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Review

Mystery behind Chinese liquor fermentation

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A B S T R A C T

Background: Chinese liquor, a very popular fermented alcoholic beverage with thousands of years' history in China, though its flavour formation and microbial process have only been partly explored, is facing the industrial challenge of modernisation and standardisation for food quality and safety as well as sustainability. Meanwhile, the hidden knowledge behind the complicated and somehow empirical solid-state fermentation process of Chinese liquor can enrich the food sector to improve our quality of life, and benefit other industrial sectors in the modern biomass-based technology, economy and society.
Scope and approach: This review reveals the traditional fermentation process and characteristics of Chinese liquor, summarises the current study progress of flavour chemistry and responsible microbial process, and addresses future improvement and research needs. We provide here a detailed, systematic and critical review on Chinese liquor to improve the current industrial practice and serve the modern society with yet incompletely explored but useful principles.
Key findings and conclusions: The hidden knowledge behind the traditional Chinese liquor production is rich in useful principles including flavour chemistry, microbial growth, solid-state fermentation, enzyme production, biocatalysis, microbial community metabolism and process engineering. Studies in a more in-depth, systematic and practical way on this look-like empirical process to explore the scientific principles behind will definitely benefit the liquor industry in particular, and the (food) biotechnology sector in general.

1. Introduction

Chinese liquor, called *Baijiu* in Chinese (in fact a transparent strong alcoholic drink), one of the oldest distilled liquors in the world, is the world's largest consumed spirit (over 4 billion litres annually) (Fan & Qian, 2006a; Xu, Wang, Fan, Mu, & Chen, 2010) and becomes more and more popular in East Asia, though little is known about Chinese liquor in western countries.

Compared to other distilled liquors (whisky and brandy in the west), Chinese liquor fermentation is a unique complex process with saccharification and spontaneous fermentation simultaneously (Fig. 1). Typically, with *Jiuqu* as starter, a sort of equivalence of Koji (Zhu & Tramper, 2013), Chinese liquor is fermented and distilled under solid-state conditions (Fig. 2 and Table 1). *Jiuqu* not only determines predominately the microbial consortium and its enzymes for liquor fermentation, but also significantly contributes to the flavour formulation (Wu, Zheng, Han, Vervoort, & Nout, 2009; Zheng, Tabrizi, Nout, & Han, 2011). Unlike enzymes from malt in the western brewing practice, exogenous microorganisms in *Jiuqu* preparation produce various enzymes for Chinese liquor fermentation. The preparation process of Koji and *Jiuqu* starter is originated from China in ancient time and later spread to Japan and other Southeast Asian countries (Zhu & Tramper, 2013). Similar starters can be found in many Asian countries, for example, Korean meju (Kim et al., 2011; Shukla, Park, Lee, Kim, & Kim, 2014) and Vietnamese banh men (Thanh, Mai, & Tuan, 2008). These starters are used for many traditional Oriental fermented foods and beverages like liquor, rice wine/Sake, vinegar and soy sauce (Chen, Xu, & Qian, 2013; Li, Aflakpui, Yu, Luo, & Lin, 2015; Liu et al., 2004; Zhu & Tramper, 2013). The unique application of *Jiuqu* starter differentiates Chinses liquor from other liquors.

Ethanol content (38 to 65%, v/v) in Chinese liquor (Han, Shi, Zhu, Lv, & Du, 2014) is mostly higher than that in other alcoholic beverages because of the spontaneous fermentation and distillation under solid-state conditions. Freshly-made liquor is stored and

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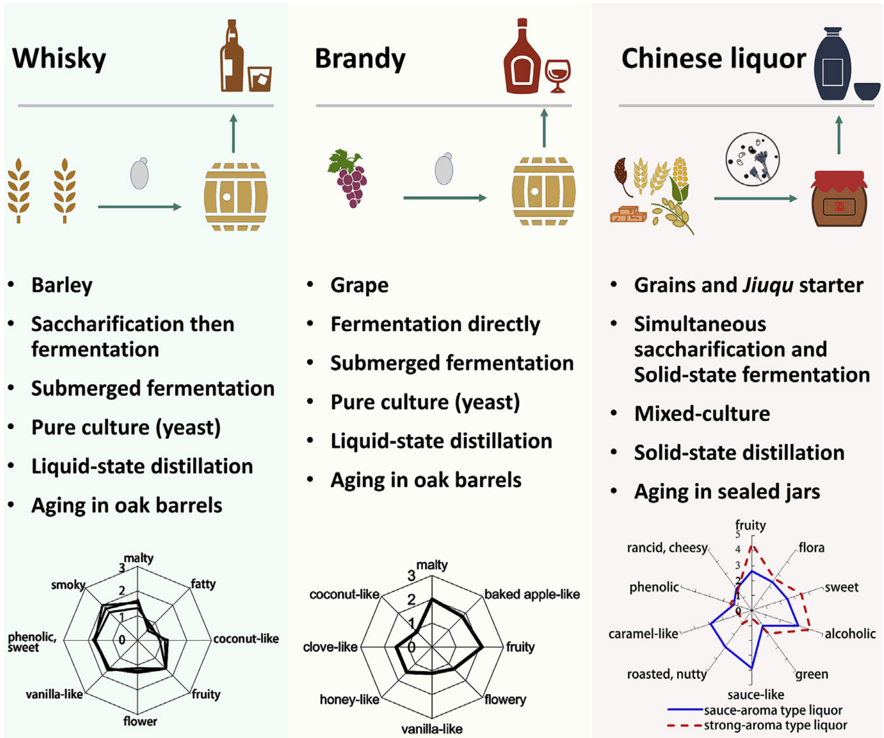

Fig. 1. Differences among whisky, brandy and Chinese liquor.
Compared to whisky and brandy, Chinese liquor differs in raw materials, manufacture process (fermentation, distillation and aging) and flavour characters (spider diagrams). Flavour characters data are from: whisky (Poisson & Schieberle, 2008), brandy (Uselmann & Schieberle, 2015) and Chinese liquor (Wang et al., 2014). Numbers from 0 to 3 in the spider diagrams of whisky and brandy mean odour intensity from not perceivable to strongly perceivable; and from 0 to 5 in the spider diagram of Chinese liquor mean odour intensity from not perceivable to strongly perceivable.

