SURVEY OF TRADITIONAL FERMENTED FOODS AVAILABLE IN INDIAN MARKET-A REVIEW

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***Abstract*:** Fermented foods in India are categorized based upon their base material. Fermented foods such as Dahi, Idli, Dosa, Sidra, Sukuti etc. As India's population increases, lactic acid fermentation is expected to become an important role in preserving fresh vegetables, fruits, and other food items for people. Among the food items, milk, fruits and vegetables are easily perishable due to their high water activity and nutritive values that are critical in our country for favoring growth of spoilage causing microorganisms. Lactic acid fermentation increases shelf life of fruits and vegetables and also enhances several beneficial properties, including an increase in nutritive value and flavors, reducing cooking time, and reduces toxicity. As a whole, the traditional fermented milk, fruits and vegetables not only serve as food supplements but also attribute towards health benefits. The objective of this review is to describe important fermented products of India and their significance.

Key words: Traditional fermented foods, Lactic acid bacteria, Dahi, Alcoholic beverage.

I. INTRODUCTION

Traditional foods generally categorized into fermented foods and beverages, produced by microorganisms, and non fermented food. Fermented foods are prepared by the action of microorganisms either naturally, or by adding pure culture (s), which modify the substrates into edible product, and are generally palatable, safe and nutritious [1]. These inexpensive acceptable traditional foods provide basic diet as staple, pickle, confectionary, condiment and alcoholic beverages, which supplement enhanced nutrition, palatability, wholesomeness of the product with acceptable flavor and texture [2]. More than twenty varieties of ethnic fermented foods and more than ten types of fermented beverages are consumed in the hilly areas in India mainly in Sikkim [3]. Traditional alcoholic beverages constitute an integral part of dietary culture and have strong ritual importance among the ethnic people in Himalayas, where social activities require provision and consumption of appreciable quantities of alcohol [1]. Alcolohic beverages are exclusively prepared from locally grown cereal grains using traditionally prepared mixed inocula or starter called 'Marcha' [4] Traditional alcohol brewing is a home based industry mostly done by rural women using their indigenous knowledge of alcohol fermentation. Yonzan and Tamang (1998) for the first time conducted a brief pattern of traditional fermented food of the Darjeeling Hills and Sikkim (Table 1).

The indigenous fermented foods constitute a group of foods that are produced in homes, villages, and small at prices within reach of a majority of the consumers in the developing world [5]. Examination of these foods therefore provide clues as to how food production and preservation can be expanded and thereby contribute to improved nutrition in the developing world [5].

Some indigenous food fermentations such as soy sauce (shoyu), Japanese miso, Indonesian tempe, Japanese sake, Indian idli and dahi, fish and shrimp sauces pastes have been intensively studied to determine optimum conditions for fermentation. The essential microorganisms, the biochemical, nutritive and flavor or texture changes that occur during fermentation and the possible toxicological problems that can arise have also been studied [6].

The huge international enzyme industry today can be traced directly to the indigenous Chinese and Japanese soy sauce and Japanese miso and sake fermentations. The monosodium glutamate (MSG) industry and relatively recent but significant nucleotide flavor enhancing industry also are outgrowths of soy sauce fermentation [7]. Tempe is produced commercially in Indonesia and Malaysia. It is also produced by factories in the United States [7, 8, 9].

Classification of Indigenous Fermented Food Fermentation

Indigenous food fermentations have been classified as follows [6]:

- Fermentations involving proteolysis of vegetable proteins by microbial enzymes in the presence of salt and/ or acid with production of amino acid and peptide mixtures with a meat like flavor (eg. are soy sauce, Indonesian Kocap, Miso, and Indonesian tauco).
- Fermentations involving an enzymatic hydrolysis of fish and shrimp or other marine animals in the presence of relatively high salt concentrations to produce meat flavored sauces and pastes.
- Fermentation in which organic acids are major products : this category includes Korean kimchi, sauerkraut, fermented milks and cheeses, African ogi und ugi, idli, dosai, tape and also tempe, in which acidification occurs during the initial soaking of the soybeans.
- Fermentation in which ethanol is a major product : this category includes rice wines, palm toddies, sugar cane wines, beers and very important nutritionally tape ketan and tape ketala.

  There is considerable overlap among the various categories of fermentation, but the classification does facilitate description and analysis of the potential usefulness of the various fermentations.

