
Practical Report

Use of “Enzyme Powder” for Inquiry Activity on Starch Digestion in Elementary School Science**Tomoko KAGA^{1)*}, Nobuyasu KATAYAMA²⁾**¹⁾ *Ritsumeikan University*, ²⁾ *Tokyo Institute of Biology Education, Japan*

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In Japan, elementary school children have been using their own saliva for experiments on starch digestion when they learn about the digestion and absorption of food in science. In carrying out these experiments, the use of their own saliva is particularly important for pupils, because they can notice that their own digestive juices digest food inside their digestive organs. However, various germs may be contained in saliva, so that considerable caution is required to avoid infectious diseases. In addition, pupils dislike providing their saliva for these experiments. To overcome such obstacles, we have introduced a step, the preparation of saliva enzyme powder by the cold ethanol precipitation method, into the experimental procedure. Since the ethanol precipitation technique is too advanced for pupils, this step must be done by the teacher as a demonstration of the method. In our trial of the laboratory class on starch digestion, at first, a solution of the saliva enzyme powder, which the teacher had prepared, and a sheet of starch-containing paper were used to confirm that the saliva enzyme powder could digest starch. Then, pupils were given a question, “Do living things other than human beings also contain digestive aids to digest starch?” To find an answer to the question, pupils examined whether starch digestion would occur by the crude enzyme powders from germinating corn grains and “kome-koji” (malted rice with *Aspergillus* sp.) which were prepared by the same method as the saliva enzyme powder. In their experiment, small polythene bags and plastic straws were used instead of test tubes and syringes, respectively, to reduce the cost of the experiment. More than four fifths of pupils expressed affirmative impressions of the laboratory class.

Keywords: *α-amylase, elementary school science, inquiry activity, saliva powder, starch digestion**** Author for correspondence:** E-mail: aureliakaga1143@gmail.com**INTRODUCTION**

In elementary school science in Japan, “degradation of starch by saliva” has been the most familiar experiment in learning about the digestion and absorption of food. It is particularly important for pupils to carry out this experiment by using their own saliva, because they can notice that their own enzymes digest food inside their digestive organs. In the experiment, one of two methods for collecting saliva, “direct (spit)

method” or “swab absorbing method,” is commonly adopted. In the former method, the donor spits into a vessel. In the latter method, the donor holds a cotton swab in his mouth to absorb saliva. Kroen (1998) has already mentioned that college students rebel against spitting into tubes. Masamoto and Serita (2007) reported university students expressed less feeling of repulsion toward the swab-absorbing method. However, according to our preliminary investigation with a

questionnaire, most elementary school children disliked both methods, because they did not want the other pupils to watch them spitting or to see their saliva. In addition, various germs may be contained in saliva, so that considerable caution is required to avoid infectious diseases (Shmaefsky, 1990). Therefore, many teachers have not put this experiment into practice in their science classes.

To overcome such obstacles, Kroen (1998) used α -amylase and amyloglucosidase commercially extracted from a bacterium and a fungus, respectively, by Sigma Chemical Company. In the present study, we invented a way to use the crude enzyme powder of saliva prepared by the cold ethanol precipitation method, which is commonly applied to the preparation of macromolecular compounds such as proteins and nucleic acids (*e.g.*, Kaga and Arai, 2004). In addition, we developed laboratory exercises on starch digestive enzymes to encourage pupils' inquiring minds. The laboratory exercises include activities to observe the degradation of starch in boiled rice grains by the crude enzyme powders of germinating corn grains and of "kome-koji" (rice malted with a mould, *Aspergillus* sp.) prepared by the same method as the saliva enzyme powder. In the present paper, we explain the methods for the preparation of crude enzyme powders and the procedures of pupil laboratory exercises, and report on a classroom trial of the exercises.

PREPARATION OF CRUDE ENZYME POWDERS

Materials

All materials used in the present study are easily available at stores in Japan. A packet of freeze-dried kome-koji (Figure 1), corn grains, and a bottle of absolute ethanol are sold at a grocery store, gardening shop, and pharmacy, respectively.

