Supplemental Materials
for
Makgeolli: Rapid Production of an Alcoholic Beverage from the Fermentation of Rice
Adam M. Kiefer¹, Caryn S. Seney¹, Alison L. Lambright¹, Kirsten A. Cottrill¹, and Virginia A. Young².*
¹Department of Chemistry, Mercer University, Macon, GA 31207,
²Department of Biology, Mercer University, Macon, GA 31207

Table of Contents
(Total pages 8)
Appendix 1: Makgeolli instructions
Appendix 2: Worksheet A and Worksheet B
Appendix 1: Makgeolli Instructions

Materials:
- Alconox®
- 2-gallon bucket (2 per group)
- 20-cup Rice cooker
- Jasmine rice
- Solo® cups (3 per group)
- Thermometer (1 per group)
- Top-loading balance
- Red Star® Pasteur Champagne Active Dry Wine Yeast (1 g per group)
- BSG™ HandCraft Amylase Enzyme Formula (40 g per group)
- Food grade large balance
- Sugar Cubes (6 per group)
- Paper towels
- Hot spring water dispenser
- Cheesecloth (1 package per group; 4.8 sq yds)
- Ethanol (70%)
- Large metal spoon (1 per group)
- 1-gallon glass jar (1 per group)
- Funnel (1 per group)
- 16 oz Flip top glass bottles (5 per group)
- Distilled water
- Large rubber band (1 per group)

Procedure:
Preparation prior to laboratory time:
A. Wash Rice: (~30 mins) One rice cooker batch (7.5 US cups of dry rice) produces enough rice for approximately 4.5 laboratory groups.
   1) Wash buckets, rice cooker pot, and measuring cup with Alconox®. Cut a piece of cheesecloth to ~2 ft x 2 ft. and line a bucket with the cheesecloth (Figure 1). Add 7.5 US cups of dry, uncooked rice on top of the cheesecloth inside the bucket.
   2) Add tap water on top of the rice until there is 1” of standing water above the rice. With a clean hand, gently agitate the rice. Gather the ends of the cheesecloth and lift the rice using the cheesecloth out of the bucket and place into the other bucket. Discard water. Open the ends of the cheesecloth and repeat the washing process until water remains clear after gentle agitation.

B. Cook Rice: (~50 mins)
   1) Transfer washed rice into the rice cooker pot. Remove cheesecloth and discard. Fill the rice cooker pot to the “10” graduation on the pot with tap water. Cook rice on the “white rice” setting.
Student Procedure- Week 1: This procedure is written to be performed in groups.

C. Yeast and Amylase Preparation: (~90 mins)

1) Fill two Solo® cups to the “5 oz” line (Figure 2a) (~150 mL) with hot water from the water dispenser (spring water). Label one cup as the “Control” and the other as “Yeast and Amylase Mixture” with your group names. Place the thermometer in the control cup. Add one heaping tablespoon of sugar to the “Yeast and Amylase Mixture” cup and swirl until dissolved.

2) Measure 0.8 g of dry yeast on the top loading balance. Set yeast aside.

3) Measure 37 g of amylase on the top loading balance. Set amylase aside.

4) When the control cup’s temperature drops below 40 ºC, add measured yeast and amylase to the “Yeast and Amylase Mixture” cup and swirl. Cover the cups with paper towels and let them sit undisturbed (Figure 2b). Record what time the yeast was added to the cup. Contents of the cup will begin to bubble (Figure 2c).

5) Let the contents sit undisturbed to cool to room temperature for 1 hr.

D. Fermentation Preparation (~30 mins)

1) Any person coming in contact with the materials in this section must rinse their hands with 70% ethanol prior to touching any materials.

2) Label the glass jar with each group member’s name.

3) While waiting for the yeast and amylase mixture, measure the cooked rice. On the large balance, record the mass of the glass jar (no lid). Add 750 g of cooked rice to the jar.

4) Using your Solo® cup to measure, add 60 oz of distilled water into your glass jar with the rice. Lightly cover the jar with the lid while waiting. Ensure rice has adequate time to cool to approximately room temperature.

