EFFECT OF DEPURATION ON THE MEAT QUALITY OF THE MARINE CLAM *SUNETTA SCRIPTA* (LINNE)

By

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THESIS

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DEPARTMENT OF PROCESSING TECHNOLOGY
COLLEGE OF FISHERIES
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1998

Dedicated to
My
Husband and Daughter

DECLARATION

I hereby declare that this thesis entitled " EFFECT OF DEPURATION ON THE MEAT QUALITY OF THE MARINE CLAM *SUNETTA SCRIPTA* (LINNE)" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other university or society.

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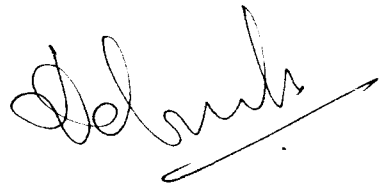

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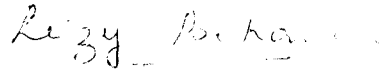

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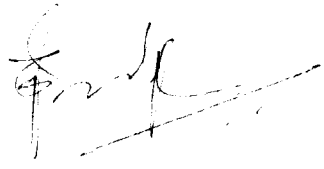

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1. INTRODUCTION

Seafood resources are dwindling at a fast rate all over the world. Although many reasons are attributed to the depletion of different stocks, over exploitation and pollution are the main reasons.

The industry has been hitherto depending largely on few varieties like shrimps, squids, cuttlefish, pomfret, seer fish etc. which earn high foreign exchange and fetch handsome returns in domestic markets. Hence it is the need of the hour to exploit many unutilised and under-utilised varieties which are copious in availability inorder to bridge the gap between demand and supply in Seafood Industry.

Molluscan fishery resources of Indian sub continent are constituted by different varieties of edible and nonedible species found in different kinds of habitats. Bivalves such as clams, mussels and oysters play a vital role in India's economy and their popularity is increasing due to their delicacy and food value.

Substantial quantities of clams are reported to be harvested in India's coastal waters, brackish waters and estuaries. Traditionally, these molluscs were harvested solely for the lime industry. Atleast 10 tons of shells are crushed daily in each of several stations in India. The rural population who were mainly engaged in this trade could find no use for the meat and was discarding it. An estimated quantity of some 1200 tons of meat from 24000 tons of raw material is reported to be wasted annually in this way. (Shenoy and Day, 1984).

Considering the vast resource potential of clams, efforts were stepped up to promote the use of this under-utilised commodity. Frozen clam meat samples were prepared and tested for product acceptability in prospective export markets. The first shipment was made to Japan in September 1981. The clam freezing and canning industry have shown a phenomenal growth in India since 1980. The country today is one of the leading exporters of clam meat as frozen, canned and pickled products to different countries. The export of clam meat started with meagre 15t in 1981 and now an average of 680t is being exported annually.

Since clams are filter feeders dwelling on muddy /sandy bottom their intestines are often loaded with mud or sand besides bacteria. The gut contents impart a muddy flavour and grittiness to the meat if retained within. Therefore a cleansing process should precede processing of clam meat.

Shell fish such as oyster and clams are filter feeding organisms and can accumulate under optimum conditions pathogenic bacteria and virus at concentrations several fold greater than their ambient sea water environment. If such shellfish are consumed raw and or improperly cooked, they may cause a risk of bacterial or viral illness . Studies by Vasconcelos (1969) shows that the number of heterotrophic bacteria in bivalve shell fish is greater than that in surrounding water.

Shellfish have a well documented history of causing food poisoning and transmitting human disease. These conditions include typhoid, paratyphoid, cholera, dysentery and gastroenteritis (Fleet, 1978b).

Between 1900 and 1986 in the United States there were 12,376 documented cases of bivalve shellfish-borne disease. This represents only 5-10 percent of the actual number of cases occurring. Out of the documented cases, 26 percent were typhoid; 11percent Infectious hepatites 11percent Norwalk virus; 2percent vibrio; 7 percent unspecified; and 43 percent gastro-enteritis, food poisoning, diarrhoea etc. (cook, 1991).

The awareness that the shellfish could be purified and thus rendered harmless goes back in history much further than the medieval times. Purification of bacteria contaminated oysters have been effected by utilising their own physiological filtration mechanism (Dodgson, 1928).

"Depuration or controlled purification" is a dynamic process where by live shellfish are allowed to purge contaminants in tanks of clean sea water. Thus this purifying step should be carried out before they are subjected to processing.

Sunetta Scripta (linne) locally known as "kadal kakka" supports a well established commercial fishery in Vypeen Island and in Calicut. During the period from 1986-1990 a total of 14,207.4 tonnes of the clam was landed at Murukkumpadam (Kripa and Joseph, 1992). In Calicut about 240 tonnes of the marine clam were exploited from the bar mouth of Korapuzha in 1992-93. The total catch of *S. scripta* from Fort Kochi during this period was 2,710 tonnes. Studies by Kripa and Joseph (1992) has brought to light that considerable quantity of clam shell was being fished every year, since their thick shell forms the raw

material for the flourishing lime based industries. However meat of the clam is sparingly utilised.

Hence a research programme intended to study the meat quality and to find out whether depuration of clam has any effect on improving bacteriological quality was undertaken which could serve to make it a source of nutritious food to the people and would forerun to explore avenues for the export of processed meat of the *Sunetta scripta*.

2. REVIEW OF LITERATURE

2.1. Nutritive Value of molluscan resources

Among commercially exploited bivalves in India, clams are of most important in terms of quantitative abundance. They are potential source of valuable protein carbohydrate and minerals. Studies on biochemical composition of different species of bivalves have because of their importance as food and their role in economy, received the attention of scientific workers in several parts of the world. Several workers have estimated the organic constituents in whole tissues of many economically important bivalves like *Meretrix casta* (Venkataraman and Chari, 1951; Wafer, 1975; Gopalakrishnan *et al.*, 1977; Salih, 1977; Lakshmanan and Nambisan, 1980) *Villorita cyprinoides* (Nair and Shynamma, 1975; Lakshmanan and Nambisan, 1980; Ansari *et al.*, 1981; Chinnamma and Gopakumar, 1995) *Meretrix meretrix* (Nagabhushanam and Deshmukh, 1976) *Paphia malabarica* (Appukuttan and Aravindan, 1995) etc. Data on biochemical and nutritional evaluation studies of yellow clams were reported by Chinnamma and Mathew (1996). Bivalve meat compares favourably with the common food fishes with regard to their calorific value and hence would be an excellent and economic source of nutrition for our people (Suryanarayanan and Alexander, 1972). Clam muscle is a rich source of protein with all essential amino acids, fat, glycogen and minerals. Carbohydrate content of clam meat is higher when compared to fish. (Chinnamma and Gopakumar, 1995).

2.2. Problems of processing molluscan resources

2.2.1. Microflora of bivalves at harvest

Bivalves due to their filter feeding habit have ability to concentrate micro-organisms from environment in which it lives. (Kelly, 1961; David, 1964; Metcalf and Stiles, 1965; Liu *et al.*, 1966a; Slanetz *et al.*, 1968; Cabelli and Heffernan, 1970a). On ingestion by humans these shellfishes may then become a vector for disease transmission. (Hart, 1945; Dougherty and Altman, 1962; Brown and Dorn, 1977; Gerba and Goyal 1978; Richards, 1988).

This uptake and elimination of micro-organisms are natural consequences of their pumping, feeding and excretory activities. The extent and rates of microbial uptake and elimination are therefore ultimately dependent on number of micro-organisms present in the water and the factors that affect pumping and feeding activities. Under optimal conditions shellfish may filter more than 10 litres/hr, so that micro-organism in surrounding water heavily contaminate the gut region (Wood, 1976).

Bivalves at harvest have a SPC of 10^3-10^5 bacteria/g.(Ayres *et al.*, 1978, Durairaj *et al.*, 1983). These levels are usually 1-2 log units greater than the numbers found in the water from which they are harvested (Vasconcelos, 1969).

Kaysner (1981) reported the incidence of faecal coliforms mainly comprised of E. coli in the clams from Bering Sea. Thison and Fleet (1980) found that edible oysters *C. Commercialis* harvested from major cultivation areas in New South Wales, were contaminated with food poisoning organisms like B. cereus, *Cl.*

perfringens, *V. parahaemolyticus* and *Salmonella*. Metcalf *et al.* (1979) found that clams bioaccumulated faeces associated natural virus mainly in their hepatic pancreas and siphon tissue. Fraiser and Kolwrger (1984) reported the incidence of Salmonella in clams and oysters in Florida. Dea pola *et al.* (1983) reported the occurrence of various strains of V. cholerae in shellfish, sediments and water along the US Gulf coast.

Studies on bacterial flora of edible oysters of Tuticorin water by Durairaj *et al.* (1983) and Pillai and Selvan (1988) shows that pathogenic bacteria were found to be absent except faecal coliforms in all the oysters samples and seawater. Similar results were obtained for mussel *Perna indica* collected from Vizhinjam. (Selvan and Pillai, 1988). The occurrence of enteric pathogen *V.parahaemolyticus* in aquatic organisms was reported by Bose and Chandrasekharan (1976) and Nair *et al.* (1980) from east coast of India. Another published report dealing with enteric pathogens in shellfishes is that of Karunasagar (1982), on the survival of *V. parahaemolyticus* in an estuarine clam. Presence of this bacterium in Cochin backwaters, on the west coast of India was reported by Pradeep and Lakshmanperumal Swamy (1984).

The microflora in the shellfish growing waters are highly variable, responding to temperature, salinity, nutrients and pollution. Therefore, bivalves harvested from various locations or from the same location at different times may show considerable variation in microflora composition and concentration and in turn pose a great risk to human.

2.2.2. Heavy metals and Pesticide residues.

The capacity of bivalves to accumulate potentially toxic heavy metals in their tissues far in excess of environmental levels is well known (Roberts, 1976) and has become the focus of increasing number of studies and restricts their use as food. Bivalves have no system in their body by which they can metabolise/ destroy the absorbed heavy metals and Pesticide residues. Nambisan and Lakshmanan (1979, and 1983) have done extensive investigations in the heavy metal content of molluscan fish and toxicity. International standards have been suggested for maximum limits for heavy metals and pesticide residues in molluscan products.

2.2.3. Shellfish poisonings

Paralytic shellfish poisoning referred to as PSP, is by far the most commonly known and widely distributed form of shellfish poisoning. This term is commonly and uniformly applied for toxins obtained from several species of bivalve molluscs distributed in widely separated areas of the world.

PSP is caused by a neurotoxin produced by certain marine dinoflagellate algae. Mussels, clams, oysters, scallops and whelks become toxic if they feed on toxigenic dinoflagellate. FDA issued a legislation that shellfish with a toxic level of 400 mouse unit/100 gm or more are unsafe for human.

Incidence of PSP has been reported from semitropical and tropical areas of both the hemispheres. India's first documented case of PSP occurred at Vayalar in Chingleput District, Tamilnadu. (Silas *et al.*, 1982). Second outbreak occurred at Arikadu near Kasargod (Karunasagar *et al.* 1984). Results of a 2 year study (1984 -

1988
86) conducted by Segar *et al.* of shellfishes along coast of Karnataka revealed the presence of PSP in some clams and oysters.

