DISTRIBUTION OF CLOSTRIDIUM BOTULINUM IN CURED FISHERY PRODUCTS

by

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ABSTRACT

A total of forty cured fish samples procured from local retail markets in and around Cochin were analysed for the moisture content, salt content, water activity (aw) and for the presence of Clostridium botulinum. Wide variations in the salt concentration and moisture level were observed in the cured fish samples examined. Fifty five percent (22/40) of the samples had water content in the range of 45-51%. The sodium chloride content of the cured fish samples varied between 16-25% in only 60 percent of the samples. The sodium chloride content of less than 7% was noticed in 8 prawn samples. The aw of the samples varied greatly. Clostridium botulinum was detected in 13% of the cured fish samples and type D was predominant (4/5) followed by type C (1/5). Clostridium botulinum spores remain viable at aw level 0.75. The incidence of C. botulinum in cured fish should emphasize the need for adequate brining and drying to protect these products until their final use.

INTRODUCTION

Cured fish is the only traditional fish product of overall commercial importance in India accounting for utilization of about 30 percent of marine fish landings (Sripathy 1983). The process of curing fish imparts a degree of microbiological stability to the product but their stability is a function of several factors including the salt level reached after brining and the amount of heat applied. Since these factors vary from product to product, the microbiological quality and safety of cured fishery products vary.

Clostridium botulinum is one of the food safety hazards associated with fish. There are several botulism hazards associated with seafoods or fish (Sakaguchi 1979, Huss 1981, Dodds 1993). Even though the salt added during the production of cured fish is inhibitory to microbial growth, there have been outbreaks of botulism associated with salt dried fish (Sakaguchi 1979, Centre for Disease Control 1987, Hauchild 1989, Weber *et al.* 1993, McClure *et al.* 1994).

Incidence of *Clostridium botulinum* was reported in fish from India (Lalitha and Iyer 1990, Lalitha and Surendran 1992). The mere presence of *C. botulinum* on a food product will not cause illness, conditions much be such that viable *C. botulinum* spores are present and are given the opportunity to germinate and produce toxin. At present there is paucity of data on the occurrence of *C. botulinum* in cured fish. This paper describes a survey on the distribution of *C. botulinum* in cured fish on sale in markets in Cochin, India.

MATERIALS AND METHODS

Cured fish/shellfish samples from the retail outlets, in and around Cochin, were aseptically collected in sterile polythene bags and transported to the laboratory for the estimation of chemical parameters such as salt content, moisture content and water activity and for the detection of *C. botulinum.*

Cured fish/shellfish samples procured from local retail outlets were analysed for sodium chloride and moisture contents. Moisture and sodium chloride contents were determined according to the methods

followed by AOAC (1990). The water activity of cured fish samples were estimated using a water activity meter (Lufft a_w - Wert-Messer, Germany).

Cured fishery products obtained from retail outlets in and around Cochin were examined for the presence of *C. botulinum*. A 3-5g sample of cured fish was inoculated into 25 ml Cooked Meat Medium (CMM) after removing dissolved oxygen by steaming 10-15 min and cooling quickly without agitation. Sterile paraffin oil was used to cover the surface. Inoculated tubes were incubated at 30°C for 6 days.

After incubation for 6 days at 30°C, cultures were centrifuged at 10000 x g and 4°C for 20 min (Remi, India) and each supernatant was adjusted to pH 6.2 with 1N HCl and frozen at -15°C overnight to eliminate non-specific mouse deaths (Baker *et al.* 1990). Supernatants were stored at -15°C until tested. Toxicity of the supernatant was tested in mice and the toxin type was identified using a procedure followed by the US Food and Drug Administration (Solomon *et al.* 1995).

RESULTS AND DISCUSSION

A total of 40 cured fish/shellfish samples procured from local retail markets were examined for the presence of *C. botulinum* and the data are summarised in table 1 and 2. *Clostridium botulinum* was detected in 13% of the samples with type D predominating (4/5) followed by type C (1/5).

Table 1. Chemical characteristics of cured fish.

Common English name of sample	Scientific name	No. of samples examined	Moisture %		Salt %		a_w	
			Average	Range	Average	Range	Average	Range
White sardine	*Escualosa thoracata*	10	45.07	39-49	16.75	13-19	0.76	0.75-0.80
Malabar Tongue sole	*Cynoglosus macrostomus*	5	45.84	40-49	17.32	10-25	0.79	0.75-0.83
Ribbon fish	*Lepturacanthus savala*	7	46.13	37-51	17.10	15-21	0.76	0.75-0.80
Malabar anchovy	*Thryssa malabarica*	8	45.35	44-48	16.69	14-20	0.75	0.75-0.76
White bait	*Stolephorus bataviensis*	2	45.76	45-46	21.51	20-22	0.75	0.75
Prawn	*Metapenaeus dobsoni*	8	18.39	12-22	4.61	3-6	0.75	0.65-0.88

