Fishery Technology
1996, Vol. 33(2) pp : 111 - 115

Microbiological Characteristics of Prawn Pickle

T. Jawahar Abraham, K. Rathnakumar and P. Jeyachandran
Department of Fish Processing Technology
Fisheries College and Research Institute
Tamil Nadu Veterinary and Animal Sciences University
Tuticorin-628 008, India

Microbiological characteristics of prawn pickle stored at ambient temperature (30±2°C) was studied. In prawn pickle with a pH around 4.75, the total viable count decreased by more than 90% in first 60 days of storage and thereafter ranged between 10^2 and 10^3 g^{-1}. Lactic acid bacteria, coliforms, *Staphylococcus aureus*, Salmonellae, Vibrios and *Clostridium perfringens* were not encountered. Anaerobic spore formers and anaerobic gas producers count increased with the storage period. The prawn pickle contained salt and acid toleant groups of bacteria at the end. The product did not show any visible signs of spoilage for a period of 270 days.

Key words : Prawn pickle, halophiles, anaerobes.

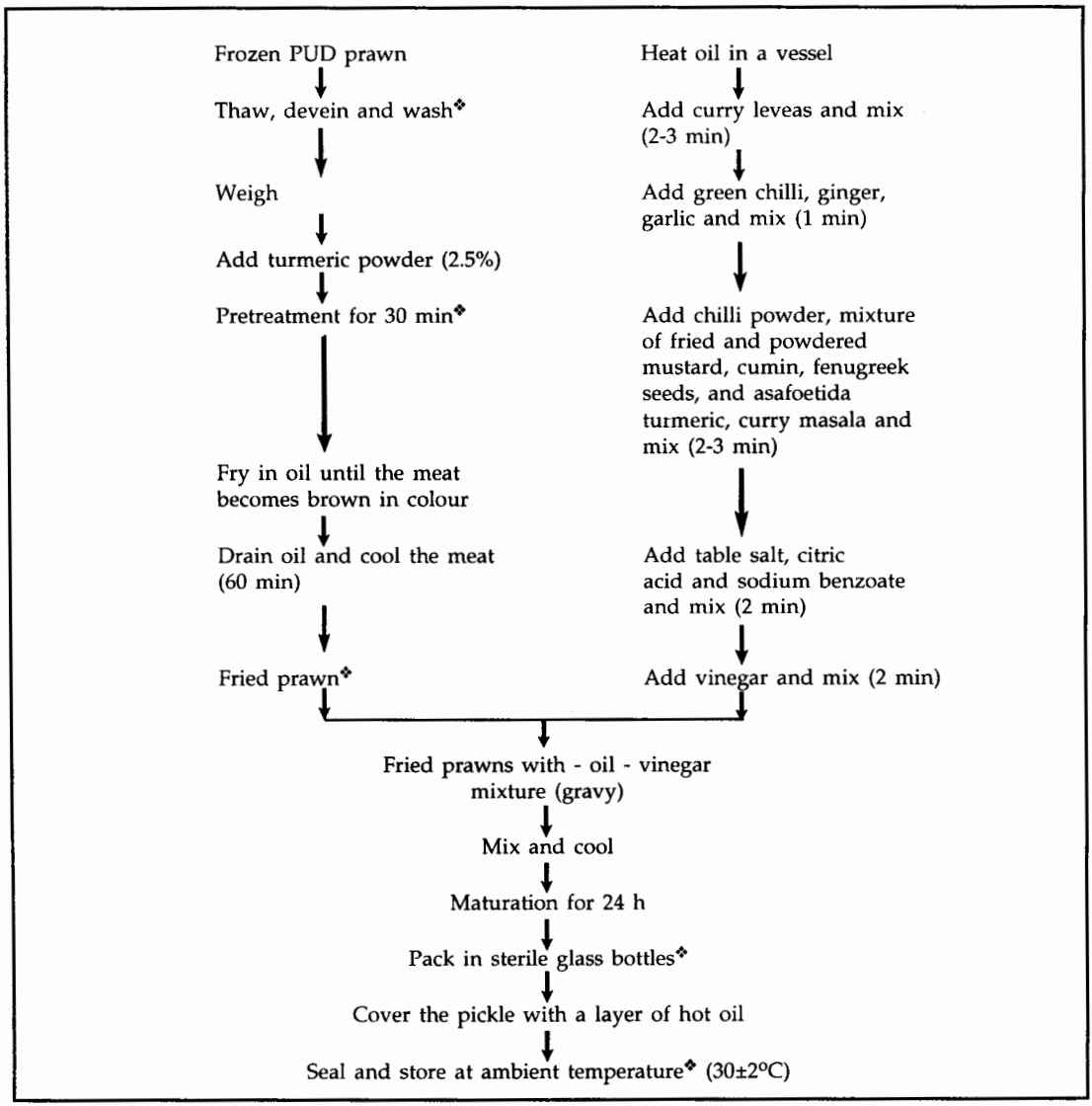
Pickling is an ancient form of food preservation. Fish in India have been pickled on a small scale in a modified form known as 'Colombo curing' using malabar tamarind (*Garcinia combogea*) or ordinary tamarind (*Tamarindus indica*), with salt as pickling agents (Nicholson, 1930, Balachandran & Muraleedharan, 1975). Currently, pickled fish and prawn are produced using organic acids as pickling agents along with spices. These pickled products are considered a delicacy and are safe without any harmful bacteria being present, maintaining their quality for more than 6 months at ambient temperature (Chandrasekhar *et al.*, 1978; Chandrasekhar, 1979). Though there are reports on the aspects of their preparation and storage studies, information on the microbiology of pickled fish products are scanty. Microorganisms associated with the spoilage of prawn pickle have been reported by Karunasagar *et al.* (1988). The present paper reports the method of preparation of prawn pickle and the bacteriological changes during various stages of preparation and storage.

