Koji

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Introduction

Ki-Koji (or yellow *koji*)[4], is the product of inoculation of the filamentous fungi *Aspergillus oryzae* to a grain in order to facilitate fermentation in the absence of necessary chemically reactive catalysts (enzymes) within the grain itself.

Other types of koji exist and are briefly mentioned in the 'Other products' box below, but for the purposes of this overview, we will focus on yellow *koji* and this is because it is the primary *koji* for the production of Japan's national drink, saké. However, for simplicity, we will refer to *ki-koji* henceforth as *koji.*

Saké Brewing Process

Key
1-7 – Commonly shared sequence of activities between breweries.
A-D – Activities which are carried out in different orders, or omitted, by different breweries.

1. Polishing
精米 [7]

Also referred to as milling, this initial process removes much of the bran, proteins, lipids and inorganic substances such as potassium and phosphate which can contribute undesirable flavours to saké[5,6,7].

The degree of polishing is a percentage of the weight of bran removed in proportion to the remaining grain[6] and the degree of polishing influences the style and categorisation of the final saké[7].

日本酒の製造工程

A. Bottling
瓶詰め [7]

Bottling can take place before or after pasteurisation and in the case of premium saké, it will often happen immediately after pressing, without filtration[7].

D. Pasteurisation
火入れ [7]

If pasteurised the saké is heated to about 65°C in order to kill any residual yeast and harmful microorganisms, inactivate enzymes, adjust the maturation velocity and stabilise flavour[5,7].

This process can occur before or after bottling or maturation[7].

2. Washing & Soaking
米洗い・浸漬 [7]

Post-polishing, rice is washed and then soaked in water for a pre-determined time[6,7]. These processes allow the rice to absorb water which is vital to the subsequent steaming and mashing stages. During the wash stage, grains absorb 9-17% of their weight and this rises to 25%-30% during steeping, thereby enhancing the ability of heat to penetrate the grain and accelerating starch and protein degradation[5].

3. Steaming
蒸米 [7]

During the steaming process, rice starch is gelatinised, protein is denatured, rice grain endosperm cell walls are partially disassembled and the grains sterilised. In addition, some glycerides are decomposed to liberate fatty acids, and free fatty acids also evaporate or partly decompose. The grains absorb more water, resulting in a gain of about 35-40% from the start of the process[5].

7. Pressing
上槽 [7]

The most common method used to separate the liquid from the solids is by using air pressure to pass the *moromi* through screens[7]. The liquid passed through is our *saké* and the separated solids can be used for making foodstuffs such as pickles[5].

B. Filtration
濾過 [7]

During filtration, saké is treated with activated carbon or charcoal to improve clarity and to eliminate undesirable flavour influences[5]. For lower grade types, filtering is often done just before bottling in order to improve the saké colour[7].

C. Maturation
熟成 [7]

During maturation, the colour of saké deepens and its taste becomes mild[5]. Maturation can take from 3 to 12 months depending on the brewery and desired properties of the final product[5,7]. This process can take place before or after pasteurisation[7].

After storage, sake is often blended and diluted with water reduce the alcohol content to 14-16%[5].

4. Making Koji
製麹 [7]

This stage is one of the most critical in the process[8]. Between 15-25% of the steamed rice is cooled (to nearly 40°C[5]) and taken to a temperature and humidity controlled room for *koji* production[5,7]. Dried spores or seed moulds of *A. oryzae* are sowed over the steamed rice and mixed,[1] then cultured at around 34-36°C for 24 to 58 hours[5,7]. *Aspergillus* is vulnerable to changes in temperature and moisture so maintaining consistency of both is a must[7].

When the temperature of the moulded rice rises to 40-42°C mycelia develop to cover and penetrate into the grains (fig. 1). The resultant product is *koji* which contains sufficient enzymes and various nutritive substances to promote mashing, yeast growth and fermentation[5].

5. The Yeast Starter
酒母造り [7]

Once the *koji* is made, a small amount of it and some steamed rice are added to water and yeast (*Saccharomyces cerevisiae* var. *saké*) for 2-4 weeks during which time rapid yeast growth occurs[6,7]. The temperature is monitored throughout[7] and maintained at around 20°C[5].