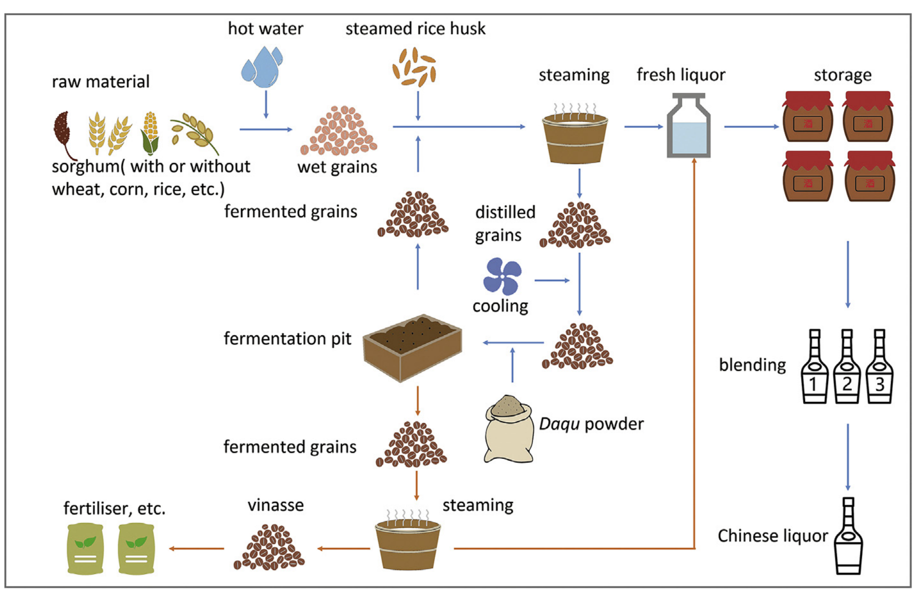

Fig. 2. Schema of traditional repeated-batch process of Chinese liquor.
Grains, mainly sorghum (and/or wheat, corn, rice and sticky rice) are soaked in hot water (about 95 °C) until the water content reaches 55% (w/w), mixed with fermented grains of the last batch from the pit and previously steamed rice husk, and the ratio depends on different processes, for example about 2:9:4 (w/w/w) during strong aroma liquor fermentation. The mixture is spade up to *Zeng* (special designed distiller) for alcohol distillation and cooking the grains simultaneously. The distilled grains are cooled to 13 to 16 °C and mixed with the *Daqu* powder (new inoculator), then fermented in the pit for the following batch. Alternatively, part of the fermented grains is distilled without adding freshly soaked grains and the distilled grain is used as fertiliser or feed. Fresh liquor is collected in a pottery jar or stainless steel vessel for storage. After aged for years, liquors are rated for blending different grade of products.

aged in sealed jars to ripen the flavour and taste. This special manufacture process determines Chinese liquor a rich flavour and strong taste. Compared to whisky with about 20 key flavour compounds (Poisson & Schieberle, 2008) and brandy with about 30 key

Table 1
Diversity of *Jiuqu* (starter).

Jiuqu	*Type	Raw material (s)	Dominant microorganisms	Main flavour compounds or precursors	References
Daqu	High-temperature(60–70 °C)	Wheat	Moulds: *Thermoascus crustaceus*, *Mucor racemosus*, *Thermomyces lanuginosus*. Yeasts: *Hanseniaspora uvarum*, *Saccharomyces cerevisiae*, *Hansenula* sp., *Candida* sp., *Pichia Torulaspora*. Bacteria: *Bacillus* (*B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens*, *B. sonorensis*), lactic acid bacteria (*Weissella cibaria*, *Weissella thailandensis*, *Lactobacillus buchneri*, *Lactococcus lactis*), *Microbacterium testaceum*, *Saccharopolyspora* sp., *Thermoactinomyces sanguinis*, *Rubellimicrobium* sp.	Tetramethylpyrazine, guaiacol, 4-vinyl guaiacol, phenylethanol propanoic acid, 1,3-butanediol, acetic acid, methyl ester etc.	(Gao, Wang, & Xu, 2010; Li, Lian, Ding, Nie, & Zhang, 2014; Liu, Guo, & Zhang, 2012; Wang, Shi, & Gong, 2008; Wu et al., 2009; Zheng et al., 2015)
	Medium-temperature(50–60 °C)	Wheat, barley and pea	Moulds: *Rhizomucor miehei*, *Absidia blakesleeana*, *Aspergillus terreus*. Yeasts: *Saccharomycopsis fibuligera*, *Pichia anomala*, *Saccharomyces exiguous*. Bacteria: *Bacillus licheniformis*, *Lichtheimia ramose*, *Weissella cibaria*, *Lactobacillus helveticus*, *Lactobacillus fermentum*, *Lactobacillus panis*.	Tetradecanoic acid ethyl ester, ethyl 9-hexadecenoate, pyrazines, guaiacol, caryophyllene, and phenylethyl alcohol etc.	(Gao et al., 2010; Wang, Gao, Fan, & Xu, 2011; Wu et al., 2009; Zheng et al., 2015)
	Low-temperature(40–50 °C)	Barley and pea	Moulds: *Rhizopus oryzae*, *Rhizopus peka*, *Amylomyces rouxii*, *Absidia blakesleeana*, *Rhizomucor miehei*. Yeasts: *Saccharomycopsis fibuligera*, *Pichia anomala*, *Wickerhamomyces anomalus*, *Saccharomyces cerevisiae*. Bacteria: lactic acid bacteria (*Weissella cibaria*, *Staphylococcus xylosus*, *Lactobacillus panis*), *Bacillus* sp., *Enterobacteriales* sp., Acetic acid bacteria sp.	Hexanal, (E)-2-octenal, (Z)-2-octen-1-ol, nonanoic acid, hexyl acetate, acetic acid, ethyl acetate, phenylethyl alcohol, ethyl alcohol, pyrazines etc.	(Le, Zheng, Chen, & Han, 2012; Wang & Xu, 2015; Wu et al., 2009; Zheng et al., 2012; Zheng et al., 2014)
Xiaoqu		Rice	Moulds: *Rhizopus oryzae*, *Rhizopus peka*, *Rhizopus chinesis*, *Absidia* sp., *Aspergillus* sp. Yeasts: *Saccharomyces cerevisiae*, *Saccharomycopsis fibuligera*, *Pichia anomala*, *Hansenula anomala*. Bacteria: *Pediococcus pentosaceus*, lactic acid bacteria (*Weissella cibaria*, *Streptococcus lutetiensis*, *Enterococcus casseliflavus*), *Deinococcus radiodurans*, *Corynebacterium variabile*, *Acinetobacter baumannii*, *Xanthomonas* sp., Acetic acid bacteria sp.	Ethyl acetate, acetic acid, ethyl acetate, phenylethyl alcohol, ethyl alcohol, pyrazines etc.	(Gou et al., 2015; Zheng et al., 2011)
Fuqu		Bran	Based on the function designed, typically: Moulds: *Rhizopus oryzae*. Yeasts: *Saccharomyces cerevisiae*, *Saccharomycopsis fibuligera*. Bacteria: *Enterococcus faecium*, *Clostridium beijerinckii*, *Bacillus cereus*, Acetic acid bacteria sp.	Based on the function designed.	(Gou et al., 2015; Zhang et al., 2009; Zheng et al., 2011)

