

IFT Experiments in Food Science Series

Microbiology in Food Systems

Activity #3

Tempeh, Soy Sauce, and Other Mold-Fermented Food Products

A Science Unit for Secondary School Curriculum



Institute of Food Technologists
The Society for Food Science and Technology

TEACHER ACTIVITY GUIDE

Tempeh, Soy Sauce, and Other Mold-Fermented Food Products

INTRODUCTION

Molds are aerobic, salt-tolerant, filamentous organisms belonging to a larger group of microorganisms known as *fungi*. Mushrooms and yeasts are other examples of fungi. Fungi differ from bacteria by their more complex structure and size. They may be multicellular or unicellular and are found everywhere in nature.

Molds contain filaments or tubes called *hyphae* which grow and elongate to form intertwined masses known as *mycelia*. Molds reproduce sexually, asexually, or by both methods through the use of spores (Visual Master 5). Molds are often associated with spoilage of foods. Although molds are not considered to be pathogenic (disease causing), some produce mycotoxins. These toxic materials can pose a potential health hazard to humans.

Fermentation was among the first methods used by man to preserve foods. While many fermentation processes commonly involve bacteria and yeasts, molds also play an important role in the fermentation of plant, dairy, and meat products (Visual Master 6). Mold fermentation of these products often extends their shelf life and improves their color, flavor, odor, texture, and nutritional value.

• **Plant Products.** Fermented grains and starch roots are an important part of the diets of people in the Far East, Africa, and Latin

America. Molds used in the fermentation hydrolyze oilseeds, grains, and roots and make these materials more digestible and more palatable by destroying undesirable enzymes, flavors, and odors and by developing desirable flavors, odors, and colors. In parts of the world where protein in the form of meat and dairy products is not available, fermented foods are used as flavoring agents for otherwise bland vegetable diets.

Miso paste is a fermented product made from rice and soybeans. It is used as a breakfast food, soup base, and seasoning in Japan and China. *Ogi* is made from corn and is a staple eaten with vegetables in Nigeria and West Africa. *Poi* is a semi-solid, fermented taro product which is eaten as a side dish with fish or meat in Hawaii. Soy sauce, also known as *shoyu* or *sho*, has been brewed in Japan with *Aspergillus* mold for more than 1,000 years. Fermented soy sauce is used in Japan, China, Philippines, and other parts of the Orient as a seasoning for meat, fish, cereals, and vegetables. *Tempeh* is prepared by cooking dehulled soybeans, inoculating them with *Rhizopus* mold, and incubating in banana leaves. The solid tempeh can be fried in oil, roasted, or used as a meat substitute in soup.

• **Dairy Products.** Blue (Bleu), Roquefort, Gorgonzola, and Stilton are important mold-ripened, blue-veined cheeses. The mold *Penicillium roqueforti*, present either naturally or through introduction of a spore powder, is allowed to grow throughout the cheese. The

growth of the mold provides these cheeses with their unique flavors and textures. In contrast, Brie and Camembert are just surface ripened with the white mold *Penicillium camemberti*. Port Salut is another surface-ripened cheese similar to Limburger or brick. The presence of *Geotrichum* mold on its surface gives Port Salut cheese its distinct flavor.

• **Meat Products.** The heavy growth of molds on the surface of European-type sausages such as Italian salami, as well as country-cured hams, helps in their preservation by inhibiting the activities of food-poisoning and food-spoilage bacteria and may also enhance their flavor development.

ACTIVITY OBJECTIVE

This experiment will illustrate to the student that:

1. Mold fermentations are important in food production.
2. Production of tempeh by fermentation with *Rhizopus oligosporus* changes the odor, texture, and flavor of soybeans.
3. Soy sauce produced by microbial fermentation has a distinctly different flavor and aroma than soy sauce produced by acid hydrolysis.

ACTIVITY LENGTH

Approximately 1 hr for preparation of tempeh plus time for the flavor evaluations.

SCIENTIFIC PRINCIPLES

Tempeh is a food consumed in Indonesia, New Guinea, and Surinam. It is made by inoculating

cooked soybeans with mold spores from *R. oligosporus*. The inoculated beans are packed into banana leaves or other suitable container and incubated at about 30°C for 24 hr. The fungus grows rapidly, with the white mold mycelia covering and penetrating the soybeans to form a compact mass. The action of the fungal enzymes destroys the unpleasant beany flavor of the soybeans, making them more palatable. In addition, the physical characteristics of the beans are altered to make slicing and frying possible, so that the cooked product can be used as a meat substitute.

Soy sauce is made from soybeans and wheat and is used as a flavoring agent on meats, fish, vegetables, and soups. In Asia, soy sauce is made using a complex fermentation process. The molds *Aspergillus oryzae* and *Aspergillus soyae* are primarily used in the fermentation, though yeasts and lactic acid bacteria are also thought to be involved. During fermentation, fungal enzymes hydrolyze the soybeans and wheat to produce a dark-brown liquid high in soluble proteins, peptides, and amino acids. It is also high in salt (18%) and contains an appreciable amount of glutamic acid, which has flavor-enhancing qualities. In the United States, most soy sauce is produced by acid hydrolysis of the soybeans and wheat. Although this lower-cost process is thought to yield a product with more uniform quality, the chemically produced soy sauce is considered by Asians to be inferior in flavor to the fermented type.

