THE BACTERIOLOGY OF DEHYDRATED FISH

I. QUALITATIVE AND QUANTITATIVE STUDIES OF THE DRYING PROCESS

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(With 4 Figures in the Text)

INTRODUCTION

In experiments at this Station designed to find the most appropriate methods for the dehydration of fish, it was early recognized that bacteriological control would be necessary in order to ensure the safety of the final product. Many preliminary experiments were performed before a suitable method was found for commercial production. In these experiments data were obtained and are given here to illustrate the general argument, although most of the results are concerned largely with the method finally adopted.

Ideally, in the manufacture of dehydrated foods, the original material should be of good quality and free from food-poisoning pathogens, no bacterial toxins should be formed during the manufacturing process, the final product should be free from intestinal pathogens, and on reconstitution for the table should have no organisms which may grow and produce poisonous products. Finally, there should be no marked spoilage of the product during processing. At this Station considerable knowledge has already been acquired of the numbers and types of organisms present in both fresh and market fish, and this knowledge and experience was of considerable value in the assessment of the changes occurring during the drying experiments. This work was carried out as well as the limited resources of the Station could provide, and although more exhaustive data would have been desirable, the general conclusions, it is believed, are valid.

METHODS

I. *The dehydration process*

(a) The procedure finally adopted for the dehydration of fish has recently been described in detail from this Station (Cutting & Reay, 1944). White fish, such as cod and haddock, are well washed to remove surface slime, etc., beheaded, skinned and filleted by hand, the flesh minced and spread on wire mesh trays for cooking at a loading of 4–5 lb./sq.ft. Cooking is accomplished in about 30 min. with live steam at 2 lb. pressure/sq.in. On removal from the retort the frame with the stack of trays is exposed to a draught which cools the fish and removes some of the moisture, after which the fish is spread on wire trays, dried in a batch drier in warm air moving at 10 ft./sec. The fish, whilst never exceeding 65–70°C. in temperature, is dried in 3–4 hr. to a water content of less than 10%. The dried fish is then collected in a large bin and packed by hand into tins. When herrings are treated, heading, gutting and filleting are done by machine; but it is not practicable to remove the skin. The fillets are cooked whole and minced once only just prior to spreading on the drying trays. Owing to the relatively high fat content of the product, compression into tins at densities up to 1·0 is readily accomplished, as well as loose packing. A special conditioning technique is necessary for the compression of dried 'white' fish.

Smoke curing of certain classes of fish, e.g. cod, haddock, whiting and herring, with subsequent cooking and drying also gives satisfactory products.

The earlier procedures, which are only considered incidentally here, consisted chiefly of drying cooked or uncooked whole fish or fillets at lower temperatures, involving longer drying times.

(b) In addition to the batch drying, three small-scale experiments using a small experimental roller drier situated at the Low Temperature Research Station, Cambridge, were also done and may be briefly mentioned. The procedure consisted of feeding the raw or cooked minced fillets of white fish on to rollers 1 ft. in diameter, 2 ft. in length, heated by steam at a pressure of about 40 lb./sq.in. to a surface temperature of about 142°C., revolving at a speed of about $4\frac{1}{2}$ r.p.m. The fish is dried in a fraction of a second, coming off the rollers as a lacework of thin white porous ribbons—a quite satisfactory product.

II. *Bacterial counts*

At first, $2\frac{1}{2}$–5 g. samples, usually in triplicate, were transferred to sterile 6 × 1 in. thick-walled test-tubes containing 15 g. sterile sand and, after adding

a measured volume of Ringer's solution, the fish was ground to a fine suspension by means of a mechanically driven glass stirrer. Although this was found satisfactory for undried muscle, difficulties were experienced with dried fish, both with regard to the mechanical operation and to sampling. For this reason it was found more expedient and accurate to take a 25–50 g. sample in a sterile mortar, grind up with sterile sand under aseptic conditions, transfer to a sterile bottle with Ringer's solution and make the appropriate dilutions in the usual way, plating out finally into horse-heart agar (pH 7·4) and incubating duplicate plates both at 20 and 37° C. In some of the early work, counts were incubated only 1–1½ days at 37° C. and 3–4 days at 20° C. Several times irregularities in the counts occurred, which appeared to even out when incubation was prolonged by 1 day at 37° C. and by 2 days at 20° C. Even so, occasionally counts were found to be higher at 37 than at 20° C., a fact already noted by Nelson (1942, 1943 *a*, *b*) in his studies on factors influencing the apparent heat resistance of bacteria. Whether these discrepancies could be eliminated by additions to the medium as found by Nelson was not investigated. It would appear, however, that the problem of the growth conditions of bacteria in fish subjected to sublethal heating requires fuller investigation.

Using the plates from the bacterial counts, qualitative studies of the flora present at various stages of the drying process were made and studied in detail with regard to their morphological and biochemical reactions.

In addition, numerous and varied samples mainly of the final dried product were inoculated into the following media, selective for organisms of the food-poisoning groups. Using the muscle suspensions, from the bacterial counts, the following cultures were made:

(1) 10 ml. were added to 10 ml. double-strength tetrathionate broth, and after incubation overnight at 37° C. smears were plated out on MacConkey's agar for salmonellas.

(2) 10 ml. were added to a fluid glucose deep agar, heated ½ hr. at 80° C. and after setting incubated at 37° C., for sporing anaerobes.

(3) 2–5 ml. were added to a blood agar plate, for streptococci and staphylococci.

(4) 1–5 ml. were added to alkaline agar (pH 10·2), for staphylococci.. This latter procedure was found very effective by Macdonald (1942) in isolating *S. aureus* from other saprophytic micrococci in impure cultures.

(5) In addition, 1–5 ml. were inoculated into a MacConkey plate to give a general idea of the extent and cleanliness in handling (see Griffiths & Fuller, 1936).

(6) A smear was made directly on desoxycholate-citrate agar, for dysentery organisms and salmonellas.

The cocci which appeared on the blood agar and alkaline agar plates were subjected to the coagulase test using horse-blood serum diluted 1 : 5 with citrate broth.

In every case where pathogens were suspected to be present, confirmatory tests were carried out at the Bacteriology Department of Aberdeen University, under the direction of Prof. J. Cruickshank.

No examination for the presence or possible action of thermophiles has been made in the course of this work. Experience here has shown that these organisms are rare in newly caught fish (Shewan, 1936).

THE BACTERIOLOGY OF FRESH FISH

I. *Newly caught fish*

The bacterial content of fish flesh stored in ice for periods up to 21 days rises steadily from sterility in the newly caught fish up to 10^7 organisms/g. after about 14–16 days and remains at about this level for the rest of the time (Shewan, 1937, 1938*a*; Stansby & Lemon, 1941). Although the flesh of newly caught fish is sterile (Proctor & Nickerson, 1935), the slime, gills, and, in 'feedy' fish, the intestines, carry large numbers of bacteria, and these invade the tissues during storage in ice. The counts vary amongst other things with the environment, the nutrient medium used for counting (Waksman & Reuszer, 1932; Zobell, 1942) and with the method of catching (line or trawl). Trawled fish usually carry a heavier bacterial load than lined fish, due to their coming into intimate contact with the bottom sludge which contains large numbers of bacteria (Waksman, Reuszer, Carey, Hotchkiss & Renn, 1933; Shewan, 1936; Zobell, 1938) and to their intestinal contents being squeezed out and spread over the surface of the fish when the heavy trawl bag is hoisted on board. The water dripping from the 'cod-end' of the net before the release of the fish on deck gave in one instance some 220,000 organisms/ml. of fluid at 20° C., whilst the neighbouring sea was almost sterile. Similar figures have been given by Lücke & Schwartz (1937). It has already been mentioned that in 'non-feedy' fish the intestines are sterile; in 'feedy' fish enormous numbers may be present, e.g. 8–20×10^6/ml. of intestinal fluid have been recorded for cod (Lücke & Schwartz, 1937).

