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#### THE BACTERIOLOGY OF DEHYDRATED FISH

### II. THE EFFECT OF STORAGE CONDITIONS ON THE BACTERIAL FLORA

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(With 3 Figures in the Text)

### INTRODUCTION

In a previous communication on the bacteriology of dehydrated fish (Shewan, 1945), data were presented concerning various aspects of the drying process, and their bearing upon commercial production was discussed. This communication reports further work, done mainly during the war, on the effect of storage conditions on the bacterial flora of various kinds of dehydrated fish products.

#### MATERIALS AND METHODS

Numerous storage experiments were performed with various products made usually from whole or filleted cod, haddock, whiting or herring that had been stored in ice for various periods before drying. Occasionally frozen and cold stored fish were used.

The raw or cooked minced flesh was dried in warm air in the Torry batch kiln (see Shewan, 1945), but on a few occasions roller drying was used.

The dried products, either as a loose mince or as a finely ground powder, or as blocks produced by compression, usually after reconditioning at various relative humidities, were stored for periods up to 2 years in tins in air or nitrogen at temperatures ranging from -10 to  $37^{\circ}$  C.  $(14-99^{\circ}$  F.) or in shallow dishes in desiccators at  $20^{\circ}$  C. at various relative humidities  $(0\cdot0-95\%)$  over CaCl<sub>2</sub> or H<sub>2</sub>SO<sub>4</sub>.

From the large number of experiments done, only those will be described which best illustrate the effects of different storage conditions on the bacteriological properties of dehydrated fish. Dried products made from different species behaved very similarly under the same or similar storage conditions.

#### RESULTS

#### (i) The effect of temperature

Experiment 23 (12 March 1948). Fillets taken from reasonably fresh market cod were air-dried in the usual way, except that the minced cooked fish, the surface of which was smeared with the heavily infected slime from the outer surfaces of several cod, was incubated overnight in the cooking retort at  $15-20^{\circ}$  C. (59-68° F.) before drying in order to increase the normally small bacterial load of the dried product. Two days after drying a qualitative and quantitative bacteriological analysis was made on representative samples of the dried product. The remainder

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was sealed in 'A1 tall' lacquered tins, half of which contained nitrogen and half air, and stored at temperatures of -10, 0, 20 and 37° C. (14, 32, 68 and 99° F.). At intervals over a period of 67 weeks, sample tins of both the air and nitrogen packs were taken, and a gas analysis and viable bacterial counts performed on each. At the end of the storage period, a qualitative analysis of the flora was again carried out.

It was evident early in the experiment that, despite normal precautions, several of the nitrogen tins were leaking. Moreover, as the experiment progressed, it was found that many of the air packs were in fact under a vacuum, probably as the result of oxygen absorption by some constituent in the fish. Only the data for the air packs will be given, but the air and nitrogen packs showed closely parallel behaviour.

The bacterial counts were performed in the usual way using 25 g. of fish and incubating the plates at 20 and  $37^{\circ}$  C. Initial counts were performed on twelve samples, but on each subsequent occasion only two from each tin were made. The results are set out graphically in Figs. 1 and 2. The qualitative analysis of the bacterial flora consisted of isolating and classifying some 250 cultures both at 20 and  $37^{\circ}$  C. from the count plates before storage and of about 120 cultures after storage. The results are set out in Table 1.

	. Por contrary	s og tille tol	Asporoger	nous rods		Micro	ococci	
Sample	Temp. of incubation (° C.)	No. of colonies examined	f Achromo- Cory ed bacter bact		Bacillus	Gram- positive	Gram- negative	
Before storage	20 37	$\begin{array}{c} 250 \\ 250 \end{array}$	2·0 1·0	$2 \cdot 0 \\ 1 \cdot 5$	0·0 0·5	$53 \cdot 0$ $95 \cdot 0$	43·0 2·0	
After storage for $67$ weeks at $-10$	$\frac{20}{37}$	115 110	43·0 5:0	11.0 2.5	3·0 2·0	<b>43</b> ∙0 31∙5	0·0 59·0	