Hashimoto (1979) noted that nontoxic bivalves become toxic within a short period when transplanted to a toxic area and toxic bivalves lost their toxicity when maintained in a nontoxic area.

2.3. Accumulation of micro-organisms by shellfishes

Data from periodic assays of individual animals suggested that accumulation of bacteria by quahauogs proceeds to an equilibrium level, which is a function of *E. coli* content of water and its overall particulate matter. Accumulation takes place in digestive gland and to a lesser extent in siphon of animal. *E. coli* accumulation factors for quahauog clams under optimal conditions were observed to be 6.5 to 8.5 and that of soft shell clams was 20 (Cabelli and Heffernan, 1970a). Perkins *et al.* (1980) observed a steady state to be rapidly attained for uptake of coliforms beyond which accumulation in gut does not occur for a given concentration of the bacteria in ambient waters.

They also noticed that maximum levels can be attained in first 6hr by some individuals but prolonged exposure increases the percentage of population before reaching a steady state. Hard clams accumulated 5×10^6 to 1×10^7 CFU of the test organisms during 15-min exposure to water containing 5×10^4 CFU/ml of bacterial suspension. This represented a contamination rate of 1×10^5 to 2×10^5 CFU/gm of clam tissue and was approximately a three fold increase over the bacterial counts per millilitre of water in the exposure tank. (Timoney and Abston, 1984).

Studies of Manzanares *et al.* (1991) on the comparative accumulation of *E. coli S. typhimurium, V. parahaemolyticus, A. hydrophila, S. faecalis, S. aureus,* and Ms-2 coliphage by the striped venus *chamelea gallina* under controlled laboratory conditions showed that micro-organisms accumulated rapidly in bivalves during the first 6hr, with accumulation rates between 3.2 to 360.5 organisms/hr depending on type of micro-organisms.

2.4. Purification of shellfish

Procedures for riding shellfish of micro-organisms of public health importance have been under investigation for centuries and have resulted in the widespread availability of depuration systems that are very effective in removal of microbial contaminants within 36-48h (Wood, 1976).

Depuration is the process of purification of shellfish in which the animals are placed in disinfected recirculating or running seawater and allowed to actively filter feed. This process leads to elimination of bacteria from bivalves (Jones *et al.*, 1991, Cook and Ellender, 1986 Timoney and Abston, 1984). In addition depuration allows sand and grit to be purged from the shell fish gut and thus rendering them more palatable to consumer (Balachandran and Surendran, 1984; Richards, 1991).

The basic principle for controlled purification or depuration of contaminated shell fish involves providing clean or purified sea water in ponds or tanks where by shell fish filter and pump such water for a period of 36-48hr or more if required. The essential components of a depuration system are tanks to hold sea water and a means of producing sterilized sea water. Tank design, size and layout are determined

largely by the number of animals to be cleansed, handling economics and amount of space available. Facilities for water aeration, heating and cooling may also be required depending upon tank design and climatic condition.

Heavily polluted oyster *C. commercialis* were cleansed to acceptable NHMRC (National Health and Medical Research Council of Australia) recommendation of 2.3 *E. coli* Cells/g oysters, which is the generally accepted international standard for depurated animals within 48hr-TPC of oysters generally decreased by a factor of 10 fold or higher depending upon initial microbial load. Tank water counts were reduced from their initial levels and maintained at about 10^3 cells/ml throughout 48hr operation (Souness and Fleet, 1979).

Commercial experiences in a number of countries have shown that a depuration cycle of 36-48hr consistently yields oysters that meet NHMRC standard and have a good public health record (Souness *et al.*, 1979). Son and fleet (1980) had suggested that this depuration time could also be sufficient to give acceptable cleansing of the contaminating levels of *salmonella, B. cereus, V. parahaemolyticus* and *C. perfringens* that are likely to be found in oysters under natural conditions.

Janseen (1974) has noted the persistence of *Salmonella typhimurium* in oysters after depuration for the standard 48h.

Studies by Timoney and Abston (1984) on the elimination of *E.coli* and *S. typhimurium* by hard clams shows that the test bacteria were rapidly eliminated at similar rates for 8hr after exposure and less rapidly thereafter. By 24 hr number of *E.coli* had declined more than *S.typhimurium.* Rodrick *et al.* (1989) subjected clams

artificially infected with *E.coli*, *V. cholerae non o1* and V.vulnificus to ultra violet rays assisted depuration in a laboratory scale system. Reduction in all the three organisms were obtained in both sea water and shellfish meat.

Putro *et al.* (1990) found on experiments conducted with ark, blood cockles and green mussels that after 3 days of depuration on a semi commercial level at 28^0 C, *E. coli* count of animals could be practically brought to zero. However *V parahaemolyticus* count remained constant for all the samples. In studies employing ultraviolet rays assisted depuration no difference were found in the oysters of the encapsulated and non encapsulated morphotypes of *V. vulnificus*. Both types were readily depurated from the oysters, While a naturally obtained microflora was shown not to depurate (Groubert and Oliver, 1994).

The mechanism of microbial elimination seems to be initially of a mechanical nature which is followed by another phase that depends upon the microbial species and their accumulation (Corre, 1990). Using a recirculating type depuration system fitted with UV sterilizer complete elimination of *E.coli* could be achieved within a period of 15hr from an initial concentration of 6.69×10^8 cfu/gm of *V. cyprinoides* (Nambudiri *et al.*, 1995).

There is strong evidence showing that moderate levels of poliovirus depurate within 3 days in hard shell clams *Mercenaria mercenaria* and soft shell clams mya arenaria (Durgin *et al.*, 1981; Liu *et al.*, 1967 a,b), pacific oysters *Crassostrea gigas* (Hoff and Becker, 1969) and Eastern oysters (Akin *et al.*, 1966; Durgin *et al.*, 1981; Hamblet *et al.*, 1969; Liu, 1968; Meinhold and Sobsey, 1982; Mitchell *et al.*, 1966;

Power and Collins, 1986; Sobsey *et al.*, 1987). Longer periods were required for the depuration of manila clams and olympia oysters (Hoff and Becker, 1969).

Results of several studies indicate that the various enteroviruses depurate at different rates even in the same shellfish species. Although inconclusive, evidence points to rapid depuration of poliovirus and slow depuration of cox sackle virus Type B4, Hepatitis A virus, and Coliphage (Liu et al 1966 b).

No review of depuration would be complete without at least a passing mention of depuration in the context of non microbial contaminants. Toxins (Blogoslawski and Stewart, 1983; Blogoslawski *et al.*, 1979), heavy metals (Denton and Burdon Jones, 1981; Okazaki and Panietz, 1981), petroleum hydrocarbons (Fossuto and Canzonier, 1976; Jackim and Wilson, 1977), and radionuclides (Clifton *et al.*, 1983; Dahlgaard, 1981) are difficult to eliminate; they either do not depurate using current procedures or they depurate so slowly that commercial steps to purify them would be uneconomical.

2.5. Water disinfection treatments

2.5.1. Chlorination

Chlorination is the oldest disinfection procedure for depuration waters. Seawater was disinfected by the addition of sodium hypochlorite and residual chlorine was inactivated by sodium thiosulphate addition. Although giving successful cleansing, process required large tanks and great volumes of water. Control of water chlorine levels was necessary, since insufficient chlorination gave inadequate

water disinfection and over chlorination yielded residuals which interfered with shellfish feeding activity and cleansing effectiveness (Kelly, 1961).

Chlorination of the water used in the blower tanks which contained artificially contaminated and shucked oysters with V. cholerae did not eliminate the organisms from the oyster meat (Motes 1982). However, Belmonte and Espinora (1984) Obtained decrease of faecal contamination levels to values significantly lower than international standards in less than 48hr of depuration in chlorinated water.

It was noticed that when water is chlorinated, shell remain tightly closed until such time that available chlorine disappeared from system (Balachandran and Surendran, 1984).

Bacterial qualities of the meat of clam *Villorita cyprinoides*, mussel *Perna indica*, oyster *Crassostrea madrasensis* and clam *Meretrix casta* were considerably improved in the case of treatment with chlorine for 2 hr after depuration in natural water for 24 hr. (Balachandran and Surendran, 1984; Selvan and Pillai, 1988; Mishra and Srikar, 1989) Eventhough chlorine is the cheapest option for sterilisation of seawater it is quickly bound up by organic materials and is difficult to maintain accurately controlled dosage. Concentrated hypochlorite solution can be a dangerous chemical to handle and combination of sodium chloride, chlorine and organic material could lead to build up of chloramines in shell fish (Thrower, 1990).

2.5.2. Ultraviolet disinfection.

It is an effective means of continuously disinfecting large volumes of water rapidly and cheaply. Extensive studies in great Britain by Wood (1961) and in USA

by Kelly (1961) have shown that UV light is most effective in disinfecting seawater for depuration. Ultraviolet light effectively reduces bacteria and viruses (Cheng and Rudo, 1976; Hill *et al.*, 1969) Numerous studies have demonstrated the effectiveness of UV light in reducing microbes in shellfish during depuration (Romagosa Vila, 1957; Vasloncelos and Lee, 1972; Wood, 1961)

Disinfection of seawater is effective provided the water is clear and the exposure time is adequate. High turbidity (Hill, 1967, , Kelly, 1961), dinoflagellate blooms (Nielson *et al.*, 1978), or the gametes produced during shellfish spawning (Buisson *et al.*, 1981; Furfari, 1966) can impair light penetration and reduce the effectiveness of UV light disinfection of seawater. UV light does not produce compounds in seawater which would affect shellfish physiology(HILL, 1970)

A slight decrease in sterilising efficiency is noticed after 48 hr circulation of depuration water and the phenomenon could be explained by a gradual selection of UV resistant bacteria in tank water (Souness and Fleet, 1979)

A great advantage of UV light is low cost and absence of residual taints and odours from chemical residues (Thrower, 1990).

2.5.3. Ozone disinfection

Ozone is a powerful oxidising agent capable of rapidly killing bacteria and virus (Thrower, 1990). Ozone levels of around 2 mg/litre are required for seawater disinfection (Fleet, 1978). Ozone has been successfully used to disinfect seawater for shellfish depuration and several such plants are in operation in France and Spain

(Furfari, 1976). However ozone depuration was inadequate due to rapid ozone decay at very high ambient temperature. (Blogoslawski and Monesterio, 1982).

2.5.4. Iodophore disinfection

Iodine has powerful antimicrobial property and is used in form of idophores as a disinfectant. When idophore was used in a recirculating system at levels between 0.1-0.4 mg/l shellfish cleansed themselves within 10hr . There was no reported adverse effects on shellfish feeding activity or on edibility characteristics for shellfish subjected to iodophor- disinfected water (Fleet, 1978 a)

2.6. Factors influencing depuration process

Depuration is a specialised application of the normal feeding mechanism of the animal and therefore is mediated by such physiological functions such as pumping rate, filtration efficiency, the rate of transport and elimination of materials through the digestive system etc. These functions in term can be influenced by a variety of environmental factors such as the temperature and salinity of water, the nature and quantity of suspended material, level and duration of contaminants in the animals and finally the environmental history of the animals.

