# PINEAPPLE JUICE AS AN AGENT FOR THE DIGESTION OF FISH PRIOR TO THE HARVESTING OF METACERCARIAE

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**Abstract.** The efficacy of crude pineapple juice obtained from *Ananus comosus* in digesting fish for the harvesting of trematode metacercariae was investigated. No significant difference was found between the total number of metacercariae detected from fish (*Cirrhina jullieni*) digested by acid pepsin and those digested by freshly prepared pineapple juice that was kept for 15 days at a temperature of either -4 ˚C or -75 ˚C. However, fewer metacercariae were found when using juice that had been kept for more than 30 days. This study showed that freshly prepared pineapple juice kept frozen for 15 days could be used instead of commercial acid pepsin to digest fish for harvesting metacercariae, some of which could be used for further biological studies.

## INTRODUCTION

Metacercaria is the infective stage of trematodes or flukes, of which *Opisthorchis viverrini*, the human liver fluke, and minute intestinal flukes of the Heterophyidae family are the two most common human parasites in Thailand (Khamboonruang, 1991; Srisawangwong *et al*, 1997; Pungpak *et al*, 1998; Radomyos *et al*, 1998). Humans acquire infection by ingesting raw or undercooked fish (the second intermediate host) that contain viable metacercariae.

The harvesting of metacercariae from fish allows the estimation of the prevalence of flukes in a given area and provides metacercariae for further biological studies. The traditional method of examining specimens for metaceracriae involved two thick glass slides, wrapping fish muscle, and examination using a dissecting microscope. One drawback of 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 (Vichaisri *et al*, 1982). Pattanapanyasat *et al* (1983) proposed a new method in which artificial acid pepsin solution was used to digest all the parts of the fish; this method has become widely used. However, acid pepsin is both expensive and must pass through demanding importation controls: an alternative substance that can digest fish is required, one that overcome the abovementioned disadvantages. An inexpensive alternative that is indigenous to Thailand has been found: some tropical fruits such as pineapple have been reported to have enzymes that have a proteolytic effect as well as aiding digestion (Kelly, 1996; Melendo *et al*, 1996). This study investigated the use of pineapple juice to digest fish in order to harvest metacercariae.

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## MATERIALS AND METHODS

The pineapples, *Ananus comosus* (Sriracha strain), were purchased from a local market in Chiang Mai, northern Thailand. A stock of 5,000 ml of crude juice extract from the fruit was prepared using a juice extractor. The solution was distributed to 5 groups prior to being used to digest the fish. These groups were: 1) freshly prepared juice kept for 15 days at a temperature of-18˚C; 2) freshly prepared juice kept for 15 days at-75˚C; 3) freshly prepared juice kept for 30 days at-18˚C; 4) freshly prepared juice kept for 30 days at-75˚C; and 5) acid pepsin solution, which was used as a control for each experiment. The acid pepsin was prepared using 1g Porcin pepsin (Sigma®): conc hydrochloric acid 1ml: 0.85% NSS 99 ml.

The cyprinoid fish (*Cirrhina jullieni*) were purchased from Mae Ngud Dam marketplace, north of downtown Chiang Mai. One kilogram of fish, from which the internal organs were removed, was minced using a mixer blender for 5 minutes. For each experiment, ten grams of minced fish was digested using 100 ml of each juice condition in a 250 ml Erlenmayer flask. The flask was then incubated in a shaking water bath set at 37˚C for 20 minutes for the pineapple juice and 1 hour for the pepsin solution. The digested material was filtered through one layer of wet gauze into a conical sedimentation glass, then 250-300 ml of 0.85% NSS were added. The digestive solution was left for 15-30 minutes to form a sediment. This procedure was repeated twice. The metacercariae were transferred from out of the sedimentation glass

and into a glass Petri dish using a transparent pipette and examined under a dissecting microscope. The total number of metacercariae was counted for each group. The identification of the metacercariae was based on Scholz *et al* (1991) and Pearson and Ow-Yang (1982).

