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DEVELOPMENT OF MARINATED FISH PRODUCTS

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Marinated fish products have been produced using locally available fish species by means of a two-stage curing process at 4°. The first stage consists of a period of 8 weeks in pickle containing 4% acetic acid and 10% salt. This was followed by 2 weeks in a weaker pickle containing 1% acetic acid and 2% salt. The finished products could be stored satisfactorily at 4° for 16 weeks. Sensory chemical and microbiological changes that take place during processing were also determined. It was concluded that satisfactory cold marinated fish products can be prepared from *Stramateus* spp. (white pomfret) followed by *Lutjanus* spp. (red snapper), *Pristipoma* spp. (sea bream) and *Cynoglossus* spp. (sole). *Pelamys* spp. and *Cybium* spp. (mackerel) were found unsuitable for this purpose.

INTRODUCTION

Fish marinades are characterized by typical odours and flavours. For their preparation, fish or parts of fish are treated with sodium chloride and acetic acid. This treatment simultaneously brings about a refinement, i.e. conversion of raw product into food [1]. With the exception of fried marinades, these products are of limited shelf life and therefore are classed as semipreserves. Cold marinated products are usually prepared by a two-stage process. In the first stage, fish fillets are immersed for upto three weeks in a relatively strong acetic acid and salt solution. At the end of this stage, fish is removed from the immersing solution, drained, and then immersed in weak acetic acid and salt solution, flavoured with sugar and spices. Onion and other vegetables may also be added at this stage [2].

Many products which may be classed as cold marinades are very popular and are prepared in Europe, particularly in Germany [2, 3, 4].

These products are unknown in Pakistan. The present study was undertaken to examine the curing conditions required to produce cold marinated products from locally available fish varieties and to compare their characteristics and acceptability.

MATERIALS AND METHODS

The following fish species were used in this study: (1) white pomfret (*Stramateus* spp.) (2) sea bream (*Pristipoma* spp): (3) flat fish (sole) *Cynoglossus* spp.), (4) mackerel (*Pelamys* spp.), (5) mackerel (*Cybium* spp.), and (6) red snapper (*Lutjanus* spp).

Fresh fish were obtained from the Karachi Fish Harbour; intact fish weighed approximately 600 g each. The fish were scaled, cleaned and filleted. The fillets, weighing approx. 200 g, were soaked in 4% sodium chloride (300 ml/fillet) for about 20 min. to remove blood.

A preliminary experiment was carried out to determine the minimum acid and salt concentration in the first pickle for an acceptable product (see results). For this purpose two fish species, viz., *Stramateus* spp. and *Pristipoma* spp. were marinaded in a one-stage process. Pickle solutions of the following variations in acid and salt concentration were used: (1) 3% acetic acid 10%, sodium chloride, (2) 4% acetic acid, 10% sodium chloride, and (3) 7% acetic acid, 14% sodium chloride. The strengths of these pickles were identical with those used by McLay and Pirie [2]. For marinading, fillets (approx. 100 g) were immersed in 100 ml of pickle in closed jars and were stored at 4°. The samples were examined for odours and appearance after 36,66 and 84-days of storage.

Following the preliminary experiment, a second more detailed experiment was performed using the following pickle strength: 5% acetic acid, 10% sodium chloride. Fish fillets were placed in 0.5 kg glass jars, containing the pickle. The ratio of the fish to the pickle was 3:2 (W/W). In this way, the individual fillets were in contact with the pickle throughout the process. After 4, 8, and 16 weeks in the first pickle (4°) samples of fish were removed from the first pickle and placed into 0.5 kg glass jars containing the second pickle. The second pickle contained 1% acetic acid and 2% salt. The ratio of fish to pickle was 1:1 (W/W). To

each jar was added one bay leaf, one whole clove, one red pepper, one white pepper and three coriander seeds and about 10 g. sliced onion. The finished marinades were stored at 4° and the samples were removed at 2, 4 and 16 weeks for subjective and objective assessment.

