

Fermentation and bio-functions of sake and Chinese rice wine: implications for ‘healthy-alcoholic industry’

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Abstract. Traditional low-alcoholic beverages were promoted to be healthy drinks with multiple bio-functions. Research into Japanese sake and Chinese rice wine (CRW) in terms of the preparation process, bacteria diversity, authentic quality control and bio functions is of significance. Japanese sake and CRW are representative alcoholic beverages which were proven to bring health benefits. For instance, sake was found to contain bio-functional components of CRW were found to obtain antioxidant activities. In this article, the production mechanisms and their bioactive components in Japanese sake and CRW were discussed. Red yeast rice is made by fermenting the rice with *Monascus*. However, commercial lovastatin (a structure that similar to monacolin K) is recently reported to be illegally added to common red yeast rice to meet drug quality standards, herein, there are many safety issues of rice wine, and it is especially important to dress the authentication assessment of the CRW. A new era of CRW as a low-alcoholic healthy beverage is coming. Prospects for the future development of CRW, with an emphasis on the absorption of the successful regulation experience from Japanese sake productions which are with high reputations worldwide, are presented.

Keywords: Japanese sake, CRW, comparison study, formation mechanisms, bioactive components, standardization, preservation, regulations.

1. Introduction

Sake (often referred to as nihonshu in Japanese), Japan’s cultural and gastronomic heritages, is progressively becoming more and more well-known globally. The history of sake is believed to be originated over 2000 years ago. In Japan, there are more than 1,500 factories of brew sake [1]. Nowadays, manufactories are paying a greater attention to the regionality and development of new styles as well as improving production techniques, to boost the sake industry with the increasing market size. Developing new products is of importance to maintain the expenditure of sake domestically and internationally. For instance, the development of sparkling sake is a prominent example of this trend to increase diversity. Rice wine is the other kind of rice based fermented beverage. It is a kind of ransparent liquid with light yellow color. In China, Shaoxing rice wine is a widely consumed type of rice-based fermented beverage [2]. Huangjiu has a long history and has been popular in China for thousands of years. The composition of variuos microbiomes in it affects on the flavour and quality of the final products. Therefore, the components, quality control parameters and flavour contributing compounds, as well as the risk of bio-hazards during the fermentation procedure are critical for maining its market size. To improve the bio-functions and the overall sensory of Huangjiu, modification of microbiome

formulations and control of the fermentation process is the trend. The future development of CRW could be taken the reference to the successful regulation experience from Japanese sake productions which would bring implications for a boosting 'healthy alcoholic industry' in China.

2. Production of Sake

Sake, a kind of popular moderate-alcoholic beverage (alcohol content ranges around 13% - 17% vol) with over 1300 - a year of history in Japan, is well known in many Asian countries [3]. With advanced sake-producing techniques, the flavour, aroma and physicochemical properties were improved rapidly, and its positive healthcare effects were validated through a diversity of scientific research. Sake is becoming a famous healthy moderate-alcoholic beverage with a high reputation worldwide. Quality assessment of sake deserves to be investigated in depth to ensure its safety, taste, and bio-functions. The composition of sake, beer and wine were compared in Table 1. The alcohol content is similar to that of wine, whereas, glutamic acid is enriched in sake. Glutamic acid in sake contributes to the overall bio-functions of sake as it is promoted to be helpful in people with diabetes, and may have functions in prevention of the nerve damages in cancerous people with chemotherapies [4].

Table 1. Composition of sake, beer and wine compared.

	Sake	Beer	White wine
Alcohol (%)	13-17	4-6	10-13
Extract (g/100ml)	3-6	3-4	2-8
Glucose (g/100ml)	0/5-4.2	0.03-0.1	0.1-3
Nitrogen (mg/l)	700-1900	250-1000	100-900
Glutamic acid (mg/l)	100-250	10-15	10-90
Titrateable acidity (g/100ml)	0.1-0.2	0.15-0.2	0.5-0.9
pH	4.2-4.7	4.1-4.4	3.0-4.1
Succinic acid (mg/l)	200-500	40-100	500-1500
Malic acid (mg/l)	100-400	50-120	250-5000
Tartaric acid (mg/l)	0	0	1500-4000
SO ₂ (total) (mg/l)	0	-20	-250

After specific pretreatments, these raw materials were mixed with water at reasonable ratios to ferment for 2 weeks to 1 month below 15°C. Sake rice, koji, and sake yeast play a key role in Japanese sake production. CRW have distinctive differences in the production process. Rice grains used in sake are polished, and the outer layers are removed. The polished rice grains are then steamed, mixed with koji, yeast, and water. Proteolytic enzymes will digest the proteins into small peptides and various free amino acids, these enzymes are mainly secreted by *A. oryzae*. After filtration, ageing, bottling, and pasteurization, the sake product is finally produced [5-7].

