



## Cleaning methods of the golden apple snail *Pomacea canaliculata* (Lamarck) for human food consumption: Basis for consumers food safety assurance



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### ABSTRACT

The golden apple snail *Pomacea canaliculata* (Lamarck) is a high protein and nutritious food. One of the solutions to protein deficiency, helpful in solving nutritional shortcomings of the less fortunate community and very helpful in mitigating food security problems. However, these snails cause damage to crops and are considered as pests to many farmers. It carries rat-lung worms and a host of chistosomiasis. But this problem can be addressed through suitable preparations. This paper presents an appropriate method of preparing and cleaning golden apple snail for food consumption. The three methods presented, namely: (1) From the point source, (2) induced starvation, and (3) dressing method are all appropriate to use in cleaning and preparing golden snail for food. Plate count by Petri Film for a point source, induced starvation, and dressing method are 78,000 CFU/g, 57,000 CFU/g, and 54,000 CFU/g, respectively. These values are very low compared to the standard maximum value of 500,000 CFU/g. The three methods for cleaning and preparing Golden Apple Snails are negative for Coliform Count and Salmonella Tests. Thus the three methods presented are safe for food consumption.

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### 1. Introduction

Food is essential for a healthy life. Good food provides the body with carbohydrates, proteins, fats and oils, water, minerals, and vitamins. Proteins especially are very important for life processes. Sources of proteins (including animals and plants) are from legumes, nuts, meat and, eggs among others (FAO, 2010).

Koneswaran and Nierenberg (2008), Hedenus et al. (2014), and Smith and Gregory (2013) emphasized that consumers will gradually have to reduce or give up the amount of animal protein they obtain from their food and most likely will need to accept more and more vegetarian sources of protein. However such a shift could come up at a cost to the nutritional state of the human population, which is why there is a need to identify alternative, easily available and cheap sources of protein, as *Pomacea*

*canaliculata* have already been accepted in many cultures, documented to be a good and high source of protein, iron, and calcium and it is known to contain almost all the amino acids needed by humans.

The golden apple snail *Pomacea canaliculata* came from South America (Brazil and Argentina via Taiwan. Its distribution has been increasing since its introduction to Asia as human food resource but perhaps also by aquarium trade (Cowie, 2002; Halwart, 1994a; 1994b; Mochida, 1991). It's utilization for food consumption is noted to China, Japan, Korea, Laos, Malaysia, Taiwan, Thailand, Vietnam, and Philippines.). Its high in nutritive value as food for human being and animals generated interest among public and private sectors to propagate the production of this organism.

Densak (2012) proudly declared that in Esan, North Eastern Thailand, they have no problem with golden snail since the farmers eat them. The snails are collected by women and children, cleaned and parboiled. They are taken from the shell, cut, cleaned in salted water, rinse with water. They are mixed with roasted rice, dried chili pepper, lime juice, and fish sauce.

Based on the study of Cowie (2013), consumption of raw or under cooked *Pomacea canaliculata* is not

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recommended as that is the primary route of infection with *Angiostrongylus cantonensis* causing *Angiostrongyliasis*. They presented the mode of preparation of the species for human consumption includes removing the shell, cleaning in saline water and boiling for several minutes.

Simberloff (2008) hold out hope that humans can become the predator of *Pomacea canaliculata*, in spite of their poor track record to culinary, thus the utilization of underappreciated nutritious *Pomacea canaliculata* could be helpful in mitigating food security problems and in solving nutritional shortcomings of the less fortunate community in the Philippines and Asian countries in general and the only way to be safe of being infected by parasites from *Pomacea canaliculata* is to clean them properly and safe methods of cleaning was introduced to find out which of the methods is free from chemicals and bacterial load through laboratory testing and analysis.

## 2. Objectives of the study

The main focus of the study was to design and formulate clean and safe method of cleaning *Pomacea canaliculata* for food consumption specifically, the study aimed to determine the chemical and bacterial load: total plate count, coliform count and salmonella count of the three methods of cleaning *Pomacea canaliculata* samples for the food safety assurance when it is considered as an alternative protein source of the less fortunate group of farmers in the Philippines and in Asian countries in general.