Sensory evaluation. Sensory evaluation was carried out by the Laboratory staff (8 judges) who were familar with the product and knew what constituted a good quality product. In the preliminary experiment the samples were examined for odour and appearance. In the detailed experiment the samples were tasted for flavour, texture and judged for appearance. The samples were presented after slicing the fillets into smaller pieces, carefully mixing them to obtain a uniform sample and served with sliced onion and spiced sauce. A scale between 9 (extremely good) and I (very poor) for odour, flavour, texture and appearance was used. The score of each parameter was calculated in terms of the average score awarded by a panel of judges to each sample. Statistical differences between final marinades were determined by the analysis of variance for flavour and texture after two weeks storage in second pickle.

Chemical evaluation. Sodium chloride was determined according to the AOAC method [5]. Acetic acid was determined by the titration of filtered aqueous extract of the flesh (10 g) with 0.1 M sodium hydroxide using phenolphthalein as indicator. The pH of the flesh was determined using a Cambridge pH meter after blending with distilled water 1:1 (W/v) for 1 min. Total volatile bases (TVB) in fish flesh was determined by the method of Cobb *et al* [6]. The thiobarbituric acid (TBA) number was determined using the distillation method of Tarladgis *et al* [7].

Microbiological evaluation. Raw fish: The aerobic plate count (APC) was determined by the method recommended by Surkiewicz [8]. To determine the microbial types a number of colonies (at least 50) were picked at random from countable plates. Gram-positive organisms were identified using the method of Baird-Parker [9] and gram-negative rods were classified by the identification scheme of Shewan *et al* [10] and Bergey's "Manual of Determinative Bacteriology" [11].

MARINADES

Sample preparation. The sample was drained in a filter funnel for 30 min, weighed aseptically into a sterile "Atomix" Jar of 200 ml capacity and homogenized for 1 min at 6,000 rpm followed by 30 sec. at 12,000 rpm. Serial dilutions of homogenate were prepared in 0.1% w/v peptone (Oxoid, Bacteriological) and 0.8% sodium chloride. The Microbiological growth was detemined using the spread technique. Plate count agar and *Lactobacillus* selective Agar (Rosaga Agar, (Merck) were used thoughout the work. In addition, a modified medium was prepared by adding 2% w/v sodium chloride and 0.15% v/v acetic acid to the plate count agar (merck). The plates were incubated at 30° and counted after 48 hr. All plates were incubated aerobically. However, a layer of the medium cooled to about 50° was poured onto the inoculated plates.

RESULTS AND DISCUSSION

Sensory evaluation of the samples in the preliminary experiment showed that after 84 days storage at 4°, samples in 3% acetic acid and 10% sodium chloride had rancid off odour and yellow colour and were considered spoiled. These samples were significantly different ($P < 0.01$) from samples in 4% acid and 10% sodium chloride and in 7% acetic acid and 14% sodium chloride which did not display similar defects and were all acceptable after the same storage. McLay and Pirie [2] and Wootton and Chuah [4] also found that below a minimum acetic acid in the first brine off odours and yellow colours developed at low temperature. It was concluded that the pickle containing 4% acetic acid and 10% salt was the minimum acid and salt content which could be used for first stage marinades. Therefore this strength was selected for the more detailed investigation.

After 4, 8 and 16 weeks in the first pickle, no rancid off odour or yellow colour was detected in any of the products. The acid and salt reached equilibrium within 4 weeks. Noteworthy is the fact that the acid exceeded the theoretical estimate of the equilibrium state if compared with the data reported by Meyer [1].

Analysis of fish in the second pickle after 2, 4 and 16 weeks storage at 4° showed that sodium choride and acid had migrated from the flesh to the weaker pickle, however, an excess over calculated equilibrium levels of acid in the flesh remained. McLay and Pirie [2] reported similar findings for marinated herring. The acid content of the fish samples varied between 1.24% and 1.74% and the salt content between 2.14% and 2.82% after two weeks storage at 4°, the corresponding figure after 4 weeks storage was found between 1.28% and 1.7% for acid and between 2.04% and 2.84% for salt. The acid and salt levels found in the product samples are comparable with those reported for similar products [1, 2, 3 and 4]. Only small changes were found in acid and salt content of fish samples even after 16 weeks storage in the second pickle. The pH of marinades was found to be 4.8 in most of the samples.

