BIOCHEMICAL COMPARISON OF RICE WINE PRODUCED USING COMMON AND GLUTINOUS RICE WITH THREE DIFFERENT TRADITIONAL STARTER CAKES

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LIST OF FIGURES

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ABSTRACT

BIOCHEMICAL COMPARISON OF RICE WINE PRODUCED USING COMMON AND GLUTINOUS RICE WITH THREE DIFFERENT TRADITIONAL STARTER CAKES.

Rice wine or 'tapai' is one of the traditional alcoholic beverages, which has been produced in Sabah for ages and is used in most of the cultural rituals and ceremonies. This study delves on the chemical characterization of rice wine produced from common and glutinous rice using three different traditional starter cakes; 1) bitter, 2) bitter sweet, 3) sweet. Each batch of fermentation involving one type of rice and starter cake lasted for 4 weeks and the wine collected were separated from its rice, and the fermented rice was extracted in ethanol to yield crude extract. Sweet starter cake had the highest yeast/LAB load followed by bitter sweet and bitter and accordingly resulted in and weight reduction of substrate at an average of 450 %, 230 % and 210 % respectively. The volume of wine produced by glutinous rice was twice the volume produced by common rice. pH of the wines was in the range of 4.3 and 4.7. Morphological changes in the rice grain during fermentation were observed through scanning electron micrographs (SEM) and the presence of yeast/LAB growth and changes to the rice grains were observed. High Performance Liquid Chromatography analysis of the wine revealed that wine produced from common rice with its respective starter cake contained much lower glucose content [1) Bitter : 120.643 $\pm$ 0.21 mg/ml, 2) Bitter-Sweet : 270.42 $\pm$ 0.32 mg/ml, 3) Sweet : 310.326 $\pm$ 5.83 mg/ml] as compared to glutinous rice [1)Bitter : 300.274 $\pm$ 0.28 mg/ml, 2) Sweet and bitter : 320.251 $\pm$ 0.00 mg/ml, 3) Sweet : 440.138 $\pm$ 29.97 mg/ml]. However, gas chromatography analysis showed that rice wine produced from common rice had comparatively higher alcohol percentage; 9 % to 12 % as compared to glutinous rice; 7 % to 8 %. The difference in wine taste could be attributed to the varying type and amounts of organic acids and metabolites contained in the starter cakes and produced during the fermentation process. Gas Chromatography analysis revealed details of this complex composition. Comparison of these chemicals between their starter cake, wine and fermented rice is discussed. Ethanolic extract of the respective fermented rice contained low glucose levels but showed strong antioxidant and potent fibrinolytic enzyme activities, with bitter sweet starter cake displaying the best of results. Antioxidant potential of fermented rice extracts of common rice displayed higher scavenging activity compared to glutinous rice, while common rice wine displayed higher antioxidant values compared to glutinous rice wine. Fibrinolytic enzyme activities were present in fermented rice extracts, and extracts of common rice showed double the activity by diameter as compared to glutinous rice for all three starters. In summary, fermentation using different rice types with different starter cakes confirms the difference in chemical properties as well as bioactive potentials of the rice wine and fermented rice.

