SPOILAGE IN ICED "PESCADA-FOGUETE" (*MACRODON ANCYLODON*) FROM SOUTH BRAZILIAN FISHING GROUNDS

(Received 1/9/61)

Ko Watanabe

I — INTRODUCTION

"Pescada-foguete" has enjoyed broad acceptance in the São Paulo fresh fish market for many years and has comprised 17.4% by weight (second to sardine) and 27.1% by value (first of any species) of the total landings in Santos during the period July 1958-June 1959 (Richardson & Moraes, 1960).

Landings of the fish till 1956 had been made mainly by the small pair-trawlers, which operate off the coast of the State of São Paulo. The boat sizes, however, have had a tendency since 1958 to become larger and the vessels have gone farther to the south, down to the coast of the State of Rio Grande do Sul as the fishery developed there (Braga, 1961). Thus each voyage takes a longer time than before and consequently the question of how many days the fish could actually be kept in edible condition in ice has become important. This question limits the length of each trip so as not to bring back unmarketable fishes, having a substantial influence on the quality of the fish arriving to the consumer.

Publ. nº 163 do Inst. Ocean. da USP.

TABLE I

Organoleptic scoring sheet for raw fish		
Score	Description of spoilage symptoms	General remark
10	Freshly caught to rigor mortis just passed, perfectly fresh with sea odor.	Excellent
8	Eyes not sunken transparent, gills red and neutral odor, surface brilliant, flesh softer.	Good
6	Eyes slightly sunken and opalescent, flesh soft, gills pinkish, surface slightly slimy.	Fair
4	Eyes sunken and turbid, flesh very soft, odor ammoniacal, surface slimy and descolored to yellowish, gills slimy and brownish with strong stale odor.	Acceptable
2	Eyes sunken, surface extremely slimy, and descolored to yellow or brown, odor putrid, gills brown and slimy, flesh very soft.	Half spoiled
0	General appearance deteriorated. Eyes like holes, surface-skin easily comes off, strongly putrid to nauseating odor, flesh milky.	Spoiled

Organoleptic scoring sheet for cooked fish			
Score	Odor	Flavour	Appearance
10	sweet, fresh	sweet	brilliant and white
8	neutral	absent of sweetness, neutral	brilliant and white
6	neutral to acidic	no flavour, slightly bitter	losing brilliancy, yellow to brown spots around bone
4	stale, slightly ammoniacal	distasteful, stronger bitter taste	general tone of flesh yellow
2	putrid, ammoniacal	sour and bitter taste strong, nauseating	putrid appearance
0	putrid, nauseating	inedible	putrid appearance

The first approach to the problem is to establish methods which can measure freshness of the fish as objectively as possible.

This pilot-scale experiment was designed to provide information on the assessment of the fish spoilage, using organoleptic, chemical and bacteriological means. The information might be useful not only for finding out the storage life of fish, but also for fisheries inspection services in view of supplying objective criteria of quality control.

II — MATERIAL AND METHODS

Three lots of "pescada-foguete" were brought into the laboratory during the period March-June 1961. Each lot consisted of 40-50 fishes, captured off the coast of the Canoa-Cidreira-Solidão region, State of Rio Grande do Sul, by a pair-trawler. Fishes were washed, packed in boxes and stored uneviscerated in sufficient ice in fish-holds during the voyages. The three lots had storage periods of 3, 8 and 13 days aboard respectively. Fishes were repacked, on arrival at the laboratory, in an insulated box with new ice, and sampling was done at intervals of 2-3 days up to 14-19 days in ice. Samples, each consisting of 6-7 fishes, were examined organoleptically, chemically and bacteriologically. Some confirmatory determinations were made on other samples with exactly known storage time during the same period and from the same fishing grounds, independently of the aforementioned runs, the results of which also were used for the analysis of the data.

Chemical tests were made with total volatile nitrogen (TVN) and trimethylamine (TMA) determinations in the fish muscle using Conway's microdiffusion method (Conway *et al.*, 1933; Jepsen, 1959), both are expressed as mgN/100 gr. fish muscle. The pH was measured with Merck special indicator paper.