Detailed analyses of the slime and intestinal flora of a variety of species of fish have been carried out both at this Station (Stewart, 1932; Shewan, 1938 *b*, *c*) and elsewhere (Reed & Spence, 1929; Griffiths, 1937; Thjötta & Sømme, 1938; Schönberg, 1938; Ferguson Wood, 1940), and the results of some such surveys are given in Table 1.

Table 1. *Bacterial flora of fish, expressed as percentage of total number of organisms isolated*

Author	Fish species		Achromo- bacter	Micro- cocci	Flavo- bacter	Pseudo- monas	Bacillus	Miscel- laneous
		(A) Aerobic groups						
Ferguson-Wood (1940)	Australian barracouta, whiting, mullet, etc.	Slime	19	48	17	7	9	—
		Intestines	30	21	1	10	35	—
Reed & Spence (1929)	Canadian haddock	Slime	23	4	8	22	24 .	18
		Intestines	4·4	1	5·6	8·7	5·7	70 (mainly Proteus)
Thjötta & Sømme (1938)	Cod (Norway)	Slime	48	14	25	5	—	8
		Intestines	55	11	—	—	—	33
Stewart (1932)	North Sea haddock	Slime	57	22	11	5	—	5
		Intestines	80	—	4·5	—	4·5	11
Shewan (1938b)	Shetland herring	Slime	43	24	13	11	—	9
		(B) Anaerobic groups						
Reed & Spence (1929)	Canadian haddock	Slime	—					
		Intestines	Members of the *Clostridium* group					
Shewan (1938c)	North Sea haddock	Slime	No anaerobes found					
		Intestines	Members of the *Clostridium* group, e.g. *Cl. sporogenes, Cl. putrificum, Cl. capitovalis, Cl. tetanis,* etc.					
Shewan (1938b)	North Sea herring, mackerel	Slime	No anaerobes found					
		Intestines	Members of *Clostridium* group, e.g. *Cl. putrificum,* etc.					

It will be noted that in every case the aerobic flora is that normally regarded as autochthonous to soil, air and water, and the similarities in distribution of these bacterial species indicate more or less their universal geographical distribution. Differences in the flora of the various species of fish are probably correlated with environment, and evidence has been accumulating at this Station to show that it may also be correlated with species and season. It is also probable that the differences between sea and river fish are more fundamental. There is no evidence to show, however, that food pathogens are members of the normal flora of newly caught fish or that fish suffer from infection due to the *Salmonella* group. Sickness resulting from the ingestion of fish, as also of vegetables, which are free from these organisms, is almost certainly the result of infection due to handling after catching (Süpfle, 1936).

It is recognized that members of the *Proteus* group have occasionally been incriminated in food-poisoning outbreaks (Süpfle, 1936; Jordan & Burrows, 1935; Cooper, Davies & Wiseman, 1941), but the evidence that they are a serious danger is very scanty. Indeed, Dolman (1943) has sought to prove that in many cases enterotoxic micrococci are the real cause, and that these may be masked or overgrown by *Proteus* before the food is examined in the laboratory.

It is known that some of the micrococci (staphylococci) have produced acute intestinal upset (enterotoxins?), but none of the micrococci from fish slime

examined here have been found to be potentially pathogenic as shown by the coagulase test.

With regard to the anaerobes these are absent from the slime (Shewan, 1938b, c; Thjötta & Sømme, 1938; Snow & Beard, 1939), but they are invariably present in the intestine (Reed & Spence, 1929; Shewan, 1938b, c). Members of the *Clostridium* group have been recorded by these workers and have been isolated from haddock, cod, herring and mackerel. *Cl. botulinum*, the only food-poisoning anaerobe, has so far not been recorded in these species of fish. van Ermengen (1897) failed to isolate it from sturgeon or salmon, but few samples were examined. Fish botulism is, however, endemic in Russia, and numerous cases have been recorded of poisoning after the ingestion of salted or smoked fish, mainly sturgeon, usually eaten raw (Zlatagoroff & Soloviev, 1927; Kurochkin & Emelyanchik, 1937). The work of Burova and her co-workers (1935a, b), as well as that of Dobrowski (1935), on live Caspian sturgeon, has shown that *Cl. botulinum* occurs in the intestines in 10–12 % of the samples examined, and it is concluded that the high incidence of botulism is due to this fact. *Cl. botulinum* has also been found by Burova *et al.* (1935b) in 2–3 % of the sea water, muds and silts examined, and the fact that the sturgeon is a sluggish bottom feeder, and common in muddy streams (Dean, 1895), is probably related to the occurrence of this organism in this species. The few recorded cases of the presence of botulism in fish (herring (Zlatagoroff & Soloviev, 1927); mackerel

(Madsen, 1908); tunny (Lang & Dean, 1934; Lang, 1935; Geiger, 1937); sprats (Hazen, 1937), and smoked salmon (Hazen, 1938; Dack, 1943)) suggest merely external contamination.

Apart from sturgeon there is no direct evidence that *Cl. botulinum* occurs in the normal flora of sea fish.

The bacterial flora of fish landed under ordinary conditions at the fishing ports will be affected both quantitatively and qualitatively by many factors, in particular by the degree of handling, damage inflicted, contacts with decks and fish rooms, the ice used for storage, by the time after catching, and the temperature of the atmosphere.

Experiments here have shown that crushed artificial ice direct from the factory may contain 100–15,000 organisms/g., comprising groups similar to

Table 2. *Bacterial groups present in artificial ice, expressed as percentage of total number of organisms isolated*

Group	Exp. I	Exp. II
Aerobes		
Achromobacter	10	35
Micrococci	30	10
Flavobacter	40	40
Miscellaneous	20	15
Anaerobes (results of five experiments)	None found	

Table 3. (Stewart, 1934). *Bacterial groups (aerobic) present in fresh and market fish, expressed as percentage of total number of organisms isolated*

Group	Fresh fish	Market fish
Achromobacter	57	64
Micrococci	22	11
Flavobacter	11	20
Miscellaneous	10	3

those on fresh fish (Table 2), but intestinal pathogens have not been found in any of the samples. In most of the recorded cases of food poisoning directly attributable to ice, natural ice formed from polluted waters has been the peccant material (Prescott & Horwood, 1935; Jensen, 1943).

