Faculty of Resource Science and Technology

A Scientific Approach to *Tuak* Making Using Rice

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A Scientific Approach to *Tuak* Making Using Rice

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A thesis submitted in partial fulfilment of the Requirement of The Degree Bachelor of Science with Honours
(Resource Biotechnology)

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Programme of Resource Biotechnology
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2022

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A Scientific Approach to *Tuak* making Using Rice

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ABSTRACT

Tuak is a type of rice wine, traditionally produced through the fermentation of rice by using a dry starter culture which contains yeast and enzymes known as *ragi*. *Tuak* is an important aspect in the indigenous Dayaks culture in Sarawak. It is often made by artisanal producers. Due to the uncontrolled fermentation settings, *tuak* usually vary in sensory characteristics, resulting in quality inconsistencies. Hence, the establishment of a controlled starter culture mixture with known strains is necessary for high quality and consistency in the commercial production of *tuak*. This study proposed to document, investigate and assess the biochemical properties of *tuak* produced from common rice (*Oryza sativa*) using traditional *ragi* and starter culture with known fungal strains namely *Rhizopus oligosporus*, *Clavispora lusitaniae*, *Saccharomyces cerevisiae*, *Saccharomycopsis fubiligera* and *Pichia kudriavzevii*. From the study conducted, it was concluded that fermentation using *ragi* produce higher amount of ethanol yield compared to fermentation using starter culture with known fungal strains. Fermentation using *ragi* also shows higher glucose consumption rate, moderate amount of acetic and lactic acid, and also has better total phenolic content. Therefore, this study suggests that the specific strains used in the fungal cultures is inefficient to produce a high quality *tuak*.

Keywords: *Tuak*, *ragi*, rice wine, starter culture, alcoholic fermentation,

ABSTRAK

Tuak merupakan salah satu jenis wain beras yang secara tradisinya diperbuat melalui proses fermentasi beras dengan bebola yis kering yang dikenali sebagai ragi. Tuak merupakan satu aspek penting dalam budaya masyarakat Dayak di Sarawak. Tuak biasanya dihasilkan oleh pembuat tuak artisanal. Dalam keadaan persekitaran fermentasi yang tidak terkawal, ciri sensori tuak yang dihasilkan adalah berbeza-beza dan mengakibatkan kualiti yang tidak konsisten. Oleh itu, penghasilan kultur kulat yang diketahui komposisinya adalah penting untuk menghasilkan tuak yang berkualiti tinggi dan konsisten. Kajian ini bertujuan untuk mendokumentasi dan mengkaji sifat-sifat biokimia dalam tuak yang dihasilkan daripada beras biasa (Oryza sativa), menggunakan dua jenis kultur pemula yang berbeza iaitu ragi dan kultur kulat yang mengandungi Rhizopus oligosporus, Clavispora lusitaniae, Saccharomyces cerevisiae, Saccharomycopsis fubiligera dan Pichia kudriavzevii. Berdasarkan kajian yang telah dijalankan, dapat disimpulkan bahawa fermentasi menggunakan ragi menghasilkan jumlah etanol yang lebih tinggi berbanding dengan jumlah etanol yang dihasilkan oleh fermentasi menggunakan kultur kulat. Fermentasi menggunakan ragi juga menunjukkan kadar konsumsi glukosa yang lebih tinggi, jumlah asid asetik dan asid laktik yang sederhana, serta mempunyai kandungan fenolik yang lebih baik. Kesimpulannya, kajian ini menunjukkan bahawa strain yang digunakan dalam kultur kulat adalah tidak efisien untuk menghasilkan tuak yang berkualiti tinggi.