Any factor which affects shellfish feeding activity will influence cleansing capability (Fleet, 1978a) The significance of these parameters varies with species and location and tolerable limits of these variables should be determined for each particular case.

2.6.1. Temperature

Maintaining the appropriate water temperature is a critical factor for any successful depuration process. Each shellfish species will pump within a range of temperature which are often substantially different among species.

If the water is too warm shellfish may become stressed or die due to lack of oxygen, if it is too cold they may go into a state approaching hibernation and slowed metabolism and feeding rate will impede the depuration process (Thrower, 1990). Results showed that depuration system conducted at temperature greater than 23^0 C caused *V.vulnificus* counts to increase in oysters. In contrast, when depuration seawater was maintained at 15^0 C, *V. vulnificus* was not detected in seawater and multiplication in oyster tissue was inhibited (Tamplin and Capers, 1992).

Heffernan and Cabelli (1970) found that there was no appreciable effects of temperatures between 10^0 C and 20^0 C in the elimination of *E.coli* by the northern quahog.

Studies were undertaken by Power *et al.*(1990) to determine the effect of temperature. On the efficiency of elimination of *E.coli* and a 2nm isohedral coliphage from experimentally contaminated mussels, *mytilus edulis* and they found that initial *E.coli* levels were reduced by 99% within 52 hr at the test temperature 5.5, 10 and 16.5^0C only.

Rajapandian *et al.* (1988) recommended temperature range between 20 and 30^0C with optimum at 30^0 C for purification of oyster suited to Indian conditions. A

slight increase in temperature from habitat temperature do not affect the pumping of shellfish and hence the purification process.

In USA minimum temperature recommended for depuration is 10^0 C (Furfari, 1966) and in Great Britain depuration is not recommended below 5^0 C (Wood, 1972).

2.6.2 Salinity

Another critical parameter for the successful depuration of shellfish involves the salinity of the seawater.

Although shellfish may be depurated over a wide salinity range depuration could be properly effected in salinity ranges in which species have been orginally thriving. (Rajapandian *et al.*,1988) while most estuarine molluscs can tolerate a variation in salt levels there will be an optimum prefered salinity which may vary with species and habitat (Thrower, 1990).

Rowse and Fleet, (1984) found that purification was clearly ineffective and incomplete at low salinity. In contrast oyster purification was very effective at higher salinites and rates of bacterial cleansing were comparable to those at normal salinities of 33ppt. Depuration was inconsistent and clearly ineffective in *C. irradeli* when salinity values were drowned to 9.9 to 14.4 ppt. Intial MPN levels did not change or even found to increase until 48 hr. However when oysters were exposed to salinity levels of 17.5-31.1ppt depuration was effective where the coliforms were reduced microbiologicaly to safe levels. The minimum salinity for successful depuration by *C. irradeli* based on this study is 17.5 ppt (PalpatLatoc *et al.*, 1986).

At 28.6 ppt *E. coli* was eliminated from mussel *Mytilus edulis* efficiently and rapidly. A final reduction of 3.01 log cycles was achieved within 52 hr. At 18.2 ppt *E.coli* was eliminated efficiently, but less rapidly than during depuration at higher salinity. A final reduction of 2.18 log cycles was achieved within 48h. (Power and Collins, 1986).

A reduction of salinity to 50-60% of original seawater completely stopped the depuration process (Liu *et al.*, 1967a).

2.6.3. Dissolved oxygen

Dissolved oxygen levels must be adequate in depuration tank water for shellfish to remain physiologically active. Recommendations in the U.S. called for DO Levels of 5 mg/L (minimum) to saturation; however a minimum DO of 50% saturation is now in effect (U.S. Public health Service 1987).

The water oxygen levels depend upon water/ oxygen ratio, water temperature and rate of flow of water through the system. With recirculating system it may become necessary to aerate the water supply as oxygen is eventually depleted by oyster activity (Wood,1961; Furfari, 1976) while oxygen concentration below certain limits are detrimental, there is also an upper limit determined by the solubility of oxygen in water which also could be harmful. The solubility of oxygen decreases with rise in temperature and with increase in salinity. When supersaturated water warms, it releases the excess oxygen in the form of bubbles and this in turn can cause death of oyster by embolism (Rajapandian *et al.*, 1988).

The dissolved oxygen levels in water should not fall below 50% saturation during conditions of maximum oyster demand (Fleet, 1978a; Thrower, 1990). Appreciable drops in depuration rates occur below 1.8 mg of dissolved oxygen/litre in oyster (Haven *et al.*, 1978).

2.6.4. Turbidity

Water turbidity can be an important factor in shellfish depuration as excessive turbidity reduces water sterilising effectiveness (Wood, 1961) and may lead to reduced shellfish feeding activity. It may clog the gills of mollusc impairing normal feeding and it can reduce penetration of UV light in sterilising unit. (Rajapandian, *et al.*, 1988).

Heffernan and Cabelli (1970) suggested that turbiditics around 25 Jackson Turbidity units enchanced bacterial depuration in hard shell clams, but not in Eastern oysters (Presnell *et al.*, 1969). Oysters depurated *E.coli* at similar rates at average water turbidites of 8.8, 19.3, 22.6 and 69.7 JTU (Presnell *et al.*, 1969). Total and fecal coliform reductions in manila clams were similar at turbidities of 0,20,43 JTU (Vasconcelos, 1969).

Furfari (1966) recommended turbidites not exceed 20 JTU for all depuration process.

2.6.5. Flow rate

Flow rates affect dissolved oxygen levels in seawater, the rate of water disinfection, and hence the rate of shellfish depuration.

For hard shell clams, a flow rate or 13ml per minute per clam provided optimal depuration of *E.coli* (Heffernan and Cabelli, 1970) and the elimination of *E.coli* was prolonged at flow rates of 3,7, and 27 ml per minute per clam. Soft shell clams effectively depurated at flow rates of 3-24 ml/minute per clam although at 3 ml per minute per clam, mortalities increased (Cabelli and Heffernan, 1970b). Goggins *et al.*, (1964) reported reduced depuration at flow rates of <1.1 l per minute per bushel. Manila clams effectively purged *E.coli* at flow rates of 20 and 30 ml per minute per clam. (Vasconcelos, 1971).

Souness and fleet (1979) compared the number of times water was cycled through UV disinfection units and found that one cycle of tank water per hour gave inadequate depuration, two cycles per hour enhanced depuration rates, whereas three cycles per hour provided optimal depuration efficiencies in their system.

2.6.6. Initial concentration of bacterica

It was shown that purification of lightly polluted shellfish, northern quahaug with virus, was achieved sooner than of heavily polluted ones. Time required to depurate the lightly polluted shellfish to non-detectable level was 24hr and that for heavily polluted one was 72 hr. This finding definitely bears out the original suspicion that the time of depuration is proportional to degree of pollution of shellfish (Liu *et al.*, 1967b)

Depuration effectiveness of soft shell clam M*ya arenaria* depended upon number of virus bioaccumulated and whether virus was closely associated. Level of

initial contamination was one of the major factors influencing success of depuration of pacific oyster *C.gigas* (Buisson *et al.*, 1981; Corre *et al.*, 1990).

3. MATERIALS AND METHODS

3.1 Raw material

The marine clam (*Sunetta scripta*)used for this study was collected from Ayyampilly beach in Vypeen Island, Kochi. Adult specimens with an average size of 3-4 cm in shell length was used for the study.

3.2 Transportation of clams

Live clams were transported to laboratory in a container full of habitat water. Date and time of harvesting, salinity of habitat water etc. were noted.

3.3 Microbiological examination

For initial microbiological analysis, clams were transported in insulated container containing ice. Care was taken to avoid direct contact of ice with shellfish. Preparation of samples for analysis was done according to APHA, 1970. The clams were scraped off all growth, loose materials from the shell and scrubbed with a sterile stiff brush under running tap water. Then they were sterilised with 70% alcohol, after which they were dried, shucked asceptically and flesh 25/g was transferred to sterile blender jar and blended for one minute. The homogenate was diluted in 0.1% peptone water to 10^{-6} .

Total plate count of the sample was determined by pour plate technique. Appropriate dilutions of the homogenate were plated using nutrient agar containing

3% Nacl. The plates were incubated at 37°C for 48 hrs in an incubator and the number of colonies counted.

The concentration of *Escherichia coli* in the sample was determined using a three tube MPN procedure. The clam homogenate was properly diluted with 0.5% NaCl and inoculated in 10ml quantities to 10 ml double strength media and in 1ml and 0.1 ml quantities to 10 ml single strength media. Then all the lactose broth were inoculated at 37°c for 24-48hrs. A small quantity of inoculum from positive tubes of lactose broth were inoculated into tubes of EC broth and incubated at 44.5°c for 24 hrs. Tubes showing gas production and turbidity were streaked on Mac Conkey agar plates and red non-mucoid colonies were subjected to IMVIC tests for confirming them as *E.coli*.

Salmonella isolation was attempted by preenrichment in lactose broth incubated at 37°c for 24hrs, followed by enrichment in selenite cystine broth and tetrathionate broth and isolation on Bismuth Sulphite agar plates. Plates were incubated at 37°c for 24hrs and examined for typical salmonella colonies. They were streaked on to nutrient agar plates for checking the purity and subjected to various biochemical tests to confirm the organism.

Vibrio parahaemolyticus was enumerated by 3 tube MPN procedure. Tubes of seawater yeast extracted broth were inoculated with clam homogenate and incubated at 37°c for 24hrs followed by plating on Thiosulphate citrate bile salt agar plates (TCBS). Suspected colonies of *Vibrio parahaemolyticus* were counted and subjected to confirmation tests such as on TSI agar, methylred, vogus proskauer

test and Indole test, growth in absence of NaCl, growth in presence of 8% and 10% NaCl etc

3.4 Biochemical Analysis

Moisture, protein, fat, ash, acid insoluble ash, glycogen, calcium and phosphorus were determined according to standard procedures.

3.4.1. Moisture

A known weight of sample was taken in preweighed tared crucible and was dried to a constant weight at 105°c in an hot air oven. Moisture was calculated from loss of weight of tissue after drying.

3.4.2. Protein

Total protein was estimated by method of Wooton (1964). Material after digestion with concentrated H_2SO_4 in presence of suitable catalyst was neutralised and treated with Nessler's reagent. Colour produced was read in spectrophotometer.

3.4.3. Lipid

A known weight of sample was extracted with hexane: Isopropanol mixture using method of Radin (1981). Extract made up to known volume using same solvent mixture. A known volume of extract evaporated off to find out percentage of total lipid gravimetrically.

3.4.4. Ash

Ash content was determined by igniting the preweighed sample at 550°c in a muffle furnace until free of carbon, allowed to cool and weighed (AOAC, 1984).