 Table 1. The bacterial flora of dehydrated cod before and after storage, expressed as a percentage of the total number of organisms examined

From Figs. 1 and 2 it will be seen that on storage at  $37^{\circ}$  C. there is a marked fall in the count both at 20 and  $37^{\circ}$  C., particularly over the first 10–14 weeks, and a definite though less spectacular fall in those stored at  $20^{\circ}$  C. At 0 and  $-10^{\circ}$  C., on the other hand, the counts at 20 and  $37^{\circ}$  C. are almost as high as those initially present. These results are in general agreement with data obtained from several similar storage experiments using herring, kipper, smoked and unsmoked haddock as well as cod.

After the first few weeks, the count increased in all the samples except one (counts at  $20^{\circ}$  C. on fish stored at  $37^{\circ}$  C.). This increase has appeared so consistently in storage experiments that it cannot be accounted for simply on the basis of sampling errors. It is suggested here that many of the bacterial cells present before dehydration were so damaged during the drying process that they could not grow immediately after dehydration when the initial counts were made, but that they recovered in time to contribute to the increased counts after the first few weeks of storage. This suggestion also probably accounts for the results of the qualitative

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0, 20 and 37° C.

survey before and after drying given in Table 1. It will be noted there that immediately after drying, the flora, both at 20 and 37° C., consisted almost exclusively of micrococci, many strains of which are known to be particularly resistant to drying. At the end of the storage period, however, 54% of the types growing at 20° C. consisted of asporogenous rods (*Achromobacter* and *Corynebacteria*), and 43% of micrococci. 90% of those growing at 37° C., however, were still micrococci. Further analysis showed that of the cocci growing on the plates incubated at 37° C., 99.5% came from tins stored at 20 and 37° C., whilst 96% of the asporogenous rods



Fig. 1. Counts at 20° C. on dehydrated fish stored at -10° C., -●--●-; 0° C., -⊡--; 20° C., -⊙--⊙-; 37° C. -■-■-.

growing at 20° C. were from tins stored at 0 and  $-10^{\circ}$  C. and were incapable of growth at 37° C. Moreover, 45% of the Gram-positive cocci growing at 20° C. came from tins stored at 20 and 37° C. To explain these results it is suggested that the flora immediately after drying consists of heat-resistant micrococci capable of growth at 20 and 37° C. and of heat-damaged cells of the asporogenous rods, most of which fail to grow at 37° C. and which are incapable of immediate growth on nutrient agar at 20° C. During storage at 0 and  $-10^{\circ}$  C., these latter cells gradually recover sufficiently well to grow again on ordinary nutrient media at 20° C., but die out on storage at 20 and 37° C. Clearly, however, all these results, including some previously recorded (Shewan, 1945) and those of other workers (Curran &

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Evans, 1937; Nelson, 1942, 1943*a*, *b*; Williams & Reed, 1942; Olsen & Scott, 1950; Murrell, Olsen & Scott, 1950) indicate that the growth conditions of bacteria subjected to sublethal heating requires fuller investigation.

As already stated, in most of the experiments there was little difference in the survival counts as between the air and nitrogen packs. A few, however, appeared to indicate better survival in nitrogen, as already reported for *Staphylococcus aureus* in dried meat (Haines & Elliot, 1944). Unfortunately, however, in most of the



Fig. 2. Counts at 37°C. on dehydrated fish stored at  $-10^{\circ}$ C.,  $-\bigcirc -- \bigcirc -;$  $0^{\circ}$ C.,  $-\bigcirc -- \bigcirc -;$   $37^{\circ}$ C.,  $-\blacksquare --\blacksquare -:$ 

experiments with dried fish no gas analyses were performed, so that it was impossible to know whether any specific tin was a 'leaker' or had suffered oxygen absorption.

## (ii) The effect of relative humidity

Since dehydrated fish may be exposed to humid atmospheres and so absorb moisture in amounts sufficient to cause microbial growth, the effect of relative humidity on the latter was studied under various storage conditions.