Kata Kunci: *Tuak, ragi, wain beras, kultur kulat, fermentasi alkohol*

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LIST OF ABBREVIATIONS

%	Percentage
µm	Micrometer
atm	Atmosphere
°C	Degree Celsius
cm	Centimeter
g	Gram
M	Molar
mg	Milligram
mg/mL	Milligrams per milliliter
min	Minutes
mL	Millilitre
mM	Millimolar
nm	Nanometer
pH	Potential of Hydrogen
rpm	Revolutions per minute
Spp.	Several species
v/v	Volume per volume

CHAPTER 1

INTRODUCTION

Fermentation is an ancient method used to maintain and improve the flavour, aroma, and nutritional value of food and drinks (Merican & Quee-Lan, 2004ch). This process has been used to preserve foods and drinks for centuries, long before pasteurisation and sterilization were invented (Chaves-López *et al*., 2014). Fermented foods and drinks are still part of the regular diet in many cultures. In Asian cultures, one of the most popular indigenous fermented product is rice wine. This alcoholic beverage goes by a variety of names for example *shiaoxing* in China, *brem* in Indonesia, *sonti* in India, *mirin* and *sake* in Japan, *tapuy* in Phillipines, *satoh* in Thailand, *ruou* in Vietnam and *samsu* or *tuak* in Malaysia (Dung *et al*., 2014). Rice wine plays a huge role and is closely intertwined in the spiritual and cultural life of many Asians (Chiang *et al*., 2006; Dung, 2013; Woldemariam, 2014; Chay *et al*., 2017).

In Sarawak (East Malaysia), *tuak* is often linked with the rice harvest festival (Gawai Dayak) which is dedicated to the rice spirits (Nik Mohd Nor *et al*., 2020). *Tuak* is often produced by fermenting rice and *ragi*, a starch based starter. *Tuak* production is mostly done on a small scale at home. The wine is obtained in non-sterile, minimally controlled settings which is highly dependent on local climatic circumstances (Nout & Motarjemi, 1997). When *tuak* is produced under conventional way in which fermentation is uncontrolled and spontaneous, the products obtained vary in sensory characteristic, resulting in quality inconsistencies (Chaves-López *et al*., 2014). To date, differences in ethanol profiles, flavour compounds of *tuak* and bacterial communities of *ragi* are also poorly understood. Hence, the establishment of a controlled starter culture mixture with known strains is necessary for high quality and consistency in the commercial production of *tuak*. This study proposed to document, investigate and assess the biochemical properties of *tuak* produced from common rice (*Oryza sativa*) using traditional *ragi* and starter culture with known strains namely *Rhizopus oligosporus*, *Clavispora lusitaniae*, *Saccharomyces cerevisiae*, *Saccharomycopsis fubiligera* and *Pichia kudriavzevii*.

1.1 OBJECTIVES

The objectives of the study are:

i. To analyze the production of *tuak* from common rice (*O. sativa*) using traditional *ragi* and starter culture with known fungal strains.

ii. To determine the biochemical properties of *tuak* produced from common rice (*O. sativa*) using *ragi* and starter culture with known fungal strains.

CHAPTER 2

LITERATURE REVIEW

2.1 *Tuak*

Tuak (**Figure 2.1**) is a traditional alcoholic beverage made particularly from rice. It is an important aspect of the Dayak culture, notably among the Iban ethnic group from Sarawak. Typically served at the harvest festival or known as Gawai Dayak by the indigenous Dayaks in Sarawak, *tuak* is also used as the main drink during the celebration (Jawol *et al*., 2018). Traditionally, *tuak* plays an important role during Gawai as it is used as a symbolic offering to the rice spirit for the plentiful harvest and in requesting the spirits for prosperity and more blessings (Nik Mohd Nor *et al*., 2020). *Tuak* is also offered to the guests as a welcoming drink during Gawai and other celebrations such as weddings (Jawol *et al*., 2018).

Figure 2.1 Commercialized *tuak* (Ting *et al*., 2017).