## 3. Materials and method

### 3.1. The survey

A survey was conducted to find out what chemical spray was used by the local farmers in the selected rice fields of Cantilan, Surigao del Sur in a random sampling. The study had utilized experimental method using *Pomacea canaliculata* as food for human consumption.

### 3.2. The experimental process

*Pomacea canaliculata* was collected in the selected rice fields of Cantilan, Surigao del Sur. The three samples were prepared 1). Collecting, washing, cleaning, cooking in 10 minutes, shelling, cleaning, weighing (20g), and packing in zip locked cellophane (from the point source); 2). Collecting, washing, cleaning, starving using induced starvation with hot pepper, cooking in 10 minutes, shelling, cleaning, weighing (20g), packing in zip locked cellophane (induced starvation); 3). Collecting, washing, cleaning, shelling, dressing (to separate the edible part from the entrails) cooking in 10 minutes, cleaning, weighing (20g), and packing in zip locked cellophane (dressed).

## 3.3. The methods of analysis

### 3.3.1. Determination of multi- pesticide residue

The received samples were prepared by homogenizing the whole sample in a robot coupe mixer. A 10 gram homogenized sample was accurately weighed in a tall glass beaker. One hundred (100) ml. of acetone is added and the mixture transferred in a blender. Ten (10) gram celite powder is added and the mixture is thoroughly blended for two minutes. Extracts are then filtered using 60 mm while the filtrate is collected in a 300 ml. eggplant flask followed by rinsing with additional 20 ml. acetone. The collected filtrate is concentrated to 10 ml by a rotary evaporator.

The concentrated extract is loaded into separator funnel, containing 100ml 10% sodium chloride solution. Seventy five (75) ml. of dichloromethane is added and the mixture is shaken (partitioned) by a mechanical shaker at 80 rpm speed for five minutes. Dichloromethane is collected. The partitioning is repeated again with additional 50 ml dichloromethane. The collected dichloromethane is filtered, concentrated to near dryness and then dissolve in 10 ml. hexane. A second partition is followed. The hexane mixture is transferred in a 300 ml. separator funnel; the container flask is rinsed twice with 10 ml. hexane and added into the funnel. Thirty (30) ml. of acetonitrile (saturated with hexane) is added into the funnel, and the mixture is shaken by a mechanical shaker for five minutes. Acetonitrile is collected in 200 ml. eggplant flask. The partitioning is repeated with additional 30 ml. acetonitrile. The collected acetonitrile is concentrated to dryness and reconstituted with 10 ml. hexane for column chromatography.

The 10 ml. extract in hexane is loaded on top of a pre conditioned SPE column (florisil connected to envicarb). A receiver is placed at the bottom end to collect the eluates. Thirty (30) ml. of 15% acetone in hexane mixture was passed to elute target pesticide (cypermethrin and lambda- cyhalothrin) followed by rinsing with 5 ml. hexane. The collected eluates is evaporated to near dryness and reconstituted with 5 ml. acetone for the final extract.

Two (2) of final extract and a standards were injected to Gas Chromatography equipped with Electron Capture Detector (GC- ECD) for determination/ quantification of cypermethrin and lambda- cyhalothrin residue. Helium, flowing at a constant rate, was used as a carrier gas in the GC system. The initial oven temperature 120 °C, which was ramped until it reached the final 300 °C. The detector temperature is at 305 °C. The limit of detection (LOD) of the method is 0.005 ug/g.

### 3.3.2. Determination of bacterial load: Total plate count, coliform count and salmonella count by Petrifilm

We have lifted top film and Hold pipette perpendicular to the plate and carefully dispense 1

ml of sample onto the center of bottom film. Release top film down onto sample. Distribute sample evenly using a gentle downward pressure on the center of the (recessed side). Do not slide the spreader across the film. Remove spreader and leave plate undisturbed for one minute to permit solidification of the gel.