Examination of the various classifications indicate that food fermentations could be used to establish the following small-scale food processing operations:

- Production of meat like flavor from vegetable proteins (soy sauce and miso)
- Production of meat like flavor from fish and shrimp and other marine animals (fish and shrimp sauces and pastes)
- Production of meat like texture in cereal legume substrates (tempe and oncom)
- Production of leavened bread-type foods without the use of wheat or rye (idli and dosai)
- Production of quick cooking food (tempe and oncom)

Production of meat like flavor from vegetable proteins [10]

The enormous pressures for protein food products in the coming decades, brought on by world population increases, will be solved through the extension of traditional animal protein foods with vegetable proteins and through the development of food products based on vegetable proteins alone. Analogs of beef, fish, poultry and other traditional animal protein products, which are based solely on vegetable proteins, are an established food category, and are expected to increase market share. Dairy analogs based on vegetable cow's milk and dairy desserts. Vegetable forms of cheese and other milk protein products are also expected to increase. Nutritional equivalence of vegetable protein products is fundamental to product design. Protein and fat content must be standardized. Vegetable proteins are blended to reach desirable protein quality. Analogs currently marketed are primarily blends of soy and wheat proteins containing lesser amounts of yeast and egg albumen. The products are fortified with vitamins and minerals to levels present in animal protein foods. Processed meat manufacturing facilities, which exist in most developed countries, can be readily adapted to produce meat analogs. The technology which has been developed to date is based on soy or soy/wheat combinations. The technology can readily be adapted to other vegetable proteins such as rapeseed, cotton-seed, sesame or sunflower. These protein sources, while in abundance in many countries, need process research which can refine them for human use. The vegetable proteins offer the world's exploding population a virtually untapped resource for its burgeoning food requirements. [10].

On the other hand, hydrolyzed vegetable or plant protein (H.V.P. or H.P.P.) are the mixtures containing amino acids and frequently other substances such as salt and peptides, obtained by hydrolysis of vegetable proteins. On an industrial scale two types of hydrolytic processes are applied: acid and enzymic hydrolysis. HVP estimated sales in the western world are at least $100 million. Most HVPs are produced for internal use by soup manufacturers, for use as vital meaty or savory flavoring ingredients in bouillons, soups, sauces, processed meat, fish and poultry products and snacks. Other HVPs are more ready-made products, or have obtained additional meat flavor value by careful blending and/or by processes based on Maillard-type reactions. Of substantially less importance is the use of HVP as whipping, foaming or areating agent, as nutritional ingredients or as bread or baking improver [11]. Most of the HVPs used for these purposes are only partially hydrolyzed by enzymes or by alkaline treatment. The legal status of HVP, food ingredient or food additive, and its safety was discussed recently at various locations. So far these discussions have resulted in the tentative conclusions that HVPs are food ingredients which need standardization, and which, at their presently used levels, can be considered as being GRAS.

In the United States, where meat dishes are mainstay of the diet, nearly every home now has its bottle of soy sauce used to add meat flavor to various dishes. There is a considerable need for meat flavors and amino acids and peptides in meat flavored sauces and pastes for modifying and formulating foods in the diets of people in the developing world.

Soy sauce and Miso fermentations [6]

The Chinese thousands of years ago showed the world how to produce meat-like flavors from vegetable protein, which was one of the great discoveries in food science and which may become of even greater importance in the future. The soy sauce and miso fermentations were originally household fermentations, and they continue to be so in parts of the Orient today even though the products are also manufactured industrially in very large quantities (21, 3337).

Miso has been made in the home and on the farm since A.D. 1600 in Japan [12]. Indigenous miso manufacture is a very interesting process. Soybeans are soaked, cooked, mashed, and then formed into balls. The balls are tied together with rice straw and suspended above a stove or heater for about 30 days, during which time the balls become overgrown with moulds naturally present in the environment. The balls are then brushed, mixed with water and salt, and packed into crocks where fermentation continues for a year or longer. The resulting meat-flavoured paste is a primitive miso. Free liquid is used as a soy sauce.

At present, the Japanese use a koji for the manufacture of both soy sauce and miso. For soy sauce, the koji is prepared by overgrowing soaked, cooked, cooled soybeans coated with ground roasted wheat with moulds belonging to the *Aspergillus oryzae* species. Production of the koji is essentially a solid-state fermentation. The soy-sauce koji contains proteases, amylases, and lipases that hydrolyse their respective substrates in the subsequent submerged fermentation in 18 per cent weight per volume salt brine. During the submerged fermentation, *Pediococcus cerevisiae*, *Lactobacillus delbruekii*, and salt-tolerant *Saccharomyces rouxii* develop.

For miso, the koji is prepared from rice, barley, or soybeans. The fermenting organism again is *Aspergillus oryzae*. Following overgrowth of the mould, the koji is mixed with hydrated, cooked soybeans and salt. Miso is essentially in a solid state during its entire fermentation [13]. Hesseltine and Shibasaki demonstrated that the only micro-organism essential for the miso fermentation (in addition to the mould *Aspergillus oryzae*) was *Saccharomyces rouxil*, which develops when the pH of the mash has fallen to below 5.0. However, miso from an earlier fermentation can be used to inoculate a new fermentation (at the time of mixing the koji with salt and soybeans).

The salt content in various miso varies from 5.5 to 13 per cent (w/v) in fresh miso [12]. Sweetness depends on the proportion of cereal koji added to the soybeans [14]. Colour depends in part on the total cooking time given the soybeans and the length and temperature of fermentation. The higher the salt content, the slower the proteolytic hydrolysis and the better the miso keeps [15]. Both soy sauce and miso are highly industrialized in Japan. Yet, by their nature, both fermentations can be conducted on a small scale at the cottage-industry level or even in the home. In the miso-dame process, the fermentation depends on moulds naturally present in the environment and on the straw used to tie the soybean-mash balls. In some places in the Orient soy-sauce manufacturers today rely on the moulds present in wheat flour to produce a soy-sauce koji.