## RESULTS

The total number of metacercariae obtained from each digestive solution was shown as a median (range). Freshly prepared pineapple juice [28 (10-33)] produced results that were not significantly different from those obtained by acid pepsin solution [31.5 (6-54)] (Mann - Whitney *U* test; p = 0.19) (Table 1). The juice that was kept for 15 days and frozen at either -18°C or -75°C gave slightly fewer metacercariae than given by the pepsin solution, but with no significant difference (Table 2). Likewise, the juice kept frozen for 30 days yielded similar results for both freezing conditions (Mann - Whitney; *U* test; p = 0.94), but produced fewer metacercariae than the acid pepsin solution (Mann - Whitney *U* test; p = 0.02).

The excystation of metacercariae was observed in the sediment when the frozen juice that had been kept for either 15 or 30 days was used, but it was not seen in the control (Table 2). More excysted worms were produced by the juice kept for 30 days. The identification of metacercariae indicated that *Haplorchis taichui* was the predominant trematode species (85.97%), followed by *Haplorchis pumilio* (6.63%); there was no *O. viverrini* (Table 3).

Table 1
Number of metacercariae [median (range)] from 100 grams of fish digested by using freshly prepared pineapple juice or acid pepsin solution.

| Digestive solution | No. of metacercariae | |
|---|---|---|
| Pineapple juice | Excysted | 1.5 (0-6) |
| | Cyst | 21.5 (5-32) |
| | Degenerated | 2 (0-3) |
| | Total | 28 (10-33)[a] |
| Acid pepsin solution | Excysted | 0 |
| | Cyst | 29 (6-52) |
| | Degenerated | 2 (0-6) |
| | Total | 31.5 (6-54)[a] |

[a] Non-significant difference at p > 0.05 (Mann - Whitney *U* test)

Unidentified (3.61%) and degenerated species (3.79%) were also found.

## DISCUSSION

The freshly prepared pineapple juice, which had been kept frozen for 15 days, and the commercial acid pepsin solution produced similar numbers of metacercariae. Following the digestion and sedimentation procedure, the relatively clear supernatant enabled the easy recovery and harvesting of the metacercariae under a stereomicroscope. Very few fine pieces of fish remained (in roughly the same quantity) after digestion by the three solutions. It has been suggested that the freshly prepared pineapple juice, kept frozen for 15 days, had proteolytic properties that were equal to those of acid pepsin solution. Fewer metacercariae were obtained using the juice kept for 30 days: this was probably due to the frozen conditions which may have protected some of the enzyme degradation processes and fermentation.

The proteolytic action of pineapple juice arises from its cysteine proteinase enzymes. Rowan *et al* (1990) reported at least four distinct cysteine proteinases from the pineapple plant (*A. comosus*), *ie* fruit bromelain, stem bromelain, ananain and comasain. The fruit bromelain was found in approximately 30-40% of the total fruit protein and represented almost 90% of the proteolytic active material of the pineapple (Rowan *et al*, 1990; Rowan, 1998). Fruit bromelain FA2 is the main proteinase component of pineapple juice (Yamada *et al*, 1976). A stem bromelain can be inactivated by exposure to light (Murachi *et al*, 1975). Although the pineapple juice was kept in a dark freezer, it could have been exposed to light when the freezer doors were opened. In order to preserve the juice's proteolytic properties, it is suggested that the juice be used immediately after preparation or kept frozen in a light-protected container.

Normally, the excystation of trematode metacercariae is reported only after incubation with specific cysteine proteases other than pepsin (Chung *et al*, 1995; Fried *et al*, 1997). In this study, some excysted juveniles were found after the pineapple juice digested the fish, but none were observed in the acid pepsin solution. Thus, the cysteine proteinases that exist in crude pineapple juice (Rowan *et al*, 1990) have the proteolytic action in both the muscle of fish and the walls of metacercarial cysts. After the fish had been digested, a higher percentage of excystation was found in the pineapple juice that had been frozen for 30 days than in the juice that had been frozen for 15 days. This could be due to the longer freezing time, which may have enhanced the proteolytic property of the enzymes