The dehydrated fish, finely ground to a meal, was exposed in thin layers in shallow trays in desiccators over either  $CaCl_2$  or  $H_2SO_4$  at R.H. ranging from 0.0 to 95% and stored in the dark, usually at 20°C., for periods up to  $l_2^1$  years. At

intervals the samples were examined for visible moulding and portions were taken for bacterial counts, and in some experiments for moisture determinations (usually by the Tate & Warren (1936) method) and for estimations of total volatile bases and trimethylamine, using the Conway technique (1947).

Experiments I and II (8 January 1943 and 11 June 1943). Dehydrated whiting was prepared from fish stored for 2 months at  $-30^{\circ}$  C.  $(-22^{\circ}$  F.), the normal procedure being altered so as to increase the bacterial load in the final meal, by using a shorter cooking time and a lower temperature and longer time in the initial stages of drying. The final product was ground to a fine meal, viable counts (at 20

# Table 2. The bacterial counts and moisture contents on whiting meals storedat 20° C. and various relative humidities

	Maintana ann tan t	(T):	(dry basis)		
Details of storage	(%)	(davs)	20° C.	37° C.	
Original meal	5.0	0	7.51		
Milled sample	_	4	7.34	7.15	
<b>в.н.:</b> 95%	$24 \cdot 5$	23	6.07	5.39	
75%	13.4	23	6.82	6.71	
50 %	11.0	23	7.35	7.01	
10 %	4.5	23	7.31	7.14	
Over CaCl <sub>2</sub> 0.0%	1.5	<b>23</b>	7.56	7.30	
Original in a lever-lid t	in 4·5	23	7.08	6.74	
<b>в.н.:</b> 95%*	31.5	51	5.66	5.71	
75 %	18	51	6.18	6.48	
50 %	11.5	51	6·84	6.95	
10%	<b>4</b> ·5	51	7.00	6.97	
Over CaCl <sub>2</sub>	1.0	51	7.12	7.18	
Original meal	4.5	51	6.99	6.83	
<b>в.н.:</b> 75 %	13	110	5.35	<3.0	
50%	8	110	6.07	5.94	
10 %	4	110	6.64	6.59	
Over CaCl <sub>2</sub>	0	110	6.74	6.79	
Original meal	4	110	6.73	6.41	
	* Moulds visibl	.e.			

(The whitings were dried on 8 January 1943, and milled on 15 January 1943.)

and  $37^{\circ}$  C.) were performed on duplicate samples and portions of the remainder were placed in shallow layers in Petri dishes over  $H_2SO_4$  at R.H. of 10, 50, 75 and 95% as well as over solid CaCl<sub>2</sub> (R.H. 0.0%). At intervals over a period of 110 days the meals were examined for visible moulding, after which samples were taken for bacterial counts and moisture determinations. These results are set out in Table 2. At the conclusion of this experiment (Exp. I) a similar one (Exp. II) was set up using the same meal, which meantime had been stored in a close-fitting lever-lid tin at room temperature. The controlled R.H. in this instance was 75, 80, 85; 90, 95 and 0.0% (over solid CaCl<sub>2</sub>); and samples were examined after 98 and 256 days. The results are given in Table 3.

Both tables show that, as storage proceeded, the viable count fell more considerably at R.H. above 50 %, and the higher the R.H. the speedier was the fall.

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Thus, whilst the counts had fallen to about one-half or one-third of the original values after 110 days over  $CaCl_2$ , at R.H. 75% (with no moulds visible), the values were only one-hundredth of the original. If moulds were present, as at R.H. of 85% and higher, the decrease might well have been the result of the antibiotic activity of the moulds.

Tarr (1945) reported bacterial multiplication in dried fish (ling cod and salmon) at R.H. just over 85%.

# Table 3. The bacterial counts and moisture contents of whiting meals stored at 20° C. and various relative humidities

(Original meal dried on 8 January 1943, milled 15 January 1943, and stored in close fitting lever-lid tin at room temperature until 11 June 1943.)

