

RESEARCH ARTICLE

Application of Pineapple Juice in the Fish Digestion Process for Carcinogenic Liver Fluke Metacercaria Collection

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Abstract

Pepsin is common digestive enzyme used for fish digestion in the laboratory to collect trematode metacercariae. In a field study, to survey the infected fish is needed a huge yield of pepsin and it is very expensive. Therefore, our purpose of this study was to investigate the candidate enzyme from pineapple juice which has a digestive enzyme called bromelain, a mixture of proteolytic enzymes, to digest fish in order to harvest metacercariae. Fish were divided into 2 groups: one group in which metacercariae were harvested using acid pepsin as a control and other groups in which the fish was digested using fresh pineapple juices. The results showed that pineapple juice is able to digest fish similarly to pepsin. The Pattavia pineapple juice had the highest number of metacercariae similar to the control. For Trat Si Thong pineapple juice, we found the number of metacercariae was less than control. This result suggests that the Pattavia pineapple juice was optimal juice for fish digestion to metacercaria collection and can be used instead of pepsin acid.

Keywords: liver fluke- metacercaria- infective stage- excysted - pineapple

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Introduction

Metacercariae are the infective stage of trematodes or flukes of which *Opisthorchis viverrini*, the human liver fluke, and minute intestinal flukes of the Heterophyidae family which are the two most common human flukes in Thailand (Khamboonruang, 1991; Pungpak et al., 1998; Radomyos et al., 1998). Trematode metacercariae can be found in cyprinoid fish species. The harvesting of metacercariae from fish allows for the estimation of the prevalence of flukes in a given area and provides metacercariae for further biological studies and other applications. The traditional method of examining specimens for metacercariae involved two thick glass slides, between which an entire fish (for smaller fish) or a portion of the fish (for larger fish) with a few drops of normal saline (NSS). The muscles were then compressed between two glass plates and examined for metacercariae under a stereomicroscope (Vichasri et al., 1982). The problem with this method was that the whole fish could not be examined, particularly the hard and thick portions of the head. Moreover, the preparatory phase was time-consuming.

Another simple method for harvesting metacercariae

from fish is pepsin digestion. This method can be used to digest all the parts of the fish. It consists of 0.25% pepsin in 0.85% NaCl and 0.15% HCl in a shaking water bath for 60 minutes at 37 °C and is widely used (Pinlaor et al., 2013; Sriraj et al., 2013). However, pepsin is expensive and sometimes must be imported from abroad. Thus, an alternative substance for digesting fish that overcomes the above mentioned disadvantages is necessary. An inexpensive alternative that is indigenous to Thailand has been found in some tropical fruits such as pineapple. These fruits have been reported to have enzymes that have a proteolytic effect and aid in digestion. Pineapple juice has several advantages. It is found in Thailand where the tropical climate means year-round availability, it is easy and inexpensive to prepare, and is non-toxic. Therefore, we investigated the use of pineapple juice to digest fish in order to harvest metacercariae.

Materials and methods

Fish preparation

The freshwater fish (*Hampala dispar*) were bought from Lao PDR's Khammoun Province in Lao PDR (Figure 1). Fish were sorted according to their characteristics,

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measured, recorded, and identified using a taxonomic reference book (Rainboth, 1996). The fish were mostly about 10 cm in length and 2 inch in width. One kilogram of fish was randomly selected to be examined for *O. viverrini* metacercariae using pepsin (Sigma, MO, USA) digestion followed by microscopic observation.

Pineapple preparation

Pineapples were divided into two groups with the same species, *Ananas comosus*, Pattavia or Smooth Cayenne and Trat Si Thong (Figure 2). These were purchased from a local market in Khon Kaen Province in northeast Thailand. The general characteristics of Pattavia are thicker skin, sweeter taste, and crunchier texture, and low fiber content. It is popular for fresh consumption in the southern part of Thailand. The general characteristics of Trat Si Thong are their cylindrical shape, thin skin and uniform yellow color, high fiber content. They are extremely sweet and mainly used for fresh consumption.

