

## Mini Review

## Vietnamese rice-based alcoholic beverages

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Varieties of traditional fermented rice-based alcoholic beverages are highly popular in various countries and as such they represent an important category of indigenous fermented products. They play a role in human spiritual and cultural life. In the present review, emphasis is laid on the alcoholic fermentation process for the preparation of rice wines and its small-scale production process in Vietnam. The processes for rice wine manufacture and alcoholic fermentation starters were described. The bioprocess including ingredients and operations as well as the roles of microorganisms involved, resulting in the final products of the rice-based alcoholic fermentation are considerably discussed. The innovation matters with recent research studies are also noted.

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**Introduction**

Fermented foods and beverages have been very popular since ancient times all over the world. In modern times fermented products are important subjects for scientific research and many aspects of fermented products have been increasingly considered and developed. Alcoholic beverages, including beers, wines and spirits are an essential type of indigenous fermented product and they play an important role in human spiritual and cultural life. Many kinds of indigenous alcoholic beverages are produced and consumed world-wide.

Like other traditional fermented alcoholic beverages in other East Asian countries (Hesseltine, 1983; Basuki *et al.*, 1996; Nout and Aidoo, 2002) rice wines are highly popular in Vietnam. In Vietnam, the production of rice wine is a source of income for farmer families in rural areas. It is mostly manufactured at home-scale using solid-state starters in tablet form and the wine is prepared under non-sterile and marginally controlled conditions.

Depending on the available regional ingredients and manufacturing procedures, rice wines are known under a variety of local names. In Vietnamese, ruou means wine. In the north and south we can find ruou de or ruou nep: these are fermented from rice or

glutinous rice, respectively, followed by distillation. In the Mekong Delta of the south there is ruou nep than (purple glutinous rice wine) which is fermented from nep than (purple glutinous rice) without distillation. In mountainous districts of the centre highlands of the country, such as Da Lat, Buon Me Thuot and Dac Lac, an ethnic minority (Thuong people) produce ruou can that is fermented from rice or maize or cassava with or without distillation.

*Traditional rice-based alcoholic beverages*

Although rice-based traditional alcoholic beverages have different compositions according to the formulation and processes used, the principle of their manufacture can be characterized as a biochemical modification of cereal starches brought about by microorganisms in which fungi (yeasts and moulds) play essential roles. Moulds produce the amylases that degrade the starch into dextrins and sugars, and yeasts convert these sugars to alcohol (Lim, 1991; Motarjemi and Nout, 1996; Nout and Aidoo 2002). Table 1 summarizes some traditional fermented alcoholic beverages from various countries. This table mentions raw materials and major functional fungi involved in the fermentation process. In line with the scope of this section, emphasis will be laid on the alcoholic fermentation process for the preparation of

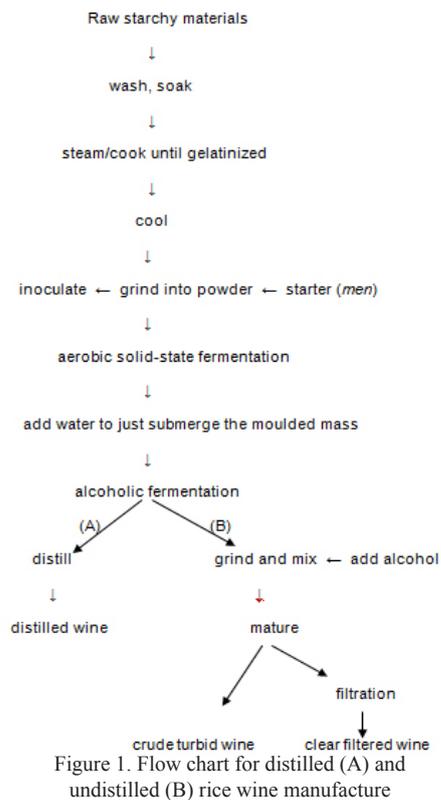
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Table 1. Traditional fermented rice-based beverages in Asian countries

