

Tapé Fermentation

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Microorganisms isolated from ragi, originally obtained from Indonesia, were selected for their ability to convert steamed glutinous rice into tapé, an Indonesian fermented food. A mixture of *Chlamydomucor oryzae* and *Endomycopsis fibuliger* had good fermentation characteristics. Prepared starters, produced by growing pure cultures on rice and drying them, were as active as pure cultures grown for 4 days on Difco mycological agar slants at 30 C.

Tapé is a popular Indonesian delicacy with a sweet-acid taste and mild alcoholic flavor. It is prepared by fermenting glutinous rice (*Oryza sativa glutinosa*) or cassave tuber (*Manihot utilisima*). To distinguish one from the other, the fermented glutinous rice is named "tapé ketan" and the fermented cassave tuber "tapé ketella" (Indonesian), "tapé tëlò" (Javanese), or "peujeum" (Sundanese). Both are produced in Indonesia on a home industry scale by traditional manufacturers or at home for family consumption. Traditional manufacturing methods were described by Vorderman (4), Went and Prinsen Geerligs (5) and Donath (2).

In the traditional method, the fermentation is initiated by addition of powdered "ragi." Ragi cakes are made of rice flour containing the required mold and yeast cells obtained by natural infection from the surroundings and equipment used by the traditional manufacturer.

In this work some selected microorganisms isolated from ragi were tested in pure culture fermentations for their ability to convert steamed glutinous rice into tapé. The changes in reducing power and pH during fermentation were recorded. The report of these preliminary results may stimulate future studies designed to develop a modernized industrial process.

MATERIALS AND METHODS

Isolation and identification of microorganisms from ragi. As a first step, eight mold and three yeast cultures were isolated from a ragi sample originally from Bandung, Indonesia. The mold isolates were first tested for their ability to carry out the tapé fermentation, which includes the transformation of steamed glutinous rice into a soft and juicy product on which mold growth is almost unnoticeable. Only two mold isolates, designated S₁₁ and S₁₄, met these requirements, and they were identified as *Chlamydomucor oryzae*. The other mold isolates were five *Mucor* species and one *Rhizopus* species,

which have not been identified further for the time being. Presumably they are not important in this fermentation, but it is not impossible that they play a secondary role. They may be considered in later studies.

Two of the yeast cultures were identified as *Pichia burtonii* (*Endomycopsis chodati*) and designated G₁₁ and G₁₂; the third one, G₁₃, was identified as *Endomycopsis fibuliger*. No attempt was made to isolate bacteria from ragi; their role in the fermentation could be considered later. Identification of the cultures was done by the kind cooperation of the U.S. Department of Agriculture, Agricultural Research Service, Northern Utilization Research and Development Division, Peoria, Ill., which designated the culture S₁₁ as NRRL A-17,199, S₁₄ as NRRL A-17,200, G₁₁ as NRRL Y-7143, G₁₂ as NRRL Y-7144, and G₁₃ as NRRL Y-7145.

Ability of cultures to convert rice into tapé. Each of the two selected mold cultures and the three yeast isolates, as well as mixtures of one mold and one yeast culture, was tested for ability to convert steamed glutinous rice into good tapé.

(i) **Preparation of glutinous rice.** Each 100-g portion of glutinous rice (Bras Ketan, Conimex N.V.) was soaked in 150 ml of distilled water overnight. It was then steamed in a rice steamer for 15 min. After the rice cake was broken up with a spoon and wetted with 25 ml of distilled water, it was steamed for another 15 min. The sticky mass was transferred aseptically with a spoon into a sterile beaker covered with the bottom of a petri dish. After cooling, it was inoculated.

(ii) **Preparation of cultures and inoculation.** The mold cultures S₁₁ and S₁₄ and the yeast cultures G₁₁, G₁₂, and G₁₃ were grown on slants of Difco mycological agar at 30 C for 4 days. A suspension of the growing microorganisms was made by adding 5 ml of sterile water into each slant. The mycelium and the yeast cells were scraped off the agar by means of an inoculating wire. They were transferred into a sterile tube, homogenized by a Whirlimixer (Fisons Ltd., London), and used to inoculate the steamed glutinous rice.

