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元　培　學　報
第十期　民國 92 年 12 月
1　～　12　頁

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標楷體 10
固定行高 18pt

Journal of Yuanpei University of
Science and Technology
No. 10, December 2003
P. 1　～　P. 12

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低頻超音波於米酒熟成之研究

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The Effects of Low Power Level of Ultrasonic Waves of Rice Wine Maturation

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(Received July 29, 2003; Revised October 3, 2003; Accepted December 16, 2003)

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摘　要：本試驗主要探討低頻超音波處理於米酒熟成之研究。米酒釀製所用之菌種為清酒酵母菌(*Saccharomyces sake*)。米酒釀製後以傳統瓦甕一年儲放及低頻超音波處理二種方式加以熟成，並分別在酒精度、pH 值、揮發性香氣成份之氣相層析及品評上做分析比較研究。結果顯示，經低頻超音波處理至某一層次後之米酒，在熟成風味上與傳統瓦甕式一年儲放的熟成酒可堪比擬至近似程度，但卻可節省大約一年的時間，值得推廣應用。本研究只試驗於單一低頻之超音波，因此建議宜再探討各種不同頻率之超音波對不同酒類的組合，研究其熟成風味上之差異性。

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關鍵字：風味、米酒、超音波、氣相層析、熟成

Abstract: Rice wine was fermented with *Saccharomyces sake*. One type of methods of rice wine maturation was matured for one year in fired clay containers (standard maturation method) and another type of methods of rice wine maturation was matured with a new ultrasonic waves process of low power level (accelerated maturation method). Comparisons were made on alcohol content, pH value, gas chromatography measurements, sensory evaluation, and the time of maturation.　Our results showed that the low power level ultrasonic waves maturation treatment was capable of maturing rice wine to a similar quality of taste as the conventional method of maturation in a much shorter period of time. However, further study is needed to learn more on applying various ultrasonic waves to age wines made of various materials.

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Key words: Flavor, Rice Wine, Ultrasonic Waves, Gas Chromatography, Maturation

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I. Introduction

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Wine plays many relatively important roles in life, especially socially but also in mood soothing, in cholesterol lowering and in cooking. Wine can be made from various materials using several processes. Wine making, however, takes time for fermenting and for maturation. Fermenting produces alcohol, flavor, and taste; maturation improves the properties that make wine pleasurable. Wine usually has several flavors and other properties which each play a subtle, intertwined, yet important, role in the tastes that humans prefer. So, many scientists have put much effort in studying the flavors, physical properties, chemical properties and constituents of wines made from various materials using different processes and maturation techniques[1-8]. There have been studies of wine maturation using different chemical methods[4,9-11], but very little work was found dealing with accelerating wine maturation by applying physical methods with a lack of verifiable data in this field. This study investigated reducing the time and space required for conventional maturation using a physical acceleration maturation method that utilized ultrasonic waves.

Suslick[8] stated that in an ultrasonic waves process, high temperature and high pressure can be generated from circulated ultrasonic waves causing chemical polymers to be broken into numerous mist particles (sub particles) and then recombined. Saterlay and Comptom[12] consolidated some information in "Sonoelectroanalysis-an Overview" of ultrasonic waves. In their special issue paper, an overview of exciting new advances in the area of sonoelectroanalysis was given and the need for ultrasound power was stated. They concluded that the ultrasonic equipment working in the range of 20-100kHz is relatively inexpensive, readily available and has proven to be an excellent enabling technology for electroanalysis. They further pointed that the introduction of ultrasound into voltammetric cells has a marked effect upon their mass transport which offers a dramatic improvements to reaction rates. Earlier than Saterlay *et al.*[12] Lindley and Mason[13] also stated the types of ultrasound which are used in chemistry for effecting chemical reactivity were broadly divided in power between 20 and 100 kHz. They stated that chemists are interested in ultrasound power because it provides a form of energy for the modification of chemical reactivity which is different from those normally used such as heat, light, and pressure. Furthermore, Lindley et al.[13] also stated that ultrasound power produces its effects via cavitation bubbles. These bubbles are generated during the rarefaction cycle of the wave when the liquid structure is literally torn apart to form micro bubbles which collapse in the compression cycle.Lindley *et al.*[13] noted further that it has been calculated that pressures of hundreds of atmospheres and temperatures of thousands of degrees are generated on collapse of the bubbles. Cocito, Gaetano and Delfini[1] studied the effects of "Rapid Extraction of Aroma Compounds in Must and Wine by Means of Ultrasound" by using 48 kHz ultrasonic waves to extract the aromatic compounds of must and wine. Matsuura, Hirotsune, Nunokawa, Satoh and Honda[14] studied the "Acceleration of Cell Growth and Ester Formation by Ultrasonic Waves Irradiation. They investigated the possible application of 43 kHz ultrasonic waves to the fermentation control of wine, beer, and sake made from a saccharified rice solution, particularly in reducing dissolved carbon dioxide levels to a better Saccharomyces cerevisiae fermentation.

