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Preservation of Traditional Chinese Shanlan Rice Wine Treated with CO_2 Top Pressure

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Abstract

CO_2 (Carbon dioxide) top pressure has been studied in brewing industry as a method for improving the wine quality. In the present study, this method was used for shanlan rice wine preservation. New made shanlan rice wine was subject to a CO2 top pressure which gradually increased from 0 to 1.3 MPa at 25°C and 5°C for 12 months respectively. Yeast was inhibited by pressure and stressed making the intracellular trehalose increase during the first 4 months. The production of higher alcohols, esters, total amino acid and ethanol except lactic acid were significantly suppressed by CO_2 top pressure. The influence of CO2 top pressure on sensory quality of shanlan rice wine at 25°C was also investigated. Residual sugar consumption was strong inhibited by pressure. The sweet, acidity, bitter taste and aroma, vinosity were kept on the same levels after 12 months pressure preservation compared to the samples at 0 month.

Keywords: Shanlan rice wine; Preservation; CO_2 top pressure; Volatile compounds; Sensory quality

Introduction

Chinese shanlan rice wine is the most popular traditional alcoholic beverage in Hainan island of China. The glutinous rice is preferred in the preparation of Chinese rice wine. Shanlan rice is a kind of glutinous rice growing in Wuzhi mountain area. This rice is used as the main materials to make wine without further distillation. The fresh made shanlan rice wine produce rich gas of CO_2 and alcohol, with mellow flavor and sweet, pleasant sensory [1].

High level of residual sugar in shanlan rice wine is not necessarily completely converted into ethanol. An appropriate ratio of sugar and alcohol will made the wine taste better. Heat treatment is usually done by flash pasteurization to terminate fermentation, where rice wine is first pasteurized and then filled into sterile glass bottles to kill all organisms in rice wine such as Chinese yellow rice wine [2]. Most conventional rice wine processing efforts aim towards the reduction or inactivation of microbial populations, which can be achieved through thermal processing using water, steam, electrical, microwave energy as a means for heat transfer [3]. However, heat treatment affects polyphenoloxidase activity and protein stability in the samples co MPared to that untreated [4]. Besides, off-flavors are easily formed in the rice wine during pasteurization.

Non-thermal methods have huge potential to eliminate the negative effect of heat on the aroma and flavors [5]. Low temperature preservation can slow down the fermentation rate of yeast and inhibit the propagation of spoilage organisms. Fresh shanlan rice wine must be stored in a sealed bottle at below 7°C, otherwise, it taste stronger bitter, alcoholic and acidic [6]. The shelf life of traditional rice wine is shorter than other industry alcohol beverages. Especially in summer, bitter taste appears in new produced rice wine without heated treatment after only 1 day at 25°C. In industry process, failure in pasteurization and storage or over-fermentation would cause bitter taste in rice wine [6]. Therefore, low temperature treatment is considered as a normal method to prolong the shelf life of rice wine.

However, new produced shanlan rice wine with atmospheric pressure kept fermentation at low temperature until the residual sugar in the wine was exhausted. In fact, new preservation technologies like high hydrostatic pressure (HHP) had been applied in many fields of liquid food processing [4]. Rice wine (*nigori-sake*) is one of the earliest HHP-treated commercial products that appeared on the Japanese market [7].

A top pressure of a sealed container is caused by the accumulation of CO_2 which generated by yeast spontaneously during alcohol fermentation. This top pressure formed from inner of fermentor play the same role as outer pressure like hydrostatic pressure cause stuck of the yeast metabolism. Yeast in medium is sensitive to the changing of fermentor top pressure during brewing process. CO_2 is evolved at the same rate throughout the entire body of fermenting wort. The fermentation is stuck when top pressure rise up to a certain value. The inactivating effect of CO_2 on microorganism is significantly effective just with a top pressure of 30 MPa [8].

Yeast cell produced intracellular trehalose through stress mechanism with pressure [9]. The yeast ability of acclimatization to changes of pressure depends on the level of intracellular trehalose kept yeast alive or even propagation for long time.

CO_2 top pressure has a huge influence on volatile compounds such as higher alcohols and esters which are the mostly effect on sensory quality of shanlan rice wine. Control of excessive higher alcohol concentration in beer can be achieved by applying top pressure during fermentation. The beer volatiles produced at 22°C with top pressure were the same as those at 15°C without top pressure [10]. The applied pressure had no effect on fermentation rate and therefore the advantage of using the higher temperature was retained. Hyperbaric conditions (7 MPa) inhibited ethanol formation. The inhibition was reversible by reducing the pressure [11].

