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ORIGINAL PAPER

Modern technology homogenizes enological traits of indigenous *Saccharomyces cerevisiae* strains associated with Msalais, a traditional wine in China

Lixia Zhu[1] · **Julan Xue**[1]

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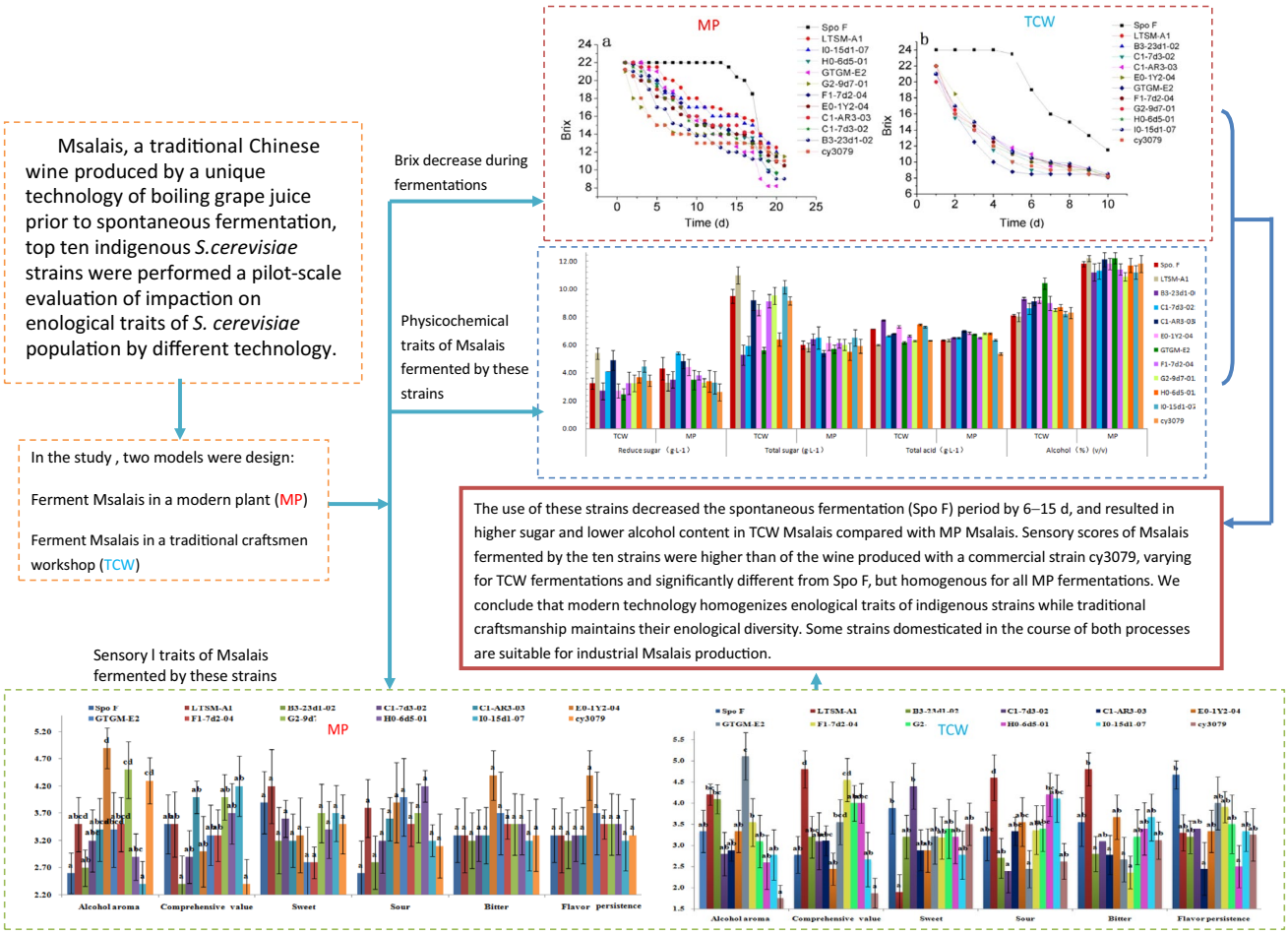
Abstract In this study, we performed a pilot-scale evaluation of the enological characteristics of indigenous *Saccharomyces cerevisiae* strains associated with Msalais, a traditional Chinese wine produced by a unique technology of boiling grape juice prior to spontaneous fermentation. Technical and sensory characteristics of top ten indigenous strains previously identified by us by screening a collection of 436 indigenous *S. cerevisiae* strains (Zhu et al. 2016) were assayed in a traditional craft workshop (TCW) and a modern plant (MP). The use of these strains reduced the spontaneous fermentation (Spo F) period by 6–15 days, and resulted in higher sugar and lower alcohol content in TCW Msalais than in MP Msalais. Sensory scores of Msalais fermented by the ten strains were higher than those of wine produced with a commercial strain cy3079, varying in TCW fermentations and significantly different from Spo F, but homogenous for all MP fermentations. Four strains were extensively screened for use in industrial Msalais production. We conclude that modern technology homogenizes enological traits of indigenous strains while traditional craftsmanship maintains their enological diversity. Some strains domesticated in the course of both processes are suitable for industrial Msalais production.