We hypothesized that the high temperature and high pressure generated from circulated ultrasonic waves might turn the acid and alcohol into ester reactions to increase the fragrance and give more flavor and/or taste to the wine. Thus by applying this physical acceleration maturation method of ultrasonic waves, the purpose of maturation would be fulfilled within a shortened time and space saved as well. Our paper reports the effects of using a low power level band width of 20 kHz ultrasonic waves to mature rice wine. In particular, the effects on alcohol content, pH value, gas chromatography measurements, sensory evaluation and the time of maturation were

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researched. The 20 kHz band width is in the range Lindley *et al.*[13] and Saterlay *et al.*[12] provided information on.It is relatively inexpensive, readily available and has been proven to provide a form of energy for the modification of chemical reactions. In this study, rice was chosen because it is relatively inexpensive, rich in starch (essential for alcohol fermentation techniques) and has less complex flavors and other properties compared to wines made from other materials for use in experimental analyzation.

II. Materials and Method

All the rice wine was made with a 1:1 ratio of whole dry grain rice (14% water, dry basis) and water. The rice/water mixture was cooked at 125℃ about one hour and cooled to room temperature (25℃). This process was done many times in our experiment in order to get enough quantity of cooked rice to produce the desired volume of rice wine for our experiments. Aspergillus awamori (CCRC 30891, 0.1% of total weight) was then inoculated into the rice in order to produce amyloglucosidase, glucoamylase, alpha-amylase, and beta-glucuronidase to break down the starch polymers into smaller starch oligo carbohydrate polymers and sugar monomers and also liquefy the rice for further yet more complete fermentation. About one hour later, when the temperature reached around 25℃, Saccharomyces sake (CCRC 20262, 0.5% of total weight) was then inoculated into the liquefied rice and kept at 25℃ for fermentation. The fermentation took place in closed fired clay containers which were vibrated 2-3 times a day. After the fermentation was completed (14 to 16 days, depending on the alcohol content desired, for example 18-20% alcohol in our experiment), the rice wine was collected by using the distillation method and ready for the ultrasonic wave treatments of the accelerated maturation process or for the 1 year standard maturation process.