Esters are produced in yeast cell through a biochemical pathway that involves ethanol or higher alcohols, acyl CoA and ester-synthesizing enzymes. The pool and availability of acetyl CoA is central role in yeast metabolism. CO_2 could affect the metabolic pathway of yeast and change the yields and types of metabolites and growth rates of yeast. The increase in top pressure from 1.05 MPa to 1.8 MPa at 16 decreased maximum yeast cells growth rates, maximal and initial ester production rates three fold [12].

Yeast could be killed with high hydrostatic pressure of 100 MPa at 40°C for 240 min [13]. The effects of top pressure treatment on inactivation of yeast grew in shanlan rice wine at different temperatures for 12 months had been studied in this paper. In addition, the effects of CO_2 top pressure on changing of volatile compounds, flavor, odour and freshness of shanlan rice wine had also been researched during preservation.

Materials and Methods

Shanlan rice and wine starter were purchased from local market in Wuzhi mountain city.

Shanlan rice pretreatment

Shanlan rice pretreatment Shanlan rice was washed and soaked in water for 12 h at room temperature (25°C), and then was drained and cooked by steam for 30 min at 100°C. The gelatinized rice was cooled to 40-45°C. The starter was ground into powder [1].

Shanlan rice wine fermentation

Shanlan rice wine fermentation According to the method described in the Yang's report [1], an advanced method was used in this study: Cooked rice (1 kg) was collected in 4-L fermenter and then 5 g starter power was added into the fermenter and well-distributed. The mixed rice was incubated for 7 d at 25°C and then the fermented mash was centrifuged at 5000 g for 20 min. The supernatant was added into the preservation system.

Shanlan rice wine preservation

Shanlan rice wine preservation Shanlan rice wine was stored in preservation system treated with CO2 top pressure at 25°C and 5°C, respectively. The Contrast was stored in the same preservation system with atmospheric pressure regulated by pressure controller at corresponding temperature (Figure 1).

Analysis of top pressure, CO2 content, biomass, survival rate, etc

Top pressure, CO_2 content, biomass, survival rate, intracellular trehalose content, pH, higher alcohols, esters, amino acids, residual sugar, ethanol and lactic acid analysis Top pressure was determined by a pressure gauge fixed on the preservation system. CO_2 content in shanlan rice wine was determined by a Huasheng CO_2 Detector (Series 7001-A, China). Biomass of yeast in the sample was determined by dry cell weight. Survival rate of yeast cell was determined by plate colony-counting method [14]. Intracellular trehalose content was determined by an anthrone colorimetry method [15]. The pH meter was used to determine pH value. Higher alcohols and esters were determined by gas chromatography [16,17]. Amino acids were determined by high performance liquid chromatography [18]. Residual sugar was determined by Fehling reagent method [19]. Ethanol content was determined by the method of Wang et al. [20]. The p-hydroxybiphenol colorimetry was used to determine lactic acid content [21].

Sensory evaluation of shanlan rice wine during preservation

A panel of thirteen volunteer panellists participated in the study. All panellists had previously participated in wine descriptive sensory analysis studies, and their performance had been assessed. Shanlan rice wines were evaluated on the basis of their aroma, vinosity, sweet, acidity and bitter.

The evaluations ranged from "negative" "weak", "strong" to "very strong" corresponding to the symbols of "-", "+", "++", "+++", respectively.

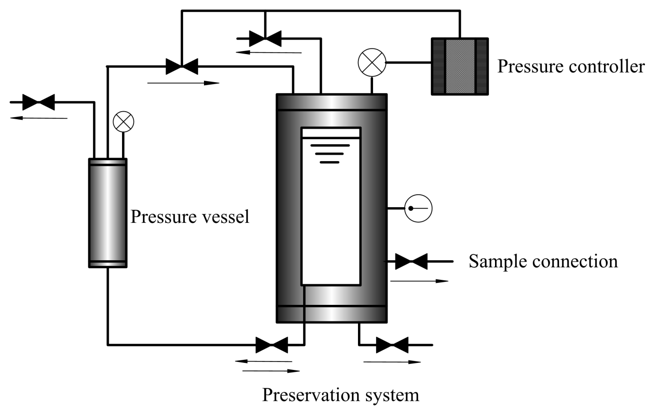

Figure 1: Apparatus for CO_2 top pressure treatment. The preservation system with a sample connection, temperature monitor, pressure controller and pressure vessel placed in a thermostat keep a constant CO_2 top pressure for shanlan rice wine preservation. The pressure controller play a pressure adjustment role and the pressure vessel act as a buffer to discharge the residual air in the main vessel.

Results and Discussion

Effects of CO_2 top pressure treatment to yeast metabolism in shanlan rice wine

Yeast grew by consumed the residual sugar in the rice wine and produced more CO_2 to aggravate top pressure during beginning stage of preservation (Figure 2A). CO_2 top pressure was build up gradually by yeast fermentation result in inhibition of physiological activities of yeast. Yeast cell and suspended matters in wine precipitated totally when the top pressure up to a certain value. CO2 top pressure rose up to 1.3 MPa and 1.0 MPa during the first 4 months storage and subsequently dropped to 1.1 MPa and 0.9 MPa at 25°C and 5°C respectively (Figure 2A). Enclosed spaces in a container limited the accumulation of CO_2 and the growth of yeast.