Pineapple digestive solution

Each pineapple was peeled, cut into small pieces,



Figure 1. Cyprinoid Fish (*Hampala Dispar*), Which is a Common Host of *Opisthorchis Viverrini*



Figure 2. Pattavia Pineapple (A) and Trat Si Thong Pineapple (B)

weighed (50, 100, 200, 300 g), and blended with 100 ml of 0.85% NaCl solution in a fruit blender. The mixture was then filtrated through a gauze filter and fiber waste was discarded. Only the supernatant was used for the digestive solution (Figure 3) as assigned group. Following this, pH indicator paper or Litmus was used to measure the pH of the pineapple juice and pepsin solutions.

The fish digestion process

The solution was divided into 2 groups: 1) the control group, acid pepsin solution was used in comparison for each experiment. The acid pepsin was prepared using 0.25% pepsin – 0.15% HCl in 0.85% NaCl solution. The experimental groups were pineapple group, the fish (200g): pineapple juice (50, 100, 200, 300 g as explained above) was 1:0.25, 1:0.5, 1:1, 1:2, and 1:3. The mixtures in each group were incubated in a shaking water bath at 37°C for 60 minutes. The digested materials were filtered through a series of sieves (1,000, 300, 250 and 106 µm mesh) and then washed with 0.85% NaCl solution in a sedimentation jar until the supernatant became clear. Finally, the metacercariae in the sediment were isolated and identified under a stereomicroscope (Figure 4).

Results

Compared pH of pineapple juice and pepsin solution

The pH of pineapple juices, Pattavia juice and Trat Si Thong juice was 4 and that of the pepsin solution was 1. The pH of the pepsin solution was lower than both pineapple solutions. The pH of pineapple juice in all groups was 4 and during incubation and final incubation pH was 5. The initial pH of the pepsin solution was 1 and during incubation and final incubation pH was 4.

Number of recovered metacercariae from fish digestion using different digestive enzymes

All different digestive enzymes could digest the fish in different level as shown in figure 5 which affected to the recovered metacercariae in different number. The total number of recovered metacercariae obtained from each digestive solution is shown in Figure 5. The group 1:0.25, the number of metacercariae (182) in Pattavia group was higher than those obtained from Trat Si Thong pineapple (11) and similar to the acid pepsin solution (130 and 295). To confirm whether metacercariae from Trat Si Thong pineapple was digested before 60 minutes or