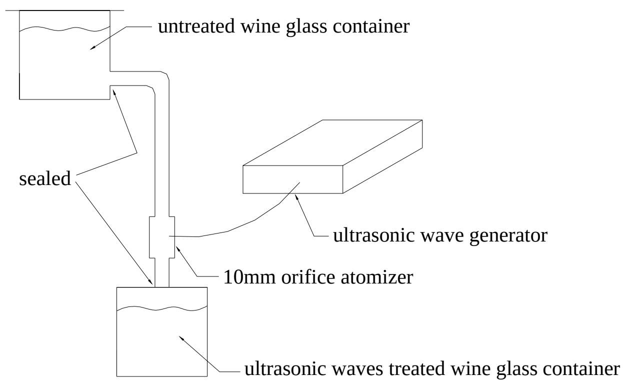

Figure. 1 Diagram of the ultrasonic waves set up

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The standard, or conventionally matured, rice wine was matured by placing it into fired clay containers for one year prior to conducting the accelerated maturation. A maturation time of one year is the standard for conventionally maturing Asian market grain wines, and analyzation at that maturation time was done. About one year later, the wine to be matured by accelerated maturation rice wine was matured by an ultrasonic process generally described as follows (Figure 1). Analyzation was done immediately after the accelerated maturation processes were completed. The accelerated matured rice wine was matured about one year after the standard maturation rice wine so that analysis of the conventionally matured wine and accelerated matured wine could take place concurrently. Moreover, the rice wine making technique for both conventional standard maturation and accelerated maturation was as closed to exactly the same as possible for all tested samples in our study in order to produce similar rice wine sample quality and to minimize the bias as much as possible. An analysis of the untreated rice wine was also done. A 20 kHz ultrasonic wave generator was used to apply ultrasound energy to the wine. The 20 kHz ultrasonic wave set up was designed and self-made by the coauthor of this manuscript. The ultrasound wave generator had piezoelectric chips which generated electrons. The electrons accumulated and vibrated in terms of ultrasonic waves. The ultrasonic nozzle orifice for this 20 kHz ultrasound was 10 mm. We chose a 10 mm nozzle because this nozzle amplitude energy was found by trial to fit our experimental needs in terms of atomizing the bulk wine liquids into a spray of smoke like mist droplets to fulfill the ultrasonic process. The rice wine volume for one ultrasonic treatment run was started with 2 liters and replicated many times until all the treated samples were sure to be fully supplied for analysis. All the ultrasonic circulation maturation treatment was in a closed chamber to prevent evaporation loss of the rice wine and assure the best collection of the misted rice wine. The rice wine was matured repeatedly by this process up to 16 times. Samples of the matured rice wine were collected after each of the 16 maturation treatments. Each sample was analyzed for alcohol content, pH value, gas chromatography measurements, sensory evaluation, and the time of maturation.Each of the experimental treatments was replicated three times for analysis, and the results were the average of the three samples.

The pH value of non-matured (fresh) rice wine, one year conventionally matured rice wine and each sample of all accelerated maturation treatments were measured by a CORNING, pH meter 240, electric pH meter. This gave an indication of acidity and the level of sour flavor of the wine; it also was an index of possible contamination of the whole study and index of possibilities of rancidity. If the pH level is out of a normal range of rice wine, further tests or efforts may need to be done to check on any problems which may have occurred, such as bad fermentation, contamination and/or rancidity.

Alcohol was measured by a KYOTO, DA-310, electric specific gravity meter. Non-matured (fresh) rice wine, one year conventionally matured rice wine and each sample of all accelerated maturation treatments were measured in room temperature (around 25 ℃). Alcohol was then calculated from the gravity measurements. The alcohol content check gave an indication of possible alcohol loss in process.

Flavor compounds were analyzed by a WHIRLPOOL 5790 gas chromatograph with a glass column that was 1.8 m long, 2 mm thick, packed with 6.6% carbowax and 20 M/80-120 mesh carbopack B Aw using a flame ion detector. The carrier gas was nitrogen with 20 ml/min flow rate. The injection temperature was 150℃ and the flame ion detector temperature was 200℃. The gas chromatograph temperature gradient started at 60℃ and stayed at the level for one minute. It then increased 5℃/min and reached 160℃ in 20 minutes where it stayed for two minutes. Each sample injection amount was 2 micro liters. Standards for the gas chromatograph were prepared as follows: 0.5 ml of 5.14% acetaldehyde, 0.4 ml of 5.01% methanol, 2 ml of 5.0% ethyl acetate, 0.2 ml of 5% 1-propanol, 0.4 ml of 5% 2-methyl-1-propanol, 1 ml of 2% acetoin, 0.5 ml of 2% 2-methyl-butanol, 1 ml of 3.04% 3-methyl-butanol, 1 ml 5% acetic acid, 0.4 ml of 5% ethyl lactate, 0.1 ml of 5% furfural, 0.2 ml of 5% 2,3-butanediol, 1 ml of 2% caproic acid, and 2 ml of 2% ethyl caproate. These chemicals were then mixed with 40% ethanol to reach 100 ml in total.

Sensory evaluations were made by 12 qualified and experienced wine sensory tasters using the Krammer Method[15] which has a statistical significance of alpha = 5% and had been applied widely for the purpose of wine tasting[16-17]. Each of the 12 tasters tasted the rice wine before any treatments and after each of the 16 accelerated maturation treatments. The tasters ranked the treated rice wine against the rice wine conventionally matured for one year. They gave the treated rice wine a ranking of 1 (best) to 5 (worst).Generally, a quality conventionally matured rice wine would be ranked 1 on this scale. The results were then calculated and analyzed from the preference priorities of each taster and ranked from best to worst flavor.