✉ Lixia Zhu
judyzhu1@sina.com

[1] College of Life Science, Tarim University/Xinjiang Production and Construction Group Key Laboratory of Agricultural Products Processing, Xinjiang South, Alar 843300, Xinjiang, People's Republic of China

Graphical Abstract



Keywords Enological characteristic · Msalais · Pilot-scale · *Saccharomyces cerevisiae*

Introduction

It is well known that native yeast strains are responsible for the production of wines of different qualities and unique flavors (Orlic et al. 2007). The use of locally selected yeast strains with strain-specific metabolic characteristics may positively affect the final quality of wine (Capece et al. 2013; Orlić et al. 2010) and ensure maintenance of characteristic sensory properties of wines derived from a specific area (Capece et al. 2014). Today, most wines are produced using selected commercial *Saccharomyces* strains, but many wine researchers and winemakers prefer to use selected autochthonous strains of *S. cerevisiae* as starters (Carrau et al. 2015; Capece et al. 2014; Gomes et al. 2007).

Msalais is a traditional wine brewed from boiled grape juice using a unique technology (Lixia et al. 2012b, 2015). We previously showed that although Msalais has a unique flavor due to the boiling of grape juice before spontaneous fermentation (Spo F); its main aroma compounds are the alcohols and esters produced by indigenous yeasts (Lixia et al. 2012a). *S. cerevisiae* strains exclusively initiate Spo F of Msalais and are dominant from the beginning of the process to its end (Lixia et al. 2012b). However, among >200 traditional Msalais producers in the main producing region of A'wati (China), the quality of traditional Msalais is quite diverse and can be unpredictable (Lixia et al. 2008, 2011). These features are ascribed to environmental parameters (e.g., fluctuating temperature), the enological characteristics of wild yeasts, as well as technological differences. For example, during Spo F of Msalais, the temperature often fluctuates between 13 and 37 °C, the fermenting period takes 15–45 days, and the fermenting equipment comprises stainless steel tanks with ~20 t capacity in modern plants (MPs) or amphora jars with <1000 kg capacity in traditional craftsmen workshops (TCWs). The final Msalais flavor characteristics vary from highly sweet and alcoholic to acidic or bitter, to high quality, harmonious, and mellow. In addition, the development of Msalais has evolved from craftsmen times to modern industry times, and the

producers change the traditional technology at will to meet the increasing demand of the market without scientific instructions or reference. This has rendered the traditional technology more complex and the quality of Msalais more unpredictable, sacrificing the traditional technology and the expected wine quality.

In this study, top ten indigenous *S. cerevisiae* strains identified in a screen of a collection of 436 indigenous strains (Zhu et al. 2016) were tested for their tehnical characteristics during fermentation and for the sensory characteristics of the produced Msalais in a pilot-scale investigation, in TCW and MP modes. We also evaluated the effect of TCW and MP on the enological traits of indigenous *S. cerevisiae* strains associated with Msalasi, with the aim of identifying strains that would produce Msalais of high quality, unique flavor, and with commercial consistency.

Table 1 Fermentation initiation time and duration

Strain	Fermentation initiation time (d)		Fermentation duration (d)	
	TCW	MP	TCW	MP
Spo F	13	5	30	16
LTSM-A1	3	1	15	8
B3-23d1-02	1	1	12	10
C1-7d3-02	1	1	7	7
C1-AR3-03	2	1	7	7
E0-1Y2-04	1	1	10	7
GTGM-E2	1	1	6	6
F1-7d2-04	1	1	10	10
G2-9d7-01	3	1	10	7
H0-6d5-01	2	1	9	10
I0-15d1-07	3	1	9	10
cy3079 (commercial strain)	1	1	6	7

Materials and methods

Preparation of strains

We previously systematically assessed 24 enological characteristics of 436 indigenous *S. cerevisiae* strains associated with Msalais production and five commercial strains in a laboratory setting (Zhu et al. 2016). We here used a pilot-scale fermentation approach to evaluate top ten indigenous *S. cerevisiae* strains and one commercial strain (cy3079) selected from the collection by Grey Relational Analysis (Ye et al. 2015) where the reference strain was artificially designed to lack H_2S and biogenic amine production, and for the highest level of alcohol production, polygalacturonase and β-glucosaccharase synthesis, the highest flocculation, autolysis, and tolerance of inhibitors,. The names of these selected strains are listed in Table 1.

