Tempe fermentation, innovation and functionality: update into the third millenium

Journal of Applied Microbiology

Nout, M.J.R.; Kiers, J.L.

https://doi.org/10.1111/j.1365-2672.2004.02471.x

Journal of Applied Microbiology 2005, **98**, 789–805 doi:10.1111/j.1365-2672.2004.02471.x

A REVIEW

Tempe fermentation, innovation and functionality: update into the third millenium

M.J.R. Nout[1] and J.L. Kiers[2]
[1]*Laboratory of Food Microbiology, Wageningen University, Wageningen, The Netherlands, and* [2]*Friesland Nutrition Research, Friesland Coberco Dairy Foods, Leeuwarden, The Netherlands*

2004/1056: received 19 November 2003, revised 8 March 2004 and accepted 9 March 2004

1. Summary, 789
2. Introduction, 789
3. Manufacturing process, 791
   3.1 Small-scale home production, 791
   3.2 Technological innovations, 792
   3.3 Industrialization, 792
   3.4 Solid-state fungal fermentation engineering, 793
   3.5 Use of alternative ingredients, 793
   3.6 Technology transfer, 794
4. Microbiological aspects, 794
   4.1 The soaking stage, 794
   4.2 Fungal starters, 795
   4.3 Growth requirements, 795
   4.4 Germination and growth, 796
   4.5 Impact on quality, 796
   4.6 Monitoring of growth, 797
5. Chemical and Nutritional changes, 797
   5.1 Fate of proteins, lipids and carbohydrates during fermentation, 797
   5.2 Antinutritional factors, 798
   5.3 Vitamins, 798
   5.4 Isoflavones, 799
6. Functionality and safety of soyabean tempe, 799
   6.1 Digestion and absroption, 799
   6.2 Diarrhoea, 799
   6.3 Intestinal growth and proliferation, 800
   6.4 Anti-oxidative properties of fermented soyabeans, 800
   6.5 Chronic degenerative diseases, 801
   6.6 Safety, 801
7. Conclusion, 802
8. References, 802

1. SUMMARY

Fermented foods represent on average one-third of total food consumption. Tempe is a major fermented soyabean food and is known for its attractive flavour, texture and superior digestibility. This present review aims at providing an overview of literature data from *ca* 1990 until present. Although traditional preparation methods are still applied at the small scale, commercial production at small and medium industrial scale have resulted in technical innovations for improved control of starters and fermentation conditions. Nevertheless, the monitoring, control and modelling of the tempe fermentation at a large scale still presents a big challenge to process engineers. The complex dynamics of the microbiological composition continuously result in new aspects being resolved including the production of enzymes and bioactive compounds. The use of tempe in food consumption has evolved from the stages of basic nutrition to the development of derived products such as burgers and salads, and in recent years health benefits are becoming an important drive for its consumption.

Correspondence to: M.J.R. Nout, Laboratory of Food Microbiology, Wageningen University, Bomenweg 2, 6703 HD Wageningen, The Netherlands (e-mail: rob.nout@wur.nl).

2. INTRODUCTION

Fermented foods of animal and plant origin are distributed worldwide, and are subject of several excellent textbooks (Steinkraus 1995). The primary objective of the fermentation of cereals and seeds is not as much their preservation, but rather the modification of their organoleptic and nutritional properties. In the Orient, the traditional art of soyabean processing by fermentation has resulted in several delicious, easily digestible nutritious and healthy food products. Tempe is one of those products and it will be the focal point of attention in this review. Tempe (Indonesian spelling) also referred to as 'tempeh', is a collective name for a sliceable mass of precooked fungal fermented beans, cereals or some other food processing by-products bound together by the mycelium of a living mould (mostly *Rhizopus* spp.). Yellow-seeded soyabeans are the most common and

Fig. 1 Soyabean tempe, packaged and labelled but not pasteurized, as sold in supermarkets in the Netherlands

preferred raw material to make tempe. Figure 1 shows soyabean tempe, as manufactured and sold in the Netherlands. Tempe most probably originated from the island of Java (Indonesia). There is no doubt (Onghokham in Hermana *et al.* 1990) that it is a typical Javanese food that fits in the habit of serving food lukewarm, if not cold. Tempe as a substitute of meat fits this way of serving completely, even better than meats, fish or even tofu. Tempe contains less fat, does not become harder and tasteless if served cold. During the early 19th century, the Javanese society experienced a strong population increase from 4·5 to tens of millions causing land scarcity. Under Dutch colonial rule, up to 80% of the Javanese peasant labour force was employed in export crop plantation areas. Consequently there was less time for hunting, fowl and cattle raising and so, the diet became meatless. Assumedly, at first tempe was made from okara, the residue from soyamilk extraction for tofu making. Later, people could afford to use the whole soyabeans to improve its quality. Tempe started its 'career' as a poor men's food and served prisoners of war to survive the World War II internment camps. At present it derives increasing appeal from its nonmeat nature, and nutritional and health functionality. Tempe is a highly nutritious, easily digestible and delicious product and as such it meets an increasing demand from consumers looking for high quality meat replacers. With its high protein content (40–50% of dry matter) it serves as a tasty protein complement to starchy staple foods such as rice, and it can replace meat or fish. In Indonesia, the estimated consumption ranges from 19 to 34 g day^{-1} per person (Sayogyo in Hermana *et al.* 1990). Tempe is not consumed raw, but heated first to develop meat-like flavours, e.g. by frying spiced and salted slices in oil, by boiling with coconut milk in soups, by stewing, by roasting spiced kababs and in peppered ground pastes. Due to microbial enzymatic activities, fresh tempe has a limited shelf life. During storage, fresh tempe eventually turns brown, the beans become visible because of senescence of the fungal mycelium, the material softens and ammoniacal odours emerge. In Indonesia, such tempe is referred to as 'tempe bosok' (ripe tempe). Although tempe bosok is unacceptable for frying or stewing purposes, it is used in the Javanese kitchen to produce strongly flavoured cookies ('mendol'). In Indonesia, tempe is of socio-economic importance, because it provides jobs and income to a large number of family-owned small-scale producer shops. Also the manufacture of tempe starter, and of tempe-derived snacks takes place at cottage scale; these activities contribute significantly to the job market. Due to this small-scale and diffuse nature of production it is not easy to estimate the annual tempe production in Indonesia. In 1986, this was estimated between 154 000 and 500 000 t, mainly by small-scale cottage industries (the largest Indonesian factory produced 800 kg tempe per day). It was estimated in 1988 that 41 000 small shops produced 765 000 t of tempe in Indonesia. During small-scale production, typically 30–50 kg soyabeans are transformed into 50–80 kg tempe on a daily basis (Soetrisno in Sudarmadji *et al.* 1997). Tempe was more (50%) consumed in urban communities because of its convenience. The KOPTI (Organization of Tempe and Tofu Producers, established in 1975 in Indonesia) is concerned with ensuring supply of good quality soyabeans, inoculum, training of producers, etc. About 40 000 home industries are members of KOPTI, and in 1997 their production varied from 10 to 2000 kg per producer day^{-1}. With production costs of 600–800 Rupiah kg^{-1} tempe (1€ = 1600 Rp), and consumer prices of 1500 Rp kg^{-1} (vs prices for meat of 14 000 Rp kg^{-1}) there is sufficient margin to make a modest income from tempe making (Pawiroharsono in Stadler and Kreysa 1997). With an estimated (1998) 150 million metric tons global annual production of soyabeans (Liu 2000) on average, *ca* 10% of soyabeans are used as human food. However, in China 58%, South Korea 23% and Japan 17% of produced soyabeans are used for human food and in the USA only 1%. Outside Indonesia, tempe becomes increasingly known as a nutritious nonmeat protein food, e.g. in Europe, Japan and the USA. It experiences a slowly growing market in Europe, and has potential for continental Asia and Africa. In the Netherlands, the 'miracle of tempe' was introduced by Indonesian immigrants. It remained confined to this group of the Dutch population for several decades. Presently, it can be purchased as fresh tempe from the refrigerated food as well as deep-frozen food sections in greengrocers and leading supermarkets. Still, from a marketing point of view, with an estimated annual sale of 1 million kg in the Netherlands and 170 t in Belgium, it is a very small commodity compared with other soyabean foods such as tahu (tofu). One of the limitations to the use of traditional tempe in Europe is that the average consumer is not well aware of how it could be used in food dishes. That is probably the reason why presently most tempe is consumed as crisps and as fried tempe in sauces. Sources of information concerning tempe include practically oriented and popular scientific papers, bibliographies (Agranoff 1999) and reviews (Hachmeister and Fung 1993); the latter is of interest because of its coverage of the use of various leguminous seeds and cereals for tempe making. Scientific reports were published already during the 1800s, and this early and subsequent literature has been covered in an excellent review (Ko and Hesseltine 1979). Later, this was complemented (Nout and Rombouts 1990) covering the microbial ecology, starter development, kinetics of fungal growth, biochemical changes and nutritional quality related to tempe fermentation. The present review connects with the latter and deals with the scientific developments of the past decade. Limitations of space do not allow the citation of all published records, so we made a representative selection of those that are innovative and of relevance for the nutritional and health functions of the product. An extended version is available from the corresponding author.

3. MANUFACTURING PROCESS

3.1 Small-scale home production

Figure 2 summarizes the process of making tempe. In most small-scale Indonesian tempe workshops, the soyabeans are dehulled in a wet process, having the advantage that no major equipment is required and that the beans suffer very