*: Type definition is based on *Daqu* maximum temperature caused by microbial metabolic heat and measured inside in the *Jiuqu* matrix.

flavour compounds (Uselmann & Schieberle, 2015), Chinese liquor has over 60 key flavour compounds (Wang, Fan, & Xu, 2014).

Every region in China has its own local special liquor flavour style and brand (Wang, Li, Qi, Li, & Pan, 2015). Along with the progress of civilisation and welfare, fermented alcoholic beverages act a pivotal role in social activities and technology (Libkind et al., 2011; McGovern et al., 2004). Chinese liquor becomes an important aspect of Chinese culture for happiness and auspiciousness (Hao, Chen, & Su, 2005). Normally consumed “neat”, Chinese liquor can always be seen at occasions like wedding, business occasions, parties and celebrations in our daily lives.

The repeated-batch fermentation (Fig. 2) is a complex process with saccharification and spontaneous fermentation simultaneously (Chen, Wu, & Xu, 2014). However, this rather old and somehow empirical fermentation process, though surprisingly still widely practiced in China, is facing the challenge of modification, standardisation and optimisation. Some issues are associated with food quality, safety, sustainability and modern industrial improvement. Therefore, it is necessary to completely study this process, move from poorly-controlled spontaneous fermentation to an inoculated fermentation under process control.

2. Chinese liquor

2.1. Traditional process

Traditional process of Chinese liquor includes starter (*Jiuqu*) preparation, substrate hydrolysis, liquor fermentation, solid-state distillation, aging and blending. These special long manufacture processes under semi-controlled conditions are unique compared with any other food and beverage fermentations. For centuries, operations are considered rather an art based on generations' experience than a technology. We describe the traditional process below.

2.1.1. Starter preparation

Starter *Jiuqu*, is in the Chinese language composed of two characters, the first character *Jiu* means alcohol and can be used as a suffix like −ol in English, and the second character *Qu* means Koji (it is the same character if the Japanese Koji is written in the traditional Kanji − a system of Japanese writing using Chinese-derived characters). *Jiuqu*, is thus a Koji special for alcohol beverages, and serves as starter and part of raw material for liquor or rice wine (Fig. 2 and Table 2) (Chen et al., 2013; Xu et al., 2010). *Daqu*, a typical derivate of *Jiuqu*, is the most commonly used *Jiuqu* starter and the preparation of *Daqu* is an important process to enrich microorganisms from the environment to produce enzymes for liquor fermentation (Zheng et al., 2011; Zhu, Wu, Luo, & Gao, 2015; Zhu & Tramper, 2013). The production process of *Daqu* is a special solid-state fermentation process in an open system, which includes ingredient formulation, shaping, incubation in *Qu*-house (a cultivation chamber with controlled temperature and moisture if possible) and maturation during storage (Fig. 3).

2.1.2. Fermentation and distillation

The actual solid-state alcoholic fermentation process happens in a special fermentation pit (about 3.4 m long, 1.8 m wide, and 2.0 m deep, and some manufacturers use pottery cylinder jars instead) between 28 and 32 °C for 60 days under anaerobic conditions (Xu et al., 2010). *Jiuqu*, enriched with various microorganisms including moulds, yeasts and bacteria, and various enzymes thereof, hydrolyses raw materials and converts them to ethanol and flavour compounds. Repeated-batch process is widely used for liquor production (Fig. 2). Fermented grains are mixed with soaked fresh grains in proper ratio and directly distilled under solid-state condition. This distillation has in fact the double effect, namely (1) distil ethanol and flavour compounds from fermented grains and (2) cook the fresh grains to make them accessible for microorganisms and enzymes. Fresh liquor is collected from condensate pipe for further grading, storage and blending. Subsequently, a wide variety of liquor product is formed with different flavour characters and ready for consumption.

2.2. Diversity of Chinese liquor

Various liquors have a wide range of diff-typical flavour characteristics and tastes, due to differences in *Jiuqu* starters, raw materials (sorghum, wheat, corn, rice, sticky rice and rice hull, all can be influenced by season, weather, storage, transport and location), manufacture processes without strict control, locations that determine the natural microorganisms, and different consumers' preferences (Fan & Qian, 2006a; Xu et al., 2010).

The starter *Jiuqu* can be sorted into *Daqu*, *Xiaoqu* and *Fuqu*, respectively with meaning in Chinese as big Koji, small Koji and bran Koji (Gou et al., 2015). *Daqu* can be classified into different types based on different process parameters such as the maximum temperature caused by microbial metabolic heat accumulated inside the *Jiuqu* matrix (Table 1). As shown in Table 1, temperature can strongly affect the dominating microorganisms enriched. Enzymes in *Jiuqu* mainly include amylases, protease and glucoamylase (Su et al., 2015; Zheng et al., 2011).

Based on flavour characters, Chinese liquor can be sorted into sauce-aroma type, strong-aroma type, light-aroma type, honey type and miscellaneous types (Fan, Fan, & Xu, 2015; Fan & Qian, 2006b). Among these, the first three types dominate the market and they all have their own characters. Table 2 gives an overview of these three types of Chinese liquor.