Organoleptic estimation was made in two ways, namely the odor and appearance of the raw fish scored in ten-point scale and the odor, flavor and appearance of the cooked fish in ten-point scale, the value of which was given by the mean of the panel members. A panel of 3-4 persons previously trained was used for the scoring of the cooked fish. Table I shows the scoring sheet. The method of the scoring itself will be discussed in detail in a future paper. The cooked fish was prepared placing beheaded and gutted fish in boiling 2% brine for 2 minutes, then allowing to stand in it for 10 minutes after taking off the fire.

Bacteriological survey was performed by making total aerobic counts on skin-surface and intestinal content of the fish, using fish extract-peptone agar (0.5% peptone, 0.8% NaCl in

TABLE II — Spoilage of "pescada-foguete" in ice

Ex. N.º	Days in ice	Organoleptic score		Chemical indices			Total aerobic counts (× 10^3)	
		raw fish	cooked fish	TMA	TVN	pH	skin surface/cm²	Intestinal contents/gr
I	3	9	8.6	3.5	14.8	6.8	52	1,200
	6	7	6.4	4.2	13.6	6.7	280	440
	8	5	5.8	4.9	16.7	6.9	92,000	49
	10	4	5.2	10.8	20.3	7.0	95,000	160
	12	3	3.6	16.6	21.7	7.0	150,000	950
	14	2	2.5	18.6	30.0	7.0	93,000	1,200
II	13	5	4.8	9.1	19.5	6.8	7,800	23
	14	4	4.5	11.3	21.2	7.0	27,000	89
	15	3	3.3	12.0	23.9	6.9	48,000	140
III	8	8	7.2	2.7	11.6	6.8	120	89
	11	6	6.3	2.8	11.6	6.8	3,800	89
	13	6	5.4	4.4	13.5	6.9	28,000	38
	15	4	4.0	7.9	19.5	6.9	650,000	1,800
	17	2	2.1	14.3	22.9	7.0	470,000	2,300
	19	0	0.9	19.2	25.4	7.0	480,000	2,500

pepsin digested fish extract basal medium) by dilution method. Counting was done after 3 days incubation at 25°C. Bacterial flora was analysed by picking up colonies at random from the count plates to follow up the changes in generic distribution due to ice storage. Tests for classification of the cultures, after being subjected to purification process, were made by observation of colonial appearance, Gram stains, morphology, motility and the triple sugar iron agar reactions, and further biochemical tests if necessary (Watanabe, 1960). For differentiation of *Pseudomonadaceae* and *Achromobacteriaceae,* the sensitivity to 2.5 IU penicillin was used (Shewan, 1954). Grand total of the isolates was 357.

III — RESULTS

Normal taste panel score range in this experiment was ± 1 unit, which was less sensitive than Shewan's (1957a) and Liston's (1961) ± 0.5 unit panel. Lowest limit of palatability was agreed by panel members to be score 4.0.

The results of organoleptic, chemical and bacteriological tests applied in the three spoilage runs is given in Table II. The organoleptic score of raw fish confirms closely to cooked ($r = 0.933$) and since the scores of cooked fish were shown in mean value of taste panel members, they are reasonably considered as a representative of the organoleptic score.

The relation between cooked fish score and days in ice is shown in Figure 1. The regression equation is: cooked fish score = 9.6 — 0.42 × days-in-ice, and most of the points lie within ± 1 score unit of the fitted line ($r = 0.946$). This equation demonstrates a close resemblance with the equation for the same relationship derived by Shewan *et al.* (1957a), which is: cooked odor = 9.2 — 0.31 × days-in-ice.

As for the relations between days-in-ice and TMA and TVN, the following approximate equations are calculated from the data:

$$10 \times \log (1 + \text{TMA}) = 4.1 + 0.46 \times \text{days-in-ice} \quad (r = 0.762)$$

$$10 \times \log (1 + \text{TVN}) = 10.2 + 0.25 \times \text{days-in-ice} \quad (r = 0.724)$$

The relations between TMA and cooked fish score showed good correlation ($r = 0.980$), the approximate equation of which is:

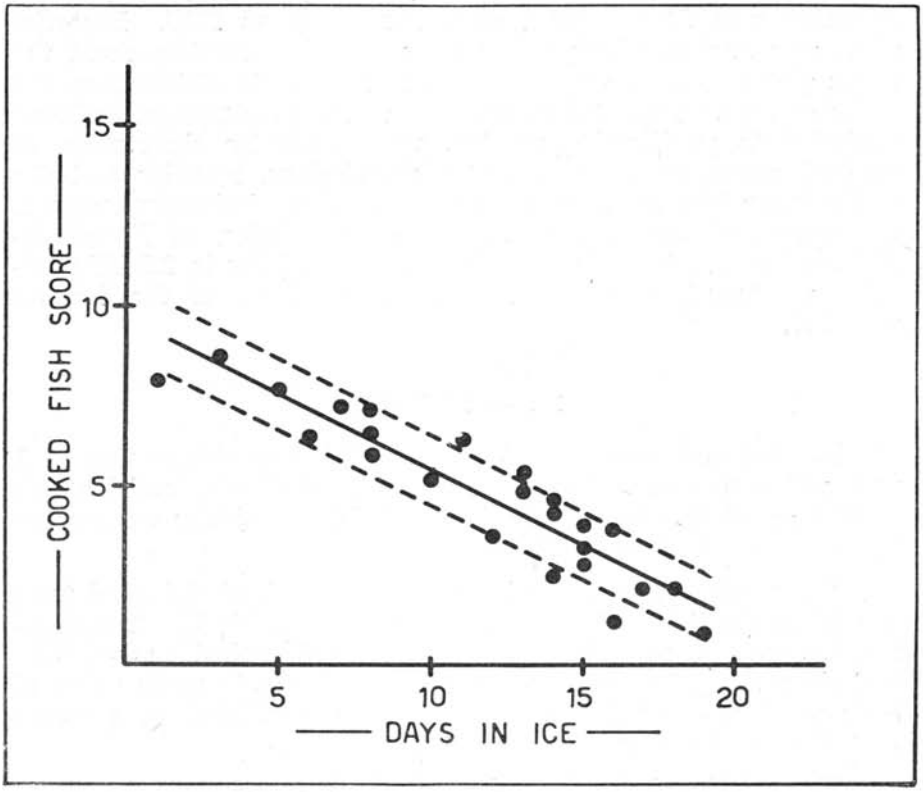

Fig. 1 — Relationship between cooked fish score and days-in-ice.

$10 \times \log (1 + \text{TMA}) = 1.46 - 1.06 \times$ cooked fish score (Fig. 2),

with most points within ± 1 TMA unit of the fitted line. The similar straight line relationship between the score and TVN is shown with regression equation of:

$$10 \times \log (1 + \text{TVN}) = 16.0 - 0.60 \times \text{cooked fish score},$$

but more points fell out of ± 1 unit ($r = 0.838$).

The pH showed small variation in the whole period of storage, from 6.8 at fresh stage to 7.0 at spoiled.

Figure 3 shows the relation between bacterial count and cooked fish score; the fitted regression equation is:

$\log$ (Bac. count) $= 9.87 - 0.54 \times$ cooked fish score ($r = 0.901$).