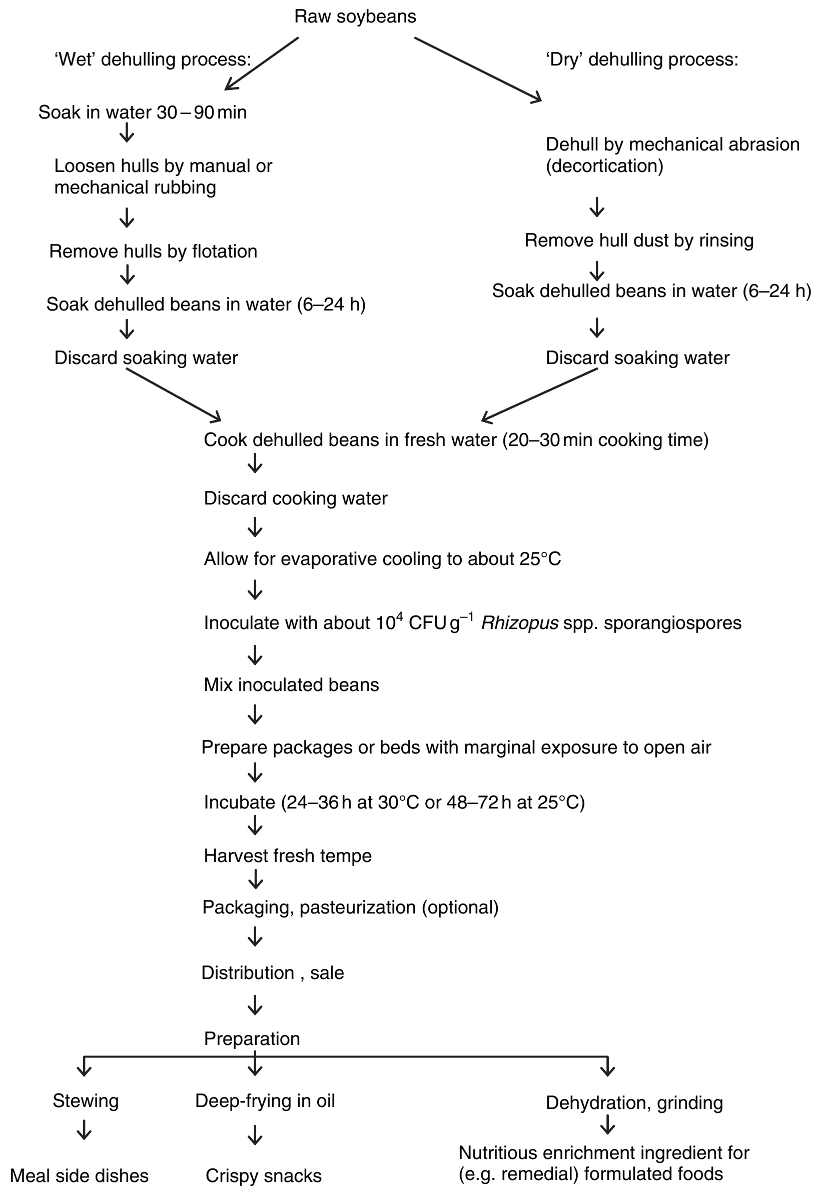

Fig. 2 Flow diagram of the tempe preparation process

little mechanical damage. Manual wet hull separation by flotation provides jobs to women who have specialized to this task. At a larger scale or when labour costs are high, dry dehulling is more economic, despite the disadvantage of higher losses of soyabean dry matter resulting from the abrasion of the soyabean hulls. Soaking has several functions: to increase the moisture content of the beans, render the beans edible, enable microbial activity during the fermentation, and extract naturally occurring antimicrobial substances (saponins) and bitter principles. Especially to remove the latter compounds, the soaking water must be discarded and the beans cooked in fresh water. After cooking, the hot water is discarded immediately, and the hot beans are spread out on trays to enable steaming-off of the beans to remove free water that could otherwise enhance microbial spoilage during the later stages of the process, and achieve a rapid cooling to *ca* 20–25°C within 10–15 min. The cooled beans are inoculated with *ca* 10^4 colony-forming units g^{-1} (CFU g^{-1} prepared substrate) using tempe starter containing fungal sporangiospores of mainly *Rhizopus oligosporus*, *Rhizopus oryzae* and sometimes *Mucor* spp. Most Indonesian tempe producers prefer to use inoculum grown on carriers of plant leaves as its quantity (leaf size) and degree of sporulation can be inspected visually.

Too low levels of starter (10^2 CFU g^{-1} or less) give irregular fungal growth, longer fermentation periods, and higher chances of bacterial spoilage. But too high levels ($\geq 10^6$ CFU g^{-1}) of starter may result in 'stormy' fermentation with excessive increase of temperature in the tempe and premature death of the mould. Beans and starter are mixed homogeneously into 3–5 cm thick beds. It is essential that a limited supply of air can reach the beans. For this purpose, packagaing materials or bed covers (polythene sheet or banana leaves) are perforated in a more or less evenly distributed manner. Incubation of 1–2 days at ambient temperature (25–30°C) is enough to allow spore germination and luxuriant growth of mycelium. Due to the restricted air supply, fungal spores are not or hardly produced and this results in an attractive creamy white tempe colour with only very little of the grey or black discolorations that otherwise would have been caused by the pigmented sporangiospores. Traditional Indonesian processing schedules were presented by Nout and Rombouts (1990) and surveyed by Haryadi in Ang *et al.* (1989). Fresh tempe is not eaten raw, but first cooked, e.g. in stews, or fried in oil to give delicious crisps (tempe kripik), or in a variety of dishes as well as applied as an enrichment ingredient for formulated foods (Vaidehi *et al.* 1996).

3.2 Technological innovations

Whereas the small-scale home production uses the traditional equipment and starters, several innovations have changed the scene of tempe making. Nowadays, wet bean dehulling is carried out mechanically using simple motor-driven concrete disc impactors. Polythene sheets have substituted the banana leaves to cover beds. Powdered starter concentrates are now commercially available.

In the Netherlands, tempe manufacturers purchase dry-dehulled soyabeans, ready for use. After removal of hulls and dust, hydration is carried out by boiling, steaming or by overnight soaking. Lactic (≤0·5% w/v) or acetic (≤0·25% w/v) acid may be added during hydration to control microbial spoilage, although acetic acid was shown to have a strong inhibitory effect on the fungal growth (De Reu *et al.* 1995a). Several tempe manufacturers prefer fermentative soaking using lactic acid bacteria, in order to improve the microbiological composition of the final product. In larger scale production, space required for adequate evaporative cooling is limited, so the cooked beans are sometimes cooled by removing the cooking water using basket centrifuges; the beans are subsequently cooled by dousing cold tapwater within the running centrifuge. Whereas this method enables rapid cooling, residual moisture levels remain relatively high and need to be absorbed by adding *ca* 2% w/w starch. This approach gives good fungal growth and tempe firmness, but the acidity in the beans is washed away, and the presence of starch can lead to 'stormy' fermentations with overheating. Several industrial tempe manufacturers prefer the use of a fan to cool the cooked beans.

3.3 Industrialization

To facilitate industrialization in Indonesia, the Government located a modern pilot plant tempe industry at Cibitung, West Java (Suharto in Sudarmadji *et al.* 1997). In commercial manufacturing practice in Europe, most tempe is produced under stationary bed conditions in small 500 g units to facilitate temperature control and retail distribution. To achieve small units, either flexible plastic bags, tubings (sausage casings) or hard plastic boxes are used. From a food safety perspective it is important to note that whenever introducing process innovations, the process should be scrutinized for its inherent safety. Poor microbiological quality of commercial tempe was attributed to the type of fermentation container (perforated plastic bags) (Vollbrecht 1997). Unfortunately no information was provided about the soaking treatment and the resulting pH of the cooked beans, as it has been shown earlier that unacidified beans allow profuse growth of pathogenic contaminants. In any case, perforation openings should be adequate to allow aerobic growth of the moulds.

Recently, some interesting concepts for semi-continuous process lines and novel products were developed that can be easily incorporated into a west european menu, such as smoked tempe, salads, burgers, 'meat' loafs and vegetarian 'bacon'. In order to obtain the reddish colour of bacon, a mixed culture is used consisting of *R. oligosporus* and *Neurospora intermedia* or *Neurospora sitophila*, the orange red mould used for the Indonesian fermented groundnut presscake 'oncom'. The product is pickled and smoked and enjoys popularity in England as 'vegetarian rashes' (E. de Wilde, pers. comm.).

3.4 Solid-state fungal fermentation engineering

Several hours after the inoculation of the beans, the germination of fungal sporangiospores starts, followed by the development of mycelium. The mycelium penetrates into several layers of soyabean cells to *ca* 25% of the width of the cotyledon (Ko and Hesseltine 1979; Varzakas 1998). Similar observations were made in a microscopical study of the growth of *R. oligosporus* on quinoa seeds, which first had to be decorticated or cracked in order to make them accessible (Penaloza *et al.* 1992b). The entanglement of hyphae results in a certain 'cohesion' of the fermented mass. As the firmness of tempe is an important quality attribute, several methods to measure or describe strength of tempe were reported (Ariffin *et al.* 1994; Manurukchinakorn and Fujio 1997a). Measurement of the strength of tempe is appropriate for quality assessment purposes, but it provides little information about the amount of fungal biomass formed. For engineering studies and fermentation monitoring, the real-time measurement of biomass accumulation is desirable. Up til now, the only real-time option is the measurement of dielectric capacitance at 0·30 MHz which directly relates to hyphal increment, as demonstrated in solid-state fermentations of soyabeans, bitter lupins and quinoa by *R. oligosporus* NRRL 2710 (Davey *et al.* 1991; Penaloza *et al.* 1992a).

Due to oxygen consumption by the mould and low rates of diffusion into the tempe bed, levels of oxygen decrease during the active stages of fermentation, typically to *ca* 2% v/v in the gaseous phase. Concomitantly, CO_2 increases to levels of ≥22% v/v. This extent of modification of the gas phase is a limiting factor to the fermentation as was shown by a comparison of 19 strains of *Rhizopus* (Soccol *et al.* 1994). Most strains tolerated 5–10% CO_2, and of some the hyphal extension was even stimulated under such conditions. Biomass growth rates μ_x (h^{-1}) of 0·043 and 0·096 at 0 and 5–10% v/v CO_2, respectively, were reported. However, concentrations of 16–35% CO_2 reduced growth rates significantly. Similarly, oxygen levels in tempe should remain higher than *ca* 0·4%; if the layer of beans is too thick, anaerobic conditions may prevail causing poor mycelium growth in the centre. Another limiting factor is the production of ammonia by degradation of nitrogenous compounds (Sparringa and Owens 1999b).

Fungal metabolic activity releases considerable heat. Due to limitations to heat- and mass-transfer in the solid (stationary) bed of beans, the temperature will increase to 40–50°C. This temperature range is too high for the mould, so control of the temperature is needed. In the traditional system, overheating of tempe can be counteracted by reducing the thickness of tempe beds, by reducing the tempe mass per incubator, and by ventilation. Heat and mass transfer could be improved considerably when the particle size is reduced. Several experiments were performed in which individual particles (cotyledons) were fermented in rotating drum fermentors. By intermittent rotation and ventilation at a 5-L scale, the temperature of fermenting soyabeans could be controlled within a band width of 1°C (De Reu *et al.* 1993). This type of process will not result in traditional tempe, but in individually fermented soyabeans that could be processed into dehydrated and particulate food ingredients.

Mixing during solid-state fermentation can also achieve a more homogenous product. But is the mould strong enough to withstand the mechanical abuse? The effect of mechanical stress caused by intermittent rotation was studied (Han *et al.* 1999) in a 0·45-m^3 capacity rotating drum fermentor on the behaviour of *R. oligosporus* and *Rhizopus microsporus* on soyabeans. *Rhizopus microsporus* tolerated agitation quite well, as judged by changes of pH, amino nitrogen, ammonia, and soluble dry matter. The other species, *R. oligosporus* was damaged by agitation. In industrial operations, a quantitative approach is desirable to enable the optimum design of fermentation containers, incubation rooms and their heating, cooling and insulation capacities. Solid-substrate fermentation can also be monitored by biomass production on the basis of ATP content, O_2 consumption or CO_2 production rates and described in mathematical models. Mathematical models serve to understand the order of magnitude and to facilitate the scaling-up of laboratory- to industrial-scale fermentations. From an engineering point of view, the traditional tempe fermentation is very difficult to control. Whereas rotating drum fermentors as mentioned earlier enable the control of temperature up to a scale of *ca* 1 m^3, modelling studies indicate that at even larger scale, additional cooling by evaporation is needed (Ueno *et al.* 1995). Loss of moisture as a result of evaporation could be prevented by spraying appropriate quantities of sterile water into the fermentor (Nagel *et al.* 2001).

3.5 Use of alternative ingredients

A large variety of raw materials can, in principle, be transformed into tempe (Nout and Rombouts 1990). The most important are pulses and leguminous seeds such as soyabeans (*Glycine max*), black gram (*Phaseolus mungo*), horse (wild) tamarind (*Leucaena leucocephala*), jack bean (*Canavalia ensiformis*), velvet bean (*Mucuna pruriens*), winged bean (*Psophocarpus tetragonolobus*) and various by-products from the food industry such as cassava fibres, soyabean hulls and oilseed presscakes. More recently the preparation and other properties of tempe made from a variety of ingredients has been reported. These include cereals such as oats (Nowak 1992), leguminous seeds including horsebean, chickpea and pea (Ashenafi and Busse 1991a), chickpea (Reyes-Moreno *et al.* 2000), common bean (Paredes-Lopez *et al.* 1990), cowpea (Kiers *et al.* 2000; Egounlety 2002), kidney beans (Kalavi *et al.* 1996), lupin (Chango *et al.* 1993; Fudiyansyah *et al.* 1995), green pea and pigeon pea. Because of their nutritional relevance, mixtures of cereals and leguminous seeds have been tested, such as finger millet with various legumes (Mugula and Lyimo 1999), maize and soyabean, rice and black beans (Rodriguez-Burger *et al.* 1998), and sorghum and common bean. Various other plants such as apricot seeds (Tunçel *et al.* 1990), African yambean (Njoku *et al.* 1991), Bambara groundnut (Amadi *et al.* 1999), groundbean (Egounlety 2002), quinoa (Penaloza *et al.* 1992a) and mixtures of sunflower seeds and soyabean were successfully transformed into tempe. In addition, food-processing by-products such as okara and rapeseed meal (Rozan *et al.* 1996) can be given added value by tempe fermentation.