Hydrostatic pressure caused by nitrogen at the same value by CO_2 almost has not inhibition effect on yeast indicated that the depression effect comes from CO_2 instead of hydrostatic pressure [22]. The CO2 content in shanlan rice wine and top pressure followed a similar pattern, however the CO2 content of the wine at 25°C was equal to which at 5°C after 12 months preservation (Figure 2A). CO2 is generally considered an inert end product of cellular metabolism. The inhibition of CO2 might contribute to retard the yeast fermentation and reduce flavors in shanlan rice wine. Inhibition mechanisms of CO_2 on yeast are available for improving the quality of fermentation process.

Deformation of yeast cells was observed through scanning electron microscopy and cytoplasmic leaking from yeast cell lead to contraction folds and collapse of cell membrane with low CO2 pressure of 1 MPa [23]. Biomass was measured during preservation of shanlan rice wine stored in the sealed fermentor system. Slightly growth of yeast was observed during the first 4 months of preservation with top pressure and without top pressure at 25°C and 5°C respectively (Figure 2B). Environment temperature affected the accumulation of biomass during preservation. The biomass of the sample treated without top pressure at 25°C had reached the maximum value of 18 mg/mL after 4 months preservation. The peak value of biomass appeared 4 months earlier than the sample treated at 5°C (Figure 2B). The requisite nutrition for yeast growth in shanlan rice wine treated with CO2 top pressure at 5°C was still sufficient after 6 months storage.

CO_2 top pressure changed the environment hydrostatic pressure and caused yeast death and reduction of survival cells. Clear decline trend of survival rate of yeast in the rice wine treated with CO_2 top pressure was observed during 12 months (Figure 2B). Corresponded to the changing of top pressure, significant descent of survival rates appeared during 4 months and 8 months respectively at 25°C and 5°C. Top pressure of 1.4 MPa (low hydrostatic pressure) inactivated yeast grew in shanlan rice wine or even killed it through long time treatment (12 months), especially at higher temperature of 25°C.

Survival rate curves of yeast corresponded to different CO_2 top pressures at different temperatures (Figure 2B). This revealed that the yeast had different sensibility and adaptability to various pressure stimulations. Lin suggested that the primary cause microorganism death with increasing CO_2 pressure is the extraction effect of dissolved CO_2. CO_2 generated carbonic acid and then combined with Ca^{2+} and Mg^{2+} forming precipitation which causing damage of cytomembrane [24]. Isenschmid suggested that there was an effective critical temperature (18°C) controlled growth or death of yeast. When the temperature rose above 18°C, the concentration of dissolved CO_2 was thought to be the key effect factor which controlling the death of yeast due to the anaesthesia effect [25].

Yeast in shanlan rice wine accumulated biomass for a long time instead is killed instantly because of the low hydrostatic pressure. Pressure increased the intracellular trehalose synthesis in yeast. Trehalose sustain irritability to resist severe environment such as the changes of pressure, temperature etc [9]. As a stress countermeasure, yeast will generate stress products such as trehalose etc. The mass fraction of intracellular trehalose has been measured in this study (Figure 2C).

Pressure has a significant effect to accumulation of trehalose in yeast. Pressure fermentation duration time has huge impact with the mass fraction of intracellular trehalose, revealed such stress products associated with growth stage of yeast [9]. Figure 2C showed a clear increase trend of intracellular trehalose in yeast during the first 4 months preservation of shanlan rice wine and a clear decline trend during the last 8 months. This decline trend means not only the decrease of trehalose in live yeast but also the reducing amount of live yeast. Sharma reported that in a strain of *S. cerevisiae* increased osmotic pressure brought about by elevated levels of sodium chloride was accompanied by increased accumulation of trehalose and increased tolerance to ethanol [26].