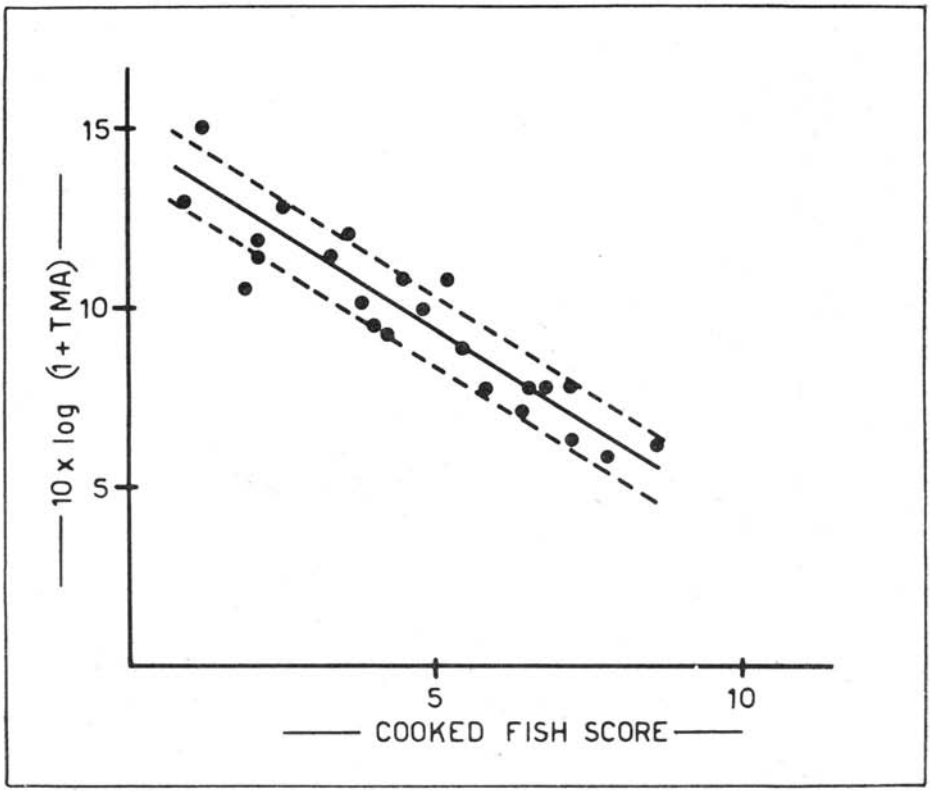

Fig. 2 — Relationship between TMA and cooked fish score.

The general pattern of bacterial growth in the Rio Grande do Sul catch is that the lag phase lasts for 5-6 days keeping the order of 10^3 — $10^4/cm^2$ bacterial count, then it turns to growth phase at 6-7 days, attaining $10^6/cm^2$ level or higher at 8-10 days, arriving static stage of the growth for further several days. As we know that bacteria are responsible for the TMA production which cause the off-odor in stored fish, and at 10^6 level the first off-odor could normally be noticed, it might be reasonable to presume that the borderline separating grade I and grade II fish of Castell *et al.* (1958) would roughly correspond to the cooked score 7, hence 4-8 day-in-ice. In intestinal contents, total aerobic counts made such a variation during storage that in the first 10-11 days it decreased almost 50 fold from the initial 10^6 level, then jumped up again to the order of 10^6 at the end the storage.

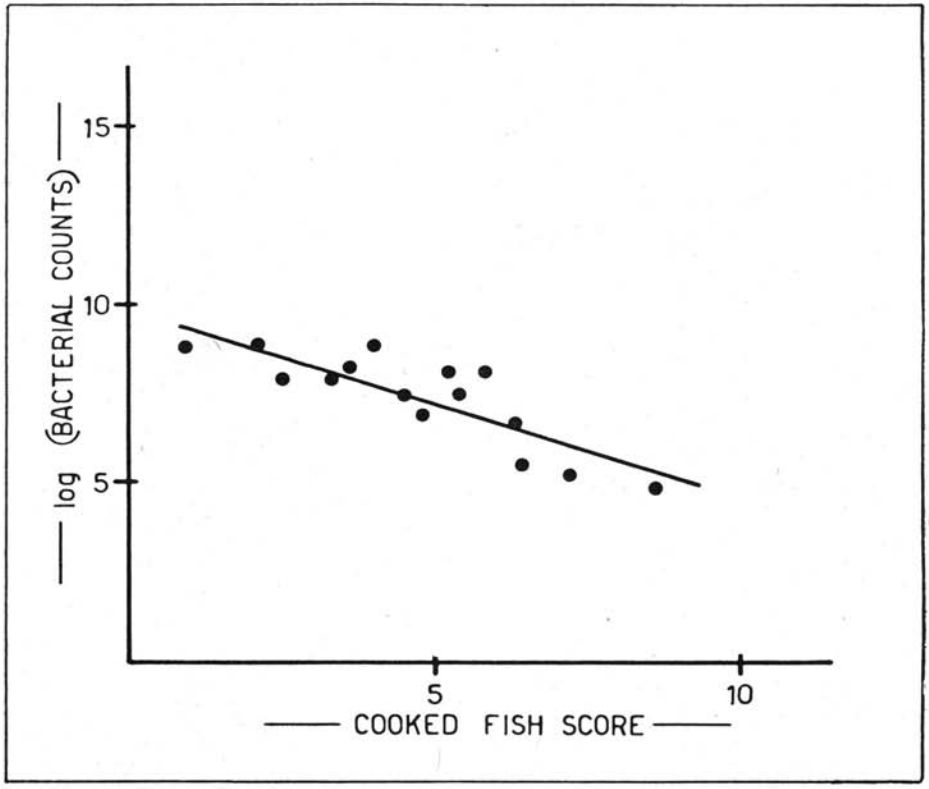

Fig. 3 — Relationship between bacterial counts and cooked fish score.

