Fermented Foods and Beverages Series

Indigenous Fermented Foods of Southeast Asia

Edited by

J. David Owens

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Indigenous Fermented Foods of Southeast Asia

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Series Preface

Natural fermentation precedes human history, and since ancient times humans have been controlling the fermentation process. Fermentation, the anaerobic way of life, has attained a wider meaning in biotransformations resulting in a wide variety of fermented foods and beverages.

Fermented products made with uncontrolled natural fermentations or with defined starter cultures, achieve their characteristic flavour, taste, consistency, and nutritional properties through the combined effects of microbial assimilation and metabolite production, as well as from enzyme activities derived from food ingredients.

Fermented foods and beverages span a wide diverse range of starchy root crops, cereals, pulses, vegetables, nuts and fruits, as well as animal products such as meat, fish, seafood, and dairy.

The science of chemical, microbiological, and technological factors and changes associated with manufacture, quality, and safety are progressing and are aimed at achieving higher levels of control of quality, safety, and profitability of food manufacture.

Both producers and consumers benefit from scientific, technological, and consumer-oriented research. Small-scale production needs to be better controlled and safeguarded. Traditional products need to be characterised and described to establish, maintain, and protect their authenticity. Medium- and large-scale food fermentation requires selected, tailor-made, or improved processes that provide sustainable solutions for the future conservation of energy and water, and responsible utilisation of resources and disposal of by-products in the environment.

The scope of the CRC book series *Fermented Foods and Beverages* includes (i) Globally known foods and beverages of plant and animal origin (such as dairy, meat, fish, vegetables, cereals, root crops, soybeans, legumes, pickles, cocoa and coffee, wines, beers, spirits, starter cultures, and probiotic cultures); their manufacture, chemical and microbiological composition, processing, compositional and functional modifications taking place as a result of microbial and enzymic effects; their safety, legislation, development of novel products, and opportunities for industrialisation. (ii) Indigenous commodities from Africa, Asia (South, East, and South-East), Europe, Latin America, and Middle East; their traditional and industrialised processes and their contribution to livelihood. (iii) Several aspects of general interest such as valorisation of food-processing by-products, biotechnology, engineering of solid-state processes, modern chemical and biological analytical approaches (genomics, transcriptomics, metabolomics, and other -omics), safety, health, and consumer perception.

The fifth book, born in the series, is titled *Indigenous Fermented Foods of Southeast Asia*. This treatise, edited by Dr. J. David Owens, deals with the indigenous fermented foods of Thailand, Vietnam, Indonesia, Malaysia, and the Philippines. The region is known for its large diversity of fermented foods and the editor has made a great effort to represent this diversity at the microbiological and ingredient levels. Thanks to his network of scientists from the Southeast Asian countries he was able to bring together much of the current knowledge and state of the art. We are convinced that this compendium will serve as an essential reference for all those scientists as well as consumers who are looking for the latest knowledge and inspiration.

Acknowledgements

I would like to express my appreciation to all the authors for the work they have put into their contributions and to Ellen Owens for doing a final proofreading and for making many helpful suggestions. I also thank the Department of Food and Nutritional Sciences, University of Reading for providing me with office space and access to computing and library facilities.

Introduction

J. David Owens

Food fermentations are noted for the creation of a multiplicity of aromas, flavours and textures from a single starting material and Southeast Asian fermented foods are no exception in creating a diversity of products from soya beans, rice, cassava as well as from various waste products of the tofu, peanut oil, tapioca and coconut industries (Table I.1). In addition, Asia, including Southeast Asia, is noted for its much wider utilisation of fungi in food fermentations than is the case in Western countries.

Despite the long history of food fermentations in Southeast Asia, they have received relatively little attention from the indigenous scientific establishment and, for many products, little has been published over the past 30 years. Even where research has occurred, in some countries, there seems to be a predilection to report findings at conferences and in reports that are not widely disseminated rather than as publications in peer-reviewed international scientific journals. This hampers research progress and does not provide encouragement to others to undertake research in the area. Consequently, many of the foods remain as artisanal products produced by small-scale backyard producers, in contrast to the situation in Japan or, increasingly, in China. While, in some cases, it is deliberate government policy to support small producers because of the employment they provide, this does not preclude upgrading the technology to produce a safer and more consistent product, which would also help to ensure the continued existence of the products and their producers. As Kiuchi and Watanabe (2004) noted, ‘Modern technology has transformed natto (*Bacillus*-fermented soya beans) from a locally distributed seasonal product of uncertain quality and safety to a consistent, nationally distributed, high-quality product that can be safely enjoyed year-round’. There are no reasons why the production of many Southeast Asian indigenous fermented foods should not be put on to a similarly sound footing.

It is unfortunate that little research has been carried out on indigenous Southeast Asian fermented foods, as many of the fermentations offer fascinating ecosystems that are ripe for investigation by newer chemical and molecular biological techniques. Additionally, researchers with direct access to indigenous producers have opportunities not easily available to researchers in other parts of the world. Also, not to be overlooked is the possible contribution to the success of local manufacturers and national economies. Seen in this light, indigenous food fermentations may offer more opportunities than following the latest fashion, be it prebiotics, probiotics or using molecular biological techniques but without addressing significant questions.

Partly because little work has been done on many of the indigenous fermented foods since the 1970s and, in particular, since the 1977 Bangkok conference (Steinkraus 1983, 1996) and partly because there are relatively few researchers active in fermented food research in the region and who are available to write reviews, this book does not offer as comprehensive a cover of products as provided by Steinkraus’ books. Nevertheless, it includes chapters dealing with examples of all the major categories of fermented foods (Table I.1). The production, microbiology, biochemistry, nutritional value and dietary roles of a wide variety of indigenous fermented foods of Southeast Asia are described. The emphasis is on the microbiological and biochemical processes in the fermentations and on the factors that influence the development of the characteristic microfloras and chemical changes induced. The classification of products is based on their microbial ecology (i.e. the predominant microbes involved; Table I.1). The rationale for this being that traditional fermentations represent solutions to the problem of how to obtain a desired microbial flora and product outcome under non-aseptic conditions and in the presence of an initially highly diverse microflora. Understanding how this is achieved is essential in establishing reliable and safe processes.