Tempe and okara-tempe were succesfully used as a partial (20%) meat substitute in hamburgers to reduce drip loss during broiling, and to reduce hardening during refrigerated storage. In addition, substitution of 10% wheat flour in cupcakes reduced fat oxidation and hardening during storage. Other fabricated foods include mixed potato/tempe snack foods, tempe spread made with grated carrot (Slamet in Hermana *et al.* 1990), tempe sausage and tempe milk (Susanto in Sudarmadji *et al.* 1997) and dry mixes based on tempe powder (incorporated at 11–17% levels) for chapatti, porridge, soup and laddoo (Vaidehi *et al.* 1996). Whereas the acceptability of these novel fabricated foods appears to be good, it was shown that in the West-African context the acceptability of tempe snacks was quite marginal; this was attributed to the West-African preference for acidic fermented foods (Egounlety 2002). The nutritional potential and superior digestibility make tempe a valuable enrichment, for e.g. starch-based formulated foods, such as infant porridges (Kodyat in Hermana *et al.* 1990).

3.6 Technology transfer

Transfer of technology, in the case of tempe manufacturing, appears to follow several routes. In Indonesia, the KOPTI cooperation and the Government play an active role in upgrading traditional technology to meet today's requirements for quality, hygiene and safety. At a global level, early workshops and courses facilitated by the United Nations University as well as research projects funded by scientific organizations such as the International Foundation for Science, have played their role in spreading the scientific message to other continents. This is evidenced by the published accounts from African countries, e.g. Ethiopia (Ashenafi 1994), Tanzania (Mugula and Lyimo 2000) and Bénin (Egounlety *et al.* 2002). As indicated in the previous section, acceptability in a different cultural environment is not always easy to secure; it is clear however that tempe has much to offer and that we may see further implementation of its technology in Africa.

4. MICROBIOLOGICAL ASPECTS

4.1 The soaking stage

The microflora of soyabean tempe is complex and its development starts during the soaking of the raw ingredients (Nout and Rombouts 1990). The microbial composition of traditional tempe is determined by ecological factors such as the acidification by lactic acid bacteria during the soaking stage, the lethal effect of the cooking operation, contamination caused by handling during cooling, the composition and vitality of the inoculum, heat and mass transfer limitations during the fungal fermentation, the incubation conditions, and the conditions under which the product is stored. Fresh tempe contains high numbers of mesophilic aerobic bacteria, as well as enterobacteria, staphylococci and yeasts; in addition psychrotrophs may develop during refrigerated storage (Ashenafi 1994). In principle, a significant lactic fermentation during the soaking stage contributes to low levels of pathogenic and spoilage micro-organisms in tempe. The predominance of lactic acid bacteria including streptococci in soaking horsebean, chick-pea and pea (Ashenafi and Busse 1991a), as well as the presence of *Lactobacillus confusus* and *Lactococcus lactis* in cowpea soak water was reported. High levels of lactic acid bacteria (up to log 9 CFU g^{-1}) were also found in tempe from Malaysia. No bacteriocin producers could be found in regular tempe, but from a spoiled 7-day-old sample of tempe, bacteriocin-producing strains of *Enterococcus faecium* and *Lactococcus lactis* ssp. *lactis* were isolated (Moreno *et al.* 2002).

Lactic acid bacteria dominate during the soaking stage of the traditional process (Mulyowidarso *et al.* 1991a). As a result, a significant increase of organic acids takes place (Table 1). Acidification and other inhibitory effects of lactic acid bacteria were also shown to suppress the natural microflora (Ashenafi 1991) such as coliforms, *Klebsiella pneumoniae* (Tunçel and Göktan 1990) and yeasts (Ashenafi 1994), and to improve the shelf life of tempe. However, because of climatic and processing differences, this lactic fermentation does not occur by itself in temperate climates. In order to ensure thorough acidification, a lactic acid

Table 1 Accumulation of major organic acids in soyabean soak water during soaks with natural, accelerated, pure culture inoculated fermentation

	pH	Lactic acid	Malic acid	Acetic acid	Reference
Natural	4·5	0·6	0·2	0·07	1
Enterococcus faecium	ND	1·0	0·02	0·15	1
Lactobacillus acidophilus	4·20	1·61	ND	0·14	2
Lactobacillus casei	ND	1·1	0·2	0·05	1
L. plantarum	4·15	1·78	ND	0·14	2
Pediococcus pentosaceus	4·24	1·56	ND	0·16	2
Accelerated	4·12	2·14	ND	0·29	2

ND, not determined.
Soaking was carried out with 300 g soyabeans in 900–1000 ml tapwater during 24 h at 30°C, without inoculum (natural), with addition of 10^4 CFU ml^{-1} soak water of pure cultures of lactic acid bacteria, or with addition of 3% v/v previously fermented soak water (accelerated). Acidity is expressed as % w/v soak water.
References: 1, Mulyowidarso *et al.* (1991a); 2, De Reu *et al.* (1995a).

bacteria starter in soyabean soak water (Nout and Rombouts 1990) enriched by inoculation of soak water with 5% of the soak water of a previous production is effective. Alternatively, pure cultures of lactic acid bacteria may be added to protect the product against pathogenic micro-organisms from an early stage. For instance, if *Lactobacillus plantarum* is added at the start of the soaking stage, it will lower the pH of the soaked beans, but hardly of the tempe. When contaminations were added on purpose, it was observed by several independant laboratories, that Enterobacteriaceae and *Bacillus cereus* were successfully inhibited (Nout and Rombouts 1990; Tunçel and Göktan 1990; Ashenafi and Busse 1991b). Challenge tests with *Listeria monocytogenes* (Ashenafi 1991) also showed that this pathogen has great difficulty to survive in tempe in the presence of active *Lb. plantarum*. However, *Staphylococcus aureus* is more versatile and will still grow in the presence of lactic acid bacteria and the acids they produce, but it is unable to produce measurable levels of enterotoxins (Nout and Rombouts 1990). It is generally held that the presence of active competitors and the absence of atmospheric oxygen disable the enterotoxin formation by staphylococci. The potential of bacteriocin-producing lactic acid bacteria such as *Enterococcus faecium* to lengthen the shelf life has been suggested (Moreno *et al.* 2002) but not yet demonstrated. The antibacterial effects of tempe appear to be rather diverse: no inhibition of enterotoxic *Escherichia coli* was observed (Kiers *et al.* 2002), whereas in different settings broad-spectrum antimicrobial effects against *B. cereus*, *E. coli*, *Bacillus subtilis*, *Proteus vulgaris*, *S. aureus* and *Salmonella typhimurium* were reported.

4.2 Fungal starters

The major genus of importance for tempe making is *R. microsporus*, with varieties *microsporus*, *oligosporus*, *rhizopodiformis* and *chinensis* (Nout and Rombouts 1990). An additional variety *tuberosus* was also described (Zheng and Chen 1998). The leaves of the Indonesian Waru tree (*Hibicus tiliaceus)* of which the leaves are used as a carrier for tempe mould starter locally known as 'usar' were examined (Nout *et al.* 1992). On leaves harvested in Indonesia, *R. oryzae* and *R. microsporus* var. *oligosporus* were found abundantly besides a mixed flora of soil fungi; on leaves of the same *Hibiscus* spp. harvested in Africa and Europe the same soil fungi were found but no *Rhizopus* spp. This suggests that the widespread use of *Rhizopus* spp. in the manufacture of tempe results in its preponderance in the air spora. Most likely, *Hibiscus* leaves provide one of its natural reservoirs. Possible mitotic recombinations between *Rhizopus* strains would be possible especially in adverse growth conditions on *Hibiscus* leaves (Arbianto in Hermana *et al.* 1990); these might support survival and predominance of the genus in its ecological niche.

It has been speculated that yeasts in tempe could affect its quality, but no experimental evidence has been published so far.

4.3 Growth requirements

In a comparative study of fungi, several strains of *R. oligosporus* and *R. oryzae* were tested for their nutritional requirements (Graffham *et al.* 1995). All strains grew in glucose–ammonium–salts medium without added vitamins but none utilized, as sole source of C, raffinose or stachyose, the main flatulence-associated oligosaccharides in soyabean. Phytic acid was not used as a C source or a phosphate source by any strain. Most strains utilized fatty acids as sole sources of C and energy. In a study with debittered lupin seeds it was observed that additional potassium stimulated the growth of *R. oligosporus* (Penaloza *et al.* 1991).

Whereas it was shown earlier that *Rhizopus* spp. can grow at low (0·2%) oxygen concentrations, it was reported (Lin and Wang 1991) that of 18 tested strains none was able to grow under absolute anaerobic conditions. It was also observed that several strains were sensitive to oxygen toxicity (H_2O_2 or O_{2-} or $OH^\bullet$) and that their growth was restored in the presence of catalase. Another important environmental parameter is the temperature of the substrate: the closer to the optimum temperature of 37°C, the shorter will be the lag time of the fungal growth. The interrelated effects of temperature, water activity and gas composition on the biomass production of *R. microsporus* var. *microsporus* and *R. microsporus* var. *oligosporus* were investigated. Optimum conditions on a model agar medium were 40°C, a_w 0·995 and a gas composition of 20% O_2 + 0·03% CO_2 for both strains. Whereas colony radial growth rates of both varieties were similar, biomass growth rates of var. *oligosporus* were higher than those of var. *microsporus* under optimum conditions (Han and Nout 2000). Quite similar results (T_{opt} 42°C, pH_{opt} 5·85, a_w 1·0, and 0·03% CO_2) were obtained (Sparringa *et al.* 2002) for *R. oligosporus* NRRL2710 using comparable measures, i.e. hyphal extension rates. The temperature-dependent growth of *Rhizopus* spp. at $a_w > 0{\cdot}98$ could be described by the Ratkowsky equation. Carbon dioxide (5–10% v/v) inhibited the growth of *Rhizopus* spp. at nonlimiting levels of oxygen. The two strains were able to grow at low (0·5% v/v) oxygen levels, but the mycelial density was rather low. No interrelation of water activity and gas composition was observed, but at high water activity the fungi were more sensitive to changes of temperature (Han and Nout 2000).

4.4 Germination and growth

The first requirement for rapid and successful fungal growth is the germination of sporangiospores. The germination process was described in several stages starting with swelling corresponding with glucose uptake and an increase of the internal pH from 5·4 to 6·2. The next stage, germ tube formation, requires some oxygen and a nitrogen source; the internal pH remains 6·2 but ATP levels increase. Newly formed mycelium (the germ tube) is sensitive towards short chain fatty acids such as acetic acid that lower the internal pH. During the earlier stages, swelling can be prevented by inhibitors such as nonanoic acid (Breeuwer *et al.* 1997).