Pilot-scale testing of the strains in TCW and MP fermentations

Cultures of the 11 strains were prepared by growing at 25 °C for 24 h in boiled grape juice (the grape was a local variety, *Vitis vinfera* He Tianhong, 24 °Brix). For MP fermentation, the initial fermentation juice was prepared by Daolang Msalasi Company as follows: the grape juice was compressed using a screw presser (YZ-10, XuZhou-Shijian Co., China), deposited in a settling pond (self-constructed, 500 L volume), and continuously transferred into 8-t capacity steam-boiling tank (self-made) to be boiled for ~8 h until sugar content reached 24 °Brix; then, it was cooled by cold running water to 35 °C before being pumped into a fermenting tank. For the experiments, 18 L of the initial fermenting juice were inoculated in 20 L glass jars with 1% (vol/vol) fresh yeast cultures, and left to ferment at ~25 °C for 7–15 days.

For TCW fermentation, the initial fermentation juice was prepared as follows. Local grape He Tianhong was squeezed using a self-made machine to imitate squeezing with feet. The grape debris was placed in a canvas sack, which was then placed inside a pot with water (weight:volume, 1:3) to be slowly boiled for 12 h. The extracted juice was mixed into compressed grape juice, slowly boiled for 12 h (final sugar up to 22 °Brix), and then spontaneously cooled down to room temperature to ferment for 24 h. In our study, 22 L of the initial fermenting juice in 25 L amphora jars were inoculated with 1% (vol/vol) fresh *S. cerevisiae* cultures, and left to ferment at room temperature (10–27 °C) for ~45 days.

Spo F in MP and TCW served as a control for pure fermentations (PMs) with the top ten indigenous strains and one commercial strain. Spo F took place in the juice previously boiled and cooled to temperature; the juice then slowly spontaneously fermented into Msalais without external interference. In PM, the boiled juice was cooled to 35 °C and inoculated with yeast cultures to quickly ferment into Msalais. During PMs and Spo F in the MP and TCW modes, sugar (Brix%) was monitored daily by a Brix refractometer. When the sugar level ceased to decrease, the fermentation was complete.

Fresh Msalais was kept in the same jar for 30 days with yeast lees, following which clarified Msalais was transferred to another jar for subsequent physicochemical and sensory analyses.

Each fermentation experiment was simultaneously performed in triplicate.

Physicochemical characterization of Msalais

Physicochemical analyses were conducted according to the analytical methods established by the National Standards of People's Republic of China (GB/T 15,038:2006). Accordingly, the alcohol content was determined by distillation, which was followed by density determination by hydrometry, with total sugars and reducing sugar content determined by a direct titration method, and total acidity determined by potentiometric titration.

Sensory analyses of Msalais

Msalais wines obtained from PMs and Spo F were tasted by ten people with extensive experience in Msalais brewing and drinking (drinking Msalais more than five times a week). The assessors scored the alcohol aroma, sweetness, sourness, bitterness, and persistence of flavor on a scale 0–5, from "none" to "strong". Every taster then assigned a comprehensive value, 0–10, to every tested sample, where 0 indicated lack of balance and unpleasing quality, and 10 indicated good balance with a pleasing overall sensation.

Statistical analysis

For statistical analysis, statistical significance was set at $P < 0.05$. One-way ANOVA was used to analyze the physicochemical and sensory differences between strains using the PC software package SPSS9.0 (IBM Corporation, NY, USA). Non-linear curve fitting mode in Origin 8.0 software downloaded from http://www.OriginLab.com was used to analyze the differences in trends of °Brix decrease between fermentations and between MP and TCW modes.