Incubate plates in a horizontal position, with the clear side up in stacks not exceeding 20 plates. Follow current total plate count standards for incubation temperature. Temperatures above 37C are not recommended. Incubate plates 48 ± 3 hr. Petrifilm Aerobic Count plates can be counted on a standard colony counter. The reduction of the tetrazolium indicator dye will cause the colonies to become red. All red dots regardless of size or intensity should be counted as colonies. The circular growth area is approximately 20 cm<sup>2</sup>. Estimates can be made on plates containing greater than 250 colonies by counting a representative number of squares and multiplying by the appropriate number to obtain an estimated count for the total 20 cm<sup>2</sup> growth area. The presence of very high

concentrations of colonies on me plates will cause the entire growth area to become red or pink in color; record results as "too numerous to count (TNTC). Occasionally, on overcrowded plates, the center may lack visible colonies but many small colonies will be seen on the edges. When this occurs records result as TNTC. Some organisms can liquefy the gel, allowing them to spread out and obscure the presence of other colonies. If a liquifier interferes with counting, an estimated count should be made by counting the unaffected areas. To isolate colonies for further identification, lift the top film and pick the colony from the gel.

#### 4. Results and discussion

Table 1 shows the result obtained by the Gas Liquid Chromatography in the *Pomacea canaliculata* samples as to pesticide residues was negative. ND means no detection or negative of pesticide or chemicals at the limit of the determination.

**Table 1:** Pesticide residue analysis of *Pomacea canaliculata* samples at the National Pesticide Analytical Laboratory, Philippines, Inc., Manila, Philippines

Laboratory Code	Identification	Pesticide Analyzed (mg/kg or ppm)
SPR 10- 0606	<i>Pomacea canaliculata</i> (Freshly Collected from the point source) Cooked in ten minutes	ND
SPR 10- 0607	<i>Pomacea canaliculata</i> , dressed, cooked in ten minutes	ND

Table 2 shows the standard plate count for the submitted *Pomacea canaliculata* samples was computed to be 78,000 colony forming unit per gram for the freshly collected from the point source cooked in ten minutes; 57,000 colony forming unit per gram for the induced starvation using hot pepper, cooked in ten minutes and 54,000 colony forming unit per gram for dressed (edible part) and

cooked in ten minutes of 500,000 colony forming unit per gram as the maximum. All the submitted samples for coliform count are less than ten. A count of less than ten colony forming unit per gram is synonymous to negative detection at 10 dilution factors. There was no detection of salmonella in the three submitted samples.

**Table 2:** Microbial analysis of *Pomacea canaliculata* samples

<i>Pomacea canaliculata</i> Samples	Parameters	Results
		78,000
Freshly collected from the point source (cooked in 10 minutes)	FTJ 05-1 Standard Plate Count, CFU/g (by Petrifilm)	
	FTJ 06- 1 Coliform Count, CFU/g (by Petrifilm)	<10
	FTJ 07-1 Salmonella Detection per 25g (Conventional Method)	Negative 57,000
Induced Starvation using hot pepper (cooked in 10 minutes)	FTJ 05-1 Standard Plate Count, CFU/g (by Petrifilm)	
	FTJ 06- 1 Coliform Count, CFU/g (by Petrifilm)	<10
	FTJ 07-1 Salmonella Detection per 25g (Conventional Method)	Negative 54,000
Dressed (entrails is separated from the edible part)	FTJ 05-1 Standard Plate Count, CFU/g (by Petrifilm)	
	FTJ 06- 1 Coliform Count, CFU/g (by Petrifilm)	<10
	FTJ 07-1 Salmonella Detection per 25g (Conventional Method)	Negative

## 5. Findings

There was no detection on the three samples of chemical residues at the limit of the determination. The total bacterial load of all samples is less than the maximum of 500,000 colony forming unit per gram. Ten minutes cooking is not enough to kill the microorganisms in *Pomacea canaliculata* meat. A combination of induced starvation with hot pepper and dressing method of cleaning came out the best and safest method of preparing ready-to-cook *Pomacea canaliculata* edible meat.

## 6. Conclusion

Based on the findings, *Pomacea canaliculata* when starved, dressed, cleaned and cooked are safe for consumption. Microbes are minimally present but certain ways such as induced starving with hot pepper; dressing (to separate the edible part from the entrails) and 40 minutes cooking can either decrease or kill the possible parasites to eliminate the risk of infection. Proper handling, sanitary preparation and right cooking time are the best solution to eliminate the risk of infection.

## Compliance with ethical standards

## Conflict of interest

The authors declare that they have no conflict of interest.

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