3. Standardization of sake in Japan

According to Japanese relative sake regulations, sake must contain less than 22% alcohol. Outline of Japanese sake brewing process was shown in Figure 1. Profiles of 24 chemical components in 20 different sakes were shown in Figure 2. One issue is that the bacteria responsible for the fermentation process present in sake should be detected, but there is a lack of control of these important organisms, to apply modern research technologies, for instance, using exhaustive DNA sequence analysis of bacterial fora and chemical components of sake should be proposed as a standardization process to improve the quality control of sake [8].

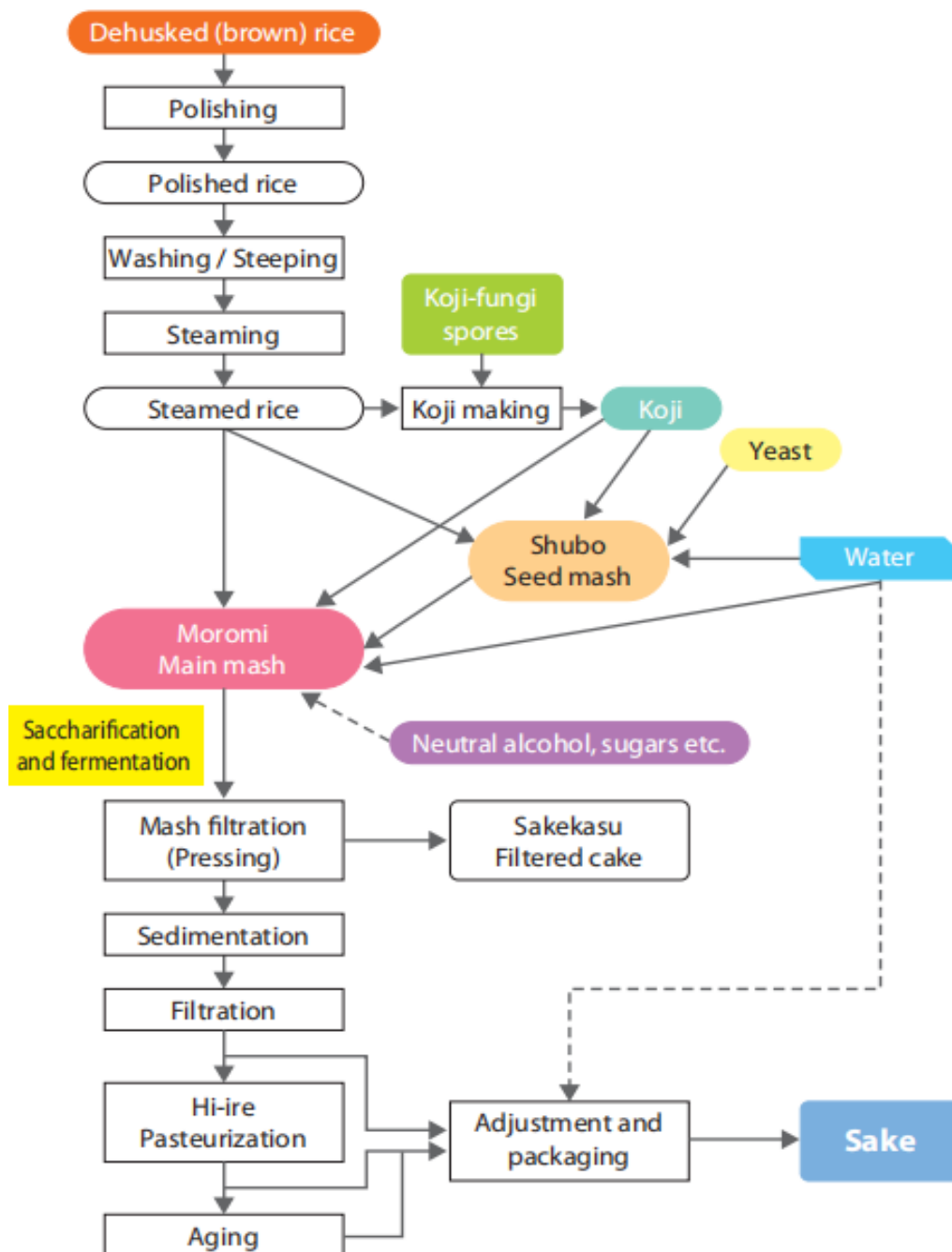


Figure 1. Outline of Japanese sake brewing process.