VOCABULARY

Fungi—a large group of microorganisms which includes molds, yeasts, and mushrooms.

Inoculum—specific microorganism(s) added to a product for fermentation.

Mycelia (or hyphae)—intertwined filaments

which make up the structural mass of molds.

Soy sauce—a flavorful sauce made from soybeans and wheat either by fermentation with *Aspergillus oryzae* or *Aspergillus soyae* or by acid hydrolysis.

Spore—a dormant or resting state of a bacterial cell, or a reproductive "seed" cell of a mold.

Tempeh—a food product prepared by fermenting cooked soybeans with the mold *Rhizopus oligosporus*.

Enzyme—Any of various proteins in plant and animal cells that act as catalysts in initiating or speeding up chemical reactions.

Hydrolysis—Splitting of a compound into smaller parts by the addition of water.

MATERIALS REQUIRED

Pre-soaked soybeans

Sauce pan and hot plate

Paper towels

Slant culture of *R. oligosporus* NRRL 2710

Preboiled water (3 mL)

Bunsen burner

Inoculating loop

Sterile beaker and pipet

Plastic gloves

Sterile Petri dishes for packing and forming the tempeh cake

Knife for slicing tempeh

Electric frying pan with vegetable oil

Soy sauce produced by fermentation (Kikkoman)

Soy sauce produced by chemical hydrolysis (La Choy)

Paper cups for tasting

STUDENT

EXPERIMENTAL PROCEDURE

1. Remove the seed coats by hand and

discard them. Place the dehulled beans in a pan, cover with tap water, bring to a boil, and boil for 30 min.

2. Drain the cooked beans and spread them on layered paper towels to absorb moisture. Allow the beans to drain and cool for 15 min. While the beans are draining, prepare the inoculum by aseptically adding 3 mL of sterile or boiled water to the *R. oligosporus* slant culture. With a flamed inoculating loop, gently mix the mold growth with the water.

3. Place some of the cooled beans into a clean container and refrigerate for later evaluation. Place the rest of the beans into a sterile beaker. Aseptically transfer the *R. oligosporus* suspension from the tube to the beans in the beaker. With a sterile pipet or glass rod, stir the inoculated beans to thoroughly distribute the inoculum.

4. Wearing sterile plastic gloves, pack the inoculated beans into sterile Petri dishes. The beans should press firmly against the cover. Each Petri dish will hold about 40 g of the original beans.

5. Incubate the Petri dishes at 30–31°C for approximately 24 hr. The fermentation is completed when a compact white cake is formed. The finished tempeh can be refrigerated or frozen for later evaluation.

6. Compare the odor and texture of the finished tempeh with that of the unfermented beans from step 3. If a proper fermentation has taken place, the tempeh will have a pleasant, fresh, mushroom-like odor. If sour, musty or other spoiled notes are present, the product is contaminated and **should not be tasted**. If the product appears to be

contaminated, skip the flavor evaluation in step 7 and proceed directly to step 8.

7. Cut the tempeh cake into thin slices (0.5 cm thick) and fry them in vegetable oil until browned. Taste immediately. Observe texture and flavor and record in the table provided.
8. Pour a small amount of the fermented soy sauce (Kikkoman) and acid-hydrolyzed soy sauce (LaChoy) into paper cups. Taste each type and note the flavor differences. Record in the table provided.

TEACHING TIPS

- Soybeans and commercial tempeh can be obtained at a health food store or some supermarkets. The commercial tempeh can be compared with the classroom-produced tempeh, or substituted for it if preparation facilities or equipment are unavailable.
- Presoak 200 g of soybeans in 600 mL of water for 2 hr.
- The *R. oligosporus* NRRL 2710 culture can be obtained free of charge by writing on school letterhead to Dr. Kerry O'Donnell, curator of the mold collection, National Center for Agricultural Utilization Research, 1815 N. University St., Peoria, IL 61604 (E-mail address: kodonnell@sunca.ncaur.usda.gov).
- If sterile plastic gloves are not available, have students wash their hands thoroughly before preparation of the tempeh.
- Small, clean, loosely covered jars or dishes or perforated plastic bags can be substituted for the Petri dishes. All

equipment and containers should be washed thoroughly with detergent and hot water.

- If an autoclave is not available for sterilizing, glassware can be covered or wrapped with aluminum foil and heated in an oven for 3 hr at 140°C, 2.5 hr at 150°C, 2.0 hr at 160°C, or 1 hr at 170–180°C. Cool thoroughly.
- To avoid airborne contamination of the tempeh, keep all equipment closed or covered when not in use.
- Videos entitled *Kikkoman, The Magic Ingredient*, *Soy Sauce from Start to Finish* and *Kikkoman, The Magnificent Ingredient* may be obtained free (except for a small handling and shipping charge) from Kikkoman's public relations firm, Ketchum Food Marketing, by contacting Beth Shaffner (phone 415-984-2207, fax 415-984-2293).