3.4.5. Acid insoluble ash

Acid insoluble ash is a measure of sand and other silicious matter in sample and was determined according to the Indian standards Institution method (IS: 3853-1966).

3.4.6. Glycogen

Glycogen was estimated colourimetricaly using the method of Hassid and Abraham (1957). The tissue was heated with strong alkali to release glycogen and precipited with the addition of ethylalcohol. The polysaccharide was then estimated colourimetricaly using Anothrone-Sulphuric acid.

3.4.7. Calcium

The titrimetric method of Clarke and Collin (1925) was used for determination of calcium. Sample was heated at 55^0C and the Calcium present in the ash was precipitated as oxalate. The amount of calcium was then determined by permanganate titration.

3.4.8. Phosphorus

Phosphorus was determined according to method of Fiske and Subbarao (1925). The phosphorus present in the sample in the form of phosphate reacts with ammonium molybdate which is then reduced by adding amnio naphthol sulphuric

acid to form a blue coloured compound whose intensity is measured colourimetricaly.

3.4.9. Aminoacids

Aminoacids analysis of the sample was carried out using a Technicon - N-C-2P amnio acid analyser.

3.4.10. Yield

$$\text{Meat yeild (\%)} = \frac{\text{Weight of shucked meat}}{\text{Weight of whole clam}} \times 100$$

3.5 Determination of optimum salinity of depuration

Eventhough *Sunetta scripta* can tolerate wide variations in salinity, there is an optimum preferred salinity at which their activity will be maximum. For determining optimum salinity at which the animals shall remain active and pathogens alive following experiments were conducted. Clams were maintained in troughs of water with varying salinities, viz. 15,20,25,30 and 35ppt using seawater. Each tray contained 3 litres of water with 15 animals of 4 replications each. They were observed for irritability and protrusion of siphon at intervals of 10 minute for 1 hour. Irritability was taken as the capacity to respond to stimuli. This was tested by prodding the animals by a glass rod and noting the animals response to it by closing the valves with a jerk.

3.6 Accumulation of *Vibrio parahaemolyticus* by *Sunetta scripta*

Accumulation pattern of *Vibrio parahaemolyticus* by the clams (*Sunetta Scripta)* was monitored by keeping 15 clams each in 3 litre portion of 30 ppt seawater seeded with *Vibrio parahaemolyticus* to a level of 10^9 organisms per ml.

The seawater used was passed through a biological fitter for a week, prior to seeding A set of 10 clams were drawn at 6 hrs interval for estimation of *Vibrio parahaemolyticus* by spread plating on TCBS agar plate and incubuting at 37^0c for 24-48 hrs. The whole experiment was repeated four times.

3.7 Depuration system

The depuration system consisted of a depuration chamber, biological filter and U V steriliser. The depuration chamber was made of fibre glass in which removable meshed platforms are arranged in rows for spreading the clams. The biological filter consisted of a chamber made of fibre glass provided with a perforated platform at the bottom to support sand and gravel. The latter served as the substratum for the attachment and colonisation of nitrifying bacteria. The U.V sterilizer is a simple device used for disinfecting water while passing from the biological filter to the depuration tank. The unit comprises of a rectangular perspex tray mounted on a metallic stand. An U.V tube with a frame fits on to the tray perfectly leaving a clearance of 3 inches from the water beneath.

In order to maintain the biological filter when the equipment was not in use, a by-pass chamber was also provided.

3.8 Depuration of clams seeded with *Vibrio parahaemolyticus*

Suneita scripta were allowed to accumulate *V. parahaemolyticus* for 6 hrs by maintaining them in plastic trays with water containing 10^9 cells of *V. parahaemolyticus* per ml. The animals were transferred to the depuration tank and the water was bye-passed through the side tank to the U.V chamber protecting the

biological filter from being contaminated with the pathogens discharged by the animals. Salinity of water was maintained at 30ppt and the animals were allowed to remain there for 48 hrs. Samples were drawn at intervals of 12 hrs and analysed for residual level of organism.

3.9 Determination of biochemical changes during depuration

50 clams which were not seeded with micro-organism were arranged in the removable mesh platforms of depuration chamber and allowed to depurate for 48 hrs. Samples were drawn at oh, 12h, 24h, 36h and 48h to find out changes in protein, ash, acid insoluble ash (sand) and glycogen content of the animal.

3.10 Sensory evaluation of depurated animals

Sensory evaluation was done by a panel of 10 judges, according to the method of (Judith, 1973). Samples were coded and presented to the judges. The judges evaluated the quality characteristics of the raw samples for odour and cooked samples for attributes like aroma, flavour and grittiness. Odour characteristic was assessed using a 10 point hedonic scale ranging from extremely fresh(10) to putrid(0) flavour and aroma ranging from excellent (10) to bad (0) for raw and cooked clam. Sensory evaluation of clam was carried out before depuration, after 24 hrs depuration and after 48 hrs depuration. Grittiness characteristic was assessed using a 5 point hedonic scale for cooked samples ranging from no sand content (5) to extremely gritty (0). A sample of the score sheet is show in Appendix 1.

3.11 Determination of water quality parameters

Before and after each depuration water samples from depuration tank were taken and the following parameters were analysed using the methods mentioned against each.

Temperature - using a graduated mercury thermometer with an accuracy of 0.1^0c

Dissolved oxygen - Winkler's method (Stickland and Purson, 1968).

Salinity - Salino-refractometer

3.12 Statistical Analysis of Data

The experiments were carried out using Completely Randomised Design (CRD). Data obtained from studies were analysed using Analysis of variance technique (Snedecor and Cohran 1968) and treatment differences studied at 5% level of significance. Pair wise comparison of treatment values were done wherever necessary using critical difference based on students 't' test at 5% level of significance.

Data on bacterial population were subjected to logarithmic transformation before analysis to contain the high variations present.

4. RESULTS

4.1. Microbiological analysis

Clams (*Sunetta scripta*) collected were transported live to laboratory in sterile containers and immediately examined for total bacterial count and pathogens such as *Escherichia coli*, *Salmonella* and *Vibrio parahaemolyticus*. The total viable count of clam was 1.64×10^3/ gm and that of the seawater was 4.4×10^2/ml . Pathogenic bacteria were found to be absent except *Escherichia coli*. *E.coli* in clams was found to be 2/g and in seawater to the 11/100ml.

4.2. Biochemical analysis

Results of biochemical analysis are presented in Table 1 and Table-2

4.3. Determination of optimum salinity of depuration

The optimum salinity level of water for the depuration of clams was determined on the basis of irritability response and protrusion of siphon exposing for one hour to varying salinity levels such as 15,20,25,30 and 35 ppt.

Irritability responses of *Sunetta scripta* maintained at various salinities are shown in Table 3

Analysis of variance of the data indicated that these salinities were significantly different ($p\leq0.05$) from one another as far as irritability response was concerned. Pair wise comparison of treatment means showed that the animals

responded maximum within short time at 30 ppt salinity. This is shown in Table (4 and fig (1).

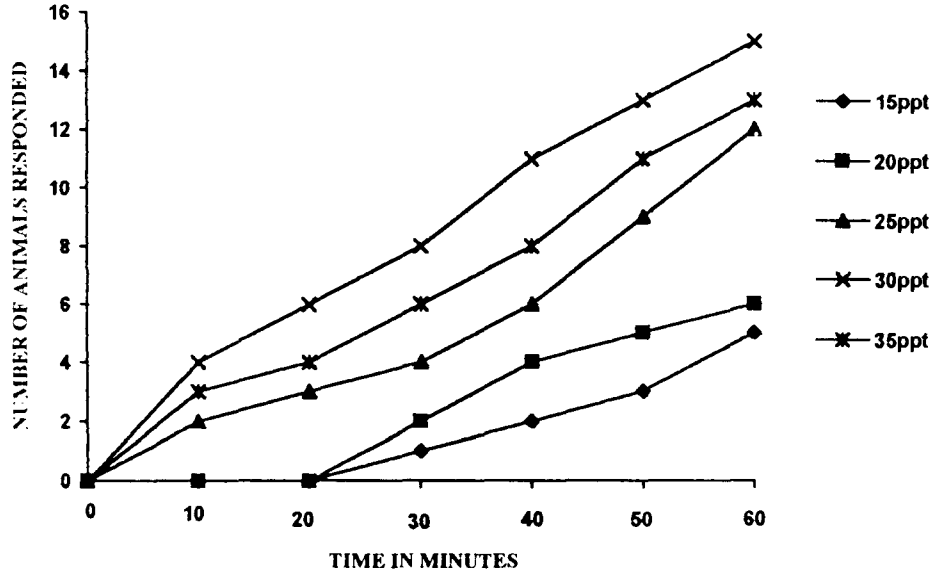
Protrusion of siphon as a response to different salinities level were studied and results given in Table (5). Analysis of the data showed that protrusion of siphon at each level of salinity was significantly different ($p \leq 0.05$) from one another (Table-6). Pair wise comparison of the treatment means show that protrusion of siphon was maximum at 30 ppt salinity.

Table-1 Biochemical Composition of *sunetta scripta* (on wet weight basis)

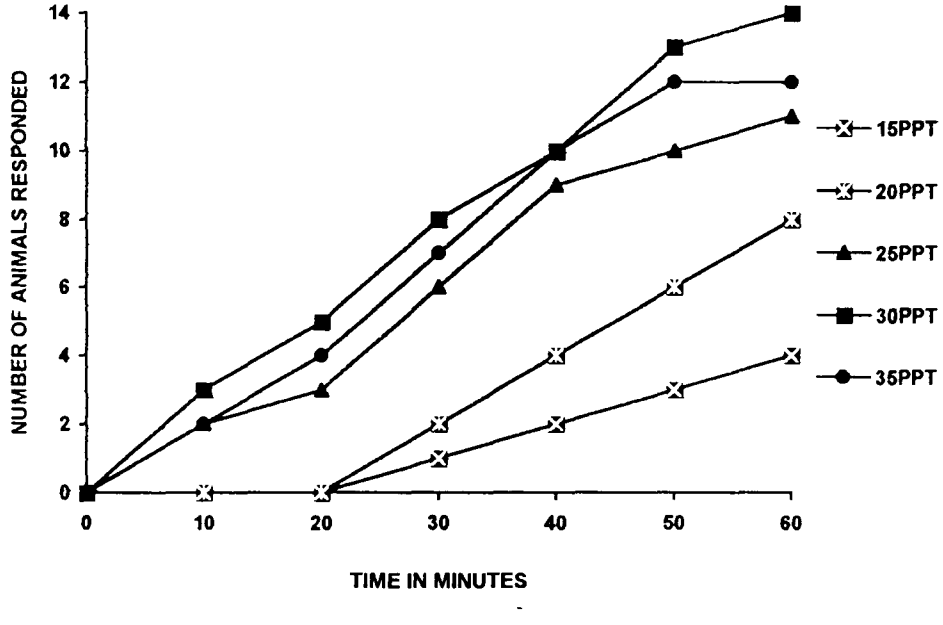
Moisture %	81.17 ± 0.649
Protein %	8.34 ± 0.373
Fat%	2.44 ± 0.061
Glycogen %	3.402 ± 0.6248
Ash %	2.12 ± 0.157
Acid insoluble ash %	0.279 ± 0.0335
Calcium mg%	50.14 ± 0.4788
Phosphorous mg%	94.92 ± 0.638
Meat yield %	9.435 ± 0.6936

Table-2 Aminoacids composition of *Sunetta scripta.*

Aminoacids	g/16g N
Aspartic acid	10.64
Threonine	4.29
Serine	4.39
Glutamic acid	16.15
Proline	2.94
Glycine	6.32
Alanine	7.55
Cysteine	1.30
Valine	4.08
Methoionine	1.11
Iso Leucine	3.97
Leucine	8.16
Tyrosine	3.81
Phenylalanie	3.58
Histidine	2.73
Lysine	5.91
Arginine	6.30
Tryphtophan	2.08



Fig(1). Irritability response of S.scripta exposed to varying salinity levels



Fig(2). Protrusion of siphon of S.scripta exposed to varying salinity levels

Table-3. Irritability Response of *Sunetta scripta* to varying salinities.