	Moisture			Log viał (dry	ole count basis)
	$\operatorname{content}$	Time	Presence or absence ~		·
Details of storage	(%)	(days)	of moulds	20° C.	37° C.
Original meal	<b>4</b> ·0		No moulds visible	6.91	6.79
<b>в.н.:</b> 75 %	16.5	98	No moulds visible	<b>4·7</b> 9	4.05
80 %	18	98	No moulds visible	4.59	3.36
85 %	19.5	98	A slight trace of mould	3.82	3.07
90 %	$23 \cdot 5$	98	Covered with moulds	3.71	3.81
95%	24.5	98	Covered with moulds	3.85	3.74
Over CaCl <sub>2</sub>	A trace	98	No moulds visible	6.76	6.70
<b>в.н.:</b> 75%	16.4	256	No moulds visible	0.0	1.40
80 %	18.4	256	Covered with moulds	3.53	1.66
85%	$21 \cdot 6$	256	Covered with moulds	4·14	3.28
90 %	24.0	<b>256</b>	Covered with moulds		
95%	$28 \cdot 8$	256	Covered with moulds		<del></del>
Over $CaCl_2$	2.8	256	No moulds visible	6.73	6.68

Table 3 shows that even after 256 days the count over  $CaCl_2$  was almost identical with that before storage. In this instance the micro-organisms present before storage probably represented the more resistant remnants of the original flora of the meal prepared 5 months previously, when a count three times greater had been obtained.

The growth of moulds naturally present in the meals was visible at R.H. 95% after 51 days, at R.H. 85% after 98 days and at R.H. 80% after 256 days, corresponding to moisture contents of 31.5, 19.5 and 18.4% respectively. No growth occurred at R.H. 75% (moisture content 16.4%) after 256 days.

As it was considered important to characterize more fully the equilibrium moisture content relation with regard to mould growth, particularly in view of the reputed xerophilous nature of some mould strains (Snow 1945), another experiment was set up with dehydrated cod.

Experiment 12 (30 June 1944). Four lots of cod, frozen and cold stored for 1-2 years at  $-30^{\circ}$  C.  $(-22^{\circ}$  F.), were filleted and dried as follows. Two lots, after being minced and spread on the trays prior to drying, were sprayed with a saline suspension of *S. aureus* containing about  $10^{10}$  cells per ml. (see Shewan, 1945), the

other two lots being left unsprayed. Half of the sprayed and of the unsprayed trays were dried at  $80^{\circ}$  C. (176° F.), the other halves at  $40^{\circ}$  C. (104° F.).

These four lots of dehydrated cod were each divided into two groups, one set over CaCl<sub>2</sub> solutions at R.H. 40, 50, 60, 70, 75, 80, 85, 90 and 95 % and the other at similar R.H. over H<sub>2</sub>SO<sub>4</sub> with a control over solid CaCl<sub>2</sub>. The intention was to observe the presence of moulds, to determine the moisture contents, bacterial counts, total volatile base and trimethylamine values in representative samples at each R.H., at intervals of 3, 6, 12 and 18 months, in the hope that the different heat treatments might be reflected in the counts during storage and that information would be gained concerning the survival of S. aureus (a potential food poisoning pathogen) under the different storage conditions. Both  $CaCl_2$  and  $H_2SO_4$  were used because Tarr (1945) had observed differences in the formation of the total volatile bases and trimethylamines in stored ling cod and salmon, which he attributed to the different natures of these solutions. The full programme was not carried out, chiefly because the original counts in the meals dried at 80° C. (176° F.) were much lower than anticipated and fell to almost nil after 3 months. The remaining data obtained for most samples after 18 months' storage in the dark at 20° C. are given in Tables 4 and 5. The equilibrium moisture contents of dried cod at the various R.H. have been calculated by averaging the eight values for the sprayed and unsprayed meals dried at 40 and 80° C. and stored over CaCl<sub>2</sub> and H<sub>2</sub>SO<sub>4</sub>, and these are shown graphically in Fig. 3. The curve obtained by Tarr (1945) for ling cod is given for comparison.