Materials and Methods

Small sized frozen prawns (peeled and undeveined) of the species, *Parapenaeopsis stylifera* and *Penaeus indicus*

Table 1. Standard recipe for prawn pickle

Ingredients	Quantity, g
Prawn meat	1000.0
Table salt (powdered)	80.0
Green chilli	100.0
Ginger	100.0
Garlic	100.0
Mustard seeds	25.0
Cumin seeds	25.0
Asafoetida	10.0
Fenugreek seeds	5.0
Turmeric powder	10.0
Curry leaves	5.0
Curry masala	10.0
Chilli powder	100.0
Citric acid	2.5
Sodium benzoate	2.0
Vinegar	300.0 ml
Double refined groundnut oil	500.0 ml



❖: Points at which samples were taken for microbiological analysis

Fig 1. Flow diagram for the preparation of prawn pickles

procured from a local shrimp processing plant were thawed, deveined and used as the main raw material. Commercially available spices and other materials (Table 1) were purchased locally. Glass bottles (250 g) with aluminium screw caps were sterilized in boiling water for 30 min., and dried at 100°C.

Prawn pickle was prepared using the standard recipe (Table 1) and methodology developed by this Institute (Fig. 1). The prawn pickle was then packed in the glass bottles, covered with caps, sealed and stored at ambient temperature (30±2°C). Before sealing care was taken to prevent the exposure of pickle to air

by a layer of groundnut oil at the top to cover the solids.

Titratable acidity and pH of the pickled prawn meat were determined by the AOAC (1975) methods. Various sensory characteristics like appearance, colour, taste, texture, odour, and overall acceptability of the prawn pickle were evaluated at intervals by a group of 9 panelists on a 9 point Hedonic scale. The limit of acceptability was fixed at 5.0.

The bacteriological changes in prawn meat during various stages of pickle preparation as shown in Fig. 1 were assessed by enumerating the total viable count and staphylococcal count (APHA, 1976). The microbiological flora of the finished product was analysed for total viable count, proteolytic count, total fungal count, Staphylococcal count, halophilic count in medium with 10% salt, aerobic spore formers, lactic acid bacterial count, coliforms, vibrios, salmonellae (APHA, 1976), *Clostridium perfringens* count (Beerans *et al.*, 1982), lipolytic count, anaerobic count, anerobic gas producers and anaerobic spore formers (Collins *et al.*, 1989).

Results and Discussion

The changes in the bacterial population and pH of the prawn meat during various stages of preparation of prawn pickle are presented in Table 2. A stepwise reduction in viable bacterial population was observed during processing. The viable count (TVC) decreased by about 1.54 and 2.27 log units from the initial count (log 5.46) after frying and acidification, respectively. The reduction in TVC after frying was not marked, indicating insufficient heat processing during frying or contamination during cooling period. The higher staphylococcal counts (SC) in fried prawns also reflect post process handling of the product. However, subsequent treatment with spices and acidification reduced the TVC and SC by 2.27 and 2.74 log units, respectively.

