

インドネシア
ボゴール農科大学・農産加工計画
カウンターパートの学位取得の経緯

1983年3月

国際協力事業団

インドネシア
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カウンターパートの学位取得の経緯

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序

MRS. Jenny Saonoは、インドネシア共和国における農科系大学の拠点校の一つであるボゴール農科大学に対するわが国の技術協力プロジェクトであるボゴール農科大学農産加工計画のカウンターパートである。同人は昭和54年から上記計画の食品微生物分野を担当するカウンターパートであり、研修員として来日するのは今回で3度目である。同人は昭和57年12月2日から昭和58年9月21日まで研修員として滞日し、この間東京農業大学農芸化学科応用微生物研究室で「ブルムワイン醸造スターター「ラギ」とその改良に関する研究」をテーマに研究を行ない、9月12日東京農業大学農芸化学科に対し論文要旨発表を行なった。研究論文は同大学大学院本委員会にて審査を受けた後、東京農業大学から学位を授与されることが正式に決定された。

本報告は、同氏の学位取得の経過及び研究論文を取りまとめたものであり、今後増大する学位取得に関する資料として活用されることを期待する。

ここに Mrs. Jenny Saono の学位取得のご指導をいただいた東京農業大学農芸化学科応用微生物研究室小崎道雄教授、外務省、文部省、農林水産省及び関係の各機関に謝意を表する。

農業開発協力部

部長 田 内 堯

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1 学位取得の経過

1) 日本における研修の概略

ボゴール農大、農産加工計画研修員 Mrs. Jenny K.D. Saono は、昭和 57 年 12 月 2 日から昭和 59 年 9 月 21 日まで、東京農業大学農芸化学科応用微生物研究室の小崎道雄教授のもとで「ブルムワイン醸造スターター“ラギ”とその改良に関する研究」を行ない、研究論文を研究論文博士号の審査のために同大学に提出した。

Mrs Jenny Saono による論文要旨発表は 9 月 12 日東京農大農芸化学科に対して行なわれ、10 月 25 日（火）に開催された大学院本委員会において投票の結果、学位（ph,Dr）の授与が正式決定された。

同氏は学位を取得するために昭和 57 年 12 月 2 日から昭和 59 年 9 月 21 日まで東京農業大学農芸化学研究室にて研修を行なったが、研修概要については次のとおりである。

① 研 修 員 Mrs. Jenny Saono

- (ア) 国 籍 インドネシア
- (イ) 現 職 ボゴール農科大学（IPB）教官
- (ウ) 学 歴 ボゴール農科大学卒業
米国ウイスクンシン大学修士課程卒業

② 研 修

- ㊦ 研究項目 ブルムワイン醸造スターター“ラギ”とその改良に関する研究
- ㊧ 研修の種類 国際協力事業団（JICA）による Ph-D 取得計画による研修
- ㊨ 研修受入先 海外農業研究教育開発協会（SAEDA）
- ㊩ 研 修 先 東京農業大学農学部農芸化学科
教授 小崎道雄
- ㊪ 研修の目的 ブルムワイン醸造に使われるラギから有用アルコール酵母、糖化菌の分離・同定を行ない、またラギ菌叢に各種植物、及ラギ保存温度の及ぼす影響を調べること。
- ㊫ 研修期間の延長 当初研修期間は昭和 59 年 9 月 1 日までであったが博士論文取りまとめのために研修期間を延長し 9 月 21 日まで延長した。

| | | | |
|---------|-----------|------------------|----------|
| 12月6～11 | 研究打合せ | 客員研究員の申請 | 場所 農大 |
| 13～18 | 分離酵母分類取扱法 | 食品工業学会誌第 2 報論文作成 | ” |
| 20～25 | ” | 〈細野助教授〉 | ” |

| | | | 場所 |
|---------|--------------------------------|------------------------|-----------|
| 27~1月1 | 休 暇 | | |
| | | 第1報は発表済み | |
| 1月 3~ 5 | 休 暇 | | |
| 6~ 8 | 培地調整 | | 農大 |
| 10~15 | 酵母同定および実施研修 | | " |
| 17~22 | 同 上 | | " |
| 24~29 | 同 上 | | " |
| 31~2月5 | 同 上 | | " |
| 2月 7~12 | 糸状菌同定および実施研修 | | " |
| 14~19 | 同 上 | (※農大入試) | " |
| 21~26 | 同 上 | この間は自己研修 | " |
| 28~3月5 | 同 上 | | " |
| 3月 7~12 | 酵母、糸状菌に関する同定のまとめ | (大学院入試) | " |
| 14~19 | 同 上 | 食品工業学会誌第3報論文作成 | " |
| 21~26 | 同 上 | (小崎先生) | " |
| | | この間小崎先生は阪大でのSeminarに出席 | |
| 28~4月2 | 農化大会(於仙台)宮城女子大 | | 仙台 |
| 4月 4~ 9 | 休 暇 | (東京農大入学式) | 農大 |
| 11~16 | 微生物(とくに細菌)取扱訓練 | | " |
| 18~23 | 信州大学(農)農化 細野助教授と打合せ(2泊3日) | | 長野県 伊那 |
| 25~30 | | | 農大 |
| 5月 2~ 7 | 論文作成及試験準備 | | " |
| 9~14 | " | | " |
| 16~21 | " | | " |
| 23~28 | " 論文発表、スライド作成 | | " |
| 30~6月4 | " | | " |
| 6月 6~11 | " | | " |
| 13~18 | 専攻内論文内容発表 Jenny Saono、試験(農化科内) | | " |
| 20~25 | | 論文審査手続 審査料¥200,000 | " |
| 27~7月2 | (受理可否) | | " |
| 7月 4~ 9 | 論文作成 | | " |
| 11~16 | " | | " |
| 18~23 | (審査報告)小崎教授 最低3部必要 | | " |

2) Jenny Saono 氏の研修日程

| | 場所 |
|-------------------|------------|
| 7月25~30 論文清書 最終提出 | 農大 |
| 8月 1~ 6 | 国際微生物学会 |
| 8~13 | 8月28日~9月3日 |
| 15~20 | 於京王プラザ |
| 22~27 学位記授与 | Dr. ガンジャール |
| 帰国準備 | " |
| 29~31 報告書提出 | " |

3) 学位論文の要約

ブルムワイン醸造スターター“ラギ”と その改良に関する研究

ジェニー サオノ (Jenny D. SAONO)

インドネシアの餅麴“ラギ”は、タペ、ブルムワイン、ブルムケーキ、アラックなどの発酵食品製造用スターターである。ラギは中国を起源とし、中国人植民者により9世紀頃にインドネシアに伝えられた (Vorderman 1893) ラギに関する研究は、オランダ人科学者により彼らが興味を示したバタビヤアラックを材料として研究が始められた (Eijkman 1894, Prinsen, Geerligs 1894, etc.)。

ラギ菌叢には、糖化及び液化をつかさどる Amylomyces, Mucor, Rhizopus アルコール及び香気を生成する Saccharomyces cerevisiae, Hansenula anomala が重要な微生物として存在する。さらに糸状酵母は、糖化だけでなく臭気、酸味も製品に与え、乳酸菌も製品に酸味を与える。

ラギの品質は、たとえ同じ製造者が作ったとしても、ラギに加える植物に由来する微生物もまちまちであり、したがって発酵製品の品質もつねに一定ではない。

ブルムワイン醸造用ラギの改良は、特に必要性が考えられている。すなわち、ブルムワインは、インドネシア政府が国産をもてなすための歓迎酒として、またバリ島への旅行者にふるまわれる酒やヒンドゥー神へのそなえものとして使われるからである。さらに将来は、19世紀のバタビヤアラックの様に重要な輸出品となることも考えられる。

マーケットで売られているブルムワインは、アルコール濃度 2.8 ~ 10.0 %、酸度 (酢酸として) 0.067 ~ 0.218 %、還元糖量 17.3 ~ 26.8 %、エキス分 24.67 ~ 39.42 % とは

ば広く、それらの品質は一定でない。

室温におけるラギの貯蔵寿命は2~3カ月位で、この時期を過ぎると高い品質の製品は望めない。これは、古いラギでは主要な微生物が死滅したり、菌叢のバランスが崩れるからだと考えられる。

1. 試料用ラギ採取及びブルムワイン発酵

ラギ及びブルムワイン共、昔ながらの方法で作られており、大工場レベルとはいってもそれは生産量の違いだけといってよい。

ラギは米粉を主原料として直径3cm程の生地を作り、これに品質のよいラギをスターターとしてふりかけ、新しいラギが作られる。この時、雑菌の繁殖を抑えたり、ラギ中の主要菌の増殖を促す目的でニンニク、コショウ、ニッケなど種々の植物粉末、あるいは抽出液が生地製作時に加えられている。その後、天日乾燥を行ない製品とする。この様にして作られたラギをスターターとして用い、黒米からブルムワインが作られる。

2. ラギからの有用アルコール酵母及び糖化菌の分離・同定

主として中、西部ジャワ、及び東ジャワ、マドゥーラ、バリ、スラベン、スマトラの各島から52種のラギを集め、ブルムワイン発酵用の米糖化液を作製し、糖濃度などから13種を有用ラギとして選出した。それらを糖化菌の分離、同定用試料とした。90株のカビを13種のラギから分離、この内から最も甘味な糖液を作る4株(W2、AV2、AU3、CB3)を選出、改良ラギ作製時の糖化菌とした。中でもAU3、CB2の糖化力、液化力は他よりも勝っていた。またアミラーゼの至適PHは4.0~4.5であった。同定の結果は原膜胞子が多いこと、アボフィズ、仮根の存在などの形態観および分離源がラギであることから4株とも Amylomyces rouxii と同定した。

主要アルコール酵母は、52種のラギ中より強い発酵力を示した7種を選択し、それらの中から88株の酵母を分離した。その内からアルコール発酵力の強い13株を選出し、さらに香气およびアルコール生産力の点で4株(K3、N3、Y5、RM1)にしぼった。同定の結果これら4株は、形態、生理学的に Saccharomyces cerevisiae であった。