As a result of work done at this Station it is recommended that gutted white fish stowed for not longer than 6–7 days in ice be used for dehydration, since bacterial spoilage with the production of 'off' flavours and odours rapidly occurs after this time (Beatty & Gibbons, 1936; Shewan, 1937, 1938a). During this period certain quantitative and qualitative changes take place in the bacterial flora. Thus a comparison of the flora of haddock and ling from the floor of Aberdeen fish market (Stewart, 1934) shows a greater predominance of *Achromobacter* and *Flavobacter*, both groups actively engaged in spoilage. It is probable that some potential patho-

gens may be found amongst the micrococci, due to human handling (gutting, stowage, etc.), but the temperature conditions in melting ice are relatively unsuited for their growth (Table 3).

It may be concluded that the likelihood of fish 0–7 days in ice containing food-poisoning pathogens is remote.

II. *Filleted fish*

After filleting even under good hygienic conditions there is a marked change in the flora of the fish flesh, in particular a large increase in the number of organisms growing at 37°C. Whereas in the slime and gills of fresh fish, of the total number of organisms growing at 20°C., only 0.5% grow at 37°C., on filleted fish the proportion may vary up to 75%. Qualitatively the most striking fact is the great increase in the micrococci, both at 20 and 37°C. A comparison of Tables 1 and 5 shows this with respect to North Sea haddock and cod. Of the cocci growing at 37°C., it was found in two experiments that 10–16% were indistinguishable from *S. aureus*, by means of the coagulase test, mannite fermentation, haemolysin production, etc. The demonstration of such a proportion of staphylococci in the filleted material is clear evidence that during handling, these organisms are derived from human sources (nose, throat, hands, etc.), although they may also be present in the air. From the food poisoning point of view it is impossible to exclude these as potential pathogens as Stritar & Jordan (1935) concluded that the power to provoke food poisoning was not limited to any recognizable variety of staphylococci. No reliable data exist, however, for the percentage which do produce 'enterotoxins', although Dolman (1934) considers that only a small number are likely to be involved, but Jones & Lochhead (1939), on the other hand, found that of the 980 micrococci (representing 50 types) isolated by them from frozen pack vegetables, 42% of the total, and 24% of the types produced enterotoxins. Roberts & Wilson (1939) also state that roughly 40% of the cultures isolated by them from various sources were enterotoxigenic as shown by the kitten test; but the reliability of the latter for the detection of enterotoxins has recently been called in question (Fulton, 1943).

The mere presence of these cocci is sufficient to emphasize the need for most careful hygienic factory conditions, both with regard to personnel and to plant (benches, floors, mincers, etc.). Moreover, it follows that the fish after filleting, mincing, spreading on cooking trays, etc., should not be allowed to stand for any but the shortest possible time at room temperature. First, there is bound to be a rapid increase in the number of organisms normally present on fish (their optimum temperature is about 20°C.), thus causing spoilage, and secondly condi-

tions are favourable for the growth of the enterotoxic micrococci. Dolman (1943), for example, states that 'even at an average temperature of a warm room (20–23°C.), enterotoxin production can in a surprisingly short period reach a level of potency sufficient to cause food poisoning in man'. Barber (1914) states that 5 hr. at room temperature was sufficient for the production of enterotoxin, although Dack & Segalove (1941), using a most favourable medium and a good enterotoxic strain, found it required 3 days at 18°C. or 12 hr. at 37°C. to produce enterotoxins (using monkeys as the experimental animals). At 9 and 15°C. none was produced in 7 and 3 days respectively, and at 4 and 6·7°C. none

of his results the whole problem of staphylococcal poisoning requires careful reconsideration. To ensure safety it would seem to be advisable either to dispatch the minced flesh quickly to the cooker or keep it in a chill room (at 5°C. or below).

III. Smoked fish

Although the flora of smoked fish was not examined in detail, a few general observations were made in the course of these experiments. Since the fish to be smoked were handled before drying to the same extent as the fillets just described, it was assumed that the flora up to this point would be

Table 4. *Log of bacterial count on fish flesh before and after filleting*

Fish species	Before filleting (computed)		After filleting		Count at 37°C. expressed as % of those growing at 20°C.
	20°C.	37°C.	20°C.	37°C.	
Haddock fillets at 0°C., not in contact with ice, handled under excellent conditions, etc.	<3·00	—	5·72	3·50	c. 1·0
Cod fillets, 1 day in ice	<2·00	—	3·90	3·78	75·0
Cod fillets 1–3 days in ice	<3·00	—	5·92	5·05	14·0
Haddock and cod (slime and gills) (60 samples)	4·85	2·5	—	—	0·5

Table 5. *Bacterial flora of fish fillets, expressed as percentage of total number of organisms examined*

Exp. no.	Fish species	Temp. of incubation °C.	No. of colonies examined	Micro-cocci	Flavo-bacter	Achromo-bacter	Bacillus	Miscellaneous (Serratia, Aerobacter, etc.)
				Aerobes				
II A	Cod	37	134	86		14 (nearly all *Flavobacter*)		—
		20	167	42	53	4	1	—
VI	Cod	37	100	96	1	—	—	—
		20	170	50	25	15	10	—

Anaerobes
None found to be present

was produced after 4 weeks (see also Dack, 1943, pp. 89–92). There can be little doubt from the numerous cases quoted by Dolman as well as by others (Jones & Lochhead, 1939), that the strains in question can elaborate toxins quite readily at 20°C., although none appears to be formed at 4–7°C. Moreover, if, as has been suggested (Jordan, Dack & Woolpert, 1931; Woolpert & Dack, 1933), these enterotoxins are heat stable, withstanding heating at 100°C. for 20–30 min., they are not likely to be destroyed during the subsequent cooking. Recent results by Fulton (1943), as well as by others (Jones & Lochhead, 1939; Jordan, 1930), suggest that these enterotoxins are indeed heat-labile and in the light

similar. Smoking causes quantitative and probably qualitative changes in the flora. From Table 6 it will be seen that there is a reduction, often quite marked, in the numbers of viable bacteria present on the fish after smoking. These results are confirmed by those of other workers (Hess, 1929; Griffiths & Lemon, 1934; Stewart, 1935). Qualitatively, the changes in the flora are characterized by the large number of moulds which are frequently found over-growing the plates to the exclusion of all else. At 37°C., the flora consists almost exclusively of cocci, whilst at 20°C. cocci, sporebearers and asporogenous rods form the bulk of the flora. This is in agreement with the few published data on

the flora of smoked fish (Griffiths & Lemon, 1934; Wundram, 1937; Brauer, 1937). Occasionally, salmonellas have been found on smoked fish, giving rise to food poisoning but these are accounted for by the insanitary conditions of the fish houses in which the smoked fish were produced (Kleeman, Frant & Abrahamson, 1942).

mental results are available to show this. It is justifiable to stress at this point that the cooking procedure is quite inadequate to kill sporebearers and in particular *Cl. botulinum*, which is known to survive 109° C. for 30 min. (McCulloch, 1936).