During the soaking of soyabeans according to an accelerated acidification method (indicated in Fig. 3 as "back-slop") organic acids were formed, resulting in a pH decrease from 6·0 to 3·9. After 24 h of accelerated acidification at 30°C, lactic acid was the major organic acid (2·1% w/v soak water), while acetic acid (0·3% w/v soak water) and citric acid (0·5% w/v soak water) were also found. During cooking in fresh water (ratio beans : water 1 : 6·5) the concentrations of lactate/lactic acid and acetate/acetic acid in the beans were reduced by 45 and 51% respectively. The effect of organic acids on the germination of *R. oligosporus* sporangiospores was studied in liquid media and on soyabeans. When pure cultures of homofermentative lactic acid bacteria (*Lb. plantarum*, *Lactobacillus acidophilus*, *Pediococcus pentosaceus*) were used in the initial soaking process, less lactic acid (1·6% w/v) and acetic acid (0·14% w/v) was formed during soaking than when the accelerated acidification method (2·14, 0·29% w/v) was used. This homolactic fermentation resulted in a reduction of the lag time of *R. oligosporus* by up to 4·7 h (Fig. 3). Furthermore, addition of ribose to *Lb. plantarum* to stimulate heterofermentation resulted in a significant acetic acid production and concomitant lag phase prolongation (De Reu *et al.* 1995a).

4.5 Impact on quality

Although both species *R. microsporus* var. *oligosporus* and *R. oryzae* are found in traditional tempe their impact on product quality is different. The mycelium of *R. oryzae* tends to be less dense, and because of its amylase activity and lactic acid formation from glucose *R. oryzae* is associated with undesirable sour off-flavours in tempe, especially those made from starch-containing raw materials.

Several other attributes of *Rhizopus* spp. have been reported that could be of relevance when selecting strains

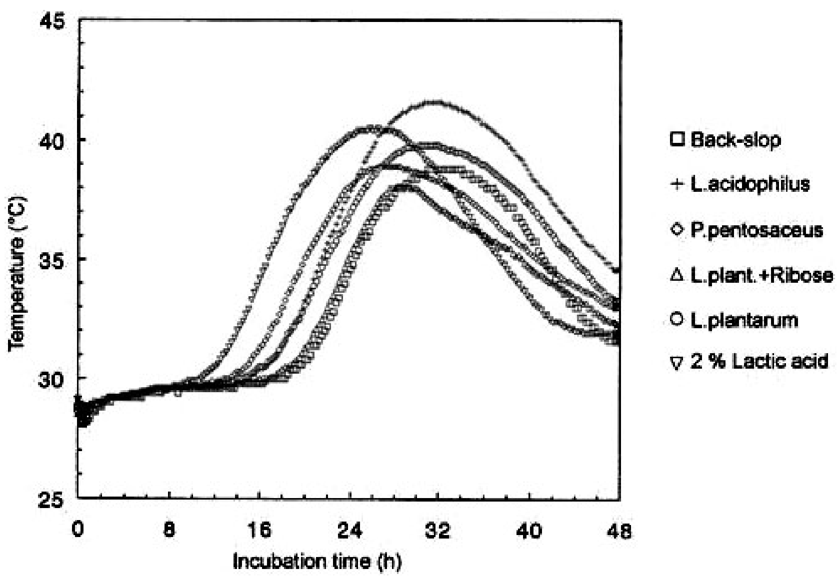

Fig. 3 Effect of organic acids on growth of *Rhizopus oligosporus* in tempe, as monitored by its temperature profile. Back-slop and *Lactobacillus plantarum* + ribose: heterofermentative fermentation with significant acetic acid production; *Lactobacillus acidophilus*, *Pediococcus pentosaceus* and *L. plantarum*: homofermentative fermentation with (mainly) lactic acid production; lactic acid: control for pure lactic acid)

for use as fermentation starter. For instance, *R. oryzae* isolated from tempe produced CO_2 (5–70% of head space) and other volatiles (acetaldehyde, ethanol, hexanal, methyl-ethyl-ketone, 2-methyl-1-propanol, 3-methyl-butanol, n-propanol and phenyl-ethanol) which had a synergistic antifungal effect with a_w on *Aspergillus flavus* (Lanciotti and Guerzoni 1993). Due to protein degradation and amino acid metabolism, significant levels of ammonia can be produced. It was shown that levels ranging from 0·42 and 1·3 mmol l^{-1} NH_3 slow down and inhibit mycelial growth and thus could be responsible for the cessation of mould growth in tempe (Sparringa and Owens 1999b). In addition to exerting an antifungal effect, volatile compounds strongly determine flavour. Using headspace GC and principal component analysis, tempe aromas could be classified according to the starters used (Supriyanto *et al.* 1991).

In addition to natural leaf-based starters, semi-pure culture starters grown on rice or soyabeans are used. The most common type of starter that is used for larger-scale tempe production, is a defined fungal culture, grown on a sterile solid substrate to obtain very high spore concentrations (*ca* 10^8 g^{-1}) (Tanuwidjaja in Hermana *et al.* 1990). Starters for tempe (*R. oligosporus*) can be grown well on cassava and rice at 37°C (Shambuyi *et al.* 1992). In dried pulverized powders (a_w 0·48) viability remained high (*ca* 10^7 CFU g^{-1}) up to 30 weeks at 5°C, and 25°C. It was observed that the viable spores only represented 5% of the total spores present in rice-grown starter. The remaining spores were not dead, but were present in a dormant state (Thanh and Nout 2002).

4.6 Monitoring of growth

The preferred method for monitoring fungal growth is in model studies on agar media or membranes that allow the separation and harvesting of microcolonies (Sparringa *et al.* 2002). Microscopical techniques have been used to describe hyphal lengthening and branching (Mitchell *et al.* 1990). In tempe, the use of an on-line capacitance measurement was reported (Davey *et al.* 1991); alternatively glucosamine is measured as a measure of cell-wall chitin production; a conversion factor of 12 g dry biomass per g glucosamine for *R. oligosporus* was proposed (Sparringa and Owens 1999a). The fungal cell wall is covered by antigenic extracellular polysaccharides (EPS); their yield is correlated with the biomass produced. Immunochemically active fractions contain mannose, fucose and protein. Using ELISA procedures it was established that antibodies raised against *Mucor racemosus* were specific for Mucorales (De Ruiter *et al.* 1992). Whereas these immunochemical approaches are useful to detect mouldy ingredients, they proved to be inadequate for quantitative biomass monitoring.

5. CHEMICAL AND NUTRITIONAL CHANGES

Primary benefits of soyabean fermentation are the improvement of organoleptic quality and nutritional value, rather than preservation. Raw soyabeans are bitter in taste. Consecutive stages of the tempe fermentation process (soaking, leaching and enzymatic modification) result in the removal of the beany flavours (Nout and Rombouts 1990). During the period of fermentation a total transformation of soyabeans occurs, unfolding a panorama of delicious new flavours and aromas, creating a unique texture and appearance, while simultaneously enhancing the nutritional value and digestibility (Karyadi in Ang *et al.* 1989). During fermentation of cooked soyabeans proteases, lipases, a variety of carbohydrases, and phytases are produced, and because of the enzymatic degradation of macromolecules into substances of lower molecular weight, the cell walls and intracellular material are partly solubilized (Nout and Rombouts 1990) contributing to a desirable texture, flavour and aroma of the product (Hachmeister and Fung 1993). In addition a decrease of anti-nutritional factors (ANF) is associated with the action of the moulds and their enzymes. Consequently, the nutritional quality of the fermented product may be improved.

5.1 Fate of proteins, lipids and carbohydrates during fermentation

During the phase of mycelial growth (0–32 h) the total dry matter decreased by *ca* 10% (w/w), accounted for by loss of crude lipid (3% of initial dry matter), protein/amino acids (0·5%), and unidentified components (6·5%) (Ruiz-Teran and Owens 1996). During the phase of mycelial senescence (60–180 h), decrease in dry matter (12% of initial dry matter) was due almost entirely to loss of crude lipid (Ruiz-Teran and Owens 1996). Protein oxidation (estimated from ammonia production) was 5 g at 28 h, 10 g at 46 h and 20 g/(kg of initial dry cotyledons) at 72 h. The total amount of soya protein hydrolysed, including that incorporated into mould biomass, was estimated to be 80 g/(kg of initial dry cotyledons) at 28 h incubation, 95 g at 46 h and 100 g at 72 h. Of the major soya proteins, conglycinin was hydrolysed faster than glycinin, which is probably related to its chemical structure; conglycinin is more sensitive towards protease activity (De Reu *et al.* 1995b). The hydrolysed protein at 46 h represented 25% of the initial protein. Of this hydrolysed protein, it is suggested that *ca* 65% remained in the tempe as amino acids and peptides (Higasa *et al.* 1996; Ruiz-Teran and Owens 1996), 25% was assimilated into mould biomass, and 10% was oxidized. The degree of hydrolysis depends strongly on the fungal strain (Baumann and Bisping 1995) and the fermentation conditions (Ikasari and Mitchell 1998). Proteases of nine strains of *R. oryzae*, *R. microsporus* var. *chinensis*, *Rhizopus stolonifer* and *R. oligosporus* comprised various isoforms of aspartic (*ca* 35 kD) and serine (*ca* 33 kD) proteases (Heskamp and Barz 1998).

Fatty acids present in glycerides decrease during fermentation, and the distribution pattern of fatty acids present in glycerides showed a slight increase of C18 : 1 (oleic) and C18 : 2 (linoleic) during fermentation at the expense of C18 : 3 (linolenic). Fatty acids are liberated resulting in hydrolysis of over 30% neutral lipid with a preferential utilization of α-linolenic acid and the total level of free fatty acids is increased in the final product (Agranoff 1999). Lipase activity and the production of free fatty acids occurred from the earliest stages of fermentation. The production of only small amounts of free glycerol indicates that triglycerides were primarily hydrolysed to partial glycerides (Ruiz-Teran and Owens 1996).

In soyabeans high levels of α-galactosides of sucrose (raffinose, stachyose) are found. These may have prebiotic properties, but they also contribute to intestinal gas production (flatulence). These oligosaccharides are removed mainly by soaking and cooking of soyabeans (Mulyowidarso *et al.* 1991b; Ruiz-Teran and Owens 1999). Several tempe-forming *Rhizopus* spp. (*R. oligosporus*, *R. microsporus* var. *chinensis*, *R. oryzae* and *R. stolonifer*) were able to utilize the flatulence-associated oligosaccharide raffinose as their sole source of carbon and energy (Rehms and Barz 1995). However, Graffham *et al.* (1995) also studied the nutritional requirements of Mucoraceous mycelial fungi and observed that *Rhizopus* spp. could not use raffinose and stachyose, nor the mineral-complexing phytic acid, as sole carbon and energy source. The fact that these substances are degraded nevertheless during the fermentation of regular tempe underlines the importance of mixed cultures of fungi as well as some of the accompanying bacterial species during the fungal fermentation. During tempe fermentation, a large range of water-soluble high molecular weight oligosaccharides are liberated by enzymic degradation of polysaccharides. Major carbohydrases of *R. oligosporus* in tempe include polygalacturonase, endocellulase, xylanase and arabinase (Sarrette *et al.* 1992), and during enzymatic maceration, predominantly the arabinogalactan and pectin fractions of the soyabean are solubilized (De Reu *et al.* 1997). Substantial glycohydrolase activities are tightly cell wall bound (Barz in Hermana *et al.* 1990). While reducing substances decrease, dietary fibre increases from 3·7 to 5·8% because of the growth of mould mycelia (Karyadi in Ang *et al.* 1989).