Table 2. Effect of processing on the bacteriology and pH of prawn meat

Stage of processing	Log count g^{-1} TVC	SC	pH
Deveined & washed prawn	5.46	3.44	7.24
Pretreated prawn (Turmeric powder treated)	5.23	3.34	7.03
Fried prawn	3.92	1.18	7.23
Pickled prawn after 24 h maturation	3.19	0.70	4.76

TVC: Total viable count; SC: Staphylococcal count

The pH of the prawn meat upon turmeric powder pretreatment decreased slightly but increased upon frying in oil. The addition of spice mixture and vinegar brought down the meat pH to 4.76. The pH initially decreased slightly after storage (90 d) to 4.71 and then increased to 4.78 at the end (270 d). A marginal increase in titratable acidity from 0.25 to 0.33% acetic acid was observed in prawn meat after 120 days and remained constant thereafter. Collins *et al.* (1989) were of the opinion that if the pH of the vinegar pickled fish is 4.5 or less, no further precaution is necessary against bacterial pathogens.

Table 3 shows the microbiological characteristics of prawn pickle during storage. The TVC decreased by more than 90% within 60 days of storage and thereafter showed a slight increase. This suggests that the initial bacterial flora of prawn pickle is highly sensitive to pickling. The proteolytic count and lipolytic count followed a trend similar to TVC. Throughout the storage the TVC was in the range of 10^3 to $10^2 g^{-1}$. Chandrasekhar (1979)

Table 3. Microbiological characteristics of prawn pickle during storage

Storage, days	Log counts g^{-1}									
	TVC	PC	LC	SC	HC	ASF	TFC	ANC*	ANGP*	ANSF*
0	3.20	2.98	3.11	1.00	2.00	2.95	<1.00	<0.30	<0.30	<0.30
30	2.74	2.54	2.70	<1.00	1.78	2.65	<1.00	0.30	0.30	0.30
60	2.00	1.78	1.90	<1.00	1.00	1.78	<1.00	1.30	0.60	1.30
90	2.08	1.78	2.00	<1.00	1.48	1.81	1.00	1.51	0.85	1.51
120	2.00	1.90	2.00	<1.00	1.85	1.90	1.18	2.44	0.85	2.44
150	2.08	1.95	2.04	<1.00	2.00	1.95	1.40	2.54	1.90	2.54
180	2.30	2.00	2.20	<1.00	2.30	2.00	1.30	2.60	1.85	2.60
270	2.64	ND	2.60	<1.00	2.60	2.45	2.28	2.54	ND	2.54

ND: Not done; TVC: Total viable count; PC: Proteolytic count; LC: Lipolytic count; SC: Staphylococcal count: HC: Halophilic count: ASF: Aerobic spore formers; TFC: Total fungal count; ANC: Anaerobic count; ANGP: Anaerobic gas producers; ANSF: Anaerobic spore formers

* By the most probable number: Black tube technique (Collins *et al.*, 1989)

reported a TVC in prawn pickle in the range of 10^3 to 10^5 g^{-1}. But Erichsen (1967), reported that pickled fish, unless spoiled, normally carry low levels of bacteria in the range of 10^1 to 10^3 g^{-1}. A few colonies of staphylococci were encountered immediately after preparation of pickle. Staphylococci have been reported in spoiled prawn pickles (Karunasagar *et al.*, 1988) and in commercial prawn pickles (Abraham & Jeyachandran, 1993).

Table 4. Sensory evaluation of prawn pickles during storage, mean scores

Storage, days	Appearance	Colour	Taste	Texture	Odour	Overall acceptability
10	7.1	7.1	7.3	6.6	6.9	6.9
30	8.3	8.0	8.3	8.1	8.4	8.3
60	7.8	8.0	7.7	7.2	8.1	8.0
90	7.4	7.8	7.7	6.9	7.6	7.5
120	7.3	7.6	7.3	6.8	7.6	7.3
150	7.3	7.5	7.3	6.7	7.5	7.2
180	7.3	7.4	7.2	6.6	7.5	7.2
270	6.0	6.0	5.6	5.0	5.6	5.2