Preparation of the crude enzyme powder of saliva

After cleaning the inside of the mouth, a clean absorbent cotton ball was put in the mouth to absorb saliva. Then, the cotton ball was transferred into a small zippered polythene bag. This operation was repeated several times and the saliva was squeezed from the cotton balls in the polythene bag. The saliva collected was dripped into a beaker containing 100 cm³ of cold absolute ethanol. Then, the beaker was kept in a refrigerator overnight until the precipitate accumulates at the bottom. The supernatant was removed gently from the beaker, and the wet precipitate was transferred into a Petri dish and kept in a refrigerator until it dried completely. The dried precipitate (hereafter we called it "saliva enzyme powder") was wrapped in a sheet of paraffin paper and kept in a small bottle with a desiccant. The bottle was kept in a refrigerator.

Preparation of the crude enzyme powder of germinating corn grains

Corn grains (Figure 2A) were soaked in water overnight (Figure 2B) and sown in a flowerpot. The flowerpot was placed under a room light. When the seedlings grew to about 5 cm in height, the sprouting corn grains were harvested and washed (Figure 2C and 2D). The leaves and roots were removed from each sprout, and the rest (scutellum and endosperm parts within the grain coat, Figure 2E) was kept in a freezer.



Figure 1: Packet of kome-koji

The frozen material was ground with a mortar and pestle to make a paste, and 50 cm³ of cold water was added to the paste (Figure 3A). After being stirred well, the mixture was filtered by a tea strainer and the filtrate was refrigerated (Figure 3B). After the filtrate separated into precipitate and supernatant (Figure 3C), the supernatant was dripped into a beaker containing 100 cm³ of cold absolute ethanol (Figure 3D). After this, the procedure for obtaining the crude en-

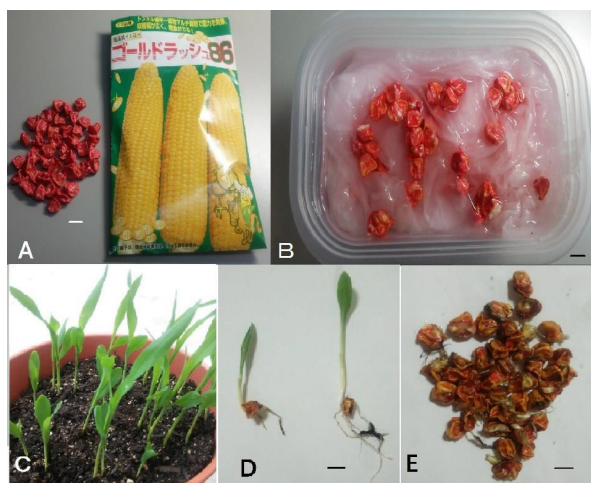


Figure 2: Preparation of germinating corn grains
 A: Corn grains (germinant-coated), B: Corn grains soaked in water overnight, C: Corn seedlings seven days after being sown, D: Harvested corn seedlings, E: Germinating corn grains, from which leaves and roots were removed.

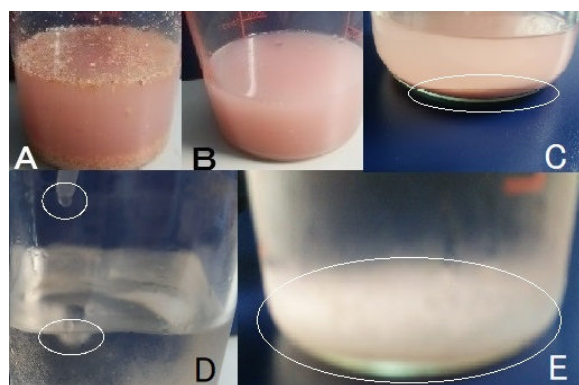


Figure 3: Preparation of germinating corn enzyme powder
 A: Suspension of ground germinating corn grains, B: Filtered suspension C: Starch precipitating at the bottom (circled), D: Supernatant being dripped into cold ethanol (circled), E: Crude enzyme precipitation accumulating at the bottom (circled).

zyme powder of germinating corn grains (hereafter we call it “germinating corn enzyme powder”) was the same as that for obtaining the saliva enzyme powder (Figure 3E).