3. ラギ菌叢に与える各種植物の影響

伝統的な方法でラギを作製する時、種々の植物粉末、抽出エキスを雑菌の汚染防止およびラギ中の主要菌の増殖を促す目的で加える。ラギより分離し、Saccharomyces cerevisiae Y5、Amylomyces rouxii W2、Saccharomycopsis fibuligera H13を用いてその効果を調べた。12.5%のレッドチリ(Capsium frutescens Linn)を米粉に混合して菌数を計ったところ、増殖を促進する効果が認められた。

18.75%ガーリック(Allium sativum Linn)は、Saccharomyces cerevisiae、Saccharomycopsis fibuligeraの増殖を抑えたが、Amylomyces rouxiiには影響を与えなかった。ラオス根(Alpinia galanga Sw)は37.5%の添加で供試した3

菌株の生育を抑えた。室温 10 週間放置後の結果は、レッドチリを含むラギについては高い菌数を認めたが、ガーリック、ラオス添加ラギでは菌数の低下が認められた。

4. ラギ保存温度の菌叢におよぼす影響

Amylomyces rouxii 3 株 (W2、AV2、AU3) を用いて、ラギを製造し、室温 (24 ~ 28 °C)、冷蔵庫 (4 °C)、冷凍庫 (-14 ~ -11 °C) で 8 ヶ月半保存して、菌数を測定した。その結果、室温保存ではすべてのカビについて減少が認められたが、冷蔵保存では、菌数減少は認められなかった。冷蔵保存で最も影響の少なかった菌株は AU3、AV2 は影響を受け易かった。また糯米を用いてラギを作用させた結果、冷凍保存しても、ラギの糖化力が強く残っていることを確認した。

5. ラギの改良およびブルムワイン醸造

市販のラギより分離した *Amylomyces rouxii* CB3、AU3、*Saccharomyces cerevisiae* RM1、K3 を用いて改良ラギ CB3-RM1、AU3-K3 の組合せを作った。ソロ市より入手した市販ラギをも使用し、ブルムワインを作製、改良ラギとの比較を行なった。市販ラギより作製したブルムワインのアルコール濃度は 8.70%、還元糖量 8.02% に対し、改良ラギ CB3-RM1、AU3-K3 で作製したブルムワインは、アルコール 10.83%、10.67%、還元糖量 7.43%、5.17% であった。

CB3-RM1 ラギで作製したブルムワインは、市販のラギで作製したブルムワインに比べてブドウ香を持っていた。AU3-K3 ラギで作製したものはフーゼル臭が強く、アルコール飲料としての価値は低かった。

伝統的手法で作られるラギであると、添加植物由来の微生物によって、ラギ菌叢をコントロールすることができなかったが、この改良ラギを使用することで、品質の一定したブルムワインを得ることが可能となった。さらに、ラギを冷蔵もしくは冷凍保存することで微生物活性を落とすことなく保てることを知った。また、純粋に培養した微生物を使用してラギを作製するため、不必要な菌を除外することが出来、常にブルムワインの品質を一定にすることが可能になった。

ラギの品質を向上することで、その生産物であるところの発酵食品の品質が向上出来、他のラギを使用する製品の品質向上へも応用可能と考える。

昭和58年10月29日

Jenny D. Saono 殿

東京農業大学大学院農学研究科委員会
委員長 杉村敬一郎

学位記授与について（通知）

先般本学院に提出された貴殿の学位論文は審査の結果、学位授与が決定しました。

つきましては、下記により学位記授与を行ないますので御出席下さい。

記

日 時 昭和58年11月29日 午後4時20分から

場 所 図書館第一会議室（1階） 大学院農学研究科委員会

なお、「学位規則」文部省令第29号第9条及び第11条の規定により、博士論文の印刷公表（予定）の内容を文部大臣宛報告しなければなりませんので当日別紙を御提出願います。

（付 記） 学位規則（文部省令第29号）

第9条 博士の学位を授与された者は、当該学位を授与された日から一年以内に、その論文を印刷公表するものとする。ただし、当該学位を授与される前に既に印刷公表したときは、この限りでない。

2 前項の規定にかかわらず、博士の学位を授与された者は、やむを得ない事由がある場合には、当該大学の承認を受けて、当該論文の全文に代えてその内容を要約したものを印刷公表することができる。この場合、当該大学は、その論文の全文を求めに応じて閲覧に供するものとする。

第11条 大学は、博士の学位を授与したときは、当該学位を授与した日から三月以内に、別記様式による学位授与報告書を文部大臣に提出するものとする。

Memperbaiki Brem Bali agar Bisa Bersaing

Jakarta, Kompas

Ny. Jenny Dewipadma Saono dari IPB berhasil meraih gelar Doktor dari Universitas Pertanian Tokyo, Jepang setelah berhasil mempertahankan disertasinya *Ragi for Brem Wine Fermentation and its improvement* (Ragi untuk peragian anggur serta perbaikannya).

Diploma gelar itu disampaikan kepadanya Kamis kemarin oleh Dutabesar Jepang, Toshio Yamazaki di gedung kedutaan dalam suatu upacara sederhana. Dalam kesempatan itu pula anggur beras hasil risetnya, disuguhkan kepada para tamu.

Gelar itu sendiri sebenarnya sudah dikukuhkan bulan November 1983 lalu, tapi baru disampaikan Kamis kemarin. Ny. Jenny Dewipadma Saono yang meraih gelar doktor dalam waktu hanya sembilan bulan ini, merupakan wanita Indonesia pertama yang memperoleh gelar itu dari Universitas Pertanian Tokyo. Ia juga merupakan orang kedua yang memperoleh gelar doktor dari hasil kerjasama RI-Jepang yang disponsori oleh *Japan International Corporation Agency* (JICA).

Dalam disertasi 104 halaman Dr Jenny menerangkan antara lain, ragi biasanya dipakai di Indonesia sebagai suatu pengantar untuk peragian pada anggur dan kue kering, badek dan arak. Akan tetapi ragi mempunyai kualitas yang berbeda-beda menurut kumpulannya, bahkan juga dalam produk yang sama pembuatnya. Hal ini disebabkan oleh karena banyaknya jumlah mikrobik yang terdapat pada bahan-bahan untuk membuat ragi tidak dapat dikontrol.

Berdasarkan pendapat tersebut, Ny. Jenny Dewipadma Saono mengadakan riset tentang cara perbaikan pengolahan ragi untuk anggur dengan memakai kulkas dan pesawat pembeku dengan suhu tertentu selama 8 - 12 bulan.



Dr Jenny Dewipadma Saono ds

Metode baru ini memungkinkan dapat dihilangkannya mikroflora yang tidak seharusnya terdapat pada ragi, dan dengan demikian anggur yang lebih baik kualitasnya dapat diproduksi.

Menaikkan pendapatan

"Tentu pertanyaan akan diajukan, kenapa anggur brem yang diutamakan," kata Dr Jenny Dewipadma Saono (44 tahun) dalam kata sambutannya. Diterangkan, karena yakin dengan adanya perbaikan dalam proses fermentasi, produksi brem Bali bisa menjadi terkenal secara internasional seperti halnya arak Batavia pada abad 19 ketika menguasai pasaran Eropa.

"Dampak dari usaha ini akan membuka lapangan kerja dan menaikkan pendapatan rakyat yang mengolah produksi ini secara tradisional," ia menerangkan. Dan bukan itu saja, dari sini diharapkan dapat pula nantinya menambah devisa.

Menjawab pertanyaan Kompas, dijelaskan, arak Batavia yang sangat dikenal di Eropa pada abad 19 itu, pada awal abad 20 menghilang dari pasaran Eropa karena tidak dapat dipelihara mutunya.

Akhir-akhir ini brem Bali dicoba diperkenalkan tapi banyak ditolak di Eropa, terutama di Jerman dan Jepang. Itu pun tidak lain karena mutu tidak dapat dijaga sehingga menurun kualitasnya yang meng-

akibatkan rasanya menjadi terlalu asam, seolah-olah basi. Dengan riset yang dilakukannya itu di Jepang, proses yang menurunkan kualitas itu dapat diatasi, sehingga ia berharap dalam waktu mendatang, brem Bali bisa bersaing di pasaran dunia.

Dr Jenny yang sudah 20 tahun menjadi dosen di IPB dan kini berpangkat IV C sebagai Pembina Utama Muda ditempat almamaternya, dipilihnya Jepang untuk tempat riset tidak lain karena keahlian dibidang fermentasi seperti sake, kecap yang terbaik terdapat di Jepang.

Pembimbing Dr Jenny dalam mempertahankan disertasinya adalah Prof Michio Kozaki.

Dikirim ke Jepang

Wakil JICA di Jakarta, H. Yamamura menerangkan dalam jumpa pers, sejak JICA aktif di Indonesia tahun 1974 hingga sekarang sudah 4.707 ahli Indonesia dikirim ke Jepang oleh badan ini. Untuk tahun 1984 menurut rencana JICA sebanyak 310 tenaga ahli Indonesia akan dikirim.

Sementara biaya yang telah dikeluarkan oleh pihak JICA dalam programnya untuk Indonesia, pada tahun 1982-83 sebesar 37,2 juta dollar AS, atau 12,2% dari anggaran internasional JICA bagi 120 negara. (ds)

Kompas

Jakarta 13 1984



WINE SIPPING, — The Japanese Ambassador to Indonesia, Mr. Toshio Yamasaki (right) tries a cup of wine processed by Ir. Jenny Dewipadma Saono (left) after she reported to the Ambassador about her achievement in Japan on Thursday. Ir Jenny was the Indonesian woman to obtain the doctorate degree at the Agriculture University of Tokyo with her paper "Research on yeast for wine fermentation and improvement." (Antara)

First Indonesian woman to receive doctorate degree in Japan

Ir. Jenny Dewipadma Saono, 44, who last Thursday received her Doctor's diploma from Japanese Ambassador to Indonesia Toshio Yamazaki, is the first Indonesian woman who has obtained a Doctor's degree from Japan.

