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Effect of Potato Tuber Extract on the Bacteriological Quality of Fish Mince

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The effect of crude potato tuber extract (PTE) on the keeping quality of fish mince at +4 and -4°C storage was studied with a view to retard the proteolytic activity in fish mince. There was significant reduction in trimethyl amino-nitrogen and total volatile base nitrogen levels in PTE treated sample than in control. The growth and activity of the bacteria were suppressed in fish mince by the addition of even small amounts of crude PTE. Incorporation of PTE and storage of fish mince at sub-zero and refrigerated temperatures enhanced the keeping quality of fish mince. A marked difference in the composition of bacterial flora on treatment with PTE was observed. The growth of *Pseudomonas* appeared to be suppressed in the treated fish mince. *Vibrio* spp. were the dominant flora in both samples at the end.

Key words : Fish mince, microbiological quality, potato extract.

Chilling and refrigeration have been recognised as the ideal way to reduce bacterial activity and retain freshness of fish. However, these alone may not be enough to completely control bacterial spoilage of fishery products. Extracellular proteases secreted by contaminant bacteria play a prominent role in fish spoilage even at low temperatures (Venugopal, 1990). It is therefore, essential to minimise bacterial activity preferably by combining other processes along with chilling to achieve maximum shelf life of stored fishery products. Interaction of factors to control microbial spoilage of refrigerated foods have been well documented (Scott, 1989). Various attempts have been made on the use of enzyme inhibitors to retard the proteolytic activity in fish. Inhibition of cathepsin, (Toyohara *et al.*, 1982), *Pseudomonas* proteases (Kato *et al.*, 1974), digestive enzymes in Anchovy (Martinez & Gildberg, 1988), alkaline proteases (Makinodon *et al.*, 1985) and serine proteinases (Orejano & Liston, 1981) by protease inhibitors from various sources have been well demonstrated. Extension of keeping quality of fish by protease inhibitors extracted from Red wood seeds, *Adenanthera pavonia* (Gowda & Karunasagar, 1985) and from potatoes (Aksnes, 1989) has also been reported. The present paper deals the effect of protease inhibitors from potato tuber (*Solanum tuberosum* L.) on the bacteriological and biochemical characteristics of fish mince obtained from *Johnius dussumieri* at refrigerated and sub-zero temperatures.

Materials and Methods

Potato tubers, *Solanum tuberosum* L., were purchased from the local vegetable market. Fresh sciaenid, *Johnius dussumieri*, procured from Tuticorin fishing harbour was used.

Crude extract of potato tubers (PTE) was prepared by homogenizing one part of potato tuber with three parts of 0.02 M phosphate buffer (pH 7.0) containing 0.15 M sodium chloride in cold for 2-3 min. The

homogenized sample was kept at 4°C for 3 h with occasional shaking and centrifuged at 7,500 x G for 15 min. The microbial load of the supernatant extract was reduced by tyndallization in a water bath at 65°C for 30 min for 3 successive days. Trypsin inhibitory activity of PTE as caseinolytic assay of trypsin was quantitatively determined (Sumathi & Pattabiraman, 1975). The protein content of the crude extract was determined by the method of Lowry *et al.* (1951).

The method of processing of fish and the details of treatment given to the fish mince are presented in Fig. 1. All the samples after thorough mixing were stored at +4 and -4°C for quality evaluation. At regular intervals, the trimethylamine nitrogen (TMAN) and total volatile base nitrogen (TVBN) contents of fish mince were determined (Conway, 1947). Total plate count (TPC) on plate count agar and proteolytic count (PC) on skimmed milk agar were determined as described by APHA (1976). The generic composition of the bacterial flora from the samples stored at 4°C were determined by identifying randomly chosen colonies, using the scheme of Surendran & Gopakumar (1981). The effect of PTE on an isolate of *Pseudomonas* sp. from fish mince was studied by inoculating 1.0 ml of a 10 fold diluted overnight culture into 100 ml nutrient broth in 250 ml Erlenmeyer flasks containing 5% PTE or 0.85% sodium chloride (physiological saline) separately. All flasks were incubated without agitation at 30°C for 48 h. Growth was assessed by turbidimetry at 530 nm and by plating on tryptone glucose yeast extract agar at intervals. Two way ANOVA was used to test any significant difference among treatments.