On removal from the retort, the fish flesh is almost sterile. During the cooling, the mass creates air

Table 6. *Bacterial count per g. fish muscle before and after smoking for 3–5 hr. temperature of fish 28–30° C.*

	Species	(a) Before smoking at 37° C.	(b) After smoking at 37° C.	% reduction in bacterial count after smoking
Herring	(1) Unbrined, undyed, split	120,000	78,000	33
	(2) Unbrined, dyed, split	77,400	24,000	71
	(3) Brined, undyed, split	51,600	21,240	60
	(4) Brined, dyed, split	10,200	3,000	70
		(a) Before smoking at 37° C.	(b) After smoking at 37° C.	
Herring	(1) Brined, undyed, split	480	120	25
	(2) Brined, dyed, split	480	160	25
		at 20° C.	at 20° C.	
	(1) Brined, undyed, split	128,000	Apparently sterile	100
	(2) Brined, dyed, split	76,800	Apparently sterile	100

Table 7. *Bacterial counts—log number of bacteria per g. of material*

Fish species	Headed and gutted		Split (machine) or hand filleted and minced before cooking		After cooking		After cooking and spreading on trays		After drying	
	20° C.	37° C.	20° C.	37° C.	20° C.	37° C.	20° C.	37° C.	20° C.	37° C.
Herring 1½ days at chill temperature	6·28	2·94	4·45	2·95	2·08	3·40	5·54	2·48	2·65	2·14
Codling: 1–4 days at 0° C.	—	—	5·92	5·05	2·75	1·48	5·84	4·25	3·30	3·47
12 hr. in ice	—	—	3·90	3·78	<1·0	2·03	3·92	3·72	3·51	3·42
8 days in ice	—	—	7·16	5·17	<1·0	1·30	3·86	3·08	3·93	3·77
12–13 days in ice	—	—	7·36	4·73	<1·0	2·11	3·49	4·39	3·84	3·77
21 days in ice	—	—	7·76	5·06	<1·0	<1·0	4·56	4·30	4·33	4·18
Herring smoked	—	—	—	—	—	—	—	—	2·71	2·53
Codling smoked	—	—	—	—	—	—	—	—	2·00	1·68

COOKING

After filleting, mincing and spreading on trays, the fish flesh is cooked for 30 min. at 2 lb. pressure. This procedure, which has been recommended (Cutting & Reay, 1944), has been found to reduce considerably the bacterial count. A few cocci, sporebearers and asporogenous rods have been found to survive. It is known that bacteria isolated from sea water and sea muds are very sensitive to heat (Zobell & Conn, 1940) and it is probable that few of the true marine types survive the cooking. It is also probable that as in canning the greater the initial load the greater the number surviving, but too few experi-

currents with the resulting deposition on the surface of large numbers of organisms, and under conditions of slow cooling there is ample opportunity for these to proliferate. The nature of the flora thus deposited depends on the environment but, if the interval between cooking and drying is less than 1–2 hr., the danger from this source is not likely to be serious. For intervals longer than this, it would probably be advisable either to cool the mass below 7° C., or keep it about 70° C.

After cooking, the fish are usually minced and spread on trays ready for drying. Experiment has shown that the bacterial load increases at this stage even under reasonably good hygienic conditions,

almost up to that originally present after filleting (before cooking) (Table 7). Moreover, the types present appear to be similar to those on the uncooked fillets (Table 8).

Of the cocci growing at 37°C. about 20% were found to be coagulase-positive. The greatest care with regard to cleanliness, handling, etc., should be exercised at this stage, since the subsequent treatment is not likely to be so drastic as the cooking in reducing the numbers of bacteria.

present in the fish after cooking, mincing and spreading on trays just before drying, two experimental runs were done using fish inoculated with a coagulase-positive strain of *S. aureus*.

Six trays of cooked minced fish muscle (2 lb./sq.ft.) were sprayed with a saline suspension of *S. aureus* (grown 18 hr. at 37°C. on agar), six similar trays of fish, unsprayed, being used as controls. Thermocouples were placed in the centre of the mass of fish on each tray, and the conditions of temperature

Table 8. *Bacterial flora of fish, minced, spread on trays after cooking, expressed as percentage of total number of organisms examined*

Exp. no.	Fish species	Temp. of incubation °C.	No. of colonies examined	Micrococci	*Flavo-bacter*	*Achromo-bacter*	Miscellaneous
6	Cod	37	80	100	—	—	—
		20	70	71	16	12	1

DRYING

During drying, there are two possible sources of increased infection, viz. (a) the air of the drier, and (b) actual bacterial growth.

(a) A preliminary examination of the air contamination of the 'Torry' drier, whilst running, was carried out as follows. Two large poured Petri plates (surface area 500 sq.cm.) and eight small ones (surface area 85 sq.cm.) were held vertically by means of wooden moulds, and the surfaces exposed against the direct air flow moving at 15 ft./sec. for 30 min. in various parts of the drier. After exposure, one large and four small Petri plates were incubated at 20°C. and a similar number at 37°C. (Table 9).

Table 9. *Total number of colonies growing at 37°C. from the air of drier running at 15 ft./sec. for 30 min.*

		Total area exposed
37°C.	89	836 sq.cm.
20°C.	96 + 39 moulds	836 sq.cm.

About 10% of the cocci growing at 37°C. were found to be coagulase-positive. It would appear from this experiment that if normal cleanliness of the drier and environment is ensured, infection from the air is unimportant.

(b) Bacterial growth during drying is a more important factor, since it is likely to occur in the wet mass during the first part of the drying process, given the proper temperature conditions. In one experiment it was found that when the temperature of the drier was in the region of 30–50°C. bacterial multiplication took place in the wet mass even during the first 3 hr. of drying—temperature of the fish 20–35°C. (Table 10).

As coagulase-positive cocci had been shown to be

and humidity of the drier could be varied at will. The conditions aimed at were maintenance of the temperature of the mass of fish at 37°C. for the first 5 hr. and of the R.H. at about 80%, so that little or no drying would occur during this time. Samples for moisture content (10–20 g.) and bacterial counts (25–50 g.) were taken from each tray every ½–1 hr. The experiment was repeated with the temperature of the fish at 50°C. The results are set out graphically in Figs. 1 and 2.

Table 10. *Increase in bacterial growth at intervals during first 7 hr. of drying*

Codling 1–2 days in ice (uncooked mince)

Time after commencement of drying hr.	Log no. of bacteria per gram of material (counts at 20°C. only)		
	Unwashed fillets	Washed fillets	Brined (80% pickle 10 min.) fillets
0	5·58	5·21	5·18
2½	5·82	5·50	5·42
7	6·28	6·16	5·64

Temperature of air of drier: 30–50°C.

It will be noted that the conditions aimed at for the first 5 hr. were practically achieved, viz. constant R.H. temperature and H$_2$O content of fish. The drops in the temperature curve are due to removal of the trays from the drier for sampling.