Results

MP and TCW fermentation analysis of indigenous *S. cerevisiae* strains

Top ten indigenous yeast strains were selected based on 24 enological characteristics from a collection of 436 indigenous *S. cerevisiae* strains in our laboratory (see "Materials and methods"). These indigenous strains and aone commercial strain (as a control) were used in TCW and MP fermentations (Table 1). Sugar content (°Brix) was continuously monitored during fermentation (Fig. 1), daily, at a fixed time, and the data were analyzed with non-linear curve fitting. All fermentations fitted well with the logistic regression model (data not shown). Trends of PM °Brix decrease in TCW or MP modes were similar (Fig. 1), but their logistic modes were different. The fermentation end-point was not obvious for strains involved

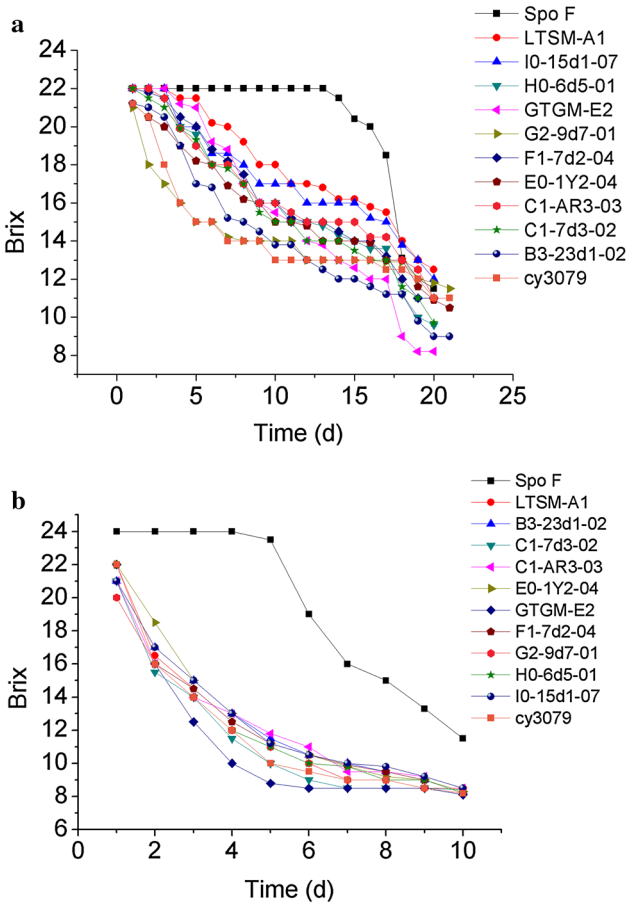


Fig. 1 Decreasing °Brix trends during TCW (**a**) and MP (**b**) fermentations. Data are presented as means ± SD (n = 3)

in TCW fermentation, with the exception of GTGM-E2 strain (Fig. 1a). Spo F °Brix decrease trend was pronouncedly different from that of PMs. Spo F fermentation was characterized by a long early lag stage with minor sugar consumption within the first 13 days in TCW mode and the first 5 days in MP mode, following which °Brix rapidly decreased, with the fermentation finished on day 30 in TCW and on day 15 in MP (Table 1; Fig. 1a, b). In contrast, PM fermentations were rapidly initiated within 3 days in TCW and 1 day in MP, and the fermentation was complete within 6–15 days and 6–10 days in TCW and MP modes, respectively (Table 1). For most PMs, the fermentation periods in TCW and MP modes were the same or slightly longer (1–3 days). The duration of fermentation with LTSM-A1 PM significantly differed in TCW mode, with the longest fermentation duration, 15 d. In contrast, GTGM-E2 PM fermentation was characterized by the most rapidly decreasing °Brix and much shorter duration, 6 d, in MP and TCW (Fig. 1a, b; Table 1).

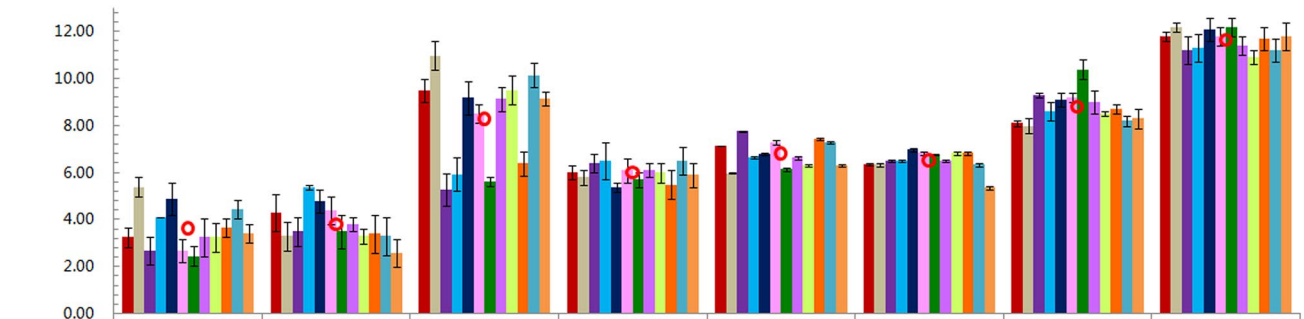
Physicochemical analysis of fermented Msalais wines