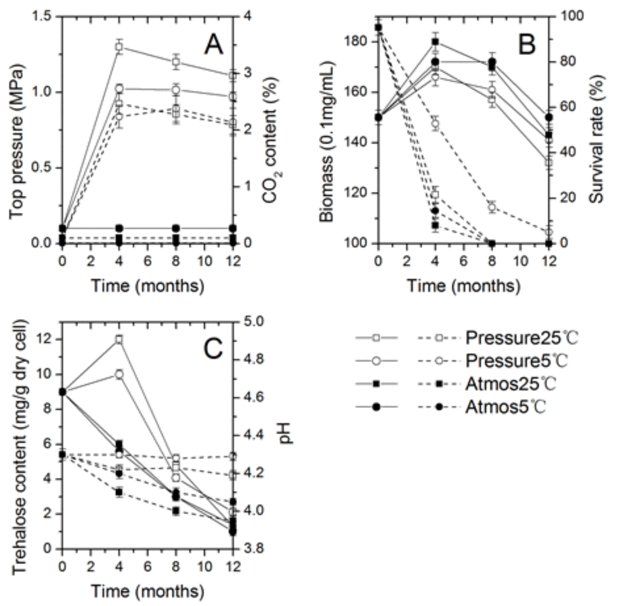

Figure 2: (A-C): Evolution of CO_2 top pressure (solid line), CO2 content (dash line), Biomass (solid line), Survival rate (dash line), intracellular trehalosein content (solid line) and pH (dash line) of shanlan rice wine during preservation under CO2 top pressure (25°C, 5°C) and atmospheric pressure (25°C, 5°C) at 25°Cand 5°C respectively. A CO_2 top pressure and CO_2 content, B biomass and survival rate, C intracellular trehalosein content and pH.

In this study, a slightly ascend of trehalose content had been observed with top pressure at different temperatures. The mass fraction of intracellular trehalose was increased by 33% at 25 while 11% at 5 (Figure 2C). With high pressure, the treatment temperature has a great impact with intracellular trehalose. Qiao found that the content of trehalose keep increase up to 11.37 mg/g (dry cell) in the company with the temperature raising until to 34°C. However, kept elevating temperature up to 39°C lead the content of trehalose reduce low to 2.22 mg/g (dry cell) instead [9].

In summary, the mass fraction of trehalose increased to reach the top values by different ranges depended on at diverse temperature during the first stage of pressurization, and then began to decrease for permanent during the last stage of preservation with certain top pressure. The mass fraction of trehalose in yeast treated with pressure was higher than contrast with atmosphere pressure (Figure 2C). There was a positive correlation between the changing of mass fraction of intracellular trehalose and yeast survival rate. In the beginning of pressure fermentation, the yeast accumulated a great number of trehalose in order to storage for live under stress. Yeast began to adapt to the changing of pressure circumstance and the trehalose reduced for the yeast growth after a period of time. Yeast accumulated trehalose inside the cell was beneficial to be suitable for a violent changing of in vitro environment.

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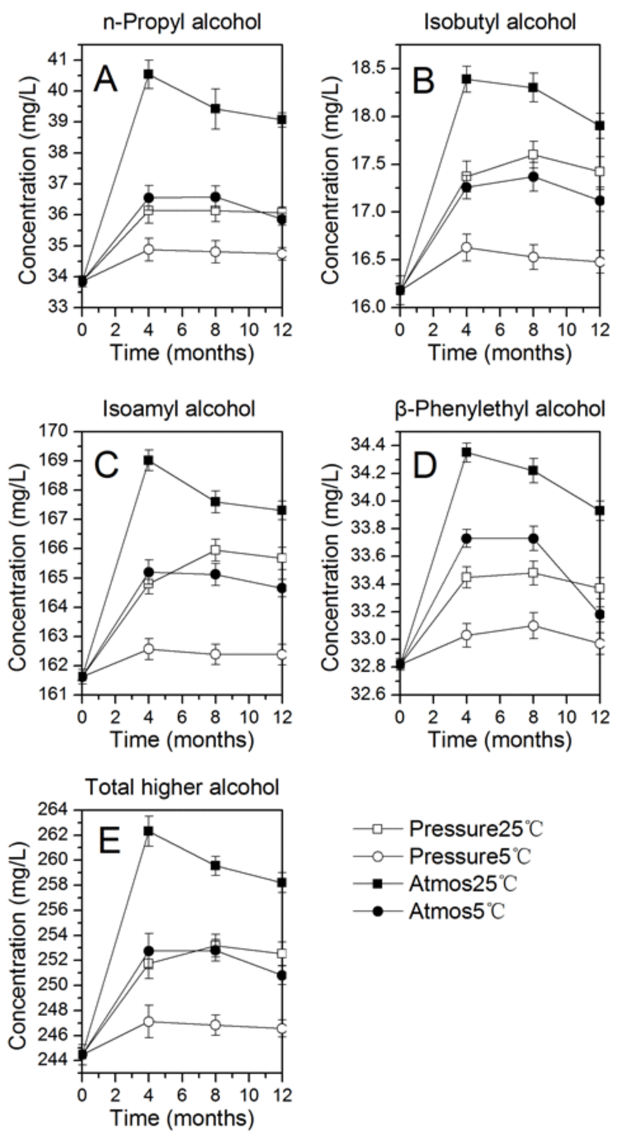

Figure 3: (A-E): Evolution of higher alcohols of shanlan rice wine during preservation under CO_2 top pressure (25°C, 5°C) and atmospheric pressure (25°C , 5°C) at 25°C and 5°C respectively. A n-Propyl alcohol, B Isobutyl alcohol, C Isoamyl alcohol, D β-Phenylethyl alcohol, E Total higher alcohols.