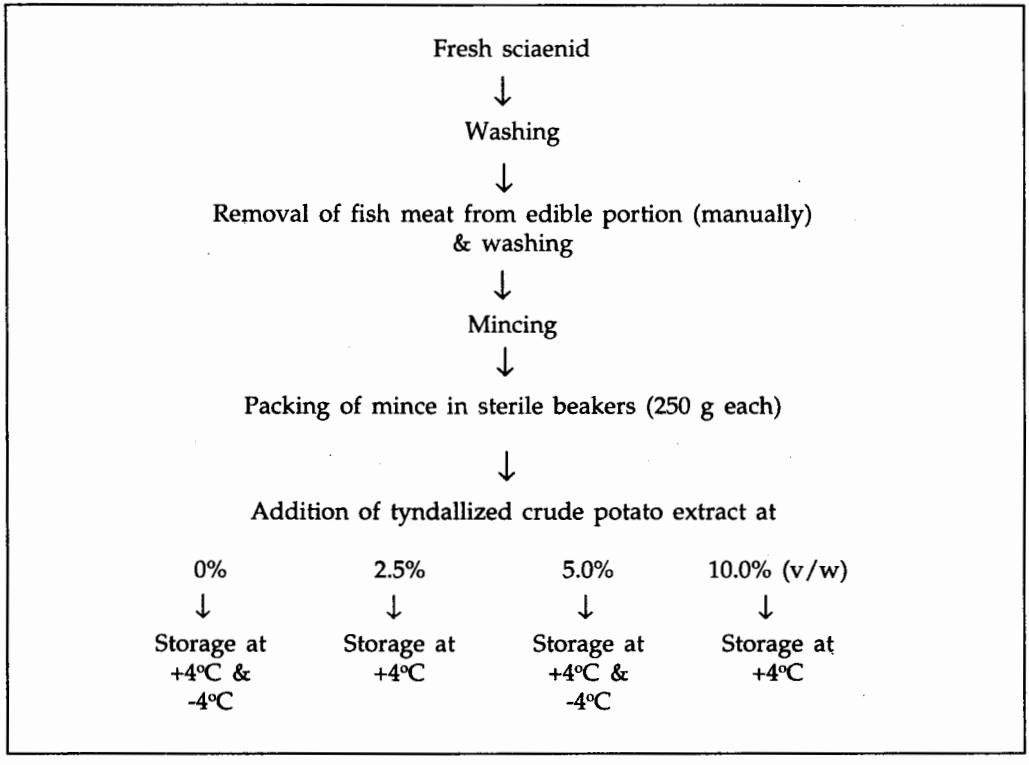

Fig. 1. Method of processing of fish and details of treatment given to fish mince

Table 1. Effect of crude potato tuber extract (PTE) on the quality of fish mince at -4°C

Storage, days	TMAN, mg 100g^{-1}				TVBN, mg 100g^{-1}				Log TPC g^{-1}			
PTE	0%	2.5%	5.0%	10.0%	0%	2.5%	5.0%	10.0%	0%	2.5%	5.0%	10.0%
0	1.15	1.15	1.15	1.15	7.17	7.17	7.17	7.17	6.32	6.32	6.32	6.32
1	3.01	2.01	1.67	1.68	7.77	7.50	7.31	7.55	6.53	6.49	6.49	6.46
3	3.20	2.02	1.77	2.01	10.67	10.37	9.44	9.74	-	-	-	-
5	3.31	2.80	2.70	2.22	17.38	10.79	10.08	10.11	6.61	6.39	6.08	6.21
7	4.25	4.26	4.25	4.07	18.57	13.70	12.15	12.46	-	-	-	-
9	5.57	4.59	4.30	4.44	21.55	17.58	17.42	16.33	7.31	7.28	6.53	6.92
11	6.89	6.20	5.83	6.08	24.80	23.10	21.87	21.27	7.44	7.40	7.08	7.16

TMAN : F 12.34; $p < 0.05$; df 18,3. TVBN : F 8.03; df 18,3. TPC : F 4.78; $p < 0.05$; df 12,3

Results and Discussion

The whole fresh fish had a TPC of log 5.18 cfu g^{-1} which increased upon mincing to log 6.32 to 6.56 cfu g^{-1}, an increase of more than one log unit. The increase in bacterial load during the process of handling, meat removal and washing has been observed earlier (Raccah & Baker, 1978). Voluntary bacteriological limits in some countries set a maximum level of bacteria at < 10^5 g^{-1} of minced fish (Bond, 1975).