3. Industrial challenge

Chinese liquor has been consumed for millennia and old traditions of fermentation practices are well preserved. The consumption grows rapidly during last decades with ever increasing

Table 2
Diversity of Chinese liquor.

Type	Flavour character	Raw materials	Fermentation process	Dominant functional microorganisms	Main flavour compounds	References
Sauce-aroma	Sauce-like, roasted aroma	High-temperature *Daqu*, sorghum	Heap fermentation then repeated-batch fermentation in pit	Moulds: *Paecilomyces variotii*, *Aspergillus oryzae*, *Aspergillus terreus*. Yeasts: *Zygosaccharomyces bailii*, *Saccharomyces cerevisiae*, *Pichia membranifaciens*, *Schizosaccharomyces pombe*. Bacteria: *Lactobacillus* sp., *Bacillus* sp.	Ethyl hexanoate, hexanoic acid, 3-methylbutanoic acid, 3-methylbutanol, pyrazines, ethyl 2-phenylacetate, 2-phenylethyl acetate, ethyl 3-phenylpropanoate, 4-methylguaiacol and γ-decalactone	(Chen et al., 2014; Fan, Shen, & Xu, 2011; Fan, Xu, & Zhang, 2007; Wu, Chen, & Xu, 2013; Wu & Xu, 2012; Wu, Zhang, Peng, & Xu, 2015; Zhu et al., 2007)
Strong-aroma	Fruity, flower, pineapple-like, banana-like, apple-like aromas	Medium- and Low-temperature *Daqu*, sorghum with or without glutinous rice, rice, wheat and corn	Repeated-batch fermentation in pit	Moulds: *Aspergillus* sp., *Rhizopus* sp., *Eurotium* sp. *Phanerochaete chrysosporium*. Yeasts: *Saccharomyces cerevisiae*, *Saccharomycopsis fibuligera*, *Talaromyces Pichia kudriavzevii*. Bacteria: *Clostridium kluyveri*, *Burkholderia* sp., *Streptococcus* sp., *Lactobacillus* sp., *Lactobacillaceae* sp.	Ethyl hexanoate, ethyl acetate, ethyl lactate, hexanoic acid, butanoic acid, ethyl butyrate, heptanoic acid, furfural, ethyl valerate, phenylethyl alcohol, ethyl heptanoate	(Cheng et al., 2013; Fan & Qian, 2005, 2006a; Hu, Du, & Xu, 2015; Tao et al., 2014; Wang, Shi, et al., 2008; Wang, Zhang et al., 2008; Xiang et al., 2013; Yao et al., 2015; Zhang et al., 2007)
Light-aroma	Pleasant fruity, floral aroma	Low-temperature *Daqu* or *Xiaoqu*, sorghum	Repeated-batch fermentation in pottery cylinder jar	Moulds: *Rhizopus oryzae*. Yeasts: *Saccharomycopsis fibuligera*, *Pichia anomala*, *Saccharomyces cerevisiae*. Bacteria: *Lactobacillus* sp., *Lactobacillaceae* sp., *Bacillus* sp.	Ethyl acetate, β-damascenone, ethyl lactate, acetic acid, 2-methylpropanoic acid and terpenoids	(Gao et al., 2014; Kong et al., 2014; Li et al., 2011; Wu, Zhu, et al., 2015)

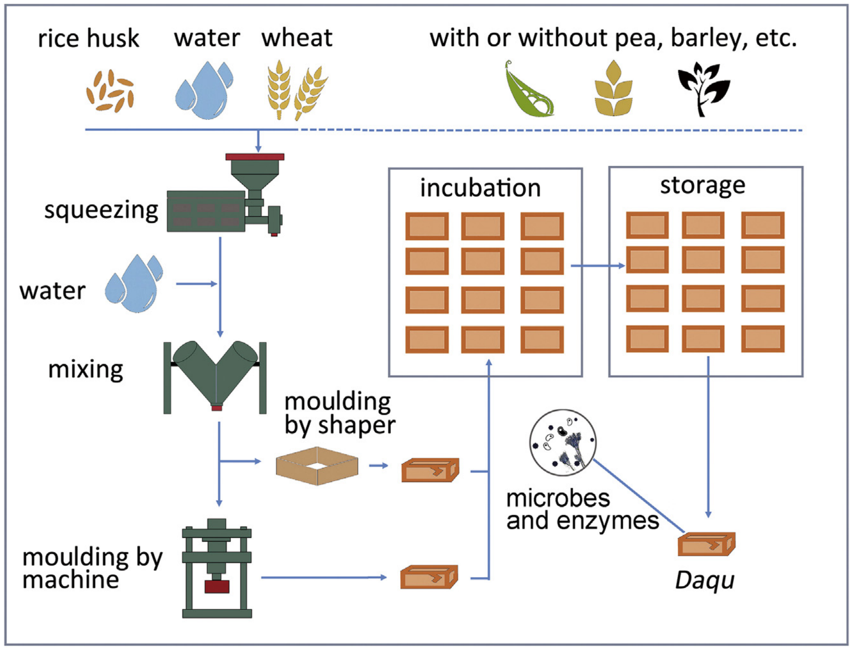

Fig. 3. Schema of *Daqu* production.
Wheat and rice husk or straw (less than 1%, w/w) (with or without peas, barley or Chinese herbs) mixed with 5% (w/w) water and squeezed. Wheat is thereby broken into three or four pieces to release starch. Then water is added to 37.5% (w/w) and mixed thoroughly. Two ways of moulding can be used, manual or mechanical. After shaped to a brick weighing 3 to 4 kg each, the bricks are moved into a *Qu* house, namely a cultivation chamber, stacked 4 to 5 layers and incubated for 28 days. Microorganisms may come from raw materials, water and air. During the incubation, workers use rice straws to cover and keep warmth for microbial growth and flavour compound formation. *Daqu* also needs maturation during storage for about 3 to 6 months before use.

living standard and welfare. However, the existing challenges of food quality, safety and modern industrial development are receiving increasing attention.