Preparation of the crude enzyme powder of kome-koji

Ten grams of freeze-dried kome-koji and 100 cm³ of water were mixed in a beaker and the mixture was stirred well. The beaker was kept in a refrigerator until the suspension separated into precipitate and supernatant (Figure 4A). The supernatant was dripped into a beaker containing 100 cm³ of cold absolute ethanol (Figure 4B). After this, the procedure for obtaining the crude enzyme powder of kome-koji (hereafter we call it “kome-koji enzyme powder”) was the same as that for obtaining the saliva enzyme powder (Figure 4C).

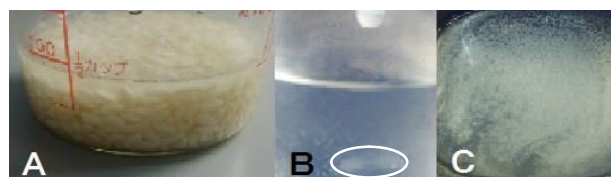


Figure 4: Preparation of “kome-koji enzyme powder”
 A: Kome-koji suspended in water, B: Supernatant dripped into cold ethanol (circled), C: Crude enzyme precipitation accumulating at the bottom.

PROTOCOLS FOR LABORATORY EXERCISES

Table 1 shows the protocols for pupil’s laboratory exercises on starch digestion which we tested at an elementary school.

TRIAL OF PUPIL LABORATORY EXERCISES

Preparation of materials

Enzyme powders: Although it is preferable that pupils carry out all steps, we prepared all enzyme powders beforehand, and at the start of the exercises we demonstrated the way of pre-

Table 1: Protocols for pupil's laboratory exercises

= Protocols for Laboratory Exercises on Starch Digestion =
<p>Materials</p> <p>Enzyme solutions (saliva, germinating corn, kome-koji, digestive aid)</p> <p>A gargle containing iodine</p> <p>A cotton swab, a sheet of starch-containing paper, a paint brush</p> <p>Five boiled rice grains, five small zippered polythene bags, six transparent plastic straws, and a polystyrene (Styrofoam) bowl with warm water of about 40°C for each group</p>
<p>Experiment 1: Observation of starch digestion in starch-containing paper</p> <ol style="list-style-type: none"> 1) Absorb saliva enzyme solution by a cotton swab. 2) By means of the cotton swab, write some letters on a sheet of paper. 3) After a few minutes, apply a diluted gargle solution to the paper with a paint brush.
<p>Experiment 2: Observation of boiled rice grain digestion by various enzyme solutions</p> <ol style="list-style-type: none"> 1) Write "saliva," "corn," "kome-koji," "digestive aid," or "water" on each of the five polythene bags. 2) Into each polythene bag, put one boiled rice grain and pour about 1 cm³ of water. 3) Zip the bags firmly, and then, rub the bags to mash the boiled rice grains thoroughly. 4) Into each of the first four bags, pour about 1 cm³ of corresponding enzyme solution. 5) Into the "water" bag, which is used as a control, pour about 1 cm³ of water. 6) Zip the bags firmly and rub them to mix their contents well. 7) Soak these bags in warm water and leave them for a while. 8) Pour about 1 cm³ of diluted gargle solution into each bag and observe the colour of the mixture in each bag.

paring saliva enzyme powder. Major reasons for doing the preparation as a demonstration are that absolute ethanol is flammable and its vapor is unsafe for pupils to handle. The demonstration is indispensable, because if the teacher uses an enzyme powder obtained by the cold ethanol precipitation method without demonstrating this preparation process, pupils may misunderstand that the powder is a kind of digestive aid.

Enzyme solutions: A small amount of each enzyme powder (saliva, germinating corn, and kome-koji) and digestive aid was dissolved into water. Prior to the class, we confirmed that each enzyme solution had enough activity to digest a boiled rice grain completely within 10 min.

