QUALITY OF RICE WINE USING FREEZE-DRIED MICROBIAL STRAINS ISOLATED FROM VIETNAM'S DRIED STARTER CULTURE

● TRINH LAN HONG

ABSTRACT:

This study evaluated the quality of rice wine using microorganisms isolated from Vietnam's dried starter culture. Five mold and three yeast strains were selected. The potential of one mold and one yeast strain identified as Mucor spp HLT01 and Saccharomyces cerevisiae, respectively for freeze dried starter culture. The physico-chemical properties and sensory attributes of resulting wines were evaluated. The general acceptability showed distilled wine and mixed wine was more preferred than the original wine. The yield of rice wine after fermentation was found 136% based on the weight of raw rice using the traditional method.

Keywords: Rice wine, Saccharomyces cerevisiae, Mucor, freeze-dried starter.

I. Introduction

The production of alcohol from rice has been a tradition in Vietnam for centuries such that it has become an indispensable feature of Vietnam's culture. One of the major problems in Vietnam, variability in quality is strongly correlated with the microbiological properties of the traditional starter cultures that are commonly used (Dung et al. 2007). In 2008, Thanh et al reported that different types of molds, yeasts are present in starter cultures not only the mixtures of useful microorganisms in starter but also opportunistic contaminants or harmful factors come from plant pathogens or environment contaminants. This drawback is because, in village, traditional ways using available natural materials which microorganisms that are not purified and undesired microorganisms are difficult to predict. The added oriental herbs from environment also contributed to unsafe product.

The research was, therefore, conducted to develop freeze-dried starter culture (men ruou) using isolated microorganisms from starters collected from three (3) regions in Vietnam (North, Central and South) for processing good quality rice wine.

II. Materials and methods

2.1. Isolation and Purification of Microorganisms from Men Ruou

10g of powdered starter samples were added to 90 mL of 0.85% sterile physiological saline solution, homogenized and a series of 10-fold dilutions were carried out. Aliquots (0.1 mL) of the appropriate dilutions were plated on PDA agar medium. Agar plates were incubated at 30oC for 48 hours, after that, representative colonies of molds and yeasts on plates were picked up and transferred onto appropriate PDA slants. These were incubated in the same condition as above.

2.2. Screening of Isolates and preparation of Freeze-dried Starter Culture

Saccharifying capability of isolated molds was determined by inoculated with cell suspension of the microbial strains on cooked glutinous rice and was fermented under aerobic condition for 3 days. The isolates found to potentially produce significantly high values of reducing sugar (%) during rice saccharificaiion were selected for preparation of freeze-dried starter culture.

2.3. Preparation of Freeze-dried Starter Culture

Freeze-dried starter culture for rice wine processing was prepared following the method described by Dizon et al (2009).

Freeze dried mold starter cultures were used (individualy or combination of strains) for saccharification of rice starch. Treatment gave the highest reducing sugar and TSS (□Brix) was selected for rice wine processing following the traditional method practiced in Vietnam.

2.4. Screening of Yeast Isolates for Alcohol Production

Another batch of the best treatment in the above study was used for saccharification of rice starch. This was placed in fermentation jars and inoculated with cell suspension of selected yeast strains separately. Then, the inoculated mixtures were fermented anaerobically for 10 days for alcohol production. After fermentation, these were filtered, placed in sterilized bottles and allowed to clarify prior to determination of alcohol content.

2.5. Identification of Selected Microbial Strains

Mold strains showing high saccharifiying ability and high alcohol producing yeast were identified through cultural, morphological, physiological and some biochemical tests. Characterization of these microorganisms was done following the methods described by Alexopoulus et al (1996), Davise (1995), Watanabe (2002) for molds. Kurtzman and Fell (1998) and Lodder (1970) for yeasts.

2.6. Determination of the Physicochemical and Phenolic Content of Rice Wine

Physicochemical and Phenolic Content of Rice Wine were determined through dertermination of pH, Total soluble solids (TSS), Total titratable acidity (TTA), Total and Reducing sugar (TS - RS), Amino-nitrogen (mg%). Alcohol content, Phenolic content.

2.7. Sensory Evaluation of Rice Wine

All products from various treatments were subjected to sensory evaluation by 15-member panelist. The samples were evaluated for their different sensory attributes (clarity, color, aroma, sweetness, flavor, acidity, alcohol content, astringency and general acceptability) using 7-point Hedonic quality scoring. The evaluators gave score for each attribute of rice wine on a scale of 1 to 7, with 7 being the highest score.

2.8. Data Analysis

All data were subjected to analysis of variance (ANOVA).

III. Results and discussion

3.1. Isolation of Dominant Microbial Strains from Men Ruou

From collected dried starter cultures, eight representative colonies were selected (5 molds and 3 yeasts). Purified isolates were properly designated with specific code based on the site of collection and type of strains.

Preliminary selection of representative mold and yeast strains was based on the cultural characteristics or growth appearance on PDA medium.