3.1. Risks in quality and safety

3.1.1. Food quality concern

Insufficient standardisation of raw materials and poorly-controlled fermentation process may cause serious quality defect and instability even though the final liquor product might be treated with blending. For example, earthy-odour of geosmin from *Streptomyces* community in *Daqu* causes serious sensory defects (Du, Fan, & Xu, 2011), though this can be controlled by two *Bacillus* strains (Zhi, Wu, Du, & Xu, 2016). Off-flavour problems like musty and feculent odour (Du et al., 2011), and bad tastes like bitter taste, are far from being solved.

In addition, as one of the most widely consumed alcoholic beverages, the price of Chinese liquor might vary from several to hundreds of US dollars per litre. The extremely high price for commercial interest results in some inferior or even fake products to appear on the market that hurt consumers' benefit and eventually also health when uncontrolled ingredients are added (Li, Song et al., 2014; Li, Wang et al., 2014; Zhen et al., 2013). Grade identification, authenticity and quality control are of great importance to protect the interests of producers and consumers.

Therefore, volatiles-based discrimination methods were developed, including spectroscopy (Cheng, Fan, & Xu, 2013; Dong et al., 2014; Li, Song et al., 2014; Li, Wang et al., 2014; Sun, Li, Wei, Zhou, & Noda, 2006; Zhu, Fan, Xu, & Zhou, 2016), electronic nose (Zhou et al., 2011) and colorimetric artificial nose (Qin et al., 2012; Ya et al., 2012). Food traceability is also available for overall control (Badia-Melis, Mishra, & Ruiz-Garcia, 2015). The protection of geographically famous brand of Chinese liquor is receiving more and more attention to protect the interest of consumers and producers (Qin et al., 2012). Though all these attempts are reliable and effective for identification, authentication and evaluation, these efforts cannot completely solve quality and forgery problems. Quality instability of traditional process needs to revolutionise for quality and safety by modern standard, validated and sustainable manufacture process.

3.1.2. Food safety risk

Some toxins may be formed during storage of fresh liquor to affect food safety. Ethyl carbamate is genotoxic and carcinogenetic, widely spread in alcoholic beverages and fermented foods, very toxic and harmful to human health (Lim & Lee, 2011; Zhao et al., 2013). Ethyl carbamate is formed by urea, cyanide and ethanol that all exist in liquor, and also found in some Chinese liquor (Wu, Pan, Wang, Shen, & Yang, 2012; Xia et al., 2014). Both fermentation techniques and chemical compounds are responsible for ethyl carbamate formation (Zhao et al., 2013). An HPLC-FLD method detected and proved that ethyl carbamate is mainly produced during storage at higher storage temperature (Li et al., 2015) with hydrocyanic acid as precursor and raw materials. Optimized storage condition as well as efficient detection and elimination techniques are needed to prevent the accumulation of ethyl carbamate in Chinese liquor.

The open and spontaneous fermentation of Chinese liquor may risk the contamination of microbial toxins. For example, Ochratoxin A, a ubiquitous mycotoxin produced by certain filamentous species of *Aspergillus* and *Penicillium* that can be found in starter *Jiuqu* and Chinese liquor fermentation environment, was detected in 9 of 76 liquor samples with a maximum concentration of 0.17 μg/L (Zhu, Ren, Nie, & Xu, 2016). Another example is toxoflavin produced by *Burkholderia* in rice straw and *Daqu* for sauce-aroma liquor fermentation. Over 8 mg/kg was found in *Daqu* sample, though no toxoflavin was detected in distilled liquor (Zhu et al., 2015). Rice straw is widely used in starter *Daqu* preparation to facilitate mass, heat and gas transfer. Contamination of rice straw used for *Daqu* may affect food safety of the final product. For safety reasons, it is essential to ensure quality of raw material without pathogenic microorganisms and to consider degrading toxic substances when they exist in the raw materials.

Higher-alcohols like isobutanol and isoamyl exist in many alcoholic drinks and contribute to the flavour and taste of Chinese liquor, though they are potential health hazard in excess amount (Han et al., 2014). The content of higher-alcohols in Chinese liquor is about 0.6 to 1.2 g/L (Zhang, Wu, Zhang, Wang, & Li, 2009). Attempts were made to reduce higher-alcohols, for example, enzymes extracted from Fuji SA-IEP apple peels can reduce higher alcohols in Chinese liquor very effectively (Han et al., 2014). Study shows that mixed starter results in relatively lower higher-alcohols, but a thinner taste and flavour (Zhang et al., 2009). Thus, the content of higher alcohol should be controlled within an appropriate range.

3.2. Environmental issues

Environmental issues are increasingly important for the food industry concerning waste management, water consumption and energy efficiency (Alsaffar, 2016; Broadbent, 1973; Hall & Howe, 2012). Chinese liquor industry, as mentioned earlier, is still mainly a traditional and primitive process with poorly controlled fermentation, distillation, and blending. Chinese liquor industry recognises the urgency of cleaner production, efficient water use and energy recycling but substantial change is still to realise (Huang, Sun, & Su, 2014). Furthermore, suspicions exist that local environment change might affect indigenous microorganisms thereby to affect product quality and safety as well as productivity. This conservative belief and practice can hardly be changed before convincible scientific principles behind the traditional process are released. Modernisation of Chinese liquor industry needs advanced environment-friendly processes, especially energy and waste management to balance sustainability.

3.3. Valorisation of wastes and by-product

Waste and by-product from food industry can be renewable resources and have great potential to produce value-added products (Federici, Fava, Kalogerakis, & Mantzavinos, 2009; Koutinas et al., 2014). Distilled grain residue is the main solid waste, consisting carbohydrates, proteins, lipids and some valuable microbial metabolites, though nowadays simply used as feed, fertiliser or culture substrate for edible mushrooms (Xu, Xu, Tao, Yuan, & Gao, 2015). Bioethanol production can be one of the possibilities to valorise liquor distillation wastes (Liu, Wu, Yang, Yuan, & Zhang, 2014; Tan et al., 2014). For example, after H_2SO_4 saccharification and fermentation, an ethanol yield of 91.9–98.9% based on glucose concentration was obtained (Tan et al., 2014).