Generally speaking these experiments show that if the conditions of drying are such that the temperature of the fish lies between 35 and 40°C., growth will occur during the first 3 hr., whilst at 50°C. even although moisture conditions (R.H., etc.) are suitable a steady decline in bacterial numbers
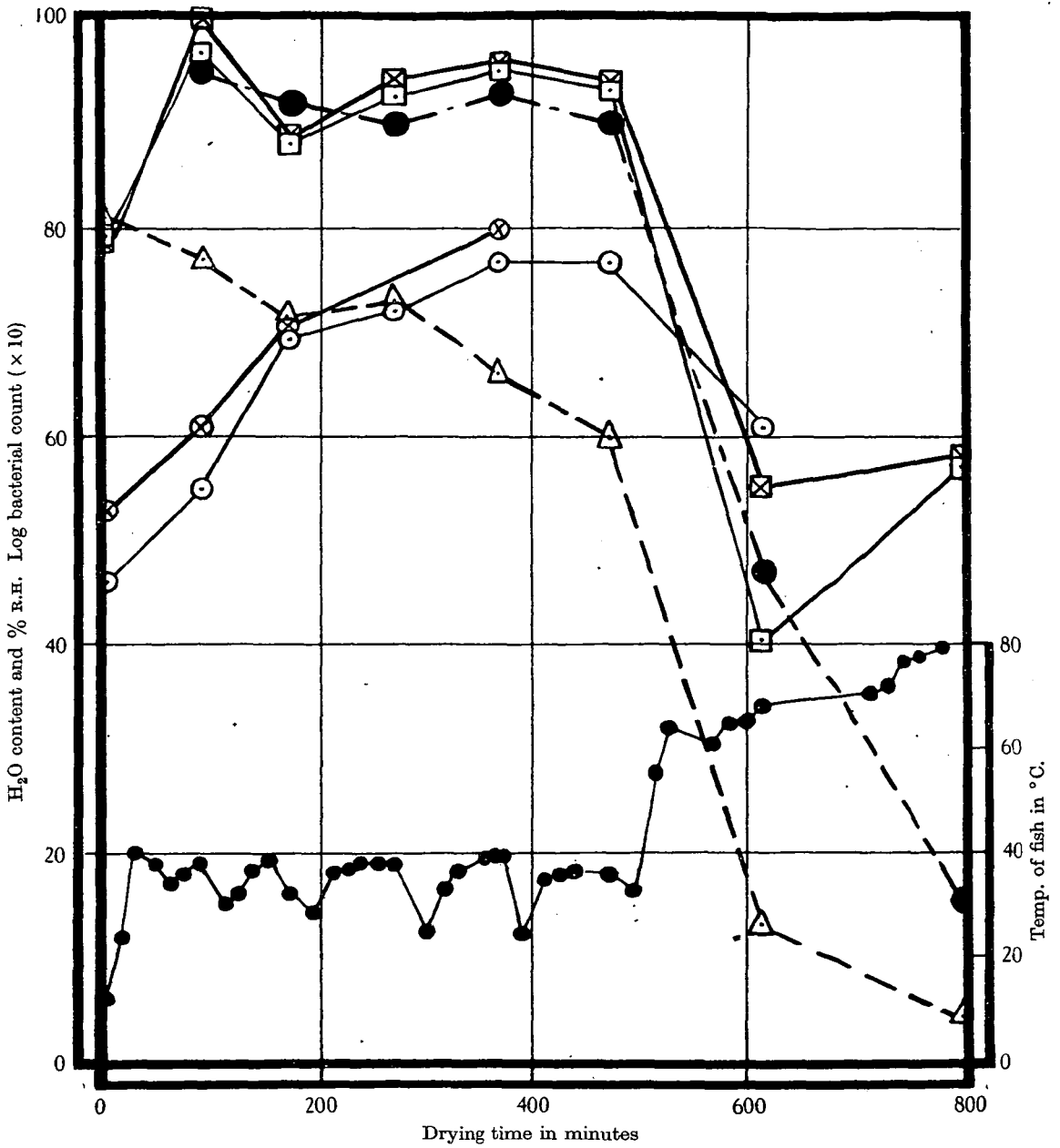
13·2

Fig. 1. Temp. of fish 37°C. for at least 5 hr.

●——●——●——● = Temp. of fish in °C.
⊙——⊙——⊙——⊙ = Log bacterial count (37°C.). Control unsprayed.
⊗——⊗——⊗——⊗ = Log bacterial count (20°C.). Control unsprayed.
△--△--△--△ = H₂O content of fish.
●--●--●--● = R.H. during drying.
▣——▣——▣——▣ = Log bacterial count (37°C.). *S. aureus* sprayed.
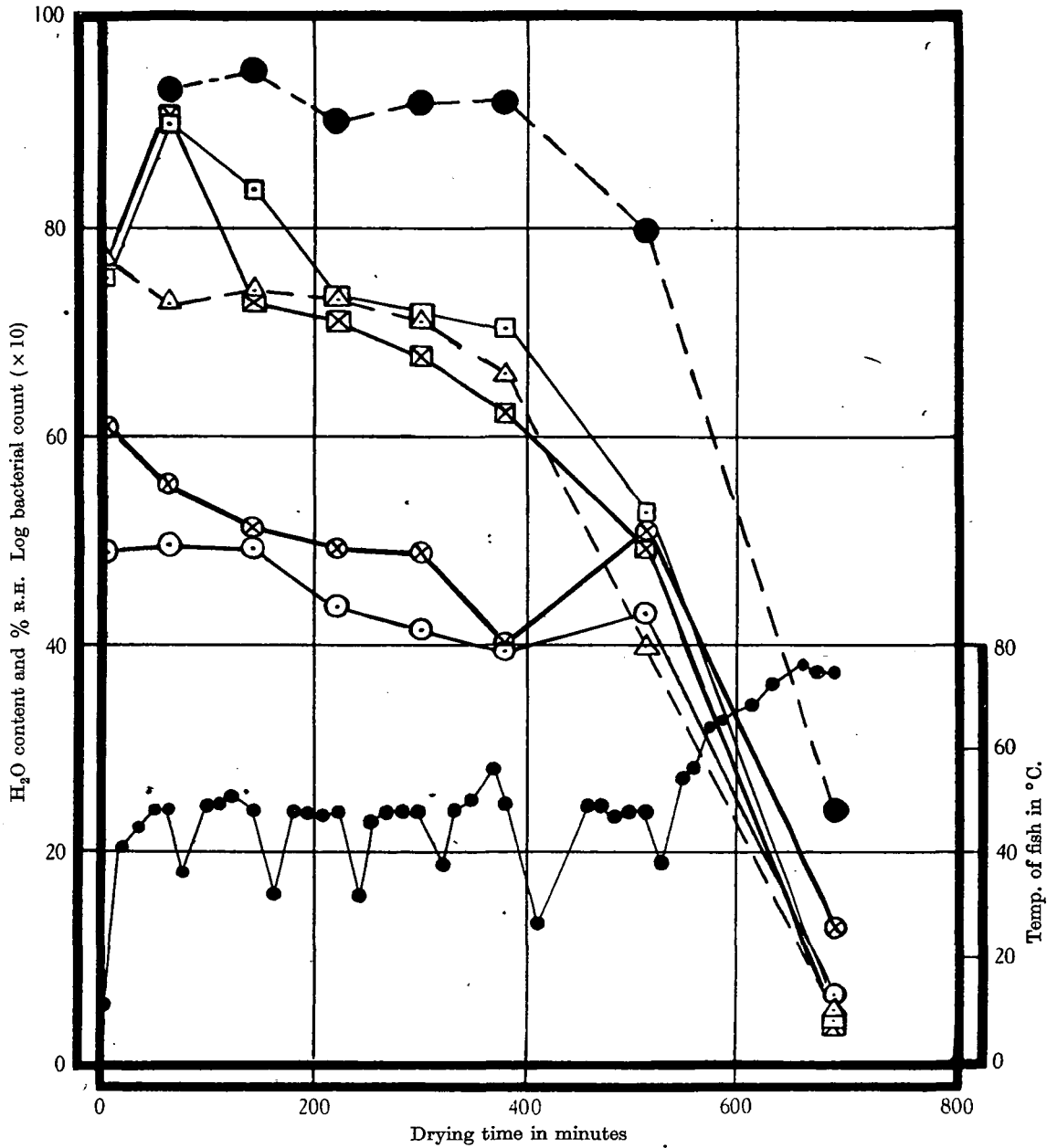⊠——⊠——⊠——⊠ = Log bacterial count (20°C.). *S. aureus* sprayed.