Conventionally, *tuak* is made by fermenting rice, typically glutinous rice with *ragi*, a dry starter culture containing yeast and enzymes, water and sugar (Ting *et al*., 2017). Despite the basic ingredients of *tuak*, the taste of *tuak* can be fairly difficult to define as it has a wide range of flavours depending on the starter cultures or *ragi* used (Dung *et al*., 2005). This is because, different starter cultures produce different volatile flavour compounds that contribute to the flavours such as heterocyclic and aromatic compound, aliphatic carbonyl compound, monocarboxylic acids and their esters and many more (WHO, 1988). However, poiled *tuak* can be easily identified by its sour taste, bad odours and cloudy solution indicating the occurrence of contamination by other bacteria which produce lactic acid and hence the taste (Jeon *et al*., 2015). Depending on the amount of sugar used in the fermentation process, *tuak* can be non-sweet, slightly sweet, or very sweet. Some people add various spices such as cinnamon, local herbs, pepper or ginger to the *ragi* to obtain unique flavours (Palaniveloo & Vairappan, 2013; Dung *et al*., 2014; Nuraida & Krusong, 2014).

2.2 The Process of Making *Tuak*

Tuak or other rice-based alcoholic beverages are made through a biological process in which rice (*Oryza sativa*) is turned into an alcoholic beverage via physical, biochemical and microbiological activities which involve steaming or cooking, grinding, inoculation with fermentation starter, and fermentation (Dung, 2013). *Tuak* is mostly manufactured at home by artisanal producers. Every *tuak* producer has their own techniques and procedures, which vary depending on their experience and the raw materials available in their region (Nout & Motarjemi, 1997). However, in general, all producers follow the same process as shown in **Figure 2.2** below.

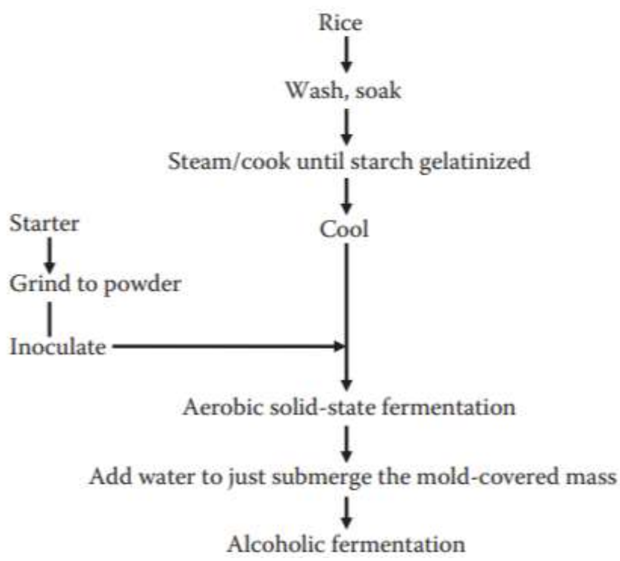

Figure 2.2 General outline of rice wine making (Dung *et al*., 2014).

Tuak is usually made by first washing and soaking the rice in tap water to hydrate and soften the starch granules before steaming or cooking the rice, depending on the producers' preference. This process is required as it increases the starch's availability for enzymatic breakdown (Snow & O'Dea, 1981; Pither, 2003; He e*t al*., 2018). Cooking is preferred by most producers since it takes less time than steaming to completely gelatinize the starch even though cooking is a bit trickier because the producers need to know the adequate amount of water needed (Dung *et al.*, 2014). The steamed or cooked rice is then left to cool. Starch-based starter which normally include yeasts, moulds and bacteria such as *ragi*, is crushed to powder by using pestle and mortar and used to inoculate the cooked rice by mixing them together (Hesseltine *et al*., 1988; Nout & Aidoo, 2013). The rice and *ragi* mixture is then incubated at ambient conditions preferably 28 to 32 °C to ferment. There is no specific time range on how long the fermentation should be done as the local producers concur that the incubation period needed for the fermentation rely on the weather, in which the hotter the weather, the shorter time it requires (Dung *et al.*, 2014). After some time, the mixture of rice and *ragi* will turn into a mould-covered mass. Next, the mould-covered mass is then merged with water to allow submerged alcoholic fermentation to take place. Traditionally, the fermentation is done in big glazed terracotta jars. However, as modernization come to pass, nowadays' producers favour polyethylene vessels as their fermentation containers as they are cheaper and more practical to use (Dung *et al.,* 2014). During the alcoholic fermentation, some producers strain the brew to separate it from its sediment a few time throughout the fermentation process to obtain clear *tuak*.