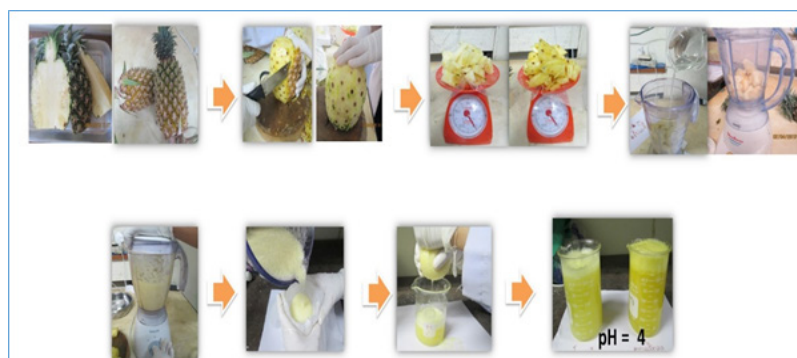


Figure 3. Pineapple Juice Preparation

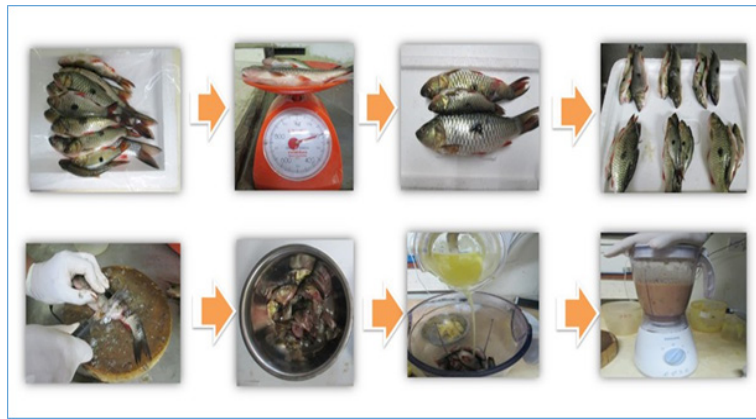


Figure 4. The Cyprinoid Fish Digestion Process

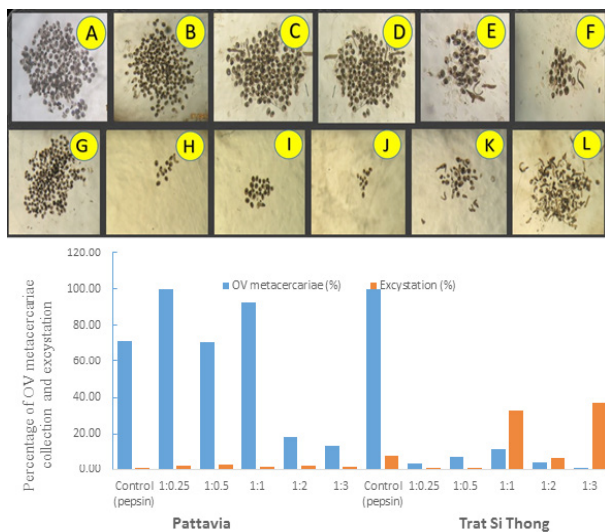


Figure 5. Recovered and Excysted Metacercariae From Digestive Solutions of Pepsin (A,G), Pattavia 1:0.25 (B), 1:0.5 (C), 1:1 (D), 1:2 (E), 1:3 (F) And Trat Si Thong At Various Concentrations 1:0.25 (H), 1:0.5 (I), 1:1 (J), 1:2 (K), 1:3 (L)

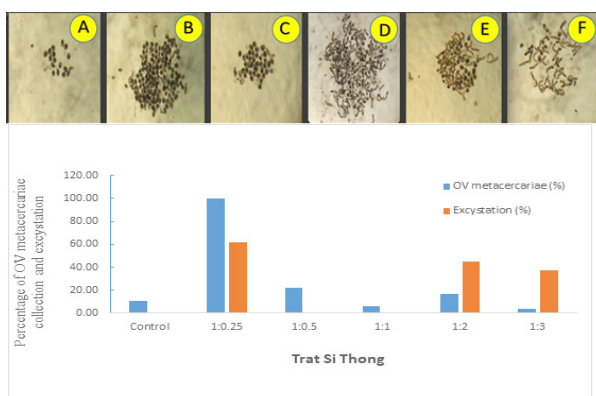


Figure 6. The Representative Number of Metacercariae from Each Group Using Pepsin (A) and Trat Si Thong Digestive Solution at 30 min (B-F)

metacercariae was packed on the sieve or in the fish meat. Surprisingly, we could observed metacercariae from Trat Si Thong pineapple group at 30 minutes (Figure 6) and moreover, metacercariae from Pattavia group was found in fish meat (Figure 7).

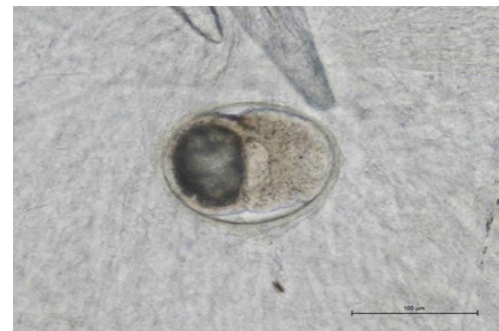


Figure 7. Metacercaria in Fish Meat

Cost

The cost of both pineapple solutions with the ratio of fish and juice was vary depended on the concentration, range from 4.1-76.9 times cheaper than pepsin. However, the optimal ratio (1:0.25) has shown the cheapest cost by 76.9 times cheaper than pepsin solution at the same condition of recovered number of metacercaria.