The presentation took place at the Japanese embassy and was only attended by several members of the embassy staff and lecturers of the Bogor Institute of Agriculture.

Ir. Jenny Dewipadma, a graduate of the Bogor Institute of Agriculture in 1964, obtained her Doctor's degree in Agriculture after defending her thesis in English, titled "Research on yeast for fermen-

tation of wine and its improvement", which she started to compile in Indonesia in 1978 and completed at Tokyo Agricultural Universities from December 1982 until November 29, 1983.

Queried by newsmen, Dr. Jenny Dewipadma said that she had chosen this title for her thesis to improve the quality of yeast production in Indonesia, so that yeast eventually could become an export commodity.

In the 19-th century "Arak Berawi", arack from Old Batavia, was very popular in Europe through the Dutch, but its quality declined in the 20-th century so that many countries in Europe and Japan refused to

import due the arack's low quality.

Through her research work on yeast, made from rice or cassava, Dr. Jenny Dewipadma had succeeded to improve the fermentation process and consequently the quality of her wine.

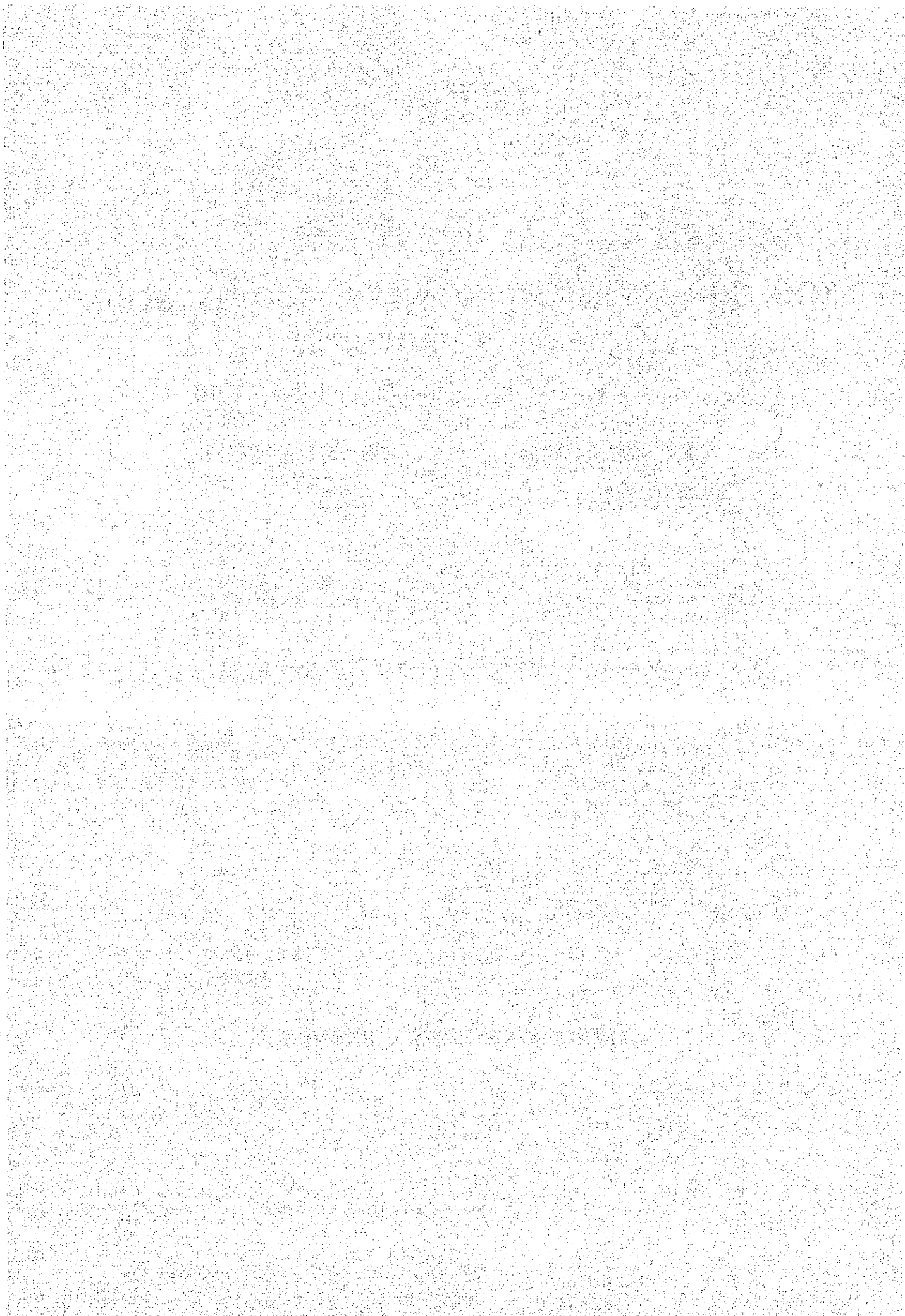
All the expenses of her research work were borne by the Japanese International Cooperation Agency (JICA), including Rp 2 million for the preparation of her thesis.

Dr. Ir. Jenny Dewipadma was born at Cilacap, Central Java, and is the eleventh Indonesian who received a Japanese Doctor's degree.

(Ant)

**THE STUDIES ON BREM WINE FERMENTATION
AND ITS IMPROVEMENT**

JENNY DEWIPADMA SAONO



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Introduction

Fermented foods originated from Central Asia. They gradually were spread to other countries and developed into indigenous fermented foods. The peoples of Southeast Asia have a variety of fermented foods, such as in Indonesia there are tempe, oncom, kecap, tauco, tape, brem dry cake, brem wine, badek, tuak, arak, cuka, sayur asin, etc. By this fermentation process, original food materials are transformed into various products which provide more variegated preference and better nutritional potentiality for the local people. On the other hand, the traditional fermentation technology provokes some problems to be solved by further investigations on fermentation processes, quality control aspects and development of improved starters. For example, the use of a more productive strains in the form of ragi available for the local people and well-designed equipments for processing may be effective for the improvements of the traditional methods of fermentation.

Among these fermentation products, brem wine has received an appreciable attention. In Bali island, brem wine in red color is used to symbolize a Hindu God, Brahma, on religious events. Raffles (1830) described in his book "The history of Java" that brem wine had been important and highly esteemed among the Javanese. President Soeharto, Indonesia, made this wine a welcome drink for state guests at his Merdeka Palace since 1979. And it may become a potential export commodity like arak in the nineteenth century.

The quality of brem wine is not always consistent, it depends mainly on the ragi used. Since the activity of the ragi is very variable with different microorganisms present, the quality of the product at this time is not fully under control. The new technique for ragi preparation could be developed with special reference to the more productive strains. Ragi for this purpose may be improved to give more liquid syrup and higher alcohol content. The main problems to be solved with brem wine are its high remaining sugar content, low alcohol yield and too sour taste.

Ragi is an Indonesian traditional starter for the fermentation of starch-rich substrates, like cassava (Manihot utilissima POHL), glutinous rice (Oryza sativa glutinosa LINN), ordinary rice (Oryza sativa LINN) and sometimes corn (Zea mais LINN), into fermentation products of tape, brem wine, brem dry cake, badek and arak.

Tape is usually made from steamed cassava or glutinous rice, and to some extent it is made from corn and ordinary rice. These food materials are fermented by ragi at the concentration of 0.5-1.0% for 2 to 3 days, yielding a soft, sweet, slightly sour, aromatic product called tape.

Brem wine, a sweet, slightly sour, red colored beverage with 3 to 10% of alcohol, is produced by ragi fermentation of black glutinous rice. Nowadays it is derived from the white variety sticky rice also. This wine is produced and consumed mainly in Bali island and therefore known as "brem Bali".

Badek is a sweet somewhat sour syrup produced by similar fermentation of black or white glutinous rice or ordinary rice, and is consumed as soft drinks, especially in Central and East Java areas.

Brem dry cake, usually called "brem", is made by ragi fermentation of white glutinous rice, from which syrup is derived, stirred to foam and sun dried into thin round cakes (Figure 1).

Arak, often called arak beras, is a colorless distilled liquor made by Chinese in Indonesia since generations by ragi fermentation of ordinary rice into rice wine, and the rice wine produced is then distilled into arak with 50-55% of alcohol content. Palm sugar or molasse may be added during the fermentation process (Figure 2). Local arak is made from distillation of palm (Borassus flabelifer) or coconut (Cocos nucifera) wine.

Ragi is principally used for the fermentation of indigenous foods mentioned above. However, in rural areas ragi is also important for local medicine. It is used in cough medicine, in herb mixture to fight worms, and in local abortiva. Asharie (1894) observed several traditional abortiva popularly used in the villages in Indonesia, in some areas the use of ragi mixed with young pineapple-fruit and black sugarcane, and also brem wine were considered very effective for this purpose.

Ragi is made from rice flour mixed with aromatic plant parts such as garlic, galanga rhizome, white pepper, red chillies, cinnamon, etc. The kind and number of aromatic plant and spices used differed from maker to maker. Teysman (1875) noted 17 herbs or spices being used for making ragi, whereas Heyne (1950) mentioned 14 kinds and Eijkman (1894) observed 13 kinds being used. All of these authors and also Vorderman (1893) were of the opinion

that ragi originated from the Chinese. Calmette (1893) described "chinesische Hefe" and its wine, and noticed 46 kind of herbs that could be used for making this ragi-like product, which number could be reduced to 10 to 12. He thought that these herbs had the purpose of inducing the wine aroma and taste.

Went and Geerligs (1894) suggested that ragi could be made from rice flour, sugar cane and rice straw only, since the important microorganisms for saccharification and alcohol production were present in these materials.

Ragi is white to creamy white in color depending on the spices being used. It may come out in different shapes and sizes. The shape may be round ball, flat ball or conical. Usually the diameter is from 1.5 to 5 cm and the thickness 0.5 to 1 cm (Figure 3).