Table 2
Number of metacercariae [median (range)] from 100 grams of fish digested by using pineapple juice kept frozen for 15 and 30 days at -18˚C or -75˚C

| Time frozen | | No. of metacercariae | |
|---|---|---|---|
| | | Kept for 15 days | Kept for 30 days |
| Pineapple juice (-18˚C) | Excysted | 1 (0-2) | 4.5 (2-12) |
| | Cyst | 9.5 (6-11) | 19 (16-25) |
| | Degenerated | 0 (0-1) | 0 (0-2) |
| | Total | 10.5 (8-18) | 23.5 (19-31)[a] |
| Pineapple juice (-75˚C) | Excysted | 1 (0-3) | 4 (2-8) |
| | Cyst | 9.5 (6-13) | 18 (14-28) |
| | Degenerated | 0 (0-2) | 1 (0-5) |
| | Total | 10.5 (8-17) | 23 (19-37)[a] |
| Acid pepsin (control) | Excysted | 0 | 0 |
| | Cyst | 12 (8-29) | 30.5 (19-57) |
| | Degenerated | 0 (0-2) | 4 (1-6) |
| | Total | 12 (10-29) | 34.5 (21-62)[b] |

[a] Non-significant difference at p = 0.94 (Mann - Whitney *U* test)
[b] Significant difference at p = 0.02 (Mann - Whitney *U* test)

Table 3
Specified metacercariae from digested fish (*Cirrhina jullieni*) using pineapple juice or acid pepsin solution.

| Digestive solution | Species of metacercariae | | | | | |
|---|---|---|---|---|---|---|
| | Ht | Hp | Ov | U | Deg | Total |
| Freshly prepared pineapple juice | 76 | 5 | 0 | 5 | 13 | 99 |
| Pepsin solution 1 | 154 | 3 | 0 | 0 | 27 | 184 |
| Pineapple juice kept for 15 days at -18˚C | 38 | 18 | 0 | 0 | 1 | 57 |
| Pineapple juice kept for 15 days at -75˚C | 38 | 18 | 0 | 7 | 3 | 66 |
| Pepsin solution 2 | 58 | 29 | 0 | 0 | 0 | 87 |
| Pineapple juice kept for 30 days at -18˚C | 173 | 1 | 0 | 15 | 0 | 189 |
| Pineapple juice kept for 30 days at -75˚C | 177 | 3 | 0 | 7 | 0 | 187 |
| Pepsin solution 3 | 285 | 0 | 0 | 8 | 0 | 293 |
| **Total (%)** | 999 (85.97) | 77 (6.63) | 0 (0) | 42 (3.61) | 44 (3.79) | 1,162 (100) |

Abbreviation: Ht, *Haplorchis taichui*; Hp = *Haplorchis pumilio*; Ov = *Opisthorchis viverrini*; U = unidentified; Deg = Degenerated.

in the pineapple juice. The *in vitro* metacercarial excystation, using pineapple juice that is frozen for longer periods of time, merits further investigation.

The commonest species of metacercariae found in this study was *H. taichui*. This parasite has been previously reported as the most prevalent trematode in both the gastrointestinal tract of humans (Pungpak *et al*, 1998; Radomyos *et al*, 1998) and cyprinoid fish in the northeast and northern regions of Thailand (Srisawangwong *et al*, 1997; Sukontason *et al*, 1999). Recently, the percentage of *H. taichui* infection in natural fish increased (Srisawangwong *et al*, 1997). Although *H. taichui* is not the most important

trematode to pose a risk to public health in Thailand, clinical disease caused by this parasite has been reported (Africa *et al*, 1935; 1937a, b). Thus, public health personnel must pay attention to the surveillance of this parasitic infection.

In conclusion, freshly prepared pineapple juice, or that kept frozen for 15 days at -18°C or -75°C, can be used instead of acid pepsin solution to digest fish for the harvesting of metacercariae in any laboratory that does not have commercial porcine pepsin powder. Pineapple juice confers several advantages: all year-round availability in tropical regions; easy and inexpensive preparation; and non-toxicity. Pineapple juice provides an alternative method for the digestion of fish prior to the harvesting of metacercariae.

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