More recently, soy sauce has been manufactured by hydrolysing soybeans with concentrated hydrochloric acids and neutralizing the product with sodium hydroxide. While this does yield a meat-like flavour, the alcohols, organic acids, and esters produced by the fermentation process are lacking and the acid-hydrolysed sauce does not have the aroma and flavour of the genuine fermented sauce. Also, tryptophan is destroyed during acid hydrolysis, resulting in a loss of nutritive value.

The soy-sauce-miso process is used to produce meat-like flavours from soybeans, wheat, rice, and barley substrates. It could probably be adapted to other substrates, such as coconut and yeast, which yield meat-like flavours by acid hydrolysis. These meat-flavoured sauces and pastes could improve the nutrition and diets in many developing countries. The demand would support small-scale factories in most countries.

During fermentation, fungal and lactic acid bacterial (LAB) growth in the miso products was inhibited, whereas soluble protein contents increased much more rapidly in the low-salt miso products supplemented with 3% ethanol and 6% NaCl than the other products. When the 4- and 8-week-fermented miso products were cooked with tofu for sensory evaluation, flavor ratings of the low-salt products were higher than that of a popular commercial product [16].

In both products, the most daidzins and genistins were hydrolyzed after 4 weeks of fermentation. The hydrolytic enzymes contributing to isoflavone transformation originated from soybeans after water soaking and from koji with mold growth. It was of merit that the low-salt fermented products were fairly acceptable in flavor rating and rich in daidzein and genistein contents after 4 weeks of fermentation.

Fish and shrimp sauces and pastes

While soy sauce was developed in northern Asia, the South East Asians made an equally great discovery concerning how to convert small, surplus fish and shrimp into meat-flavoured sauces and pastes. In fact, the soy-sauce and fish sauce fermentations both depend on proteolytic enzymes to hydrolyse the proteins in the substrate to the constituent amino acids and peptides. In soy sauce, fungal enzymes are used, while in fish sauce, enzymes in the fish tissues, particularly the gut tissues, are involved. Both fermentations are carried out in concentrated salt brine (18 per cent for soy sauce; 23 per cent or higher for fish sauce). In their highest qualities, soy sauce and fish sauce are quite comparable in flavour: both are similar to beef broths. The processes are amenable to the use of surplus marine animals of many types (non-commercial) and could serve as a base for small-scale factories.

Over the past few years a number of accelerated processes for manufacturing fish sauces and pastes have been developed. Some of these involve the addition of vegetable proteases such as bromeli or papain to the fermenting fish [17]. Ismail [18, 19] developed a "rapid" process for fish sauce in which he produced a koji by growing selected strains of *Aspergillus oryzae* on soybeans and then mixing the koji with an equal weight of fish, thus effectively producing a fish-soy sauce.

Many new products are technologically possible by combining known fermentations with new substrates or new microorganisms. Much of the world has not as yet adopted fish-shrimp sauces or pastes to its diet, although they present an excellent opportunity to utilize surplus marine animals as substrates. The processes offer an opportunity to entrepreneurs who wish to start new businesses wherever surplus marine animals are available.

Meat Substitutes (analogues)

Large Western food companies have invested millions of dollars in developing processes by which soybean protein is extracted and concentrated to purities above 90 per cent and then by chemical modification and extrusion through platinum dies spun into protein strands which can be formed into pieces with meat-like texture. With added fats and meat flavors, the products are called meat analogues (a sophisticated name for imitation meats) and there is no doubt that these vegetable protein products will be an important part of "meat" consumption in the Western world in the future. Several products are already on the market [20, 21, 22, 23].

Similarly, large meat packers have developed processes in which soybeans are flaked, tempered, formulated, and extruded so that the products are subjected to high pressure and temperature for a short time and emerge from the extruder as chewy, protein-rich, meat-like nuggets that supply the flavour, texture, and nutritive value of meats in a number of Western dishes. In England, Rank, Hovis, and McDougall developed a process wherein mould mycelium is grown on low-cost carbohydrate, recovered by filtration, and formulated with added fat, flavour, and other components to produce meat analogues in which the mould mycelium provides the fibrous texture [24]. This process entails modern sophisticated food science and technology.

Nutritional Value of Meat Analogues [25]

With so many different meat alternatives available to consumers, the nutritional value of these foods varies considerably. Generally they are lower in fat than the foods they replace, although meat alternatives themselves vary a lot in fat content. With the increasing consumer demand for low-fat products, a number of "lite" or low-fat soy meat alternatives are appearing on the market. Consumers can buy nonfat hot dogs or lowfat sausages, for example. Most meat alternatives made from soybeans are excellent sources of protein, iron and B vitamins. Meat alternatives are sometimes fortified with other nutrients, such as vitamin B12. Taking a vitamin B12 supplement is often necessary for a vegetarian, depending on how restrictive our diet is. If we do not eat fortified vegan products, such as soy milk or faux meats, it is essential to get this vitamin elsewhere [26]. There are vegan-friendly multivitamins available, as well as vitamin B12 as a supplement itself. As vegetarianism and veganism rises in popularity, it's becoming easier to find these supplements made without gelatin, which comes from animals. These supplements can be found in chain and local health food markets, such as Whole Foods, as well as conventional grocery stores, pharmacies and supplement stores.