Time interval (Mts.)	Number of animals responded				
	15ppt	20ppt	25ppt	30ppt	35ppt
10	0	0	2	4	3
20	0	0	3	6	4
30	1	2	4	8	6
40	2	4	6	11	8
50	3	5	9	13	11
60	5	6	12	15	13

Each value is the mean of 4 replications

Number of animals used in each tray - 15

Table-4 Analysis of variance of the data on irritability response of *Sunetta scripta* exposed to varying salinity levels.

Source of variation	S.S	D.F	M.S.S	F Value
Treatment	2072.8	29	71.475	148.9*
Salinity	948.55	4	237.13	494.020*
Time	1030.7	5	206.14	429.4*
Interaction	93.55	20	4.677	9.74
Error	44	90	0.48	
Total	2116.8	119		

* Significant at 5%

C.D at 5% - 0.332

Salinity means S_{30} - 41.66

S_{35} - 32.66

S_{15} - 11.1

S_{20} - 15

S_{25} - 27.55

Table-5 Observations on the protrusion of siphon of *Sunetta scripta* as a response to varying salinity

Time interval (mts)	Number of animals responded				
	15ppt	20ppt	25ppt	30ppt	35ppt
10	0	0	2	3	2
20	0	0	3	5	4
30	1	2	6	8	7
40	2	4	9	10	10
50	3	6	10	13	12
60	4	8	11	14	12

Number of animals responded -15

Each value is the average of 4 replications

Based on the above results 30 ppt was taken as the optimum salinity for further studies.

4.4 Accumulation of *Vibrio parahaemolyticus* By *Sunetta scripta*

The pattern of accumulation of *Vibrio parahaemolyticus* by the clam is shown in Table.٦. It is seen that there was a progressive accumulation during the period of 24 hrs as shown in fig 3.

Analysis of variance of the data indicated that there was significant difference at 5% level in time intervals in accumulating the micro-organism. Pair wise comparison of the data reveals that accumulation of micro-organism was not significantly different between 18 hrs and 24 hrs duration. Others were significantly different from this.

4.5 Depuration studies

4.5.1 Biochemical changes during depuration

Clams were kept in depuration tank containing seawater of 30 ppt salinity and depurated for 48 hrs. Samples of clams were taken out from the tank at intervals of 0h, 12h, 24h, 36h, and 48h and analysed for changes in protein , glycogen, ash and acid insoluble ash.

Results of above experiments are shown in Table ٩

Table-6 - Analysis of variance of the data on protrusion of siphon of *S. scripta* exposed to varying salinity levels.

Source of variation	S.S	D.F	M.S.S	F Value
Treatment	2084.97	29	71.89	167.18*
Salinity	908.136	4	227.034	527.98*
Time	1064.37	5	212.874	495.05*
Interaction	112.464	20	5.6232	13.07
Error	39	90	0.43	
Total	2123.97	119		

* Significant at 5%

C.D at 5% - 0.31

Salinity means S_{30} - 39.33

S_{35} - 34.83

S_{25} - 31.83

S_{20} - 17.16

S_{15} - 10.5

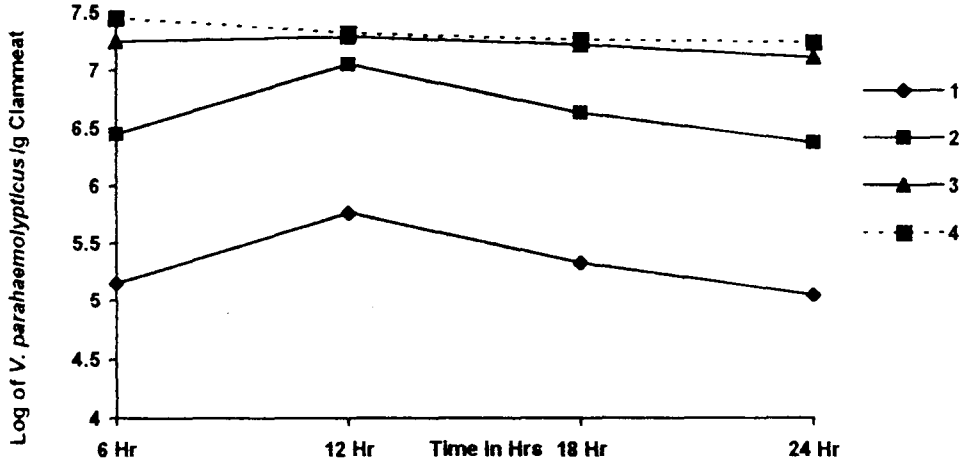


Fig (3). Accumulation pattern of Vibrio parahaemolyticus by Sunetta scripta

Table-7 Accumulation of *Vibrio parahaemolyticus* by *Sunetta Scripta*

Duration in hours	Number of organisms cfu/g^{-1}			
	Replication I	Replication II	Replication III	Replication IV
6	1.42×10^{5}	5.8×10^{5}	2.14×10^{5}	1.12×10^{5}
12	2.81×10^{6}	1.12×10^{7}	4.26×10^{6}	2.32×10^{6}
18	1.44×10^{7}	1.96×10^{7}	1.64×10^{7}	1.28×10^{7}
24	2.85×10^{7}	2.10×10^{7}	1.82×10^{7}	1.73×10^{7}

Table 8 Analysis of Variance of the data on accumulation rate of *Vibrio parahaemolyticus* by *Sunetta scripta*

Source of variation	S.S	D.F	M.S.S	F
Treatment	9.9869	3	3.3290	64.02*
Error	0.6240	12	0.0520	
Total	10-6109	15		f (5%)-8.74

* Significant at 5%.

C.D at 5% level- 0.29

Treatment means - 5.3238, 6.6226, 7.1931, 7.3187

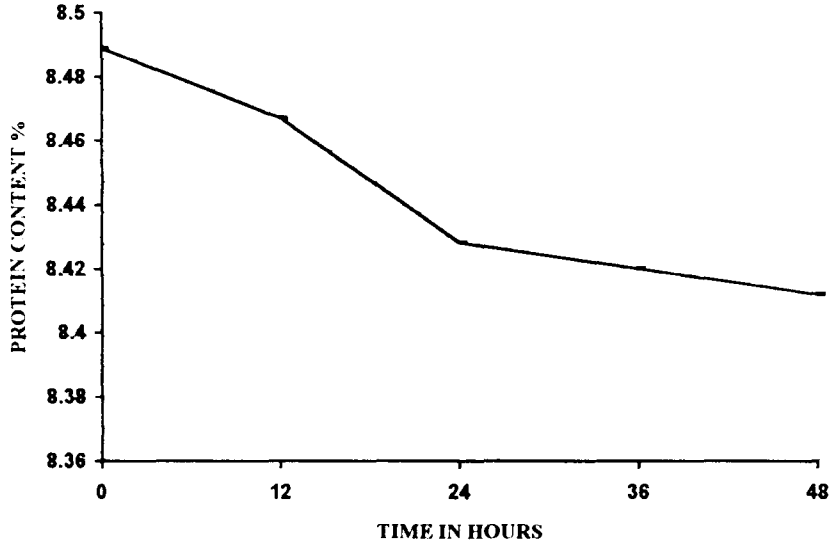
D= 7.3187

C=7.1931

B=6.6226

A=5.3238

Average initial content of protein was 8.489%. From the Fig (4) it can be seen that protein content does not vary much and it declined only to 8.412 % even after 48 hrs of depuration. Analysis of variance, Table 16 showed that there is no significant difference in protein content with different periods of depuration at 5% level of significance.



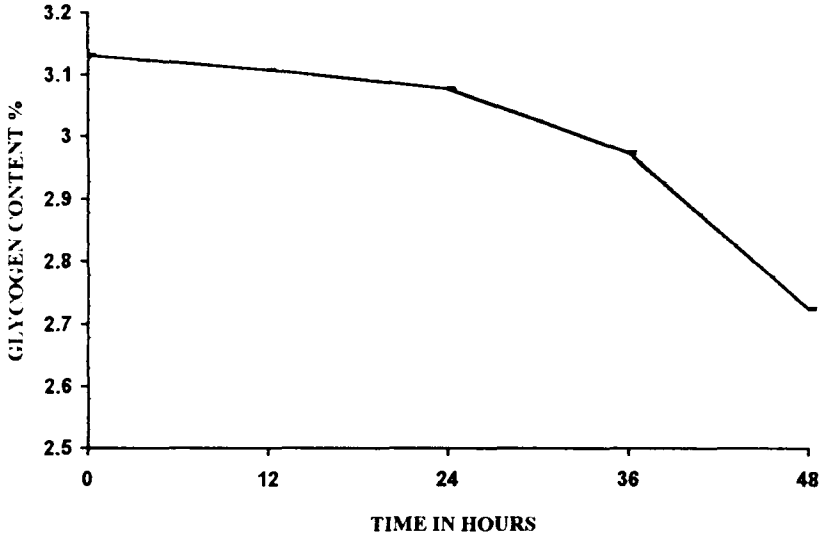
Fig(4) Showing Protein content during 48 hrs depuration

Table-9 Biochemical changes during depuration

Time interval in hrs / Parameter (%)	0	12	24	36	48
Protein	8.489	8.467	8.428	8.42	8.412
Glycogen	3.131	3.107	3.076	2.973	2.725
Ash	2.12	2.072	1.137	1.025	0.846
Acid insoluble ash (sand)	0.2677	0.073	0.0245	0.01725	0.01575

Each value is the average of 4 replications

Glycogen content during 48 hrs depuration is shown in Fig (5). Analysis of variance of data reveal that there is no significant difference in glycogen content with different periods of depuration.(Table 10)