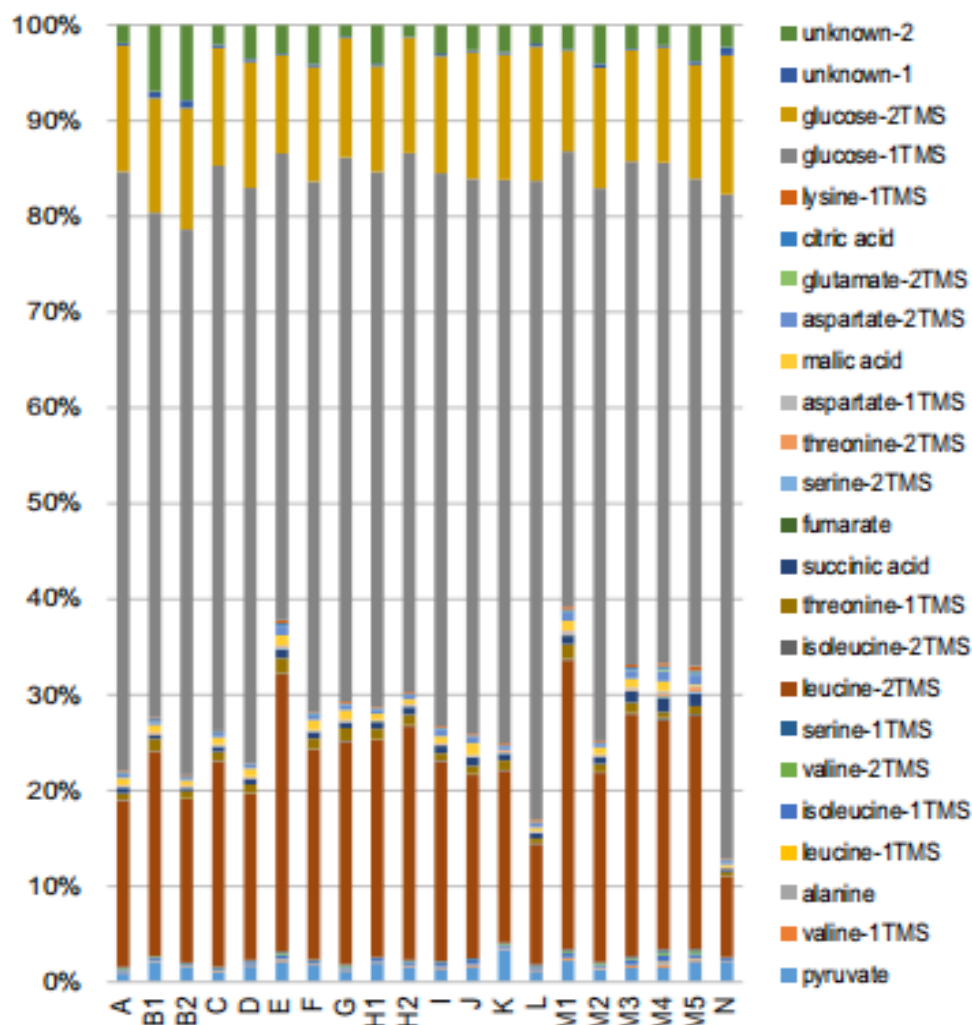


Figure 2. Profiles of chemicals in various sakes (20 analytes). X axis indicated sake production company. Numbers indicated various sake brands. Y axis indicated the percentage of each chemical component in sake, individual chemical compound was measured through the gas chromatography equipped with a fame ionization detector (GC-FID) [8].