Traditional Tempe fermentation

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 soybean 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 [27]. 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 soybeans are the most common and activities, fresh tempeh has a limited shelf life. During storage, fresh tempeh eventually turns brown, the beans become visible because of senescence of the fungal mycelium, the material softens and ammoniacal odors emerge.

Microbiological aspects

The soaking stage

The microflora of soyabean tempeh is complex and its development starts during the soaking of the raw ingredients [28]. The microbial composition of traditional tempeh 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 tempeh contains high numbers of mesophilic aerobic bacteria, as well as enterobacteria, staphylococci and yeasts; in addition psychrotrophs may develop during refrigerated storage [29]. In principle, a significant lactic fermentation during the soaking stage contributes to low levels of pathogenic and spoilage microorganisms in tempeh. The predominance of lactic acid bacteria including streptococci in soaking horse-bean, chickpea and pea, 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 tempeh from Malaysia. No bacteriocin producers could be found in regular tempe, but from a spoiled 7-day-old sample of tempeh, bacteriocin-producing strains of *Enterococcus faecium* and *Lactococcus lactis ssp. lactis* were isolated [30]. Lactic acid bacteria dominate during the soaking stage of the traditional process [31]. As a result, a significant increase of organic acids takes place
(Table1). Acidification and other inhibitory effects of lactic acid bacteria were also shown to suppress the natural microflora [32] such as coliforms, *Klebsiella pneumoniae* [33] and yeasts [29], and to improve the shelf life of tempeh. 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 bacteria starter in soybean soak water [28] 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 tempeh. When contaminations were added on purpose, it was observed by several independent laboratories, that *Enterobacteriaceae* and *Bacillus cereus* were successfully inhibited [28, 33, 34]. Challenge tests with *Listeria monocytogenes* [35] also showed that this pathogen has great difficulty to survive in tempe in the presence of active *L. 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 [28]. 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 [36] but not yet demonstrated. The antibacterial effects of tempe appear to be rather diverse: no inhibition of enterotoxic Escherichia coli was observed [37], whereas in different settings broad spectrum antimicrobial effects against B. cereus, *E. coli*, *Bacillus subtilis*, *Proteus vulgaris*, *S. aureus* and *Salmonella typhimurium* were reported.

Fungal starters

The major genus of importance for tempe making is *R. microsporus*, with varieties microsporus, oligosporus, rhizopodiformis and chinensis [28]. An additional variety tuberosus was also described [38]. 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 [39]. 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 spores. 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 [40]; 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.

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-flavors 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 for use as fermentation starter.

Chemical and nutritional changes

Primary benefits of soybean fermentation are the improvement of organoleptic quality and nutritional value, rather than preservation. Raw soybeans are bitter in taste. Consecutive stages of the tempe fermentation process (soaking, leaching and enzymatic modification) result in the removal of the beany flavours [28]. During the period of fermentation a total transformation of soybeans occurs, unfolding a panorama of delicious new flavors and aromas, creating a unique texture and appearance, while simultaneously enhancing the nutritional value and digestibility [41]. During fermentation of cooked soybeans proteases, lipases, a variety of carbohydrates, 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 [28] contributing to a desirable texture, flavor and aroma of the product [42]. 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.

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 (0Æ5%), and unidentified components (6Æ5%) [43]. 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 [43]. 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 hydrolyzed, 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 [44]. The hydrolyzed protein at 46 h represented 25% of the initial protein. Of this hydrolyzed protein, it is suggested that 65% remained in the tempe as amino acids and peptides [43, 45], 25% was assimilated into mould biomass, and 10% was oxidized.

The degree of hydrolysis depends strongly on the fungal strain [46] and the fermentation conditions [47]. 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 [48]. 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 a-linolenic acid and the total level of free fatty acids is increased in the final product. 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 [43].

In soyabeans high levels of a-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 [43, 49]. Several tempe-forming *Rhizopus spp*. (*R. oligosporus*, *R. microsporus* var. *chinensis*, *R. oryzae* and *R. stolonifer*) were able to utilize the flatulenceassociated oligosaccharide raffinose as their sole source of carbon and energy [50]. However, [51] 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 [52], and during enzymatic maceration, predominantly the arabinogalactan and pectin fractions of the soyabean are solubilized [53]. Substantial glycohydrolase activities are tightly cell wall bound [40]. While reducing substances decrease, dietary fibre increases from 3Æ7 to 5Æ8% because of the growth of mould mycelia [49].