Although PM °Brix decreasing trends were similar, differences were noted in the duration of the lag phase, °Brix decrease rate, duration of the fermentation period, etc. These demonstrated different technological traits of the indigenous strains, most probably leading to the varying final quality of Msalais. Most of our final TCW-brewed Msalais had high reducing sugar content and low alcohol content, mainly because of a gradually decreasing temperature between late October and late November. This was especially evident in Msalais obtained with LTSM-A1 and GTGM-E2. The slowest °Brix decrease was observed during LTSM-A1 PM TCW fermentation, and the obtained Msalais had the lowest total acid content (7.0 g/L) but the highest total sugar and reducing sugar contents (10.98 and 5.4 g/L, respectively) (Fig. 2). Its comprehensive sensory value was also highest among the obtained Msalais (Fig. 3). However, Msalais fermented by GTGM-E2 had the highest alcohol content (10.4%), lowest total sugar and reducing sugar contents (6.1 and 4.9 g/L, respectively) (Fig. 2), and the lowest flavor persistence value (Fig. 3), most likely because of the shortest lag in starting the fermentation and the most rapid decrease of °Brix within the shortest fermentation period.

MP fermentation with GTGM-E2 PM was characterized by the most rapid and greatest °Brix decrease, and the shortest duration (6 days) (Fig. 1b; Table 1). The initiation time and duration were typically shorter and °Brix decrease was more pronounced in MP fermentations than in TCW fermentations at low temperature. This resulted in higher alcohol content (10.9–12.2%) and lower total sugar and reducing sugar contents (5.50–6.50 and 2.50–3.10 g/L, respectively) (Fig. 2). The total acid content of wine was also lower in MPs than in TCWs (Fig. 2).

Sensory analysis of the fermented Msalais wines

In Msalais obtained with PMs and Spo F, significant differences were observed in alcohol aroma ($P < 0.01$), comprehensive value ($P < 0.01$), and sourness ($P < 0.05$). No significant differences in sensory characteristics except for alcohol aroma ($P < 0.05$) were seen in MPs (Fig. 4). This indicated that traditional fermentation favors diversification of the quality of Msalais produced by different wild indigenous strains, while modern fermentation technology can homogenize the wine quality. In both TCW and MP, the sensory quality of Spo F Msalais was average or low compared with PMs (Figs. 3, 4), except for flavor persistence of Spo F, which was longer than that of PMs in TCW (Fig. 3). Compared with the selected indigenous strains, the commercial cy3709 strain exhibited relatively weak enological characteristics, as assessed by sensory examination of Msalais. This indicated the great potential of the use of



	TCW	MP	TCW	MP	TCW	MP	TCW	MP
	Reduce sugar (g·L-1)		Total sugar (g·L-1)		Total acid (g·L-1)		Alcohol (%) (v/v)	
Spo. F	3.25	4.30	9.50	6.00	7.13	6.34	8.10	11.80
LTSM-A1	5.40	3.30	10.98	5.80	5.99	6.34	8.00	12.20
B3-23d1-02	2.69	3.50	5.28	6.40	7.77	6.50	9.30	11.20
C1-7d3-02	4.10	5.40	5.93	6.50	6.64	6.50	8.60	11.30
C1-AR3-03	4.90	4.80	9.18	5.40	6.80	6.99	9.10	12.10
E0-1Y2-04	2.70	4.40	8.52	6.10	7.29	6.83	9.20	11.80
GTGM-E2	2.46	3.50	5.61	5.70	6.15	6.77	10.40	12.20
F1-7d2-04	3.25	3.80	9.13	6.10	6.64	6.50	9.00	11.40
G2-9d7-01	3.25	3.30	9.52	6.00	6.29	6.83	8.50	10.90
H0-6d5-01	3.68	3.40	6.39	5.50	7.45	6.83	8.70	11.70
I0-15d1-07	4.44	3.30	10.15	6.50	7.29	6.34	8.20	11.20
cy3079	3.43	2.60	9.15	5.90	6.31	5.36	8.30	11.80
mean	3.63	3.80	8.28	5.99	6.81	6.51	8.78	11.63