5.2 Antinutritional factors

Raw soyabeans contain significant levels of ANF, such as trypsin inhibitors and phytic acid. Many are leached out or destroyed during soaking and cooking, but also during fermentation (Tawali *et al.* 1998). Soaking and boiling reduces trypsin inhibitor activity (Prinyawiwatkul *et al.* 1996). The decrease of phytic acid is very important because it inhibits minerals availability. While *Rhizopus* spp. could not use phytic acid as sole source of carbon and energy (Graffham *et al.* 1995), tempe fermentation reduces levels of phytic acid significantly, resulting in significant increases of calcium, zinc and iron (Astuti in Agranoff 1999), (Macfarlane *et al.* 1990; Tawali and Schwedt 1998). Iron-deficient rats consuming tempe achieved higher liver iron levels than those fed unfermented cooked soyabeans (Kasaoka *et al.* 1997). Despite their antinutritional effect, protease inhibitors and phytic acid can also have positive health effects (Anderson and Wolf 1995) such as suppression of carcinogenesis (Kennedy 1995).

5.3 Vitamins

The increased content of some vitamins of the B group, especially riboflavin, niacin, vitamin B6, and vitamin B_{12}, because of fungal and bacterial metabolic activities has been extensively examined (Bisping *et al.* 1993; Keuth and Bisping 1993; Denter *et al.* 1998). An issue of specific interest is the production of vitamin B_{12}. In the past, the use of inadequate bioassay methods for vitamin B_{12} determination gave an overestimation of this vitamin (Nout and Rombouts 1990). Using more specific methods (Okada *et al.* 1985), the vitamin B_{12} levels in tempe are estimated in the range of 2–40 ng g^{-1} (Areekul *et al.* 1990; Bisping *et al.* 1993; Keuth and Bisping 1993). There is now a general concensus that not the mould, but the naturally occurring (or added) bacteria *K. pneumoniae* and *C. freundii* are responsible for vitamin B_{12} production (Keuth and Bisping 1994; Wiesel *et al.* 1997).

Carotenoids are formed in small amounts during tempe fermentation, although β-carotene is not produced by all strains. Soyabean did not contain ergosterol in detectable amounts, but it was produced in concentrations of up to 750 $\mu g\ g^{-1}$ tempe dry weight during a 34-h fermentation period by all 14 *Rhizopus* strains studied (Denter *et al.* 1998). During fermentation the total amount of vitamin E remained constant but the content of free (not esterified) tocopherols decreased. The content of vitamin K_1 in soyabeans is not strongly affected by tempe fermentation with pure cultures of *Rhizopus* sp. (Denter *et al.* 1998).

Soaking of cowpeas resulted in decreased folic acid content but did not affect thiamine, niacin and riboflavin. Boiling soaked seeds sharply decreased these B-vitamins, however losses were largely recovered during fermentation except for thiamine (Prinyawiwatkul *et al.* 1996).

5.4 Isoflavones

Of much interest is the modification of soyabean isoflavones, substances with antioxidant and radical-scavenging activity that could have health-promoting effects, by microbial activity (Cassidy *et al.* 2000). For instance, the formation of polyhydroxylated isoflavones from biochanin A and genistein by *Micrococcus* and *Arthrobacter* spp. isolated from tempe was demonstrated (Klus and Barz 1998), and 3-hydroxyanthranilic acid (HAA) is formed by fungal transformation of soyabean flavonoids (Matsuo *et al.* 1997). *Rhizopus* species were shown to metabolize 5-hydroxyisoflavones (genistein, biochanin A) and 5-deoxyisoflavones (daidzein, formononetin) (Barz in Hermana *et al.* 1990).

6. FUNCTIONALITY AND SAFETY OF SOYABEAN TEMPE

6.1 Digestion and absorption

During World War II, prisoners of war suffering from dysentery could not tolerate soyabeans but were able to subsist on tempe; an experience that underscores the easy digestibility of tempe. This is related with enzymatic degradation of soyabean polymeric substances resulting in soluble solids, such as soluble nitrogenous compounds. Macromolecules are degraded into oligomeric and smaller units improving the tempe digestion (Matsuo 1996). Digestibility of cereals and legumes increases during cooking and fermentation (Kiers *et al.* 2000). Cooking improved the total *in vitro* digestibility of both soyabean and cowpea from 37 to 45% and from 15 to 41% respectively. Subsequent fungal fermentation increased total digestibility only *ca* 3% for both soyabean and cowpea. Digestibility was influenced by fungal strain and fermentation time. Although total digestibility of cooked legumes was only slightly improved by mould fermentation, the level of water-soluble dry matter of food samples increased spectacularly from 7 to 27% for soyabean and from 4 to 24% for cowpea. From this it can be concluded that mould fermentation already 'predigested' the soyabean macronutrients to a significant extent. Fermentation was nearly capable to reach nutrient availability to the level obtained after *in vitro* digestion of cooked soyabeans.

Early *in vivo* trials using rats and neonatal pigs showed minor improvements of growth, daily weight gain and protein efficiency when fermented soyabeans and cowpeas were fed compared with unfermented controls (Nout and Rombouts 1990; Hachmeister and Fung 1993; Steinkraus 1995). This would suggest that fermentation of legumes using *Rhizopus* spp. would hardly improve their *in vivo* digestibility in healthy rats and piglets. However, tempe fermentation of a maize–soyabean mixture appeared to have resulted in increased protein digestibility, protein efficiency ratio and net protein utilization (tested in rats), which were almost similar to values for skimmed milk (Tchango 1995). Tempe showed higher dry matter and total solute absorption than cooked soyabean during perfusion of piglet small intestinal segments (J.L. Kiers, unpubl. data). When total net solute absorption was corrected for sodium, chloride and potassium only tempe showed a positive balance, probably reflecting the uptake of easily accessible nutrients and/or minerals (Macfarlane *et al.* 1990; Kiers *et al.* 2000). In fact, beneficial effects of fermented soyabeans were observed *in vivo* on growth and feed efficiency in early weaned piglets, indicating improved nutrient availability/digestibility (Kiers *et al.* 2003).

Feeding fermented soyabean could have more distinct beneficial effects in individuals suffering from a decreased gastrointestinal digestive and/or absorptive capacity. The use of tempe in the rehabilitation of children suffering from protein-energy malnutrition in Indonesia was shown to have a greater nutritional impact than food mixtures containing cooked but unfermented soyabeans. Protein-energy malnutrition is highly prevalent in developing countries because of the decline in breast-feeding, use of complementary foods that are low in energy and nutrients, and a high prevalence of diarrhoea and infections (Abiodun 1991). Fermentation of soyabean–cereal mixtures has great potential for application in complementary foods. A significant higher growth rate, shorter duration of diarrhoeal episodes and shorter rehabilitation period was reported in children suffering from protein-energy malnutrition, supplemented with a porridge containing tempe and yellow maize, compared with a similar porridge made of milk and yellow maize (Kalavi *et al.* 1996). These limited data indicate that tempe is of particular interest in patients suffering intestinal digestive defects. Tempe-based foods could therefore play a role as sources of easily available nutrients for individuals suffering from malnutrition and/or acute diarrhoea for whom the need for easily digestible rehabilitation foods is high. An ideal food for the prevention and management of malnutrition and diarrhoea should be of high nutritive value, easily digestible, acceptable, well tolerated and preferably should have additional anti-diarrhoeal properties.

6.2 Diarrhoea

As early as the 1960s, tempe was reported to contain an antibacterial substance, confirmed as demonstrated antimicrobial activity against selected species of Gram-positive bacteria (Rachmaniar in Hermana *et al.* 1990) (Kobayasi *et al.* 1992; Kiers *et al.* 2002). Recent work shows that several tempe extracts were able to inhibit adhesion of *E. coli* to piglet small intestinal brush border membranes (Fig. 4). Tempe appeared to interfere with *E. coli* adhesion *in vitro*

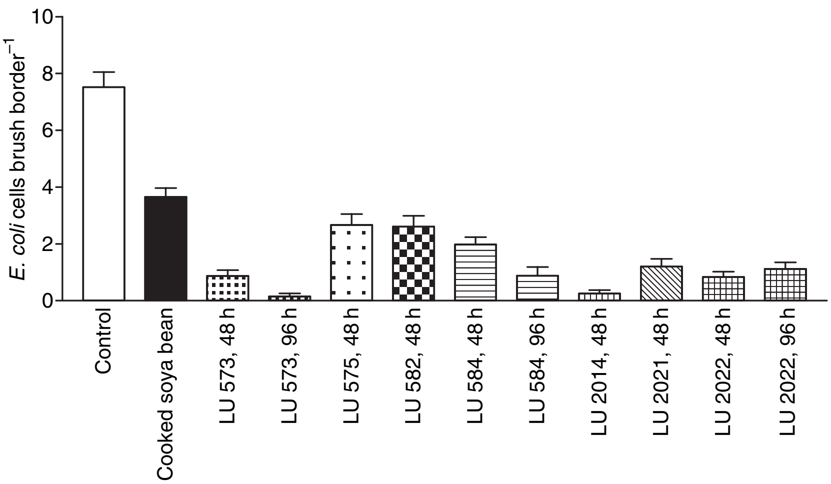

Fig. 4 *In vitro* inhibition of adhesion of enterotoxigenic *Escherichia coli* to intestinal brush border membranes (Kiers *et al.* 2002)

and might therefore have a protective effect against *E. coli* infection (Kiers *et al.* 2002).

Rabbits infected with enteropathogenic *E. coli* were fed on tempe during four weeks and showed reduced diarrhoea (36%) compared with rabbits fed diets without tempe (50–64%). In the tempe group, no *E. coli* was found in the small intestines and histopathological examinations indicated no enteritis (Karmini in Sudarmadji *et al.* 1997). The effect of processed and mould-fermented soyabean products on net absorption in uninfected and enterotoxigenic *E. coli* (ETEC)-infected piglet small intestine was studied (J.L. Kiers, unpubl. data). Both cooked soyabean and tempe showed a high protection against fluid loss caused by ETEC infection. The protective effect of tempe might be related to the production of a high molecular weight fraction (>5 kDa) by the specific mould (Kiers *et al.* 2001). The effect on the occurrence and severity of diarrhoea in ETEC K88+ challenged weaned piglets was determined (Kiers *et al.* 2003). Severity of diarrhoea was significantly less on the diet with tempe compared with the control diet containing toasted soyabeans (Table 2).

Various beneficial effects of tempe in disease prevention and treatment, principally in diarrhoea management and positive nutritional impact in Indonesian children have been reported (Soenarto in Sudarmadji *et al.* 1997) (Karyadi and Lukito 1996, 2000). An immune modulating effect of tempe

Table 2 Average diarrhoea incidence, average diarrhoea severity and days with diarrhoea for groups of piglets fed toasted soyabeans or tempe (Kiers *et al.* 2003)

	Toasted soyabeans	Tempe
Average diarrhoea incidence (%)	46 ± 22	33 ± 12
Average diarrhoea severity	2·3 ± 1·1[a]	1·7 ± 0·6[b]
Days with diarrhoea (days)	6·2 ± 3·1	4·3 ± 3·5

Values are given as mean ± S.D.
Values with different superscript alphabets differ significantly.

was suggested, but further evidence will have to be sought (Karmini in Agranoff 1999). Tempe can also be very useful as a nutritional supplement in oral rehydration therapy (Sudigbia in Agranoff 1999). Because of its protective effects during infection and its improved digestibility/nutrient availability, tempe may be beneficial in case of (postweaning) diarrhoea and accelerating the recovery of young animals and young children, who are most at risk for enterotoxic diarrhoea and malnutrition.