Digestive aid (a powder/granulated one) and a gargle containing iodine (or Lugol's solution) were purchased at a pharmacy. The gargle, of which iodine content is 0.7%, was diluted into

10% for Experiment 1 and 1% for Experiment 2 with water.

A sheet of paper was confirmed to include starch beforehand, because some sorts of paper contain a synthetic paste instead of starch.

Plastic straws of 6 mm in diameter were used. These were marked at 3.5 cm from an end to indicate the volume of about 1 cm³. By immersing this end in a liquid to the mark and blocking up the other end with a fingertip, a 1 cm³ liquid can be taken.

Experiment 1

Although it is preferable that pupils carry out all experiments, this experiment was conducted by the teacher as a demonstration, because of time limitations. At first, the teacher led pupils to have a group discussion on what they had learnt about the working of saliva in the previous class through the textbook. Then, he explained an outline of Experiment 1 and that the paper to be used included starch and led pupils to predict

the result of the experiment.

This experiment could allow pupils to ascertain the working of saliva and the method of detecting it.

Experiment 2

Experiment 2 was carried out by the pupils as a group activity. At first, the teacher posed a question, “Do living things other than human beings also contain digestive aids to digest starch?”. To find the answer, pupils were advised to mull over whether starch digestion would occur in plants and moulds. Then, they were introduced to Experiment 2 where the enzyme solutions of a plant (germinating corn grains) and a mould (kome-koji) were used. A saliva enzyme solution and a solution of digestive aid were also used as comparatives.

Pupils were asked to predict the results of the experiment. Then, they carried out the experiment by following the protocols.

Results

In Experiment 1, a few minutes after writing letters, the saliva enzyme solution dried and the traces of letters left wrinkles in the paper (Figure 5A). When the diluted gargle solution was applied, the paper was dyed bluish purple except the parts where the letters had been written with the saliva enzyme solution remained colourless (Figure 5B). The result allowed pupils to understand

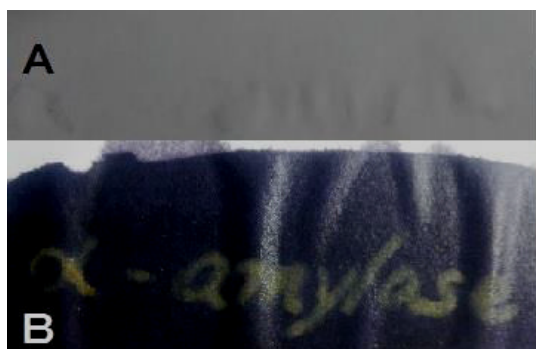


Figure 5: Starch digestion in starch-containing paper

A: Starch-containing paper with letters written in saliva enzyme solution, B: Same paper with diluted gargle solution applied.

that saliva can digest starch.

In Experiment 2, by rubbing the bags in step 3), the boiled rice grain in each bag was mashed into tiny fragments suspended in liquid. In step 8), when the diluted iodine solution was poured in, the mixtures in the bags marked “saliva,” “corn,” “kome-koji,” and “digestive aid” remained colourless, while that in the “water” bag turned bluish purple (Figure 6). The results indicate that, along with saliva and digestive aid, the crude enzyme powders of plants and moulds can digest starch.

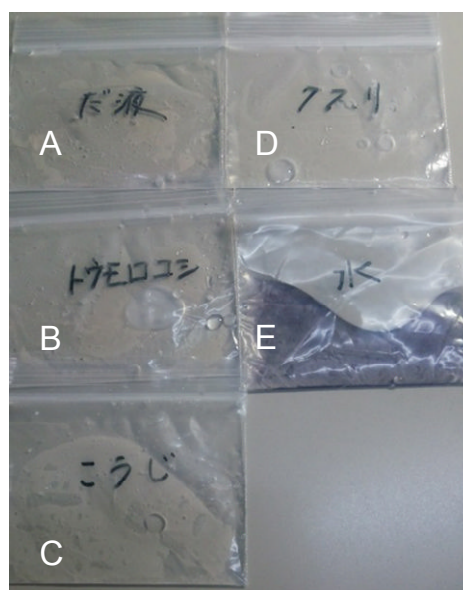


Figure 6: Digestion of boiled rice grains by various enzymes

A: Saliva, B: Germinating corn grain, C: Kome-koji, D: Digestive aid, E: Water (control)