Fig(5) Showing glycogen content during 48 hrs depuration

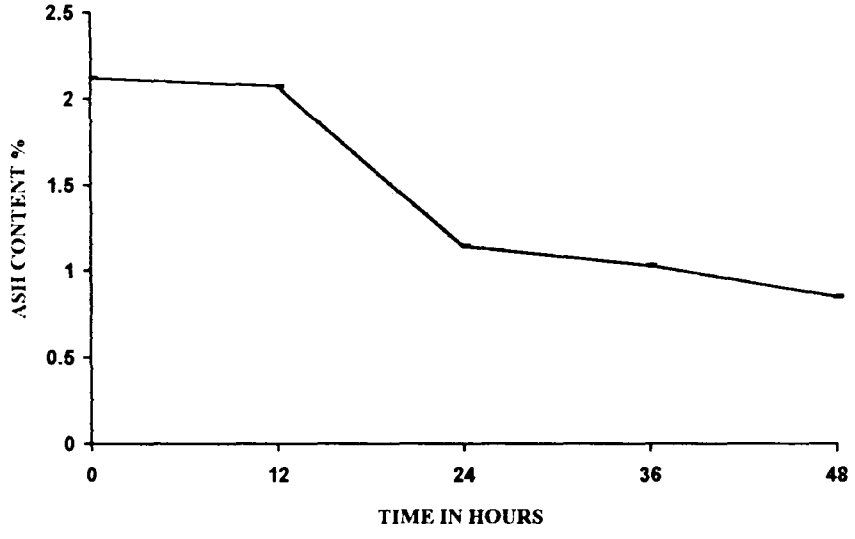
Table 10 Anova table showing change in protein content during 48 hr depuration

Source of variation	S.S	D.F	M.S.S	F value
Treatment	0.0179	4	0.0045	0.04
Error	1.6320	15	0.1088	
Total	1.6499	19		F(5%) 3.055

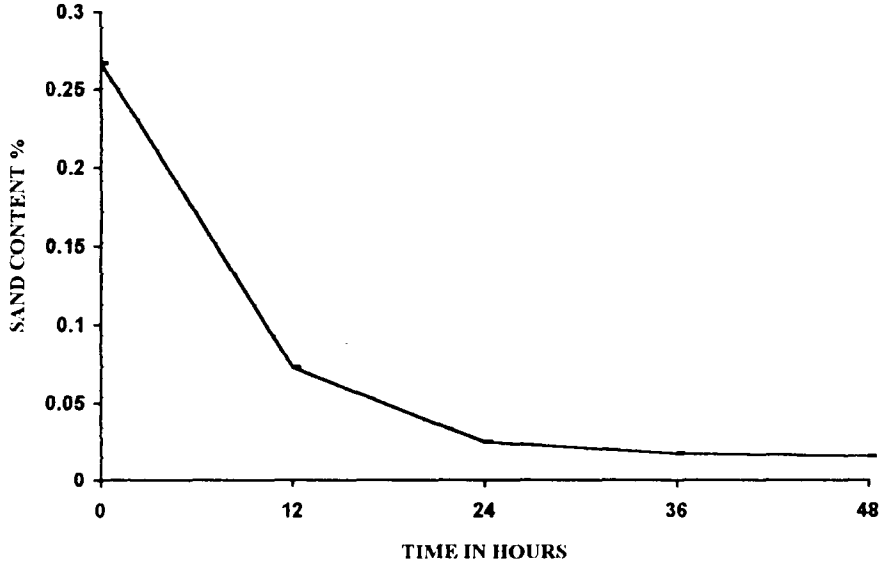
From the Table 12 and Figs (6&7) it is evident that depurated samples showed significant reduction in ash content and acid insoluble ash which represent the sand content in the gut of clams. Sand content reduced from initial 0.26775% to 0.01575%. Analysis of variance Tables 12 & 13 showed that there is significant difference in ash and acid insoluble ash content at 5% level of significance. But no sgnificant difference exist between 24h and 48h

Table 11 Anova table showing change in glycogen content during 48 hrs depuration

Source of variation	S.S	D.F	M.S.S	F value
Treatment	0.4436	4	0.1109	0.19
Error	8.8418	15	0.5895	
Total	9.2854	19		F(5%) -3.055



Fig(6) Showing ash content during 48 hrs depuration



Fig(7) Showing Sand content during 48 hrs depuration

Table 12 Anova table showing change in ash content during 48 hr depuration

Source of variation	S.S	D.F	M.S.S	F value
Treatment	5.9718	4	1.4930	12.05*
Error	1.8578	15	0.1239	
Total	7.8297	19		F(5%) -3.055

Significant at 5% level C.D at 5% - 0.43

Treatment means - 2.120, 2.072, 1.113, 1.025, 0.8467

4.5.2 Depuration of clams seeded with *Vibrio parahaemolyticus*

Clams are allowed to accumulate on an average 3.1×10^5 *Vibrio parahaemolyticus* cells/gm of clam within 6 hrs exposure period in the laboratory. The seeded animals were depurated for 48 hrs in seawater sterilised by U.V.

The *Vibrio parahaemolyticus* content of *Sunetta Scripta* depurated for 48 hrs is shown in Table 14 . Average initial levels of 3.1×10^5 cells/gm were reduced to 3.5×10^1 after 48hrs of depuration . Data obtained are plotted together with line fitted as shown in Fig 8.

Table 13 Anova table showing changing in acid insoluble ash during 48 hrs depuration

Source of variation	S.S	D.F	M.S.S	F value
Treatment	0-1858	4	0.0464	35.48*
Error	0-0196	15	0.0013	
Total	0-2054	19		F(5%) -3.055

* Significant at 5% level C.D at 5% - 0.04

Treatment means - 0.2677, 0.073, 0.0245, 0.0172, 0.01575

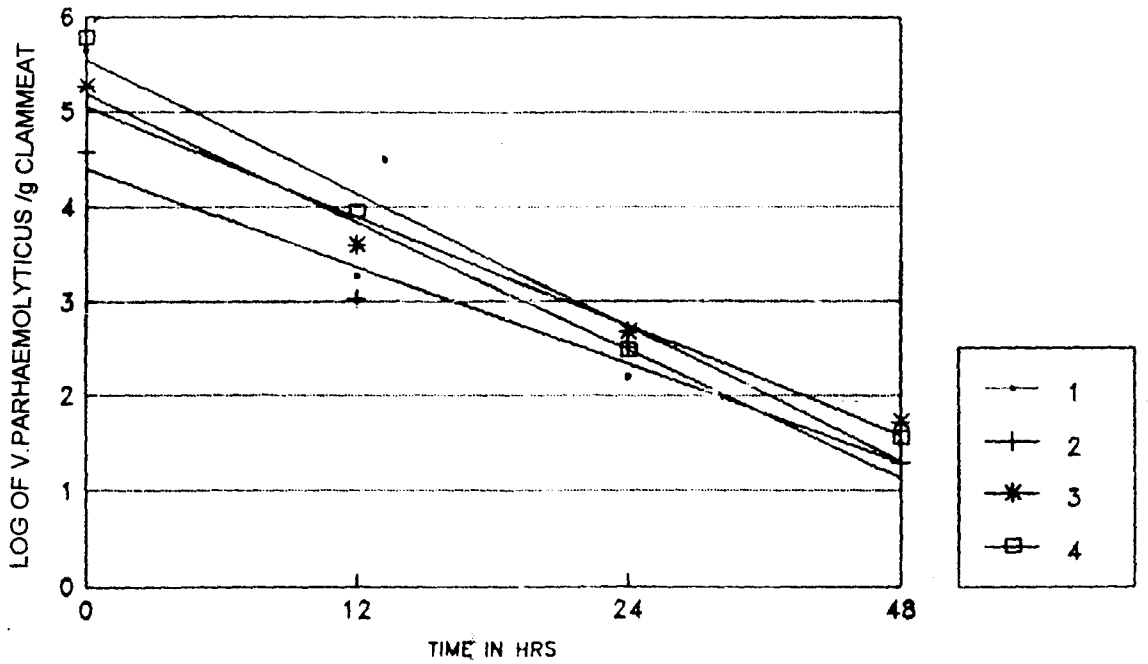


Fig (8). Depuration of Sunetta Scripta seeded with V.parahaemolyticus

A final reduction of 3.84 log cycle in Depuration I , 2.88 log cycle in Depuration II , 3.36 log cycle in Depuration III and 4.32 log cycle in Depuration IV were achieved within 48 hrs.

Analysis of variance of data (Table 15 showed that there was significant change in the load of micro-organism with different periods of depuration at 5% level of significance. Pair-wise comparison showed significant difference among each of the durations.

4.5.3 Sensory evaluation of depurated clams

To assess the effect of depuration on the quality characteristics, clam samples subjected to different periods of depuration were evaluated by a panel of 10 Judges.

There is an improvement in organoleptic qualities after depuration as indicated by sensory Scores (Table 16 & Figs 9-12)

Analysis of Variance shows that significant difference exist at 5% level between different treatment periods with respect to grittiness, odour, flavour and aroma of the sample. (Table 17, 18, 19 & 20) Pair wise comparison of data reveals that there is no significant difference in the grittiness of clam with respect to 24 and 48 hrs of depuration.

Table 14 *Vibrio parahaemolyticus* content of *Sunetta Scripta* depurated for 48 hrs

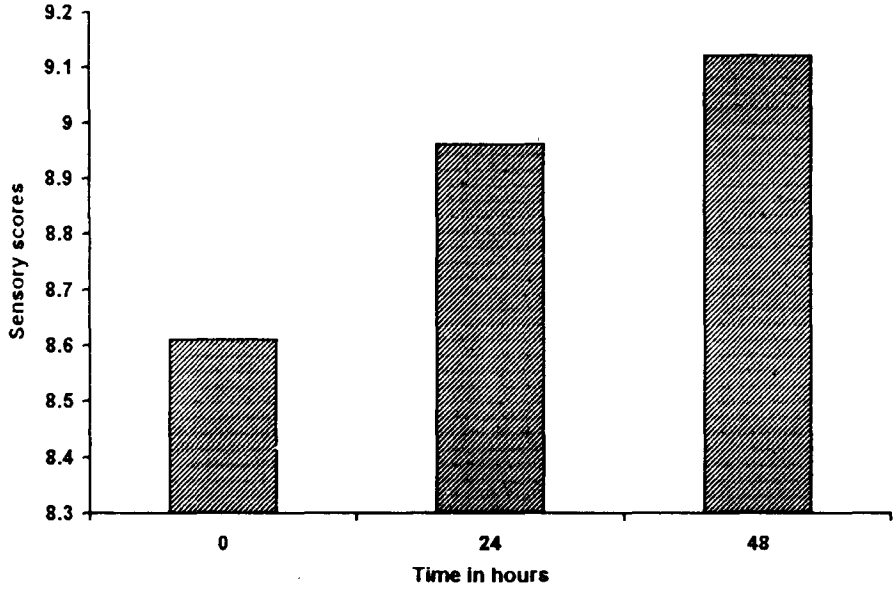
Duration in hrs	Number of organisms cfu g^{-1}			
	Depuration I	DepurationII	Depuration III	Depuration IV
0	4.45×10^5	3.71×10^4	1.85×10^5	5.99×10^5
12	1.89×10^3	1.05×10^3	3.92×10^3	8.96×10^3
24	1.58×10^2	3.23×10^2	4.67×10^2	2.98×10^2
48	32	20	54	36