6.3 Intestinal growth and proliferation

Weaning is often associated with marked histological and biochemical changes of the small intestine (e.g. villous atrophy and crypt hyperplasia), causing decreased digestive and absorptive capacity and contributing to postweaning diarrhoea. Biopsies from the small intestinal mucosa showed improved repair after intestinal inflammation as a result of tempe supplementation (Sudigbia in Agranoff 1999). Tempe, compared with cooked soyabeans, contains high levels of easily accessible compounds such as peptides and free amino acids and possibly other factors which could affect intestinal growth and cell proliferation.

6.4 Anti-oxidative properties of fermented soyabeans

Soyabeans contain natural antioxidants. Tocopherols and phosphatides can be found in soyabean oil, while the non-oil compound contains many isoflavones. Of the isoflavones, 99% occur as 7-0-monoglucosides. Of these, three isoflavones predominate: genistin, daidzin and glycitin (Berghofer *et al.* 1998). Their aglycones are genistein, daidzein and glycitein respectively.

Fermentation of soyabean foods causes increased anti-oxidative capacity (Berghofer *et al.* 1998). During fermentation at least a partial cleavage or change in the glucosides takes place associated with increased glucosidase and glucuronidase activities (McCue and Shetty 2003), releasing potent anti-oxidant substances by transformation of flavonoids (Hein in Stadler and Kreysa 1997). Besides the above mentioned, also factor 2 (6,7,4′-trihydroxyisoflavone) is found in tempe (Hoppe *et al.* 1997). The level and type of isoflavones generated during fermentation depends on the composition of the inoculum; for instance, it was reported that not only *Rhizopus* sp. but also bacterial species, e.g. *M. luteus* and *Bacillus epidermis* determine the formation of specific isoflavones (Siregar in Sudarmadji *et al.* 1997) (Klus and Barz 1995). Hence, the addition of specific bacteria during the tempe fermentation process could increase the level of factor 2 and of other isoflavone compounds in tempe. Another anti-oxidative substance formed in tempe was identified as HAA (Esaki *et al.* 1996). Of several soyabean foods (Anderson and Wolf 1995) tempe had somewhat lower isoflavone content than tofu but contained elevated levels of the aglycones formed by enzymatic hydrolysis during fermentation (Wang and Murphy 1996). Fermentation of soya increased the human bioavailability of isoflavones. It was also shown *in vivo* with eight women aged 20–41 years, that isoflavones (daidzein & genistein) from soyafoods including tempe were retained for *ca* 75% (Xu *et al.* 2000).

6.5 Chronic degenerative diseases

In addition to the role of antioxidants in protecting the food against oxidative spoilage, antioxidants in soyabean (tempe) are of medical interest because of their protective role against oxidative stress which is related to the pathogenesis of various chronic degenerative diseases.

Soya (protein) has a potential role in the prevention and treatment of chronic diseases, most notably cancer (Kennedy 1995) and heart disease (Messina 1995), but also osteoporosis and menopausal symptoms (Liu 2000). Isoflavones in soyabeans also have favourable effects on the postmenopausal reproductive system and bone health. Soyabean protein has been known for many years to have a hypocholesterolaemic effect. It is therefore not surprising that tempe has also been found to lower blood cholesterol levels (Guermani *et al.* 1993) and that it may thus be of benefit as a protective agent against cardiovascular disease. Copper induced human blood LDL oxidation in the presence of tempe isoflavonoids showed that factor 2 was the most active inhibitor (Jha in Sudarmadji *et al.* 1997). Karyadi and Lukito (1996) described the available studies from Indonesia on the hypolipidemic properties of tempe. In a number of clinical intervention trials, total cholesterol and low-density lipoprotein cholesterol were significantly reduced in subjects treated with tempe (Brata-Arbai in Sudarmadji *et al.* 1997), whereas HDL cholesterol was raised (Brata-Arbai in Agranoff 1999) (Karyadi and Lukito 1996). These findings raised the question whether tempe, directly or indirectly, has anti-atherogenic characteristics. Studies in mice showed a reduction in serum cholesterol and inhibition of atherogenenesis. Tempe, similar to other soyafoods and related products, has beneficial effects on metabolic outcome insofar as lipid metabolism is concerned, and probably inhibits atherogenesis as well (Karyadi and Lukito 1996, 2000). Hypocholesteraemic effects may be due to the protein, fibre, carotenoids, sitosterol, isoflavones, calcium, lecithin, niacin, and/or unsaturated fatty acids in tempe (Brata-Arbai in Agranoff 1999). The possible difference between a hypocholesterolaemic effect of soyabean and of tempe has to our knowledge not been investigated.

Okara is an insoluble residue of soyamilk manufacture. Tempe made from okara is of interest as a high fibre and low energy food. Okara or okara tempe as well as soyabean protein and fibre, reduce plasma cholesterol levels in rats significantly compared with casein (Matsuo and Hitomi 1993). Moreover, okara tempe achieved reduced liver cholesterol and increase of cholesterol excretion in feces, most probably as a result of its increased water-soluble dietary fibre levels.

Whereas it has been reported that HAA had a cytocidal effect, and induced apoptosis to a human hepatoma derived cell line (Matsuo *et al.* 1997), further study is needed to assess the relevance of HAA and its absorption from tempe through the digestive tract, and its resulting levels in blood and/or tissues. It was demonstrated that tempe, especially its glucolipids, inhibits the proliferation of tumour cells in mice (Kiriakidis *et al.* 1997). Epidemiological studies relating to tempe consumption and the prevalence of cancer, particularly in Indonesia, have not yet been conducted.

Superoxide dismutase (SOD) is a free radical scavenger that increased during soyabean fermentation concomitant with the growth of the tempe mould. Rats consuming tempe had increased SOD activity in serum and liver (Astuti in Sudarmadji *et al.* 1997) and decreased lipid oxidation.

Gamma-aminobutyric acid (GABA) has been overproduced in soyabean tempe by means of a short anaerobic fermentation stage following the aerobic main fermentation. The GABA-enriched tempe was reported to have an antihypertensive effect on rats (Aoki *et al.* 2003).

6.6 Safety

Most traditional fermented foods are generally regarded as safe. Many traditional Asian fermented foods rely on growth of moulds of which strains are known that can produce potent mycotoxins. However, certain strains used in fermented foods production appear to have been domesticated over thousands of years. *Rhizopus oligosporus* is considered to be a potential human pathogen because of its ability to grow at 37°C. Inhalation of its sporangiospores might potentially cause pathogenic reactions, but this has not been studied *in vivo*; neither have pathological cases been reported. There have been no reports of poisonings, other than from a tempe-like product made of coconut presscake (tempe bongkrek). No reports of food poisoning or aflatoxin contamination in soyabean tempe exist, although some species of *Rhizopus* were found to be toxic when tested in animals. Levels of biogenic amines were found to be low, whereas ethyl carbamate could not be detected (Nout *et al.* 1993). *Rhizopus* spp. have even been shown to detoxify toxic substances in seeds (Kuo *et al.* 1995). Nevertheless, for modernized industrial-scale processing, toxicological screening of *Rhizopus* strains grown on various substrates is recommended (Hachmeister and Fung 1993). The long use of tempe at all stages of life, without recognized adverse effects, suggests that it poses no undue safety risk at the levels of intake known in Central Java.

7. CONCLUSION

During the past decade, significant scientific discoveries and technological innovations relating to the fermentation of soyabean tempe have been achieved. There is an increased interest in nutritional and health effects of food and feed. An important question is what fermentation has to offer in this field. A variety of indigenous fermented foods exist, many of which date back hundreds or even thousands of years. An important function of the micro-organisms involved in the fermentation process is the synthesis of enzymes that hydrolyse the food ingredients and contribute to the development of a desirable texture, flavour and aroma of the product. Fermentation also decreases anti-nutritional constituents, and the nutritional quality and digestibility of the fermented products is improved. Fermented foods could enhance the barrier function of the gastrointestinal tract, possibly resulting in reduced diarrhoeal disease risks. The potential impact of diarrhoeal episodes on nutritional status seems obvious, and the synergistic interactions of diarrhoea and malnutrition are well recognized. Fermented soyabean foods being nutritious, easily digested and absorbed, culturally acceptable, palatable and possibly protective against diarrhoea might break the vicious cycle of diarrhoea and malnutrition. Furthermore, the present or potential role of fermented soyabean foods in prevention and treatment of chronic diseases merits increasing attention.

8. REFERENCES

Abiodun, P.O. (1991) Use of soya-beans for the dietary prevention and management of malnutrition in Nigeria. *Acta Paediatrica Scandinavica Supplement* **374**, 175–182.

Agranoff, J. (ed.) (1999) *The Complete Handbook of Tempe*. Singapore: American Soybean Association.

Amadi, E.N., Uneze, R., Barimalaa, I.S. and Achinewhu, S.C. (1999) Studies on the production of bambara groundnut (*Vigna subterranea*) tempe. *Plant Foods For Human Nutrition* **53**, 199–208.

Anderson, R.L. and Wolf, W.J. (1995) Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. *Journal of Nutrition* **125**, S581–S588.

Ang, H.G., Nga, B.H., and Lim, K.K. (ed.) (1989) *7th World Congress of Food Science and Technology*. Singapore: Singapore Institute of Food Science and Technology.

Aoki, H., Furuya, Y., Endo, Y. and Fujimoto, K. (2003) Effect of gamma-aminobutyric acid-enriched tempeh-like fermented soybean (GABA-tempeh) on the blood pressure of spontaneously hypertensive rats. *Bioscience Biotechnology and Biochemistry* **67**, 1806–1808.

Areekul, S., Pattanamatum, S., Cheeramakara, C., Churdchue, K., Nitayapabskoon, S. and Chongsanguan, M. (1990) The source and content of vitamin B12 in the tempehs. *Journal of the Medical Association of Thailand* **73**, 152–156.

Ariffin, R., Apostolopoulos, C., Graffham, A., MacDougall, D. and Owens, J.D. (1994) Assessment of hyphal binding in tempe. *Letters in Applied Microbiology* **18**, 32–34.

Ashenafi, M. (1991) Growth of *Listeria monocytogenes* in fermenting tempeh made of various beans and its inhibition by *Lactobacillus plantarum*. *Food Microbiology* **8**, 303–310.

Ashenafi, M. (1994) Microbiological evaluation of tofu and tempeh during processing and storage. *Plant Foods for Human Nutrition* **45**, 183–189.

Ashenafi, M. and Busse, M. (1991a) The microflora of soak water during tempeh production from various beans. *Journal of Applied Bacteriology* **70**, 334–338.

Ashenafi, M. and Busse, M. (1991b) Growth of *Bacillus cereus* in fermenting tempeh made from various beans and its inhibition by *Lactobacillus plantarum*. *Journal of Applied Bacteriology* **70**, 329–333.

Baumann, U. and Bisping, B. (1995) Proteolysis during tempe fermentation. *Food Microbiology* **12**, 39–47.

Berghofer, E., Grzeskowiak, B., Mundigler, N., Sentall, W.B. and Walcak, J. (1998) Antioxidative properties of faba bean-, soybean- and oat tempeh. *International Journal of Food Sciences and Nutrition* **49**, 45–54.

Bisping, B., Hering, L., Baumann, U., Denter, J., Keuth, S. and Rehm, H.J. (1993) Tempe fermentation – some aspects of formation of gamma-linolenic acid, proteases and vitamins. *Biotechnology Advances* **11**, 481–493.

Breeuwer, P., De Reu, J.C., Drocourt, J.-L., Rombouts, F.M. and Abee, T. (1997) Nonanoic acid, a fungal self-inhibitor, prevents germination of *Rhizopus oligosporus* sporangiospores by dissipation of the pH gradient. *Applied and Environmental Microbiology* **63**, 178–185.

Cassidy, A., Hanley, B. and Lamuela-Raventos, R.M. (2000) Isoflavones, lignans and stilbenes – origins, metabolism and potential importance to human health. *Journal of the Science of Food and Agriculture* **80**, 1044–1062.