Discussions

Our present study found that Pattavia pineapple juice was able to digest fish and collect the trematode metacercaria similar to pepsin solution, as evidenced by observation of the similar number of recovered metacercariae. Moreover, using pineapple juice for digestion of fish is the cheapest pepsin solution.

Pineapple (*Ananas comosus*) is grown in several tropical and subtropical countries, including Hawaii, India, China, Kenya, South Africa, Malaysia, the Philippines and including Thailand (Pavan et al., 2012). In Thailand, there are 27 pineapple types. Pattavia or Smooth Cayenne is the most popular used in canning and for fresh consumption. It has also been used as a medicinal plant in several indigenous cultures. Pineapple also contains a digestive enzyme called bromelain, which is a mixture of proteolytic enzymes and has been chemically known since 1876 (Taussig and Batkin, 1988). A study by Pavan et al. (2012) found that pineapple protease (bromelain), which is a crude extract from pineapple that contains various closely-related proteinases, among other compounds (Pavan et al., 2012).

The proteolytic action of pineapple juice occurs due

to its cysteine proteinase enzymes. Rowan et al.(1990) reported that the pineapple plant contains at least four distinct cysteine proteinases (*A. comosus*) i.e., fruit bromelain, stem bromelain, ananain and comasain. Fruit bromelain was found in approximately 30-40% of the total fruit protein and represented almost 90% of the pineapple's active proteolytic enzymes (Rowan et al., 1990). Fruit bromelain FA2 is the main proteinase component in pineapple juice (Yamada et al., 1976). Pineapple has been reported to contain enzymes that have a proteolytic effect as well as supporting indigestion. This study investigated the use of pineapple juice to digest fish in order to harvest metacercariae. We found that pineapple juice has the ability to digest fish similar to pepsin solution. In comparing the pH of pineapple juice and pepsin solution we found that pepsin solution has quite the same pH with pineapple juice (pH 4 and 5 respectively). Pineapple juice was able to digest fish within a shorter time period and was cheaper than pepsin solution.

The highest number of recovered metacercariae at 60 minutes was found in Pattavia subsequence to the control group and Trat Si Thong respectively. In the case of the same weight of Trat Si Thong and Pattavia, Trat Si Thong has high fiber with low juice but Pattavia has low fiber with high juice. Thus, Trat Si Thong has very concentrated enzyme leading to the early digest at 30 minutes. The optimal ratio of fish and Pattavia juice for fish digestion was 1:0.25. For other high concentrations could digest not only fish meat but also metacercariae. However, the recovered metacercaria depend on the number of metacercaria, or the metacercaria intensity in each cyprinoid fish. Therefore, we tried to control this factor by 1) used the same size and species of fish and 2) the same location and same date of collection. Our present study found results similar to those of a previous study, which found that freshly prepared pineapple juice frozen for 15 days could be used to harvest a similar number of metacercariae as commercial acid pepsin solution (Prawang et al., 2002).

In this study, some excysted juveniles were found after the pineapple juice had digested the fish, but were rarely observed in the acid pepsin solution. We found that pineapple juice was also cheaper than pepsin solution and can, thus, reduce the cost of digestion of fish. This present study suggests that Pattavia pineapple may be used as a candidate enzyme for fish digestion and metacercaria collection in field study.

In this study, we described techniques for digestion of fish by using pineapple instead of artificial pepsin solution. The result from this study of fish digestion by pineapple juice is more economically benefit than artificial pepsin solution and it is more easily available. However, further additional methods are needed to research for fish digestion in every time and everywhere.

Conflict of interests

No competing financial interests exist.

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