Table 15 Analysis of variance of the data on reduction of *Vibrio parahaemolyticus* in *Sunetta Scripta* on depuration

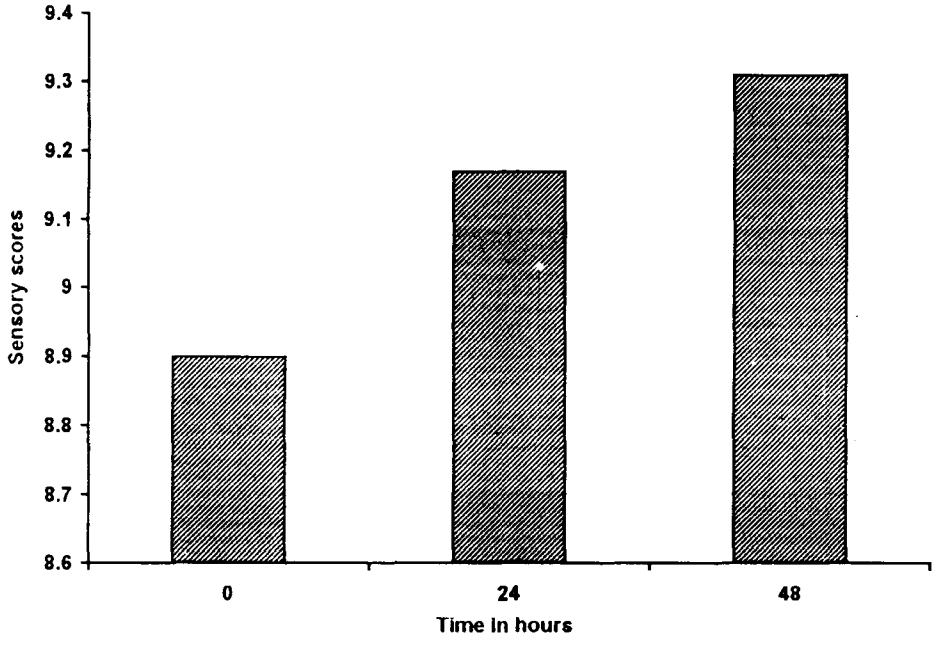
Source of variation	S.S	D.F	M.S.S	F value
Treatment	31.5887	3	10.5296	80.03*
Error	1.5788	12	0.1316	
Total	33.1675	15		f(5%)-8.74

*Significant at 5% level C.D at 5% - 0.46

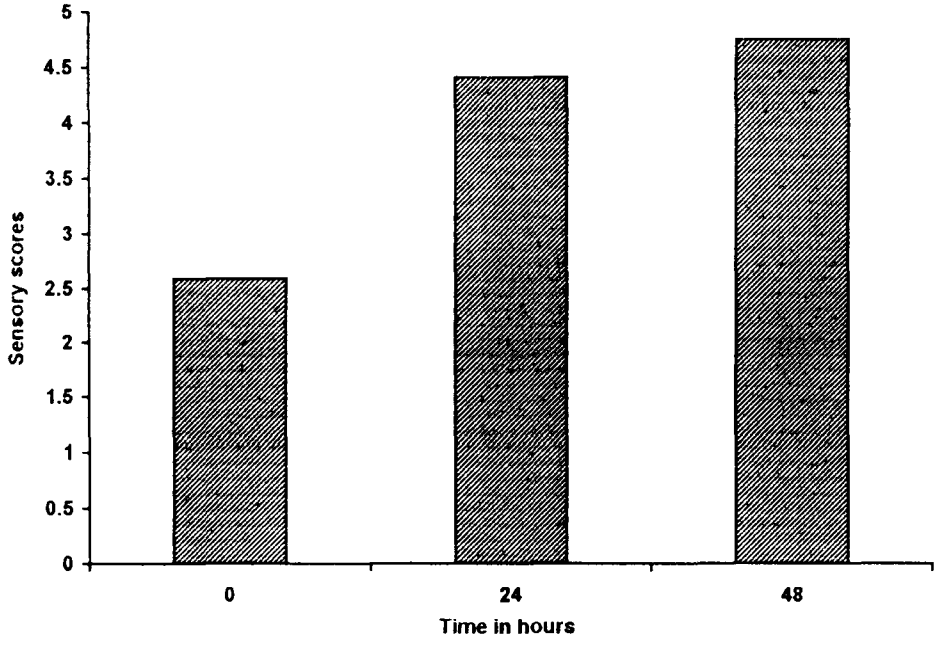
Treatment means - 5.3155, 3.4607, 2.4628, 1.5235



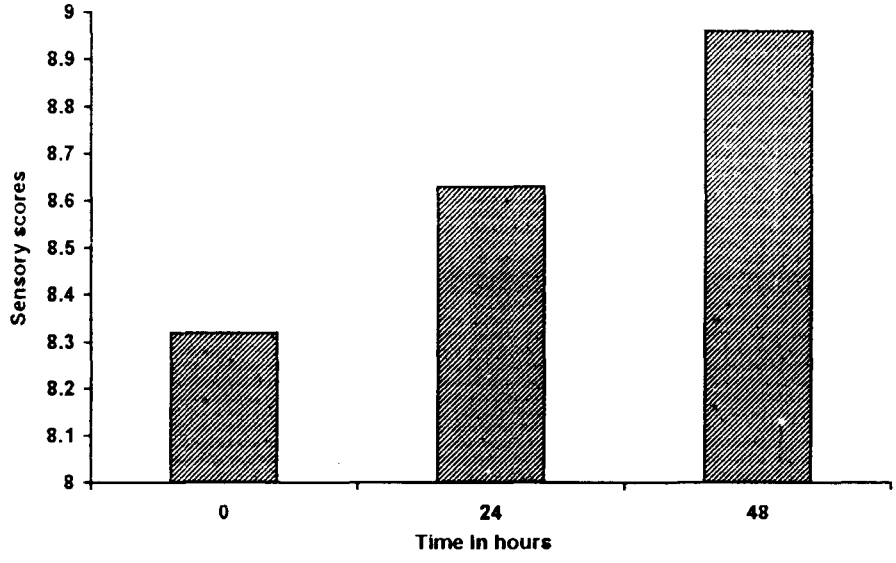
Fig(9) Aroma of clam depurated for different periods



Fig(10) Flavour of clam depurated for different periods



Fig(11) Grittiness of clam depurated for different periods



Fig(12) Odour of clam depurated for different periods

Table 16 Sensory score of clams depurated for 48 hrs.

Time period (hrs)	0	24	48
CHARACTERISTIC	SENSORY SCORE		
ODOUR	8.32	8.63	8.96
FLAVOUR(Cooked)	8.9	9.17	9.31
AROMA	8.61	8.96	9.12
GRITTINESS(Cooked)	2.59	4.41	4.75

Each value is the average of 4 replication

Table 17 Analysis of Variance of the data on odour of the clam during different periods of depuration

Source of variation	S.S	D.F	M.S.S	F value
Treatment	0.8386	2	0.4193	6.57*
Error	0.5741	9	0.0638	
Total	1.4127	11		f(5%) - 4.256

* Significant difference between treatments.

C.D at 5% level - 0.32

Treatment means 8.32,8.63,8.96

Table 18 Analysis of Variance of the data on Aroma of the clam during different periods of depuration

Source of variation	S.S	D.F	M.S.S	F value
Treatment	0.5557	2	0.2779	4.83*
Error	0.5176	9	0.0575	
Total	1.0733	11		f(5%)-4.256

*Significant differene between treatments.

C.D at 5% level- 0.30

Treatment means- 8.61, 8.96, 9.12.

Table 19 Analysis of Variance of the data on Flavour of the clam during different periods of depuration

Source of variation	S.S	D.F	M.S.S	F value
Treatment	0.3528	2	0.1764	4.97*
Error	0.3193	9	0.0355	
Total	0.6722			f(5%)-4.256

* Significant differene between treatments.

C.D at 5% level- .24

Treatment means- 8.9,9.175,9.312

Table 20 Analysis of Variance of the data on grittiness of the clam during different eriods of depuration

Source of variation	S.S	D.F	M.S.S	F value
Treatment	10.8378	2	5.4189	61.99*
Error	0.7868	9	0.0874	
Total	11.6246	11		f (5%)-4.256

* Significant difference between treatments

C.D at 5% level = 0.38

Treatment means - 2.59, 4.4175, 4.7525

5. DISCUSSION

5.1 Microbiological analysis

The total viable count of clam was 1.64×10^3/g and that of the seawater was 4.4×10^2/ml. Pathogenic bacteria were found to be absent except *E. coli*. *E.coli* in clams was found to be 2/ g and 11/100ml in seawater. These findings are in agreement with the findings of Durairaj *et al.* (1983) and Selvan and Pillai (1988). At the time of sampling, habitat salinity was 30 ppt. Coliform counts of water were reported to be maximum under low salinity conditions (Presnell and Kelly, 1981). Chandrika *et al.* (1986) and Chinnamma and Gopakumar (1995) have observed high values for total bacterial count, faecal coliforms and faecal streptococci in *Meretrix casta* and *Villorita cyprinoides* respectively.

5.2 Biochemical analysis

Results of biochemical analysis shows that the clam *S.scripta* is a good source of protein, fat, carbohydrate and minerals. Moisture content was higher and protein slightly lower with medium fat and high proportion of sand. It is also a source of all the essential aminoacids. Chinnamma and Gopakumar (1995) have reported similar results for the clam *V.cyprinoides*. According to Mannan *et al.*, (1961), important factors which influence the composition of any particular species of fish are the abundance of food, spawning cycles, spawning migration, and age. The wide variations observed in the values are probably due to seasonal changes.

5.3 Optimum salinity for depuration

Salinity is an important parameter affecting structural and functional responses in marine invertebrates. Bivalve molluscs are able to isolate and protect themselves from a variety of adverse external conditions by closing the valves (Coleman and Trueman, 1971; Bayne, 1973; Akberali, 1978; Akberali and Davenport, 1981;1982). Bivalves such as *Modiolus* (Pierce, 1971), *Mytilus edulis* and *Glycmeris glycmeris* (Gilles, 1972) *Crassostrea cucullata* (Nagabhushanam and Bidarkar, 1975), and *Scrobicalaria plana* (Akberali, 1978) close their valves when subjected to adverse salinity change.

Davenport (1979) showed the closure of the exhalent siphon, thus preventing pumping, was the crucial event which largely isolates the mantle cavity of mussel from falling external salinities; shell valve closure occurred at rather lower salinities to produce virtually a complete isolation. The same was also observed for *Katelysia opima* (Mane, 1974), *Scrobicularia plana* (Akberali and Davenport, 1981) and *Donax denticulatus* (Genovea *et al.*, 1988).

Salinity tolerance experiments of *S.scripta* showed that animal is capable of tolerating considerable variations in salinities (Latha Thampuran, 1986). Shell valve movements of *S.scripta* in different salinities were studied by Supriya *et al.*, (1990) and they observed that when the ambient medium alters from 30% salinity the animals showed behavioural change by altering the pattern of valve movement and in extreme by closing it.