Chango, A., Bau, H.M., Villaume, C., Mejean, L. and Nicolas, J.-P. (1993) Effets de la fermentation par *Rhizopus oligosporus* sur la composition chimique des lupins blanc doux, jaune doux et jaune amer (Effects of fermentation by *Rhizopus oligosporus* on chemical composition of sweet white lupine, sweet yellow lupine and bitter yellow lupine). *Sciences des Aliments* **13**, 285–295.

Davey, C.L., Penaloza, W., Kell, D.B. and Hedger, J.N. (1991) Real-time monitoring of the accretion of *Rhizopus oligosporus* biomass during the solid-substrate tempe fermentation. *World Journal of Microbiology & Biotechnology* **7**, 248–259.

De Reu, J.C., Zwietering, M.H., Rombouts, F.M. and Nout, M.J.R. (1993) Temperature control in solid substrate fermentation through discontinuous rotation. *Applied Microbiology and Biotechnology* **40**, 261–265.

De Reu, J.C., Rombouts, F.M. and Nout, M.J.R. (1995a) Influence of acidity and initial substrate temperature on germination of *Rhizopus oligosporus* sporangiospores in tempe fermentation. *Journal of Applied Bacteriology* **78**, 200–208.

De Reu, J.C., Ten Wolde, R.M., De Groot, J., Nout, M.J.R., Rombouts, F.M. and Gruppen, H. (1995b) Protein hydrolysis during soybean fermentation *Rhizopus oligosporus*. *Journal of Agricultural and Food Chemistry* **43**, 2235–2239.

De Reu, J.C., Linssen, V. A. J. M., Rombouts, F.M. and Nout, M.J.R. (1997) Consistency, polysaccharidase activities and non-starch polysaccharides content of soya beans during tempe fermentation. *Journal of the Science of Food and Agriculture* **73**, 357–363.

De Ruiter, G.A., Van Bruggen-Van der Lugt, A.W., Nout, M.J.R., Middelhoven, W.J., Soentoro, P.S.S., Notermans, S.H.W. and Rombouts, F.M. (1992) Formation of antigenic extracellular polysaccharides by selected strains of *Mucor* spp., *Rhizopus* spp., *Rhizomucor* spp., *Absidia corymbifera* and *Syncephalastrum racemosum*. *Antonie Van Leeuwenhoek* **62**, 189–199.

Denter, J., Rehm, H.J. and Bisping, B. (1998) Changes in the contents of fat-soluble vitamins and provitamins during tempe fermentation. *International Journal of Food Microbiology* **45**, 129–134.

Egounlety, M. (2002) Sensory evaluation and nutritive value of tempe snacks in West Africa. *International Journal of Food Properties* **5**, 247.

Egounlety, M., Aworh, O.C., Akingbala, J.O., Houben, J.H. and Nago, M.C. (2002) Nutritional and sensory evaluation of tempe-fortified maize-based weaning foods. *International Journal of Food Sciences and Nutrition* **53**, 15–27.

Esaki, H., Onozaki, H., Kawakishi, S. and Osawa, T. (1996) New antioxidant isolated from tempeh. *Journal of Agricultural and Food Chemistry* **44**, 696–700.

Fudiyansyah, N., Petterson, D.S., Bell, R.R. and Fairbrother, A.H. (1995) A nutritional, chemical and sensory evaluation of lupin (*L. angustifolius*) tempe. *International Journal of Food Science and Technology* **30**, 297–305.

Graffham, A.J., Gordon, M.H., Westby, A. and Owens, J.D. (1995) Nutrition of tempe moulds. *Letters in Applied Microbiology* **21**, 223–227.

Guermani, L., Villaume, C., Bau, H.M., Nicolas, J.P. and Mejean, L. (1993) Modification of soyprotein hypocholesterolemic effect after fermentation by *Rhizopus oligosporus* spT3. *Sciences des Aliments* **13**, 317–324.

Hachmeister, K.A. and Fung, D.Y.C. (1993) Tempeh – a mold-modified indigenous fermented food made from soybeans and/or cereal grains. *Critical Reviews in Microbiology* **19**, 137–188.

Han, B.Z. and Nout, M.J.R. (2000) Effects of temperature, water activity and gas atmosphere on mycelial growth of tempe fungi *Rhizopus microsporus* var. *microsporus* and *R. microsporus* var. *oligosporus*. *World Journal of Microbiology & Biotechnology* **16**, 853–858.

Han, B.Z., Kiers, J.L. and Nout, M.J.R. (1999) Solid-substrate fermentation of soybeans with *Rhizopus* spp.: comparison of discontinuous rotation with stationary bed fermentation. *Journal of Bioscience and Bioengineering* **88**, 205–209.

Hermana, H., Mahmud, M. K. M. S., and Karyadi, D. (ed.) (1990) *Second Asian Symposium on Non-salted Soybean Fermentation*. Bogor, Indonesia: Nutrition Research and Development Centre.

Heskamp, M.L. and Barz, W. (1998) Expression of proteases by *Rhizopus* species during tempeh fermentation of soybeans. *Nahrung – Food* **42**, 23–28.

Higasa, S., Negishi, Y., Aoyagi, Y. and Sugahara, T. (1996) Changes in free amino acids of tempe during preparation with velvet beans (*Mucuna pruriens*). *Journal of the Japanese Society for Food Science and Technology – Nippon Shokuhin Kagaku Kogaku Kaishi* **43**, 188–193.

Hoppe, M.B., Jha, H.C. and Egge, H. (1997) Structure of an antioxidant from fermented soybeans (tempeh). *Journal of the American Oil Chemists Society* **74**, 477–479.

Ikasari, L. and Mitchell, D. (1998) Mimicking gas and temperature changes during enzyme production by *Rhizopus oligosporus* in solid-state fermentation. *Biotechnology Letters* **20**, 349–353.

Kalavi, F.N.M., Muroki, N.M., Omwega, A.M. and Mwadime, R.K.N. (1996) Effect of tempe yellow maize porridge and milk yellow maize porridge on growth rate, diarrhoea and duration of rehabilitation of malnourished children. *East African Medical Journal* **73**, 427–431.

Karyadi, D. and Lukito, W. (1996) Beneficial effects of tempeh in disease prevention and treatment. *Nutrition Reviews* **54**, S94–S98.

Karyadi, D. and Lukito, W. (2000) Functional food and contemporary nutrition-health paradigm: tempeh and its potential beneficial effects in disease prevention and treatment. *Nutrition* **16**, 697.

Kasaoka, S., Astuti, M., Uehara, M., Suzuki, K. and Goto, S. (1997) Effect of Indonesian fermented soybean tempeh on iron bioavailability and lipid peroxidation in anemic rats. *Journal of Agricultural and Food Chemistry* **45**, 195–198.

Kennedy, A.R. (1995) The evidence for soybean products as cancer preventive agents. *Journal of Nutrition* **125**, 733S–743S.

Keuth, S. and Bisping, B. (1993) Formation of vitamins by pure cultures of tempe moulds and bacteria during the tempe solid substrate fermentation. *Journal of Applied Bacteriology* **75**, 427–434.

Keuth, S. and Bisping, B. (1994) Vitamin B-12 production by *Citrobacter freundii* or *Klebsiella pneumoniae* during tempeh fermentation and proof of enterotoxin absence by PCR. *Applied and Environmental Microbiology* **60**, 1495–1499.

Kiers, J.L., Nout, M.J.R. and Rombouts, F.M. (2000) In vitro digestibility of processed and fermented soya bean, cowpea and maize. *Journal of the Science of Food and Agriculture* **80**, 1325–1331.

Kiers, J.L., Nout, M.J.R., Rombouts, F.M., Koops, B.C., Van Laere, K.M.J., Wissing, E., Hagemann, R.J.J. and Van der Meulen, J. (2001) Process for the manufacture of a fermented health-promoting product. European Patent Application 01201510.3–2110 Nutricia.

Kiers, J.L., Nout, M.J.R., Rombouts, F.M., Nabuurs, M.J.A. and Van der Meulen, J. (2002) Inhibition of adhesion of enterotoxic

Escherichia coli K88 by soya bean tempe. *Letters of Applied Microbiology* **35**, 311–315.

Kiers, J.L., Meijer, J.C., Nout, M.J.R., Rombouts, F.M., Nabuurs, M.J.A. and Van der Meulen, J. (2003) Effect of fermented soya beans on diarrhoea and feed efficiency in weaned piglets. *Journal of Applied Microbiology* **95**, 545–552.

Kiriakidis, S., Stathi, S., Jha, H.C., Hartmann, R. and Egge, H. (1997) Fatty acid esters of sitosterol 3 beta glucoside from soybeans and tempe (fermented soybeans) as antiproliferative substances. *Journal of Clinical Biochemistry and Nutrition* **22**, 139–147.

Klus, K. and Barz, W. (1995) Formation of polyhydroxylated isoflavones from the soybean seed isoflavones daidzein and glycitein by bacteria isolated from tempe. *Archives of Microbiology* **164**, 428–434.

Klus, K. and Barz, W. (1998) Formation of polyhydroxylated isoflavones from the isoflavones genistein and biochanin A by bacteria isolated from tempe. *Phytochemistry* **47**, 1045–1048.

Ko, S.D. and Hesseltine, C.W. (1979) Tempe and related foods. In *Microbial Biomass*, Vol. 4. ed. Rose, A.H. pp. 115–140. London: Academic Press.

Kobayasi, S.Y., Okazaki, N. and Koseki, T. (1992) Purification and characterization of an antibiotic substance produced from *Rhizopus oligosporus* IFO 8631. *Bioscience Biotechnology and Biochemistry* **56**, 94–98.

Kuo, Y.H., Bau, H.M., Quemener, B., Khan, J.K. and Lambein, F. (1995) Solid-state fermentation of *Lathyrus sativus* seeds using *Aspergillus oryzae* and *Rhizopus oligosporus* sp T-3 to eliminate the neurotoxin beta-ODAP without loss of nutritional value. *Journal of the Science of Food and Agriculture* **69**, 81–89.

Lanciotti, R. and Guerzoni, M.E. (1993) Competitive inhibition of *Aspergillus flavus* by volatile metabolites of *Rhizopus arrhizus*. *Food Microbiology* **10**, 367–377.

Lin, M.-S. and Wang, H.-H. (1991) Anaerobic growth and oxygen toxicity of *Rhizopus* cultures isolated from starters made by solid state fermentation. *Chinese Journal of Microbiology and Immunology* **24**, 229–239.

Liu, K. (2000) Expanding soybean food utilization. *Food Technology* **54**, 46–58.

Macfarlane, B.J., Van der Riet, W.B., Bothwell, T.H., Baynes, R.D., Siegenberg, D., Schmidt, U., Tal, A., Taylor, J.R. *et al.* (1990) Effect of traditional oriental soy products on iron absorption. *American Journal of Clinical Nutrition* **51**, 873–880.

Manurukchinakorn, S. and Fujio, Y. (1997a) Development of method for measuring the degree of maceration of soybean fermented with *Rhizopus*. *Journal of the Faculty of Agriculture Kyushu University* **41**, 223–230.

Matsuo, M. (1996) Digestibility of Okara-tempe protein in rats. *Journal of the Japanese Society for Food Science and Technology – Nippon Shokuhin Kagaku Kogaku Kaishi* **43**, 1059–1062.

Matsuo, M. and Hitomi, E. (1993) Suppression of plasma cholesterol elevation by Okara-Tempe in Rats. *Bioscience Biotechnology and Biochemistry* **57**, 1188–1190.