Fig. 2 Physicochemical characteristics of Msalais generated in the course of this study. Data are presented as means ± SD of three assessments

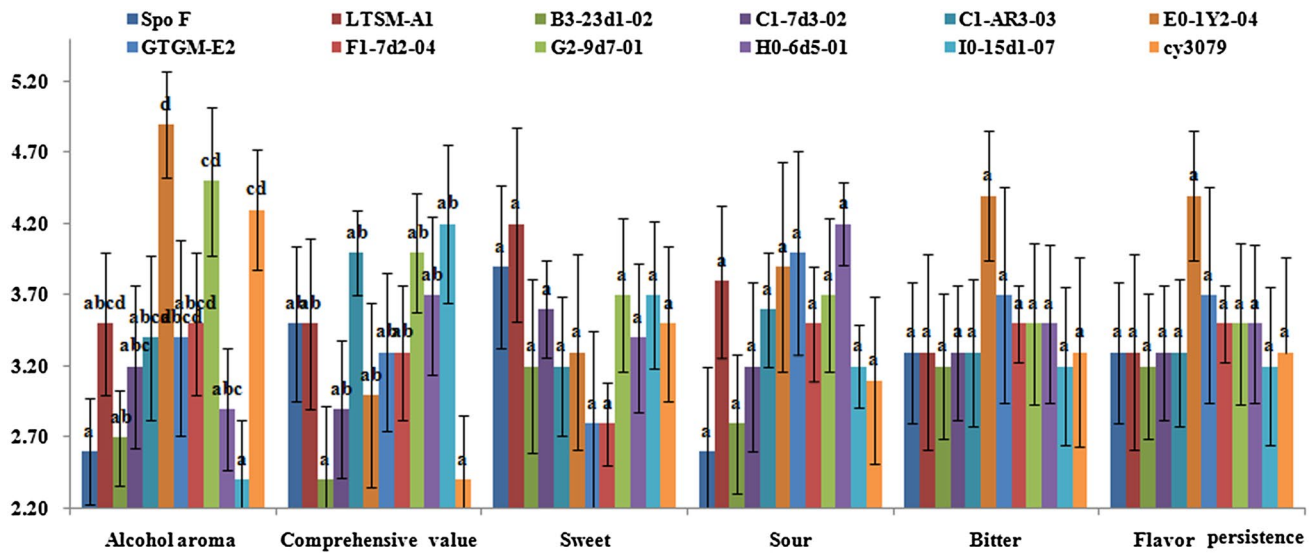


Fig. 3 Sensory analysis of Msalais from TCW fermentation. Data are presented as means ± SD of ten assessments