Similar starter as ragi are also available in other Asian countries. In Thailand it is known as "look-paeng", in the Philippines - "budod", in India - "bakhar" and "ranu", in Nepal - "murcha", in Bhuta - "champha", "chang-phap", in Korea - "nurook" and in China it is called in different dialects such as "chiu-yaw", "chu", "chiu-piang" and "pekhak".

The Indonesian word ragi means yeast. This is similar to the word "gist" in Dutch, "Hefe" in German and "levure" in French indicating foam or scum. The Greek word for yeast is "zestos" which means boiling. However, in Indonesia, the white cake starter containing yeasts is called ragi too.

Ragi, when used as a single word, indicates the creamy white colored cake starter for making tape, brem, etc. But, nowadays the term ragi is also used to indicate starter for other fermentations such as tempe, kecap, tauco, etc. called ragi-tempe, ragi-kecap, ragi-tauco, etc. In certain places ragi-tape is used to substitute ragi, and others used the term ragi-beras (Ko 1965) which means ragi made of rice.

As mentioned above, ragi in Indonesia is considered to be originated from China. It may have come with the Chinese settlers arriving in Indonesia in the ninth century (Raffles 1930). These people took along their food habits of consumption and making rice wine using ragi-like starter.

Prinsen-Geerligs (1894) who studied the Javanese ragi noted that it was very similar to the "levure Chinoise" of Calmette (1893) in Chocin-China, which Wehmer (1900) introduced as "chinesische Hefe", in its preparation and its molds population. Both starter cakes contained mainly molds of the genera Amylomyces and Mucor capable to convert starch to dextrose. Also Chrzaszcz (1901) and Saito (1904) described the molds they isolated from the Chinese starter cake.

Vorderman (1893) was the first to suggest the study of the microflora of ragi since he was interested in the quality of arak produced in Batavia (now Jakarta) area. At that time Batavia arak was outstanding in Europe and capable to compete in the European market (Raffles 1830). However, Eijkman (1894^a; 1894^b) was the first to observe ragi mold capable in converting rice starch into sugar in the arak fermentation process and named it Amylomyces rouxii. In the previous year, Calmette (1893) isolated A. rouxii from chinesische Hefe, which according to Eijkman should be called Mucor amylomyces rouxii, since he proved it to be a Mucor.

Went and Geerligs (1894; 1895) were the pioneers who studied ragi microorganisms. They isolated Clamydomucor oryzae, Rhizopus oryzae, Saccharomyces vordermanii and Monilia javanica from arak fermentation by ragi. Prinsen-Geerligs (1905) concluded that C. oryzae and S. vordermanii were the microorganisms responsible for making good arak, whereas M. javanica resulted in a poor quality arak. They further considered that Calmette's Amylomyces rouxii was very similar to their Clamydomucor oryzae but differed in the physiological properties, and therefore suggested the name Clamydomucor rouxii for Calmette's mold. They also noticed that their C. oryzae and R. oryzae had the same physiological properties.

Later, Wehmer (1900; 1901; 1904) restudied C. oryzae and R. oryzae of Went and Geerligs and determined that C. oryzae was a sporeless variety of R. oryzae capable to form clamydospores only. These clamydospores still survived after 5 years, considered very important for ragi activity. They also isolated Mucor javanicus and M. dubius, both are strong alcohol formers from sugar.

Half a century thereafter Boedijn (1958) isolated R. clamydumucor and concluded that this species was synonymous with A. rouxii Eijkman and C. oryzae Went et Geerligs. He suggested that it should be named Rhizopus clamydosporus because

of the presence of fascicles of abortive sporangiophores clearly indicating that it is a Rhizopus.

More recently, Ellis et al. (1976) proposed a more correct name for the predominant molds in ragi and Chinese yeast belonging to the Mucorales that propagated primarily by clamydospores, should be named Amylomyces rouxii Calmette. These molds include A. rouxii Calmette 1892, C. oryzae Went et Geerligs 1896, C. rouxii (Calmette) Went et Geerligs 1896, C. rouxianus (Calmette) Wehmer 1907, C. javanicus Yamasaki 1919, and Rhizopus clamydosporus Boedijn 1958.

The arak yeasts S. vordermanii Went et Geerligs is now included to Saccharomyces cerevisiae; and Monilia javanica Went et Geerligs had been renamed to Candida javanica (Heyne 1950) and now included to Hansenula anomala (Hesseltine 1965; Lodder 1970).

In 1909 De Kruffyff discovered two important yeasts contributing to the good aroma in the Batavia arak, those microorganisms were Dematium arakii, which had been described by Vorderman (1893) and observed by Eijkman (1894), originated from molasse, and Torula indicum which came from ragi. D. arakii was later named Schizosaccharomyces asporus (Heyne 1950), and now belongs to Saccharomyces pombe (Lodder 1970), and T. indicum was later called Willia indica (Heyne 1950).

After 1970, many scientists from USA, Japan and Indonesia were getting more and more interested in ragi and tape, and further research had been carried out since then. Enzymological studies had been also made and become increasingly active.

Dwidjosepoetro (1970^a; 1970^b) isolated and identified two new species of yeast from ragi, Candida lactosa and Hansenula malanga. Ko in 1972 studied Endomycopsis fibulgera and Clamydomucor oryzae isolated from ragi and concluded that if combined they can make good tape. In 1974, Saono et al. collected ragi samples from West Java and studied their microflora. Figure 4 shows the microorganisms isolated and identified.

Soedarsono (1972) studied the effect of spices garlic, ginger, galanga, cardamom, clove, pepper on the microorganisms isolated from ragi. He concluded that garlic inhibited the Rhizopus oryzae, Aspergillus niger and Bacillus subtilis, whereas ginger inhibited the amyolytic molds Mucor sp. and Clamydomucor sp., but A.

niger, S. cerevisiae and B. subtilis were not affected.

Kato et al. (1976) isolated a yeast from ragi, that produces high glucoamylase and identified it as Endomycopsis fibulgera. They also succeeded in the purification of glucoamylase into crystalline form.

Hadisepoetro et al. (1979) isolated 13 strains of molds, 9 strains of yeasts and 17 species of acid-producing bacteria from ragi samples made in Central Java. Among the 13 strains of mold, 11 strains were Zygorhynchus moellerie Vuillein, since the strains formed zygosporangia of which the spores were heterogenous as they paired with big and small ones, and produced dark brown zygosporangia 24 to 66 micrometer in diameter; and the 2 other strains were Rhizopus cohnii and R. oryzae. Among the 9 strains of yeasts isolated, 8 were Candida sp. and one strain was Torula sp.; and all the acid-producing bacteria were Pediococcus pentosaceus.

Toyota and Kozaki (1978) also studied the bacteria found in ragi and reported that in new ragi both P. pentosaceus and Bacillus sp were present, but in old ragi Bacillus disappeared.

Short description of the nature of ragi and its microorganisms has been compiled by several authors, such as Hesseltine (1965), Frazier and Westhoff (1978), Beauchat (1978), Van Veen (1972) and Ko (1982).

The scientists that paid great attention to and studied ragi molds extensively were Boedijn (1958) and Hesseltine (1976; 1981). Whereas Cronk (1975) and his coworkers (1977; 1979) investigated the biochemical aspect of tape fermentation.

Similar product as ragi, tape and rice wine in other countries has been described, such as bubod and binobudan (Del Rosario 1980) and tapuy (Kozaki 1976) from the Philippines; look-paeng and kao-mak (Dhamcharee 1981) from Thailand; and chiupiang and lo-chiu (Vorderman 1893) or chiu-yaw, chiu-niang and mi-chiu from China.

However, on brem wine little attention has been paid. The only research published was on its aroma components by Soedarmo (1970).

Brem wine is very important in Bali island where Hindu is the religious belief of the inhabitants. The Balinese considered blood offering of great importance in their religious rites and has been practicing it since early times. They believe that blood is the most important sacrifice they can offer to their Gods in order to live a peaceful life. In ancient time human blood were used, however, time has changed and they used the blood of household animals instead. In the later period a sweet and red brem wine has been used to substitute blood. Further, they also believe that in order to be in good health one is required to offer 5 liquids different in color symbolizing the 5 liquids in the human body. These are: (1) brem wine, red, as the symbol of red blood, (2) milk, white, the symbol of white blood, (3) honey, black, the symbol of gal juice, (4) arak, yellow, the symbol of enzyme juice, and (5) water, colorless, the symbol of urine (Tabuh rah 1976).

In a survey by Rachtamianto (1980) on brem wine marketed in Jakarta, the alcohol content varied from 2.8 to 10.0% and the reducing sugar content from 17.3 to 26.3%. Presence of artificial coloring was also indicated in some of the samples. Soeratman (1979) tried to improve brem wine in reducing the high acidity by developing a cabinet fermentor.

The quality of brem wine can not be consistent due to several factors:

- (1) The ragi for brem wine preparation is not consistent in its fermentation ability.
- (2) The microorganisms involved or their importance in this fermentation process are not known.
- (3) The microorganisms present in ragi is dependent upon the raw materials for ragi preparation.
- (4) The traditional making of brem wine is simple.

This means, extensive studies on the method of making ragi and brem wine are necessary, so that improvements of ragi and brem wine making could be carried out.

In these studies the following objectives were conducted:

- (1) Survey of the traditional ragi preparation method at home scale and factory scale.
- (2) Survey of the traditional brem wine making at home scale and factory scale.
- (3) Collection of ragi samples and determinations of fermentation activity.
- (4) Isolation and selection of the productive microorganisms.
- (5) Identification of the selected microorganisms.
- (6) Design of a method for the improvement in ragi preparation.
- (7) The improvement of brem wine fermentation.