The quality of fish mince stored at -4°C with different levels of crude PTE is presented in Table 1. In the untreated sample, the TPC increased by 1.12 log units in 11 days while in treated samples the increase was in the range of 0.76 to 1.08 log units during the same period. The TMAN and TVBN levels increased steadily throughout the period of storage in all samples. But a reduction in TMAN levels was noticed with the increase in PTE levels. There existed a significant difference in TMAN levels ($p < 0.05$) between PTE treated (5% and 10%) and untreated samples. Among PTE treated samples no significant difference was noticed. A significant suppression ($p < 0.05$) of bacterial growth and bacterial activity was evidenced by plate counts and TMAN and TVBN levels, respectively. Similar observation has been reported by Aksnes

Table 2. Effect of crude potato tuber extract (PTE) on the quality of fish mince at +4°C

Storage, days	TMAN, mg 100g^{-1}		TVBN, mg 100^{-1}		Log TPC g^{-1}		Log PC g^{-1}	
PTE	5%	0%	5%	0%	5%	0%	5%	0%
0	1.38	1.38	7.19	7.19	6.56	6.36	4.00	4.00
1	4.75	5.51	16.59	17.23	6.76	6.92	6.00	6.40
3	10.95	16.20	17.79	20.25	7.04	7.20	6.29	6.68
5	13.76	20.73	24.99	31.79	7.31	7.28	6.57	6.81
7	15.74	25.92	33.04	40.32	7.84	7.68	7.29	7.37
9	22.71	32.70	66.06	76.30	8.65	9.53	8.24	9.25
11	32.38	36.16	103.81	116.92	9.77	9.78	9.51	9.61

TMAN : F 11.83; $p < 0.05$; df 6,1. TVBN : F 9.53; $p < 0.05$; df 6,1.
TPC : F 1.27; $p < 0.05$; df 6,1. PC : F 1.27; $p < 0.05$; df 6,1.

Table 3. Changes in the bacterial profile of potato tuber extract treated and untreated fish mince at 4°C (represented as percentage of total microflora)

Microorganisms	Fresh fish (%)	Fish mince on 1st day		Fish mince on 11th day	
		Treated	Untreated	Treated	Untreated
Acinetobacter	24.0	31.0	3.0	9.0	2.0
Aeromonas	24.0	5.0	3.0	21.0	7.0
Alcaligenes	2.0	2.0	3.0	ND	ND
Arthrobacter	2.0	7.0	7.0	ND	9.0
Bacillus	7.0	15.0	16.0	21.0	9.0
Corynebacterium	2.0	7.0	6.0	5.0	ND
Enterobacteriaceae	7.0	3.0	ND	2.0	5.0
Micrococcus	10.0	14.0	26.0	2.0	ND
Moraxella	2.0	ND	ND	ND	ND
Pseudomonas	3.0	5.0	17.0	2.0	21.0
Staphylococcus	12.0	5.0	16.0	8.0	2.0
Vibrio	5.0	3.0	3.0	28.0	45.0
Unidentified	ND	3.0	ND	2.0	ND
Total gram negative bacteria	67.0	49.0	29.0	62.0	80.0
Total gram positive bacteria	33.0	51.0	71.0	38.0	20.0

ND = Not detected

(1989) in PTE treated ground herring stored at 6°C. The results of the TMAN content of this experiment suggest that the PTE can keep fish mince in good condition for 9 days at -4°C whereas untreated fish tend to become unacceptable by this period.

The changes in the bacteriological and biochemical characteristics of fish mince stored at 4°C are presented in Table 2. The increase in TPC was rather slow initially, about one log unit in seven days, followed by 2 log units increase in subsequent four days of storage. Similarly there was an increase in PC by 5 log units in both the samples in 11 days. The increase in PC was very sharp immediately after storage (1 day) by more than 2 log, and after 7 days of storage. A significant difference ($p < 0.05$) in proteolytic counts was observed between treated and untreated samples throughout the storage.

TMAN was detectable even in fresh fish and increased by more than 2.4 folds on the very first day itself. Significant difference ($p < 0.05$) were noticed in TVBN ad TMAN levels between treated and untreated fish mince throughout the storage.