Matsuo, M., Nakamura, N., Shidoji, Y., Muto, Y., Esaki, H. and Osawa, T. (1997) Antioxidative mechanism and apoptosis induction by 3 hydroxyanthranilic acid, an antioxidant in Indonesian food tempeh, in the human hepatoma derived cell line, HUH 7. *Journal of Nutritional Science and Vitaminology* **43**, 249–259.

McCue, P. and Shetty, K. (2003) Role of carbohydrate-cleaving enzymes in phenolic antioxidant mobilization from whole soybean fermented with *Rhizopus oligosporus*. *Food biotechnology* **17**, 27–37.

Messina, M. (1995) Modern applications for an ancient bean: soybeans and the prevention and treatment of chronic disease. *Journal of Nutrition* **125**, 567S–569S.

Mitchell, D.A., Greenfield, P.F. and Doelle, H.W. (1990) Mode of growth of *Rhizopus oligosporus* on a model substrate in solid state fermentation. *World Journal of Microbiology and Biotechnology* **6**, 201–208.

Moreno, M.R.F., Leisner, J.J., Tee, L.K., Ley, C., Radu, S., Rusul, G., Vancanneyt, M. and De Vuyst, L. (2002) Microbial analysis of Malaysian tempeh, and characterization of two bacteriocins produced by isolates of *Enterococcus faecium*. *Journal of Applied Microbiology* **92**, 147–157.

Mugula, J.K. and Lyimo, M. (1999) Evaluation of the nutritional quality and acceptability of fingermillet-based tempe as potential weaning foods in Tanzania. *International Journal of Food Sciences and Nutrition* **50**, 275–282.

Mugula, J.K. and Lyimo, M. (2000) Evaluation of the nutritional quality and acceptability of sorghum-based tempe as potential weaning foods in Tanzania. *International Journal of Food Sciences and Nutrition* **51**, 269–277.

Mulyowidarso, R.K., Fleet, G.H. and Buckle, K.A. (1991a) Changes in the concentration of organic acids during the soaking of soybeans for tempe production. *International Journal of Food Science and Technology* **26**, 607–614.

Mulyowidarso, R.K., Fleet, G.H. and Buckle, K.A. (1991b) Changes in the concentration of carbohydrates during the soaking of soybeans for tempe production. *International Journal of Food Science and Technology* **26**, 595–606.

Nagel, F.J.J.I., Tramper, J., Bakker, M.S.N. and Rinzema, A. (2001) Temperature control in a continuously mixed bioreactor for solid-state fermentation. *Biotechnology and Bioengineering* **72**, 219–230.

Njoku, H.O., Ofuya, C.O. and Ogbulie, J.N. (1991) Production of tempeh from the African yam bean (*Sphenostylis stenocarpa* Hams). *Food Microbiology* **8**, 209–214.

Nout, M.J.R. and Rombouts, F.M. (1990) Recent developments in tempe research. *Journal of Applied Bacteriology* **69**, 609–633.

Nout, M.J.R., Martoyuwono, T.D., Bonné, P.C.J. and Odamtten, G.T. (1992) Hibiscus leaves for the manufacture of Usar, a traditional inoculum for tempe. *Journal of the Science of Food and Agriculture* **58**, 339–346.

Nout, M.J.R., Ruikes, M.M.W., Bouwmeester, H.M. and Beljaars, P.R. (1993) Effect of processing conditions on the formation of biogenic amines and ethyl carbamate in soybean tempe. *Journal of Food Safety* **13**, 293–303.

Nowak, J. (1992) Oats Tempeh. *Acta Biotechnologica* **12**, 345–348.

Okada, N., Hadioetomo, R.S., Nikkuni, S. and Itoh, H. (1985) Isolation of bacteria producing vitamin B12 from fermented soybean tempeh from Indonesia. *Report of the National Food Research Institute [Shokuryo Kenkyusho Kenkyu Hokoku]* **46**, 15–20.

Paredes-Lopez, O., Harry, G.I. and Gonzalez-Castaneda, J. (1990) Sensory evaluation of tempeh produced by fermentation of common beans. *Journal of Food Science* **55**, 123–126.

Penaloza, W., Davey, C.L., Hedger, J.N. and Kell, D.B. (1991) Stimulation by potassium ions of the growth of *Rhizopus oligosporus* during liquid- and solid-substrate fermentations. *World Journal of Microbiology & Biotechnology* **7**, 260–268.

Penaloza, W., Davey, C.L., Hedger, J.N. and Kell, D.B. (1992a) Physiological studies on the solid-state quinoa tempe fermentation, using on-line measurements of fungal biomass production. *Journal of the Science of Food and Agriculture* **59**, 227–235.

Penaloza, W., Hedger, J.N. and Thomas, B.J. (1992b) Low-temperature scanning electron microscopy of hyphal colonisation of *Chenopodium quinoa* during fermentation with *Rhizopus oligosporus*. *Annals of Applied Biology* **121**, 559–565.

Prinyawiwatkul, W., Eitenmiller, R.R., Beuchat, L.R., McWatters, K.H. and Phillips, R.D. (1996) Cowpea flour vitamins and trypsin inhibitor affected by treatment and fermentation with *Rhizopus microsporus*. *Journal of Food Science* **61**, 1039–1042.

Rehms, H. and Barz, W. (1995) Degradation of stachyose, raffinose, melibiose and sucrose by different tempe-producing *Rhizopus* fungi. *Applied Microbiology and Biotechnology* **44**, 47–52.

Reyes-Moreno, C., Romero-Urias, C.A., Milan-Carrillo, J. and Gomez-Garza, R.M. (2000) Chemical composition and nutritional quality of fresh and hardened chickpea (*Cicer arietinum* L) after the solid state fermentation (SSF). *Food Science and Technology International* **6**, 251–258.

Rodriguez-Burger, A.P., Mason, A. and Nielsen, S.S. (1998) Use of fermented black beans combined with rice to develop a nutritious weaning food. *Journal of Agricultural and Food Chemistry* **46**, 4806–4813.

Rozan, P., Villaume, C., Bau, H.M., Schwertz, A., Nicolas, J.P. and Mejean, L. (1996) Detoxication of rapeseed meal by *Rhizopus oligosporus* sp-T3: a first step towards rapeseed protein concentrate. *International Journal of Food Science & Technology* **31**, 85–90.

Ruiz-Teran, F. and Owens, J.D. (1996) Chemical and enzymic changes during the fermentation of bacteria-free soya bean tempe. *Journal of the Science of Food and Agriculture* **71**, 523–530.

Ruiz-Teran, F. and Owens, J.D. (1999) Fate of oligosaccharides during production of soya bean tempe. *Journal of the Science of Food and Agriculture* **79**, 249–252.

Sarrette, M., Nout, M.J.R., Gervais, P. and Rombouts, F.M. (1992) Effect of water activity on production and activity of *Rhizopus oligosporus* polysaccharidases. *Applied Microbiology and Biotechnology* **37**, 420–425.

Shambuyi, M., Beuchat, L.R., Hung, Y.C. and Nakayama, T. (1992) Evaluation of substrates and storage conditions for preparing and maintaining starter cultures for tempeh fermentation. *International Journal of Food Microbiology* **15**, 77–85.

Soccol, C.R., Raimbault, M. and Pinheiro, L.I. (1994) Effect of CO_2 concentration on the mycelium growth of *Rhizopus* spp. *Arquivos de Biologia e Tecnologia* **37**, 203–210.

Sparringa, R.A. and Owens, J.D. (1999a) Glucosamine content of tempe mould, *Rhizopus oligosporus*. *International Journal of Food Microbiology* **47**, 153–157.

Sparringa, R.A. and Owens, J.D. (1999b) Inhibition of the tempe mould, Rhizopus oligosporus, by ammonia. *Letters in Applied Microbiology* **29**, 93–96.

Sparringa, R.A., Kendall, M., Westby, A. and Owens, J.D. (2002) Effects of temperature, pH, water activity and CO_2 concentration on growth of *Rhizopus oligosporus* NRRL 2710. *Journal of Applied Microbiology* **92**, 329–337.

Stadler, P., and Kreysa, G. (ed.) (1997) *15th Dechema Biotechnology Conference*. Frankfurt am Main, Germany: DECHEMA.

Steinkraus, K.H. (1995) *Handbook of Indigenous Fermented Foods*, 2nd revised and expanded edn. New York: Marcel Dekker.

Sudarmadji, S., Suparmo, S., and Raharjo, S. (ed.) (1997) *Proceedings, International Tempe Symposium*. Den Pasar, Bali, Indonesia: Indonesian Tempe Foundation, Jakarta, Indonesia.

Supriyanto, Wada, K., Hayakawa, I. and Fujio, Y. (1991) Principal component analysis of aroma profiles of tempeh produced by using various starters. *Hakkokogaku Kaishi* **69**, 331–336.

Tawali, A.B. and Schwedt, G. (1998) Change of iron-species during processing of soy beans to its fermented product Tempeh. *Nahrung – Food* **42**, 29–31.

Tawali, A.B., Hain, J.U. and Schwedt, G. (1998) Determination of phytic acid content of soybeans during tempeh production using capillary electrophoresis. *Deutsche Lebensmittel-Rundschau* **94**, 28–30.

Tchango, J.T. (1995) The nutritive quality of maize-soybean (70:30) tempe flour. *Plant Foods for Human Nutrition* **47**, 319–326.

Thanh, N.V. and Nout, M.J.R. (2002) Biomass and yield, viability and dormancy of sporangiospores of *Rhizopus oligosporus* as a function of tempe starter preparation conditions. *Food Microbiology* **19**, 91–96.

Tunçel, G. and Göktan, D. (1990) Effect of different methods of soaking soya beans on the growth of *Bacillus cereus*, *Klebsiella pneumoniae* and *Staphylococcus aureus* in tempeh. *Journal of the Science of Food and Agriculture* **53**, 287–296.

Tunçel, G., Nout, M.J.R., Brimer, L. and Göktan, D. (1990) Toxicological, nutritional and microbiological evaluation of tempe fermentation with *Rhizopus oligosporus* of bitter and sweet apricot seeds. *International Journal of Food Microbiology* **11**, 337–344.

Ueno, T., Maekawa, T. and Kimura, T. (1995) Control of water content through evaporative cooling during solid state fermentation of potato processing waste. *ASAE Publication* **7**, 449–454.

Vaidehi, M.P., Sumangala, S.G. and Vijayakumari, J. (1996) Tempe-based ready-to-prepare food mixes of high nutritional value. *Journal of Food Science and Technology, India* **33**, 506–509.

Varzakas, T. (1998) *Rhizopus oligosporus* mycelial penetration and enzyme diffusion in soya bean tempe. *Process Biochemistry* **33**, 741–747.

Vollbrecht, D.E. (1997) Health risks for consumers of tempeh fermented using the plastic bag method. *Advances in Food Sciences* **19**, 87–89.

Wang, H.J. and Murphy, P.A. (1996) Mass balance study of isoflavones during soybean processing. *Journal of Agricultural and Food Chemistry* **44**, 2377–2383.

Wiesel, I., Rehm, H.J. and Bisping, B. (1997) Improvement of tempe fermentations by application of mixed cultures consisting of *Rhizopus* sp. and bacterial strains. *Applied Microbiology and Biotechnology* **47**, 218–225.

Xu, X., Wang, H.J., Murphy, P.A. and Hendrich, S. (2000) Neither background diet nor type of soy food affects short-term isoflavone bioavailability in women. *Journal of Nutrition* **130**, 798–801.

Zheng, R.Y. and Chen, G.Q. (1998) *Rhizopus microsporus* var. *tuberosus* var. nov. *Mycotaxon* **69**, 181–186.