6. The Main Mash
醪造り [7]

Steamed rice (cooled to around 10°C), *koji* and water are added to the yeast starter for mashing, the volume of the additions being around twice that of the starter[5]. At this stage the temperature is around 12°C. After 2 days double quantities of rice, *koji* and water are added to the mixture and the temperature lowers to about 9-10°C. The remainder of the ingredients are added the next day lowering the temperature again to 7-8°C[5]*. The final alcohol concentration of this *moromi* is 20%-22%[6].

The simultaneous process of *koji* saccharification and yeast fermentation is called 'multiple parallel fermentation'[2].

Table 1: Enzymes involved in Saké brewing.
(Adapted from Okuda *et al 2019*, Iwano *et al* 1986 & Zhang, Wu & Oin 2020)

Enzyme	Function	Optimal pH	Optimal Temp
α-amylase	Hydrolyses α-1,4 glycosidic bonds in amylose and amylopectin of rice starch into glucose, dextrins, oligo- and mono-saccharides	4.2 ~ 5.5	50°C
Glucoamylase	Hydrolyses α-1,4 glycosidic bonds from the non-reducing end of the rice starches into glucose	3.2 ~ 6.0	60°C
Acid protease	Hydrolyses peptide bonds of rice proteins into peptides assisting amylase activity.	3.0	50°C
Acid carboxypeptidase	Hydrolyses peptide bonds of proteins forming amino acids	3.0 ~ 3.5	50°C

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**Timings and phases may vary between breweries.*

Koji flavour contributions

As referenced in table 1, amylase enzymes form glucose, dextrins and other saccharides in a process known as *saccharification*. This process provides yeast with the sugars required for ethanol formation via fermentation[2]. The role of the proteases is, according to *kurabito* Andrew Russell (2020), "to provide *umami*". The peptides and amino acids formed by the proteases which provide nutrients to yeast and encourage growth, but also impart their own flavour into the saké.

During fermentation yeast then forms higher alcohols, esters and organic acids which all contribute to flavour. Regarding flavours derived from the rice proteins by *koji*, these are divided into amino acids (table 2) which can be a source of *umami*[2,8,9] and, peptides which bring a round and mellow quality to saké[2].

Table 2: Amino Acid flavour contributions.
(Adapted from Furukawa. 2012)

Amino Acids	Flavour
Arginine & Threonine	Gentle sweet
Alanine	Very sweet
Glycine	Fresh sweet
Proline	Sweet and Sour
Glutamic Acid	Strong *umami* and sour
Leucine	Faintly Sweet

Saké flavour is also be influenced by the degree of *koji* propagation[8,9].

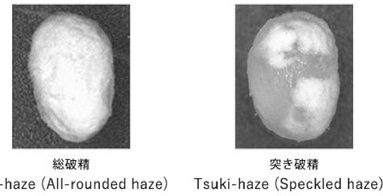

Figure 1: Two main states of koji propagation.
(Image reproduced from Sake Street, 2020)

The relative differences in propagation result in different rates of enzyme activity within the koji. *So-haze* has high levels of enzyme activity and imparts a character of sweetness and *umami*. Whereas *tsuki-haze* has less enzyme activity and can produce saké with a drier taste[3,9].

Other Products

Other varieties of rice *koji* are available for use in the production of alcoholic beverages. *Kuro-koji* (black *koji* from *Aspergillus luchuensis*) is required by law to be used in the production of *Awamori,* which is a unique type of *shochu* from Okinawa, and *shiro-koji* (white *koji* from *Aspergillus kawachii*) is generally used for most *shochu* production. Both have high levels of citric acid which confer anti-bacterial properties[4].

In addition, *koji* is used for fermentation of soy beans, wheat and chestnuts (and more) in a variety of products including soy sauce[2], *natto*, *mirin*[2] *miso*[2] and a chestnut *shochu* which is a local speciality in my future home prefecture.