Recovery of flavour compounds from distilled solid waste is also attractive. By using supercritical carbon dioxide extraction, 55.17 g ethyl (9Z)-9-octadecenoate per litre extract was obtained and can be used as solvent and food additive (Xu et al., 2015). However, valorisation of waste and by-product in Chinese liquor industry is still at a pioneering stage and the complex process causes inefficiency and embarrassment of the traditional industry. Modern industry, modern biotechnology, and sustainable development will enforce the progress in this sector.

3.4. Traditional process facing modern industrial challenge

Many drawbacks in the traditional fermentation are directly or indirectly caused by lack of control and standardisation. No exception also in this sector, modern industrial development is imperative. Traditional fermentation process evolution and modernisation succeeded in many fermented foods, such as soy sauce in the East (Zhu & Tramper, 2013) and cheese in the West (Settanni & Moschetti, 2010; Tramper & Zhu, 2011). The traditional fermentation and manufacturing methods exist for centuries and strongly rely on individualoperation skills and experiences. Fig. 4a gives a glimpse of an old producing site (over 400 years) from *Luzhou Laojiao* (a famous brand of strong-aroma liquor) Museum. In an empirical manufacture process, individual capability and skills, as well as raw materials, environment and climate factors, can affect the productivity and quality consistency. In the last few decades, developments in modern biotechnology and related fields improved the traditional methods and led to numerous technological innovations. Many breweries in the Chinese liquor industry have transformed to semi-mechanised operations (Fig. 4b), but still a considerable percentage remain unchanged.

Nowadays, with the development of modern society, Chinese liquor industry faces challenge and opportunity: process modernisation, environment-friendly production, better process control,

Fig. 4. Site of old traditional process and mechanised process.
a. Old traditional process in *Luzhou Laojiao* Museum.
b. Mechanised process of Roasted-sesame-aroma liquor.
Photos kindly provided and permitted to reproduce by Luzhou Laojiao Co. Ltd. (Luzhou, Sichuan Province, China) and Jiangsu King's Luck Brewery Joint-stock Co. Ltd. (Huai'an, Jiangsu Province, China).

standardised operation and consistent product quality and safety. Semi-automation is already used for some new type liquors (like Roasted-sesame-aroma liquor) fermentation (Fig. 4b), all showing the shift of primitive operation to industrial modernisation. However, the innovation of Chinese liquor is a complex and long-term process, and needs to study basic principles to optimise the process. Modern food biotechnology has facilitated the evolution of food fermentations from empirical process to advanced techniques (Holzapfel, 2002). No exception for Chinese liquor, scientists have made efforts in related disciplines. We will highlight the advances below and meanwhile address relevant perspectives.

4. Progresses and perspectives

Chinese liquor production covers the processes of microbial growth, enzyme production, hydrolysis, bioconversion, flavour formation, fermentation, distillation, storage and blending, covering relevant disciplines of microbiology, biotechnology, biochemistry/enzymology, food chemistry, analytical chemistry, flavour chemistry, chemical engineering and bioprocess engineering. Study on Chinese liquor began in the 1960s and had many breakthroughs in past few decades. Researchers' interests in Chinese liquor can be categorised into two main directions: flavour chemistry and the associated microbial processes under solid-state fermentation conditions.

4.1. Recent advances

4.1.1. Flavour chemistry

Consumer flavour sensation is the key factor that defines a successful and acceptable food product (Carrau, Gaggero, & Aguilar, 2015), and so as to Chinese liquor (Wang et al., 2015). The quality and value of Chinese liquor are critically related to complex flavour compounds that determine the organoleptic properties though they count for only about 1 to 2% (v/v) of whole liquor (Li et al., 2012; Li, Wang, Raghavan, & Vigneault, 2011). Volatile and non-volatile compounds including their interactions constitute Chinese liquor complex flavour characteristics.

The study on flavours of Chinese liquor began with the identification of flavour compounds. So far more than 1000 volatile compounds have been detected in Chinese liquor including alcohols, esters, fatty acids, pyrazines and polyphenols (Wu & Xu, 2013; Zhu et al., 2007) and new compounds continue to emerge with more advanced analytical techniques. With gas chromatography–olfactometry, quantitative measurement and flavour contribution analysis, main flavour compounds are characterised in many diff-flavour-types Chinese liquor (Fan et al., 2015; Gao, Fan, & Xu, 2014; Wang et al., 2014). Table 2 lists the main flavour compounds in the three dominant aroma-type liquors.

Further studies focus more on the interactions of various flavour compounds and even that between volatile and non-volatile compounds, because volatile composition alone is not enough for overall flavour construction. For example, lichenysin, a non-volatile compound (molecular weight >1000 Da.) isolated from Chinese liquor, can significantly decrease volatile phenols whereas contributes significantly to the volatility of other volatile flavour components in liquor (Zhang, Wu, & Xu, 2014; Zhang, Wu, Xu, & Qian, 2014).

More recently, efforts are made to clarify where the flavour compounds come from. This opens theoretically the possibility to control the profile of various compounds, volatile or non-volatile. Flavour compounds may come from raw materials, microbial metabolism and chemical reaction during fermentation, storage and formulation, as illustrated in Fig. 5, the association of all factors that can affect the final flavour profile. *Jiuqu* starter can also partly provide flavour compounds and precursors (Table 1 and Fig. 5) including pyrazines (Zheng et al., 2011), glycerol, malate, trimethylamine, mannitol, lactate (Wu et al., 2009), β-damascenone and 2-phenylethanol (Gao et al., 2014). However, microbial process under solid-state fermentation conditions plays a key role and studies are going on continuously, which we will address below.

4.1.2. Microbial process

Microbial process under solid-state fermentation conditions determines the unique outcomes of Chinese liquor. With *Jiuqu* as starter that is a complex mixture of various enriched microorganisms and enzymes thereof, and influenced by factors including raw materials (grains), pit mud and the open environment (natural microflora, air and water), Chinese liquor fermentation undergoes a microbial process where microbial diversity contributes to the delicate balance and functions for stability, quality and productivity.