The samples showed no signs of spoilage up to 5th day inspite of higher TMAN and TVBN levels. Slight off-odour and signs of spoilage were noticed on the 7th day of sampling. The immediate sharp rise in TMAN levels could be attributed to the sharp increase in PC by more than 2 log units on the 1st day. Apart from this, maceration of fish meat might have released cellular materials thereby making the fish mince a better substrate for bacterial growth and chemical reaction than the intact raw material.

Table 4. Effect of crude potato tuber extract on *Pseudomonas* sp. in liquid medium

Storage period, h	Log *Pseudomonas* count ml^{-1}		Absorbance at 530 nm	
	Treated	Untreated	Treated	Untreated
0	5.40	5.48	0.010	0.010
12	8.06	8.18	0.065	0.070
24	9.40	9.79	0.103	0.120
36	9.79	9.85	0.150	0.160
48	9.82	9.86	0.160	0.173

Pseudomonas count: F 4.595; $p < 0.01$; df 5,1. Absorbance: F 9.10; $p < 0.05$; df 4,1.

It is worth mentioning here that organoleptically the shelf life of mechanically deboned fish meat was reported to be 5 days at 2°C with a corresponding TPC of 1.0 to 5.0 x 10^8 cfu g^{-1} (Raccach & Baker, 1978).

A marked difference in the composition of bacterial flora in fresh fish and fish minces on treatment with PTE at 4°C for 11 days was observed (Table 3). *Acinetobacter* and *Aeromonas* among gram negative, and *Staphylococcus* and *Micrococcus* among gram positive bacteria were the dominant groups in fresh fish accounting for 70% of the total population. The process of washing, filleting, meat removal and mincing changed the composition of bacterial flora. Both treated and untreated samples had varying composition of bacterial flora on the first day. In treated sample *Acinetobacter* was dominant followed by *Bacillus* and *Micrococcus*, while in untreated sample *Micrococcus* was dominant followed by *Pseudomonas*, *Bacillus* and *Staphylococcus*. The fish mince which was almost spoilt on the 11th day was dominated by *Vibrio* in both samples. Spoilage of fish is reportedly caused by microbial population dominated by *Pseudomonas* and related bacteria like *Vibrio* (Liston, 1982; Chandrasekaran *et al.*, 1984). This was evident in untreated fish, where *Pseudomonas* and *Vibrio* accounted for 21% and 45% of the total population, respectively. In treated fish, on the other hand, the growth of *Pseudomonas* group appeared to be suppressed and accounted only 2% of the total flora. Similar suppression of *Pseudomonas* with protease inhibitors (PI) from *Adenanthera pavonia* treated fish has been reported (Gowda & Karunasagar, 1985). The suppression of growth of *Pseudomonas* was also confirmed when grown in liquid medium with and without crude PTE (Table 4). The addition of PTE has been reported to inhibit autolysis and reduce the liberation of free amino acids (Aksnes, 1989) thereby making the fish mince an unsuitable substrate for immediate bacterial growth and activity.

The protease inhibitory activity of crude PTE was measured in an assay for trypsin and it was found to have 14.29 Trypsin Inhibitory Units (TIU) activity mg^{-1} protein. Sumathi & Pattabiraman (1975) recorded 18.66 TIU mg^{-1} protein in potato and Aksnes (1989) observed variation in the activities of PI from different cultivars of potato. The lower Trypsin inhibitory activity in this case may be because of the heat treatment given to reduce the microbial load. Although the potato tubers were reported to contain inhibitors for chymotrypsin, carboxypeptidases A and B and Cathepsin D (Pearce *et al.*, 1982; Aksnes, 1989) no attempt was made in this study to confirm the same. Potato tuber was reported to show greater inhibitory activity against α - Chymotryp-

sin and the PI activity of potato was completely destroyed when heated for 20 min at 95°C (Sumathi & Pattabiraman, 1975).

Thus, addition of crude extract of potato tuber containing protease inhibitors to fish mince decreased the production of TMAN and TVBN and suppressed the growth and activity of bacteria. Since the PI from potato has been reported to be inactivated on heat treatment, it can be expected that any residual protease inhibitor in treated fish would be destroyed during cooking and the fish would hence be safe for human consumption. Storage of fish mince at sub zero temperature would further help enhance its keeping quality. Extracts from potato tuber could thus be effectively used to prolong the shelf life of fish mince at refrigerated storage temperatures.

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