4. Health benefits of sake

To date, sake is believed to contribute to a holistic treatment of various diseases. Amino acids are enriched in sake which serve the shaping of sake flavor. Sake obtains 2 to 5 times of amino acids than that of ordinary wine. The alanine, arginine, glutamate, valine, proline, lysine, and leucine have different bio-functions. Peptides (short chains formed by amino acids) in brewed wines are important functional components. Pyroglutamyl peptides are especially enriched in sake. The processing procedures including enzymatic modifications and fermentation could generate a group of pyroglutamyl peptides [9-16]. Its hepatoprotective effects have been well studied [17]. A low stroke risk was indicated in sake intaken group which may be owed to the antioxidants in sake. Theories suggest that sake correlates with a better digestion ability and helps to improve the immune system. An increase of IgA and mucin levels was detected in sake intaken mice model [18]. Sake has moderately calories and sugars. The possible health benefits of sake are not suitable to a weight-loss diet. Many of the unique properties in sake come from the specific yeast strains and koji activity. Some researchers believe these properties can positively impact the body on the cellular level. Protein is a major macromolecular substance of Japanese sake

[19]. Scientists have conducted numbers of experiments *in vitro* and *in vivo* to test the healthy benefits of sake [20]. Studies also reported that a specific amount intake of sake presented an anxiolytic effect and can help to improve sleep. In addition, sake contains a specific sugar (α -D-glucoside ethyl ester) which has been reported to inhibit chronic alcoholic liver injury by suppression of the GalN-induced elevation of ALT and AST activities. In addition to contributing to the overall sensory including the flavor, aroma, and mouth feel of alcoholic beverages, proteins are involved in the development of brewed wine turbidity, and partially converted into corresponding small-molecule metabolites which are responsible for the beneficial effects [21-23].

5. Rice wine: starter cultures and production process-in comparison to sake

There are numbers of rice wine worldwide, the functional yeast and mold in starter cultures vary a lot which lead to a diversity of properties of rice wine (Table 2).

Table 2. Rice wine production in various countries, the starter culture and the functional yeast used in starter cultures.

Country	Type of rice wine	Functional yeast and mold present in starter cultures
China Mie-chiu, Shaohing, Huangjiu	Mie-chiu, Shaohing, Huangjiu	Saccharomyces cerevisiae, Wheat koji
Japan	Sake	A. Oryzae, A. awamorii, Saccharomyces cerevisiae
Korea	Yakju, Takju	Yeast, Wheat barn koji, Nuruk (traditional natural starter with bacteria, yeast, and fungi)
India	Sonti	Acidovorax, Herbaspirillum, Methylobacterium, Pantoea, Pseudomonas, Stenotrophomonas, Staphylococcus, Micrococcus, Acinetobacter
Malaysia	Tapai	Amylomyces rouxii, Rhizopus sp., Endomycopsis sp.
Vietnam	Ruou Nep	Mucor sp., Rhizopus sp., Aspergillus sp., Saccharomyces cerevisiae, Torulopsis candida
Thailand	Sato, Krachae	Mucor sp., Rhizopus sp., Candida sp., Saccharomyces sp.
Phillipine	Tapuy	Aspergillus sp., Endomycopsis sp., Hansenula sp., Rhodotorula glutinis, Candida parapsilosis

Rice wine has a unique role in Chinese cultures. It also has functional and nutritional health benefits which is similar to sake [24-25]. CRW (Shaoxing rice wine) polysaccharide has been proved to engage in the immune activities as shown in an *in-vivo* study. There are numerous types of CRWs, with differences in ingredients and methods of production. On the basis of the starter, wheat “Qu” and “rice wine Qu” were the major differences of the CRW starter source. Shaoxing rice wine is the most representative rice wine. Flow chart presents the fermentation process of Chinese Huangjiu was shown in Figure 3.