Anti-nutritional factors

Raw soybeans 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 [54]. Soaking and boiling reduces trypsin inhibitor activity [55]. 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 [51], tempeh fermentation reduces levels of phytic acid significantly, resulting in significant increases of calcium, zinc and iron [54, 56, 57]. Iron-deficient rats consuming tempeh achieved higher liver iron levels than those fed unfermented cooked soybeans [58]. Despite their anti-nutritional effect, protease inhibitors and phytic acid can also have positive health effects [59], such as suppression of carcinogenesis [60].

Vitamins

The increased content of some vitamins of the B group, especially riboflavin, niacin, vitamin B6, and vitamin B12, because of fungal and bacterial metabolic activities has been extensively examined [46, 61, 62]. An issue of specific interest is the production of vitamin B12. In the past, the use of inadequate bioassay methods for vitamin B12 determination gave an overestimation of this vitamin [28].

Using more specific methods [63], the vitamin B12 levels in tempe are estimated in the range of 2–40 ng/1 [61, 64, 65]. There is now a general consensus that not the mould, but the naturally occurring (or added) bacteria *K. pneumoniae* and *C. freundii* are responsible for vitamin B12 production [61, 66]. Carotenoids are formed in small amounts during tempeh fermentation, although b-carotene is not produced by all strains.

Feeding fermented soybean could have more distinct beneficial effects in individuals suffering from a decreased gastrointestinal digestive and/or absorptive capacity. The use of tempeh 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 soybeans. 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 [67]. Fermentation of soybean–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 porridge containing tempeh and yellow maize, compared with a similar porridge made of milk and yellow maize [68]. These limited data indicate that tempeh 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 which 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.

Anti-oxidative properties of fermented soybeans

Soybeans contain natural antioxidants. Tocopherols and phosphatides can be found in soybean 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 [69]. Their aglycones are genistein, daidzein and glycitein respectively. Fermentation of soybean foods causes increased antioxidative 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 [70], releasing potent anti-oxidant substances by transformation of flavonoids [71]. Besides the above mentioned, also factor 2 (6, 7,4¢-trihydroxyisoflavone) is found in tempe [72]. 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 [73, 74]. Hence, the addition of specific bacteria during the tempeh fermentation process could increase the level of factor 2 and of other isoflavone compounds in tempeh. Another anti-oxidative substance formed in tempeh was identified as HAA [75]. Of several soybean foods [59] tempeh had somewhat lower isoflavone content than tofu but contained elevated levels of the aglycones formed by enzymatic hydrolysis during fermentation [76]. 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 soy-foods including h were retained for ca 75% [77].

A Process for Raising the Protein Content of High-starch substrates

Millions of people in the world today, particularly the poor, use cassava (manioc, yucca) as a staple in the diet [6]. It is an excellent source of calories but is entirely too low in protein to provide the needs of the consumers. The Indonesians centuries ago developed a fermentation process whereby the protein content of cassava or any other substrate rich in starch can be increased, improving its overall nutritive value. The product is called tape ketan when rice is the substrate and tape ketela when cassava is the substrate.

The essential micro-organisms are *Amylomyces rouxli* and a yeast of the *Endomycoosis burtonii* type [78, 79]. The organisms are available in the markets of most South East Asian countries as a ragi cake. The micro-organisms utilize a portion of the starch, and the protein content of the tape ketan reaches approximately 16 per cent (dry basis) - about double the initial protein content of the rice. Of considerable nutritional importance, lysine-the first limiting amino acid in rice-is selectively synthesized, increasing about 15 per cent, and thiamine, which is very low in polished rice, increases 300 per cent, reaching a level close to that found in unpolished rice.

If cassava is the substrate, the tubers are peeled and steamed and inoculated with the powdered ragi cake. The tubers become sweet-sour and alcoholic. Again a portion of the starch is utilized, and the protein content on a dry basis can reach as high as 3 per cent, or even higher if the microorganisms use a higher proportion of the starch in the tuber. The product can be sun-dried and used as an ingredient in soups.

The tape fermentation, which provides a means of raising the protein content of high starch substrates and also increases the lysine and thiamine content of starchy substrates, is a potentially valuable industrial resource. It should be noted that tape ketan would very likely be a highly acceptable new food in many developing countries if it were produced by small factories and distributed in an attractive preserved form.

II. CONCLUSION

The developing world is rich in indigenous food fermentations that can contribute significantly to world small-scale food processing and consumption over the next 20 to 50 years as population reaches six to eight billion. The world needs low-cost methods of providing nutritious protein rich meat analogues for its millions of consumers. The Indonesian tempeh fermentation can serve as a model. A bacterium present in commercial tempeh can be used to add vitamin B_{12} to other vegetarian foods. Fuel requirements for cooking can be decreased by applying a fungal fermentation of the tempeh to legume substrates. The world needs high-quality meat flavours derived from vegetable protein. The soy-sauce (kecap)/miso (tauco) processes and the fish shrimp sauce and paste processes can be modified to yield a wide variety of meat-like flavours for use in formulating new foods. The protein content of high-starch substrates can be increased by applying the Indonesian tape fermentation. Leavened sourdough bread like products can be produced without the use of wheat or rye flours, using the Indian idli-dosa fermentation process. Production of such foods by small-scale food processors will contribute both to the economy of the country and to the nutritional improvement of consumers.