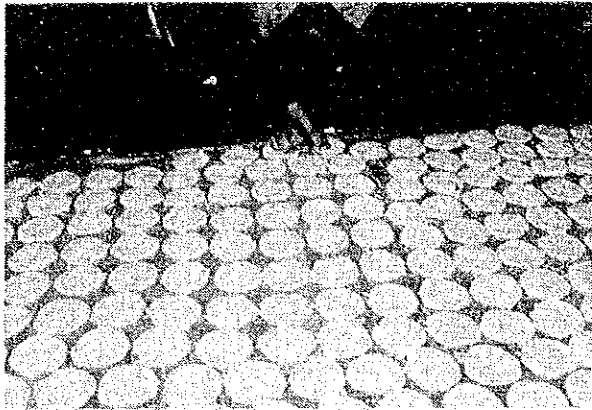


Figure 1.
The molding of brem dry cake by pouring whipped liquor obtained from ragi-fermented glutinous rice.



Figure 2.
Palm sugar is added to the rice saccharified by ragi during arak beras fermentation.

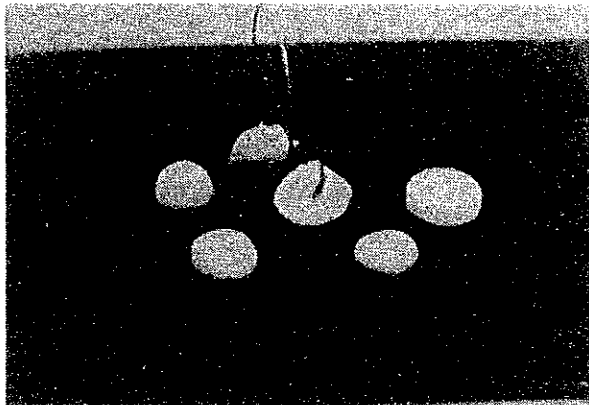
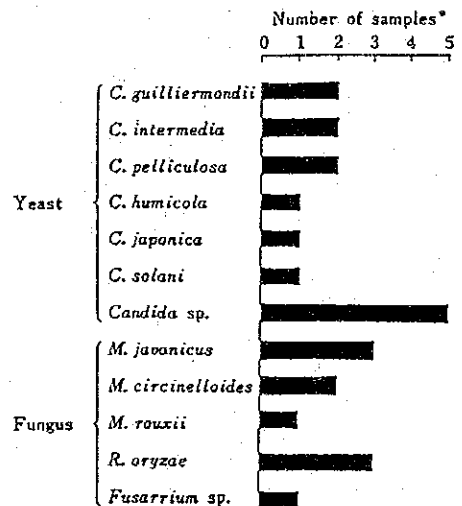


Figure 3.
Different shapes and sizes of ragi.



* The number of "ragi" samples investigated were 14.

Figure 4. Microflora of "ragi"

PART I - TRADITIONAL METHOD OF RAGI AND BREM WINE PREPARATION

1. Ragi preparation

Ragi is prepared in home-scale industries in the villages of West and Central Java with a producing capacity of 1 to 50 kg of ragi per day. Whereas in the city vicinity of Solo (Central Java) there are several larger ragi factories owned by Indonesians of Chinese descendant with a production capacity of 75 to 100 kg of ragi per day. These ragi preparation process still follows the traditional method which has been inherited from generation to generation.

A. The home-scale method

Twenty four home-scale ragi makers, mostly located in West Java and only two in Central Java, were surveyed on their method of ragi preparation. The ingredients were weighed, and expressed as % for the weight of the rice flour used.

The procedure of ragi preparation by the local people is as follows:

- a. One to 50 kg of rice is soaked overnight, drained and pounded.
- b. Laos rhizome and garlic are peeled and sliced, then these spices are pounded together with red chilies and rice flour in the ricepounder (Figure 5) and sieved.
- c. White pepper, black pepper, cinnamon and other spices are finely ground and added to the rice mixture.
- d. Cooled boiled-water is added in a quantity just enough to form balls.
- e. Molding into ragi balls is done by hands (Figure 6).
- f. The formed balls are inoculated with ragi powder (Figure 7).
- g. The powdered balls are arranged on a layer of rice straw on bamboo trays (Figure 8). Only two ragi makers, AE and AP, used clean cloth instead of the rice straw; this considered to avoid black mold growth.
- h. Then the ragi balls are kept or incubated at room temperature (25-30°C) for 2 days. Temperature in Cipanas, where ragi AA and AB are made, may reach as low as 15°C, whereas in Pekalongan, where ragi AX is made, may go up to 32°C. Burning cane sugar is usually used to smoke the AA

and AB ragi before incubation (Figure 9). This smoking process is believed to result in better ragi. Perhaps due to the better growth of the molds at an elevated temperature.

- i. Finally, the ragi is dried in the sun for 2-3 days. During this drying period usually black spores develop (Figure 10), which is esthetically undesirable and therefore such ragi is brushed off the black spots.
- j. The finished ragi may be packed in plastic bags.

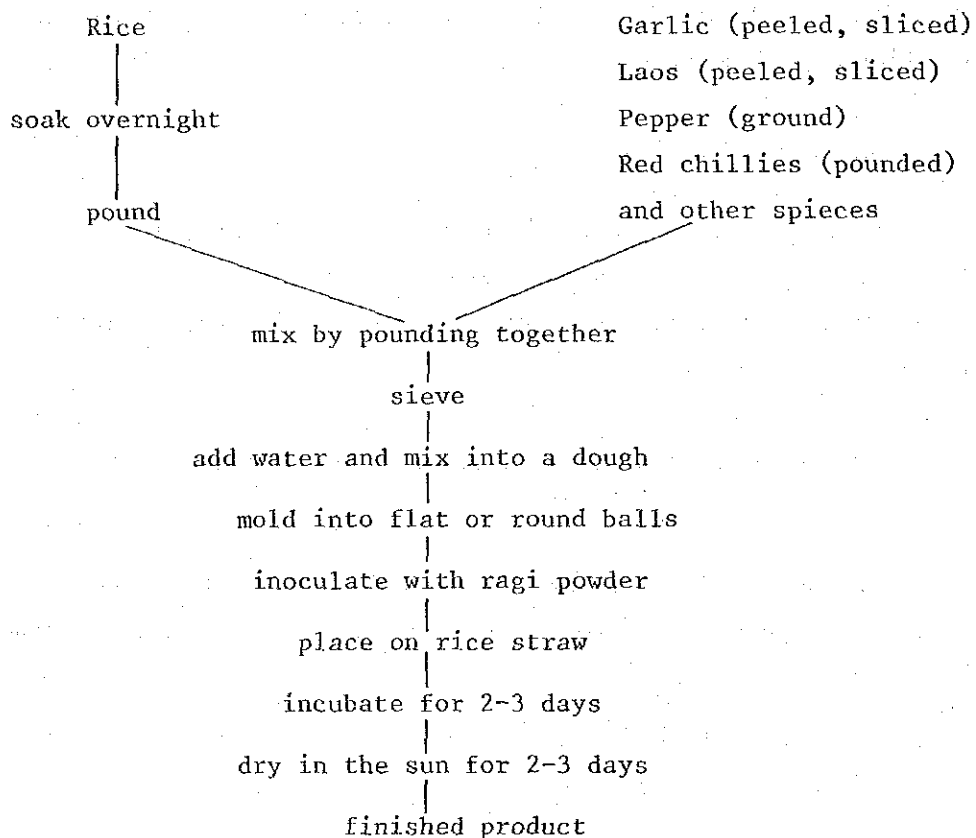


Figure 11. Scheme of ragi preparation.

The recipe for 24 ragi made by village people and the resume of the ingredients and their percentage are presented in Tables 1 and 2. These ragi were mainly composed of rice flour, garlic, laos, white pepper and red chillies. And some may add other spices as desired. According to Calmette (1893) the purpose of these aromatic plant parts in the Chinese-yeast was to give good aroma to the rice wine. It shows further, that only few makers put cinnamon, black pepper and adas. Sugar cane and coconut juice are

Table 1. Receipt of 24 raji made by local people in home-scale industries, in grams.

| | Rice | Laos | Garlic | White Pepper | Red Chillies | Cinna- mon | Black Pepper | Adas | Sugar Cane | Lemon | Coconut juice |
|----|--------|-------|--------|--------------|--------------|---------------|--------------|------|------------|-------|---------------|
| AA | 8,000 | 1,000 | 500 | 500 | 250 | - | - | - | - | - | - |
| AB | 8,000 | 1,000 | 500 | 500 | 500 | - | - | - | - | - | - |
| AC | 1,000 | 60 | 40 | 5 | - | - | - | - | - | - | - |
| AD | 4,000 | 1,000 | 250 | 100 | 10 | - | - | - | - | - | - |
| AE | 15,000 | 1,000 | 500 | 50 | - | 50 | - | - | 150 | - | - |
| AF | 800 | 50 | 20 | 10 | 25 | - | - | - | - | - | - |
| AG | 2,400 | 150 | 30 | 5 | 20 | - | - | - | - | - | - |
| AH | 50,000 | 6,000 | 3,000 | 25 | - | 25 | - | - | - | - | - |
| AI | 800 | 75 | 45 | - | - | - | - | - | 150 | 20 | - |
| AJ | 800 | 400 | 100 | - | 50 | - | - | - | - | - | - |
| AK | 8,000 | 1,000 | 300 | 50 | - | - | - | - | - | - | - |
| AL | 8,000 | 1,500 | 500 | 100 | 50 | - | - | - | - | - | - |
| AM | 400 | 100 | 75 | 5 | - | - | - | - | - | - | - |
| AN | 400 | - | 50 | 7.5 | - | - | - | - | - | - | - |
| AO | 800 | 250 | 100 | 10 | 50 | - | - | - | - | - | - |
| AP | 100 | - | - | - | - | - | - | - | - | - | 50 |
| AQ | 800 | 50 | 5 | 4 | 10 | - | - | - | - | - | - |
| AR | 500 | - | 5 | 15 | - | 17.5 | 12.5 | 15 | - | - | - |
| AT | 5,000 | - | 125 | 125 | - | - | 125 | 125 | - | - | - |
| AU | 2,000 | 50 | 75 | 5 | - | 1.0 | - | - | - | - | - |
| AV | 8,000 | 500 | 300 | 25 | - | - | - | - | - | - | - |
| AW | 400 | 200 | 75 | - | 50 | - | - | - | - | - | - |
| AX | 20,000 | 1,000 | 1,000 | 5 | - | - | - | - | - | - | - |
| AY | 8,000 | 200 | 400 | 50 | 50 | 50 | - | - | - | - | - |

