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Applications of biotechnology to traditional fermented foods

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Upgrading Traditional Biotechnological Processes

M. J. R. Nout

TRADITIONAL FOOD FERMENTATION

The general aims of food technology are to exploit natural food resources as efficiently and profitably as possible. Adequate and economically sound processing, prolongation of shelf life by preservation and optimization of storage and handling, improvement of safety and nutritive value, adequate and appropriate packaging, and maximum consumer appeal are key prerequisites to achieving these aims.

Fermentation is one of the oldest methods of food processing. The history of fermented foods has early records in Southeast Asia, where China is regarded as the cradle of mold-fermented foods, and in Africa where the Egyptians developed the concept of the combined brewery-bakery. The early Egyptian beers were probably quite similar to some of the traditional opaque sorghum, maize, or millet beers found in various African countries today (1).

In technologically developed regions, the crafts of baking, brewing, wine making, and dairying have evolved into the large-scale industrial production of fermented consumer goods, including cheeses, cultured milks, pickles, wines, beers, spirits, fermented meat products, and soy sauces.

The introduction of such foreign "high-tech" fermented products to tropical countries by early travelers, clergymen, and colonists was followed by an accelerated demand during the early postindependence period. Their high price ensured status, and their refined quality guaranteed continued and increasing consumption.

In contrast, many of the traditional indigenous foods lack this image; some may even be regarded as backward or poor people's food. Factors contributing to such lack of appeal include inadequate grading and cleaning of raw materials, crude handling and processing techniques,

and insufficient product protection due to lack of packaging. Such unhygienic practices are easily translated into a fear of food-borne diseases. From a nutritionist's point of view, many traditional starchy staples are deficient in energy, protein, and vitamins. Variable sensory characteristics (quality) and lack of durability (shelf life) reduce convenience to the consumer: time needs to be spent selecting products of adequate quality, whereas perishable products require frequent purchasing and result in increased wastage. In addition, ungraded heterogenous products, inconvenient unpacked bulk foods, or unattractive presentation inhibit consumers to develop regular purchasing attitudes.

The contrast outlined here serves as a general guideline to the major targets for upgrading the present status of traditional indigenous fermented foods. The latter are part of the regional cultural heritage; they are well known and accepted by consumers and consequently provide an appropriate basis for development of a local food industry, which not only preserves the agricultural produce but also stimulates and supports agroindustrial development.

DECENTRALIZED SMALL-SCALE PROCESSES

In most African countries, 70 percent or more of the population lives in rural areas. However, if the present trend in urbanization continues (urban growth rates of 5 to 10 percent annually), 50 percent of the African population will be living in cities by the year 2000. Governments become increasingly aware that rural industrialization is a worthwhile investment because it creates job opportunities, improves agricultural productivity, and helps to check urbanization. But even at the present urbanization rate, a rapidly increasing low-income population will be located in urban areas. The resultant uncoupling in place and time of primary production and food consumption necessitates the manufacture of wholesome, low-cost, nutritious products that can withstand low-hygiene handling.

Agro-allied industries are closely linked to regions of primary production, and it is particularly in the field of food processing, with low-cost perishable raw materials, that establishment of a rural network of small-scale processing facilities is most appropriate. Home-or village-scale enterprises require only modest capital investment, which should be made available on a "soft loan" basis. Against this background, some basic process improvements that increase the appeal of traditional fermented foods and that can be carried out by simple means will be outlined (2).

BASIC PROCESSING OPERATIONS

In food manufacturing several operations are required to prepare raw materials, handle and process them into products, and finally prepare the finished product for distribution and sale by preservation and/or packaging. One might think of sorting, grading, cleaning, disinfection, grinding, or packaging. The establishment and success of some indigenous enterprises in Nigeria and Kenya show that the appeal and marketability of such products as beans, peas, *gari*, and spices, formerly sold in bulk, increase significantly when they have "only" been sorted, cleaned, graded, sometimes ground, labeled, and packaged in simple polythene bags.

NUTRITIVE VALUE

The nutritive value of traditional fermented foods needs improvement. The energy density of starch-based porridges is inadequate, particularly when used for weaning purposes. Root crop- or cereal-derived products have rather low protein contents, and the quality of their protein is limited by the amount of lysine present. Various antinutritional factors, including polyphenols, phytic acid, trypsin inhibitors, and lectins, are present in legumes and cereals.

Composite products (legume additions to starchy staples) offer an opportunity to improve protein quantity and quality. Combinations of simple unit operations, including roasting, germination, and fermentation, afford increased energy density in porridges and reduce antinutritional factors considerably (3).

STABILIZATION OF NATURAL FERMENTATIONS BY INOCULUM ENRICHMENT

Most traditional fermented products result from natural fermentations carried out under nonsterile conditions. The environment resulting from the chemical composition of the raw materials, fermentation temperature, absence or presence of oxygen, and additives such as salt and spices causes a gradual selection of microorganisms responsible for the desired product characteristics.