Product	Country	Raw material	Functional yeasts and moulds
Brem Bali, Tuak, Ciu		Rice, glutinous rice, sap of palm trees, cane-sugar	<i>Amylomyces</i> spp., <i>Mucor</i> spp., <i>Rhizopus</i> spp., <i>Candida</i> spp., <i>Saccharomyces</i> spp.
Bubod, Lambanog, Tuba, Tapok, Tapuy		Rice, roselle fruit, palmyra juice	<i>Aspergillus</i> spp., <i>Endomycopsis</i> spp., <i>Hansenula</i> spp., <i>Endomycopsis fibuliger</i> , <i>Rhodotorula glutinis</i> , <i>Debaromyces hansenii</i> , <i>Candida parapsilosis</i> , <i>Trichosporon fennicum</i> , <i>Saccharomyces ellipsoideus</i>
Bujju, Takju, Yakju		Rice, glutinous rice, barley, wheat, millet	<i>Aspergillus oryzae</i> , <i>Aspergillus sojae</i> , <i>Mucor</i> spp., <i>Rhizopus</i> spp., <i>Saccharomyces cerevisiae</i> , <i>Hansenula anomala</i> , <i>H. subpelliculosa</i> , <i>Torulopsis sake</i> , <i>T. inconspicua</i> , <i>Pichia polymorpha</i>
Fenni, Sonti, Ruhi, Madhu, Jnard		Rice, Cashew apple	<i>Mucor</i> , <i>Rhizopus</i> , <i>Aspergillus oryzae</i> , <i>Rhizopus</i> spp., <i>Saccharomyces cerevisiae</i> , <i>Aspergillus oryzae</i> , <i>Aspergillus awamori</i> , <i>Saccharomyces sake</i> , <i>Hansenula anomala</i>
Mie-chiu, Shaoxing		Rice, wheat, barley	<i>Mucor</i> spp., <i>Rhizopus</i> spp., <i>Aspergillus</i> spp., <i>Saccharomyces ellipsoideus</i> , <i>Saccharomyces cerevisiae</i> , <i>Endomycopsis</i>
Mirin, Sake, Shochu, Umeshu		Rice, maize, barley, plum	<i>Mucor</i> spp., <i>Rhizopus</i> spp., <i>Aspergillus</i> spp., <i>Saccharomyces ellipsoideus</i> , <i>Saccharomyces cerevisiae</i> , <i>Endomycopsis</i>
Ruou De, Ruou Nep, Ruou Nep Than, Ruou Can, Ruou Vang		Rice, (purple) glutinous rice, maize, cassava	<i>fibuliger</i> , <i>Hansenula anomala</i> , <i>Torulopsis candida</i>
Sato, Ou, Nam-Khao		Rice, glutinous rice	<i>Mucor</i> spp., <i>Rhizopus</i> spp., <i>Candida</i> spp., <i>Saccharomyces</i> spp.
Tapai, Sameu		Rice, glutinous rice	<i>Amylomyces rouxii</i> , <i>Rhizopus</i> spp., <i>Endomycopsis</i> spp.

\* based on data of Hesselstine, 1983; Luong, 1998; Haard *et al.*, 1999; Nout and Aidoo, 2002



rice wines and its small-scale production process in Vietnam.

### Production methods of rice wines

The manufacture of rice wine can be characterized as a biological process whereby rice (*Oryza sativa* L.) is converted into wine by physical, microbiological and biochemical operations, including steaming, inoculation with starter, mashing and fermentation. Depending on the fermentation performance, the alcohol content may reach up to 15% (v/v). By

distillation, products with approximately 50-60% (v/v) alcohol can be obtained. The general outline of traditional production processes is shown in Figure 1.