From the 1960s and even till rather recently, studies have focused on the separation and identification of microorganisms from samples. Most dominant functional microorganisms in the production of *Jiuqu* starters and Chinese liquor have been identified (Tables 1 and 2). With the development of modern molecular biology, a more comprehensive understanding of the microbial diversity realized in the last decade. A typical example is the detection of uncultured microorganisms like *Clostridia* in pit mud, revealed by an improved PCR-based denaturing gradient gel electrophoresis method (Hu, Wang, Wu, & Xu, 2014). The microbial community may dynamically change during fermentation (Tao et al., 2014). For example, by studying dominant bacterial community, Zhang et al. indicate (Zhang et al., 2005; Zhang et al., 2007) that bacterial diversity decreases with fermentation time and finally *Lactobacillus acetotolerans* becomes the predominant species during strong-aroma liquor fermentation (Wang, Zhang, Zhao, & Xu, 2008).

Microbial diversity has various functions, in particular in a very complex microbial system like liquor fermentation. First, diversity of microbial community accomplishes industrial microbial ecosystem (Beyter et al., 2016), thereby provides a stable microenvironment so that various functional microorganisms can exercise respective and/or synergic functions. Second, microorganisms release diverse enzymes that influence the microbial fermentation or bioconversion and liquor flavour (Huang, Wu, & Xu, 2014). More importantly, microorganisms generate directly flavour compounds that determine the fermentation result of liquor. For example, *in situ* analysis of yeast flavour metabolisms showed that *Pichia anomala* is responsible for ethyl lactate, octanoic acid, and ethyl tetradecanoate in light-aroma liquor (Kong, Wu, Zhang, & Xu, 2014). Evidence from biosynthetic mechanism reveals that *Saccharomyces cerevisiae* can form terpenoids by using cereals containing terpenoids precursors (Wu, Zhu, Wang, & Xu, 2015). Also bacteria are important for flavour formation. Microarray profiling evidences proved that heat-resistant strain *Bacillus licheniformis* CGMCC3962 produces metabolites like tetramethylpyrazine and 2,3-butanediol that are likely related to sauce flavour of liquor (Wu & Xu, 2012). These findings give us useful information of flavour-producing microorganisms and their metabolisms that can be potentially controlled for enhancing desired metabolites (flavours) while eliminating undesired metabolites/intermediates such as off-flavours and hazards.

Microbial interactions are yet another primary factor that affects the success and safety of food fermentations to obtain desired product (Ivey, Massel, & Phister, 2013; Smid & Lacroix, 2013). The interactions among different microbial strains can have both positive and negative effects. For example, the intrinsic functional yeasts contribute to flavour formation, and the extrinsic strains can

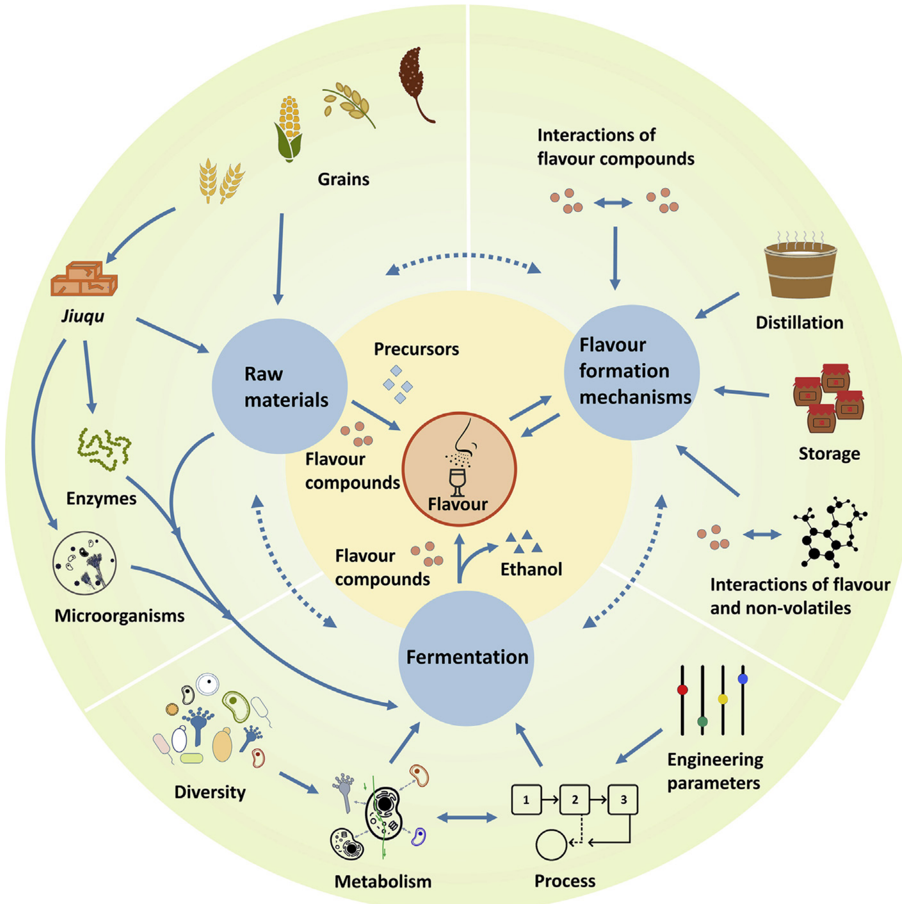

Fig. 5. Association of all factors influencing Chinese liquor flavour formation.
Raw materials and microbial fermentation influence flavour formation of Chinese liquor (solid-line arrows), and interactions among various factors (dashed arrows). Raw materials (including starter *Jiuqu*) supply flavour compounds and precursors, enzymes and microorganisms for fermentation. Microbial community produces flavour compounds and ethanol, and influenced by solid-state fermentation process. Flavour compounds interact with each other and also with non-volatiles. Flavour profile may dynamically change during the whole manufacture process.

regulate and improve the growth and metabolisms (Meng et al., 2015; Wu, Kong, & Xu, 2016). However, geosmin-producing (off-flavour) *Streptomyces* sp. inhibits the growth of functional yeasts and moulds, and consequently, decreases the formation of flavour metabolites (Du, Lu, & Xu, 2015).

Moreover, microbial community structure and metabolisms are strongly influenced by external factors like environment conditions. During solid-state fermentation process, microorganisms grow and metabolise in extreme environment (extremely high local temperature due to metabolic heat production with poor heat transfer, acidic and ethanol stresses due to acid and ethanol production, low oxygen due to the lack of agitation and aeration, low water-activity due to evaporation for heat transfer and inhomogeneous due to lack of agitation) and evolve to exhibit unique metabolic traits. For example, *Saccharomyces cerevisiae* MT1 isolated from sauce-aroma liquor fermentation can simultaneously use various sugars for alcohol production (Lu, Wu, Zhang, & Xu, 2015).