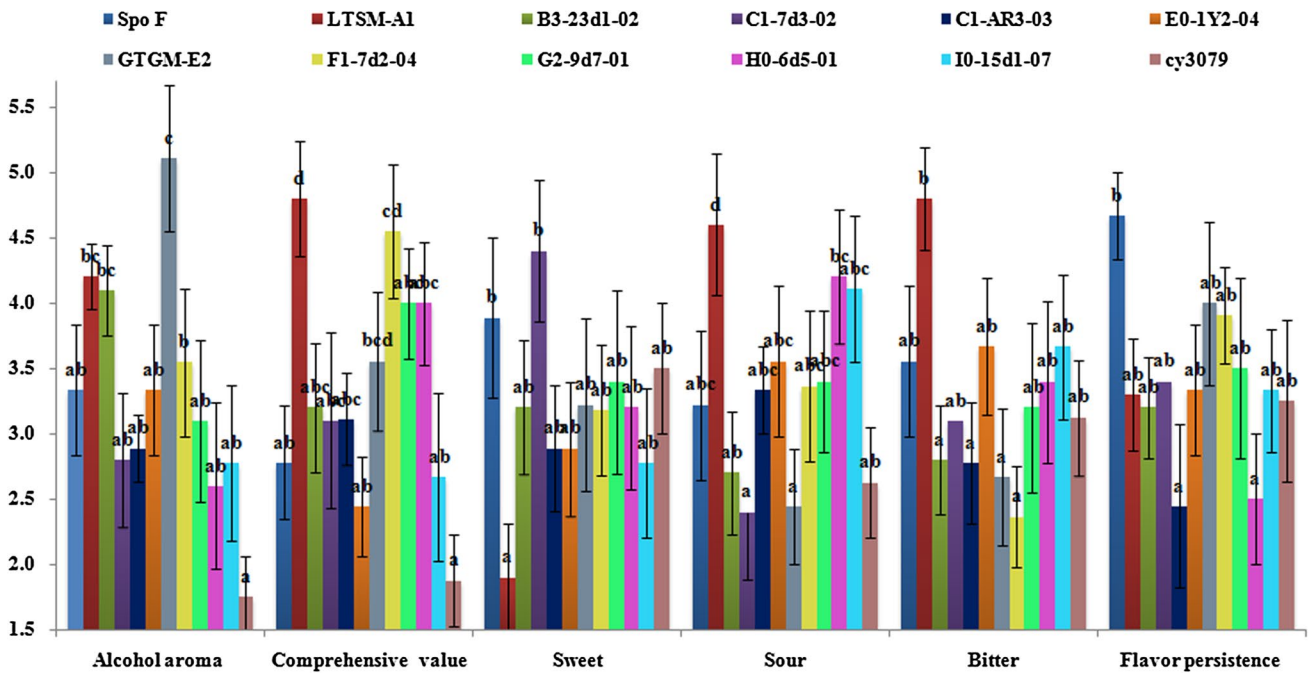


Fig. 4 Sensory analysis of Msalais from MP fermentation. Data are presented as means ± SD of ten assessments

indigenous wild *S. cerevisiae* strains to improve the quality of Msalais; meanwhile, their use was also associated with the considerable advantage of shortening the fermentation period compared with Spo F (Table 1).

To further analyze the sensory differences in Msalais obtained in TCW and MP fermentations, we used one-way ANOVA to compare Msalais wines fermented by the same strain in TCW and MP modes (Table 2). Msalais of Spo F significantly differed in flavor persistence ($P < 0.05$); Msalais fermented by LTSM-A1 significantly differed in sourness ($P < 0.05$) and flavor persistence ($P < 0.01$); Msalais fermented by F1-7d2-04 significantly differed in bitterness ($P < 0.05$); Msalais fermented by cy3079 and B3-21d3-02 significantly differed in alcohol aroma ($P < 0.01$); and that fermented by E0-1Y2-04 significantly differed in alcohol aroma ($P < 0.05$) (Table 2).

The above differences between Msalais fermented by Spo F and PMs, and the difference between TCW

Table 2 Differences in sensory characteristics of Msalais fermented in TCW and MP modes

Strain	Alcohol aroma	Comprehensive value	Sweetness	Sourness	Bitterness	Flavor persistence
Spo F	0.25	0.32	0.99	0.47	0.74	0.04*
LTSM-A1	0.58	0.17	0.48	0.02*	0.33	0.00**
B3-23d1-02	0.01**	0.28	1.00	0.88	0.55	1.00
C1-7d3-02	0.61	0.81	0.23	0.32	0.80	0.91
C1-AR3-03	0.45	0.07	0.66	0.62	0.47	0.30
E0-1Y2-04	0.02*	0.48	0.64	0.72	0.30	0.13
GTGM-E2	0.07	0.75	0.65	0.09	0.29	0.77
F1-7d2-04	0.95	0.09	0.53	0.85	0.03*	0.39
G2-9d7-01	0.10	1.00	0.74	0.70	0.73	1.00
H0-6d5-01	0.73	0.69	0.82	1.00	0.91	0.22
I0-15d1-07	0.61	0.09	0.25	0.16	0.56	0.86
cy3079	0.00**	0.39	1.00	0.54	0.84	0.96

P values of ten assessments are shown (one-way ANOVA)

*P < 0.05; **P > 0.01

and MP, indicated that the indigenous wild yeasts had a greater impact on the sensory quality of Msalais than the technology.

Discussion

As established by pilot-scale testing, °Brix decrease trends in all the fermentation reactions fit well with the logistic mode, with differences between PMs and Spo F, and also between the individual PMs. Spo F °Brix showed the slowest decrease, leading to the longest overall fermentation period of 30 days (TCW) and 15 days (MP). However, PM °Brix decreased rapidly, resulting in shorter fermentation time than that of Spo F, by 6–15 days in TCW and 6–10 days in MP. TCW and MP fermentation periods of the majority of selected strains, including cy3079, were the same or slightly longer (by 1–3 days). Of course, inoculation of fresh yeast cultures into the initial boiled grape juice (with almost all microbes killed by boiling) resulted in more yeast cells during the early fermentation in PMs than in Spo F. This also resulted in shorter fermentation periods in PMs than in Spo F. The shorter fermentation period with PMs was also associated with good adaptation to Msalais fermentation with strong fermenting vigor, and good tolerance of high sugar content and fluctuating temperature.