This phenomenon could be accounted for by the disappearance of *Enterobacteriaceae* in thirteen day and steep increase of *Pseudomonas* percentage from 0% to 80% in 10 days, which is shown in Table III as change in "pescada-foguete" flora during the storage expressed as the percentage of genera in total flora. There also is demonstrated that on the skin-surface flora the proportion of *Pseudomonadaceae* predominated, in the longer stored samples in ice, at the expense of *Achromobacteriaceae,* a tendency which again confirmed the statement of Shewan *et al.* (1957b).

IV — DISCUSSION

Inasmuch as the taste panel score played an important role in this experiment in judging the fish quality as well as in establishing the relationship between organoleptic and objetive tests,

TABLE III — Change in generic distribution of bacterial flora of "pescada-foguete" during storage in ice, expressed as percentage of total number of organisms isolated

Specimen	Days in ice	Total colonies tested	Entero-bacteria-ceae *	Pseudomo-nas/vibrio	Achromo-bacter/Al-kaligenes	Flavo-bacterium	Micro-coccus	Bacillus	Coryne-bacterium
Skin surface	3	24	4.1	33.3	29.2	12.5	12.5	0.0	8.4
	8	20	0.0	25.0	15.0	15.0	5.0	10.0	30.0
	10	14	0.0	82.8	10.0	0.0	0.0	7.2	0.0
	11	8	0.0	72.5	2.5	0.0	12.5	12.5	0.0
	12	8	0.0	87.5	0.0	0.0	0.0	0.0	12.5
	13	35	0.0	74.2	19.2	0.0	6.6	0.0	0.0
	15	39	0.0	79.5	2.6	2.7	5.1	7.6	2.5
	17	33	0.0	87.7	0.0	0.0	6.3	2.9	3.1
Intestinal contents	3	14	78.6	0.0	0.0	0.0	7.2	7.1	7.1
	8	26	5.0	71.9	15.4	0.0	3.9	0.0	3.8
	10	26	3.8	80.9	3.9	0.0	7.7	3.7	0.0
	11	10	10.0	70.0	20.0	0.0	0.0	0.0	0.0
	12	18	5.6	78.0	16.4	0.0	0.0	0.0	0.0
	13	21	0.0	52.7	36.8	0.0	0.0	10.5	0.0
	15	35	0.0	68.4	13.3	0.0	12.5	5.8	0.0
	17	26	0.0	70.3	3.6	0.0	15.4	3.6	7.1

* Family name.

the reliability of the taste panel score should be as high as possible. For this reason, training of the panel members to achieve a uniformity in the assessment was performed. At present, however, the variability of the score is ± 1 unit, while it has been demonstrated that it is possible to attain the ± 0.5 unit level. So, a more elaborate method of training is being planned to obtain better uniformity in our panel, which will be discussed in more detail elsewhere.

Among those six relations of chemical, bacteriological and organoleptic tests, TMA-cooked-fish-score relation is the best correlated, showing a value in excess of 10 for TMA number as an evidence of grossly spoiled, inedible fish. The appliance of this criterion to the governmental fish inspection services on a national scale, however, should be very cautious because the fishery industry also has a right to be shown that the standard to be promulgated will produce consistent results in the hands of a large group of government inspectors. In this connection, it is worthwhile to note that TMA value has been found to oscillate according to season and fishing grounds (Castell *et al.*, 1961; Shewan *et al.*, 1957a). Other factors such as feeding condition and maturity seem to have influence on the value. A large scale investigation is indispensable, therefore, to verify the consistency of the results before putting them into practical use.