Studies on microbial process give us important information of the role of a single microorganism or a microbial community in flavour formation and associated factors to affect this role. For example, mixed–culture fermentation of different combination of five dominant species proved that they can be directly used for pure-culture starter preparation of sesame-aroma liquor production (Wu, Ling, & Xu, 2014). However, current studies are mainly theoretical, thus, comprehensive studies on the roles of individual microorganism and microbial community will provide more insights and prospects.

4.1.3. Solid-state fermentation

As one of the most important environmental factors that could be theoretically well controlled, solid-state fermentation is crucial for Chinese liquor production. Compared to submerged fermentation, it is eco-friendly, resource-saving and high yielding, but difficult in upscaling and process control (Nagel, Tramper, Bakker, & Rinzema, 2001; Thomas, Larroche, & Pandey, 2013). The unique solid-state fermentation favours the formation of distinguished enzymes, higher concentration of ethanol and flavours. However, the very complicated triangle association among microbial growth and metabolism, temperature and water activity is hardly studied, although similar studies are intensively done on lab-scale using model fungus (*Aspergillus oryzae*) and substrates (wheat) (te Biesebeke et al., 2002).

One of the pioneer studies on liquor solid-state fermentation is process simplification on lab-scale using artificial pit with online measurement to explore the association of temperature and gas change with alcohol content (Yue, Zhang, Yang, Zhang, & Liu, 2007). However, it was too simple for in-depth study though the system simulated the fermentation environment. In particular, in a spontaneous solid-state fermentation process without strict process control, various dynamic changes including microbial growth, glucose and oxygen consumption, metabolites formation, temperature change and moisture loss, each of them is critical for the quality and productivity. Better understanding and control of the solid-state fermentation process, will help control an optimized environment for liquor flavour formation, as mentioned earlier (see also Fig. 5).

4.2. Perspectives

Although the traditional fermentation of Chinese liquor is rather successful for thousands of years, it faces critical challenges as we mentioned earlier (Part 3 of this article). The progresses in the past half century prove the possibility and necessity to uncover the hidden knowledge behind the process and to improve it in a scientific manner. Studies need to tackle the challenges and meanwhile the hidden principles may drive modern biotechnologies' development conversely (Zhu & Tramper, 2013).

4.2.1. Future research needs

Any modernisation and innovations cannot succeed without basic research. As we indicated earlier, liquor production covers the basic knowledge of microbiology, biochemistry, biotechnology, process engineering, among others. A very successful example to refer is the Sake production in Japan. High operational standards strictly based on scientific and technical principles define and standardise raw materials, microorganisms and manufacture process to assure quality and productivity, and even further promote associated laws to safeguard the interests of consumers and producers (Kanauchi, 2013). Although knowledge on Chinese liquor is rapidly accumulating, we are still far from completely understanding the principles behind this traditional product.

Concerning microorganisms, an ideal manufacture process should undergo with starters of pure microbial cultures or at least defined microbial consortium, use consistent or relatively defined raw materials under controlled process conditions to maintain quality, safety and stability of liquor product. Therefore, various aspects of further studies are imminent.

Concerning flavour formation, modern advanced detection methods provide faster, cheaper and more precise high throughput analytical methods. Thus, to detect and identify should not be a crucial challenge in coming decades. We need more insights into trace substances, non-volatile compounds and the interactions contributing to flavour as well as taste.

Concerning the role of microbial fermentation in flavour forming, the use of next-generation sequencing techniques, high-throughput "-omics" techniques (including flavour-omics, genomics, transcriptomics, proteomics, metagenomics and metabolomics), and simulation and reconstruction fermentation can help open a window into the enormous taxonomic, evolution and *in situ* and *in vitro* functions in more details for potential control of the microbial metabolism.

Last but not the least, as Chinese liquor uses solid-state fermentation, understanding process engineering aspect of the solid-state fermentation will enable process optimisation (Thomas et al., 2013). Simulation approach, mathematical modelling, and Big data-based techniques will provide efficient alternative solutions to better understand complex process dynamics, control and prediction.

4.2.2. Perspectives beyond liquor production

Food fermentation is an ancient bioprocessing and probably the simplest and most economical way to improve nutrients, sensory properties and functions of foods (Blandino, Al-Aseeri, Pandiella, Cantero, & Webb, 2003; Marsh, Hill, Ross, & Cotter, 2014). Exploring the mystery behind Chinese liquor production will provide both scientific and practical values.

Flavours are often the main characters of fermented foods (Carrau et al., 2015) and essential for consumers' criteria (Aprotosoaie, Luca, & Miron, 2016). Understanding chemical composition of flavours, interactions of various flavour compounds and factors influencing flavour formation gives us useful information for quality control and process optimisation. Exploring, controlling and optimising a complex microbial community involved in flavour generation and the associated biochemical pathways will provide insights into similar complex traditional food fermentation processes.

Furthermore, exploring mystery behind traditional food fermentations can be valuable as a model for studying microbiome characteristics in less tractable ecosystems (Wolfe & Dutton, 2015). Ecological principles in this traditional food fermentation system give us useful advice to understand the evolution strategies of special function microbes and microbiotas, which can serve as a source for specific applications. For example, the repeated-batch fermentation technique can be served as reference for bioethanol or other value-added products that need to undergo multistep processing stages including pre-treatment, hydrolysis and bioconversion (Tan et al., 2014; Xu et al., 2015).

5. Conclusion

Traditional Chinese liquor fermentation remains so far semi-controlled and empirical. New challenges in food safety and quality, microbial technology and process engineering need to be tackled to meet the requirements of the modern society. Flavour formation and corresponding microbial fermentation, in particular the application of pure cultures, better process control and standardisation will be the key issue in near future. Meanwhile, exploring the principles behind the complex spontaneous process will not only benefit the liquor industry in particular, but food and biotechnology sector in general. To achieve all these goals, a multidisciplinary approach is absolutely necessary.

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