Time of maturation for the wines aged by the accelerated process was counted as the time needed to complete the whole ultrasonic waves maturation process versus the one year conventional maturation process.

III. Results and Discussion

Alcohol content: The alcohol content in the rice wine matured with ultrasonic waves decreased as the number of maturation treatments increased, giving a less spicy flavor of the rice wine (Figure 2). The alcohol content of the samples matured 16 times (52 %) was a little lower than the alcohol content of samples matured by the standard 1-year process (54 %).

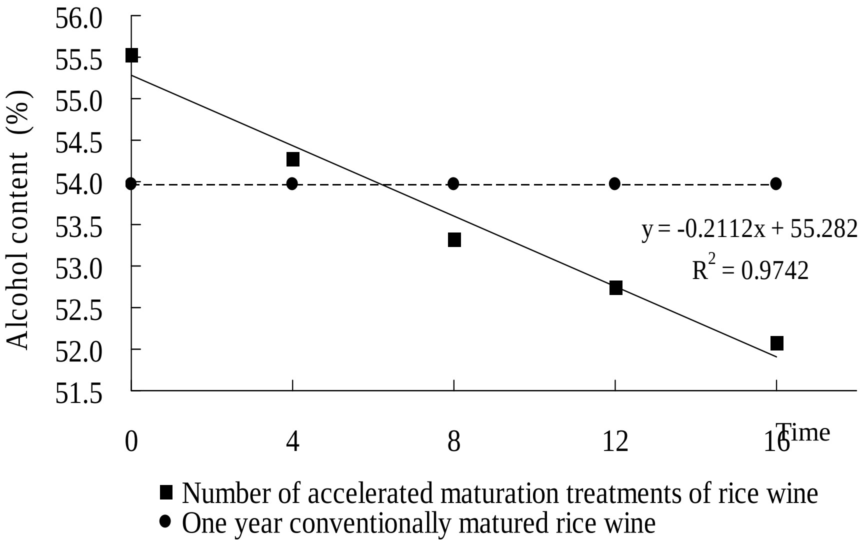

Figure. 2 The alcohol content of ultrasonic waves matured rice wine with different treatments vs. one year conventionally matured rice wine and fresh rice wine (non-matured)

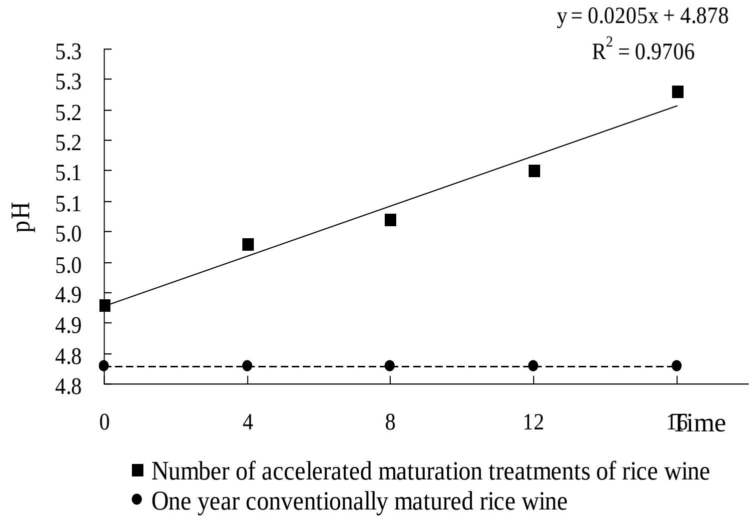

Figure. 3 The pH value of ultrasonic waves matured rice wine with different treatments vs. one year conventionally matured rice wine and fresh rice wine (non-matured)

pH: The pH value of the accelerated maturation rice wine increased slightly as the number of maturation treatments increased, giving it a less sour taste (Figure 3) that was preferred by the sensory evaluators (Table 2). The pH value check of all studied samples also showed that it was in the normal range of rice wine, which indicated the right fermentation process, no rancidity, and no contamination of our work in this whole study.