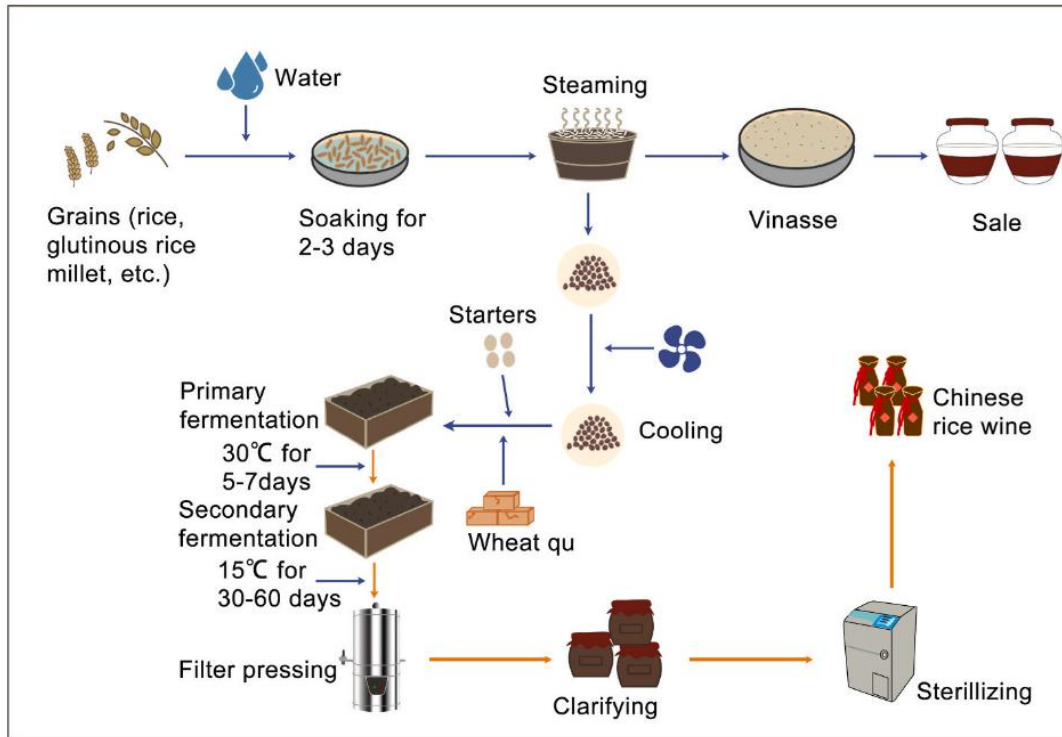


Figure 3. Flow chart presents the fermentation process of Chinese Huangjiu.

CRW is yellow or brown in color with a pure and pleasant taste, and an elegant flavor. CRW has an abundance of completed amino acids which are essential to human health. Vitamin related components, oligo-saccharides from sugars, short peptides from proteins, and polyphenol antioxidants are enriched in CRW. The contents of peptides range from 156 to 242 mg/l in sake, in comparison, the peptides ranging from 1,770 to 4,210 mg/l in CRW. Huangjiu has thousands of years' history. The grains chosen for its fermentation include glutinous rice, rice, or millet. After soak of the rice in water for 2 to 3 days, steaming and cooling, wheat Qu, the starters and the cooled rice will be mixed and fermented at 30 °C for another 5-7 days, the mash will be opened and cooled to a lower temperature at around 15 °C, then it will be maintained at 15 °C for 1 to 2 months until the fermentation is complete. The fermented liquid part will be filtered, clarified, sterilized and aged to generate the final rice wine. The differences of the production process were compared in Figure 4. The starting materials differ a lot which define the final properties of the rice wine.

Nowadays, Ganoderma, corn, bitter vegetable mixed with black glutinous rice, black and glutinous rice mixed sweet wine were developed in China. Importantly, Chinese researchers investigate the effect of fermentation time on the fermentation performance of a four-rice mixed rice wine, the results showed that the most suitable concentration of the mold used in the culture should be 1 g in 240 g materials, and the fermentation time was suggested to be 14 days. Fermenting glutinous rice wine with aroma rice was able to increase the aroma as a consequence.