The main advantage of natural fermentation processes is that they are fitting to the rural situation, since they were in fact created by it. Also, the consumer safety of several African fermented foods is improved by lactic acid fermentation, which creates an environment that is unfavorable to pathogenic Enterobacteriaceae and Bacillaceae.

In addition, the variety of microorganisms present in a fermented food can create rich and full flavors that are hard to imitate when using pure starter cultures under aseptic conditions.

However, natural fermentation processes tend to be difficult to control if carried out at a larger scale; moreover, the presence of a significant accompanying microflora can accelerate spoilage once the fermentation is completed. Particularly with increased holding periods between product fermentation and consumption when catering for urban markets, uncontrolled fermentations under variable conditions will cause unacceptable wastage by premature spoilage.

Techniques to stabilize fermentations operating under nonsterile conditions would therefore be appropriate in the control of natural fermentations. For this purpose the use of pure culture starters, obtained either by laboratory selection procedures or genetic engineering, offers no realistic solutions because they are expensive and require sterile processing conditions. A more feasible approach is to exploit the ecological principle of inoculum enrichment by natural selection. This can be achieved by the sourdough process, in which some portion of one batch of fermented dough is used to inoculate another batch. This practice is also referred to as "back-slopping" or inoculum enrichment. The resulting starters are active and should not be stored but used in a continuous manner.

Sourdoughs from commercial sources, having been maintained by daily or weekly transfers during 2 or more years, contain only two or three microbial species, although they are exposed to a wide variety of potential competitors and spoilage-causing microorganisms each time the sourdough is mixed with fresh flour for a transfer. It can take as long as 10 weeks of regular transfers before a sourdough population becomes stabilized. Such populations could contain a yeast, *Saccharomyces exiguus*, and one or two *Lactobacillus* species, namely *Lb. brevis* var. *linderi* II and *Lb. sanfrancisco*. Although the mechanism of the stable coexistence of sourdough populations is not yet fully understood, lack of competition for the same substrate might play an important role. Other factors besides substrate competition, such as antimicrobial substances produced by lactic acid bacteria, might play an important role in the stability of such stable populations, obtained by "back-slopping" (4).

Similar experiments in the field of *tempe* manufacture showed that the first stage of the *tempe* process—soaking of soybeans—can be rendered more predictable in terms of acidification of the beans, by simple inoculum enrichment. Depending on soaking temperatures, stable soaking water populations were obtained after 30 to 60 daily transfers, containing *Leuconostoc* spp. at 14° and 19°C, yeasts and *Lactobacillus* spp. at 25°C, *Lactobacillus* spp. at 30°C, or *Pediococcus*

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and *Streptococcus* spp. at 37° and 45°C. *Tempe* made With Well-acidified beans contained fewer undesirable microorganisms and was more attractive (5).

Based on the same principle of inoculum enrichment, the intrinsic microbiological safety of composite meals of cereals and legumes can be improved significantly by lactic fermentation (6). This offers interesting possibilities in the manufacture of food for vulnerable consumer groups, such as infants, malnourished patients, and the elderly (7).

Although development of such gradually evolved and stable fermentation starters will be an attractive proposition for use in small-scale fermentations under nonsterile conditions, they will not be the most appropriate in all cases. This is exemplified by the sauerkraut (lactic acid fermented cabbage) fermentation, during which flavor development is determined by a succession of *Leuconostoc* and *Lactobacillus* species occurring during the course of the fermentation. Practical experience in the sauerkraut industry in the Netherlands has shown that carryover of previous sauerkraut into a fresh batch of cabbage will cause a rapid domination of homofermentative *Lactobacillus* spp., which should normally only dominate during the final stage of fermentation. The result is an excessively sour-tasting product that lacks the flavor otherwise produced by the heterofermentative *Leuconostoc* and *Lactobacillus* spp.

In the exercise of upgrading traditional food fermentation techniques, it would therefore be worthwhile to investigate the effect of inoculum enrichment on product characteristics and consumer acceptance.

MULTISTRAIN DEHYDRATED STARTER

A different tool to stabilize fermentations under nonsterile conditions is the use of multistrain dehydrated starters, which can be stored at ambient temperatures, enabling more flexibility. Such homemade starters are widely used in several Asian food fermentations. Examples are the manufacture of *tempe* (mainly from soybeans) and *tapé* (from glutinous rice or cassava). Indonesian traditional *tempe* starters (*usar*) are essentially molded hibiscus leaves that carry a multitude of molds, dominated by *Rhizopus* spp., including the *Rh. oryzae* and *Rh. microsporus* varieties. Instead of using *usar*, Indonesian *tempe* production is increasingly carried out with factory-prepared "pure" starters consisting of granulated cassava or soybean fiber carrying a mixed population of *Rhizopus* species (5). These starters are more homogenous and their dosage is convenient, but because they are manufactured under nonsterile conditions, some are heavily contaminated with

spoilage-causing bacteria and yeasts. This requires quality monitoring of the inoculum and of the fermentation process in which it is used.