Gas Chromatography: Acetaldehyde which has the lowest boiling point of rice wine flavor compounds and has an unpleasant stingy flavor. The gas chromatography analyses showed that the evaporating flavor compounds generally decreased slightly as the number of maturation treatments increased. By decreasing the amount of acetaldehyde (Figure 4), other pleasant flavor compounds become more prominent and give the accelerated maturation rice wine a better taste (Table 2). Ethyl acetate, which is the fragrant compound in wine, slightly increased in the accelerated maturation rice wine (Figure 5). This combined with the decreased unpleasant acetaldehyde (Figure 4) and the decreased oil flavor of polyols (Figure 6 and Table 1), increased the ethyl acetate contribution to taste in the accelerated maturation rice wine (Table 2). Also, polyols (polyhydric alcohol) such as; 1-propanol, 2-methyl-1-propanol, 2,3-dimethyl-butanol, and methanol often have rice-oil flavors and cause a greasy feel in the mouth. Their decrease (Figure 6 and Table 1) along with the decrease of sour mouth feel (Figure 3), as the number of ultrasonic wave treatments increased, resulted in a smoother tasting wine with a less greasy feel in the mouth. The R square of linear regression equations of alcohol content, pH value, acetaldehyde content, ethyl acetate content, and polyols content versus the number of ultrasonic wave treatments were all very close to 1, indicating linear relationships.

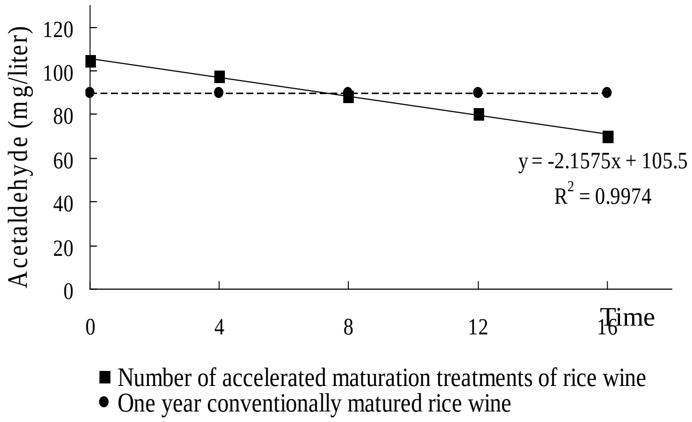

Figure. 4 The acetaldehyde content of ultrasonic waves matured rice wine with different treatments vs. one year conventionally matured rice wine and fresh rice wine (non-matured)

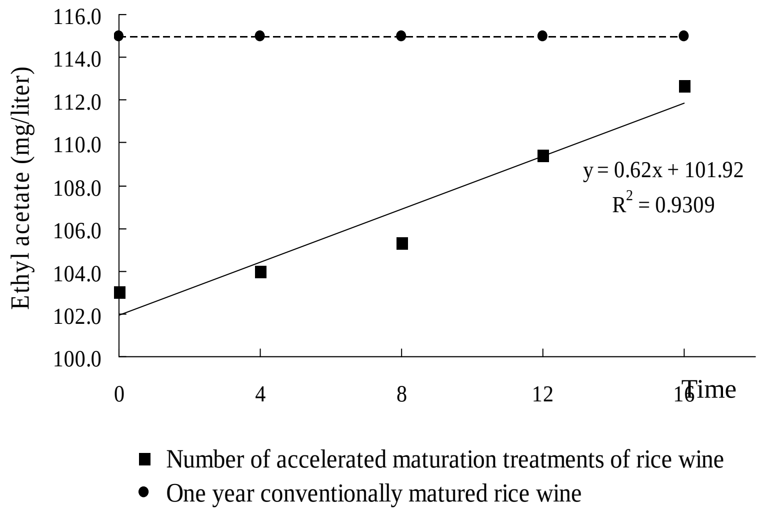

Figure. 5 The ethyl acetate content of ultrasonic waves matured rice wine with different treatments vs. one year conventionally matured rice wine and fresh rice wine (non-matured)

