavy sporulation will occur at temperatures below 26 °C. Bright yellow patches may appear; these are due to β-carotene biosynthesis by *R. oligosporus,* and indicate that light is reaching the fermentation.
11. *R. oligosporus* should be inoculated onto 3% malt slopes at least 7 days before the spores are required and incubated at 30 °C. Slope cultures more than 1 month old should not be used.
12. Add 1 drop of "Tween 80" to l00 ml of sterile distilled water.

MATERIALS

1. *Rhizopus oligosporus* culture. Several strains are available in this department. Alternatively, cultures are available from the Northern Regional Research Laboratory, A.R.S., Peoria, IL 61604, USA (culture no. NRRL 2710).
2. 3% malt medium (malt extract 30.0 g, agar 15 g, distilled water 1 litre).
3. Soya beans *(Glycine max).*
4. Saucepan or glass beaker for boiling beans.
5. Bucket or large plastic beaker for soaking beans.
6. Plastic bags, e.g. 18.0 x 23.0 cm.

7. Kitchen sieve.
8. Large plastic spoon or spatula.
9. Plastic trays.
10. Artist's paintbrush (sterilize by autoclaving in grease-proof paper).
11. Gas ring to boil beans.
12. Clips or rubber bands.

SPECIFIC REQUIREMENTS

Day 1
7 day old *R. oligosporus* culture
10 ml pipettes (sterile)
250 ml sterile distilled water + "Tween 80"
autoclaved artist's paintbrush
sterile centrifuge tube
low speed centrifuge
2 clean beakers (500 ml)
balance
soaked soya beans (overnight in plastic bucket)
saucepan or glass beaker (2 litres)
gas ring
kitchen sieve
large plastic spoon or spatula
tray
plastic bags
clips
[rubber bands
Petri dishes]
incubation facilities (25–38 °C)

Days 2 and 3
refrigerator or deep freeze

FURTHER INFORMATION

In Indonesia, tempe is either sold fresh, to be cooked by slicing and frying, or as processed biscuits or crisps. It forms an important part of the diet of many poor people and indeed may supply much of their protein.

There is some controversy in the literature over the food value of tempe, as against unprocessed soya beans, but there seems to be some evidence that the PER (protein efficiency ratio) is increased and content of the B vitamins rises (growth of *Klebsiella pneumoniae* in Indonesian tempe may be responsible for this increase). In addition, the trypsin inhibitor and phytic acid present in soya bean, which reduce the nutritional value by, respectively, inhibiting gut enzymes and chelating important metal ions in the intestine, are destroyed by the fungus. *R. oligosporus* also appears to produce an antioxidant which prevents the dried product becoming rancid. These and other aspects are well reviewed by Djien and Hesseltine (1979).

This method of tempe production can be used as a basis for further experimental work. We have used the bacterium *Lactobacillus casei* var. *rhamnosus* NCIB No. 6375 to bio-assay the accumulation of riboflavin during the fermentation (Roelofsen and Talens, 1964). The effect of temperature, light level and aeration upon the fermentation also provides useful variations.

R. oligosporus can be used to ferment other substrates. Wang and Hesseltine (1966) describe wheat tempe, and it is also possible to ferment a variety of pulses other than soya to produce tempe-like products (e.g. peanuts, etc.).

A frequent question which arises is the potential problem of mycotoxin production. *R. oligosporus* is not known to produce any mycotoxin. However, fermentation at too low a temperature (25 °C) could allow growth of *Aspergillus flavus* and other mycotoxin producers to

take place. In practice, even in Indonesian cottage industries, soya bean tempe is rarely reported to contain any mycotoxin.

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