3.2. Screening of Isolated Strains for their Functional Properties

Selected mold isolates were utilized in the screening for their ability to saccahrify Vietnamese glutinous rice. Initial screening showed the isolates TB-M2, TH-M1, and DL-M gave the best results based on the percentage of total sugar and reducing sugar content of the saccharified rice.

3.3. Saccharification efficiency of freeze-dried mold starter culture

The treatment with the highest percentage reducing sugar, TB-M2 was chosen as the most promising mold starter. The TH-MŒŒ1 sample that obtained second highest reducing sugar was also utilized for the succeeding studies in evaluating the capacity of selected yeast strains for their alcohol production.

3.4. Screening of yeast isolates for alcohol production

The alcohol contents from treatments TH-M1 fermented with three selected yeasts were lower, ranging from 9-11% compared to treatments from TB-M2 utilizing the same yeast strains with alcohol content ranging from 10-13%, with the highest

value obtained from DL-Y. Thus, the final mold and yeast strains for further studies are TB-M2 and DL-Y, respectively. (Table 1).

3.5. Identification of Selected Microbial Strains

3.5.1. Identification of mold isolate coded TB-M_2

Mucor and Rhizopus were the two most considered which have been common in starch saccharification (Alexopoulos 1959). The description of isolate's characteristics pointed out that it had branched sporangiophores and no rhizoids thus, this isolate was identified as Mucor sp. and will be named as Mucor sp. HLT_{01}.

Table 1.

TREATMENT	ALCOHOL CONTENT (%)	
	Mold	Yeast
TH – M1	TB – Y	11.0[bc]
	TH – Y	9.0[b]
	DL – Y	11.0[bc]
TB – M2	TB – Y	12.0[bc]
	TH – Y	10.0[bc]
	DL – Y	13.0[d]

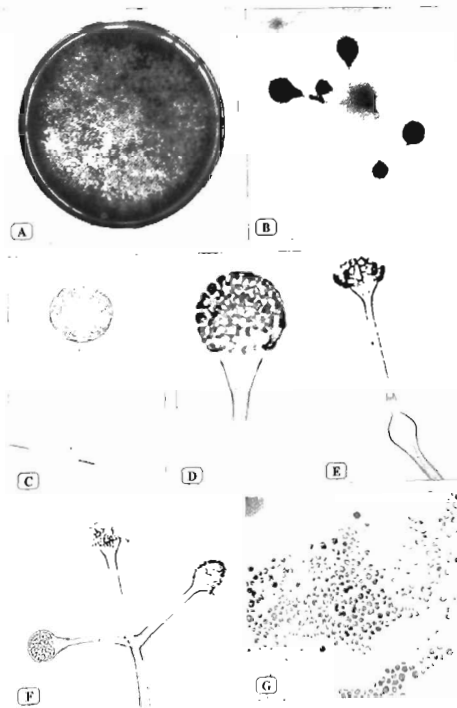

Fig 1: Cultural and morphological characteristics of isolated Mucor sp.HLT_{01}

(A) Cottony growth on plated PDA;

(B) Fruiting bodies as viewed under dissecting microscope;

(C) The globose sporangial head and sporangiophore;

(D) A mature sporangium with sporangiospores around;

(E) Swelling along sporangiophore;

(F) Branched sporangiophore and its apical portion bearing fruit body;

(G) Globose, hyaline, and smooth sporangiospores.

3.5.2. Identification of yeast isolate coded DL-Y

Identification of genus. The DL-Y strain was found belong to sub-family Saccharomycetoideae. To find out which genus of DL-Y possibly belonged, a step by step diagram was made by elimination until only one genus was left. Results showed that the strain belonged to the genus Saccharomyces.

Identification of Saccharomyces species. For identification of species, a dichotomous diagram was done and the final identity of the yeast strain (DL-Y) pointed to the properties of Saccharomyces cerevisiae.

3.6. Application of Freeze-dried Starter Cultures for Rice Wine Fermentation

Based on the previous study, there were two promising strains chosen for rice wine fermentation, Mucor HLT01 and Sacchraromyces cerevisiae were used in the preparation of new batch of freeze-dried starter culture. The starter culture was also checked for viable cell count and found to have count of 106 – 107 CFUml-1. This is crucial since initial population plays a very significant role on fermentation.

The freeze-dried mold starter culture was used in rice wine making. After the fermentation, the yield of wine was also measured based on the weight of raw rice (500 g) and result showed yield of 136%. All three wine samples were analyzed for physicochemical properties and subjected to sensory evaluation. (Table 2).

Table 2. Physicochemical properties and phenolic contents of rice wine

PARAMETER	TREATMENT		
	Undistilled Rice Wine	*Mixed Wine	Distilled Rice Wine
pH	4.1	4.2	4 4
Total Titratable Acidity (%)	3 69	3.24	2.83
TSS (oBrix)	10	9 3	9
Total Sugar (%)	3.01	2.92	2.86
Reducing Sugar (%)	2.62	2.57	2.49
Amino-nitrogen (mg%)	0.031	0 029	0.026
Alcohol content (%)	14.0	25.0	45.0
Phenolic content mg /L GAE	110	135	198
Yield (%)	136		

* 1part rice wine undistilled : 1 part distilled rice wine

3.7. Sensory Evaluation

The three rice wine samples, undistilled wine, mixed wine, and distilled wine, were subjected to sensory evaluation. Fifteen judges were selected to evaluate the samples. The panelists also gave their opinion on the overall acceptability of the wine samples. (Table 3; 4).