Rice wines are produced predominantly at artisanal home- or cottage-level. Though each producer has his own way for making wine, depending on his individual experience and regional available raw materials, in principle, all producers use the same process. Powdered starch-based starter (about 1% - 2% of the raw starchy materials) is mixed with steamed or cooked gelatinized rice, which is then incubated under ambient conditions. In the Mekong Delta of the south that is the leading production area of a variety of rice wines, the typical ambient temperatures (approx. 28-32°C) are favourable for the incubation. After an initial period (2 - 3 days) of uncontrolled aerobic solid-state fermentation, the now moulded mass is mixed with water and allowed to undergo for further 3 - 4 days of a submerged alcoholic fermentation. The ratio of added water to the moulded mass is about 3:1. Regular rice wine is made from white rice or white glutinous rice and is distilled after alcoholic fermentation, yielding a colorless liquor with a bland taste. Other kinds of rice wine, such as purple glutinous rice wine, are fermented and not distilled and may be sold as a crude cloudy wine containing sediment or as a clear filtered wine. Normally, the alcohol content in undistilled wines is approximately 7-10% (v/v; about 6-8% w/v), which is insufficient to preserve the wine from any possible contamination or spoilage made by undesired microorganisms. Therefore, a volume of distilled concentrated alcohol is often added to the wine to increase the alcohol concentration to 13-16% (v/v) depending on producers' and consumers' requirements for the level of alcohol and the required storage life. Purple glutinous rice wine is particularly interesting with its brown-red color and sherry-like flavor which make it a very attractive and characteristic product compared with the colorless and neutrally flavored regular distilled wines. Purple glutinous rice wine is twice as expensive as colorless wines because of the more high cost of purple glutinous rice.

### Starchy raw materials

Depending on available ingredients and preferences in the region, different kinds of agricultural starchy materials may be utilised. The most popular materials are dehulled rice including whole or broken rice (*Oryza sativa* L.), glutinous rice and purple glutinous rice. The rice, composed of amylose and amylopectin molecules associated by hydrogen bonding (Leach, 1965; Wasserman and

Table 2. Starters for alcoholic beverages used in Asian countries

Product	Country	Functional moulds and yeasts
Bakhar		<i>Mucor</i> spp., <i>Rhizopus</i> spp., <i>Amylomyces</i> spp.
Bubud	Korea	<i>Mucor</i> spp., <i>Rhizopus</i> spp., <i>Amylomyces</i> spp., <i>Candida</i> spp.,
Look-pang		<i>Saccharomyces</i> spp., <i>Endomycopsis</i> spp., <i>Torulopsis</i> spp.
Men ruou,		<i>Aspergillus oryzae</i> , <i>Rhizopus</i> spp.
Men com ruou		<i>Mucor</i> spp., <i>Chlamydomucor oryzae</i> , <i>Candida</i> spp.
Murcha		<i>Saccharomycopsis</i> .
Nurook		<i>Mucor</i> spp., <i>Rhizopus</i> spp., <i>Aspergillus</i> spp., <i>Penicillium</i> spp.,
Ragi		<i>Saccharomyces</i> spp., <i>Endomycopsis</i> spp., <i>Hansenula</i> spp.,
Tapai		<i>Torulopsis</i> spp., <i>Rhodotorula</i> spp.
		<i>Rhizopus</i> spp., <i>Mucor</i> spp., <i>Saccharomyces cerevisiae</i> ,
		<i>Endomycopsis fibuliger</i> .
		<i>Aspergillus oryzae</i> , <i>A. niger</i> , <i>Rhizopus</i> spp., <i>Penicillium</i> spp.,
		<i>Mucor</i> spp., <i>Hansenula anomala</i> , <i>Pichia anomala</i> .
		<i>Mucor</i> spp., <i>Rhizopus</i> spp., <i>Amylomyces</i> spp., <i>Aspergillus</i>
		spp., <i>Penicillium</i> spp., <i>Candida</i> spp., <i>Saccharomyces</i> spp.,
		<i>Endomycopsis</i> spp., <i>Hansenula</i> spp., <i>Torulopsis</i> spp.,
		<i>Rhodotorula</i> spp.
		<i>Amylomyces rouxii</i> , <i>Rhizopus</i> spp., <i>Endomycopsis</i> spp.