Undistilled ferments typically have an alcohol content of 7-11% (v/v), which is not enough to protect *tuak* from spoilage by undesirable microbes (Dung *et al.,* 2014; Saranraj *et al*., 2017). In order to solve this issue, *tuak* producers typically increase the alcohol content of *tuak*, by adding sugar before the alcoholic fermentation take place in order to secure final alcohol concentrations of 15–35% (v/v) depending on the level of alcohol and the desired storage life as demanded by producers and customers (Dung *et al.,* 2014).

2.3 *Tuak* Fermentation Process

Tuak fermentation process involves two main steps which occur simultaneously without any effort to control the fermentation (Kofli & Dayaon, 2010). The first step is saccharification of steamed starchy resource or rice in an aerobic solid-state fermentation (SSF) (Blandino *et al*., 2003; Sujaya *et al*., 2004; Dung *et al*., 2007).

SSF is a typical method in producing microbial metabolites in which bacteria grow on a moist solid substrate (**Figure 2.3**) with a low moisture content without access to free-flowing water (Abdul Manan & Webb, 2017; Farooq *et al*., 2021). The water content in SSF is absorbed by the substrate in a solid matrix and aids in the oxygen transfer for the growth of microorganism. Meanwhile the solid matrix itself act as support, as well as to supply carbon and nutrient for the production of enzymes by the microorganisms (Carboué *et al*.,2018; Srivastava *et al*., 2019).

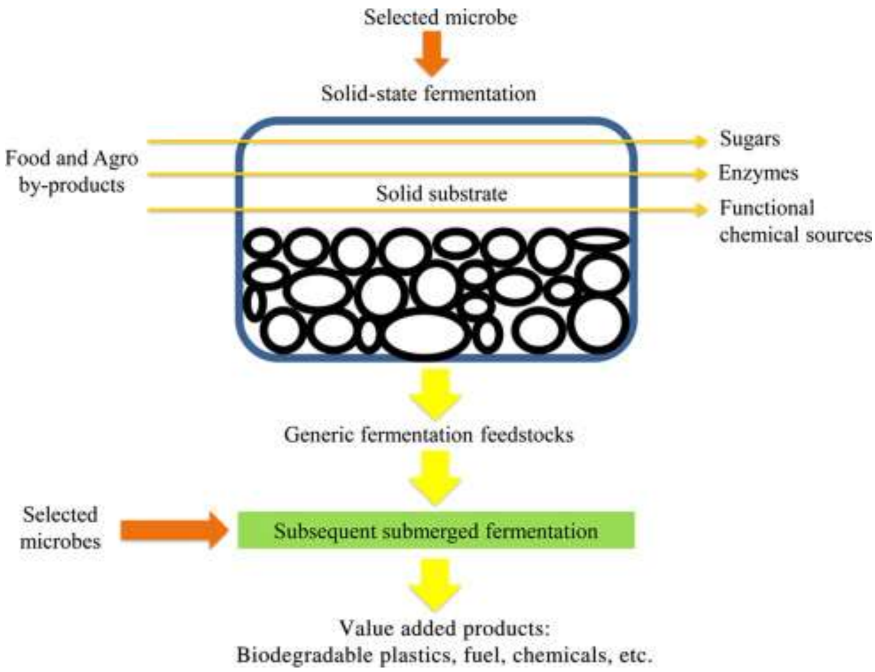

Figure 2.3 Solid-state fermentation process (Abdul Manan & Webb, 2017).

During the saccharification stage, the microbial metabolites are obtained by extracting sugars and hydrolyzing cellulose using amylolytic microorganisms or enzymes such as glucoamylase (also called amyloglucosidase) and α-amylase (Crabb, 1999; Nout & Aidoo, 2002; Toksoy Öner *et al*., 2005). The hydrolysis causes the formations of fructose syrup, glucose syrup and maltose. The fructose syrup is widely employed in the beverages industry as an alternative sweetener which can be made by the isomerizing the high glucose syrup with the aid of glucose isomerase as shown in **Figure 2.4** below (Uthumporn *et al*. 2010).