DISCUSSION

Saliva (salivary amylase) has commonly been used in student laboratory exercises on carbohydrate (starch) digestion (e.g., Germann, 1991). The use of saliva is simple, easy, and cheap, but as mentioned in the Introduction, there are two issues in using human saliva that should be surmounted. Freeland (1974) and Wyatt (1974) proposed the use of the digestive organ of a freshly killed animal, but schoolchil-

dren may feel this to be repulsive. As proposed in this report, the cold ethanol precipitation method is applicable not only for preparing saliva enzyme powder but also for preparing crude enzyme powders of various living materials. By the ethanol treatment, any living germs can probably be eliminated from enzyme preparations.

It is well-known that the activity of saliva amylase varies considerably with individual, and even with the same person the activity is changeable dependent on body conditions. This is the same with the other living materials. Crude enzyme powders prepared by the cold ethanol precipitation method from various organisms can be kept in a refrigerator for a long time. Once teachers determine a suitable amount of these powders to be dissolved in water, they can use them without examining it as the need arises.

The use of a sheet of starch-containing paper for detecting starch digestive enzyme(s) is convenient and practically costless, so that this is suitable for any school setting, even those in developing countries. For Experiment 2, teachers can also use the paper, but we recommend using a starch-containing staple food in each country. Brown (1994) in the U.S.A. used McMush in her student laboratory to analyze the organic compounds found in an ordinary fast-food lunch. In Asian countries, boiled rice is more familiar to schoolchildren than McMush, easily available, and almost costless. Furthermore, the use of small polythene bags and plastic straws instead of test tubes and syringes, respectively, can also reduce the cost of the experiment.

To determine the activities of starch digestive enzymes, Davis (1977) used the radial diffusion method. As his method is suitable for a quantitative assay of the amylolytic enzymes and it takes much time to prepare the agar disks for the experiment, it would be better to introduce it into biology laboratories at the secondary level.

Laboratory exercises proposed by Hogue (1972) and Law (1996) are also suitable for student laboratories at the secondary level to deepen student understanding of digestion and absorption in our digestive organs, because the procedures of these experiments seem to be somewhat complicated and unsuitable for schoolchildren.

The latest Course of Study for Elementary Schools and its guidelines were announced in 2017 by the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT; See its website, also refer to Asia Society website). The purpose of the subject “Science” mentioned in the Course of Study is “to allow children to foster their talents and abilities for solving questions about natural things and phenomena scientifically.” For achieving the purpose, schoolteachers are asked to let pupils get close to nature, to make the most of the scientific way of observing and thinking, to carry out an observation or an experiment with insight, etc. In addition, pupils are expected to nurture the feeling of love toward nature, to understand natural things and natural phenomena, to acquire basic skills of observation and experimentation, to foster the ability of problem-solving, and to develop (independent) positive attitudes toward problem-solving. In our classroom trial, more than four fifths of pupils expressed affirmative impressions of the laboratory activity. Examples of pupil comments are as follows:

“I am glad I could carry out hands-on laboratory activities on the working of saliva;”

“Through the observation and experimentation, I can easily understand what I have not understood.”

However, to know what skills and what knowledge pupils can achieve through the inquiry activities we have proposed, further investigations are required. Germann (1991) proposed DIAL (SPS)², the Directed Inquiry Approach to Learning Science Process Skills and Scientific Problem

Solving, which can be adopted to student laboratories possibly at secondary and tertiary levels, to help students develop problem-solving skills. With directed inquiry activities as introduced in the present paper at the primary level, pupils can acquire basic science processing skills and some background knowledge which is required for proceeding to the further inquiry activities which Germann (1991) described.

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