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CONTENTS

3. Soy Sauce and Miso
BRIAN J. B. WOOD

4. Breadmaking
ERKKI OURA, HEIKKI SUOMALAINEN and RISTO VISKARI

5. Cheeses
B. A. LAW

6. Fermented Milks
EBENEZER R. VEDAMUTHU

7. Fermented Vegetables
H. P. FLEMING

8. Coffee
ROBERT O. ARUNGA

9. Cocoa
J. G. CARR

10. Yeast Extracts
H. J. PEPPLER

species of lactic-acid bacteria as for manufacture of *idli* (Pederson, 1979; Sanchez, 1977).

3. *Fermented Cassava*

In several countries of West Africa (Nigeria, Ghana), *gari* is an important staple food. For its manufacture, fresh cassava (*Manihot utilissima*) is peeled, grated and the greater part of the juice squeezed out. The remaining pulp is left for three or four days for natural fermentation to occur. It was suggested that this is a two-stage fermentation. In the first stage, *Corynebacterium* spp. attack the starch with production of organic acids. The lower pH value causes hydrolysis of a cyanogenic glucoside with liberation of gaseous hydrocyanic acid. Organic acids also make conditions favourable for growth of *Geotrichum candidum*. This micro-organism produces a variety of aldehydes and esters, which are responsible for the characteristic taste and aroma of *gari* (Collard and Levi, 1959). Other reports also mentioned the probable role of *Leuconostoc* spp. (Okafor, 1977) and *Streptococcus faecium* (Abe and Lindsay, 1978).

The fermented pulp is finally fried in iron or earthenware pans. Sometimes the pan is greased with palm-oil, in which case the *gari* comes out yellow. The resulting product is dry and granular and has the property of swelling up in cold water. It can be consumed simply with water, without any cooking. Sugar may be added, or it may be mixed with spices (pepper) or other foods like fish or egg (Whitby, 1968).

E. Fermented Plant Juice

Throughout the tropics, the sugary sap of palm trees of many species is spontaneously fermented into alcoholic beverages. The microflora isolated during this fermentation are rather complex, but it is considered certain that *Zymomonas* spp. are largely responsible for the alcoholic content (4–5%) and carbon dioxide formation. The fruity odour and the taste of palm wine may be also due to production of some acetaldehydes by *Zymomonas* spp. Lactic-acid bacteria contribute to the acidity by producing small amounts of lactic and acetic acids (Swings and de Ley, 1977).

Similar bacterial alcoholic fermentation occur during fermentation of

the juice of certain species of *Agave* in Mexico to produce the alcoholic beverage *pulque* (Sanchez-Marroquin, 1977). Only small numbers of yeast cells are usually found in these alcoholic beverages. Because formation of alcohol and carbon dioxide from sugar fermentation is a characteristic of yeasts, alcoholic fermentation by bacteria is not a common process (Borgstrom, 1968).

IV. FOODS FERMENTED BY A MIXTURE OF MOULDS AND YEASTS

A. Ragi

Ragi itself is not a food; it is an inoculum used to induce fermentation of certain food products which will be described in this chapter. It is known in many Asian countries under different names (Table 4). In this review, it will be designated as ragi. Most probably, its origin is China, where *ch'ü* was already described in the old Chinese classics as the most important ingredient in the manufacture of alcoholic beverages. Later records gave thorough descriptions of its preparation and applications (Yamazaki, 1932). It is made of rice flour, which is moulded into flattened round cakes of 2–3 cm diameter.

Table 4

Names given in various countries to an inoculum used to manufacture certain food products

Name	Country
Bubud	Philippines
Ch'ü	China
Luk-paeng	Thailand
Nurook	Korea
Ragi	Indonesia
Ragi	Malaysia

B. Micro-Organisms

Numerous fungi and yeasts have been isolated from ragi (Boedijn, 1958; Dwidjoseputro and Wolf, 1970; Ko, 1965, 1972; Saono and Basuki, 1978, 1979). Of the many mould species which have been isolated from ragi, *Mucor* and *Rhizopus* species are the most important. They are

amylolytically, lipolytically as well as proteolytically active (Saono and Basuki, 1978). *Chlamydomucor oryzae*, recently re-identified as *Amylomyces rouxii* (Ellis *et al.*, 1976), plays a major role.

Yeast strains isolated from ragi included species of *Candida*, *Endomycopsis* and also *Saccharomyces*. They produce alcohol from the sugar which is produced from starch by the mould. It was reported that most yeasts isolated from ragi were amylolytically active, but only some were lipolytically active and none was proteolytically active (Saono and Basuki, 1978). It was found that, for a good fermentation of glutinous rice into good quality *tapé* (see Table 5), a combination of *Chlamydomucor oryzae* and *Endomycopsis chodati* was essential (Ko, 1972). This combination of micro-organisms was the starting point for later studies on *tapé* fermentation (Cronk, 1975; Cronk *et al.*, 1977, 1979).

C. Fermented Starch-Rich Raw Materials

When ragi is inoculated into a starch-containing substrate like steamed rice, cassava, maize or sorghum, a soft juicy product with a sweet, mild sour taste and mild alcoholic flavour is obtained after two to three days incubation at 25–30°C.

1. Fermented Rice

Such a product made of rice is known in different countries under different names (Table 5). It is consumed as such without any preparation and is considered as a delicacy. In China, *lao-chao* has a

Table 5

Names given in various countries to fermented glutinous rice (*Oryza sativa glutinosa*)

Name	Country
Binuburan	Philippines
Lao-chao Chiu-niang	China
Khao-mak	Thailand
Tapai pulut	Malaysia
Tapé ketan	Indonesia

unique place in the diets of new mothers. It is believed that *lao-chao* helps them regain their strength (Wang and Hesseltine, 1970).

2. Brem

In Indonesia, a confectionery is made by separating the juice which is produced during fermentation of *tapé ketan*. Traditionally, the liquid is dried in the sun until a solid cake is obtained. It is considered a delicacy, particularly for children, and is known under the name of *brem*.

3. Rice Wine

When fermentation of rice is extended to several weeks, more alcohol and more liquid are produced. To produce rice wine, the liquid is expressed and kept for clarification and ripening during several months. The beverage is well known under different names in different countries (Table 6). Depending on the fermentation time, the alcohol content differs and a concentration of 15% can be reached. By distillation, a beverage can be obtained with approximately 50% alcohol.

Table 6

Different names given in various countries to rice wine

Name	Country
Brem Bali	Indonesia
Mie-chiu	China
Saké	Japan
Sato	Thailand
Sonti	India
Tapoi	Philippines
Yakju	Korea

4. Tapé-ketella

In Indonesia, peeled, washed and steamed cassava tubers are fermented by inoculation with ragi to produce *tapé-ketella* or *peuyeum*. The final product is a food with a soft texture, a lightly sour and sweet taste and a mild alcoholic flavour. It is considered as a delicacy and is usually consumed without additional preparation. Sometimes it is shortly deep-fried in coconut-oil before consumption. It may also be mixed with other ingredients to make a kind of pie.

Species of *Rhizopus*, *Mucor* and *Aspergillus* are used for fermentation of foods throughout the Orient, with the exception of Japan. In Japan, it is restricted to species of *Aspergillus* including *A. oryzae* and *A. soyae*. The difference between Japan and other Asian countries in the application of mould species is thought to be due to the development of tane koji and its general use as a starter for food fermentations in Japan. Tane koji is made by growing moulds on steamed rice.

In other Asian countries, on the other hand, ragi-type starters (Table 4, p. 27) are in common use. These starters are made by cultivating moulds on cakes made of rice- or wheat-flour which has not been steamed or cooked. The difference in preparing growth substrates for manufacture of the two types of inocula is thought to be the cause of a natural selection of the mould species which were developing in each of the type starters over many centuries, when they were produced with non-aseptic, traditional methods (Sakaguchi, 1972). Hesseltine (1979b) suggested that the relative humidity of the areas may be an important selective factor, so that fungi in the order Mucorales are found in the tropics while *Aspergillus* species grow in semi-tropical countries such as China and Japan. Exceptions in Asian countries are *Monascus purpureus* for manufacture of *angkak* from rice, and *Neurospora* species for production of *oncom* from peanut presscake. In Occidental countries, only *Penicillium* species are used for fermentation of foods including Roquefort and Camembert types of cheeses and certain fermented meat products (Mintzlaff and Christ, 1973).