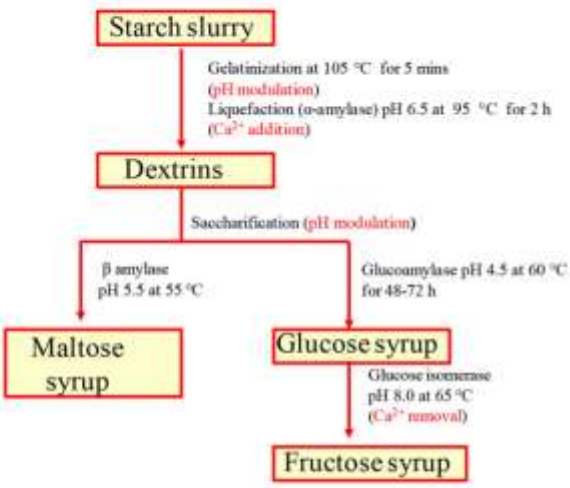

Figure 2.4 Starch saccharification process (Parashar & Satyanarayana, 2018).

The second step is ethanol fermentation or specifically submerged alcoholic fermentation where sugar is converted by yeasts forming ethanol and carbon dioxide as the by-products (Toksoy Öner *et al*., 2005; Ly *et al*., 2018). This is represented in **Figure 2.5**. Ethanol fermentation uses anaerobic pathway, which means the fermentation process does not require oxygen (Serafim & Lanças, 2018).

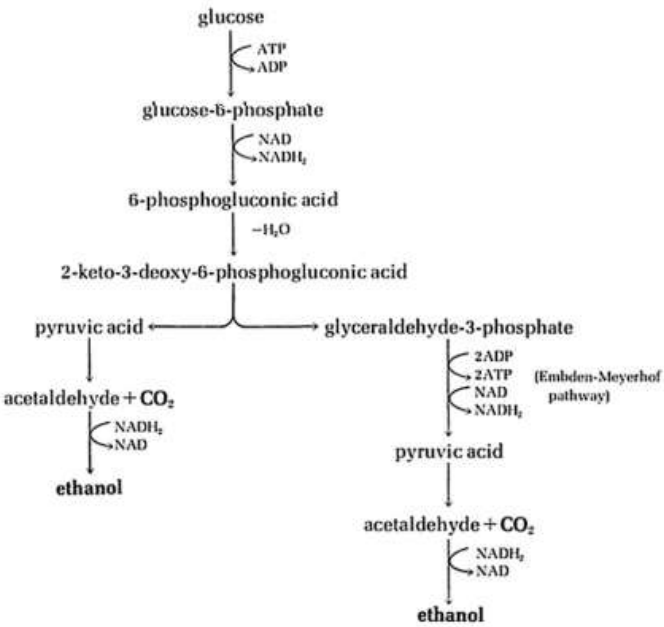

Figure 2.5 Glucose conversion to ethanol during alcoholic fermentation (Rupasinghe *et al*., 2017).

As shown in **Figure 2.5** above, glucose conversion to ethanol during fermentation starts with glycolysis. In this process, the yeast breaks down one molecule of hexose in the form of glucose to form two molecules of triose namely pyruvate and glyceraldehyde-3-phosphate (G3P) (Serafim & Lanças, 2019). G3P can be converted into pyruvate via Embden Meryenhof pathway (Rupasinghe *et al*., 2017). In anaerobic conditions, the pyruvate molecules produced will be decarboxylated to form acetaldehyde and the acetaldehyde is then reduced to form ethanol (Malakar *et al*., 2019).