Yu, 2003) is first soaked to hydrate and soften the starch granules prior to subsequent gelatinization by steaming or cooking which makes the starch more available to enzymic hydrolysis (Snow and O'Dea, 1981). Either steaming or cooking may be used but most producers prefer cooking because it takes less time than steaming to completely gelatinize the starch, although operators need to be experienced to add the appropriate amount of water. Enzymes which hydrolyse starch are available from animal, plant, and microbial sources; microbes are increasingly and widely used as important enzyme sources for production of starch-degrading enzymes.

In our recent research (Dung and Phong, 2011), in the context of the aim to pave the way for applying the experimental superior fungal starter into practice, the ability of defined starter was tested in the winemaking from different available starchy materials (including rice, glutinous rice and purple glutinous rice) in the region and its validation in comparison with commercial starters were assessed in the winemaking. By assessing the alcoholic contents of the final wine as the main factor indicating the starter ability, the results show that in the treatments of commercial starter inoculation the alcohol contents differently varied depending on the kinds of starchy materials employed, whereas the high levels of alcohol contents were stably achieved in all treatments of defined starter performance. This can be indicated that the experimental mixed-culture fungal starter granules can be able to employed effectively as the inoculum for the rice wine fermentation from different kinds of available agricultural starchy materials in the

region. The defined starter also received the highest performance for its function during the alcoholic fermentation process, as compared to this of other commercial starters.

#### Alcoholic fermentation starters

The preparation and the use of fermentation starters as a source of inoculum is important in the manufacture of rice wine. It is recognized by winemakers that the choice of starter tablets influences the yield and quality of the wine. A number of local processors claim that using a combination of two or three different starters yields wine of better quality with a stronger sweet alcoholic taste and more attractive flavor than is obtained with a single starter. There experience is that each different starter has its own advantages and disadvantages. These dried starters normally include yeasts, moulds and bacteria and convert starchy materials to fermentable sugars and subsequently to alcohol and organic acids (Hesseltine *et al.*, 1988; Nwosu and Ojmelukwe, 1993; Luong, 1998; Nout and Aidoo, 2002). A variety of starter cultures is available in the markets in most Asian countries (Table 2).

The raw ingredients for the preparation of starter tablets can be either rice flour or cassava flour or combinations of rice flour and cassava flour. However, the mixed flours are preferred by local producers. These mixtures are ground and thoroughly mixed with spices and herbs that are believed to play a role in preventing the growth of undesirable microorganisms. The spices and herbs used include mixtures of garlic, pepper, onion, rhizomes and root of oriental herbs and producers jealously guard their own secret recipes. The ratio of ground rice to mixed spices is about 14:1 by weight. Water is added to form a dough-like mass with a moisture content of 55-60% which is inoculated with dry powdered starter from previous batches, followed by thorough mixing. The inoculated dough is shaped into small flattened or ball-shaped cakes about 4 cm in diameter and 1 cm thick. The cakes are placed on a bamboo tray and are then covered with a thin layer of rice husks. According to producers this reduces overheating and facilitates aeration. The tray is covered with a cloth and incubated in a ventilated place at ambient temperature (approx. 28-32°C) for 2-5 days during which time the dough rises slightly and becomes covered with fungal mycelium. The cakes are air- or sun-dried and then have a shelf life of several months. The traditional process of starter production is summarized in Figure 2.