The water activity (a_w) of samples varied greatly (Fig. 1). Eighty eight percent of the samples had a_w values in the range of 0.71-0.80 while 10% had a_w levels of 0.81 to 0.88. The analysis of the water content of the cured fishery products revealed that 55% (22/40) samples had water content in the range of 45-51% and in 25% of the samples, water content varied between 36-45%. In cured prawn samples, water content was still lower (<22%). Sodium chloride content of the cured fish samples varied between 16-25% in only 60 percent of the samples. 20% had sodium chloride content in the range of 11-15%. Sodium chloride content of less than 7% was noticed in 8 prawn samples in the present study. Wide variations in the salt concentration and moisture level were observed in the cured fish samples examined (Fig. 2 and 3).

Table 2. Occurrence of *Clostridium botulinum* in cured fish.

Sample	No. of samples examined	No. of samples positive for C. botulinum	C. botulinum Type identified
Escualosa thoracata	10	1	C
Cynoglossus macrostomus	5	1	D
Lepturacanthus savala	7	1	D
Thryssa malabarica	8	1	D
Stolephorus bataviensis	2	-	-
Metapenaeus dobsoni	8	1	D

Hauschild (1989) reviewed the prevalence of *C. botulinum* in fresh processed fish. Salted fish from the Caspian Sea were 29 percent positive while smoked fish carried spores in 1.1-20 percent of samples. Dodds (1993) reported incidence of *C. botulinum* in 63% of salted carp from the Caspian sea and type E spores were found at the level of <60/10g in salted fish from the Caspian sea and 490/10g in salted carp. A low incidence of *C. botulinum* (13%) was found in cured fish in the present study.

The viability of *C. botulinum* spores at water activity level 0.75 was shown in the present study by the toxic enrichment cultures of cured fish with water activity 0.75. The viability of *C. botulinum* spores for long periods in many foods of low water activity have been reported earlier (Troller and Christian 1978; Genigeorgis and Riemann 1979; ICMSF 1980). These spores are of great concern after rehydration of such foods (ICMSF 1980). There have been outbreaks of botulism associated with salt dried fish (Troller and Christian 1978; Sakaguchi 1979; Huss 1981; McClure *et al.,* 1994).

It is noted that 56% of the cured fish samples (18/32) had moisture content in the range of 46-51% and 44% (14/32) had moisture contents in the range of 37-45%, values higher than the Bureau of Indian Standards (BIS) (IS: 2882, 1964; IS: 5198, 1969 and IS: 2883, 1976) which prescribe a range of 10-35% moisture normally and 40-45% moisture in respect of certain big fish (Gopakumar and Devadasan 1983). Most of the samples do not conform to the BIS levels of salt (IS: 594, 1962). It is evident from the results of the present study that the majority of the cured fish at the retail level contained inadequate salt levels and high moisture levels as reported earlier (George Joseph *et al.,* 1986, 1988; Kalaimani *et al.,* 1988; Prasad *et al.,* 1994). Kalaimani *et al.,* (1988) reported a_w levels of less than 0.80 in 30 percent of the cured fish examined from Quilon, Vizhinjam and Tuticorin. In the present study nearly 90% of the samples had a_w values in the range of 0.71 to 0.80. Wide variations were also observed in the a_w levels of cured fish examined in the present study.

A survey of the cured fish sold at the retail markets in Cochin revealed wide variation in the salt concentration indicating inadequate control of the brining process in a number of products. Recently outbreaks of botulism from *Kapchunka* (salt-cured, air dried uneviscerated white fish) in USA and Israel (CDC 1987; Slater *et al.,* 1989; Telzak *et al.,* 1990) and from aseikh (uneviscerated fish) in Egypt (Weber *et al.,* 1993) have been reported and attributed to a poorly controlled salting process. *C. botulinum* types B and E were involved in the above outbreaks.

The predominance of *C. botulinum* types C and D in fresh fish have been reported from tropical areas (Tanasugarn 1979; Haq and Suhadi 1981; Lalitha and Surendran 1992). *C. botulinum* types C and D are the predominant types in sediment samples from Bangladesh, Indonesia, Thailand and India (Huss 1980; Haq and Suhadi 1981; Tanasugarn 1979; Lalitha and Surendran 1993) and soil samples from Indonesia and Thailand (Tanasugarn 1979; Hayashi *et al.*. 1981). The finding that types C and D are the predominant types in cured fish as shown by the present study is as expected.