Fig. 2. Temp. of fish 50°C. for at least 5 hr.

● ● ● ● = Temp. of fish in °C.
⊙ ⊙ ⊙ ⊙ = Log bacterial count (37°C.). Control unsprayed.
⊗ ⊗ ⊗ ⊗ = Log bacterial count (20°C.). Control unsprayed.
△ △ △ △ = H_2O content of fish.
● ● ● ● = R.H. during drying.
☐ ☐ ☐ ☐ = Log bacterial count (37°C.). *S. aureus* sprayed.
⊠ ⊠ ⊠ ⊠ = Log bacterial count (20°C.). *S. aureus* sprayed.

ensues. The sprayed fish at 37° C. show an increased bacterial count after the first hour (incubation temperature 37° C.), but there is relatively little increase in the count over the 5 hr. period. This is in contrast to the unsprayed lot. This may, in part, be due to experimental error (sampling, counts, etc.), but alternately it may be due to maximal number for the subtrate having been reached. It may be concluded, however, that with the young growing culture of *S. aureus*, growth occurred during the first hour or so, when drying was done at 37° C. and that none probably occurred at 50° C. It is of interest to note that Jensen (1941) in the examination of frankfurters held at 28·9° C. found a lag phase of 3 hr. for *S. aureus*, and at the higher temperature (37° C.), which is the optimum for *S. aureus*; no doubt this time would be reduced.

The important practical conclusion to be drawn from these experiments is that in order to avoid growth and the possible production of toxins the temperature of the fish should be raised at least to 55° C. within the first hour and thereafter rise steadily to the dry bulb temperature during the subsequent drying.

FINAL DRIED PRODUCT

Table 7 shows that the final dried product is not sterile. It also appears that the fresher the fish the lower the count. This is in keeping with the results of canning practice that the thermal death-rates are slower the higher the initial contamination (Baumgartner, 1943).

The variation in the bacterial count of the dried product was done on two occasions and is given in Table 11. The samples were kept reasonably clean throughout, and these counts may serve therefore as a standard by which such products might be judged. By proper planning of the equipment and special attention to cleanliness these figures could be improved upon.

Table 11. *Variation in the bacterial count on dried fish*

| | No. of bacteria per gram of dried fish | |
Sample	20° C.	37° C.
Cod 1–3 days in ice, dried standard procedure (21 samples)	16,300 ± 5400	9000 ± 4400
Cod 1–2 days in ice, dried standard procedure (18 samples)	2,500 ± 1500	2000 ± 1370

Table 12 shows that the flora of dried fish is somewhat similar to that of the fish just before drying. It will be noted that in nearly every experi-

ment the cocci constitute the predominant type, more particularly of those growing at 37° C. At 20° C. the asporogenous rods (*Flavobacter, Achromobacter*) form a small but consistent part of the flora, with consequent drop in the percentage of cocci present. Of the cocci growing at 37° C. the percentage which is coagulase-positive varies from 3, to 29 %. Examination of many samples of dried fish, either by the use of glucose agar deeps or by cultures in an anaerobic jar, have shown that anaerobes are very seldom encountered. Only on one or two occasions have anaerobic sporebearers been found, and these were shown to be non-pathogenic, consisting exclusively of *sporogenes-putrificum* types.

In addition to the detailed analysis of the dried fish given in Table 12 which consist almost exclusively of dried haddock and cod, some sixty-seven varied samples of dried fish were examined by the use of the selective media (Table 13). For the sake of interest, several samples of fish dried elsewhere are included as well as samples of fresh-fish slime, commercial ice and fish (herring) before drying. The most significant fact emerging from these results is that apart from sample No. 17c no growth has been found in any of the media used with the exception of the blood agar and alkaline agar plates. Examination of these showed that of the sixty-seven samples tested, twenty (29 %) contained coagulase-positive staphylococci (in 0·2–0·5 g.).

ROLLER DRYING

It has already been mentioned that three small-scale dryings were done using a laboratory roller drier at the Low Temperature Research Station, Cambridge. The bacterial counts of these products are given in Table 14. Comparison with the figures for fish dried in the batch drier shows that they are much lower both at 20 and 37° C. No detailed examination of the flora was made, nor was use made of the selective media.

RECONSTITUTED FISH—AS FISH CAKES

It has already been stated that the final dried product is not sterile and that at least one-third of the samples contain coagulase-positive cocci and hence are potentially capable of producing enterotoxins. As the most suitable way of utilizing the dried fish is by incorporating it with potatoes in the form of fish cakes or fish pie, and as these might be kept in warm canteens, etc., before being fried or consumed, it was thought important to investigate the bacterial growth in such fish cakes both fried and unfried, stored at normal room temperatures. Two such storage experiments were set up, at 15 and 20° C., using dried cod with and without *S. aureus*, obtained from the drying experiments described

Table 12. *Bacterial flora of dried fish*

Exp. no.	Sample	Total no. of colonies examined at	Bacterial flora (aerobic) expressed as percentage of total no. of colonies examined						% of cocci growing at 37°C. which is coagulase-positive
			Micro-cocci	Asporogenous rods			*Bacillus*	Anaerobes	
				Flavo-bacter	*Achromo-bacter*	Miscel-laneous			
I	Haddock and whiting 1–3 days in ice, brined, minced, uncooked, dried at 50°C. in 24–30 hr.	37°C. 241	95	—	—	—	}	Negative	15
		20°C. 203	76	9	1	4	}		
II	Haddock and whiting, cooked (C) and uncooked (A), dried at 50°C. 12–24 hr. or in steam-jacketed pot at 150°C. (B) 7 hr.	A 37°C. 35	100	—	—	—	}	Negative	10
		B 37°C. 9	100	—	—	—	}		
		C 37°C. 50	84	16	—	—	—		
		A 20°C. 26	92	8	—	—	—	}	—
		C 20°C. 29	93	7	—	—	—	}	—
IIA	Haddock, whole or filleted, cooked and uncooked, dried at 50°C. in 12 hr.	37°C. 236	40	—	60	—	}	Negative	5
		20°C. 458	38	—	62	—	}		
III	Haddock, headed, cooked, dried at 50°C. in 12 hr.	37°C. 70	64	3	10	23	—	Negative	0
IV	Haddock and whiting cooked whole, minced, dried at 50°C. in 12 hr.	37°C. 30	100	—	—	—	}	Negative	—
		20°C. 44	99	1	—	—	}		
V	Cod fillets, cooked, dried 90–60°C. in 3 hr.— good hygienic handling, 0–21 days in ice	37°C. 16 hr. in ice 72	90	9	1	—			29
		7 days in ice 85	88	10	2	—			
		12 days in ice 46	93	—	—	7		Negative	
		21 days in ice 41	99	—	—	1			
		20°C. fresh only —	83	8	8	1			
VII	Cod fillets, 1–2 days in ice, dried 90–70°C. in 3 hr.	37°C. 227	84	6	4	6	} 1	Negative	3
		20°C. 92	59	26	8	6	}		