One of the aims of a review is to detect deficiencies in knowledge or understanding in order to aid researchers in identifying areas for future research. The contributions here have all attempted to address a series of basic questions, including the following: (i) What are the dominant/desired microbes and what factors in the processing and environment select for them? (ii) What other microbes are commonly present? (iii) What compounds are utilised as major carbon and energy sources and, in particular, what are the sources of fermentable carbohydrates? Since rice is often an ingredient, it has frequently been assumed that rice serves as the main source of fermentable carbohydrates but Paludan-Müller et al. (1999) showed that in Thai low-salt fish fermentations garlic fructans are a major source of fermentable carbohydrate. (iv) What are the main biochemical activities and chemical changes in the fermentation? (v) What is the true yield of product per kg of initial raw materials? Yield has a large impact on the economics of a process and it is important to understand what causes losses of materials and how these may be minimised. (vi) What possible hazards may be associated with a product and how may they be minimised or eliminated?

In the cases of many indigenous Southeast Asian fermented foods, even these quite basic questions have not been answered unambiguously and there are, therefore, many opportunities to undertake good research in answering them. The aim in every case should be to have a sufficiently good understanding of a process to be able to ensure the production of a consistently high-quality and safe product. If this is not done, the danger is that many of these traditional products will be lost and/or displaced by imported variants. If this book plays any role in encouraging such research activities and in promoting the development of indigenous Southeast Asian fermented foods, then I will be happy that it has served its purpose.

Table I.1 Some Indigenous Fermented Foods Produced in Southeast Asia

CATEGORY AND NAME OF PRODUCT	MAIN METABOLIC ACTIVITIES	SUBSTRATE	APPEARANCE AND MODE OF CONSUMPTION	SE ASIAN COUNTRIES WHERE MADE	REFERENCE
1. AEROBIC MOULD FERMENTATIONS					
Tempe	Mould growth (*Rhizopus* spp.)	Cooked, dehulled soya beans	Cotyledons bound into a solid cake Fried; used in soups, curries	Indonesia	Chapter 1
Oncom merah (red oncom)	Mould growth (*Neurospora intermedia*)	Waste materials from production of soya bean curd, tapioca	As tempe but pink	Indonesia	Chapter 1
Oncom hitam (black oncom)	Mould growth (*Rhizopus* spp.)	Wastes from production of peanut oil, tapioca, coconut milk	As tempe but grey-black	Indonesia	Chapter 1
Dage	Mould growth (*Mucor* spp.)	Wastes from production of peanut oil, coconut milk, cassava starch	As tempe but grey	Indonesia	Chapter 1
Koji	Mould growth (*Aspergillus oryzae*); production of amylase and protoases	Whole soya beans or defatted soya bean meal + wheat grains	Beans/grains covered with fungal mycelium. Used to make soya sauce and other products	All	Fukishima (2004)
Red rice (ang kak)	Mould growth (*Monascus purpureus*); production of red pigment	Rice	Red rice grains. Food colouring agent	?	Steinkraus (1996)
Mould-ripened soya bean curd (sufu)	Surface growth of mould(s); protein $\rightarrow$ peptides, amino acids	Soya bean curd, salt, rice wine, sugar, spices	Cubes covered with fungal mycelium in brine–ethanol–wine–sugar–spices solution. Relish	Thailand	Han et al. (2001)

2. STARTER CULTURES					
Ragi tape	Growth of moulds and yeasts	Rice flour, spices, coconut water or sugar cane juice	Dried balls or discs containing moulds and yeasts. Starter for tape, rice wines and so on	Indonesia, other countries	Chapter 2
Loog paeng	Growth of moulds and yeasts	Rice flour + herbs	Dried balls or discs containing moulds and yeasts. Starter for tape, rice wines and so on	Thailand	Chapter 2
Ragi tempe (laru)	Mould growth	Cassava solid waste or rice flour	Dry white-grey powder. Starter for tempe	Indonesia	Chapter 2
Ragi tempe (usar)	Mould growth	Cooked soya beans inter-leaved with hibiscus leaves	Dried leaves. Starter for tempe	Indonesia	Chapter 2
3. SWEET, SOUR, ALCOHOLIC SOLID-SUBSTRATE FUNGAL FERMENTATIONS					
Tape ketan (Indonesian)	Starch → sugars, ethanol, lactic acid	Cooked glutinous rice	Sweet, slightly sour, slightly alcoholic cooked rice dessert	All	Chapter 3
Tape singkong (tape ketela)	Starch → sugars, ethanol, lactic acid	Peeled, steamed cassava roots	Sweet, slightly sour, slightly alcoholic, cassava roots with whitish fungal growth. Dessert	Indonesia	Chapter 3
Brem	Starch → sugars	Tape ketan liquid	Sweet, solid rectangular or circular cakes	Indonesia	Steinkraus (1996)
4. ALCOHOLIC DRINKS					
Rice wine	Starch → sugars → ethanol	Rice	Alcoholic drink, usually distilled or fortified	Vietnam	Chapter 4
Satoh	Starch → sugars → ethanol	Rice	Non-distilled alcoholic drink	Thailand	Chapter 4
Cui	Starch → sugars → ethanol	Molasses	Distilled alcoholic drink	Indonesia	Chapter 4