Three main kinds of Vietnamese traditional starters (men) are known: starters without oriental

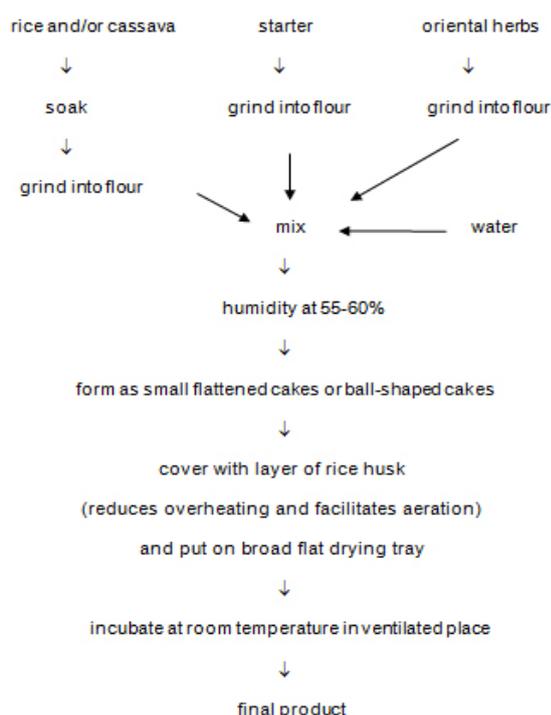


Figure 2. Traditional process for preparing rice wine starter

medicinal herbs; starters supplemented with oriental medicinal herbs; and starters supplemented with leaves containing aromatic essential oils. In the Mekong Delta region, starters supplemented with herbs predominate. While these herbs are primarily added to starters for the fragrant flavor which they convey to the rice wine, they may also have antibacterial properties and protect the rice wine fermentation against failure. The effects of the herbs used in traditional starter preparations on the starter microflora has been studied (Phuc, 1998; Dung *et al.*, 2005). Various combinations of herbal extracts and single extracts were examined for their effect on growth of the mould and yeast. Fermentation medium without any herbal extract served as a control. It was suggested that some kinds of herbs have a stimulatory effect on biomass and also on yeast count. Particularly the herbs “Tieu Hoi” (Fennel: *Foeniculum vulgare* Miller) and “Dinh Huong” (Clove: *Syzygium aromaticum* L.) prove to be stimulatory in biomass production of mould and yeast. Both these two herbs are commonly applied in the traditional process of rice wine making in Vietnam because of their assumed antibacterial properties and their fragrant flavour. It is unknown whether or not this is of relevance to the fermentation. Probably of more importance is their contribution to the flavor of the wine.

#### *Fermentation and the roles of microorganisms involved*

The two essential stages involved in rice wine production are the saccharification of starch in an aerobic solid-state fermentation and an alcoholic

fermentation. Starters for rice wine fermentation generally include mycelial fungi, yeasts and bacteria but the mycelial fungi and yeasts receive most attention as they are crucial for starch degradation and alcoholic fermentation. During the solid-state fermentation the moulds bring about saccharification of the rice starch and the sugars thus formed are fermented into alcohol by the yeasts and the quality of the final products depends mainly on the activities of these microorganisms.

The major moulds in traditional starters are *Amylomyces rouxii*, *Rhizopus* spp. and *Mucor* spp., and the commonly present yeasts are *Saccharomyces cerevisiae*, *Hansenula* spp., *Endomycopsis fibuligera* and *Candida* spp. (Table 2). The moulds produce  $\alpha$ -amylase and amyloglucosidase (also called glucoamylase) that hydrolyse starch to dextrins and maltose but mainly to glucose (Cook *et al.*, 1991; Crabb, 1999; Nout and Aidoo, 2002). The raw starch is gelatinized by cooking, liquefied by  $\alpha$ -amylase and saccharified to glucose by amyloglucosidase.  $\alpha$ -amylase cleaves starch randomly at 1,4- $\alpha$ -glycosidic bonds, giving maltooligosaccharides as final products. Amyloglucosidase liberates single D-glucose monomers in the  $\beta$ -form from the non-reducing end of starch and preferentially hydrolyses 1,4- $\alpha$ -glucosidic linkages (Schindler *et al.*, 1998).