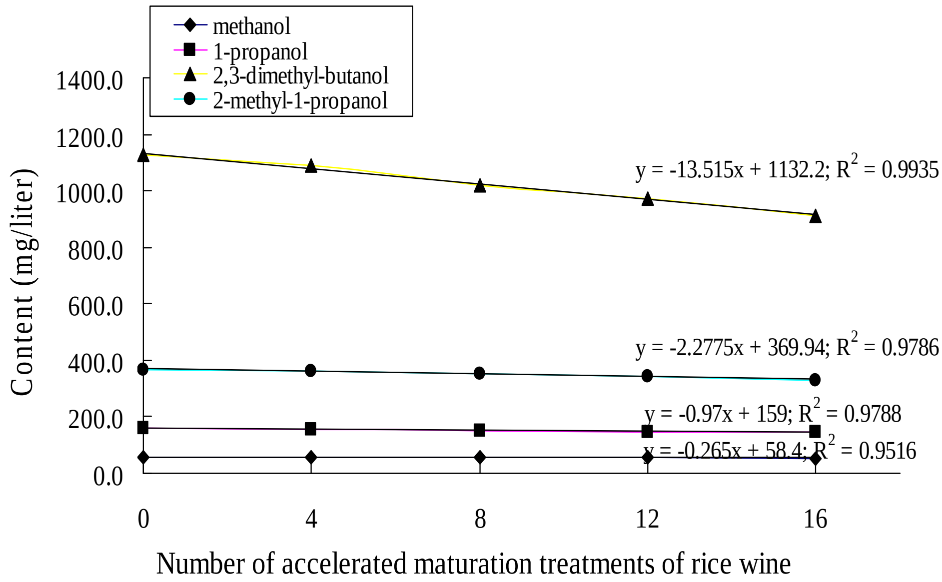

Figure. 6 The polyols content of ultrasonic waves matured rice wine with different treatments

Table. 1 The polyols content of ultrasonic waves matured rice wine with different treatments vs. one year conventionally matured rice wine and fresh rice wine (non-matured)

Number of accelerated maturation treatments	Methanol (mg/liter)	1-Propanol (mg/liter)	2,3-Dimethyl-butanol (mg/liter)	2-Methyl-1-propanol (mg/liter)
0 (non-matured)	58.0	160.0	1126.4	368.0
4	57.6	154.6	1088.1	361.2
8	56.8	150.1	1018.6	354.5
12	55.0	147.2	974.5	343.7
16	54.0	144.3	912.9	331.2
One year conventionally Matured rice wine	54.0	154.0	933.0	354.6

Table. 2 The results of sensory evaluation of ultrasonic waves matured rice wine with different treatments vs. one year conventionally matured rice wine and fresh rice wine (non-matured)

taster	Non matured	Number of accelerated maturation treatments	One year

rank	0	4	8	12	16	conventionally matured rice wine
A	5	4	3	2	1	1
B	5	4	3	2	1	1
C	5	4	4	3	1	1
D	5	5	4	3	1	1
E	5	5	4	2	1	1
F	5	4	3	2	1	1
G	5	4	3	2	1	1
H	5	4	3	2	1	1
I	5	4	3	2	1	1
J	5	4	3	2	1	1
K	5	4	3	2	1	1
L	5	4	3	2	1	1
Rank summation	60	50	39	26	12	12
Preference	5	4	3	2	1	1

Sensory Evaluation: Wines usually have several flavors and other properties that each plays a subtle, intertwined, yet important role in terms of human preferences. However, some are not detectable in gas chromatography and thus human sensory evaluations are needed as references. Also, wine is for humans to taste as a final product.Results of sensory evaluations showed that the taste of the accelerated maturation rice wine improved with each of the 16 maturation treatments. The rice wine that was matured 16 times had a taste equivalent to the conventional 1-year matured rice wine (Table 2). Ultrasonic waves treatment showed potential as an alternative method of maturing wine.However, further study is needed in wine quantity control, in processing and in promotional possibilities.

Time of maturation: The ultrasonic waves process of maturation wine is much faster than the 1-year conventional process of maturation wine. Rice wine could potentially be matured to a quality taste within one week by using ultrasonic waves.

IV. Conclusion

Ultrasonic waves treatment of rice wine was capable of maturing wine to a similar quality of taste as the conventional method of maturation. The ultrasonic waves process is a much faster maturation process for rice wine (1 week versus 1 year), yet the ultrasonic waves process can save precious room for a better utilization of space.

V. Acknowledgements

The authors is very grateful to Dr. Fa Chun Chen, Associate Professor of Mechanical Engineering of National Ilan university , Taiwan for his help with this research.

References

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