continued

Table I.1 (continued) Some Indigenous Fermented Foods Produced in Southeast Asia

CATEGORY AND NAME OF PRODUCT	MAIN METABOLIC ACTIVITIES	SUBSTRATE	APPEARANCE AND MODE OF CONSUMPTION	SE ASIAN COUNTRIES WHERE MADE	REFERENCE
Brem bali	Starch → sugars → ethanol	Tape ketan	Sweet, alcoholic beverage	Indonesia	
Various	Starch → sugars → ethanol		Alcoholic drinks	All	
5. LACTIC ACID BACTERIAL VEGETABLE AND FRUIT FERMENTATIONS					
Various	Sucrose/glucose/fructose → lactate, acetate, ethanol, CO_2	Various vegetables	Fermented vegetables	All	Steinkraus (1996)
Budu pakis	Sucrose/glucose/fructose → lactate, acetate, ethanol, CO_2	Fern fronds, salt	Sour, slightly salty fern fronds, consumed as a side dish	Brunei	Chapter 5
Growol	Sucrose/glucose/fructose → lactate, acetate, ethanol, CO_2	Cassava root	Sour dough. Alternative to rice	Indonesia	Chapter 5
Tempoyak	Sucrose/glucose/fructose → lactate, acetate, ethanol, CO_2	Durian flesh	Fried with spices. Condiment or appetiser	Indonesia, Malaysia	Chapter 5
6. LACTIC ACID BACTERIAL CEREAL FERMENTATIONS					
Kanomjeen	Starch → sugars → lactate, acetate, ethanol	Rice	Fermented rice noodles eaten with soups and curries	Thailand	Chapter 6
7. LACTIC ACID BACTERIAL FISH AND SEA FOOD FERMENTATIONS					
Burong isda	Starch → sugars → lactate, acetate, ethanol	Fish, cooked glutinous rice, salt	Cooked and consumed with rice and vegetables	Philippines	Chapter 7
Tinapayan	Starch → sugars → lactate, acetate, ethanol	Dried, salted mudfish fillets, fermented rice, spices	Dry, fried flakes, consumed as a meat substitute	Philippines	Chapter 7

Balao balao	Starch → sugars → lactate, acetate, ethanol	Fresh water shrimps, cooked rice, salt	Sautéed in oil and spices and served as a condiment	Philippines	Chapter 7
Pla-som	Garlic fructans → fructose; starch → sugars → lactate, acetate, ethanol, CO_2	Minced fish, ground boiled rice, garlic, salt	Consumed raw or cooked on its own or as part of a main meal	Thailand	Paludan-Müller et al. (1999)
8. LACTIC ACID BACTERIAL MEAT FERMENTATIONS					
Nham	Starch/garlic oligosaccharides → sugars → lactic acid	Pork, garlic, cooked rice, salt, spices, nitrite	Pork sausage, eaten raw or cooked on its own or as part of a main meal	Thailand	Chapter 7
Belutak	Sugar → lactic acid	Cow or buffalo small intestines, meat, salt, sugar	Slightly acidic, salty and chewy fermented sausage. Fried with chillies and onions, with rice	Brunei	Chapter 7
9. LACTIC ACID BACTERIAL DAIRY FERMENTATIONS					
Dadih	Lactose → lactate, ethanol, CO_2	Unheated buffalo milk	Yoghurt-like	Indonesia	Akuzawa et al. (2011)
Tairu	Lactose → lactate	Heated cow milk	Yoghurt-like drink, and used in cooking	Malaysia	Steinkraus (1996)
10. MIXED LACTIC ACID BACTERIAL AND YEAST FERMENTATIONS					
Soya sauce	Proteins → amino acids; sugars → lactate, acetate, ethanol, CO_2	Koji (soya bean ± wheat)	Pale brown liquid with umami and salty taste. Flavouring agent	All	Fukishima (2004)
Kecap	Proteins → amino acids; sugars → lactate, acetate, ethanol, CO_2	Koji, palm sugar	Dark brown, sweet liquid. Flavouring agent	Indonesia	Chapter 9
Tauco	Proteins → amino acids; sugars → lactate, acetate, ethanol, CO_2	Cooked, dehulled soya beans, palm sugar, salt	Brown paste used in soups and side dishes	Indonesia	Steinkraus (1996)

continued

Table I.1 (continued) Some Indigenous Fermented Foods Produced in Southeast Asia

CATEGORY AND NAME OF PRODUCT	MAIN METABOLIC ACTIVITIES	SUBSTRATE	APPEARANCE AND MODE OF CONSUMPTION	SE ASIAN COUNTRIES WHERE MADE	REFERENCE
11. *BACILLUS* FERMENTATIONS					
Thua nua	Protein → amino acids → cells, NH_4, CO_2	Soya bean	Brown paste or dried disks; flavouring agent	Thailand	Chapter 8
Cabuk	Protein → amino acids → cells, NH_4, CO_2	Sesame seed press cake	Black-brown, slightly sticky balls. Flavouring agent or snack	Indonesia	Chapter 8
Semayu	Protein → amino acids → cells, NH_4, CO_2	Coconut residue after extraction of milk	Black-brown, slightly sticky balls. Flavouring agent or snack	Indonesia	Steinkraus (1996)
12. ACETIC ACID BACTERIAL FERMENTATIONS					
Vinegar	Ethanol → acetic acid	Rice wine	Sour liquid. Condiment	Probably all	Steinkraus (1996)
Nata de coco	Sugars → cellulose	Coconut water	Translucent jelly-like cubes in sugar solution. Dessert	Philippines, Thailand	Steinkraus (1996)
Nata de pinea	Sugars → cellulose	Dilute mashed pineapple	Translucent jelly-like cubes in sugar solution. Dessert	Philippines, Thailand	Steinkraus (1996)

References

Akuzawa, R., T. Miura and I.S. Surono. 2011. Asian fermented milks. In *Encyclopedia of Dairy Sciences*, Volume 2, second ed., edited by J.W. Fuquay, P.F. Fox and P.L.H. McSweeney. Amsterdam: Elsevier and Academic Press.

Fukishima, D. 2004. Industrialization of fermented soy sauce production centering around Japanese shoyu. In *Industrialization of Indigenous Fermented Foods*, second ed., edited by K.H. Steinkraus, pp. 1–98. New York: Marcel Dekker.

Han, B.-Z., F.M. Rombouts and M.J.R. Nout. 2001. A Chinese fermented soybean food. *International Journal of Food Microbiology* 65:1–10.

Kiuchi, K. and S. Watanabe. 2004. Industrialization of Japanese natto. In *Industrialization of Indigenous Fermented Foods*, second ed., edited by K.H. Steinkraus, pp. 193–245. New York: Marcel Dekker.

Paludan-Müller, C., H.H. Huss and L. Gram. 1999. Characterization of lactic acid bacteria isolated from a Thai low-salt fermented fish product and the role of garlic as a substrate for fermentation. *International Journal of Food Microbiology* 46:219–229.

Steinkraus, K.H. ed. 1983 and 1996. *Handbook of Indigenous Fermented Foods*, first and second ed. New York: Marcel Dekker.