Yeasts are significant in winemaking because they conduct the alcoholic fermentation but some may also spoil wines during storage through changes of biochemical activities (eg. metabolism of carbohydrates, nitrogen compounds, organic acids, or degradation of lipids, production of polyols) or have negative affects on wine quality through autolysis (Fleet, 1993). Some representatives of spoilage yeasts in alcoholic fermentation include *Pichia* spp., *Zygosaccharomyces* spp., *Kluyveromyces* spp. Ethanol is the main product of glucose fermentation by yeast; it is important to note that at certain concentrations ethanol inhibits growth and viability of yeasts. Ethanol has been reported to have a variety of inhibitory effects on yeast cells (Casay and Ingledew, 1986; D'Amore *et al.*, 1990; Sharma, 1997). One of the major target sites is the plasma membrane of yeasts and other microorganisms. At certain high concentrations, ethanol causes altered membrane organization and permeability and leakage of cell components. Besides, a number of other factors also affect yeast fermentation performance, such as osmotic pressure, substrate feeding, nutrient supplementation, temperature, and intracellular ethanol accumulation (Sharma *et al.*, 1996; Peres and Lalue, 1998; Wang and Sheu, 2000).

In practice, all local producers agree that the

incubation period required for the fermentation depends on the weather. The hotter the weather, the shorter the incubation time. In the Mekong Delta region of South Vietnam, particularly known as a leading area for the production of rice wine, the temperature is normally around 30-33°C and ranges from 28°C to 40°C. Normally, an initial period of aerobic solid-state fermentation is about 2 – 3 days and the further anaerobic alcoholic fermentation takes about 3 – 4 days. Recently, producers tend to prefer polyethylene vessels instead of the old-fashioned large glazed terracotta jars as fermentation containers because the former are cheap, and more convenient to use.

#### *The final product of rice wine fermentation*

For rice wine manufactured without distillation, the product is filtered after the submerged alcoholic fermentation to obtain a clear liquid containing a mixture of residual glucose, accumulated ethanol and other soluble matter. The alcohol content of this kind of undistilled wine is approximately 7-10% (v/v), which is quite low and not adequate for preservation. As noted, in practice it is manufactured at home-scale using solid-state starters that comprise different mixed undefined cultures, and the wine is prepared under non-sterile and marginally controlled conditions. To overcome this problem, producers use increase the alcohol content, either by adding refined or crude cane sugar after the initial aerobic solid-state fermentation or by adding distilled concentrated alcohol to the final product to obtain final concentrations meeting the consumers' requirements. Some producers mix the moulded rice-mass with distilled alcohol instead of water, followed by submerged alcoholic fermentation for a few months and filtration. To this clear filtered liquid, cane sugar can be added and the solution is cooked and filtered again; this kind of wine can be kept for a year without spoiling and keeps its taste. The range of alcohol concentrations in such wines (undistilled added with distilled concentrated alcohol) vary quite large due to regional consumers' preference, approximately 15 to 35% (v/v).

For the manufacture of distilled rice wines, the distillation equipment employed is hand made and simple including three main parts: a boiling pot, a condenser with cooling water, and a receptacle to receive the condensed alcohol distillate. Normally, the distillation is performed in two stages. The alcohol level can reach up to 65% (v/v) in the first stage and approximately 25 - 30% (v/v) in the latter stage. This is due to the principle during the distillation process. At the beginning of the distillation, the materials need a certain time to be heated, once the materials

are stably heated and boiled, consequently the rate obtaining the distillates is rapid and steady resulting high concentrated alcohol level obtained and this stage is remarked as first stage in distillation process. Then the rate is gradually decreased and in the final period of distillation the low alcohol levels are obtained. In practice, based on the experience the local producers decide when is the first sampling and when is the last sampling. Normally they try to get the distilled volumes as much as possible with high alcoholic levels in order to get more income, and that's why having a problem because of the presence of harmful by-products in the final distilled fermented wine.

