REFERENCES

(1) Kesten, Mulinos and Pomerantz, *J. A. M. A.*, 109, 1509 (1937).

(2) H. G. O. Holck, *Ibid.*, 109, 1517 (1937).

(3) Geiling, Coon and Schoeffel, *Ibid.*, 109, 1532 (1937).

(4) Hanzlick, Seidenfeld and Johnson, *J. Pharmacol. and Exper. Therap.*, 41, 387 (1931).

(5) Haag and Ambrose, *Ibid.*, 59, 93 (1937).

A Preliminary Study of the Anthelmintic Activity *in Vitro* of Fresh Pineapple Juice*

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INTRODUCTION

Recently Berger and the author (1) published a short note pointing to the anthelmintic activity exhibited *in vitro* by fresh pineapple juice.

The purpose of the present paper is to report some further experiments, as well as to summarize the literature related to bromelin, the proteolytic enzyme probably responsible for the digestive activity of the juice.

The existence of a proteolytic enzyme in pineapple juice was discovered by the Venezuelan chemist Marcano (2) in 1891. The name bromelin, derived from *Bromeliaceæ*, the family to which pineapple belongs, was given to this enzyme. Soon afterward a concern in Detroit, Michigan,[1] patented the use of pineapple juice for the preparation of pre-digested foods (3), (4), (5), (6).

Later in that same year (1891) Chittenden (7) learned of Marcano's discovery and undertook a series of experiments with pineapple juice. He showed that the active enzyme is concentrated in the protein precipitate that can be separated by saturation of the juice with ammonium sulfate, sodium chloride or magnesium sulfate. In a second paper (8) published in 1893, particular attention was paid to the nature of the isolated enzyme as well as to its action on different proteins. He reports that inactivation of the enzyme takes place at 60–70° C.

Vines (9) observed that peptolysis as well as fibrin digestion by pineapple juice occurred both in acid and alkaline media.

Caldwell (10) studied the effect of metallic salts upon the action of bromelin, finding that silver salts were most poisonous. He also suggested the presence of two proteolytic enzymes in bromelin.

Willstätter, Grassmann and Ambros (11) showed that bromelin is activated by HCN as well as by H_2S. They report that the optimum cleavage of gelatin takes place at p_H 4.5–5. In the case of albumin peptone the p_H is 5. They describe in detail a method for obtaining very active bromelin preparations.

Ambros and Harteneck (12) claim that the juice from the green fruit is inert toward peptone. According to them the ripe fruit alone yields the activated enzyme. It is their theory that during the process of maturation the natural activator present in the cell juice from the interior of the fruit increases considerably. The inactive enzyme is present in the outer layers of the fruit; the combination of these two principles on crushing the tissue yields then the active bromelin. On the other hand Tanaka (13) found that the largest yields of crude bromelin are obtained from unripe fruits. According to this investigator the proteolytic activity of the juice decreases with increased maturity.

Maschmann (14) has recently studied the effect of different activators on plant proteases including bromelin.

Bergmann and co-workers (15) after experimenting with different synthetic substrates concluded that bromelin contains two enzymes which they have designated as Bromelin I and Bromelin II. These same investigators have effected synthesis using bromelin as promoter (16).

There are records in the literature of the use of pineapple juice as an anthelmintic by the native population of Brazil (17) and India (18). However, up to the present