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References

1. Tamang, J.P., Thapa, N., Rai, B., Thapa, S., Yonzan, H., Dewan, S., Tamang, B., Sharma, R., Rai, A., and Chettri, R. 2007. Food Consumption in Sikkim with special reference to Traditional Fermented Foods and Beverages: A Micro-level Survey. Journal of Hill Research. 20(1).
2. http://www.nehu.ac.in/BIC/Fermented_Food_Final/introduction.htm.
3. Jeyaram, K., Singh, Th. A., Romi, W., Devi, A.R., Singh, W.M., Dayanidhi, H., Singh, N.R., and Tamang, J.P. 2009. Traditional fermented food of Manipur. Ind. J. of Traditional Knowledge. 8(1):115-121.
4. Tamang, J.P., and Fleet, G.H. 2009. Yeasts Diversity in Fermented Foods and Beverages. Yeast Biotechnology: Diversity and Applications. 169-198.
5. http://www.unu.edu/unupress/food/8F072e/8F072E04.htm
6. Steinkraus, K.H. 1983. Handbook of indigenous fermented foods: Marcel Dekker, New York.

7. Steinkraus, K.H. 2004. Traditional food fermentations as industrial resources. Acta Biotechnologica. 3(1): 3 – 12.
8. Lambrecht, H.S., Nielsen, S.S., Liska. B. J., and Nielsen, N. C. 1987. Effect of soybean storage on tofu and soymilk production. *J. of Food Quality*. 19(3**)**: 189 – 202.
9. http://jn.nutrition.org/cgi/reprint/125/3_Suppl/570S.pdf
10. Welsh, T. L. 2007. Meat and dairy analogs from vegetable proteins, analogs from vegetable proteins. J. of the American Oil Chemists' Society. 56(3): 404-406.
11. Olsman, H. 2007. Hydrolyzed and autolyzed vegetable proteins as functional food ingredients. J. of the American Oil Chemists' Society. 56(3): 375-376.
12. Shurtleff, W., and Aoyogi, A. 1976. The Book of Miso (Autumn Press), Kanagawa-ken, Japan.
13. Hesseltine. C.W., and Shibasaki, K. 1961. III Pure Culture Fermentation with Saccharomyces rorixli. Appl. Microbiol. 9: 515-518.
14. Ebine, H. 1977. Japanese Miso, Symposium on Indigenous Fermented Foods, Bangkok, Thailand. 21-27.
15. Robin Y., Chiou, Y. and Cheng, S. L. 1901. Isoflavone Transformation during Soybean Koji Preparation and Subsequent Miso Fermentation Supplemented with Ethanol and NaCl. J. Agr. Food Chem. 49 (8): 3656–3660.
16. Palo, L.J. Andrada, M.A., Anglo, L.G., and Argulles, L. 1969. *New and Quick Process of Manufacturing* Patis and Bagoong. Baens-Arcega. Philippine Patent No. 4133.
17. Ismail, M.S. 1977. Accelerated Fermentation of Fish-Soy Paste and Fish-Soy Sauce. Ph.D. thesis, Cornell University, Ithaca, N.Y., USA.
18. Ismail, M.S. Accelerated Fermentation of Fish-Soy Paste and Fish-Soy Sauce by Using *Aspergillus oryzae*. NRRL 1989, Symposium *on* Indigenous Fermented Foods, Bangkok, Thailand. 21 -27 Nov. 1977.
19. Hesseltine, C.W. (1965). A Millennium of Fungi Food and Fermentation. Mycologia. 57: 149-197.
20. Odell, A.D. (1968). Incorporation of Protein Isolates into Meat Analogues in R. l. Mateles and S.R. Tannenbaum, eds,, Single-Cell Protein. MIT Press, Cambridge, Mass., USA.
21. Skinner, J.J. 1975. Single-Cell Protein Moves toward Market. Chem. Eng. News. 53 (18): 24-26.
22. Wanderstock, J. J. 1968. Food Analogs, Cornell H. R.A, Ouarterly. 22-23.
23. Spicer, A. 1971. Synthetic Proteins for Human and Animal Consumption. Vet. record. 89: 482-486.
24. http://www.soyfoods.com/soyfoodsdescriptions/meatalternatives.html.
25. http://www.ehow.com/about_5373627_vitamin-foods-vegetarians.html.
26. Nout, M.J.R., and Kiers, J.L. 2005. Tempe fermentation, innovation and functionality: update into the third millennium (A Review). J. of Appl. Microbiol. 98: 789–805.
27. Nout, M.J.R. and Rombouts, F.M., Recent developments in tempe research, J. of Appl. Bact. 69, pp. 609–633, 1990.
28. Ashenafi, M. 1994. Microbiological evaluation of tofu and tempeh during processing and storage. Plant Foods for Human Nutrition. 45: 183–189.
29. 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*. J. of Appl. Micro. 92: 147–157.
30. Mulyowidarso, R.K., Fleet, G.H., and Buckle, K.A. 1991 a. Changes in the concentration of organic acids during the soaking of soybeans for tempeh production. Int. J. of Food Sci. and Technol. 26: 607–614.
31. Ashenafi, M. and Busse, M. 1991a. The microflora of soak water during tempeh production from various beans. J. of Appl. Bac*t*. 70: 334–338.
32. Tunc¸el, G. and Go¨ktan, D. 1990. Effect of different methods of soaking soya beans on the growth of Bacillus cereus, *Klebsiella pneumoniae* and *Staphylococcus aureus* in tempeh. J. of the Sci. of Food and Agr. 53: 287–296.
33. Ashenafi, M. and Busse, M. 1991 b. Growth of *Bacillus cereus* in fermenting tempeh made from various beans and its inhibition by *Lactobacillus plantarum*. *J. of Appl. Bact*.
70: 329–333.
34. Ashenafi, M. 1991. Growth of *Listeria monocytogenes* in fermenting tempeh made of various beans and its inhibition by *Lactobacillus plantarum*. Food Microbiol. 8: 303–310.