2.4 Ethanol

Ethanol or commonly known as ethyl alcohol, is a colourless, clear liquid. It is an essential industrial chemical and often used as a solvent in the production of a variety of items, from cosmetic products to pharmaceutical products, to paints and other organic chemicals and also as a fuel additive (Barbulova *et al*., 2015). Its solubility in water and other organic compounds enable it to be a good solvent. It is also the main components in most alcoholic beverages such as wine, beer and brandy. Even so, different types of alcoholic beverage have different ethanol concentration in them (Banarjee, 2014). In alcoholic beverages, ethanol is present as a product of carbohydrates fermentation using yeast (WHO, 1988). Although ethanol can also be made by using ethylene from cracked petroleum hydrocarbons, the alcoholic beverages industry has decided not to utilize the synthetic ethanol because it contains impurities (WHO, 1988). Production of ethanol in alcoholic beverages is achieved through the fermentation of sugar crops such as sugarcane and beets and grain crops such as corn and rice. During the fermentation of ethanol, some volatile acids may present for example, acetic acid. Acetic acid can be produced by the yeast itself but only in a small amount. High amount of volatile acidity in alcoholic beverages indicate the activity of spoilage microorganisms (WHO, 1988).

Ethanol is naturally toxic to human body as it affects the central nervous system (Vale, 2007; Banarjee, 2014). When consumed in a moderate amount, ethanol relaxes the muscles and generate an obvious stimulating effect by supressing the inhibitory functions of the brain (Valenzuela, 1997; Vale, 2007). However, if ethanol is consumed too much, it wreaks havoc on coordination and judgement which can lead to coma and death (Cherpitel, 1993).

2.5 *Ragi*

Ragi is a traditional Asian dry starter culture that is commonly employed in the fermentation of a variety of foods such as fermented cassava or glutinous rice (*tapai* or *tape*), fermented peanut press cake (*oncom*) and fermented soy bean (*tempe*) (Nuraida & Krusong, 2014). A starter culture is a preparation employed prior to the process of making fermented foods which contain at least one microorganism with a large number of cells to be added on a raw material for speeding up and controlling the fermentation process (Leroy & Vuyst, 2004). *Ragi* is dry, off-white coloured and comes in a variety of shapes and sizes, but is most commonly produced into round (**Figure 2.6**) and flattened hemispherical balls about 2–6 cm in diameter and 0.5-1 cm thick (Merican & Quee-Lan, 2004; Nuraida & Krusong, 2014; Roslan *et al*., 2018).

Figure 2.6 Traditional *ragi*.

In Malaysia, ragi are usually available in markets in a plastic bag without any brand name labelling as they are still produced on a small scale by cottage industries (Merican & Quee-Lan, 2004; Nuraida & Krusong, 2014). Although the methods of manufacture vary by region, basic ingredients of *ragi* comprises of rice flour, spices and water or sometimes coconut water or sugar cane juice (Kofli & Dayaon, 2010; Nuraida & Krusong, 2014).

To make *ragi*, the rice flour mixture is pounded and thoroughly mixed with powdered or ground spices and herbs that are said to inhibit the growth of unwanted bacteria or promote desirable microorganisms' growth (Hesseltine, 1983). The spices and herbs that are usually used are chilli, onion, cinnamon, pepper, garlic, galangal, and oriental herbs roots. The proportion of spices used vary among different *ragi* producers (Nuraida & Krusong, 2014). However, according to Dung *et al*., (2014), the ratio of mixed spice to ground rice by weight is around 1:14. Water is added to the mixture forming a thick paste and then inoculated with *ragi* from a previous batch that is grounded into powder mixing them thoroughly (back slopping system) (Merican & Quee-Lan, 2004; Dung *et al*., 2014). The inoculated dough is shaped by hand into flattened balls. They are then set on bamboo trays with rice husks, muslin cloth or banana leaves on top to reduces overheating and facilitates aeration (Merican & Quee-Lan, 2004; Nuraida & Krusong, 2014; Roslan *et al*., 2018). After 2-5 days being incubated at ambient temperature (28–32 °C), the dough slowly dessicate and becomes covered with fungal mycelium. The microorganisms thrive and reproduce while the paste is still wet, and hence when the ball is dehydrated, the total viable count of fungi and yeasts has dramatically elevated. The dough is then air- or sun-dried at room temperature (25–30 °C) for 3–4 days forming dried cakes that can be stored for in dry, airtight containers for a long time (Merican & Quee-Lan, 2004). The flowchart in **Figure 2.7** summarizes the production of *ragi*.