While most esturaine molluscs can tolerate wide variations in salinities, there will be an optimum preferred salinity which may vary with species and habitat (Thrower, 1990). For *C. madrasensis* 30 ppt (Usha, 1993) and *V.cyprinoides* 10 ppt (Nambudiri *et al.*, 1995) was found to be optimum.

In the present study, protrusion of siphon and irritability response of the clam was found to be maximum in 30 ppt salinity and it was found to be significantly different from other salinities tested. Hence 30 ppt salinity was taken as the optimum salinity for *S.scripta* and chosen for further studies.

5.4 Accumulation of *Vibrio parahaemolyticus*

Microbial accumulation by the shellfish occurred mainly in the first 6-12hr of exposure to the micro-organisms tested depending on the micro-organism considered. After initial exposure and accumulation, an equilibrium existed between the seawater and shellfish concentrations of the micro-organisms. (Borrego *et al.*, 1990). A steady state is known to be rapidly attained for uptake of coliforms beyond which accumulation in gut does not occur for a given concentration of the bacteria in ambient waters. Maximum levels can be attained in first 6h by some but prolonged exposure increases the percentage of population before reaching a steady state (Perkins *et al.*, 1980). Results of present study is in agreement with above findings. In this study when clams were exposed to bacterial concentration of 10^9 cells/ml of water, there was a progressive accumulation of *V.parahaemolyticus* during the 24hr exposure period. Maximum concentrations were attained within 12hr and then a steady state followed.

Borrego *et al.* (1990) studied the comparative accumulation for several micro-organisms by the striped venus under controlled laboratory conditions. He found out that the accumulation period of the shellfish varied depending on the microbial species used. Thus for *E.coli* this period was between 0 and 12h, the maximal accumulation level being obtained at this moment. Similar results were obtained for *S.faecalis*. The kinetics of accumulation of *S. lyphimurium*, *S. aureus*, and *V.parahaemolyticus* were similar, with accumulation by the 3rd h and levels are maintained during the 12h.

However Groubert *et al.* (1994) obtained values which were not significantly different from each other, suggesting *C.virginica* does not differentiate in its uptake of opaque and translucent morphotypes of *V.vulnificus*. They were exposed to a concentration of 10^{8-9} cells/ml for 4h following "piggy-back" method in which the bacteria were presented to the oysters along with phytoplankton cells. This method causes a concentration of bacteria within the oyster rather than an equilibrium between the oyster and the surrounding seawater (Steslow, 1987).

A significant feature in all accumulation studies is that levels above environmental titters have been achieved is by the use of flow through system. Accumulation of both bacteria and viral particles is generally poor in standing water system (Hedstrom and Lycke, 1964; Hoff and Beekar, 1969).

An accumulation level of 2.6×10^{5} cells/gm was sufficient for depuration studies and hence 6h exposed period was taken for seeding.

5.5 Biochemical changes during depuration

These studies were conducted to find out whether any change in proximate composition during depuration since it involve direct feeding and pumping activities. The intestine of bivalves are often loaded with mud and sand due to the filter feeding habit, which impart grittiness to the meat if retained within. Acid insoluble ash is a direct measure of the sand content present in the meat.

Results of experiment shows that there is no significant change in the content of protein and glycogen after depuration. But the depurated samples showed significant difference in the level of ash and sand compared to undepurated samples. Ash content reduced from initial 2.1% to 0.8% and sand from 0.26775% to 0.015%. Sensory evaluation studies also shows the absence of grittines within 24 hr of depuration.

Results of depuration conducted by Surendran and Balachandran (1988) on *V.cyprinoides* and *P.indica* shows that acid insoluble ash could be brought to insignificant level within 24hr of depuration.

A depuration time of 24hr reduced substantially the sand content in oysters (Chellappan, 1991). Moisture, protein and lipid contents of depurated clams did not change significantly after depuration but glycogen decreased significantly. Total ash and sand contents could be brought down to insignificant level by depuration in *Meretrix casta* (Mishra and Srikar, 1989).

Thus the results of the present study is in agreement with above findings and process is sufficient for removal of grittiness in *S.scripta.*

5.6 Bacterial depuration

Several studies of depuration have indicated that diverse bacterial species are eliminated at different rates by molluscan shellfish (Canzonier, 1971; Power and collins, 1986; Power et al, 1989)

In the present study though complete elimination of *Vibrio parahaemolyticus* did not occur from clam, by 48h initial contaminating loads had been reduced by more than 1000 fold to levels generally less than 35 cells/g. On an average a final reduction of 3.6 logcycle was achieved within 48h.

Son and Fleet (1980) also found that laboratory contaminated *V.parahaemolyticus* were readily cleansed from oysters during depuration and by 3days initial load of 9×10^5/g had been reduced to 8 cells/g. Similarly *B.cereus* and *S. typhimurium* were cleansed to undetectable levels.

It was found that contaminated oysters cleansed themselves to NHMRC standard of 2.3 *E.coli* cells/g of oyster within 48h using water continuously recirculated through UV steriliser. (Wood, 1961; Mitchell *et al.*, 1966, Fleet, 1978b; Souness and Fleet, 1979). Son and Fleet (1980) had reported that cleaning of *E.coli* on depuration could be used to indicate the cleaning of other pathogenic bacteria like *Salmonella* sp., *Bacillus cereus, Clostridium perfringens* and *Vibrio parahaemolyticus*. However depuration process may be of limited use in controlling presence of pathogenic vibrios in *C. commercialis* (Eyles and Davey, 1984) and *V. vulnificus* in *C. virgnica*. Longer depuration time was required for more heavily contaminated oysters with Salmonella. (Souness and Fleet, 1979).

Oysters naturally contaminated with *V. parahaemolyticus* showed no significant difference in mean counts between nondepurated and depurated oysters (Eyles and Davey, 1984). Shellfish naturally contaminated with *V. vulnificus* and non-01 *V. cholerae* appear to depurate more slowly than laboratory-contaminated shellfish (Steslow *et al.*, 1987). However these reports are in contrast to the findings of Son and Fleet (1980), Manzanares *et al.* (1991) and Groubert and Oliver (1994). These conflicting results may be related to use of laboratory versus environmentally contaminated specimens.

Studies suggest that large number of laboratory acquired vibrios may be readily purged because they become entrapped within faeces and migrate through digestive tract (Rowse and Fleet, 1982). However, the closer association of some of the vibrios with cells of the hepatopancreas and digestive diverticula may permit vibrio colonisation within the cells and produce persistently infected shellfish (Capers *et al.*, 1990; Eyles and Davey 1984; Greenburg *et al.*, 1982). Richards (1988) suggests that natural populations of vibrios may be more resistant to the effects of depuration than vibrios grown in the laboratory. Similarly Tamplin and Capers (1992) have observed that laboratory and natural strains of *V. vulnificus* show different survival patterns under depuration and dry-storage conditions.

The greater depuration of the artificially infected oysters over natually infected oysters suggests that naturally introduced bacteria, including vibrios, have become a part of the oysters normal flora. The failure to depurate normal flora bacteria may be a result of the ability of such bacteria to attach to oyster tissue, to

colonise oyster tissue previously inhabited by other bacteria or to grow at rates exceeding that of depuration. (Greenberg *et al.*, 1982; Jones *et al.*, 1991).

Usha (1993) and Nambudiri *et al.* (1995) using the same depuration system employed in this study have achieved complete elimination of *E.coli.* But in the present study complete elimination of *V.parahaemolyticus* couldn't be attained using the same system. This is in agreement with findings of Greenburg *et al.* (1982) that clams depurated vibrios more slowly than *E.coli.* The rapid reduction of *V.parahaemolyticus* occurred in this study in contrast to the findings of Eyles and Davey (1984), and Greenburg *et al.* (1982) may be due to the use of laboratory contaminated clams.

Thus the findings of this study are in concurrence with previous studies which have suggested that artificial infection of animals of in the laboratory may not equate to infection of animals in the natural environment. (Vasconcelos and Lee, 1972; Son and Fleet, 1980; Eyles and Davey, 1984; Blake *et al.*, 1985; Steslow, 1987).

In view of the above, there is absolute necessity of more detailed examination of the kinetics of uptake and elimination of specific food poisoning bacteria by shellfishes.

6. Summary

The objective of the study was to find out effect of depuration on the meat quality of the marine clam *Sunetta scripta* .

1. The study included

a) Microbiological, biochemical and organoleptic analysis of clams before depuration

b) Determination of optimum salinity of the clam for depuration

c) Seeding studies on *Sunetta scripta* with *Vibrio parahaemolyticus.*

d) Bacterial depuration of seeded clam.

e) Determination of biochemical changes during depuration and sensory evaluation of depurated clam.

2) On microbiological examination total viable count of the clam was found to be 1.64×10^3 / gm of clam meat. Pathogenic bacteria were absent except *E.coli* and that too in low numbers.

3) Among the different salinities tested viz. 15, 20, 25, 30 and 35 ppt, 30 ppt was found optimum for the animal based on irritability response and protrusion of siphon. This salinity was chosen for further studies.

4) Studies on artificial contamination of the clam with *Vibrio parahaemolyticus* revealed that there was progressive accumulation of the organism up to 12 hrs and

then rate slowed down. A 6hrs exposure period was sufficient to attain 3.1×10^5 cells/gm

5) Results of depuration studies indicate that there was no significant change in protein and glycogen content but significant fall observed in ash and acid insoluble ash content during 48 hrs depuration period. Sand content reduced to insignificant level even by 24 hrs depuration.

6) It was observed from bacterial depuration that though the accumulated micro-organism was not completely eliminated by the clam, the number had significantly reduced within 48hrs depuration period.

7. Sensory evaluation of depurated clams showed that there was significant difference in organoleptic qualities and grittiness of meat between depurated and undepurated samples.

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APPENDIX-1

SENSORY EVALUATION

The clam samples provided to you for evaluation have been subjected to depuration for different time period periods as a part of a study to assess the effect of depuration on the quality characteristics of the clam. Your valuable opinion will be of immense relevance in this study. Thank you.

Time:

Date:

Name:

Kindly evaluate the various quality characteristics of the given clam meat. You may make a 'tick' mark in the appropriate coloumn for all the three samples.

ATTRIBUTES				
ODOUR (RAW)	SCORE	A	B	C
EXTREMELY FRESH	10			
VERY FRESH	8			
MODERATELY FRESH	6			
NEUTRAL	5			
STALE	4			
VERY STALE	2			
PUTRID	0			

ATTRIBUTES COOKED		EXCELLENT (10)	GOOD (8)	FAIR (6)	BORDER LINE (5)	POOR (4)	VERY POOR (2)	BAD (0)
AROMA	A							
	B							
	C							
FLAVOUR	A							
	B							
	C							
GRITTNESS	A	0						5
	B	0						5
	C	0						5

Remarks if any:

Key of grittiness

No sand/ No grittiness - 5

Slight sand content - 4

Moderate sand content - 3

High sand content - 2

Very high sand content - 1

Extremely gritty & noticeable sand content - 0