35. 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*, J. of Appl. Micro. 92: 147–157.
36. Kiers, J.L., Nout, M.J.R., Rombouts, F.M., Nabuurs, M.J.A. and Vander Meulen, J. 2002. Inhibition of adhesion of enterotoxic Escherichia coli K88 by soya bean tempeh. Letters of Appl. Microbiol. 35: 311–315.
37. Zheng, R.Y. and Chen, G.Q. 1998. *Rhizopus microsporus* var. *tuberosus* var. nov. Mycotaxon. 69: 181–186.
38. Nout, M.J.R. and Rombouts, F.M. 1990. Recent developments in tempe research. J. of Appl. Bact. 69: 609–633.
39. 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.
40. Ang, H.G., Nga, B.H., and Lim, K.K. (ed.) 1989. 7th World Congress of Food Science and Technology. Singapore, Singapore Ins. of Food Sci. and Technol.
41. 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 Microbiol. 19: 137–188.
42. Ruiz-Teran, F. and Owens, J.D. 1996. Chemical and enzymatic changes during the fermentation of bacteria-free soya bean tempeh. J. of the Sci. of Food and Agr. 71: 523–530.
43. De Reu, J.C., Ten Wolde, R.M., De Groot, J., Nout, M.J.R., Rombouts, F.M., and Gruppen, H. 1995 b. Protein hydrolysis during soybean fermentation *Rhizopus oligosporus*. J. of Agr. and Food Chem. 43: 2235–2239.
44. Higasa, S., Negishi, Y., Aoyagi, Y. and Sugahara, T. 1996. Changes in free amino acids of tempe during preparation with velvet beans *(Mucuna pruriens)*. J. of the Japanese Society for Food Sci. and Technol. – Nippon Shokuhin Kagaku Kogaku Kaishi 43: 188–193.
45. Baumann, U. and Bisping, B. 1995. Proteolysis during tempeh fermentation. Food Microbiol. 12: 39–47.
46. Ikasari, L. and Mitchell, D. 1998. Mimicking gas and temperature changes during enzyme production by *Rhizopus oligosporus* in solidstate fermentation. Biotechnol. Letters. 20: 349–353.
47. Heskamp, M.L. and Barz, W. 1998. Expression of proteases by *Rhizopus* species during tempeh fermentation of soybeans. Nahrung – Food. 42: 23–28.
48. Mulyowidarso, R.K., Fleet, G.H. and Buckle, K.A. 1991 b. Changes in the concentration of carbohydrates during the soaking of soybeans for tempeh production. Int. J. of Food Sci. and Technol. 26: 595–606.
49. Rehms, H. and Barz, W. 1995. Degradation of stachyose, raffinose, melibiose and sucrose by different tempeh-producing *Rhizopus* fungi. Appl. Microbiol. and Biotechnol. 44: 47–52.
50. Graffham, A.J., Gordon, M.H., Westby, A. and Owens, J.D. 1995. Nutrition of tempeh moulds. Letters in Appl. Microbiol. 21: 223–227.
51. 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. Appl. Microbiol. and Biotechnol. 37: 420–425.
52. 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 tempeh fermentation. J. of the Sci. of Food and Agr. 73: 357–363.
53. 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.
54. 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 microspores*. J. of Food Sci. 61: 1039–1042.
55. Agranoff, J. (ed.) 1999. The Complete Handbook of Temp, Singapore: American Soybean Association.
56. Macfarlane, B.J., Van der Riet, W.B., Bothwell, T.H., Baynes, R.D., Siegenberg, D., Schmidt, U., Tal, A., Taylor, J.R. 1990. Effect of traditional oriental soy products on iron absorption. American J. of Clinical Ntr. 51: 873–880.
57. 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. J. of Agr. and Food Chem. 45: 195–198.

