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Research review paper

Koji – where East meets West in fermentation

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ABSTRACT

Almost all biotechnological processes originate from traditional food fermentations, i.e. the many indigenous processes that can be found already in the written history of thousands of years ago. We still consume many of these fermented foods and beverages on a daily basis today. The evolution of these traditional processes, in particular since the 19th century, stimulated and influenced the development of modern biotechnological processes. In return, the development of modern biotechnology and related advanced techniques will no doubt improve the process, the product quality and the safety of our favourite fermented foods and beverages. In this article, we describe the relationship between these traditional food fermentations and modern biotechnology. Using Koji and its derived product soy sauce as examples, we address the mutual influences that will provide us with a better future concerning the quality, safety and nutritional effect of many fermented food products.

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1. Introduction

A millennium of fungi, food, and fermentation was the title of the inaugural address by Clifford W. Hesseltine in his position as the new President of the Mycological Society of America. He gave this presentation at the society's meeting in Colorado on 25 August 1964 and stated (Hesseltine, 1965): "I propose to discuss an area of industrial mycology that has been neglected in most courses of mycology and industrial microbiology, and in which there is little or often misleading information. This is the field of the fermentation of raw agricultural products into foods. … What I shall discuss are some of the foods of the world with which most of you are unfamiliar – foods prepared by the action of diverse species of fungi on various animal and plant materials. The fermentations vary from the most simple to such highly specialized processes as the production of shoyu [soy sauce] in the Orient. The largest companies involved in shoyu fermentation compare favourably with our best fermentation companies in the size of their operation, in the capabilities of their research staff, in their quality controls, and in their modern marketing and advertising programs. Today Japan stands among the top countries in fermentation and, I predict, may eventually assume the leadership in the world in this field." Hesseltine was quite

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right in his prediction and whole processes have been exported from Japan to the Western world in the last two decades.

In fact, Japanese technology had already been initiated in the Western fermentation industry at the end of the nineteenth century by Jokichi Takamine. He introduced and adapted a traditional Japanese fermentation process, i.e. the Koji process, for making starch-hydrolysing enzymes (amylases), in the American beer and whiskey manufacturing industry, quite the reverse of what was usual at that time (Bennett and Yamamoto, 2004). Traditional Japanese Koji consists of with-fungi-fermented soybeans and/or grains such as wheat or rice. For over a thousand years *Aspergillus* strains have been extensively used as starter cultures in the manufacture of Koji in the Japanese traditional fermentation industry, involving the production of rice wine (sake), soy sauce (shoyu), soybean paste (miso), and distilled spirits (shochu). The most widely used fungus for Koji production is *Aspergillus oryzae* and this strain has even been recognised as Japan's national microorganism in much the same way as cherry blossom (sakura) is the national flower (Kitamoto, 2002). In addition to being a carbohydrate- and protein-hydrolysing agent, Koji, also contributes to the characteristic colour, flavour and aroma of oriental fermented foods.

The use of Koji as a digestive aid was already described 2500 years ago in a Chinese classic book entitled *Zuo-Zhuan*, in English *Chronicle of Zuo* or *Commentary of Zuo* (Sailey, 1992). In the description, wheat-based Koji was used to treat digestion problems; from the modern biochemical view it is clear that this is the action of amylases and proteases, coincidently the scientific discovery and commercial success of Takamine 100 years ago. The application of Koji for starch hydrolysis in grains and/or soybeans is the key element in the production of classical fermented foods, beverages and seasonings in the Orient (Murooka and Yamshita, 2008). Soy sauce, derived from Koji, was also invented in China over 2500 years ago and the recipe was brought to Japan in the thirteenth century by Buddhist monks (Fukushima, 2004). The Japanese word Koji (麹) still uses the Chinese character that means (wheat) grains fermented by fungi.

At the end of the twentieth century two leading Japanese soy sauce companies exported their technology to the Western world. In June 1992 Yamasa Corp. established a USA production and distribution facility in Salem, Oregon. In October 1997 Kikkoman Corp. (Box 1) did so in Sappemeer, the Netherlands, with the aim of supplying the European market; until then this had been done from Singapore. Both companies cite the presence of clean drinking water as an important reason for their choice of location. Interestingly, in the Dutch-Japanese factory of Kikkoman there is a mix of Japanese and Dutch equipment. The Dutch part consists primarily of pieces of apparatus from the malting industry, malt being the Western counterpart of Koji. The management is largely Japanese and the production team a mix of Dutch and Japanese people, with the first Dutch operators having had training in Japan to acquire hands-on experience.

Box 1
The hexagonal logo on the Kikkoman products represents the shell of a turtle containing the Japanese character meaning ten thousand. According to Japanese tradition the turtle lives ten thousand years and as such is a symbol of happiness and a long life.

"Kikko" means turtle in Japanese and "man" the word for ten thousand. At the time Kikkoman was therefore chosen as a trademark for the best soy sauce of the family Mogi, founders of Kikkoman soy sauce. Later it also became the name of the company (Janssen, 1997).

2. Forerunners of modern biotechnology

Fermentation biotechnology is older than documented history, even outside Asia. To give an example, malting and brewing were already taking place in Mesopotamia (current-day Iraq) in 4000 BC (Tramper and Zhu, 2011). Traditional fermentations of foods such as bread, beer, cheese, wine and soy sauce, are the forefathers of modern biotechnology (Fig. 1). These products still occupy a key place in our life today. The developments and improvements in making these foods were empirical before the nineteenth century and are now largely science-based. A prominent example is soy sauce of which the fermentation technology has been improved with experience through the ages. From the 1950s on, however, a more scientific approach was taken, resulting in entirely different production techniques and facilities. Today, soy sauce is produced using ultramodern and sophisticated methods and facilities (Fukushima, 2004).

Fermented foods were first thoroughly reviewed in English by Hesseltine (Hesseltine, 1965). Since then, many reviews and books describing various traditional food fermentations have appeared, for example the books *Industrialization of indigenous fermented foods* by Steinkraus and *Microbiology of fermented foods* by Wood (Steinkraus, 2004; Wood, 1998). Fig. 1 shows a few selected, representative fermented foods worldwide (Nout et al., 2007). In this paper the primary focus is on Koji. Koji is the starting point of numerous oriental traditional fermented foods in China, Japan and other Southeast Asian countries, and is nowadays utilized worldwide. The secondary focus is on soy sauce, which as mentioned previously is an example of an important oriental product manufactured in a complex, highly specialised, ultra-modern process all over the world.

Historically, Koji is a solid fermented mixture of grains and/or soybeans containing not only starch-hydrolysing enzymes (amylases), but also proteinases, cellulases, and phytases, among others (Liu et al., 2004; Machida et al., 2008; Singh et al., 2008). The main microorganisms in traditional Koji are fungi such as *Aspergillus*, *Rhizopus*, *Mucor*, *Monascus* and *Penicillium*. Each type of Koji has its own microbial flora. In a typical traditional process steamed rice and/or grain is/are mixed with fungal spores and put on porous plates, on cloths or in wooden containers. To achieve the right conditions for fungal growth and the desired enzyme production, air is passed through these systems. In modern industrial applications, most Koji is prepared using pure cultures of fungi, temperature- and humidity-controlled air, and in sophisticated fully-automated Koji-machines to ensure the quality and safety of the products (Fig. 2).

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