ABSTRAK

Tapai atau 'rice wine' merupakan salah satu minuman beralkohol tradisional yang telah dihasilkan di Sabah sejak berkurun lamanya dan digunakan dalam kebanyakan upacara dan keramaian. Kajian ini melibatkan penyelidikan terhadap ciri-ciri kimia tapai yang dihasilkan daripada beras putih dan pulut menggunakan tiga ragi berbeza; 1) pahit, 2) pahit manis, 3) manis. Setiap set penapaian yang melibatkan salah satu beras dan ragi mengambil masa 4 minggu. Tapai yang dihasilkan telah diasingkan daripada nasi yang seterusnya diekstrak dengan ethanol untuk mendapatkan ekstraknya. Ragi manis mempunyai kandungan yis/LAB tertinggi diikuti ragi pahit manis serta ragi pahit dan dalam urutan sedemikian perubahan berat substrat untuk penapaian adalah dalam purata 450 %, 230 % dan 210 % masing-masing. Isipadu arak yang dihasilkan beras pulut adalah dua kali ganda arak yang dihasilkan oleh beras biasa. pH semua arak berada dalam julat 4.3 dan 4.7. Perubahan morfologi butiran nasi sewaktu penapaian diteliti melalu mikrograf pengimbasan elektron (SEM) dan kehadiran yis/LAB serta perubahan terhadap butiran nasi juga diteliti. Analisis Kromatografi Cair Kinerja Tinggi menunjukkan alkohol yang dihasilkan menggunakan beras biasa dengan ragi berlainan mengandungi kandungan glukosa yang lebih rendah [1) Pahit : 120.643 ± 0.21 mg/ml, 2) Pahit manis : 270.42 ± 0.32 mg/ml, 3) Manis : 310*.326 ± 5.83 mg/ml] berbanding beras pulut [1) Pahit : 300.274 ± 0.28 mg/ml, 2) Pahit manis : 320.251 ± 0.00 mg/ml, 3) Manis : 440.138 ± 29.97 mg/ml]. Bagaimanapun, analisis kromatografi gas menunjukkan tapai yang dihasilkan dari beras biasa mempunyai kandungan alkohol yang lebih tinggi; 9 % hingga 12 % berbanding beras pulut; 7 % hingga 8 %. Perbezaan dari segi rasa pula boleh dikaitkan dengan jumlah dan kepelbagaian asid organik dan unsur- unsur yang terkandung dalam ragi dan yang dihasilkan semasa proses penapaian. Analisis kromatografi gas telah memberi data yang lebih mendalam berkenaan komposisi kompleks ini. Perbezaan kandungan kimia antara ragi, tapai dan nasi hasil penapaian juga telah dikaji. Ekstrak ethanol nasi hasil penapaian mengandungi kandungan glukosa yang rendah tetapi menunjukkan keupayaan antioksidan dan aktiviti enzim fibrinolitik yang kuat dengan hasil ragi pahit manis memberi keputusan terbaik. Keupayaan antioksidan ekstrak nasi biasa menunjukkan kebolehan 'scavenging' yang lebih tinggi berbanding ekstrak nasi pulut, manakala tapai beras biasa memberi bacaan antioksidan yang lebih tinggi berbanding hasil beras pulut. Aktiviti enzim fibrinolitik adalah ketara pada ekstrak nasi dan ekstrak nasi biasa menunjukkan aktiviti dua kali ganda lebih kuat dari segi diameter daripada ekstrak nasi pulut bagi ketiga- tiga ragi. Secara kesimpulan, penapaian menggunakan jenis beras berbeza dengan ragi berbeza pastinya menunjukkan perbezaan dari segi kandungan kimia dan potensi bioaktif tapai dan nasi hasil penapaian.*

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LIST OF ABBREVATIONS AND SYMBOLS

C-B	Common Rice, Bitter Starter Cake
C-BS	Common Rice, Bitter Sweet Starter Cake
C-S	Common Rice, Sweet Starter Cake
G-B	Glutinous Rice, Bitter Starter Cake
G-BS	Glutinous Rice, Bitter Sweet Starter Cake
G-S	Glutinous Rice, Sweet Starter Cake
%	Percent
°C	Degree Celsius
CFU	Colony Forming Unit
AA	Ascorbic Acid
cm	centimeter
g	gram
GCMS	Gas chromatography Mass Spectrometry
HPLC	High Performance Liquid Chromatography
mg	milligram
mL	milliliter
µg	microgram
µL	microliter
NaCl	Sodium Chloride
RT	Retention Time
RI	Retention Index
R_f	mobility relative to front
PTLC	Preparative Thin Layer Chromatography
TLC	Thin Layer Chromatography
Tol	Toluene
UV	ultra violet
w/v	Weight over Volume

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CHAPTER 1

INTRODUCTION

1.1 FOOD AND FERMENTATION

Man had practiced the art of food fermentation for centuries even before its basic principle were really understood. The study of fermentation in a scientific approach only took place in the 1850s with the isolation of amyl alcohol by Louis Pasteur (El-Mansi *et al.*, 2007). According to Pasteur, fermentation is associated with the life and structural integrity of the yeast cells whereby yeasts cells play the role as the ferment. Various theories were proposed by various researches regarding fermentation but generally fermentation is understood as an enzyme catalyzed, energy producing process which generally uses sugar as substrate to transform it into products such as ethanol, lactic acid, hydrogen and at the same time resulting in the production of other minor organic acids (Cornish-Bowden, 1997). Fermentation was also described as a sequence of events whereby oxygen from sugar was transferred from one molecule of sugar to the other to form a highly oxidized and reduced product. Finally, the concept of fermentation was modified to support the idea that fermentation was a function of a living entity involving a chain of multiple reactions catalyzed by various enzymes produced by microorganisms (El-Mansi *et al.*, 2007). Therefore, fermentation can be considered as the breaking of food into small components for easy consumption and digestion as well as proper assimilation of nutrient by the body.