58. Anderson, R.L. and Wolf, W.J. 1995. Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. J. of Ntr. 125: S581–S588.
59. Kennedy, A.R. 1995. The evidence for soybean products as cancer preventive agents. J. of Ntr. 125: 733S–743S.
60. Keuth, S. and Bisping, B. 1993. Formation of vitamins by pure cultures of tempeh moulds and bacteria during the tempeh solid substrate fermentation. J. of Appl. Bact. 75: 427–434.
61. Denter, J., Rehm, H.J. and Bisping, B. 1998. Changes in the contents of fat-soluble vitamins and provitamins during tempe fermentation. Int. J. of Food Microbiol. 45: 129–134.
62. 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.
63. Bisping, B., Hering, L., Baumann, U., Denter, J., Keuth, S. and Rehm, H.J. 1993. Tempeh fermentation – some aspects of formation of gamma-linolenic acid, proteases and vitamins. Biotechnology Advances. 11: 481–493.
64. Areekul, S., Pattanamatum, S., Cheeramakara, C., Churdchue, K., Nitayapabskoon, S. and Chongsanguan, M. 1990. The source and content of vitamin B12 in the tempehs. J. of the Medical Association of Thailand. 73: 152–156.
65. Wiesel, I., Rehm, H.J. and Bisping, B. 1997. Improvement of tempeh fermentations by application of mixed cultures consisting of *Rhizopus sp.* and bacterial strains. Appl. Microbiol. and Biotechnol. 47: 218–225.
66. 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.
67. Kalavi, F.N.M., Muroki, N.M., Omwega, A.M. and Mwadime, R.K.N. 1996. Effect of tempeh yellow maize porridge and milk yellow maize porridge on growth rate, diarrhoea and duration of rehabilitation of malnourished children. East African Medical J. 73: 427–431.
68. Berghofer, E., Grzeskowiak, B., Mundigler, N., Sentall, W.B. and Walcak, J. 1998. Antioxidative properties of faba bean-, soybeanand oat tempeh. Int. J. of Food Sci. and Ntr. 49: 45–54.
69. McCue, P. and Shetty, K. 2003. Role of carbohydrate-cleaving enzymes in phenolic antioxidant mobilization from whole soybean fermented with *Rhizopus oligosporus*. Food biotechnol.17: 27–37.
70. Stadler, P., and Kreysa, G. (ed.) 1997. 15th Dechema Biotechnology Conference. Frankfurt am Main, Germany: DECHEMA.
71. Hoppe, M.B., Jha, H.C. and Egge, H. 1997. Structure of an antioxidant from fermented soybeans (tempeh). J. of the American Oil Chemists Society. 74: 477–479.
72. Klus, K. and Barz, W. 1995. Formation of polyhydroxylated isoflavones from the soybean seed isoflavones daidzein and glycitein by bacteria isolated from tempeh. *Archives of Microbiol*. 164: 428–434.
73. Sudarmadji, S., Suparmo, S., and Raharjo, S. (ed.) 1997. Proceedings, International Tempe Symposium. Den Pasar, Bali, Indonesia. Indonesian Tempe Foundation, Jakarta, Indonesia.
74. Esaki, H., Onozaki, H., Kawakishi, S. and Osawa, T. 1996. New antioxidant isolated from tempeh. J. of Agr. and Food Chemistry. 44: 696–700.
75. Wang, H.J. and Murphy, P.A. 1996. Mass balance study of isoflavones during soybean processing. J. of Agr. and Food Chemistry. 44: 2377–2383.
76. 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. J. of Ntr. 130: 798–801.
77. Cronk, T.C., Steinkraus, K.H., Hackler, L.R., and Mattick, L.R. 1977. Indonesian Tape Ketan Fermentation. Appl. Fnviron. Microbiol. 33 (5): 1067-1073.
78. Ko, S.D. 1972. Tape Fermentation. Appl. Microbiol. 23: 976-978.

Table legend.

Table 1. Ethnic fermented foods of India (especially in Hilly areas)

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Products	Substrate	Nature and use	Major consumer	References
Kinema	Soybean	Sticky soybeans curry	Non-brahmin Nepalis	Tamang (2001)
Maseura	Black lentil	Dry-ball like condiment	Newar	Tamang (2005)
Gundruk	Leafy vegetable	Dried, sour soup/pickle	All	Tamang et al (2005)
Mesu	Bamboo shoots	Sour : Pickle	All	Tamang and Sarkar (1996)
Somar	Cow milk/ Yak milk	Paste, flavored condiment	Sherpa	Dwean and Tamang (2007)
Dahi / Shyow	Cow milk/ Yak milk	Curd: Savory	All	-do-
Ghee	Cow milk	Butter	All	Tamang (2007)
Sidra	Fish	Dried fish curry	Non-brahmin Nepalis	Thapa et al (2006)
Sukuti	Fish	Dried, salted	Non-brahmin Nepalis	Thapa et al (2006)
Kargyong	Beef/ Yak/ Pork	Sausage :Curry	Bhutias,Lepehas	Tamang (2005)