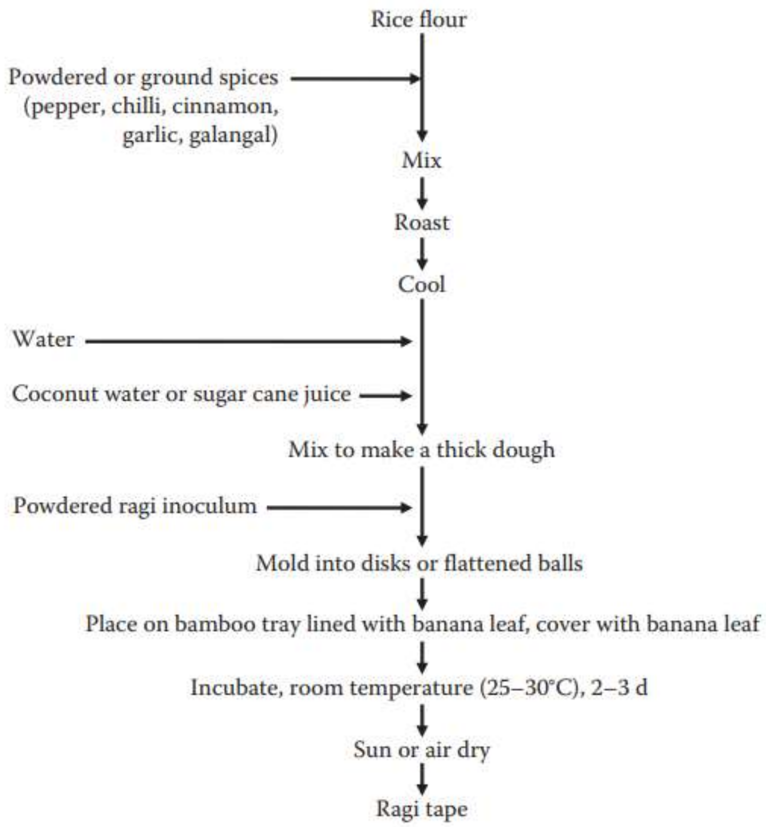

Figure 2.7 Process of making *ragi* (Nuraida & Krusong, 2014).

2.6 Microbiology of *ragi*

Ragi contains starch which can be further broken down into fermentable sugar such as glucose and maltose by using the enzyme amylases. According to a study done by Wanyo *et al*., (2009), rice flour is composed of 80% carbohydrate with amylose content of about 35%. The enzyme amylase, needed to degrade the starch can be found in the *ragi* itself as it is provided by the bacteria and other microorganisms present in the *ragi* (Roslan *et al*., 2018). Since the production of *ragi* is still conducted at small scale, most of the time, none of the environmental factors control is performed. This also means, any microorganisms that land on the freshly made *ragi* will reproduce and proliferate. Due to this uncontrolled condition, different places and different *ragi* producers will have different microbial load content in their *ragi* (Merican & Quee-Lan, 2004). A good *ragi* is defined based on by the existence of pre-existing load in both the containers and environment, as well as the prevention of other undesirable organisms' growth (Merican & Quee-Lan, 2004).

The intriguing aspect about *ragi* microbiology is that they invariably contain an amylolytic molds, such as *Aspergillus* or *Amylomyces* (Merican & Quee-Lan, 2004). The presence of *Amylomyces* in *ragi* and other starters has been reported by Hesseltine *et al*., (1985), Ardhana & Fleet, (1989), Djien, (1972) and Saono *et al*., (1974). The most common *Amylomyces* species found is *Amylomyces rouxii*. Amylolytic strain is important in *ragi* as they provide amylases, a type of hydrolase enzymes capable of hydrolysing glycosidic bonds in starch and degrade them into polymers made up of glucose subunits (Souza & Magalhães, 2010). If the fermentation was allowed to carry on until it was finished, the glucose subunits will then be converted to alcohol, aldehydes, aromatic esters and acids (Merican & Quee-Lan, 2004). Amylases obtained from microorganisms are more preferable over other sources because of their high production rate and their ability to be manufactured in a huge quantity, making them more cost-efficient (Sundaram & Murthy, 2014). Another amylase producing