Clostridium botulinum types C and D have been reported to cause intoxications only in animals (Smith 1990; Hauschild 1993). However, cases of human botulism due to types C and D have occurred earlier (Roberts and Gibson 1979; Hauschild 1993). Sonnabend and Sonnabend (1981) detected *C. botulinum* type D and toxin in patients who died unexpectedly with severe respiratory difficulty and dysphagia. Fastidiousness of type C in experimental and captive monkeys (Dolman *et al.*, 1961; Barns and Mead 1986) and recent infant botulism by type C in Japan (Oguma *et al.*, 1990) indicate a potential risk. The possible hazard due to salted fish is highlighted by the incidents of botulism associated with it.

The present study indicates a low incidence of *C. botulinum* (13%) and predominance of type C and D in cured fishery products. Clostridium botulinum spores remain viable at water activity (a_w) level 0.75 and are able to grow and produce toxin after rehydration of such foods. The detection of *C. botulinum* in cured fish indicate either post processing contamination or the ability of *C. botulinum* strains to survive the salting process during the manufacture of cured fish. Wide variations in the salt concentration of cured fishery products indicate inadequate control of the brining process. Therefore, the incidence of *C. botulinum* in 13 percent of the cured fishery products should emphasize the need for adequate brining and drying to protect these products until their final use. Proper hygienic conditions in the curing yards are to be encouraged for the production of good quality products.

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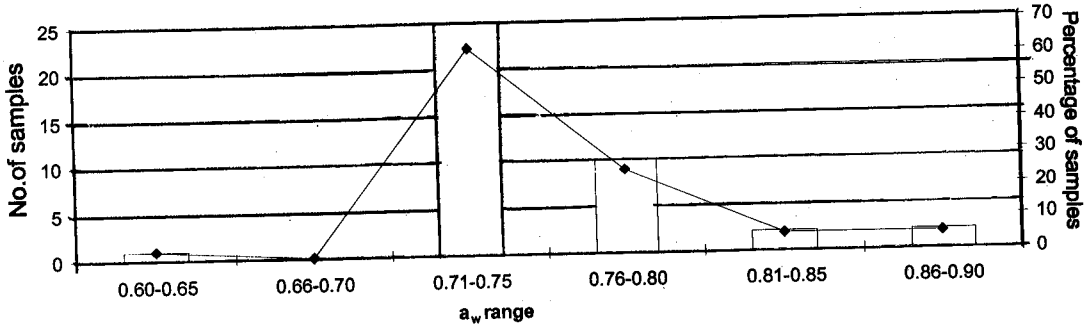

Fig.1. a_w in cured fish.

No. of Samples %of Samples

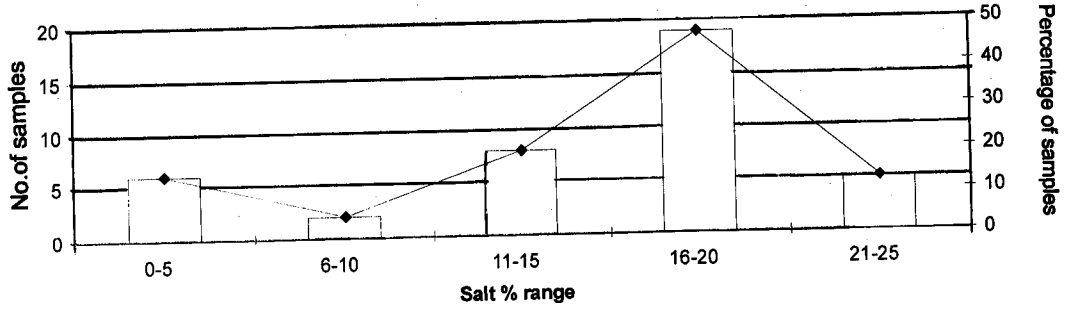

Fig.2. Percentage of salt in cured fish.

No. of Samples %of Samples in each salt range

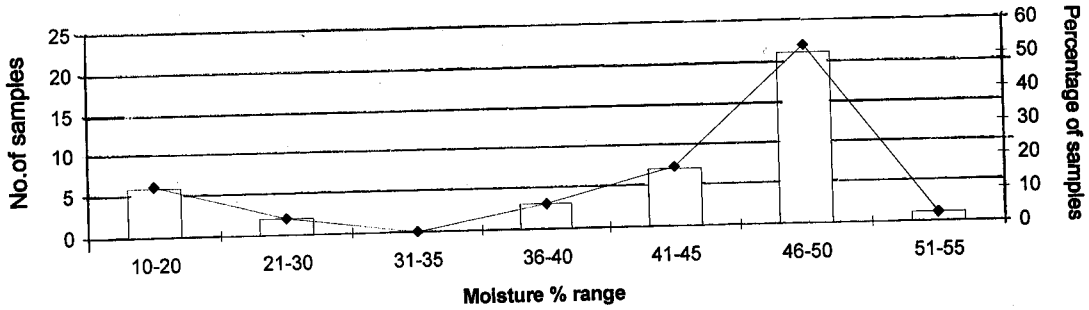

Fig.3. Percentage of moisture in cured fish.

No. of Samples %of Samples