Table 2. The percentage of ingredients in 24 raji samples.

| Ingredients: | No. of raji. | % (compared to rice) |
|---|--------------|----------------------|
| Rice <u>Oryza sativa</u> Linn | 24 | 100.0 |
| Garlic <u>Allium sativum</u> Linn | 23 | 0.5 - 18.75 |
| Laos root <u>Alpinia galanga</u> Sw | 20 | 2.5 - 50.0 |
| White pepper <u>Piper nigrum</u> Linn | 20 | 0.05 - 6.25 |
| Red chillies <u>Capsicum frutescens</u> Linn | 11 | 0.25 - 6.25 |
| Cinnamon <u>Cinnamomum burmani</u> BL | 5 | 0.05 - 3.5 |
| Black pepper <u>Piper retrofractum</u> Vahl | 3 | 0.3 - 2.5 |
| Adas seed <u>Foeniculum vulgare</u> Mill | 2 | 2.5 - 3.0 |
| Sugar cane <u>Saccharum officinarum</u> Linn | 2 | 1.0 - 12.5 |
| Lemon juice <u>Citrus auranticum auranti- folia</u> var <u>fusca</u> Linn | 1 | 2.5 |
| Coconut juice <u>Cocos nucifera</u> Linn | 1 | 50.0 |

seldom used, whereas, these ingredients may support the non-amylolytic yeasts at the early stage of incubation, e.g. the growth of the fermentative Saccharomyces cerevisiae necessary for brem wine fermentation. Lemon juice is not important to most makers, even though it may suppress the development of bacteria and favor the mold growth. The incubation and drying of the ragi are done mostly on a layer of rice straw. To get ragi to produce good sweetness, the rice straw should be at least 2 months old. This is probably due to the certain molds present or survived on stored dry straw such as the amylolytic molds. Went and Geerligs (1894) suggested that one could make ragi only from rice flour, water and rice straw, since the amylolytic molds were present in rice straw and rice.

However, the author thinks that the spices or aromatic plant parts may be important in controlling certain microflora which are desired or undesired for tape fermentation. Garlic was reported to inhibit the Rhizopus oryzae, Aspergillus niger and Bacillus subtilis, whereas ginger inhibited the amylolytic molds Mucor and Clamydomucor but did not affect Saccharomyces cerevisiae (Soedarsono 1972). As seen in Table 2, laos and garlic are used in quite large amount; on the other hand, the other spices are added in very small quantities.

One ragi, AP, is quite unique and consisted only of rice and coconut juice, without any spices added. This ragi is used for making a bread-like cake called "kue bika ambon". In this type of ragi the fermentative yeasts are expected to be dominating.

B. Analysis of the local ragi

Materials and Method

Ragi samples were collected from the ragi makers for further investigations. Analysis of ragi was done for: (1) fermentative ability, by inoculating 0.1 g of ragi into 8 ml of yeast extract malt extract broth containing 3% of glucose, where the production of high quantity of gas within 24 hours at 25 - 28°C indicated a high fermentative ability; (2) content of amylolytic microorganisms, by the plating method on starch agar medium of the finely ground ragi and incubated at 25-28°C for 1-2 days; (3) counts of lactic-acid bacteria, by the plating method on glucose yeast extract peptone agar containing 1% of CaCO₃ and with a pH of 6.5, incubated at 37°C for 3 days;

(4) usefulness for tape making (sensory test of the tape made), by inoculating a 250 g of steamed cassava tuber with 1% of ragi powder and incubated at 25-28°C for 3 days in plastic bag. Scoring was done for sweetness in taste, softness in texture, and pleasantness in aroma. Score ranges from 1 to 5.

Results and Discussion

The results are indicated in Table 3 and the resume in Table 4. They show that only 10 ragi produced good sweet fermentation product of tape, 6 ragi resulted in soft textured tape, which means that the liquifaction activity was high, 10 ragi gave nice aromatic tape. And, in the whole, only 10 ragi could make good quality tape. The fermentative test showed that 3 were highly fermentative and 7 non-fermentative at all. The fermentative ability of ragi was related with the alcohol production in the tape. And, the non-fermentative ragi usually produces a sweet product (also in Table 5).

The microbiological analysis showed that all the ragi had amylolytic molds mostly around 10^4 to 10^5 , most of them had very high numbers of amylolytic filamentous yeasts, few had amylolytic bacteria which seemed to be Bacillus sp. originating from the rice flour, and only 4 ragi had considerable counts of lactic-acid bacteria.

The amylolytic molds are responsible for the conversion of starch to sugar, the amylolytic yeasts and bacteria affected the flavor (unpublished experiment by the author), and the lactics could cause the sour taste in the fermentation product if allowed to develop.

From Table 1 and 3 it seems that the ragi producing excellent tape, AM, AU, AV and AX, consisted mainly of rice flour, laos (2.5-25%), garlic (2.5-18%) and white pepper (0.03-1.2%). AV ragi had an additional cinnamon of 0.05%. However, it is difficult to conclude that these ingredients made good ragi, since AC and AK having similar ingredients of laos (6-12.5%), garlic (3.7-4%) and white pepper (0.5-0.6%) could not produce good tape. The kind and the quantity of ingredients used to prepare the ragi do not necessarily determine the quality of the ragi. The environmental are most determinative, such as the water content of the ragi during incubation, the quality of the straw, the quality of the rice, the temperature of incubation, the cleanliness during preparation, and the kind and number of

Table 3. Fermentative test, number of microflora, and fermentation activity on cassava substrate of 24 ragi samples.

| Ragi | Fermentative test | Number of Molds | Amylolytic Yeasts | Number of Bacteria Total | Bacteria Lactics | Cassava sweet-ness | Fermentation soft-ness | Aroma |
|------|-------------------|-----------------|-------------------|--------------------------|------------------|--------------------|------------------------|-------|
| AA | - | 10^6 | 10^8 | L * | L | 3 | 3 | 3 |
| AB | + | 10^5 | 10^8 | L | L | 3 | 2 | 3 |
| AC | ++ | 10^5 | L | L | L | 4 | 2 | 4 |
| AD | + | 10^5 | 10^7 | L | L | 4 | 3 | 2 |
| AE | ++ | 10^4 | 10^7 | 10^4 | L | 2 | 3 | 2 |
| AF | +++ | 10^6 | 10^7 | 10^5 | L | 1 | 2 | 3 |
| AG | ++ | 10^5 | 10^7 | 10^4 | L | 4 | 3 | 3 |
| AH | - | 10^4 | 10^5 | 10^4 | L | 3 | 3 | 2 |
| AI | ++ | 10^4 | 10^7 | L | L | 3 | 2 | 4 |
| AJ | - | 10^4 | 10^5 | 10^4 | 10^3 | 2 | 2 | 1 |
| AK | + | 10^5 | 10^7 | 10^5 | L | 2 | 1 | 4 |
| AL | +++ | 10^6 | 10^7 | L | L | 1 | 1 | 4 |
| AM | - | 10^5 | 10^7 | 10^5 | L | 5 | 5 | 4 |
| AN | +++ | 10^4 | 10^8 | L | L | 4 | 5 | 4 |
| AO | ++ | 10^6 | 10^8 | 10^3 | L | 4 | 4 | 3 |
| AP | ++ | 10^3 | 10^6 | 10^4 | L | 1 | 1 | 1 |
| AQ | ++ | 10^7 | 10^7 | 10^4 | L | 2 | 3 | 2 |
| AR | - | 10^4 | 10^6 | 10^5 | 10^3 | 1 | 3 | 1 |
| AT | ++ | 10^6 | 10^7 | 10^3 | 10^3 | 2 | 1 | 3 |
| AU | ++ | 10^5 | 10^5 | 10^4 | L | 5 | 5 | 4 |
| AV | - | 10^5 | 10^7 | 10^3 | L | 5 | 5 | 4 |
| AW | - | 10^5 | 10^5 | 10^4 | 10^3 | 3 | 2 | 3 |
| AX | ++ | 10^4 | L | 10^4 | L | 5 | 5 | 4 |
| AY | ++ | 10^6 | 10^7 | 10^5 | L | 4 | 3 | 5 |

* L : Low, less than 10^2

Table 4. Analysis of fermentation activity and numbers of microorganisms in 24 ragi.

| <u>Fermentative test:</u> | <u>Number of ragi</u> | <u>Number of:</u> | <u>Number of ragi</u> |
|---------------------------|-----------------------|-----------------------------|-----------------------|
| non-fermentative | 7 | <u>Amylolytic mold</u> | |
| low | 3 | low $10^2 - 10^3$ | 1 |
| moderate | 11 | moderate $10^4 - 10^5$ | 16 |
| high | | high $10^6 - 10^7$ | 7 |
| | | <u>Amylolytic yeast</u> | |
| <u>Cassava-tape:</u> | | low $10^2 - 10^3$ | 2 |
| Taste/sweet | | moderate $10^4 - 10^5$ | 4 |
| good (score 4-5) | 10 | high $10^6 - 10^8$ | 18 |
| moderate (3) | 5 | <u>Total bacteria</u> | |
| poor (1-2) | 9 | non or less than 10^4 | 10 |
| Softness of texture | | moderate $10^4 - 10^5$ | 14 |
| soft (score 4-5) | 6 | <u>Lactic-acid bacteria</u> | |
| moderate (3) | 8 | non or less than 10^3 | 20 |
| hard (1-2) | 10 | low $10^3 - 10^4$ | 4 |
| Odor/aroma | | | |
| good (score 4-5) | 10 | | |
| moderate (3) | 7 | | |
| poor (1-2) | 7 | | |

microflora present in the ingredients which are able to grow in the rice containing spices. The concentration of spices and aromatic plants used may affect the survival and dominance of certain microorganisms growing in the ragi. For example, AA and AB are produced by one maker but AB has twice chillie content if compared to AA. Similarly AK and AL are made by one maker and in this case AL contains chillie whereas AK has none. From the Tables 1 and 3 it can be observed that the presence of higher concentration of red chillies increases the fermentative ability of the ragi.