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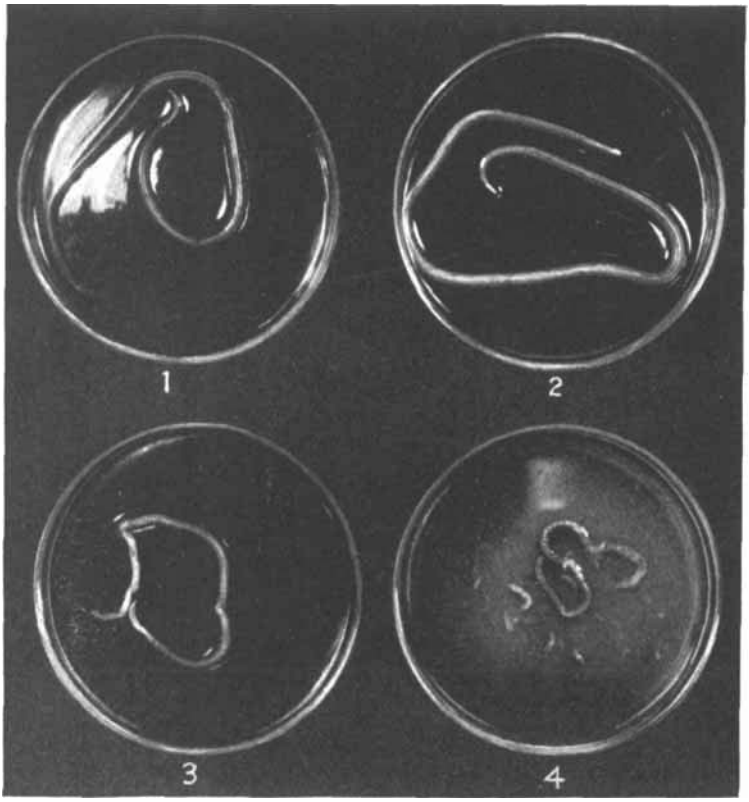
[1] Mosquera-Julia Food Co.

time no investigation has been conducted to ascertain the validity of this folk use of pineapple juice.

EXPERIMENTAL

Action of the Juice on Live Parasites.—Common round worms (*Ascaris lumbricoides*) obtained from hogs were incubated at a temperature of 37° C. with fresh pineapple juice, obtained from Cuban pineapples. At the end of 12 hours the parasites were dead and partly digested, while at the end of 24 hours they were totally digested. The results of this initial experiment are well illustrated in the photograph above.



Effect of pineapple juice on *Ascaris* worms: (1) Control in saline solution. (2) Control in pineapple juice heated to 100° C. (3) After 12 hours in fresh pineapple juice. (4) After 24 hours in fresh pineapple juice.

The same experiment was conducted using *Macracanthorynchus hirundinaceus*, another parasite found in hog's intestines, and similar results were obtained.

Minimum Time Necessary for Initial Digestion to Take Place.—Thirty-two lively *Ascaris* were incubated at 37° C. with fresh pineapple juice. Every half-hour four worms were taken out, examined carefully and then dropped into water at 45° C. Living worms when in contact with warm water move actively. At the end of 3 hours the surface of the worms was slightly wrinkled but they were still alive. At the end of three and one-half hours small ulcers were apparent and at the end of four hours large ulcers were visible to the naked eye. The parasites were still alive.

This experiment plainly showed that the digestion of the parasite precedes its death. Apparent signs of digestion can be observed in three to four hours.

Action of Heat on the Proteolytic Activity of the Juice.—The thermal inactivation point of the enzyme present in the juice was found to be in the neighborhood of 65° to 70° C. This is in agreement with the findings of other workers.

The gelatin hydrolysis was measured by means of the formol titration.[2] The decrease in worm

[2] The technique followed in the formol titration throughout this work is the following: 2% gelatin solution containing 1% toluene as a preservative is used as a substrate. To 10 cc. of this gelatin solution 1 cc. of the juice to be tested is added. One-cc. samples are taken from this mixture for titration. The indicator used is a solution containing 0.2% phenolthalein in 50% alcohol. One cc. of 40% formaldehyde is added to each volume of mixture to be titrated. The strength of the sodium hydroxide solution is 0.01*N*. One cc. of this solution is equivalent to 0.14 mg. of amino nitrogen. The tests are run for a period of 24 hours at an incubation temperature of 37° ± 2° C. No buffer is used. The natural p_H of the mixture is in the neighborhood of 4.5 to 5.

TABLE I.—EFFECT OF HEAT ON PROTEOLYTIC ACTIVITY OF JUICE

Temperature in ° C. to Which the Juice Was Pre-heated for 5 Minutes	Action on *Ascaris* after 24 Hours Incubation at 37° C.	Milligrams of Amino Nitrogen Liberated after 24 Hours from 1 Cc. Gelatin Reaction Mixture
25	Digested	0.24
45	Digested	0.28
50	Digested	0.30
55	Digested	0.24
60	Digested	0.22
65	Slight digestion	0.13
70	No digestion	0.01
75	No digestion	0.00

digestive activity runs parallel to the decrease in gelatin hydrolysis.

Dilution at Which the Juice Will Digest Ascaris.—*Ascaris* is digested at a concentration as low as 15 per cent juice. Worms incubated in solutions containing 10 per cent juice were not digested after 24 hours. Distilled water was used as diluent.

Anthelmintic Activity of Juices from Different Geographical Origins.—Juices obtained from Cuban, Puerto Rican and Mexican pineapples exhibited the same degree of anthelmintic activity *in vitro*, when tests were performed on *Ascaris* worms.

Action of Canned Juice.—*Ascaris* worms incubated at 37° C. with canned pineapple juice were not digested.

DISCUSSION

The fact that pineapple juice has been found to digest intestinal parasites *in vitro* gives some scientific support to the use of this juice as an anthelmintic by the native population of Brazil and India.

It has been experimentally demonstrated that the latexes of some species of Ficus (19), (20), (21) are very effective anthelmintics *in vitro* as well as *in vivo*. Their anthelmintic activity is due to the enzyme ficin which has been recently obtained in the crystalline form (22).

As the pineapple juice acts, *in vitro*, on parasites, in a fashion similar to the latexes, there is a probability that it may have the same effect *in vivo*. However, not until experiments under controlled conditions are performed with animals and human beings can this question be answered definitely.

SUMMARY

1. Fresh pineapple juice digests parasites *in vitro*. The time required for visible signs of digestion to take place is from three to four hours. Digestion takes place on the live parasite.

2. The juice maintains its digestive activity on *Ascaris* at a concentration of 15%.

3. A temperature of 65° C. or above inactivates the enzyme present in the juice.

4. Juice from Cuban, Puerto Rican and Mexican pineapples exhibits the same digestive activity on parasites *in vitro*.

5. Canned juice has no digestive activity on parasites.

REFERENCES

(1) Berger, J., and Asenjo, C. F., *Science*, 90, No. 2335, 299 (1939).

(2) Marcano, V., *Apoth. Ztg.* (1891), quoted in *Polytechn. Notizbl.*, 46, 159, Frankfurt (1891).

(3) Anonymous, *Pharm. Zentralhalle*, 32, 230 (1891).

(4) Anonymous, *Ibid.*, 32, 682 (1891).

(5) Anonymous, *Reichs.-Med.-Anz.*, 153 (1891); through reference No. 4.

(6) Anonymous, *Bull. of Pharm.*, 77 (1891).

(7) Chittenden, R. H., *Trans. Conn. Acad. Sc.*, 8, 281 (1891).

(8) Chittenden, R. H., *J. Physiol.*, 15, 249 (1893).

(9) Vines, S. H., *Annals of Botany*, XIX, 177 and 184 (1905).

(10) Caldwell, J. S., *The Botanical Gazette*, XXXIX, 409 (1905).

(11) Willstätter, R., Grassmann, W., and Ambros, O., *Z. Physiol. Chem.*, 151, 286 (1926).

(12) Ambros, O., and Harteneck, A., *Ibid.*, 181, 24 (1929); 184, 93 (1929).

(13) Tanaka, S., *J. Agr. Chem. Soc. Japan*, 454–455 (1930).

(14) Maschmann, E., *Z. Physiol. Chem.*, 228, 141 (1934).

(15) Bergmann, M., Fruton, J. S., and Fraenkel-Conrat, H., *J. Biol. Chem.*, 119, 35 (1937).

(16) Bergmann, M., and Fraenkel-Conrat, H., *Ibid.*, 119, 707 (1937).

(17) Peckolt, T., and Peckolt, G., *Historia das Plantas Medicinaes e Uteis do Brazil*, I, 178, Rio de Janeiro (1888).

(18) Nadkarni, K. M., *The Indian Materia Medica*, 62, Bombay (1927).

(19) Robbins, B. H., *J. Biol. Chem.*, 87, 251 (1930).

(20) Robbins, B. H., and Lamson, P. D., *Ibid.*, 106, 725 (1934).

(21) Caldwell, F. C., and Caldwell, F. L., *Am. J. Trop. Med.*, 9, 471 (1929).

(22) Walti, A. J., *J. Biol. Chem.*, 119, *Sc. Proc. Soc. Biol. Chem.*, xxxi, CI (1937).