Safety tip: Work quickly to move to the next step (addition of yeast-amylase mixture) once the rice has cooled to room temperature. The growth of the food-borne pathogen Bacillus cereus can occur when rice sits too long at room temperature.
5) Once the yeast has sat undisturbed for one hour, pour the yeast and amylase mixture into the glass jar with the water and rice. Ensure that the water and rice are not hot when adding the yeast.

6) Fold a piece of cheesecloth (4’ x 2’) so that it has eight layers. Thoroughly soak the cheesecloth in 70% ethanol then air dry. Once mostly dry, cover the glass jar with the cheesecloth (all eight layers) and place a rubber band around the lip of the jar to hold the cheesecloth in place (Figure 3).

7) Transport your jar to the fermenting location (25 ºC). Make sure to keep the jar steady to prevent getting the cheesecloth wet.

E. Fermentation (~10 mins daily)

1) At the same time each day, stir the contents of the glass jar. After rinsing your hands with 70% ethanol, rinse a large, clean metal spoon with 70% ethanol and allow to air dry. Carefully remove the cheesecloth, but do not place it down on the table. Stir the makgeolli mixture for ~30 s then reattach the cheesecloth on top of the jar. Make sure the cheesecloth does not touch any contaminated surfaces. If desired, remove a small aliquot of liquid and use a pH strip to measure the pH before reattaching the cheesecloth.

2) Repeat this process everyday for one week.

Student Procedure- Week 2:

F. Straining (~45 mins)

1) Wash five glass bottles, a bucket, and a funnel with Alconox®, then rinse each with 70% ethanol. Leave ethanol in the containers until they are used.

2) Take the remaining cheesecloth (~4’ x 6’) and soak in 70% ethanol. Do this by folding the cheesecloth into a small rectangle and pouring 200 mL of 70% ethanol on top. Ensure all cheesecloth is soaked. Unfold and allow to air dry without touching any contaminated surfaces.

3) Fold the cheesecloth in half and line the bucket (two layers) (Figure 1).

4) Remove the rubber band and cheesecloth attached to the glass jar. Pour the makgeolli mixture on top of the cheesecloth in the bucket ensuring that the cheesecloth catches all solid material (Figure 4a).

Figure 4. (a) Pour the fermented mixture through cheesecloth into a bucket. (b) Collect the corners of the cheesecloth to remove solid. (c) Squeeze the remaining liquid out of the solid.
5) Lift the edges of the cheesecloth to remove the solid rice mash from the bucket. Using a sanitized hand, squeeze the solid mash to filter any remaining liquid into the bucket (Figure 4b,c).

G. **Bottling (≈45 mins)**
1) Using a new Solo® cup and funnel, fill each glass bottle to the “shoulders” of the bottle (Figure 5a,b). Dip the Solo® cup into the bucket to transfer the liquid into the bottles.
2) As soon as a bottle is filled, loosely cap to prevent any bacteria from entering the bottle (Figure 5c).

H. **Carbonation**
1) Add one tablespoon of sugar using a clean metal spoon (Figure 5d). Add the crushed sugar to one bottle of makgeolli. Tighten the cap.
2) Cover the bottles with a plastic bag and allow the makgeolli to sit at room temperature for 3 to 7 days.

![Figure 5](image-url) (a) Transfer the liquid makgeolli into bottles using a funnel. (b) Fill the bottles to the “shoulder” of the bottle. (c) Loosely cap the bottles. (d) Grind sugar cubes using a spoon.
Appendix 2: Worksheet A and Worksheet B
Worksheet A for Makgeolli Exercise

Names:_______________________________________________________________________________________

No more than three students per group. Any sources, if used, should be cited following the Name-Year system.

1. What is fermentation?
Fermentation is the oxidation of an organic molecule (often glucose) that involves the use of an endogenous electron acceptor to regenerate the oxidized form of the electron carrier NAD+.