Other examples of durable home-prepared starter materials used in Asian food fermentations are Indonesian *ragi* and Vietnamese *men* tablets (8). Depending on their specific purpose, these dehydrated tablets, prepared from fermented rice flour, contain mixed populations of yeasts, molds, and bacteria. *Ragi* tablets can be stored up to 6 months and constitute a convenient starter material for application in home and small-scale industrial fermentations of rice or cassava, for example.

Especially in the fermentation of neutral pH, protein-rich substrates, such as legumes, one should be extremely careful with the use of substandard inoculum. If the process lacks factors that control microbial development, pathogens may survive or produce toxins in such products. *Tempe* manufacture is a good example of a process with intrinsic safety. The preliminary soaking of the beans results in an acidification that inhibits the multiplication of bacterial contaminants during the mold fermentation stage. Also, antimicrobial substances of *Rhizopus oligosporus* would play a protective role against outgrowth of several genera of microorganisms. Moreover, near-anaerobic conditions and microbial competition during the fermentation stage, and the usual cooking or frying of *tempe* prior to consumption, strongly reduce the chances of food-borne illness (5).

Nevertheless, the introduction of fermentation processes in regions where they are not traditionally mastered requires adequate guidance, supervised processing, and monitoring of product safety.

ENZYME PRODUCTION BY KOJI TECHNIQUE

Not only microorganisms but also enzymes play an important role in the manufacture of traditional fermentation processes. In cassava processing the naturally occurring enzyme linamarase is able to degrade potentially toxic cyanogenic glycosides (e.g., linamarin). This enzymatic detoxification has always been an integral part of traditional cassava fermentations, such as in *gari* and *lafun*. Under certain conditions the detoxification of linamarin is accelerated by linamarase addition (9). It is conceivable that there will be commercial applications for the enzymatic process of linamarin decomposition, which could be used to detoxify cassava without having to ferment it; the result would be a neutral and bland-flavored product.

Enzyme sources for African traditional beer brewing are mostly germinated sorghum and millet varieties, whereas sorghum and millet malts possess adequate diastatic power with α -amylase, resulting in

poor conversion of dextrans into maltose (10). The availability of cheap technical-grade α -amylase preparations could lead to the development of novel brewing processes utilizing home-grown starch sources instead of imported barley malt.

In East Asia, *koji* is used as a source of enzymes in the manufacture of soy sauce and rice wine. *Koji* is obtained by solid-substrate fermentation of cereals or soybeans with fungi (e.g., *Aspergillus oryzae* and *Asp. soyae*). Depending on the particular substrate to be degraded, selected strains of molds are used, often as mixed cultures. Their enzymes include amylases, proteases, and cellulolytic enzymes. During fermentation the enzymes are accumulated into the *koji*. The enzymes produced are subsequently extracted from the *koji* using brine solutions. *Koji* fermentations are carried out in East Asia at a small home scale, as well as in the large-scale industrial manufacture of soy sauce and rice wine (11). Although mycotoxin-producing molds such as *Aspergillus flavus* and *Asp. parasitiosus* occur in *koji* as natural contaminations, they have not been observed to produce aflatoxins under the given conditions.

The principle of fungal solid-substrate fermentation may be used to prepare enzyme concentrations for conversion of starch, detoxification of cyanogenic glycosides, and other applications.

DRY MATTER BALANCE

Food fermentation is advantageously used for food preservation and to obtain desirable flavor and digestibility. However, some processes are rather wasteful. For instance, prolonged soaking and microbial respiration of organic matter may lead to considerable losses of valuable raw material dry matter. Examples can be found in the traditional process of *ogi* manufacture (fermented maize cake) and the *tempe* process, during which up to 30 percent of the raw material may be lost by leaching during soaking steps. Encouraging research has been carried out by Banigo et al. (12) in the field of Nigerian *ogi* manufacture, aimed at reducing these raw material losses by omitting soaking stages. It would certainly be worthwhile to investigate dry matter balances of traditional fermentations with a view to reducing losses of raw material by implementing "dry" instead of "wet" processing.

IMPLEMENTATION

No matter how much research is carried out on improved traditional processes or novel products, the ultimate aim is implementation.

Unfortunately, a wide gap exists between research data published in scientific journals and the practice of food processing. Much attention should be given to the extent of usefulness of new products to the end user. To this effect, not only should the sensory, nutritional, and other quality characteristics of newly developed products or processes be taken into account, but they should also be integrated with sound price calculations, market surveys, and extension efforts. Only a competitive process has good chances of being implemented.

In conclusion, the importance of a business-oriented approach and close contact between researchers and food processors, working together toward mutual benefit, must be stressed.

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