When a single culture was tested, a suspension prepared from one slant was used to inoculate 100 g

of steamed glutinous rice.

When mixed cultures were tested, the suspension from one slant of S_{11} or S_{14} was mixed with 1 ml of suspension of G_{11} , G_{12} , or G_{13} and then used for the inoculation of 100 g of steamed glutinous rice.

Inoculation was accomplished by adding the mold or mixed suspension little by little to the rice mass in a beaker while aseptically mixing as well as possible with a flamed spoon. The inoculated steamed rice was then incubated at 30 C.

Prepared inoculum. When larger quantities of inoculum are needed, growing the mold and yeast cultures on agar slants is complicated and insufficient. An attempt was made to prepare larger quantities of inoculum at one time by the following method.

One hundred grams of rice and 40 ml of distilled water in a 400-ml beaker covered by a petri dish were sterilized for 20 min at 120 C. The grains which were then packed together were broken up aseptically with a flamed spoon. Then the rice was inoculated with one of the mold or yeast isolates, which had been grown for 4 days at 30 C on a slant of mycological agar (Difco) and suspended in 5 ml of sterile water.

The inoculated rice was incubated for 4 days at 30 C and broken up every day to promote uniform growth. It was then dried by replacing the petri dish cover with absorbent paper (Kleenex) and placing it in an aerated incubator at 37 C for 5 days. To make it easy to use, the dried material was pulverized in a coffee grinder and kept in a glass-stoppered bottle in a refrigerator.

When needed for a fermentation, one or more grams of the powder, containing either the mold or yeast culture, or a mixture of the powders, was suspended in 5 ml of sterile water. The suspension was then mixed with 100 g of steamed glutinous rice, which was prepared as described above. A similar method could be applied for the preparation of larger quantities of inocula.

Progress of fermentation. The progress of the fermentation was determined by the degree of decomposition of the rice starch, as measured by its reducing power, according to a modification of Bernfeld's method (1) for the determination of amylase activity. Glutinous rice was prepared and inoculated according to the methods described above. At intervals, 10-g portions of fermenting rice were drawn aseptically from the fermentation beaker. They were diluted with 90 ml of distilled water, homogenized for 5 min, and centrifuged for 30 min at $1,500 \times g$. The supernatant solution was decanted. At the beginning of the fermentation the solution was usually not yet clear, and it was centrifuged again for 30 min. The supernatant solution was then filtered through filter paper. The filtrate was assayed for its reducing power by modification of Bernfeld's method for the determination of amylase activity.

To 1 ml of filtrate was added 1 ml of dinitrosalicylic acid reagent. The color due to the reducing sugars was developed by heating in a boiling water bath for 5 min and rapid cooling to room temperature. The absorbance of the liquid or its dilution was

determined at 540 nm against the blank in a Hitachi-Perkin-Elmer spectrophotometer.

A calibration curve established with maltose (0.2 to 2 mg of maltose in 2 ml of water) was used to convert the colorimeter readings into micromoles of maltose per gram of fermented product. By this method the progress of the fermentations was recorded, with the following inocula used per 100 g of steamed glutinous rice: (I) one slant of S_{11} ; (II) 1 g of prepared S_{11} ; (III) as II, but 1 g of prepared G_{11} was added; (IV) as II, but 2 g of prepared G_{11} was added; (V) as II, but 5 g of prepared G_{11} was added; (VI) 1 g of prepared inoculum of S_{11} , which had been kept at room temperature (ca. 20 C) for 5 months. In II, III, IV, and V the term "prepared" means prepared as described above and kept in a refrigerator less than 1 month.