B. Lactic-Acid Bacteria

In vegetable, fish as well as some soybean fermentations, one or more species of lactic-acid bacteria play an important role. Their production of organic acids not only contributes to the desired taste and flavour of the final product, but it also makes the substrate unfavourable for proliferation of spoilage and other undesirable micro-organisms. At the same time, the acids make the substrate more suitable for growth of desirable micro-organisms which improve the properties of the food. The role of carbon dioxide produced by *Leuconostoc mesenteroides* is in different products not the same. In vegetable fermentations, it provides an anaerobic condition which inhibits proliferation of aerobic micro-

organisms. In fermentation of *idli* and *puto*, carbon dioxide is essential for leavening the dough.

C. Yeasts

When yeasts are involved in the fermentation process, production of alcohols improves the aroma of the product. In addition, alcohol at a certain concentration makes the substrate unsuitable for micro-organisms which may create undesirable properties in the product. Combined with the organic acids that are produced by lactic-acid bacteria, the inhibitory effect of alcohol on undesirable micro-organisms is increased.

D. Salt

Application of salt in fermentation of vegetables, fish and soybeans inhibits proliferation of putrefactive micro-organisms. Salt also affects the development of pathogenic and toxin-producing species. Growth of *Salmonella* spp. is prevented by concentrations of about 6% sodium chloride (Ingram and Kitchell, 1967). *Clostridium botulinum* is the species to which is attached most interest because of the fatal toxin produced when it multiplies in food, but all types of *Cl. botulinum* are inhibited by 10–12% salt (Mackie *et al.*, 1971). *Staphylococcus aureus* is able to resist up to 15% salt or occasionally even 20%, but 5% is the highest salt concentration at which toxin formation is recorded (Ingram and Kitchell, 1967). *Pseudomonas cocovenenans* died in 1% soya peptone medium containing 3.5% sodium chloride. Although 2% salt in a coconut medium did not suppress its growth, toxin production is completely suppressed (Ko *et al.*, 1977). On the other hand, some useful and unharmful species, including lactic acid-producing bacteria and some yeast species, are less affected by salt. *Lactobacillus delbrueckii* can adjust to growing readily on media containing 18% sodium chloride (Wood *et al.*, 1973).

The salt concentrations in many fermented foods are too high for micro-organisms to cause undesirable effects. Fermented fish pastes (Table 3, p. 22) contain 15–25% salt (van Veen, 1965; Mackie *et al.*, 1971; Soedarmo, 1972).

For the manufacture of fish sauces (Table 2, p. 22) approximately 30% salt is applied (van Veen, 1965; Saisithi *et al.*, 1966).

In the fermentation of vegetables, moderate salt concentrations of between 4 and 6% are used in the brine (Hsio Hui Chao, 1949). Notwithstanding the marginal salt concentrations, if the required conditions are supplied, lactic acid-producing bacteria will soon proliferate and produce enough acids. The combination of salt and acids will protect the product against undesirable effects of harmful micro-organisms. Considering also the protective role of certain mould species in fermented foods, it may be concluded that fermentation of indigenous foods is surrounded by many safety factors, provided the procedure of preparation is properly followed.

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REFERENCES

Abe, M.G. and Lindsay, R.C. (1978). *Journal of Food Production* **41,** 781.
Akinrele, I.A. (1970). *Journal of the Science of Food and Agriculture* **21,** 619.
Batra, L.R. and Millner, P.D. (1976). *Developments in Industrial Microbiology* **17,** 117.
Beuchat, L.R. (1978a). *Food Technology* **32**(5), 193.
Beuchat, L.R. (1978b). *In* 'Food and Beverage Mycology' (L.R. Beuchat, ed.), p. 224. Avi Publishing Company, Westport, Connecticut.
Bhumiratana, A., Flegel, T.W., Glinsukon, T. and Somporan, W. (1980). *Applied and Environmental Microbiology* **39,** 430.
Boedijn, K.B. (1958). *Sydowia* **12,** 321.
Boorsma, P.A. (1900). *Geneeskundig Tijdschrift van Nederlands Indie* **40,** 247.
Borgstrom, G. (1968). "Principles of Food Science", vol. 2. The Macmillan Company, New York.
Burkholder, L., Burkholder, P.R., Chu, A., Kostijk, N. and Roels, O.A. (1968). *Food Technology* **22** (10), 76.