Table 13. *Bacterial flora of dried fish*

Sample no.	Origin, etc.	Total no. of samples tested	Time after preparation before testing	Count at		Growth on selective media						Presence of coagulase-positive cocci
				20°C.	37°C.	Desoxy-cholate citrate agar	MacConkey's agar	Tetrathionate broth and subsequent plating on MacConkey's agar	Glucose agar deep	Blood agar	Alkaline agar pH 10.2	
1	Surface slime from haddock, cod, etc. 0–18 hr. in sterile box at 0°C. after catching	6	—	61,000	117	−ve in 1 sq.cm.	−ve in 1 sq.cm.	−ve in 1 sq.cm.	−ve in 1 sq.cm.	−ve in 1 sq.cm.	+ve in 1 sq.cm.	Not tested
2	Commercial ice, direct from ice factory	5	—	—	—	−ve in 2 c.c.	−ve in 2 c.c.	−ve in 10 c.c.	−ve in 10 c.c.	Some haemolytic organisms, no streptococci in 2 c.c.	−ve in 2 c.c.	−ve in 2 c.c.
3	Dried cod: Fillets, 0–3 days in ice	1	Directly after preparation	2,000	2,950	—	—	—	—	—	+ve in 0.2 g.	+ve in 0.2 g.
4	Fillets, 1–20 days in ice	4	Do.	3,000–20,000	2,600–15,000	—	—	—	—	—	Do.	+ve in all 4 samples
5	Fillets, stored 11 months at −10°C. after preparation	2	11 months	—	—	−ve in 0.2 g.	−ve in 0.2 g.	−ve in 1 g.	−ve in 1 g.	+ve in 0.2 g. (few haemolytic staphylococci, no streptococci)	−ve in 0.2 g.	−ve in 0.2 g.
6	Fillets, stored room temp for 2 months and then 7 months at −10°C.	1	9 months	—	—	Do.	Do.	Do.	Do	Do.	+ve in 0.2 g.	+ve in 0.2 g.
7	Fillets, stored at −10°C. after preparation	2	8 months	—	—	Do.	Do.	Do.	Do.	Do.	−ve in 0.2 g.	−ve in 0.2 g.
8	Fillets, from cod 0–3 days iced, stored in air and nitrogen at 25 and 37°C.	8	8 months	5–75	5–150	Do.	Do.	Do.	Do.	Do.	+ve in 0.2 g.	+ve in 0.2 g. in 3 out of 8 samples
9	Dried smoked cod: fillets	1	7 months	—	—	−ve in 0.2 g.	−ve in 0.2 g.	−ve in 1 g.	−ve in 1 g.	−ve in 0.2 g.	−ve in 0.2 g.	−ve in 0.2 g.
10	Do.	1	15 months	—	—	Do.	Do.	Do.	Do.	Do.	Do.	Do.

No.	Description	No. of samples	Period									
11	Herring flesh: Herring, 18 hr. iced, brined and unbrined, before and after smoking	4	—	312,000 to 570,000	10,000 to 120,000	−ve in 1 g.	−ve in 1 g.	+ve in 2 g. (*aerogenes* coliforms)	−ve in 2 g.	−ve in 0·2 g.	+ve in 2 g.	+ve in 2 g. in 1 sample
12	Dried herring: Herring, 1 day uniced after catching, stored at temp. from −30 to +37°C. over air or nitrogen	10	9 months	10–190	10–115	−ve in 0·2 g.	−ve in 0·2 g.	−ve in 1·0 g.	−ve in 1·0 g.	3 samples gave haemolytic col., no streptococci in 0·2 g.	+ve in 0·2 g.	+ve in 0·2 g. in 3 samples
13	Herring, split or nobbed, 1–9 days in ice—stored at −10°C.	10	3–12 months	—	—	Do.	Do.	Do.	Do.	Do.	Do.	+ve in 0·2 g. in 5 out of 10 samples
14	Dried smoked herring: Herring, 1 day uniced after catching then smoked and dried	10	9 months	30–70	20–105	Do.	Do.	Do.	Do.	−ve in 0·2 g.	−ve in 0·2 g.	−ve in 0·2 g.
15	Split and nobbed herring, 1–9 days in ice before smoking and drying	5	12–14 months	—	—	Do.	Do.	Do.	Do.	Do.	+ve in 0·2 g.	+ve in 0·2 g. in 1 out of 5 samples
16	Dried mackerel: Whole fish and fillets	1	4 months	3,240	1,980	Do.	Do.	Do.	Do.	+ve in 0·2 g.	Do.	−ve in 0·2 g.
	Fillets	1	4 months	318	132	Do.	Do.	Do.	Do.	Do.	Do.	+ve in 0·2 g.
17	Dried mullet from U.S.A. (*a*)	3	3–4 months	9,700 to 460,000	9,600 to 82,000	Do.	Do.	Do.	Do.	−ve in 0·2 g.	−ve in 0·2 g.	Do.
	(*b*)					Do.	Do.	+ve in 1·0 g. (coliforms and *Proteus* only)	Do.	Do.	Do.	−ve in 0·2 g.
	(*c*)					Do.	+ve in 0·2 g.		Do.	+ve in 0·2 g. (staphylococci and no streptococci)	+ve in 0·2 g.	+ve in 0·2 g.
18	Stockfish from South Africa	2	—	0–90	15–20	−ve in 0·2 g.	−ve in 0·2 g.	−ve in 0·2 g.	−ve in 1·0 g.	−ve in 0·2 g.	−ve in 0·2 g.	−ve in 0·2 g.
19	Dried cod from U.S.S.R.	1	—	6	258	Do.	Do.	Do.	Do.	Do.	+ve in 0·2 g.	+ve in 0·2 g.
20	Dried crawfish from South Africa	4	—	5–93	10–80	Do.	Do.	Do.	Do.	Do.	−ve in 0·2 g.	−ve in 0·2 g.

Table 14. *Bacterial count in roller dried fish*

		No. of organisms per gram			
		Bacterial count		Aerobic spores	Anaerobes
Exp. no.	Species	37°C.	20°C.		
4A	Whiting fillets 0–6 days iced, skin on—frozen (−30°C.) for 1 week before drying	100	< 100	—	Negative (2 g.)
			25°C.		
6A*	Cod fillets (skinned) at 0°C. for 4 days, and in ice for 1 day before drying	100	40	10	Positive (1 g.) Negative ($\frac{1}{10}$ g.)
6B*	Cod fillets, 2 days in ice before drying	30	< 10	< 10	Negative (1 g.)

* Counts done by Miss E. Elliott, Department of Pathology, Cambridge, to whom the author's thanks are due.

Log bacterial count at 20° C.