Editor

J. David Owens graduated in microbiology and chemistry from the University of Bristol, UK in 1962 and earned his PhD at the University of Reading, UK, in 1966 for research on soil and herbage coryneform bacteria. After postdoctoral research at the Johns Hopkins University, Baltimore, Maryland on the ecology of bacteria in an estuarine bay in Jamaica, he took up a lectureship at the West of Scotland Agricultural College and research on the biological treatment of farm wastes. In 1973 he moved to the Science University of Malaysia, Penang, Malaysia and worked on marine pollution problems and various aspects of food microbiology and local fermented foods. In 1976 he joined the University of Sydney, Australia and initiated research on the evolutionary ecology of methylamine-utilising bacteria. After a year at the Australian National University he returned to Reading in 1980. At Reading he expanded his interests in indigenous fermented foods and the physiology of lactic acid bacteria and conducted research on yoghurt, Philippine fermented fish/rice mixtures, Mexican fermented maize dough, Indonesian tempe, Nepalese and African *Bacillus* fermentations, Papua New Guinean fermented taro/coconut gruel, Tanzanian cassava fungal fermentation and Zambian fermented maize beverage. He also investigated the mechanisms of conductance changes in microbial cultures and invented the Indirect Method for the conductimetric assay of microbial populations. Although formally retired since 2005, he continues to do some teaching at the Department of Food and Nutritional Sciences, University of Reading.

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1

Tempe and Related Products

J. DAVID OWENS, MARY ASTUTI
AND KAPTI RAHAYU KUSWANTO

Contents

1.1 Tempe

J. David Owens and Mary Astuti

1.1.1 Description of Product

Tempe is a natural product made from soya beans or other pulses that are dehulled, hydrated, cooked and inoculated with *Rhizopus* spp. moulds, without the addition of salt or other ingredients. The mould

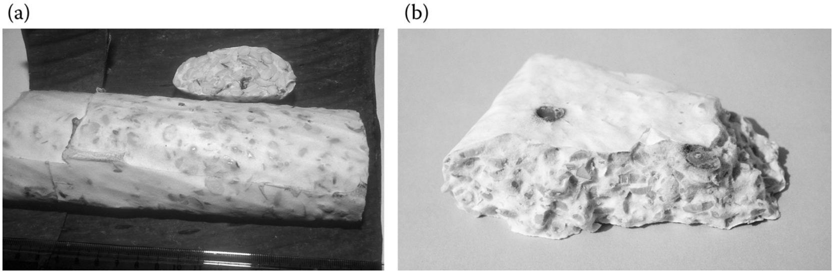

Figure 1.1 Tempe, showing cooked soya bean kernels tightly bound together by mould mycelium. (a) Indonesian tempe; (b) laboratory-made tempe with location of aeration hole delineated by a ring of black fungal spores. (Courtesy of J.D. Owens.)

growth binds the cooked bean kernels into a solid cake covered by a matt of white mycelium on its surface and without yellow spots or evidence of black sporulation. The cake is sufficiently firm, such that it does not disintegrate when cut with a knife (Figure 1.1). Tempe has a slightly beany flavour and an aroma characteristic of mould mycelium and boiled soya beans. Tempe is a traditional product from Indonesia, especially Java, and is now widespread over the world.

The word tempe, pronounced 'tempay' (tĕmpā, U.S. Dictionary transcription) in Indonesian and, based on the 1996 international agreement in Bangkok, the English spelling is tempe rather than tempeh (Anonymous 1996).

Different kinds of raw materials may be used to prepare tempe and, commonly, the name of the product includes reference to its raw material. Thus, *tempe kedele* is soya bean (*Glycine max*), yellow or black, tempe and the term tempe alone usually refers to soya bean tempe. Black soya bean tempe may be referred to as *tempe kedele hitam* (Figure 1.2). Among other pulses that may be used to prepare tempe are velvet bean (*Mucuna pruriens*; *tempe benguk*; Handayani 1997), sword bean (*Canavalia gladiata*; *tempe koro*; Figure 1.3), winged bean (*Psophocarpus tetragonolobus*; *tempe kecipir*), pigeon pea (*Cajanus cajan*; *tempe gude*), lablab bean (*Lablab purpureus*; *tempe kacang komak* or *tempe koro wedus*) and *Leucaena leucocephala* bean (*tempe lamtoro*). In addition, tempe may be made from the waste left over from tofu manufacture, generally called *tempe gembus* (Subchan and Rukmi 2007) or a mixture of tofu waste and waste of defatted peanut, *tempe menjes*.

The most popular and widely consumed is tempe made from soya bean. Tempe lamtoro, from *Leucaena leucocephala* bean, is only found

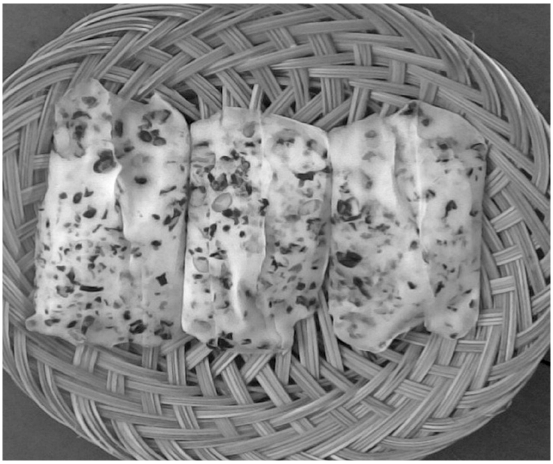

Figure 1.2 Black soya bean tempe (tempe hitam). (Courtesy of M. Astuti.)

in areas with unfertile land, such as in Wonogiri (southeast Central Java province) and Gunungkidul (southeast Jogyakarta province) districts. Tempe koro and tempe benguk can only be found in certain areas of Central Java, East Java and Yogyakarta province.