C. The Factory-Scale Method

Two biggest ragi factories, factory A and factory B, were selected among the six ragi factories in Solo. These two brands of ragi are the most popular and most widely distributed throughout Indonesia. In Bali, where brem wine is made and consumed daily, the factory B ragi (symbolized as "B") is used in much higher quantity than the ragi from factory A (symbolized as "A"). The "A" ragi could not make good brem wine and could be used only for making tape or brem dry cake. Therefore, in Java, where tape and brem cake are made daily, the "A" ragi is much used. Factory A has a producing capacity of 3,000 kg of ragi per month and factory B 1,875 kg.

The procedure of making ragi in large factories is as follows:

- a. Seventy-five to 100 kg of rice of the non-sticky variety is ground in motor-driven mill. In factory B the rice is soaked overnight to make pounding easier, since in this factory a rice pounder is used.
- b. Garlic is ground in a meat grinder (Figure 12).
- c. Spices are ground in a grinder and added to the rice-garlic mixture. The spices used are white pepper, cinnamon, adas, jinten and black pepper. In "B" ragi ginger is added.
- d. Cooled boiled-water is added in a quantity just sufficient to obtain a consistency for forming rather-dry ragi balls.
- e. The molding into ragi discs is done by syringelike metal device. It is conducted manually by young girls (Figure 13).
- f. Then the ragi is inoculated by finely ground ragi and pressed afterwards (Figure 14).
- g. The ragi is arranged on rice straw and incubated at room temperature (28-32°C) for 2-3 days.

h. Finally, the ragi is sun-dried for 2-3 days (Figure 15).

i. The finished ragi is packed in plastic bags (Figure 16).

It is interesting to note, that all of the brem dry cake makers in Central Java prefer to use the "A" ragi. The "B" ragi is not successful for making brem cakes. On the contrary, the brem wine makers in Bali can not use the "A" ragi, and instead, they would use the "B" ragi.

During a survey on popular ragi marketed in Java, Madura and Bali, it was indicated that the most demanded ragi were the "A" and "B" ragi. The "A" ragi was most popular in Java and Madura, whereas "B" ragi was more used in Bali. The results of the analysis of the ragi samples are given below.

Table 5. Analysis of "A" and "B" ragi

| Ragi | Place of collection | Fermen- tative test | Number of microorganisms | | | Taste of cassa- va tape of two- days fermenta- tion |
|------|---------------------|---------------------------|--------------------------|--------------------------|----------------------------|--|
| | | | Lactics | Amyloly- tic molds | Filamen- tous yeasts | |
| "A" | Bali | - | 10^3 | 10^4 | ND | very sweet |
| "A" | Madiun | - | 6.1×10^4 | 10^5 | ND | very sweet |
| "A" | Surabaya | - | 8.5×10^5 | 10^4 | ND | sweet |
| "A" | Magelang | - | 1.1×10^5 | 10^5 | ND | sweet |
| "B" | Bali | +++ | 1.2×10^5 | 10^4 | ND | alcoholic |
| "B" | Semarang | ++++ | 4.0×10^4 | 10^5 | ND | alcoholic |
| "B" | Solo | +++ | 4.9×10^5 | 10^6 | 4.8×10^9 | alcoholic |
| "B" | Solo | ++++ | 3.3×10^4 | 10^5 | 1.2×10^9 | alcoholic |

All "A" ragi samples collected from different places had no gas formation in the YM broth-3% glucose, whereas all "B" ragi produced enormous gas. These indicated that the "A" ragi did not have the alcohol producing ability and the "B" ragi had the alcohol fermentation activity. The cassava tape fermentation also showed that "A" ragi produced a sweet tape with no alcohol, whereas "B" ragi produced an alcoholic one. That is the main difference between these two ragi.

In a two-days fermentation of tape, the high number of lactics in the ragi did not cause sourness. At the early stage the amylolytic molds were the only important ones. The filamentous yeasts, present in very high number in the "B" ragi may later affect the brem wine fermentaion. Cronk (1975) observed that at the early stage of tape fermentation, the yeasts had no chance to develop yet.



Figure 5.
Rice pounding and spice grinding
during ragi preparation.



Figure 6.
Ragi molding is done by hands.



Figure 7.
Inoculation of ragi by powdering
them with ragi powder of previous
batch.



Figure 8.
Arranging inoculated ragi on a layer
of rice straw.



Figure 9.
Smoling of ragi by burning cane sugar.

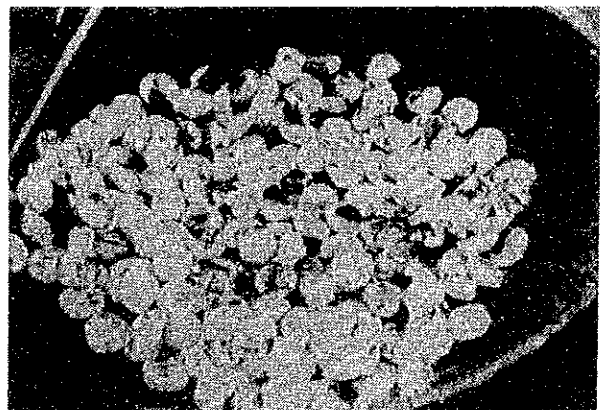


Figure 10.
Black spores development during the drying
process of ragi.



Figure 12.
Grinding of garlic during ragi preparation
in NKL ragi factory.



Figure 13.
Molding of ragi balls by metal tube
and inoculating them by ragi seed
powder layer on a metal tray.



Figure 14.
Pressing of ragi by foot-stamping

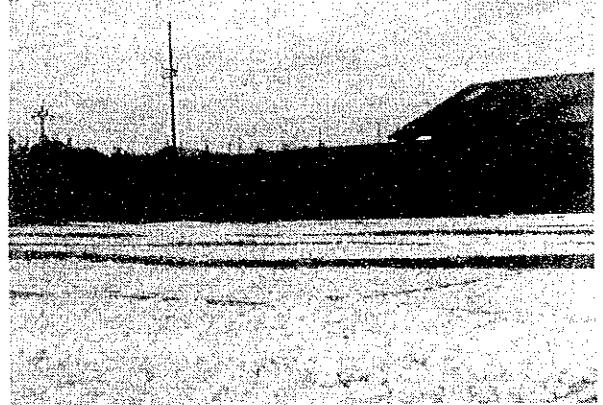


Figure 15.
Sun drying of ragi at a ragi factory.



Figure 16.
Popular commercial-ragi in Central
Java.



Figure 17.
Brem wine fermentation in a Balinese
home.

2. Brem wine preparation

In Bali brem wine is much used for offerings and daily consumption. The brem wine was made traditionally by village people in the homes. Nowadays, this beverage is scarcely made in the homes, since factory-made brem wine is available on the market. Only few villagers still make their own brem wine.

There are several brem wine factories in Bali, among them two are the biggest ones: (1) A company located in Singaraja with a producing capacity of 20,000 liters of brem wine per month, and (2) B company located in Sanur, Denoasar, with a producing capacity of 1,500 liters of brem wine per month.

A. The home-made brem wine

Brem wine makers located in the village of Sebelanga, near Denpasar, were visited and observed on their brem wine preparation.

The procedure of brem wine making in the homes is as follows:

- a. Two liters of black glutinous rice is steamed for one hour until half cooked, then the hot steaming water is poured onto the rice at a concentration of approximately 2 liters of hot water and held so for about half of an hour. During this time the water is absorbed by the rice.
- b. The rice is further steamed until soft.
- c. The cooked rice is spread on bamboo basket to cool down.
- d. Two ragi balls, each ragi weighs 3.1-3.2 g, are ground and inoculated onto the cooled rice.
- e. The inoculated rice is covered with waru (Hibiscus tiliaceus L) leaves and kept in the kitchen for incubation.
- f. The liquor produced will drip and is received in an earthen bown (Figure 17).
- g. After 5-6 days the liquor is harvested and put in a bottle to continue alcohol fermentaion. This process is usually completed in 4-6 days. The average yield of brem wine is around 1.5 liters per 2 liters of rice.

B. The factory-made brem wine

B company was visited and observed on its method of brem wine preparation. The brem wine from this company is most wellknown and is made fully in this factory. The brem wine from A company on the other hand, is made partly by people and the factory collects the saccharified liquor from those people to further ferment and fortify into brem wine, without any aging treatment. The A company brem wine is sold on the market at a lower price, Rp 250/200 cc, than the B company brem wine, Rp 400/200 cc.

The procedure of brem wine preparation at B company is as follows:

- a. Firstly, 50 liters of black or white glutinous rice is cooked in a steamer containing 50 liter of steaming water for one hour.
- b. Then, the colander containing the half cooked rice is dumped into the hot steaming water in which 0.1% of NaHSO_3 has been added; at this time the rice is stirred (Figure 18).
- c. The steaming process of the rice is continued for about 2 hours until the rice turns soft.
- d. The cooked rice is transfered into bamboo baskets having a capacity for about two andhalf hours. The temperature reaches 30.5°C .
- e. Afterwards, the "B" ragi is ground and inoculated onto the rice at the proportion of 4 ragi to 3 kg of rice (1 liter of rice is 750-800g). (Figure 20)
- f. The inoculated rice is then transfered into an aluminium steamer (Figure 21) and kept for 4-5 days at the ambient temeperature ($28-32^\circ\text{C}$).
- g. During the 5 days of incubation period brem liquor drips through the holes of the steamer and is collected in the pot. The yield of the liquor is usually around 50% of the weight of the raw rice; this liquor is called the first quality young brem.
- h. The remaining fermented mass is wrapped in cloth and pressed in a screwtype presser (Figure 22), so that more liquor is squeezed out. The liquor obtained here is approximately 75% of the weight of the original raw rice, and is called the second quality young brem.
- i. These liquor are put in large bottles (Figure 23) to continue the alcohol fermentation and cell settling processes.