Mean values within row with the same superscripts are not significantly different at $p \leq 0.05$.

* 1part undistilled rice wine : 1 part distilled rice wine

Table 4. Mean scores for the general acceptability of treatments

TREATMENT	GENERAL ACCEPTABILITY SCORE	
	Mean Score	Std. Deviation
Undistilled wine	4 0a	± 1.0
Mixed wine*	5.3b	± 0.8
Distilled wine	5 1b	± 1.0

Mean values within column with the same superscripts are not significantly different at $p \leq 0.05$.

Based on the data for general acceptability and other parameters evaluated, the undistilled wine or sample not added with distilled wine was evaluated as a "not interesting" rice wine. On the other hand, the other two wine samples with higher alcohol contents were judged as more clear due to the absence of impurities and both were acceptable. Therefore, it can be concluded that both treatments can be used for processing acceptable rice wines. In addition, these treatments contained higher alcohol level so that their shelf-life will be longer as far as the preservation and maintenance of quality are concerned.

IV. Summary and conclusions

Preliminary screening and further tests suggested the potential of one mold, coded TB-M2 and one yeast strain, coded DL-Y for freeze dried starter culture in rice wine processing. The mold TB-M2, identified as Mucor spp. gave the highest values for total sugar and reducing sugar at 44.13% and

Table 3. Mean scores for different sensory attributes of treatments

SENSORY ATTRIBUTE	UNDISTILLED WINE	MIXED WINE*	DISTILLED WINE
Clarity	2.5a	4 8b	5.9c
Color	5.5a	3 7b	1.7c
Aroma	5.0a	4.1a	4 6a
Sweetness	5 1a	4.1b	2.3c
Flavor	4 6a	3 4b	2 3b
Acidity	4.8a	3.5b	2.5b
Alcohol Content	2.2a	4 9b	6.1c
Astringency	2.1a	4 5b	5 8c

33.01%, respectively The yeast coded as DL-Y, on the other hand, gave the highest alcohol content of 13% and was identified as Saccharomyces cerevisiae.

The mold strain was applied in making freeze-dried starter culture and utilized for rice wine fermentation using the traditional method of Vietnam. After alcohol fermentation, the wine was divided into three treatments; undistilled (original wine), mixed wine (1 part distilled and 1 pat undistilled), and the distilled wine. Results showed that the undistilled wine produced a pH value of 4.1; total soluble solid (TSS) of 10oBx; total titratable acidity of 3.69 %; total sugar of 3.01%; reducing sugar of 2.62%; amino-N of 0.031 mg%; alcohol content of 14% and phenolics of 110 mg/L of GAE; while the mixed wine and distilled wine have pH of 4.2 and 4.4; TSS of 9.3 and 9.0oBx; TTA of 3.24 and 2.83%; TS of 2.92 and 2.86%; reducing sugar of 2.57 and 2.49%; amino-N of 0.029 and 0.026 mg%; alcohol content of 25 and 45%, and phenolics of 135 and 198 mg/L of GAE, respectively.

All three wine treatments were evaluated by 15 panelists in terms of color, clarity, flavor, aroma, sweetness, acidity, alcoholic taste, astringency and general acceptability. Results showed that the high alcohol concentration of distilled wine contributed considerable effect on the other attributes. The general acceptability of distilled wine and mixed wine were more preferred by the judges than the original wine (undistilled) because of higher alcohol contents, clear and sparkling quality ■

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NÂNG CAO CHẤT LƯỢNG RƯỢU GẠO ỨNG DỤNG VI SINH VẬT ĐÔNG KHÔ ĐƯỢC PHÂN LẬP TỪ CÁC LOẠI MEN RƯỢU VIỆT

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TÓM TẮT:

Nghiên cứu đánh giá chất lượng rượu gạo sử dụng vi sinh vật phân lập từ các loại men rượu khác nhau ở Việt Nam. Sau khi phân lập, có 5 chủng nấm mốc và 3 chủng nấm men được lựa chọn và tiếp tục sàng lọc. Chủng nấm mốc và nấm men được đánh giá là tiềm năng nhất để sản xuất men rượu đông khô đã được định danh tương ứng là Mucor spp HLT01 và Saccharomyces cerevisiae. Hiệu suất lên men rượu là 136%. Rượu thành phẩm được phân tích đánh giá chất lượng lý hóa và cảm quan cho thấy rượu qua chưng cất và rượu pha cho chất lượng tốt hơn rượu gốc sau lên men.

Từ khóa: Rượu gạo, Saccharomyces cerevisiae, Mucor, men rượu đông khô.