1.1.2 History of Tempe

1.1.2.1 Origin of Usar Tempe Inoculum The question of where tempe inoculum or *usar* came from was raised by Widagdo in an article

Figure 1.3 White sword bean (*Canavalia gladiata*) tempe (tempe koro). (Courtesy of M. Astuti.)

about how to make tempe in *Majalah Guru Desa*, the village teacher magazine, published in 1915. None of the many usar producers to whom he asked knew of its origin, but all agreed that it had been handed down for many generations. The raw materials for usar preparation are black soya beans and leaves of *Hibiscus tiliaceus*. The soya beans are first boiled well, allowed to cool, dehulled and then soaked overnight in water. Half of the soakwater is removed and is re-used to boil the next batch of soya beans. The cooked, dehulled kernels are inoculated with usar from a previous batch, using two to four usar leaves per kilogram wet weight of soya beans. Next, new clean hibiscus leaves are selected. They must not be washed or wiped as this would damage the fine hairs that are important for growth of the mould. Banana leaves are then cut into small pieces (5–6 cm^2), small holes are punched in them with a sharp twig and they are then placed on the hibiscus leaves (hairy undersurface uppermost). A thin layer of the inoculated soya bean kernels is then spread over the banana leaves and covered with more hibiscus leaves, their hairy undersurface in contact with the soya beans. Layers of beans and leaves are built up in this way until 10 or so layers have been created. The pile of leaves is then rolled and tied with rice straw. This roll is placed in a gunny bag or basket and left for 2–3 days at ambient temperature. The usar is ready when the white fungal mycelium appears on the surface of the banana leaves and out through the holes. At this stage the roll is opened up. The hibiscus leaves, which will have a layer of sporulating mycelium on their hairy under surface, are taken and dried for 2 days in a well-ventilated place. When black spores appear over the surface of the leaves the drying is stopped and the usar is ready to be sold in the market (see Chapter 2, Figure 1.3d).

Almost 100 years after Widagdo had speculated on the origins of usar, Ogawa et al. (2004) noted that *Rhizopus oryzae* was commonly present on fresh leaves of *Hibiscus tiliaceus*. This led to the suggestion that the origin of usar and tempe lay in the 'accidental' discovery that cooked soya bean kernels wrapped in hibiscus leaves became a solid mass that could be utilised in a variety of ways.

Usar exists in the market and some tempe producers use it as a source of mould. Modern tempe inoculum is a dried powder made by growing a mixture of *Rhizopus oligosporus* and *R. oryzae* on a substrate of rice or cassava powder.

The use of an inoculum to 'steer' a fermentation in the desired direction can be a very important factor in determining the outcome of that fermentation. Indonesian tempe is primarily a mould fermentation of non-salted soya beans in contrast to the non-salted soya bean fermentations found in Japan, Thailand, Korea, China, Myanmar and Bhutan, where the causative organism is *Bacillus subtilis* (see Chapter 10). The *Bacillus* fermented products, such as Japanese natto and Thai thua nao, are quite different from tempe. They are greyish or brownish in colour with a sticky texture, a strong flavour and a musty, ammoniacal smell. The traditional *Bacillus* fermentations are not inoculated, suggesting that one factor promoting a tempe mould fermentation is inoculation. Undoubtedly, other environmental factors are also involved in obtaining mould fermentation rather than a *Bacillus* one.

1.1.2.2 Soya Beans in Indonesia Soya beans originated in north China and from there have spread around the world. The word for soya bean in Indonesian is *kedelai*, from the Javanese *kadele*. Among old Javanese manuscripts, the word *kadele* is first recorded in the *Serat Sri Tanjung* manuscript (Prijono 1930), believed to have been written in the twelfth or thirteenth century. The word for soya bean is also found in the *Serat Centhini* manuscript, written in 1814 (Kamajaya 1986). Soya beans are not only consumed as tempe but also have a role in the Javanese marriage ceremony, where the groom gives presents of food, including rice, maize, black soya beans and long beans, as a symbol of his responsibility for the welfare of the family. Black soya beans also appear in the dish, *nasi udug* (rice cooked with coconut milk), and their use has not been replaced by yellow soya beans. Black soya beans are always used as the raw material for making usar. Thus, black soya beans continue to have a great significance in Javanese culture.

Rumphius (1747) described a type of soya bean plant, *Phaseolus niger*, which the Javanese called *kadele*, the Chinese *authau*, and the Dutch *zwarte boontjes* (black beans). His description of the plant is as follows: 'The soybean plant resembles a small shrub with pointed leaves, yellow flowers and seeds in pods. In Java and Bali, soybeans are widely cultivated and harvested by pulling up the plant with its pods. To store them, the leaves are first washed, then, 8–10 plants are tied together and hung up in a bunch. When they are required for eating, the entire plant is put into boiling water, or the beans are separated from the pods and boiled. Eating the boiled beans alone is not frequently done as they are quite hard and have a bitter flavour. The soybean plant grows in Java and Bali but is seldom found in Ambon'.

Black soya beans were cultivated by the Javanese and mainly sold to the Chinese, who ground them into flour from which they manufactured *laksa* or *tautsjian* (a type of flat noodle). In addition, soya beans were processed by heating, removing the black husks and milling the beans into flour for tofu production. Rumphius (1747) does not make any mention of the use of soya beans in tempe, possibly because he did not observe soya bean processing in rural areas. A magazine published in the 1900s stated that Javanese people made tempe from black soya beans and Chinese people made tempe from yellow soya beans (M. Astuti, unpublished data). Black soya beans were cheaper than yellow soya beans. The Chinese used imported yellow soya beans for making tempe and tofu. Tempe made from black soya beans is very rarely found in the market nowadays and almost all tempe is made from yellow soya beans. Tempe made from black soya beans is now priced higher than that made from yellow soya beans.

Before Indonesian independence in 1945, black soya bean was the dominant type cultivated and studied in Indonesia (Anonymous 1996). Black soya beans were mainly used as raw material for fermented soya sauce and were made into tempe. However, preferences shifted to yellow soya beans when they began to be imported from China and America and the black traditional variety has almost disappeared (Agranoff 2001).

1.1.2.3 Javanese and Tempe The earliest written record of the word tempe, comes from the 1814 *Serat Centhini* manuscript (Kamajaya 1986). The manuscript includes a description of the journey of Mas Cebolang when he traveled between Prambanan temple and Pajang, via Tembayat in the Klaten sub-district of Central Java province. Here, Cebolang was served a lunch, described in its entirety, that included a dish of tempe in coconut milk and a tempe sauce made from over-fermented tempe (cf. section on Indonesian dage below; Gandjar and Hermana 1972). At that time, tempe made from soya beans was an ordinary food found only in rural areas but tempe is currently popular, found everywhere, not only in rural areas but also in the cities. Rather surprisingly, Raffles (1817 and 1830) makes no mention of tempe.