The preliminary study of the bacteriology of marinades demonstrated that normal spoilage bacteria were inhibited by the acid and salt. In raw fish the *Pseudomonas* group was the most predominant bacteria. The next dominant genus was *Micrococcus* which comprised 15 to 40% of the total flora. These were followed by *Moraxella, Vibrio,* and *Bacillus* spp. After processing, however, only *Bacillus* and *Micrococcus* genera were isolated. The total bacterial count showed 2 to 3 log cycle reduction during processing. The total count ranged between $5.3 \times 10^5 - 9.2 \times 10^6$/g in the starting raw material which was reduced to $1.8 \times 10^3 - 5.3 \times 10^4$/g in the finished product. Homofermentative *Lactobacilli* and gram positive *Cocci* were isolated at various stages of processing.

These organisms are being characterized and their role in the production of typical aroma of fish marinades will be reported in another communication.

A survey of the literature shows that the physical limits for growth and toxin formation for *Clostridium botulinum* are pH < 4.6 and temperature $< 3.3°C$. Thus botulism may be a potential problem if the marinades are handled in such a way that allow *Cl-botulinum* to grow.

Changes in TVB and TBA number in marinades are given in Table 1. TVB was used to compare the formation of volatile substances perhaps as a result of chemical and enzymatic changes. TVB however, is of a little value as an index of quality of marinated fish due to the inhibition of normal spoilage flora [4].

It may be seen that TVB increased only slightly from that of the fresh fish irrespective of fish species and very little change occurred during the second stage brining of 2 and 16 weeks. The TBA number was found to vary between 0.09 and 2.98 at 2 weeks storage and between 0.8 and 5.1 at 16 weeks storage in the final marinades. Initially the TBA number was zero in all cases indicating that good quality raw material was used for the preparation of the marinades. Yu and Sinnhuber [12] have reported that good quality fish had TBA numbers less than 3. On the basis of this observation the experimental products except for the two mackerel samples may be regarded as being of good quality. While increases in TBA number were apparent as a result of the longer storage time, no evidence of rancid off flavour or yellow colour in brine or flesh was noted in the samples except for the two with a high TBA number.

Tables 2 and 3 give the taste panel assessment of final marinades. The samples prepared from the white pomfret, sea bream, flat fish and red snapper were significantly ($P < 0.01$) better than those prepared from the mackerel in both flavour and texture. For both flavour and texture, the samples treated for with 8 weeks in strong brine and 2 weeks in weak brine ($P < 0.05$) were preferred. The longest brining (16 weeks) give products which had a soft texture and strong flavour. The shortest brining period (4 weeks) gave products which had a tough, rubbery texture and a raw flavour. Final marinades were also examined by the taste panel after 16 weeks. Relatively little deterioration was indicated by sensory ratings. If examined in terms of the species of fish used, the best product was obtained form white pomfret followed by red snapper, sea bream

Table 1. Changes in TVB and TBA number in final marinades[a]

Type of Marinade	Initial TVB mg/100g	Initial TBA Number	TVB mg N/100 g						TBA Number					
			4[b]		8		16		4		8		16	
			2[c]	16	2	16	2	16	2	16	2	16	2	16
White pomfret	21.2	0	24.2	28.2	27.2	29.8	26.8	29.6	0.28	2.1	0.42	2.6	2.88	2.8
Sea bream	18.9	0	22.6	24.8	23.4	26.2	24.2	27.2	2.12	1.9	0.15	2.2	0.46	2.3
Flat fish (Sole)	15.4	0	17.1	26.2	18.1	20.2	18.2	20.8	0.09	0.8	0.12	2.0	0.72	2.4
Mackerel (*Pelamys* spp)	25.4	0	27.2	30.6	26.0	28.8	27.8	29.8	1.86	3.8	2.2	4.1	2.98	5.1
Mackerel (*Cybium* spp.)	28.6	0	27.4	31.2	29.6	30.8	29.7	32.6	0.82	1.8	0.98	2.8	2.06	4.6
Red snapper	20.8	0	22.8	24.6	23.6	25.2	23.8	24.9	0.24	1.2	0.82	2.1	0.82	2.1

[a] Samples were marinated in strong pickle for period shown ([b]weeks), followed by curing in weak pickel ([c]weeks).