- j. Then, for aging process, the brem is transferred into fiber-glass tanks with a capacity of 1250 liter (Figure 24) and kept in a storage room with an uncontrolled temperature of around 32°C for 6 to 7 months. During this stage, arak or distilled rice wine is added at a concentration of 0.2%. The arak is purchased from arak distillers in Bali.
- k. Finally, bottling of the wine into crown-capped bottles is done (Figure 25).

The flow sheet of the brem wine making is given in Figure 26.

The temperature of the rooms in the factory is not controlled and it usually reaches as high as 32°C. This high temperature is especially obvious in the aging room. At such a high temperature, unwanted enzymatic reactions may be accelerated. For example, the discoloration of the red pigments in brem wine into brownish color. This is especially important since this brem wine does not receive any pasteurization treatment before the aging process. The aging temperature for sake and most wines in other countries are at a much lower temperature, near 15°C.

It was only recently that B company has fortified its brem wine with arak, which seems to improve its quality. By adding arak, the alcohol content of the brem wine increases and so inhibiting the undesirable microbial growth and reaction.

The whole process of brem wine fermentation is principally dependent upon the ragi used. From a poor quality ragi no good brem wine could be expected. And, the inconsistency of ragi quality is acknowledged by the maker of the outstanding "B" ragi. It is difficult to get a consistent quality of ragi if the quality of the raw materials was uncontrollable. The ragi maker considers that the ragi quality depends mainly on the quality of the rice used for ragi preparation.



Figure 18.
Cooking and stirring of glutinous rice at a brem wine factory.



Figure 19.
Cooling of the cooked rice in bamboo basket before inoculation with ragi powder.

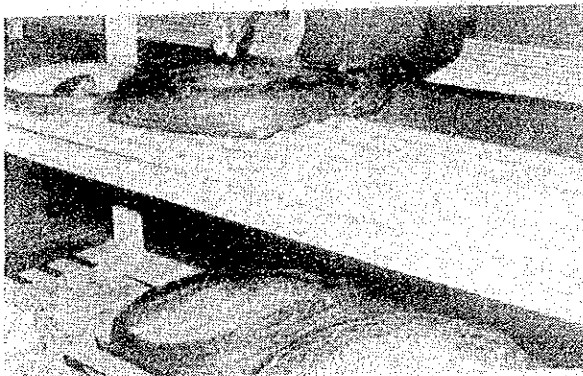


Figure 20.
The inoculation of glutinous rice with ragi powder.



Figure 21.
The saccharification of rice in aluminium steamer.

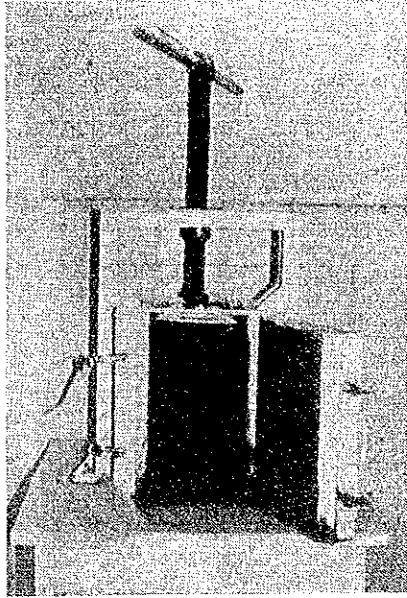


Figure 22.
Screw presser for squeezing out
brem liquor from fermented rice.

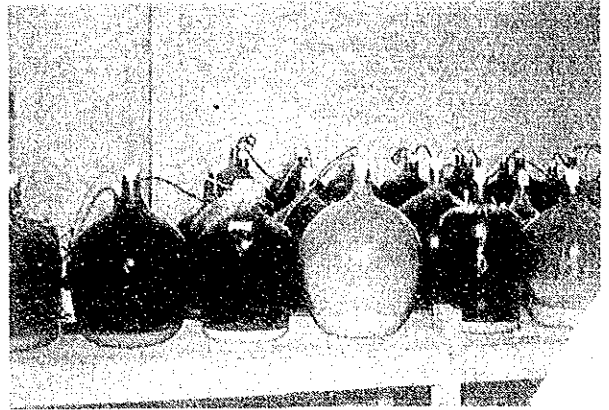


Figure 23.
Alcohol fermentation of brem wine.

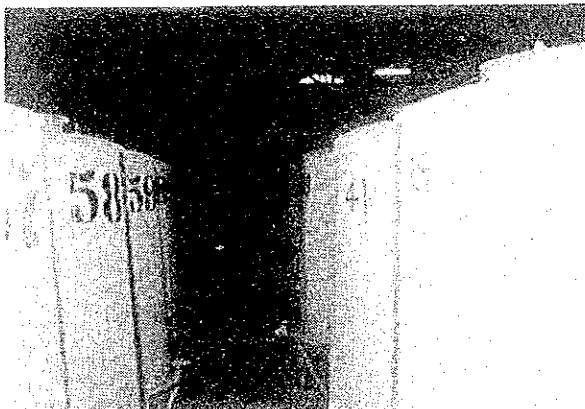


Figure 24.
Aging of brem wine in fiber-glass tanks.

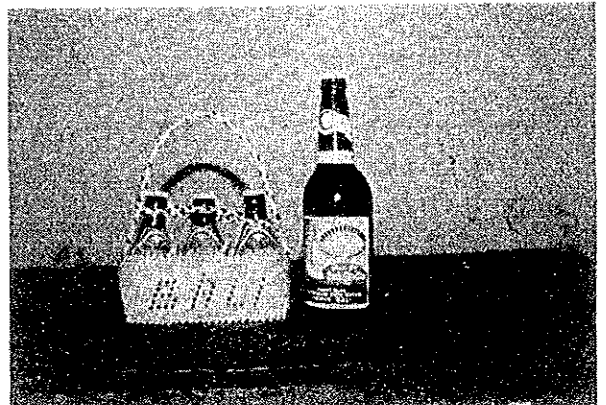


Figure 25.
Brem wine products in crown-capped
bottles of 200 cc and 650 cc capacities.

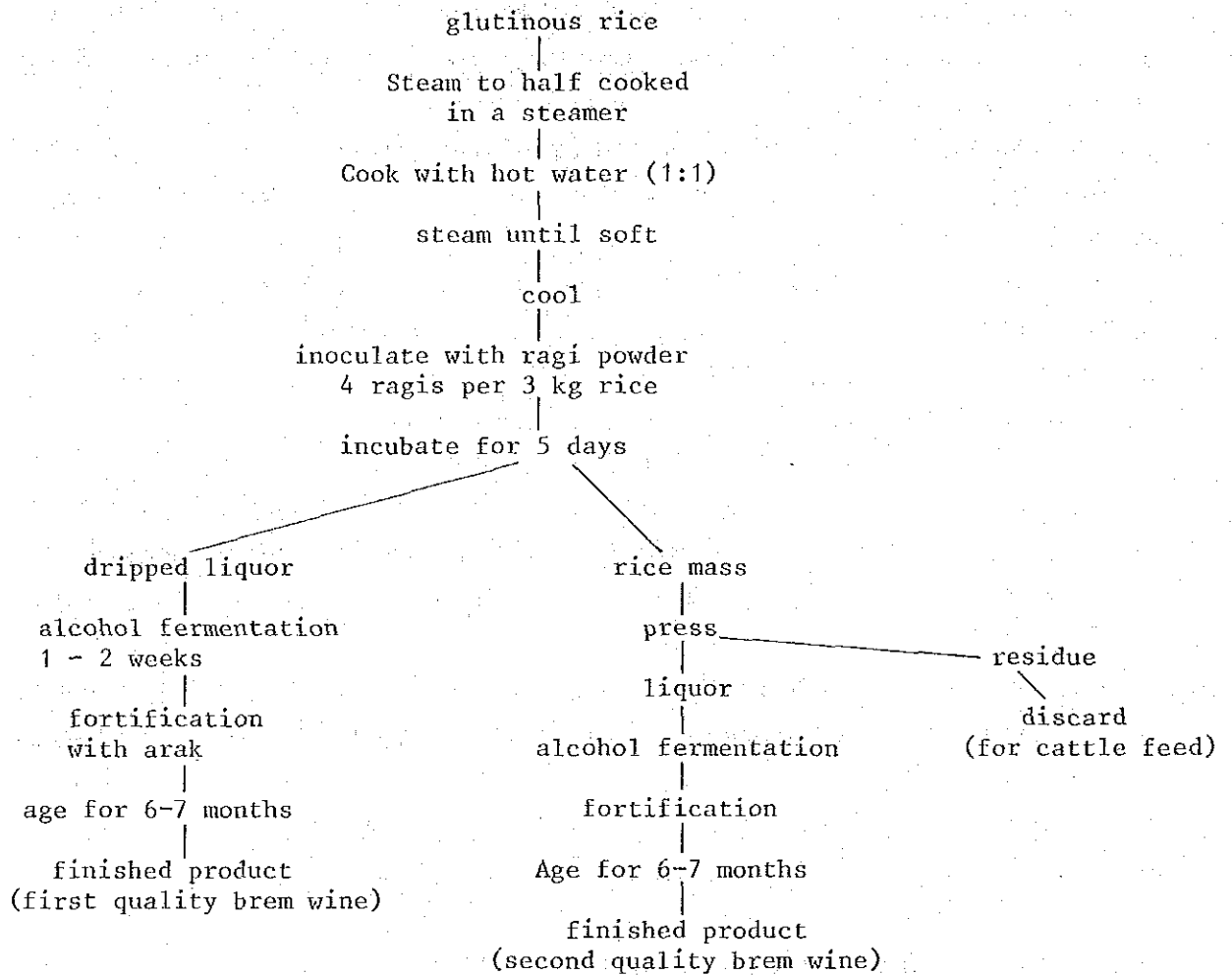


Figure 26. Scheme of brem wine making