The halophilic count followed a trend similar to TVC. Their proportion was lower (6.45%) initially, but increased to more than 90% of the total population (TVC) at the end. It would seem that the final surviving bacterial population of prawn pickle are salt and acid tolerant. Karunasagar *et al.* (1988) have reported a viable count in the range of 10^6 to 10^9 g^{-1} in spoiled prawn pickles of which 10^6 to 10^7 g^{-1} were halophiles. The aerobic spore formers comprised more than 50% of the viable bacterial population throughout the storage period. These have been reported to be the dominant group in fish pickle (Chandrasekhar *et al.*, 1978). Lactic acid bacteria, coliforms, salmonellae, vibrios, and *Clostridium perfringens* were not encountered in prawn pickle immediately after preparation and up to 60 days. Hence, no quantitative estimation was attempted thereafter. These organisms are reported to be either killed or fail to multiply in acetic acid fish preserves (Emberger, 1972) and in fish pickle (Chandrasekhar *et al.*, 1978) No fungal colonies were observed up to 60 days.

These results are in accordance with those of Behanan *et al.* (1990). The initial low counts of moulds and yeasts could be due to the preservative action of sodium benzoate and the maintenance of anoxic conditions in the pickle.

The counts of anaerobes (ANC) and anaerobic spore formers (ANSF) increased with period of storage. The layer of oil and sealed cap of pickle bottles are conducive to a partially anaerobic climate in the pickle favouring the growth of anaerobes. The ANC and ANSF counts were similar, which suggest that the anaerobes present in prawn pickle were spore formers, of which considerable population produced gas (Table 3). Occurrence of *Clostridium botulinum* in pickled herring (Dolman *et al.*, 1950), *C. perfringens* type organisms in oil based egg pickles (Swamy *et al.*, 1978) and anaerobic spore formers in commercial prawn pickles (Abraham & Jeyachandran, 1993) are well documented.

The mean panel scores for all the sensory characteristics remained within the acceptability limit throughout the storage period (Table 4). The product had maximum scores on the 30th day and the scores showed a decreasing trend with period of storage. The texture of the pickled prawn meat received low scores towards the end. As a whole the product was microbiologically sound and did not show any visible signs of spoilage for a period of 270 days.

The authors thank the Dean, Fisheries College and Research Institute, Tuticorin for his keen interest and encouragement.

References

Abraham, T.J. & Jeyachandran, P. (1993) *Fish. Technol.* **30**, 81

AOAC (1975) *Official Methods of Analysis*, 12th Edn. Association of Official Analytical Chemists. Washington DC, USA

APHA (1976) *Compendium of Methods for the Microbiological Examination of Food.* American Public Health Association, New York, USA

Balachandran, K.K. & Muraleedharan, V. (1975) *Fish. Technol.* **12**, 145

Beerans, H., Romond, C.H., Lepage, C. & Criquelion, J. (1982) in *Isolation and Identification Methods of Food Poisoning Organisms* (Carry J.E.L., Roberts, D. & Skinner, F.A., Eds), p. 137, Academic Press, London, UK

Behanan, L., Saleema, M., Sudharma, D., Mukundan M.K. & Malika, V. (1990) *Fish. Technol.* **27**, 40

Chandrasekhar, T.C. (1979) *Seafood Export J.* **11**, 15

Chandrasekhar, T.C., Rudrasetty, T.M., Laxmanreddy, P.T. & Ashwathnarayana, C. (1978) *Fish. Technol.* **15**, 125

Collins, C.H., Lyne, P.M. & Grange, J.M. (1989) *Microbiological Methods.* 6th edn. Butterworths, London, UK

Dolman, C.E., Chang, H., Kerr, D.C. & Shearer, A.R. (1950) *Can. Public Health J.* **41**, 215

Emberger, O. (1972) *Prum. Potravin.* **23**, 347

Erichsen, I. (1967) *J. Microbiol. Ser.* **33**, 107

Karunasagar, K., Venugopal, M.N., Jeyasekharan, G., Sekar, K. & Karunasagar, I. (1988) *J. Food Sci. Technol.* (India) **25**, 103

Nicholson, F.S. (1930) *The Preservation and Curing of Fish.* Government Press, Madras, India

Swamy, V., Gowda, A.R. & Krishnamurthy, S. (1978) in *Proceedings of the Symposium on Fats and Oils in Relation to Food Products and Their Preparations.* p. 56, CFTRI, Mysore, India