2. What type of fermentation is occurring when we make makgeolli?
Ethanol fermentation

3. In the procedure, which enzyme is added to the rice? What does the enzyme do?
The amylase enzyme is added to the rice; it breaks 1,4 glycosidic bonds in starch, releasing maltose, maltotriose, and dextrin, etc. The small sugars can be transported into the yeast cell for use in catabolism.

4. What is the fate of the sugars produced by enzyme action—i.e., how do they get into the cell and how do they feed into a glycolytic pathway? Which metabolic pathway is used to produce ATP for the yeast cell? How does the pathway benefit the yeast cell?
Yeast cell imports maltose via a maltose transporter. Maltose is broken into two glucose molecules by maltase.
The glucose goes through glycolysis to form 2 pyruvates. The pyruvates are decarboxylated to acetaldehyde (the release of CO₂), and the acetaldehyde receives electrons from the electron carrier NADH. Once NADH reduces acetaldehyde to make ethanol (a step catalyzed by alcohol dehydrogenase), NAD+ is now available for use in glycolysis, allowing more glucose molecules to be partially oxidized to pyruvate.
The yeast cell benefits from this process because it gets ATP molecules from the partial oxidation of glucose; additionally, it can continue to extract energy from glucose molecules by regenerating the oxidized form of the electron carrier (NAD+) by undergoing fermentation.

5. Look at the procedures for wine-making and for producing beer in your textbook or from credible online sources; if you use an online source, be sure to cite it. Based on the procedure you followed for making makgeolli, explain why makgeolli is really a rice beer instead of a rice wine.
Beer production involves a malting stage in which the grains (e.g., barley) germinate and the enzymes of the grain are activated so that soluble carbohydrates are released from the starch of the grains. The mashing step then allows for additional enzymatic action to further break down starch into usable carbohydrates. This is analogous to what we did when we added amylase to the rice to allow the starch to be broken down into glucose so that the yeast can take it up and ferment into ethanol. This differs from the wine-making process in that the juice being fermented during wine-making does not have to be broken down into simple sugars first; the grape juice already exists as a simple sugar upon which the yeast cells can act. Therefore, makgeolli is a rice beer because the amylase
transformation of complex carbohydrates in the rice into simple sugars most closely mirrors the malting and mashing steps in beer production (1).
No more than three students per group. Any sources, if used, should be cited following the Name-Year system.

1. Over the week, you monitored the pH of the makgeolli. What happened to the pH over time?
The pH decreased over the week.

2. Based on the reactions occurring inside the yeast cells (as described in #4 on Worksheet A), explain what happened to the pH over the week. (i.e., what product is causing the pH to change?)
The pH decreased over the week period. The CO₂ produced by the ethanol fermentation becomes carbonic acid in aqueous solution, thereby lowering the pH over time.

3. If we had bacterial cells present in the fermentation, we might also see similar trends in pH over time. What lab technique(s) could you perform to assure yourself that the predominant microorganism present in the makgeolli was yeast? Explain what result you would see that would convince you that your primary fermenter was yeast.
Gram stain of a sample taken from the makgeolli; yeast cells should be larger than any bacteria that could be visualized. Yeast often undergo budding, as well, and their shape is more variable than that seen with bacterial cocci. These things—larger size and more variable shape (even sometimes seeing budding)—should provide convincing evidence that only yeast cells are present. Other answers may be acceptable (e.g., genome sequencing and small subunit rRNA sequencing of the cells present have been mentioned by students), but I encourage students to consider simple techniques that we have used in lab during the semester.

4. State four common elements of microbial fermentations. You should consider the following questions: What compound is oxidized? In what ways is fermentation different from aerobic respiration? What acts as the electron acceptor?
NADH is oxidized to NAD⁺.
NADH often donates its electrons to pyruvate or a pyruvate derivate.
Oxygen is not needed.
During fermentation, an electron transport chain is not used to regenerate NAD⁺.
Fermentation is a partial oxidation of the substrate. ATP yields from fermentation are very low, relative to what can be produced during aerobic respiration, and the ATP is generated by substrate-level phosphorylation (1).

References: