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STUDIES ON THE EFFECT OF CALAMANSI (*CITRUS MITIS*) JUICE ON THE PRESERVATION OF SUAHE OR SHRIMPS (GENUS *PENAEUS*)

By

**S.V. BERSAMIN, A.S. LEGASPI
and N.G. MACALINCAG**

ABSTRACT

Two sets of experiments (packing in calamansi juice ice, dipping in calamansi juice and packing in ordinary ice) conducted to determine the efficacy of calamansi (a type of citrus fruit similar to lemon) juice in prolonging the freshness of shrimps showed that calamansi juice inhibited the growth of micro-organisms on the flesh of shrimps, retarded proteolytic decomposition which was usually characterized by the development of fishness, blackening of the head and tail and loosening of shells, and inhibited development of strong off-odors.

INTRODUCTION

One of the major problems in the commercial handling of fishery products is the prevention of the deteriorative changes which make them unacceptable to the discriminating consumers. Fish starts to deteriorate immediately after catching, and no method of preservation has yet been devised to keep them absolutely fresh. For this reason, it is necessary that some precautionary measures be taken in order to slow down the deteriorative changes that take place in fish and other fishery products. Deterioration in fish is primarily due to enzymes and bacteria present in them. Among the various methods of preservation, icing is the most practical, especially if the fish is to be sold fresh. However, icing preserves fishery products for only a limited time, especially when fish is subject to too much handling and transporting.

* Presented at the IPFC 10th Session, 1962.

The accompanying experiment aims to improve the icing method now being employed by commercial handlers of fishery products, through the use of an inexpensive native fruit, *Citrus microcarpa* Bunge or *Citrus mitis*, commonly known as calamansi. The tree is widely cultivated in the Philippines and bears fruits the year round. At a certain season it grows in abundance and becomes very cheap. It is readily available in most markets and stores in the Philippines.

Analysis of calamansi (per 100 grams):*

Edible portion, 40%	
Moisture, g.	89.9
Fat, g.	0.6
Carbohydrates, g.	0.3
Ash, g.	8.9
Calcium, mg.	22.0
Phosphorus, mg.	12.0
Iron, mg.	1.5
Sodium, mg.	2.4
Potassium, mg.	800.0
Thiamin, mg.	0.02
Rivoflavin, mg.	0.01
Niacin, mg.	0.01
Ascorbic acid, mg.	40.00
Citric acid	3.6-3.7 g./100 g. of juice.

The Philippine calamansi is the counterpart of lemon in other countries. Calamansi and lemon have more or less the same acid contents (ascorbic and citric) which are responsible for their preservative effect.

In a detailed study on the chemistry of shrimp which was published by the Sunkist Growers, Ontario, California, ("The Effect of Lemon Juice in Shrimp Processing"), the factors associated with the aging of shrimp and the breakdown of shrimp tissues resulting in a strong fishy ammoniacal odor were determined. The properties of lemon juice which make it an effective preservative were explored and conclusions were made that: lemon juice is an effective anti-

* Data supplied by the Food and Nutrition Center and the Bureau of Plant Industry, Manila.

oxidant and a buffered acidic system capable of great dilution; it inhibits the growth of micro-organisms which affect shrimp flesh and that lemon juice retards the enzymatic action producing the darkening of sea-foods associated with proteolytic breakdown. Other conclusions were: certain ingredients in lemon juice are good sequestering agents and will suppress the effects of metals such as iron and copper in promoting unwanted chemical reactions in sea foods; lemon juice neutralizes or keeps lobster or shrimps from becoming alkaline thus preventing the disagreeably strong "fish-like" odors from developing; certain combinations of ingredients in lemon juice contribute to the freshness and flavor enhancement in seafoods; and that lemon juice has a very low surface tension which makes possible rapid penetration in solid foods and increase its effectiveness.

An experiment done by Luther and Grawal (1946) revealed that citric acid and ascorbic acid added to apples retained their natural color. The experiment also revealed that citric acid inactivates the enzymes of the fruit. The combined use of citric and ascorbic acids produced improved anti-oxidant properties over citric acid alone. This natural combination of citric and ascorbic acids are found in the local calamansi.

In another experiment conducted by Watts and Techmann (1952) on the use of ascorbic acid on meat, it was revealed that the addition of ascorbic acid to refrigerated ground meat resulted in the "brightening" of the meat surfaces after a few days storage in the refrigerator.

Costilow (1955) states that ascorbic acid in 0.1 per cent concentration was found to have an inhibitory effect on the strains of *Pseudomonas* sp. Growth of micro-organisms in a medium containing ascorbic acid is inhibited by the increase in acidity and release of hydrogen peroxide in the medium.

Faulkner *et al* (1954) states that "black spots" is a condition in shrimp characterized by the development of a black coloration which starts from the head and spreads to the tail, where it forms black bands outlining the sections of the tail region. Unless they are iced immediately, blackening may begin within a few hours after death of the shrimps. However, even with continuous icing, blackening may be far advanced before the catch can be marketed. This is a predicament usually encountered in the handling of shrimps in the Philippines.

Hardening and darkening of the cuticles of lobsters were due to the enzyme tyrosinase. Fieger (1951) presented the first evidence for the enzymatic nature of the blackening reaction in shrimps. Fieger established that bacteria was not instrumental in bringing about blackening. If it could be definitely established that blackening in shrimp is due to the enzyme tyrosinase, measures found effective in inhibiting the enzyme in other sources might be adopted to control the black spots in shrimps. The work herein presented was undertaken to obtain further evidence for the enzymatic nature of the black spots and to explore methods of retarding the reaction in shrimps.

It is surmised that calamansi which is a cheap source of ascorbic and citric acids, hence a reducing agent, would be a controlling measure for the retardation of darkening in shrimps. Refrigerated shrimps may be able to retain their quality when covered by lemon juice solutions. From the work of Faulkner (1954), it was found that ascorbic acid and sodium ascorbate in concentrations ranging from 0.05% to 0.5% were effective in preventing darkening of cuticle extracts in the presence of added phenolic substrate as well as inhibit blackening of heads or whole shrimps as long as the latter remain in solution. In another instance, lowered pH of shrimp medium decreases the activity of the enzyme tyrosinase.

The foregoing experiments tend to prove that calamansi juice lengthens the freshness of refrigerated shrimps.

EXPERIMENTAL METHODS

Seven hundred pieces of ripe calamansi fruits and 4 kg. of shrimps or suahe (*Penaeus*) were purchased from a local market. The juice was extracted from the fruits and the various concentrations needed in the experiment were made using tap water as the diluent. Calamansi ice was prepared by mixing one part of calamansi juice to forty parts of water and placed in a deep freezer where the solution was converted to ice. The shrimps were washed thoroughly with tap water, drained and divided into four batches, and the initial total bacterial count taken from each batch. The first batch was packed in ordinary ice to serve as control. The second batch was packed in calamansi ice (1:40 calamansi solution frozen into ice). The third batch was dipped in calamansi solution (1:20) for a period of 10 minutes and packed in ordinary ice.

The total bacterial count (TBC) of the different experimental batches were taken at periodic intervals. Organoleptic observations on raw and cooked samples were also made daily throughout the period of the investigation.

Two sets of experiments were made. The first aimed at finding the concentration of calamansi solution for dipping and the proportion of calamansi juice in the preparation of calamansi in preserving the freshness of shrimp. The second experiment served to confirm the result of the first. Due to the toughening effect, which was noticed after the seventh day of observations of calamansi ice on the shrimp, the confirmatory experiment was altered.

Experimental groups for the confirmatory experiment were the following:

Batch No. 1 was packed in ordinary ice (control).

Batch No. 2 was dipped in a 1:30 calamansi solution and packed in ordinary ice.

Batch No. 3 was dipped in a 1:20 calamansi solution and packed in ordinary ice.

Batch No. 4 was dipped in a 1:10 calamansi solution and packed in ordinary ice.

The TBC and organoleptic observations on raw and cooked samples were made daily on the experimental batches. The shrimps used in this experiment having been purchased from a local market were not in a very fresh state. The samples were refrigerated at a temperature ranging from -5.0°C to 8.0°C . Ice was replaced when melted. Proximate chemical analyses were made on the samples before and after the completion of the experiments.

For purposes of identification a code was made to represent the various sample batches used in the two experiments:

Batch No. 1	C	C
Batch No. 2	CI	C ₃₀
Batch No. 3	D	C ₂₀
Batch No. 4	DCI	C ₁₀

RESULTS AND DISCUSSIONS

The results are shown in Tables I, II, III and IV, and Fig. 1 and 2.

TABLE I
The Effect of Dipping in Calamansi Solution and the Use
of Calamansi Ice on the Total Bacterial Count and Physical
Appearance of Shrimps

Condition of Storage	Number of days stored	pH of melted ice	Total Bacterial Count	ORGANOLEPTIC		
				RAW		
				Color	Texture	Odor
As it comes from the market	0		35,125	Brownish	Loose	Good (fresh odor)
Immediately after dipping for 10 min. in calamansi solution	0			Brownish	Firm (improved)	Good
Control	1	7.8	120,072	Brownish	Firm	Smells like sea water.
CI	1	5.0	19,000	"	"	Good
D	1	7.6	153,670	"	"	Good
DCI	1	5.0	62,000	"	"	Odor of calamansi perceived
Control	2	8.0	37,212	Brownish	Firm	As of fresh shrimp
CI	2	5.0	29,210	"	"	"
D	2	7.8	17,780	"	"	"
DCI	2	5.0	35,560	"	"	"
Control	4	8.2	77,212	Head portion becoming dark	Firm	As of fresh shrimp
CI	4	5.0	61,845	Brownish	"	"
D	4	8.2	82,550	"	"	"
DCI	4	5.0	46,343	"	"	"
Control	7	8.2	119,650	Head portion becoming dark	Firm	As of fresh shrimp
CI	7	4.8	77,310	Brownish	"	"
D	7	8.2	115,170	"	"	"
DCI	7	4.8	100,330	"	"	"
Control	8	8.4	97,512	Head portion becoming black	Shell becoming loose	Fishiness perceived
CI	8	4.4	100,965	Brownish	Firm	As of fresh shrimp
D	8	8.4	83,500	"	Shell becoming loose	Fishiness perceived
DCI	8	4.4	124,727	"	Firm	As of fresh shrimp

TABLE I Continued

Condition of Storage	Number of days stored	pH of melted ice	Total Bacterial Count	ORGANOLEPTIC		
				RAW		
				Color	Texture	Odor
Control	9	8.4	137,795	Head portion darker	Soft	Fishiness more pronounced
CI	9	4.4	83,058	Pinkish	Tough	Shrimp odor no longer perceived
D	9	8.4	83,500	Brownish	Firm	Fishiness is perceived
DCI	9	4.4	61,087	Pinkish	Tough	Shrimp odor no longer perceived
Control	11	8.4	107,101	Head portion darker	Soft	Ammoniacal odor perceived.
CI	11	4.4	81,242	Pinkish	Tough	Shrimp odor no longer perceived
D	11	8.4	27,967	Brownish	Firm	Ammoniacal odor perceived
DCI	11	4.4	34,744	Pinkish	Tough	Shrimp odor no longer perceived
Control	14	8.5	1,543,050	Head portion becoming dark	Soft	Ammoniacal odor developed
CI	14	4.2	619,587	bluish	Tough	Odorless
D	14	8.5	742,950	Brownish	Firm	Ammoniacal odor developed
DCI	14	4.2	128,807	Bluish	Tough	Odorless
Control	16	8.5	7,543,800	Head portion becoming dark	Soft	Ammoniacal odor more intense
CI	16	4.0	Not sampled	Bluish	Tough	Odorless
D	16	8.5	565,785	Brownish	Firm	Ammoniacal odor perceived
DCI	16	3.8	Not sampled	Bluish	Tough	Odorless
Control	17	8.6	8,045,450	Head portion blacker	Soft	Ammoniacal odor more intense
CI	17	4.0	Not sampled	Bluish	Tough	Odorless
D	17	8.5	2,171,700	Head portion becoming black	Soft	Ammoniacal odor perceived
DCI	17	3.8	Not sampled	Bluish	Tough	Odorless
Control	18	8.6	49,277,600	Head portion blacker	Soft	Ammoniacal odor perceived
CI	18	4.0	Not sampled	Bluish	Tough	Odorless
D	18	8.5	7,610,475	Head portion becoming black	Soft	Ammoniacal odor perceived
DCI	18	0.8	Not sampled	Bluish	Tough	Odorless
Control	21	8.6	47,777,400	Head portion blacker	Soft	Ammoniacal odor more intense
CI	21	3.8	Not sampled	Bluish	Tough	Odorless
D	21	8.5	28,862,000	Head portion becoming black	Soft	Ammoniacal odor more intense
DCI	21	3.8	Not sampled	Bluish	Tough	Odorless

TABLE II

Proximate Chemical Analysis of Shrimp Samples Representing the Five Experimental Groups in Table I after Seventeen Days of Storage

Treatment and Condition of Storage	Moisture, %	Protein, %	Ash, %	Fat, %
Initial	78.93	19.51	1.37	1.03
(Control) C	87.44	10.20	0.612	0.808
(Dipped) D	87.31	10.68	0.527	0.206
CI	75.92	20.71	0.525	1.02
DCI	75.45	19.08	0.555	1.117

TABLE III

The Effect of Dipping in Different Concentrations of Calamansi Solution and the Use of Ordinary Ice on The Total Bacterial Count and Physical Appearance of Shrimps

Treatment and Condition of	Number of days stored	pH of water	Total Bacterial Count/g.	ORGANOLEPTIC		
				RAW		
				Color	Texture	Odor
As it came from the market.	0		175,580	As of fresh shrimp Brownish black	Firm	As of fresh Shrimp
C	1	7.8	149,897	As of fresh shrimp Brownish black	Firm	As of fresh shrimp
C ₃₀	1	7.7	175,440	"	"	"
C ₂₀	1	7.6	103,362	"	"	"
C ₁₀	1	7.4	133,604	"	"	"
C	2	8.0	528,955	Brownish black	Firm	As of fresh shrimp
C ₃₀	2	8.0	527,050	"	"	"
C ₂₀	2	8.0	342,900	"	"	"
C ₁₀	2	8.0	330,200	"	"	"
C	3	8.2	116,205	Brownish black	Firm	As of fresh shrimp
C ₃₀	3	8.2	79,000	"	"	"
C ₂₀	3	8.2	111,760	"	"	"
C ₁₀	3	8.1	107,950	"	"	"
C	6	8.2	182,765	Head portion slightly pinkish Brownish black	Firm	As of fresh shrimp
C ₃₀	6	8.1	121,722	"	"	"
C ₂₀	6	8.1	103,505	"	"	"
C ₁₀	6	8.0	92,055	"	"	"
C	7	8.3	440,055	Pinkish, head portion with black spots Brownish black	Soft	As of fresh shrimp
C ₃₀	7	8.2	101,925	"	"	"
C ₂₀	7	8.2	98,760	"	Firm	"
C ₁₀	7	8.1	103,610	"	"	"
C	8	8.3	312,204	Pinkish, head portion with black spots Brownish	Soft	Fishiness perceived
C ₃₀	8	8.3	121,920	"	"	"
C ₂₀	8	8.2	71,325	"	Firm	As of fresh shrimp
C ₁₀	8	8.1	88,090	"	"	"

TABLE III Continued

Treatment and Condition of	Number of days stored	pH of water	Total Bacterial Count/g.	ORGANOLEPTIC		
				RAW		
				Color	Texture	Odor
C	9	8.4	819,150	Pinkish, head portion with black spots	Soft, shell becoming loose	Ammoniacal odor perceived
C ₃₀	9	8.4	1,060,450	"	"	"
C ₂₀	9	8.3	1,428,750	Head portion pinkish kish	Firmness ceased	Good
C ₁₀	9	8.2	404,495	"	"	"
C	10	8.5	1,727,200	Pinkish, head portion with black spots	Soft, shell becoming loose	Ammoniacal odor perceived
C ₃₀	10	8.5	450,850	"	"	"
C ₂₀	10	8.4	439,864	Slightly pinkish	Slightly soft	Fair
C ₁₀	10	8.4	414,337	"	"	"
C	12	8.7	3,136,900	Pinkish, head portion with black spots	Soft, shells becoming loose	Ammoniacal odor more pronounced
C ₃₀	12	8.7	2,543,300	Head portion with black spots	Soft, shells becoming loose	"
C ₂₀	12	8.6	590,550	Slightly pink	Soft	Fair
C ₁₀	12	8.6	717,550	"	"	"
C	13	8.7	No Sampling	Pinkish, head portion blackish	Soft, shells loosened	Ammoniacal odor more pronounced
C ₃₀	13	8.7		"	"	"
C ₂₀	13	8.6		"	"	"
C ₁₀	13	8.6		"	"	"
C	14	8.7	1,371,600	Pinkish, head portion blackish	Soft, shells loosened	Ammoniacal odor more pronounced
C ₃₀	14	8.7	1,060,450	"	"	"
C ₂₀	14	8.6	1,358,900	"	"	"
C ₁₀	14	8.6	908,050	"	"	"
C	15	8.7	No Sampling	Pinkish, head portion blackish	Soft, shells loosened	Ammoniacal odor more pronounced
C ₃₀	15	8.7		"	"	"
C ₂₀	15	8.7		"	"	"
C ₁₀	15	8.6		"	"	"
C	16	8.7	3,571,875	Pinkish, head portion blackish	Soft, shells loosened	Ammoniacal odor more pronounced
C ₃₀	16	8.7	3,657,600	"	"	"
C ₂₀	16	8.7	4,386,250	"	"	"
C ₁₀	16	8.6	692,150	"	"	"

TABLE IV

Proximate Chemical Analysis of Shrimp Samples Dipped in Calamansi Solution then stored for Sixteen Days in Ordinary Ice

Treatment and Condition of Storage	Moisture, %	Protein, %	Ash, %	Fat, %
Initial	76.37	18.62	1.797	0.832
C ₂	81.83	9.11	1.37	0.432
C ₃₀	82.22	9.02	2.70	0.157
C ₂₀	83.85	9.14	2.65	0.115
C ₁₀	84.42	11.30	1.26	0.20

Experiment 1 showed the effect of dipping shrimps in 1:20 calamansi solution and the use of calamansi ice for packing on the preservation of shrimp. The length of the experimental period was 16 days after which a proximate chemical analysis was made on all the samples. The control samples showed signs of deterioration such as blackening of the head and tail, loosening of shells, and development of fishiness characteristic of shrimp spoilage after the third day. The protein content showed a marked reduction from 19.51% to 10.20%. Signs of spoilage were evident on the samples dipped in 1:20 calamansi solution and packed in ordinary ice after the eighth day. The protein content was reduced to 10.68%. The samples which were packed in calamansi ice and those dipped in 1:20 calamansi solution and packed in calamansi ice showed no signs of spoilage up to the seventh day. On the eighth day, the shrimp meat was observed to toughen and to turn pink. The normal shrimp odor was no longer perceived. On the ninth day deteriorative changes began to take place and on the sixteenth day, they were positively spoiled. The protein content was more or less maintained.

Experiment 2 showed the effect of different kinds of dips without packing in calamansi ice on shrimps. This procedure was done to eliminate the toughening effect of calamansi ice on shrimps with the hope that the protein content would also be maintained. The control samples showed evidences of spoilage after the third to the fifth day and the protein content was reduced from 18.62% to 9.11%. The samples dipped in 1:30 calamansi solution were considered fresh up to the 6th day. The protein content was reduced from 18.62% to 9.02%. Those samples which received 1:20 and 1:10 dips were considered fresh up to the eighth day. On the ninth day deteriorative characteristics began to be evident such as pink discoloration of the flesh, development of black spots on the head and tail, loosening of shells and the development of an ammoniacal odor. Protein content in the C₂₀ batch was reduced to 9.14% and that of the C₁₀ batch went down to 11.30%. No toughening effect was observed in all the samples.

Calamansi juice in any form applied to shrimps was capable of lowering the total bacterial count as seen in the accompanying tables and graphs. The characteristics of spoilage in the raw samples were still evident after they had been cooked.

Organoleptic examination of cooked samples of Experiment 1 showed no difference in taste, color, texture and odor until the fifth

day of observations, after which the head portion of the control samples developed blackish discoloration. However the taste, texture and odor were normally maintained. On the eighth day, samples that were packed in ordinary ice (dipped and not dipped in calamansi solution) became tough, lost the shrimp taste and calamansi taste was perceived. Samples that were dipped in calamansi solution packed in ordinary ice maintained normal characteristics within a period of 12 days.

Similarly, another organoleptic examination made on cooked samples of Experiment 2 showed no difference in taste, odor, color and texture within a period of 8 days. On the ninth day the head portion of the control samples and samples dipped in 1:30 calamansi solution developed blackish discoloration and the taste became flat. Samples dipped in 1:20 and 1:10 calamansi solution maintained their normal characteristics within 11 days. On the 15th day all samples tasted flat with significant off-odors.

SUMMARY AND CONCLUSIONS

Two experiments were conducted to determine the efficacy of calamansi juice in prolonging the freshness of shrimps. The first consisted of dipping the shrimp samples with a 1:20 calamansi solution and packed with calamansi ice formed by freezing 1:40 calamansi solution. The second consisted of dipping the shrimp samples in different concentrations of calamansi solution and then packed with ordinary ice.

In Experiment 1, the controls were considered fresh up to the third day while those which were dipped in a 1:20 calamansi solution and packed with ordinary ice showed signs of deterioration, blackening of the head and tail, loosening of shells and development of a fishy odor, characteristic of shrimp spoilage after the eighth day. A proximate chemical analysis revealed that the protein content was reduced to almost one half of the initial protein content. The samples which were packed with calamansi ice together with those which were dipped in a 1:20 calamansi solution and then packed with calamansi ice showed no signs of deterioration up to the seventh day; however, after the eighth day, there was pink discoloration and toughening of the flesh. This characteristic was also evident in the cooked samples. After this period, the shrimp odor was no longer perceived. However, the protein content was maintained. All the samples which

received a calamansi treatment, in the form of a dip, of calamansi ice and a combined treatment of both, showed a reduced TBC as compared with the control samples.

Experiment 2 was planned to eliminate the toughening effect of calamansi ice. Three batches of shrimp samples were dipped in different concentrations of calamansi solution. They were all packed with ordinary ice. The control samples showed signs of deterioration after the third to the fifth day. Those dipped in a 1:30 calamansi solution indicated signs of spoilage after the 6th day and those which were treated with 1:20 and 1:10 solutions, after the eighth day. No toughening was observed in all these samples.

In all cases, the TBC of samples treated with calamansi juice was lower than those which were not treated. Proximate chemical analysis showed that there was a loss in the protein content in all shrimp samples; however the samples treated with higher concentrations of the calamansi solution showed a lower reduction of protein content.

The use of calamansi ice is more effective than the use of ordinary ice, if applied for short periods. Calamansi juice, because of its ascorbic and citric acid content was capable of retaining and maintaining the physical freshness and protein content of the shrimp. However, the latter is dependent upon the treatment to which the shrimp samples are subjected. Calamansi juice inhibits the growth of micro-organisms on the flesh of shrimps. Results of the experiment indicate that there was a significant reduction in the TBC of those samples dipped in calamansi solution. Proteolytic decomposition which is usually characterized by the development of fishiness, blackening of the head and tail and loosening of shells was retarded. The acid content of calamansi juice neutralizes the flesh of shrimp and prevents the development of strong off odors. Improvement in the flavor was noted in the cooked samples. Enzymatic activity was retarded because calamansi juice has the property of lowering the pH.

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Fig. I. Showing the effect of dipping in Calamansi solution and the use of Calamansi ice on the total bacterial count of Shrimps

TOTAL BACTERIAL COUNT
IN MILLIONS

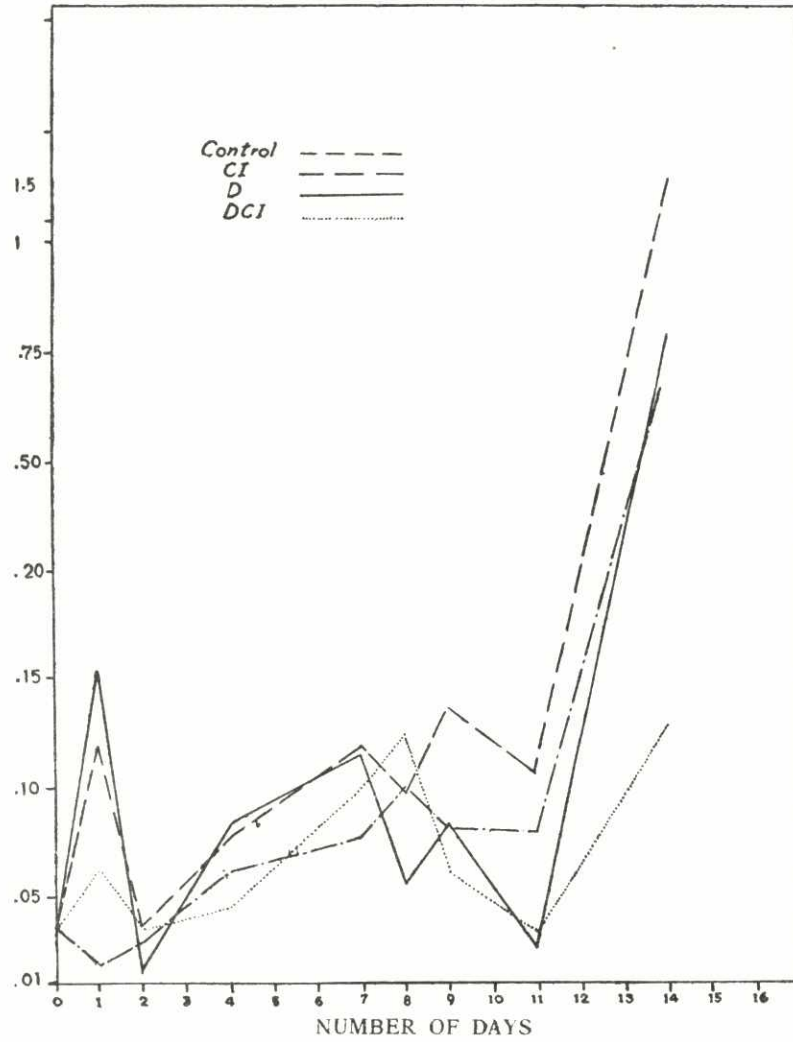


Fig. II. Showing the effect of dipping in different concentrations of Calamansi solution and the use of ordinary ice on the total bacterial count of Shrimps

TOTAL BACTERIAL COUNT
IN MILLIONS