The pH of shanlan rice wine was measured during 12 months. Figure 2C showed that the values of pH of the samples treated with CO_2 top pressure were higher than those with atmospheric pressure both at 25°C and 5°C. Miedener reported a number of analytical differences between beers from normal and pressurised fermentations. The pH of the latter was higher, possibly a consequence of increased yeast shock excretion of nucleotides and amino acids in response to the higher pressure [27].

CO_2 inhibits the bioactivity of yeast in the medium lead to the fluctuation of pH does not mainly caused by the yeast metabolism but outer physical parameters changing. The first possible causes was that some CO_2 dissolved in the wort resulted in a slight decrease of pH, however, inorganic salt ions come from the medium or yeast metabolism played a buffer role to retard the decline of pH. Another reason was yeast cells autolysis due to the raise top pressure operation. Some alkaline substances leaked from the corrupted yeast cells into fermentation medium were used for neutralization reaction with acidic medium, result in pH raising.

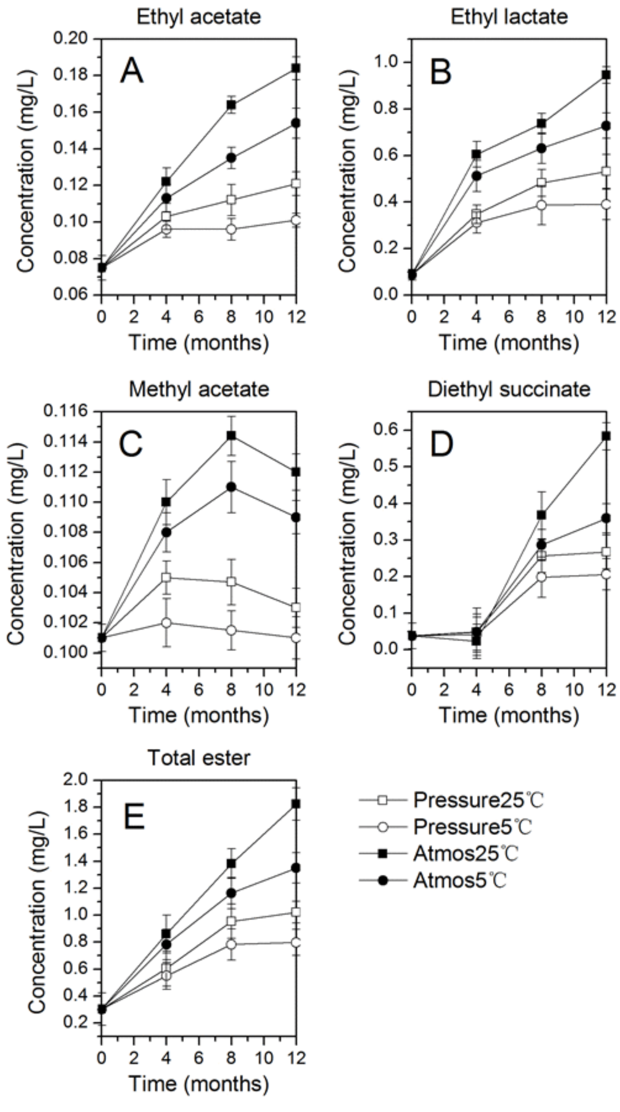

Figure 4: (A-E): Evolution of esters of shanlan rice wine during preservation under CO_2 top pressure (25°C, 5°C) and atmospheric pressure (25°C , 5°C) at 25°C and 5°C respectively. An ethyl acetate, B ethyl lactate, C methyl acetate, D diethyl succinate, E total ester.

Evolutions of higher alcohols and esters in shanlan rice wine during preservation

The growth of yeast was stuck accompany by an insufficient ability to produce higher alcohol due to apply of CO_2 top pressure during preservation. A sharper increase of higher alcohols in the wine preserved with atmospheric pressure co MPared to those treated with pressure had been observed after 4 months preservation. And the trend of the samples treated without pressure then became decline (Figures 3A-3E). Ethyl lactate, ethyl acetate and higher alcohol composed the main aroma of yellow rice wine [28]. Bao made a quantitative analysis of isoamyl alcohol and β-Phenylethyl alcohol in Chinese yellow wine by direct injection GC. A reduction of isoamyl alcohol and β-Phenylethyl alcohol were found with the preservation years increasing. The contents of isoamyl aldehyde, furfural and benzaldehyde were found reduction with the preservation years increasing by the method of GC/MS method by dynamic Headspace Sample Injection [29]. Yeast in Chinese rice wine will not stop fermentation until the fermentable sugar is exhausted, producing tyrosol, tryptophol and some higher alcohols which mainly gave bitter taste through metabolism under anaerobic condition [6]. Anaerobic fermentation is favorable for the accumulation of higher alcohols [30]. However, such an anaerobic condition is favorable for yeast growth without extreme case like top pressure, high temperature, or high salt and alkali etc.