Each fermentation with the same composition of inoculum was repeated three to six times, with the exception of fermentation V, which was not repeated. In the fermentations with the same inoculum, the time of sampling was varied and the results were plotted together to make one graph only.

RESULTS AND DISCUSSION

Ability of cultures to produce tapé. Single cultures of G_{11} , G_{12} , or G_{13} showed a white growth on the rice grains, but no other changes of the rice were observed. S_{11} or S_{14} made the rice soft and juicy, and a sweet taste was developed; no alcoholic flavor was observed. No mycelium growth was noticeable.

Mixtures $G_{11} + S_{11}$, $G_{11} + S_{14}$, $G_{12} + S_{11}$, and $G_{12} + S_{14}$ changed the sticky glutinous rice into a soft and juicy mass with a sweet and mild alcoholic flavor; this is considered to be good tapé.

Mixtures $G_{13} + S_{11}$ and $G_{13} + S_{14}$ softened the rice, but not much juice was produced; the product had a sweet-sour taste and sourstinging flavor. It could not be considered as tasty tapé.

These results indicate that the cooperation of *Chlamydomucor oryzae* and *Endomycopsis fibuliger* was necessary to convert steamed glutinous rice into good tapé. Presumably *C. oryzae* initiated the fermentation by conversion of the rice starch into sugars, and *E. fibuliger* continued the process by the production of alcohols and flavor components. The chemistry of the produced compounds as well as the eventual role of *Mucor rouxii*, *M. javanicus*, and *Hansenula anomala*, which are often present in ragi (3), in the tapé fermentation will be considered in future studies.

Progress of fermentation. The graphs in Fig. 1 show the progress of fermentation with less than 1-month-old prepared inoculum of S_{11} only (II) compared with fermentations in which increasing amounts of prepared G_{11}

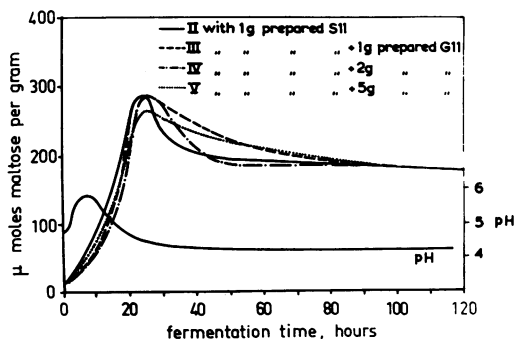


FIG. 1. Progress by fermentation of glutinous rice at 30 C by a prepared inoculum of *Chlamydomucor oryzae* (S_{11}) mixed with various amounts of prepared inoculum of *Endomycopsis fibuliger* (G_{11}).

were added (III, IV, and V). All graphs have a similar shape. After a relatively short induction period in which the mold began to grow, it showed a fast increase of reducing substances during the period between approximately 10 to 30 hr of fermentation. A rather sharp decrease followed during the following 10 to 15 hr, after which it remained approximately constant. Apparently the addition of G_{11} did not affect the amount of reducing substances during the fermentation.

The changes in the pH of all fermentations had the same pattern. In Fig. 1 the pattern is represented by the pH changes in fermentation II. A small increase in the pH was observed in the first 5 hr of fermentation, followed by a decrease; after 30 hr of fermentation the pH reached approximately 4 and remained more or less constant. It is of interest that the time at which a maximum content of reducing substances was observed coincided with the time at which pH 4 was reached. The

pattern of results with fermentations I and VI are not presented because they were approximately the same as those reported above.

All together, it appeared that inocula of *C. oryzae* and *E. fibuliger* grown on rice and dehydrated were as active as cultures grown on Difco mycological agar slants. Furthermore, the prepared inoculum of *C. oryzae* stored at least 5 months at room temperature of approximately 20 C was as active as a newly prepared one. These results indicate that it is possible to produce large quantities of pure culture starter for tapé fermentation on an industrial scale. The culture could easily be packed, stored, and transported. When needed, it could be easily added in the desired quantities.

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