Although the TMA-days-in-ice relation showed good correlation, it could be supposed to show differences in storage condition, such as bacterial load, temperature, etc., from lot to lot.

The result that the fish can be kept in edible condition for 11-16 days-in-ice leads us to the conclusion that Santos based boats operating in the Rio Grande do Sul waters should be advised to make a voyage shorter than 15 days, involving 4 days to and from the grounds. By so doing, the landings will comprise fish with storing time ranging from 2 to 13 days in ice, still leaving reasonable time to distribute fish even from the initial catch of the voyage to consumers in edible conditions.

It is generally said that fishes from tropical waters might be preserved in ice longer than those from cold waters on the ground that the spoilage bacteria at the former would not adapt themselves to low temperatures so well as those from cold waters do. Nevertheless, the results of this experiment, where fishes from waters of 20°C were used, showed almost equal storage time as those of cod and haddock. In so stating we do not imply that the same type of spoilage occurs, and closer examination in bacterial flora of fishes from waters differing in temperature is required before comments on this matter can be made.

V — ACKNOWLEDGEMENT

The author is sincerely grateful for the co-operation of "Sociedade de Pesca Taiyo Ltda.", at Santos, in providing the fish samples and valuable information on the fishery.

VI — SUMMARY

Chemical, bacteriological and organoleptic variables were measured during the spoilage of fish stored in ice up to 19 days. Among the six relationships obtained, significant correlation was found between trimethylamine value and cooked fish score. The fishes were stored in edible condition for 11-16 days in ice and showed a trimethylamine value of 10 or more using microdiffusion method.

VI — RESUMO

A pescada-foguete (*Macrodon ancylodon*) tem tido larga aceitação nos mercados de peixe fresco de São Paulo, figurando nas estatísticas em primeiro lugar, quanto ao valor (27,1% de qualquer espécie), e em segundo lugar, depois da sardinha, em volume (17,4%), durante o período de julho 1958-junho 1959 (Richardson & Moraes, 1960).

Tem sido, entretanto, assinalada (Braga, 1962) uma tendência cada vez mais acentuada, desde 1958, dos barcos componentes das parelhas santistas que efetuam a pesca dessa valiosa espécie, em aumentar sua tonelagem e realizar viagens cada vez mais longas, alcançando as costas do Rio Grande do Sul, onde se têm intensificado grandemente essas operações pesqueiras.

Com o aumento do número de dias gastos nessas viagens o problema da conservação do pescado a bordo, em condições satisfatórias para o consumo ulterior, tem-se tornado cada vez mais importante. Êsse problema, com efeito, atua como fator limitante da duração de cada viagem, uma vez que os prazos mais dilatados de armazenamento a bordo exercem uma influência prejudicial sôbre a qualidade do peixe entregue ao consumidor.

A primeira abordagem dêsse problema consistia, por conseguinte, em estabelecer métodos os mais objetivos possíveis pelos quais fôsse permitido medir o estado de frescor do peixe.

A pesquisa descrita a seguir, realizada em escala-piloto, foi levada a efeito no intuito de obter dados sôbre a avaliação da deterioração do peixe, usando processos organolépticos, químicos e bacteriológicos.

Três lotes, compostos cada um de 40-50 exemplares de pescada-foguete, provenientes da área pesqueira riograndense, foram examinados em laboratório durante o período de março-junho 1961.

Os três lotes de peixe, acondicionados sem eviscerar em caixas com gêlo, haviam sofrido um armazenamento nos porões dos pesqueiros de períodos equivalendo respectivamente a 3, 8 e 13 dias.

Ao chegar ao laboratório foram os peixes reacondicionados com gêlo fresco, em caixa isotérmica, procedendo-se então à amostragem em gêlo, a intervalos de 2-3 dias, até o limite de 14-19 dias. As amostras, cada uma consistindo de 6-7 peixes, foram a seguir examinadas do ponto de vista químico, organoléptico e bacteriológico.

Os testes químicos foram realizados pela determinação do nitrogênio volátil total (TVN) e da trimetilamina (TMA) no músculo do peixe, pelo método de microdifusão de Conway.