The hypothesis that tempe originated in Java is supported by the fact that tempe can be found in every corner of the island, with variations only in terms of the type of substrate used to manufacture it. The production and consumption of tempe are integral to the Javanese lifestyle and the bond between tempe and the Javanese is so strong that the two have become inseparable. Wherever there are Javanese, there is sure to be a source of tempe too.

The spread of tempe outside Java began with the migration of the Javanese to other regions, both within Indonesia as well as abroad. Within Indonesia, many transmigrants settled in Lampung, Sumatra, taking with them their tempe technology. Interestingly, although these Javanese passed on their knowledge of tempe-making to natives of Lampung, the failure of the industry to develop among the Lampungese illustrates the special affinity that only the Javanese seem to have for this food. Trade links between Java and China as well as with Western nations have existed over several hundreds of years. The Javanese migrated to places such as Malaysia, Thailand, Surinam and the Netherlands and took with them their traditions as well as their knowledge of tempe production. As a result, tempe manufacture can now be found in Malaysia, Thailand, Surinam and the Netherlands and, more recently, has been taken up in many western countries, including the United States, Japan, Australia and Europe.

1.1.3 Places and Scale of Production, How Tempe Is Consumed and Its Role in the Diet

1.1.3.1 Distribution of Tempe Producers Indonesia comprises 33 provinces, 399 districts and 98 municipals, and tempe factories are found in all of them, with the greatest number being in Java. Java comprises six provinces and is home to 241 million people (National Population and Family Planning Board 2013). Tempe factories exist from Sabang, Aceh Special District in western Sumatra to the eastern most parts of Papua. In Indonesia there are more than 100,000 tempe producers with 10,000 tempe producers in Yogyakarta province alone (Anonymous 2010).

The amount of soya beans processed into tempe varies between 5 kg and 2 tonnes per day in factories classified as micro and small enterprises. These manufacturers are protected by law and are highly promoted by the government because of the high level of employment they provide. Tempe production in these small factories is mostly carried out by hand, using simple instruments and machines rather than complex ones. Producers with capacities of 1 tonne per day may use some stainless-steel equipment but smaller producers tend to use cheaper aluminium items. Machine packaging is rarely used. Tempe production workers and tempe makers are usually members of the Indonesia Tempe Makers Cooperative Enterprises (*Koperasi Perajin Tempe Indonesia/*KOPTI). KOPTI estimated that in 2012 the tempe industries contributed about USD700 million to the Indonesian economy.

The increasing demand for tempe, due to economic growth and the increasing population, has led to increases in tempe production and greater importation of soya beans to supplement locally grown soya beans.

1.1.3.2 Consumption of Tempe in Indonesia Generally, tempe is consumed as a source of protein in the Indonesian diet. Historically, tempe was viewed as a protein source of the lower classes and animal protein was categorised as high-class food. Although tempe was considered a home food and was rarely served in large restaurants or high-class hotels, people of all economic classes and of all ages consumed tempe and it was a favourite traditional food, served as a daily side dish in every household, especially in Java island. Currently, tempe has gained wider acceptance and is increasingly available in supermarkets and high-class restaurants.

Tempe is rarely consumed fresh but is incorporated into other dishes. It is suggested that there are more than 100 tempe recipes in Indonesia, with a wide diversity of tastes and appearances. Generally, dishes made from tempe can be classified as side dishes, snack foods or foreign-adapted foods. Tempe can be sliced or cut into cubes as either the main or as an additional ingredient of side dishes. It can be stir fried or seasoned and boiled, alone or with vegetables. Tempe dishes include *oseng-oseng tempe* (stir-fried tempe), *besengek* (whole tempe cooked with spices in thick coconut milk), *sambel goreng tempe* (sliced

Figure 1.4 Tempe restaurant dish, *mendoan* (tempe dipped in thin flour batter and fried). (Courtesy of J.D. Owens.)

tempe cooked with chili and spices in coconut milk), *sambel kering tempe* (sliced tempe and peanuts dry-fried and then cooked in oil with spices, chili, tamarind and coconut sugar), *tempe bacem* (savory, dark brown tempe made by boiling with spices and coconut sugar followed by frying), *tempe penyet* (fried tempe pressed flat onto the frying pan surface) among others. Most of these are Javanese cuisine specialties. Snack foods made from tempe include *mendoan* (tempe is dipped in thin flour dough and then fried; Figure 1.4) and *keripik tempe* (crunchy tempe crisps; Figure 1.5). There are also foreign-adapted products, such as tempe nuggets and tempe burgers.

Deep-fat frying, which takes only 3–5 min, is the most popular cooking method for serving tempe at a meal or as a snack food (Figure 1.6). The temperature for frying should be about 180°C. During frying,

Figure 1.5 Tempe crisps. (Courtesy of J.D. Owens.)

Figure 1.6 Deep-fat-fried tempe. (Courtesy of J.D. Owens.)

the colour changes from white to a golden brown but, if the temperature is too high, an unpleasant, dark brown colour is produced.

Tempe is generally consumed along with staple foods, such as rice, corn or cassava. Although tempe has a high protein content, it is estimated that it supplies less than 20% of total protein in the Indonesian diet (Table 1.1). Cereals, which are consumed in much greater quantities, supply around 60% (Central Bureau of Statistics 2012). In Yogyakarta province, the frequency of tempe consumption is ~50 times a month. This means that, on average, people consume tempe almost twice a day (M. Astuti, unpublished data). Although the quality of tempe protein is lower than that of animal protein, mixing rice and tempe in the ratio 7:3 improves the quality of protein (Astuti 1992).

Table 1.1 Consumption of Protein Food Sources in Indonesia in 2012

FOOD	CONSUMPTION (KG WET WT CAPITA^{-1} Y^{-1})
Fresh fish and shrimp	13.5
Chicken	9.3
Tempe	7.0
Tofu	7.0
Broiler, local chicken meat	4.0
Beef, buffalo meat	0.36

Source: Central Bureau of Statistics. 2012. *Food Balance Sheet*. Jakarta: Central Bureau of Statistics.