Log bacterial count at 37° C.
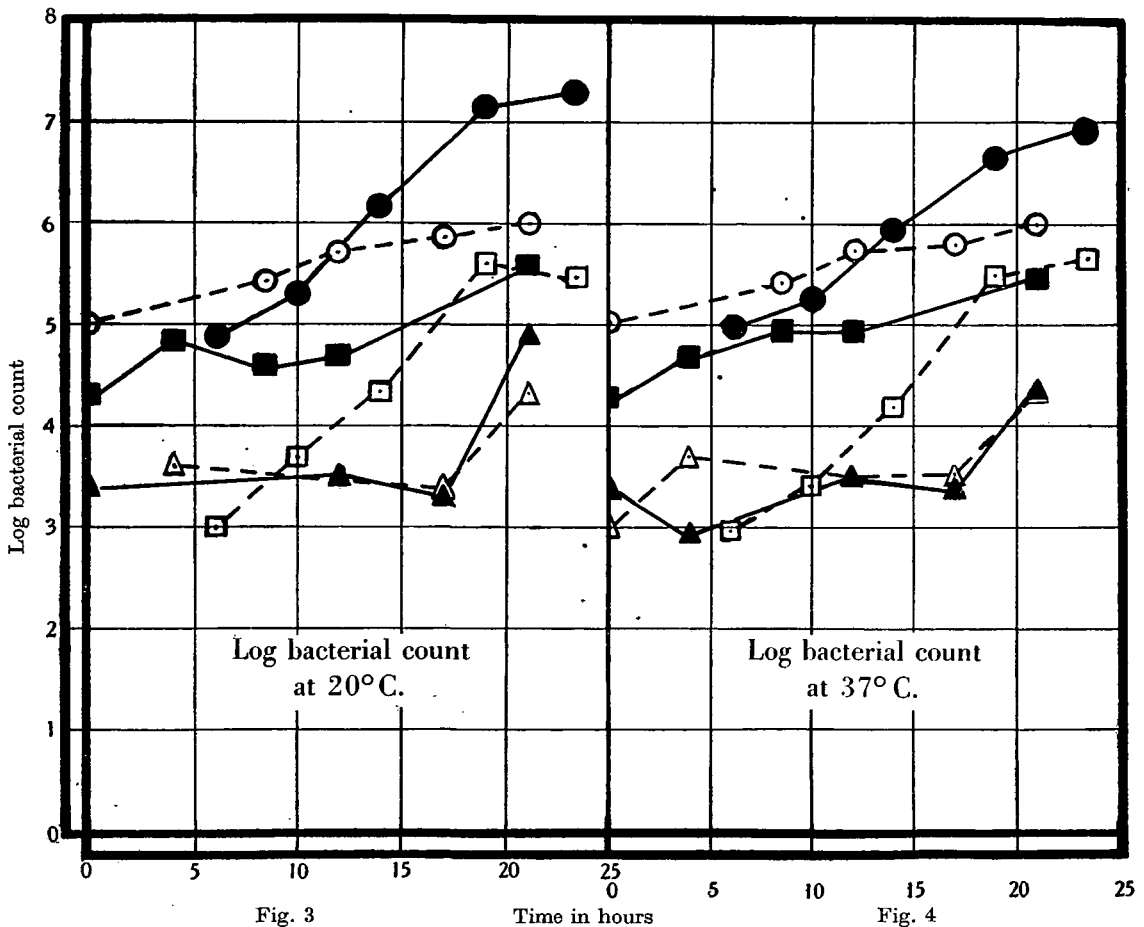
Fig. 3   Time in hours   Fig. 4

Figs. 3 and 4. Moisture content of cakes (before frying) = 62 %.

●—●—●—● = Cakes with *S. aureus* stored at 20°C.
□—□—□—□ = Control cakes stored at 20°C.
■—■—■—■ = Cakes with *S. aureus* stored at 15°C.
⊙—⊙—⊙—⊙ = Control cakes stored at 15°C.
▲—▲—▲—▲ = Cakes with *S. aureus* (fried 5 min. 80°C.) stored at 15°C.
△--△--△--△ = Control cakes (fried 5 min. 80°C.) stored at 15°C.

above (p. 199). Fish cakes unfried and each containing 15 g. dried fish, 35 c.c. of 3 % NaCl solution, and 60 g. of mashed boiled potatoes were placed singly in sterile screw-capped jars. In the storage experiment at 15° C., cakes were also fried in deep fat for 5 min. (temperature of fat 190–124° C., temperature of cakes after 5 min. cooking 80° C.) and then stored singly in jars as in the uncooked samples. In addition, one or two cakes, both fried and unfried, were stored at 0° C., but only the results of the former are available.

After incubation, one cake was taken, for each point on the curve, ground up with sterile sand and Ringer's solution and appropriate dilutions made for counts at 20 and 37° C. The results of these experiments are shown graphically in Figs. 3 and 4. It will be seen that in unfried cakes, both with or without *S. aureus*, stored at 20° C., active growth is occurring even after 5 hr., and within 15–20 hr. considerable increase in the numbers of bacteria growing at 20 and 37° C. has taken place. As might be expected growth in the unfried cakes stored at 15° C. is not so rapid as at 20° C., but some growth is occurring after 7–10 hr. In the corresponding fried cakes (at 15° C.), little seems to have occurred until after 16–17 hr., when there is a sudden increase in the numbers present.

Although no figures are available for unfried fish cakes stored at 0° C., those obtained for the fried samples (Table 15) suggest that no growth would occur at least over a period of 21 hr.

Table 15. *Growth of bacteria in fish cakes (fried) stored at 0° C.*

Sample	Time in hr.	Log no. of bacteria per gram	
		20° C.	37° C.
Cake with *S. aureus*	0	3·38	2·50
	21	2·64	2·68
Cake without *S. aureus*	0	3·00	2·0
	21	3·35	2·82

In view of the fact that Jordan & Burrows (1935) found starchy media excellent for enhancing the production of staphylococcal enterotoxins, and that growth occurs even at 15° C., it would appear from these few results that fish cakes made from reconstituted dehydrated fish should never be kept unfried in a warm room, but should be cooked immediately. If storage overnight is contemplated, they must be kept in a cool place (below +7° C.).

CONCLUSIONS

1. Dehydrated fish, made from good-quality fresh material, under reasonable hygienic conditions, is not sterile, but may contain up to 10^5 organisms/g.

2. Apart from a small percentage of asporogenous rods, the flora consists chiefly of micrococci and thus contrasts sharply with that of the original fresh fish.

3. Of the cocci growing at 37° C., 0–29 % are coagulase-positive and hence potentially capable of producing enterotoxins.

4. The main change in the flora occurs immediately after filleting the fresh fish, and is due to human handling, contacts with benches, etc.

5. The cooking process results in almost complete destruction of the bacteria present, but re-infection up to that present on the raw fillets occurs immediately afterwards, in the remincing, spreading on trays, etc.

6. If the temperature of the fish during the first 3–4 hr. of drying is in the region of 35–40° C., rapid increase in the bacterial counts occurs, but at a temperature of 50° C., on the other hand, a steady decline.

7. The drying process results in a considerable decrease in the numbers of bacteria present in the final product.

8. Fish cakes (uncooked) made from the reconstituted fish, and stored at 20 and 15° C., showed increased bacterial counts after 5 and 7–10 hr. respectively, whilst fried cakes, at 15° C., showed increases after 16–17 hr. Up to 21 hr. no growth occurred at 0° C.

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