A estimativa organoléptica foi efetuada por dois processos:

a) cheiro e aparência do peixe cru avaliados numa escala de 10 pontos;

b) cheiro, aparência e sabor do peixe cozido avaliados por uma escala de 10 pontos, cujo valor representava a média obtida por intermédio dos membros de um "panel" de 3-4 pessoas prèviamente treinadas para a apreciação do peixe cozido. A Tabela I apresenta os resultados desta avaliação.

O exame bacteriológico foi efetuado com auxílio de contagens aeróbicas globais de culturas de material retirado da superfície da pele e conteúdo intestinal dos peixes, em meio agar-extrato-de-peixe-peptona. A Tabela II apresenta os resultados dos testes químicos, bacteriológicos e organolépticos realizados com as três amostras.

Dentre as seis relações obtidas dos referidos testes a que evidenciou melhor correlação foi o índice peixe-cozido-TMA, apresentando um valor superior a 10 para a TMA, referente a peixe positivamente estragado, impróprio para o consumo. Entretanto, a adoção eventual dêste padrão para os serviços governamentais de inspecção sanitária do peixe, em escala nacional, teria de ser realizada com muita cautela uma vez que a indústria pesqueira tem o direito de reclamar garantias suficientes de que semelhante norma irá produzir resultados consistentes e uniformes, quando aplicada por um vasto grupo de inspetores governamentais. É necessário assinalar, outrossim, ter sido constatada uma oscilação do valor da TMA, de acôrdo com as estações do ano e com a zona de pesca. Outros fatôres, tais como as condições de alimentação e estádio de maturação dos peixes, parecem também ter influência sôbre o mesmo valor.

Por conseguinte, antes da adoção de quaisquer medidas práticas nesse sentido, faz-se indispensável uma pesquisa em larga escala, para a verificação da constância dos resultados.

Das pesquisas efetuadas ficou demonstrado que é possível conservar o peixe, em condições próprias para o consumo, durante 11-16 dias em gêlo, levando à conclusão que os mestres dos barcos pesqueiros santistas, operando nas costas do Rio Grande do Sul, devem ser aconselhados a realizar viagens de menos de 15 dias, incluindo 4 dias do percurso de ida e volta aos bancos de pesca.

Desta forma as descargas em Santos compreenderão peixes com períodos de armazenamento no gêlo indo de 2 a 13 dias, deixando uma margem razoável de tempo para a distribuição aos consumidores, em condições satisfatórias de consumo, até de exemplares capturados no início da viagem.

VIII — BIBLIOGRAPHY

BRAGA, A. S.
1962. Métodos de compilação e computação de dados estatísticos de desembarque de pescado no pôrto de Santos. Bol. Inst. Ocean., vol. XII, n.º 2, p. 39-61, tabs.

CASTELL, C. H. *et al.*
1958. Grading fish for quality, 1. J. Fish. Res. Bd. Can., vol 15, n.º 4, p. 701-716.
1961. Grading fish for quality, 4. J. Fish. Res. Bd. Can., vol. 18, n.º 3, p. 303-310.

CONWAY, E. C. *et al.*
1933. The micro-determination of ammonia. Bioch. J., vol. 27, p. 419-429.

JEPSEN, A.
1959. The spoilage of fish and the examination of fish. WHO, Regional Office for the Western Pacific. Seminar on Veterinary Public Health. WPR/VPH/9, 31/3/1959.

LISTON, J. *et al.*
1961. The spoilage of Pacific coast rockfish I. Food Technol., vol. XV, n.° 1, p. 19-22.

RICHARDSON, I. D. & MORAES, M. N.
1960. A first appraisal of the landing and mechanism of the Santos fishery. Bol. Inst. Ocean., vol. XI, n.° 1, p. 10-11.

SHEWAN, J. M. *et al.*
1954. A method for the rapid differentiation of certain nonpathogenic, asporogenous bacilli. Nature (London), vol. 173, p. 208-209.

1957a. Quality of iced North Sea cod. J. Sci. Food Agric., vol. 8, p. 227-231.

1957b. The microbiology of fish and meat curing brine. London, H.M.S.O.

WATANABE, Ko
1960. Bacterial flora of commercial fresh codfish. Nordisk Veterinaer-Medicin, vol. 12, p. 541-554.