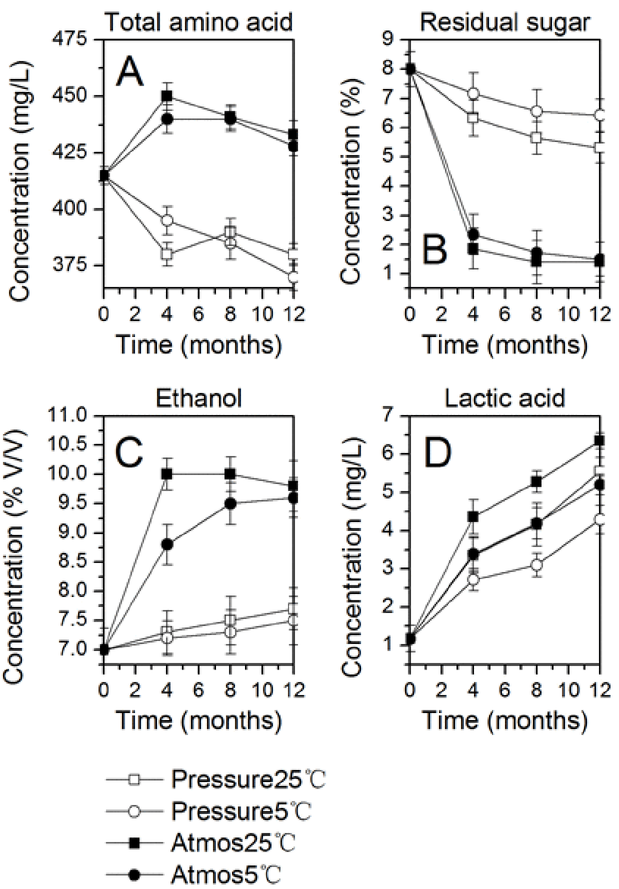

Figure 5: (A-D): Evolution of total amino acid, residual sugar, ethanol, lactic acid in shanlan rice wine during preservation under CO_2 top pressure (25°C, 5°C) and atmospheric pressure (25°C , 5°C) at 25°C and 5°C respectively. A total amino acid, B residual sugar, C ethanol, D lactic acid.

The growth of Yeast in shanlan rice wine treated with CO2 top pressure was stuck and produced less higher alcohols than in an open fermentation system. Figures 3A-3D showed a slow growth of n-Propyl alcohol, Isobutyl alcohol, Isoamyl alcohol, β-Phenylethyl alcohol and Total higher alcohols content during the begun 8 months of preservation, slight fluctuation and decline during the end 4 months respectively.

Temperature affects the assimilation of amino acid and sugar metabolism and also controls higher alcohol production [31]. The higher temperature, the higher alcohols production no matter treated with pressure or without (Figures 3A-3D).

Pressure fermentation has been used successfully to compensate for the effects on beer flavour of elevated temperature at production scale. A pressure of 0.12 MPa was applied gradually during fermentation. The reduced volatile components co MPared to fermentations with atmospheric pressure. Both esters and higher alcohols were influenced but to varying degrees [32]. CO_2 top pressure was used to reduce the concentrations of higher alcohols performed at elevated temperatures. Corresponding to the elevated temperature, the release of CO_2 was restricted and a top-pressure of roughly 0.18 MPa was allowed to build up [27].

While ethyl lactate increased by 2.45 fold (Figure 4A) and methyl acetate increased by 10% only (Figure 4C). In comparison, top pressure made lower level productions of esters (Figures 4A-4E). A raise of content of ethyl lactate was found with the preservation years increasing in Chinese yellow wine [29]. However, a decrease of production of total ester and ethyl acetate while a huge increase of production of ethyl decanoate were observed when yeast ferment in a sealed container [33].

Temperature enhanced the CO_2 pressure impacts to esters forming. The esters of the wine stored at 5°C were measured by lower level than those at 25°C (Figures 4A-4E). Raising top pressure may eliminate the influence of negative flavour substance caused by heating up operation [34]. Various styles of beer might be produced by even the same wort and yeast strain through CO_2 top pressure and temperature regulation to control the ester forming [35]. Appropriately changing pressure and temperature has a certain influence on yeast growth, CO_2 producing and concentration of high alcohol and total ester, even on production kinetics. Forming a harmonious fermentation circumstance may make special flavour of beer [36].

Volatile compounds in shanlan rice wine can be toxic to yeast cells, and consequently cause various effects to the productivity. The sensory diversities of shanlan rice wine depend on what method been taken during the storage period.