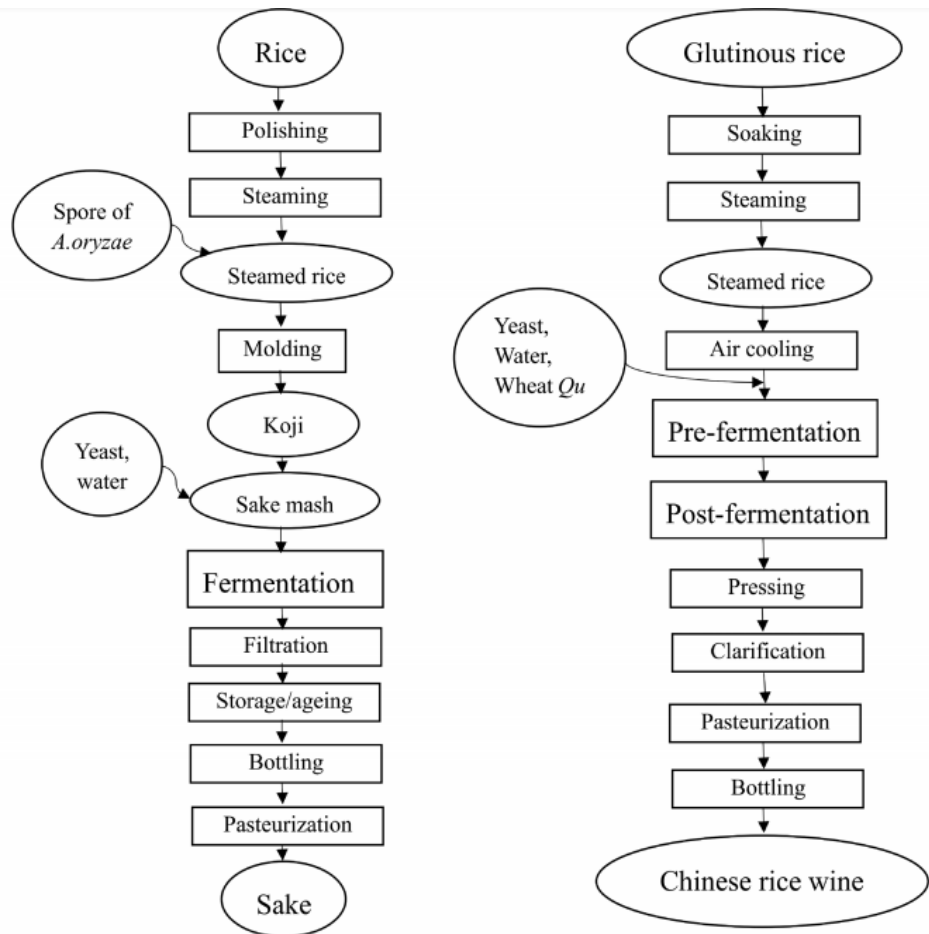


Figure 4. Production processes of Japanese sake and CRW.

6. Standardization of CRW

Being an attractive aspect, the composition of the microbiome in Huangjiu has been studied for years as the diversity of microbiome has a strong impact on the flavor and quality of the final products. Bacteria, yeasts, and fungi are commonly used in rice wines, microbial spoilage hazards may exist during the fermentation process, the determination of the microbiome quantities is important. It is also critical to control the microbial population of rice wines through advanced preservation techniques, and the commercial shelf life is determined by the microbial safety [26]. In the fermentation stage, nitrate-reducing bacteria is needed, however, the number of lactic acid bacteria requires to be controlled strictly. Compared to the pasteurization which is commonly to be applied in the elimination of pathogens and extending shelf life, plant additives such as bamboo leaf extracts won't reduce the sensory of rice wine. Ultra-high-temperature processing (UHT) is able to sterilize and kill bacteria while it will bring side impact on flavor. The high hydrostatic pressure, a kind of sterilization methods, can well ensure the sensory quality. A combination of ultraviolet irradiation (UV), pulsed electric fields (PEF) and electron beam irradiation (EB) technologies can prolong the storage period. In addition, nanotechnology applied in food preservation, transportation and processing technologies can be explored further in the yellow rice wine industry [27].

To determine the geographical origins is an important aspect for a better standardization of CRW. To date, rice wine is classified and identified mainly according to polyphenolic compounds, volatile compounds, minerals, and their trace elements. High-performance liquid chromatography (HPLC), gas chromatography-mass spectroscopy (GC-MS), electronic nose, atomic absorption spectroscopy (AAS) were employed in the detection system [28]. Near-infrared (NIR) spectroscopy has been developed to

rapidly analyze CRW products [29]. The authenticity of rice wine should be more clearer to standardize the rice wine industry in China.

7. Conclusion and future perspectives

CRW has regional authentic or traces issues which is more complex than Japanese sake. The raw material and water in different regions will provide different grades of flavour and nutrients. CRW companies should consider it in terms of the protection of the local rice wine origins. Identification and classification of specific rice wine with no preservatives, colouring agents, or flavoring agents added. Substances added should be approved by the relative rules such as the salts that could be used in the processing procedures. Increasing the diversity of CRW in flavours and for special health chronic populations is suggested for the whole industry. Drinking rice wine with seafood is a hot topic to be investigated. As research showed that drinking sake with seafood could help reduce the fishy senses. Finally, labelling of quality and ingredients, age and preservation shelf life should be standardized, and quality control, especially in microgram safety should be improved, which sake has set a good example for the whole industry.

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