At present, there are various types of food that are fermented for human and animal consumption. Fermented food are the end product from biochemical processes caused by microorganisms whereby their enzymes hydrolyze polysaccharides, proteins and lipids to nontoxic products with better flavorings, aromas and texture so that it is much attraction for human consumption (Streinkraus, 2002). Originally fermented food was only restricted to household

consumption and was produced in limited quantity but as demand increased, these food production transformed to cottage industry and is now produced in large scale industrially.

1.2 BENEFITS OF FERMENTATION

Fermentation of food has been known to provide multiple benefits to consumers and animals. Fermentation has the potential to enrich the food by developing a variety of flavors, aromas, and textures from the various chemical processes and fine chemicals contributed by the microorganisms. In the production of wine, the final product can taste sweet, bitter or sour depending on the combination of ingredients used and produced.

Fermented food as a whole is much more nutritious as the process of fermentation enriches the end product biologically with vitamins, proteins, amino acids and fatty acids. As an example, starch from rice is converted into sugar then fermented into alcohol; there would be an increase in protein level and free amino acid content (Steinkraus, 2002). However, the variation in nutritional quality and quantity at the end of the fermentation process depend on the kind of microorganisms employed and the parameters governing the process.

During the process of fermentation, detoxification takes place through the continuous hydration of the raw material and by subjecting the materials to extreme condition (either acidic or alkaline), which removes potential toxins in food. At the same time, the specific extreme pH condition created by the yeast/Lactic acid bacteria eliminates unwanted microorganisms present that might spoil the fermented product, thus making it last longer. As such, fermentation also helps to preserve food so that it can be stored long without deteriorating its quality and taste. Preservation of food is best through lactic acid, alcohol, acetic acid and alkaline fermentations (Steinkraus, 2002).

On the contrary, though fermented food takes time to be produced very little energy is required to produce it. Fermented food is produced naturally with the reaction of microorganism under suitable conditions to provide hygienic, health

beneficial food ready for consumption. Therefore, fermentation is an environment friendly process as energy is conserved (Steinkraus, 2002).

1.3 RICE WINE OF SABAH

Today, indigenous fermented food has become a new research interest since these are believed to contain therapeutic properties beneficial to the health of non-traditional consumers (Chiang *et al.*, 2006). The application of fermentation is not restricted to a particular region but is practiced all over the world thus widespread research had been conducted to understand the benefits of fermented food. Some example of fermented food are the Indonesian "tempe", Chinese soy sauce, Malaysian "belacan" and "tempoyak", Cambodian "prahoc", Middle-Eastern yoghurts, Nigerian "gari", Indian jackfruit wine, Zambian maize beer and Kenyan "busaa", among the many varieties available today.

In this regard, the East Malaysian states in the island of Borneo are extremely rich in nature as well as culture with the most number of indigenous ethnic races with their unique cultural values. With the diversity of culture comes various traditional food and techniques for their preparation. Specifically, in Sabah, the local community has the knowledge to transform harvested rice into an alcoholic beverage. The rice wine which is locally known as 'tapai' is one of such product and has been produced for ages by the local community in small quantities, mainly for household consumption as well as cultural ceremonies and rituals. This wine has a variety of names, which differ with the ethic races, for instance certain Dusun slangs call it 'hiing', whereas others call it 'kinomol', 'segantang', 'kinarung', 'kinopi', and 'linahas'.

The produced wine has an alcoholic aroma with a mixture of bitter sweet taste and a sparkling feel. The freshly produced wine comes in faded yellow color but turns bright golden yellow when stored for a long duration. This alcoholic beverage is the end product of rice fermentation with the aid of a starter cake or yeast/lactic acid bacteria, which is locally known as 'ragi' or 'sasad'. The starter cake, which is extremely important in the production of wines, is made from rice, spice and yeast. A variety of rice is used to make rice wine and the starter cake.