In Indonesia, tempe is one of the cheapest sources of protein. Tempe prices in 2013 (USD) per kg were as follows: tempe, 1.0; chicken eggs, 1.75; and beef, 14.5. On a per kg protein basis it is as follows: tempe, 5.1; fresh water fish, 7; sea water fish, 8; tofu,11; egg, 14; and beef, 97.

1.1.4 Traditional and Current Production Methods

There are many variations in the methods for making tempe in Indonesia, although they all share the same basic process which may be used for most types of beans. Only the preparation of *tempe gembus* (from tofu waste) and *bungkil* (residue from pulses, especially peanut, after extraction of oil) bean tempe are a little different.

The traditional method described by Widagdo (1915) is as follows: 'First of all, the whole soya beans are boiled until cooked, then allowed to cool. When cool, the hulls are removed by treading underfoot several times (Figure 1.7) until they float easily to the surface of the water (Figure 1.8). The hulls can then be easily separated and discarded, more water being added to clean the soya beans. The beans are then left to soak overnight until the soak water begins to smell bad (acid smell). At this stage they are boiled until soft, drained and left to cool on a bamboo or woven leaf mat. When cool they are mixed with 'usar'. The way this is done is by tearing up the usar into tiny pieces

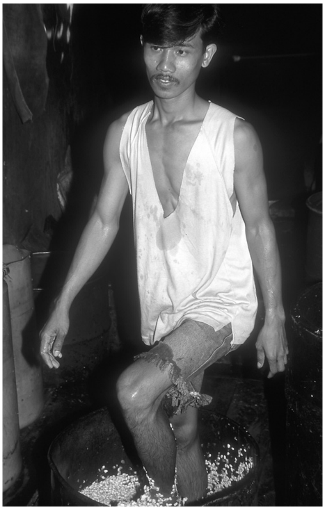

Figure 1.7 Dehulling cooked soya beans by treading, Bogor 1995. (Courtesy of J.D. Owens.)

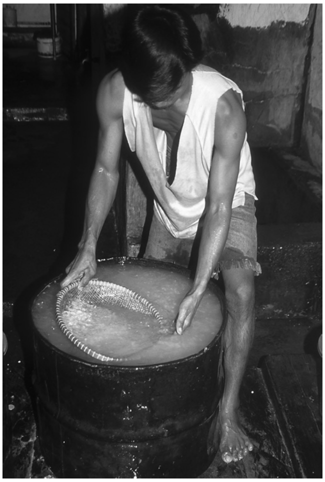

Figure 1.8 Separating hulls by flotation, Bogor 1995. (Courtesy of J.D. Owens.)

and placing these on a clay pot which is heated over a fire, without letting the usar burn. When the leaf fragments are fully dried, they are agitated until the mould becomes detached. The mould is then mixed with the cooled soya beans. The beans are wrapped in leaves and fermented in a gunny sack for 48 hours until they become tempe.'

The traditional method, as described by Widagdo, is still used in current tempe preparation (Table 1.2, procedure A), but the removal of the soya bean coat is done differently. In current methods, tempe producers use a soya bean dehuller to separate the testa and cotyledons of the soya beans (Figure 1.9). Use of tempe inoculum in the form of usar is now very limited and producers have changed to using powdered tempe inoculum (Figure 1.10). Perforated plastic bags are now used to contain the fermenting soya beans and have largely replaced natural wrapping materials, such as banana or teak leaves. For the fermentation, only very small-scale tempe producers still use a gunny sack and the bigger manufacturers use a fermentation rack made from bamboo, wood or metal (Figure 1.11).

Basic tempe processing involves two stages, preparation and fermentation, and the variations occur only in the preparatory stage (Saono et al. 1986; Steinkraus 1996; Shurtleff and Aoyagi 1985). The preparation stage changes the raw, hulled soya beans into cooked, dehulled kernels/cotyledons that provide a suitable substrate for growth of the mould. A survey of the methods of tempe preparation

Table 1.2 Some Traditional Methods for Preparing Tempe

	PROCEDURE						
STEP	A	B	C	D	E	F	G
1	Clean	Clean	Clean	Clean	Clean	Clean	Clean
2	Boil	Boil[b]	Boil	Soak[a]	Soak	Soak	Dehull
3	Cool	Soak	Soak	Dehull	Boil	Boil	Wash
4	Dehull	Dehull	Dehull	Wash	Wash	Cool	Boil
5	Wash	Wash	Wash	Boil	Boil	Dehull	Drain
6	Soak	Boil	Drain	Drain	Cool	Wash	Cool
7	Boil	Drain	Inoculate	Cool	Dehull	Soak	Inoculate
8	Drain	Cool	Wrap	Inoculate	Mash	Drain	Wrap
9	Cool	Inoculate[c]	Incubate	Wrap	Drain	Drain	Incubate
10	Inoculate	Wrap		Incubate	Inoculate	Cool	
11	Wrap	Incubate			Wrap	Inoculate	
12	Incubate				Incubate	Wrap	
13						Incubate	

Source: Modified from Saono, S., R.R. Hull and B. Dhamcharee. 1986. *A Concise Handbook of Indigenous Fermented Food in the Asia Countries*. Jakarta, Indonesia: The Indonesian Institute of Science.

[a] If only once, 60–120 min, if twice, first 30 min, second 90–120 min.

[b] 10 h to overnight.

[c] 36–48 h at room temperature (26–30°C).

in Indonesia conducted by Saono et al. (1986) revealed seven variant processes (Table 1.2). It can be seen that there are between 9 and 13 stages in the whole process. However, the procedures described by Saono omit the cleaning of the beans. Cleaning is important to remove materials, such as stones, metal and other impurities.

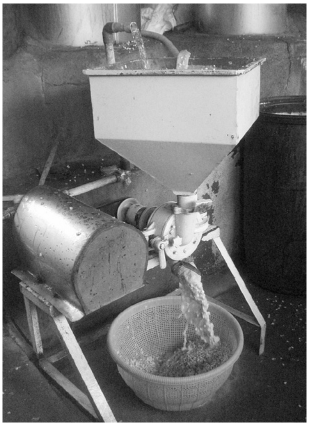

Figure 1.9 Mechanical dehulling machine. (Courtesy of M. Astuti.)

Figure 1.10 Powdered ragi tempe inoculum. (Courtesy of M. Astuti.)