Evolution of flavor amino acids, residual sugar, ethanol and lactic acid in shanlan rice wine during preservation

Chinese rice wine contains rich amino acids ranking the highest content amino acids of alcohol beverages all over the world. All kinds of amino acids show their own taste and bring various flavors to the rice wine. In all kinds of amino acids, bitter amino acids accounted for 44%, and the threshold levels of these bitter amino acids were generally lower than others. It was suggested that the bitter in Chinese rice wine mostly comes from the amino acids due to the hydrophobic groups in it [37].

Shanlan rice wine is nutritious account of the rich amino acids content. All kinds of amino acids displayed a multiple flavor such as palatable, sweet, bitter, astringent etc. The compatible proportion of different amino acids and the controlling of the amount of total amino acids will make a combination of flavor and improve the sensory quality of shanlan rice wine. Bitter amino acid was the dominant of total amino acids had been shown in Table 1, accounting for 66%. Total amino acids of shanlan rice wine slightly declined in the level.

An increasing and then decreasing trend of total amino acids was observed in the sample of shanlan rice wine treated with atmosphere pressure. Lower temperature kept a lower level of amino acids during preservation (Figure 5A). Part of reduced amino acids was used to build yeast cell and produce higher alcohol [31]. The higher the CO_2 pressure, the more original amino acids were retained in the wine which was kept a fresh flavor and taste when the pressure was discharged. Increasing the pressure and the content of CO_2 could change the patterns of amino acids absorption and that could be related to changes in the formation of flavor volatiles [38].

Amino acid	**Concentration (mg/L)**	**Proportion of flovor amino acid (%)**	**Flavors presentationa (38)**			
			Bitter taste	Acidity taste	Palatable taste	Sweet taste
Asp	67.47 ± 0.02		-	+	+	-
Ser	15.01 ± 0.01		-	-	-	+
Glu	4.57 ± 0.05		-	+	+	+
Gly	9.24 ± 0.03		-	-	-	+
His	14.21 ± 0.02		+	+	-	-
Arg	12.45 ± 0.02		+	-	-	-
Thr	0.62 ± 0.05		-	-	-	+
Ala	17.09 ± 0.04		-	-	-	+
Pro	14.20 ± 0.01		-	-	-	+
Cys	1.16 ± 0.01		-	+	-	-
Tyr	58.50 ± 0.03		+	-	-	-
Val	17.19 ± 0.02		+	-	-	+
Met	11.91 ± 0.03		-	-	-	+
Lys	41.23 ± 0.05		+	-	+	-
Ile	18.79 ± 0.05		+	-	-	-
Leu	49.02 ± 0.04		+	-	-	-
Phe	62.52 ± 0.02		+	-	-	-
Total amino acid	415.19	-				
Bitter amino acid	273.92	65.98			-	
Acidity amino acid	87.41	21.05		-		-
Palatable amino acid	113.27	27.28	-			
Sweet amion acid	89.82	21.63				

Table 1: The concentration of amino acids in shanlan wine after 7 d fermentation and the flavors presented by each amino acid. The results are means of duplicate analyses ±standard deviation. a Flavors presentation + (positive), - (negative).

Shanlan rice wine is also known as sweet wine and a kind of folk fermented beverage which tasted sweet, acidic, bitter and alcoholic. The fermentation of shanlan rice wine was suspended by sealed the ferment vessel and the product was turned into sweet wine. The sweet wines produced in other region of China were all sugary by the total sugar content of 25-30% in Guangxi sweet wine [14] and 27% in Hubei sweet wine [39] respectively. The total sugar of Shanlan rice wine was only measured as 8% after 7 d fermentation (Figure 5B), but with a much higher content of alcohol counting by 7% (v/v) (Figure 5C) than Guangxi sweet wine and Hubei sweet wine which counting by 4.5% (v/v) and 2.7% (v/v) respectively. Alcohol yield of Chinese sweet rice wine is usually about 4% (v/v) to 10% (v/v). Japanese mirin compositions consist of approximately 10-13% (v/v) alcohol [40]. During Shanlan rice wine preservation, a slight decrease of residual sugar low to 5.5% with pressure at 5°C and a sharply decrease low to 1.5% with atmospheric pressure at 25°C were observed (Figure 5B). Alcohol slight increase up to 7.7% with pressure at 25°C and a sharply increase up to 10% with atmospheric pressure at 25°C were observed (Figure 5C).

Lactobacillus fast yielded lactic acid utilizing oligosaccharides and pH sharply decreased to 4.0 at the starting phase [41]. The rice was degraded by microorganism in starter through a synergistic effect of saccharification and fermentation. The organic acid such as lactic acid was generated by rhizopu and lactobacillus caused the acidity increase. While the fermentation of yeast degraded the lactic acid into small molecular substance such as alcohol etc. results in the acidity decrease during the post-fermentation [41]. The lactic acid content kept increase no matter in pressurised preservation or none. The preservation temperature of 25°C and 5°C were applied, the higher temperature, the faster growth of lactic acid (Figure 5D).