In my research (Dung, 2009) on improved distilling system, the distillates were collected in a 500mL glass bottle, for the first two sampling (200mL/sample) were obtained, then 500mL/sample for the next samplings and the final sampling was made when the distilled wine became turbid. A total of samples (approx. 13-16 samples) were numbered following the order from starting to ending the distillation. We did this experiment with the aim to obtain the high alcohol contents as much as possible but minimize the contents of harmful by-products in the final distilled wine according to Vietnamese standard regulation. Depending on the preferences of producers and customers, as well as local commercial demands, the final distilled wine obtained during these two distilling stages can be mixed or kept separate.

#### *Current developments*

Nowadays, the evaluation of the quality of traditional fermented rice-based products, including rice wine, are required for professional examination using officially acknowledged and standardized techniques when they are commercialized. While it is recognized that the choice of starter strongly influences the yield and quality of the wine, there is not much knowledge of the relationship between the microbiological composition of starters and their performance. The limited knowledge about traditional starters poses an obstacle to industrial development and, thus, these starters has attracted the attention of researchers in food microbiology and technology and studies concerning the selection of safe and storable superior starters for small-scale fermentation processes. Advantages of defined mixed starter cultures have been described (Holzapfel, 1997; Ndip *et al.*, 2001; Siebenhandl *et al.*, 2001) and include enhanced yield, improved hygiene, predictability of fermentation processes and control of safety and quality.

A series of studies (Dung *et al.*, 2005, 2006, 2007) have addressed the problem of poor and variable

quality of traditional starter tablets by understanding and quantifying the impact of microflora in these starters, concentrating on mycelial fungi and yeasts and by assessing the option of preparing stable mixed cultures of selected compatible strains. Of pure isolates from Vietnamese rice wine starters *Amylomyces rouxii* (deposited as CBS 111757) and *Saccharomyces cerevisiae* (deposited as LU 1250) were selected as a powerful glucose producer and a superior fermentative strain, respectively. These were shown to be compatible in mixed cultures, which is of importance for the production of starters with good quality. The development of a laboratory-scale process to formulate defined mixed-culture starter granules was established. The wine produced with experimental dehydrated defined mixed-culture starter was assessed as superior compared with commercially available rice wines, particularly because of its flavor and overall acceptability. As a logical continuation, the feasibility of pilot-scale manufacture of defined fungal starter and its application in rice wine production from different local starchy materials were also investigated. It was indicated that the defined starter performed well in rice wine fermentations using different starchy resources of rice, glutinous rice and purple glutinous rice (Dung and Phong, 2011).

Another important factor affecting the yield and quality of the final distilled wine, particularly the chemical composition, including the contents of alcohol, acetaldehydes, esters, methanol and furfural, is the distilling method. Based on field work at local manufacturers and some preliminary screening tests, it was realized that the boiling temperature and the condensed liquid output strongly affected the yield and quality of the final product. By using the improved distilling system, the study (Dung, 2009) investigated the favorable pressure levels (P) and the volumes of condensed water output (Q) during the distillation. The two treatments including P=0.5kPa combined with Q=100L/h and P=0.4kPa combined with Q=100L/h were found favorably for the distillation with the initial amount of final fermented products at 25-40L. This conclusion based on the good results of high yield, less time and work labor, and achievement of the physicochemical standard regulation. The validation results showed that distillation of fermented rice wines, prepared from both improved defined starter and commercial starters, using improved distillation methods led to a significant reduction in the content of harmful by-products, particularly of acetaldehydes, in the final distilled wine.

## Conclusions

In nearly all regions of the world fermented alcoholic beverages – strongly linked to people's culture – are produced in diverse forms and tastes and are consumed since ancient times. Nowadays, the development of small-scale technology to process agricultural products by fermentation is one of the major parts of the programme to improve the socio-economic situation in Vietnam. Stimulating innovation for controlled manufacture of fermented rice wine to achieve consistent good quality and high yields, the essentially important factors to be considered include starter inoculation, fermentation conditions and the distillation operation. Home-scale production units are convenient for trialing new procedures and expanded marketing would benefit the small-scale rice wine producers.

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