Table 2. Taste panel assessment of final marindes[a]

Name of Fish	Flavour						Texture					
	4[b]		8		16		2		8		16	
	2[c]	16	2	16	2	16	2	16	2	16	2	16
White pomfret	6.2	5.8	6.8	6.4	6.6	6.3	5.8	5.6	6.2	6.0	5.6	5.4
Sea bream	6.0	5.6	6.4	6.3	5.5	6.2	5.6	5.4	5.8	5.8	4.5	5.6
Flat fish (Sole)	5.5	5.4	5.8	5.7	5.2	5.4	5.2	5.1	5.3	5.0	5.2	5.0
Mackerel (*Pelamys* sp.)	2.2	2.1	2.6	2.4	2.8	2.2	2.0	1.8	2.2	1.8	1.7	1.6
Mackerel (*Cybium* sp.)	3.0	2.2	2.8	2.3	2.6	2.4	2.2	2.4	2.6	2.4	2.2	2.0
Red snapper	5.8	5.6	5.7	5.6	5.9	5.4	5.8	5.6	5.7	5.6	5.4	5.5

[a] Samples were marinated in a strong pickle for period shown ([b] weeks), followed by curing in a weak pickel ([c] weeks).

Table 3. Taste panel assessment of final marinades and comparision after two weeks storage in second pickle[a]

Name of Fish	Flavour						Texture					
	4[b]		8		16		4		8		16	
	2[c]	16	2	16	2	16	2	16	2	16	2	16
White pomfret	6.2	5.8	6.8	6.4	6.6	6.3	5.8	5.6	6.2	6.0	5.6	5.4
Sea bream	6.0	5.6	6.4	6.3	5.5	6.2	5.6	5.4	5.8	5.8	4.5	5.6
Flat fish (Sole)	5.5	5.4	5.8	5.7	5.2	5.4	5.2	5.1	5.3	5.0	5.2	5.0
Mackerel (*Pelamys* spp.)	2.2	2.1	2.6	2.4	2.8	2.2	2.0	1.8	2.2	1.8	1.7	1.0
Mackerel (*Cybium* spp.)	3.0	2.2	2.8	2.3	2.6	2.4	2.2	2.4	2.6	2.4	2.2	2.0
Red snapper	5.8	5.6	5.7	5.6	5.9	5.4	5.8	5.6	5.7	5.6	5.4	5.5

Analysis of variance: (After 2 weeks storage in second pickle)

Source of variation	DF	Sum of squares	Mean squares	F	DF	Sum of Squares	Mean Square	F
Fish	5	45.62	9.124	240.10[o]	5	46.676	9.335	225.04*
Storage	2	0.42	0.21	5.52**	2	0.708	0.354	9.672**
Error	10	0.38	0.038		10	0.366	0.0366	
Total	17	46.42			17	47.75		

[a] Sample were marinated in a strong pickle for the period shown ([b] weeks), followed by curing in a weak pickle ([c] weeks).

*Significant at the 1% level ($P < 0.01$)

**Significant at the 5% level ($P < 0.05$)

and flat fish (sole). Mackerel (*Pelamys* spp.) gave the worst product both in terms of texture and flavour followed by Mackerel (*Cybium* spp.) This may be due to the fat contents of these species.

It may be concluded that satisfactory cold marinades can be prepared from white pomfret, red snapper, sea bream and flat fish (sole). Final product underwent little deterioration in quality as measured by sensory acceptability, TBA number and TVB determinations during storage at 4° for 16 weeks.

The following process produced an acceptable product: a first-stage treatment in pickle containing 10% salt and 4% acetic acid at 4° for 8 weeks, followed by a second-stage treatment in pickle containing 2% salt and 1% acetic acid at 4° for 2 weeks. At this stage suitable species are added and the product should be stored at 4°. The result of this investigation indicates the feasibility of cold marinated products production based on fish available in Pakistan.

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