Effect of CO_2 top pressure treatment to sensory quality of shanlan rice wine

Sweet is the primary taste in Shanlan rice wine in which the sugar content accounting by 8% after 7 d fermentation. The sweet taste in the wine persisted during whole 12 months preservation treated with CO_2 top pressure. However, the sweet taste of those samples stored in the vessel with atmospheric pressure tented to weaken till to vanish by the day when the sugar in the wine was exhausted instead. Once the carbon source (carbohydrates) exhausted, the increase of bitter taste was observed by sensory quality test (Table 2).

The acidity reflected the sour degree of shanlan rice wine, which initially increase before then decreased in all samples (Table 2). Lactic acid is a significant flavor in shanlan rice wine. The overabundance or shortage both result in disharmony sensory of the wine. In the case of overabundance, the more ethyl lactate will be produced by redundant lactic acid and lead to sour and astringent taste in the wine. In the case of shortage, a lack of lactic acid leads to the sensory monotony. The content of lactic acid was the highest in all organic acids, which dominated the sour flavors.

The appearance of bitter could be delayed by extended the logarithmic growth phase of yeast [6]. The bitter substances in rice wine consist of the following categories: higher alcohol, bitter amino acid and tyrosol. The higher alcohols in wine such as butyl alcohol, butyl alcohol, isobutyl alcohol and isobutyl alcohol were all bitter taste. The bitter amino acids accounted for a majority of all amino acids, which played an important role in wine flavors. The tyrosol was generated by tyrosine decomposition, which smelled delightful with pleasant aroma while tasted strong bitter.

The normal bitterness endows the flavor style for sensory to each production like beer, yellow rice wine, coffee and green tea etc. The abnormal bitterness tasted strong bitter and keeping for a long period in mouth without vanishing [37]. The bitter was found weaken during 12 months pressure preservation both at 25°C and 5°C, while it turned to be stronger and prolong the unpleasant bitter taste in the wine with atmospheric pressure preservation (Table 2).

Some substance such as higher alcohols and aldehydes in rice wine make peoples who have drunk dizziness, headache and dry throat. Higher alcohol is a component of flavors of wine while it is harmful to people when the content is exorbitant.

Preservation time (month)	**Appearance of shanlan wine**				**Sensory Quality**									
	Clarification		Color		Aroma		Vinoscity		Sweet		Acidity		Bitter	
	Pc	Nd	P	N	P	N	P	N	P	N	P	N	P	N
1	+	+	+	+	+++	++	++	++	++		+	++	++	++
2	++	++	+	+	+++	++	++	++	++	-	+	++	+	++
3	+++	++	+	+	+++	+	++	+++	++	-	+	++	+	++
4	+++	++	+	+	+++	-	++	+++	++	-	+	++	+	++
5	+++	+++	+	++	+++	-	++	+++	++	-	+	++	-	+++
6	+++	+++	++	++	+++	-	++	+++	++	-	++	++	-	+++
7	+++	+++	++	++	++	-	++	+++	++	-	++	++	-	+++
8	+++	+++	++	+++	++	-	++	+++	++	-	++	++	-	+++
9	+++	+++	++	+++	++	-	++	+++	++	-	++	++	-	+++
10	+++	+++	++	+++	++	-	++	+++	++	-	++	++	-	+++
11	+++	+++	+++	+++	+	-	++	+++	++	-	++	++	-	+++
12	+++	+++	+++	+++	+	-	++	+++	++	-	++	++	-	+++

Table 2: Appearance and sensory quality of shanlan wine preservation (at 25°C). a levels of clarification and color of shanlan wine from + (little) to +++ (very clear) and + (light yellow) to +++ (dark yellow) respectively. b levels of aroma, vinosity, sweet, acidity, bitter respectively ranging

from + (weak) to +++ (very strong). c shanlan wine was treated with top pressure of carbon dioxide. d shanlan wine was treated with atmospheric pressure. e not dectected.

A mellow wine taste and gentle vinosity had been observed during pressure preservation for 12 months, however, the overabundant higher alcohol taste turned to stronger with storage time accompany with the bitterness increase during atmospheric pressure preservation. Obviously, the temperature impact on the alcoholic sensory and the higher the temperature, the sharper the fusel taste (Table 2).

Conclusion

The production of higher alcohols, esters, total amino acid and ethanol except lactic acid were significantly suppressed by CO2 top pressure. After 12 months low pressure preservation, the sweet, acidity, bitter taste and aroma, vinosity was kept on the same levels compared to the samples at 0 month.

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