QUESTIONS & ANSWERS

1. How do the texture, odor, and flavor of tempeh differ from those of the original soybeans? What caused these changes?
Ans. The soybeans have firm texture and a beany, grass-like odor. The tempeh has a firm, matted texture that can be sliced and fried. It has a pleasant, fresh, mushroom-like odor and bland flavor. Fried tempeh is similar in flavor to fried potatoes. The changes are due to the growth and activity of the R. oligosporus mold during fermentation.
2. How do the flavor and odor of soy sauce produced for fermentation compare to those of the soy sauce prepared by chemical means? Which type of soy sauce do you prefer?

Ans. The fermented soy sauce has a more-rounded, less-harsh flavor and is often perceived to be less salty than the chemically produced sauce.

Ans. The soy sauce can become contaminated by using dirty equipment or dirty hands, leaving the equipment or the tempeh open to the air, adding insufficient mold culture or culture that is not viable, and/or incubating at too high a temperature.

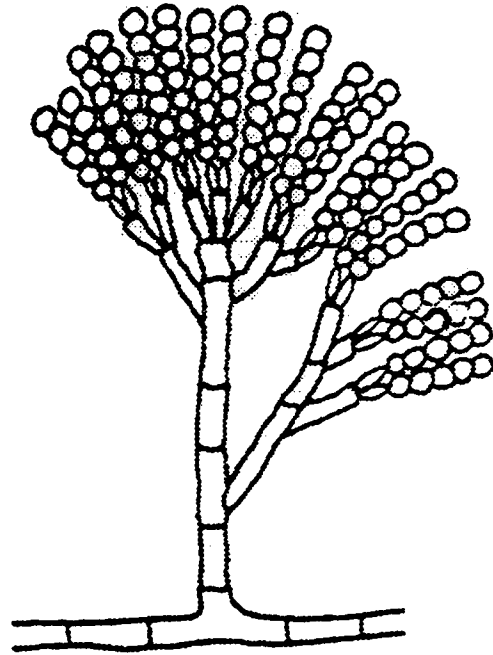
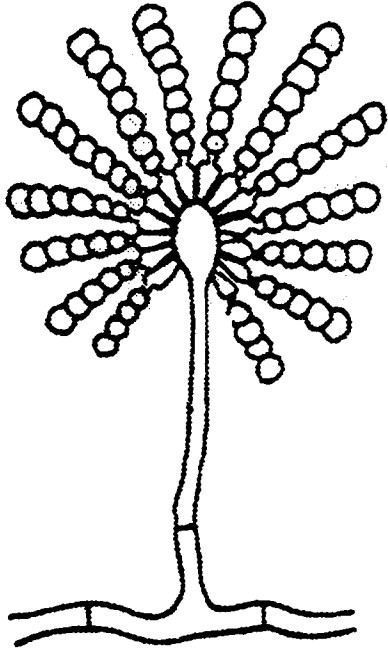
3. What can cause the tempeh to become contaminated or spoiled?

DATA TABLE—Texture and Flavor of Tempeh and Soy Sauces

Product	Texture	Flavor
Tempeh		
Fermented soy sauce (Kikkoman)		
Acid-hydrolyzed soy sauce (La Choy)		

Visual Master 5

Mold Fruiting Body



Visual Master 6

Some Food Products Which Involve Mold Fermentation

Raw material	Microorganisms involved	Foods produced	Where produced
Milk	<i>Penicillium roqueforti</i> , lactic acid bacteria (LAB)	Blue (bleu) cheese	France, U.S.
	<i>Penicillium camemberti</i> , LAB	Brie, Camembert cheese	France
	<i>P. Roqueforti</i> , LAB	Gorgonzola cheese	Italy
	<i>Geotrichum</i> spp., LAB, yeasts	Port Salut cheese	France
	<i>P. Roqueforti</i> , LAB	Stilton cheese	England
Beef, pork	<i>Scopulariopsis</i> , <i>Aspergillus</i> , <i>Penicillium</i> spp., LAB	Aged dry salami	Europe
Pork hams	<i>Aspergillus</i> , <i>Penicillium</i> spp	Country-cured hams	Southern U.S.
Rice, soybeans	<i>Aspergillus oryzae</i> , LAB, yeasts	Miso	Japan, China
Corn	<i>Aspergillus</i> , <i>Penicillium</i> , LAB	Ogi	Nigeria, West Africa
Taro corms	<i>Geotrichum candidum</i> , LAB, yeasts	Poi	U.S. (Hawaii)
Soybeans, wheat	<i>A. oryzae</i> or <i>A. soyae</i> , LAB, yeasts	Soy sauce	Japan, China
Soybeans	<i>Rhizopus oligosporus</i>	Tempeh	Indonesia