Although details of preparation methods vary from one place to another and from one producer to another, essentially, they are all similar. It involves three basic steps: soaking, dehulling and cooking.

1.1.4.1 Soaking Some producers use a slow method which includes two periods of soaking. Others use a rapid method which only includes one soaking. The soaking stage allows full hydration of the

Figure 1.11 Bags of tempe on incubation rack. (Courtesy of M. Astuti.)

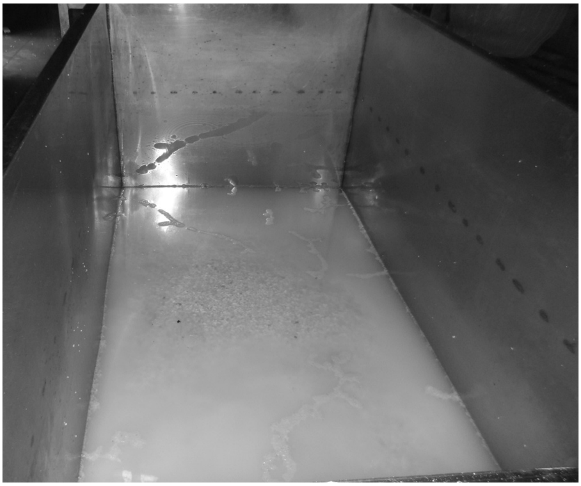

Figure 1.12 Soaking soya beans. (Courtesy of M. Astuti.)

soya beans, softens the beans and improves their texture for eating (Figure 1.12).

1.1.4.2 Cooking Cooking can be done by steaming or by boiling (Figure 1.13). It serves to eliminate contaminant vegetative bacteria and fungi from the beans, to soften the beans and to inactivate trypsin inhibitor and other anti-nutritional factors. Cooking in water is easier than steaming, but loss of water-soluble nutrients is higher with boiling than with steaming. The temperature of steaming is slightly higher than cooking in excess water. The soya beans may be boiled once or twice, depending on which system is preferred. If they are

Figure 1.13 Boiling soya beans in small-scale industry. (Courtesy of M. Astuti.)

boiled twice, the first boil is often short, about 30 min, and the second boil then lasts about 60–90 min (Figure 1.13). However, there is much variation in boiling times and Efriwati et al. (2013) observed a process in which the first boiling was for 2–3 h and the second was for ~2 h.

1.1.4.3 Dehulling Dehulling soya beans is now generally performed using a mechanical dehuller machine as this is somewhat more hygienic and economical for those who process in excess of 50 kg soya beans per day (Figure 1.9). Dehulling may be performed with a simple roller mill, adjusting the distance between rollers to somewhat less than the size of the soaked soya beans. The pairs of cotyledons are slightly pressed from each other by rotating cylinders and at the same time the hulls detached. The hulls can then be separated from the kernels by floating them off in running water. However, an equipment that separates all of the skins is not available and so around 10 – 15% of seed coats of the beans remain in the tempe. Nurrahman et al. (2011) suggested that tempe is still acceptable with around 15% of the seed coat with the cotyledons. If the seed coat content was higher than 20%, the taste of the tempe was not accepted by consumers.

It is also possible to dehull the dry beans (Steinkraus 1996) and dry dehulling of soya beans, using a mechanical dehuller, is done in the Tempe Murni factory in Yogyakarta province. However, tempe produced from dry dehulled soya beans can have a lower quality than tempe prepared from wet dehulled soya beans (M. Astuti, unpublished data).

When tempe producers use black soya beans as raw material some of the seed coat is visible on the surface of the tempe as black specks, but if yellow soya beans are used the seed coat is not easily seen. This is one reason why consumers, and producers, prefer yellow soya bean tempe to black soya bean tempe.

1.1.4.4 Washing Washing with clean water removes dirt attached to the beans and in the soaking water and replaces the acidic soaking water with clean water so that the beans do not become too sour.

1.1.4.5 Draining and Cooling The cooked kernels are drained to remove excess water and cooled, to obtain material that is dry on the surface and sufficiently cool to allow fungal growth. Spreading the hot kernels in thin layers allows evaporative cooling to achieve this (Figure 1.14). A suitable temperature for growth of the mould is 30°C, close to ambient temperature in Indonesia. The cooked kernels are not sterile and may contain bacteria, both contaminants and bacterial spores that survive the cooking, but in the environment offered by the kernels the growth of bacteria is slow and growth of the mould dominates.

The kernels need to be drained thoroughly since excessive water, and especially surface water can promote bacterial growth. Usually, the moisture content of the kernels is around 50–60%. If the moisture content is higher than 60%, bacterial growth in the free water on the kernels is liable to affect growth of the mould.

1.1.4.6 Inoculation Cooled beans at approximately room temperature can be inoculated with starter and homogenised. Some tempe manufacturers still use usar. One usar leaf is used for 2–4 kg of cooled soya beans. The amount of usar used is influenced by the environment. If the room contains a lot of tempe mould spores, only one piece of usar is needed per 10 kg of kernels. Most tempe producers use tempe inoculum in the form of a powder. The powder consists of ground rice and *R. oligosporus* and *R. oryzae* and is mixed with the cooked and cooled kernels (Figure 1.14). Usually, 1 g of inoculum powder is used per 1 kg of cooled beans. In the rainy season, the amount of inoculum

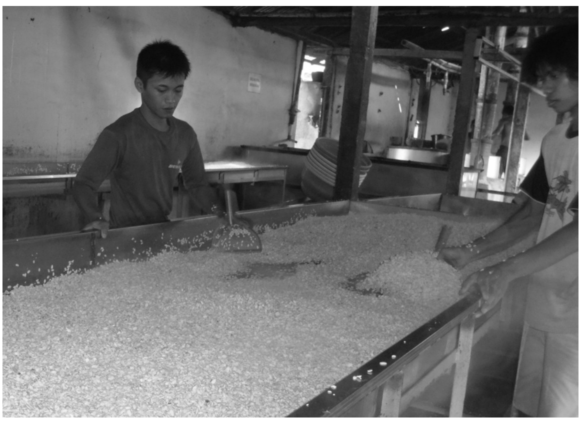

Figure 1.14 Draining, cooling and inoculating cooked cotyledons. (Courtesy of M. Astuti.)