With respect to the final Msalais product, the total sugar content was higher in TCW than in MP mode, and variable in TCW fermentations; however, the alcohol content was lower in TCW than in MP fermentation, with no differences between individual fermentations. The other physicochemical characteristics were similar between fermentations and between TCW and MP modes. The sensory characteristics of wines were significantly different between TCW fermentations but not between MP fermentations, except for the alcohol aroma and comprehensive value. The sensory quality of Spo F was obviously different from PMs, with the best flavor persistence in TCW fermentation. The commercial strain cy3079 produced wine of a lower sensory quality than other fermentations, specifically, with the lowest comprehensive value in both TCW and MP, except for a strong alcohol aroma after MP fermentation. For individual Msalais wines, the comprehensive value and sweetness were not significantly different between TCWs and MPs, but five strains, including cy3079 and Spo F, produced wine that significantly differed either only in alcohol aroma, sourness, bitterness, or flavor persistence.

Considering the basic enological and fermentation characteristics, and the contribution to the final Msalais product, the indigenous strains LTMS-A1 and F1-7d2-04 were selected to brew high-quality Msalais by the traditional technology, and GTGM-E2 and I0-15d1-07 were selected to make high-quality Msalais by modern technology. GTGM-E2 and LTMS-A1 strains have been deposited in the China General Microbiological Culture Collection Center (CGMCC), accession numbers CGMCC 7513 and CGMCC 7512, respectively. They are currently successfully employed in Msalais production at Daolang Msalais Company and in Guzuofang workshop.

Because the boiling process reduces the effect of grape variety and quality on the final quality of Msalais and kills non-*Saccharomyces* yeast and bacteria, no significant difference in the final comprehensive sensory quality of the individual Msalais wines produced by MP and TCW fermentations was observed. Although it would be tempting to use pure fermentation with selected indigenous *S. cerevisiae* strains to produce Msalais on an industrial scale, the homogenization of Msalais quality,

sacrificing the wine's characteristic flavor, with the use of MP technology will be problematic. Fortunately, to avoid such homogenization and to increase complexity or obtain a typical flavor of wine, wine enologists and scientists are gradually turning their attention to a combined fermentation; they co-inoculate *S. cerevisiae* and some non-*Saccharomyces* strains, after characterizing their enological characteristics, interactions, and even specific yeast ecology (Azzolini et al. 2015; Blanco et al. 2013; Ciani and Comitini 2015; Contreras et al. 2015; Jolly et al. 2014; Renault et al. 2013; Rossouw and Bauer 2009; Zara et al. 2014). This constitutes a promising approach for fully utilizing our indigenous *S. cerevisiae* strains for Msalais production in the future and such work is currently under way in our laboratory.

In this study, for Msalais fermented by the same strain, the overall comprehensive value and sweetness were not significantly different between TCW and MP fermentations, and ten strains (including cy3079 and Spo F) fermented wines with significantly different alcohol aroma, or sourness, or bitterness, or persistence of flavor, in TCW versus MP comparisons. Furthermore, although the total sugar and alcohol contents were slightly different between TCW and MP wines, no differences were observed for other physicochemical characteristics. This is probably associated with the fact that the same basic producing technology was used and that the TCW and MP are located in the same area, within ~10 km^2. However, the differences in individual Msalais wine sensory characteristics can be explained by different enological traits of the individual strains and different TCW and MP microenvironments.

The selection and assaying of indigenous strains for a certain application, e.g., as a starter culture, to reduce alcohol content, enhance the aroma, generate a characteristic wine flavor, etc., is a popular approach in winemaking (Álvarez-Pérez et al. 2014; Medina et al. 2013; Romano et al. 2015; Steensels and Verstrepen 2014). However, it is apparent that no single indigenous strain possesses all the desirable enological characteristics. Many studies are concerned with comprehensive assaying of enological characteristics of strains to reduce random selection of indigenous strains, employing advanced microbiology technologies and big data mining methods (Bovo et al. 2015; Elmacı et al. 2014; Ortiz et al. 2013; Petruzzi et al. 2014; Steensels and Verstrepen 2014). Previously, we evaluated enological characteristics of a large collection of indigenous strains and used data mining methods for their comprehensive analyses, revealing a unique ecology of indigenous Msalais-associated *S. cerevisiae* strains, with typical enological characteristics typical for different TCWs or MP (Zhu et al. 2016). Here, we used top ten indigenous *S. cerevisiae* strains from our collection in a real fermentation scenario, in TCW and MP modes, to verify their industrial potential. This approach provides a reference for selecting high-quality strains, minimizing random sampling.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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