The most interesting feature of the experiment is the occurrence of moulds in samples stored at R.H. 70% and above. From this it may be deduced that the limiting R.H. for mould growth on dehydrated cod lies somewhere between 70 and 80%, the corresponding moisture contents being 9.6 and 14.7% respectively.

It will also be seen from Tables 4 and 5 that irrespective of whether storage was over  $CaCl_2$  or  $H_2SO_4$ , the total volatile base and trimethylamine values (compared with those of the original meal) increased significantly only at R.H. 75% and above, i.e. only in those samples where mould growth became well established. At R.H. below 75% where mould growth was probably scant or non-existent the values were usually lower than those in the original meal. These results are somewhat different from those of Tarr (1945), who found significant increases in both the total volatile bases and trimethylamine in dehydrated ling cod and salmon stored at R.H. below 75%. The cause of these discrepancies is at present unknown, but one may be the higher temperature of storage (25° C.) used in Tarr's experiments. A few individual estimations of total volatile bases and trimethylamine made on samples stored at Torry Research Station at 37° C. and at R.H. below those capable of supporting active mould growth have given values larger than those quoted in Tables 4 and 5. It appears, however, as if the nature of the stabilizing fluid (CaCl<sub>2</sub> or H<sub>2</sub>SO<sub>4</sub>) had little effect on the development of these volatile bases.

Experiment 24 (29 March 1948). Another experiment at R.H.75 % and below was set up in order to determine more closely the limiting R.H. equilibrium moisture content level at which moulding occurs after prolonged storage and also to investigate the claim of Watts (1945) that for some micro-organisms maximum survival counts during storage at R.H. 0.0-100 % occur at 25 %.

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Table 4. The appearance, moisture contents, total volatile bases, and trimethylamine values in cod meal after storage for 549 days at 20° C. and at various relative humidities over CaCl<sub>2</sub> solutions

	Moisture	TVB (mg.	(CH <sub>3</sub> ) <sub>3</sub> N (mg.	Presence	
	$\operatorname{content}$	$N_2$ per 100 g.	$N_2$ per 100 g.	or absence	Remarks on appearance,
Sample	(%)	meal)	meal)	of mould	odour, etc.
S*, 40†	6.0	34.0	7.0		
S, 80†	6.0	40.6	21.7		
<b>U*, 4</b> 0	7.0	29.0	9.0		
U, 80	5.0	29.4	12.6		
					Colour dark brown with moist
S, 40-95‡	45.5	<b>204</b>	54.0	+)	appearance, soft and semi-
U, 40-95	52.5	138	64.0	+	fluid in places; odour is
S, 80–95	45.0	198	$52 \cdot 0$	+ (	strongly ammoniacal; dark
<b>U</b> , 80–95	38.0	94	<b>44</b> ·0	+)	brown and red mould present
					on all samples
S, 40-90	42.0	130	66.0	+ )	
U, 40-90	<b>47·0</b>	68	28.0	+	Similar to R.H. 95, perhaps not
S, 80–90	49.5	32	32.0	+ (	so wet looking; odour and
U, 80–90	37.0	<b>32</b>	34.0	+ ]	colour similar
S, 40–85	28.0	86	12.0	+ )	Moist looking, with patches of
Ú, 40–85	25.5	64	10.0	+1	reddish and white moulds;
S, 80-85	$22 \cdot 5$	48	14.0	+ }	odour is more musty than
<b>U</b> , 80–85	25.5	40	6.0	+)	ammoniacal
S. 40-80	21.5	38	6.0	+ Ì	
U. 40-80	17.0	64	16.0	<u>+</u>	Mould in patches, but colour
S, 80-80	21.5	43	12.0	+ }	nearer normal fish meal;
<b>Ú</b> , 80–80	20.0	44	12.0	+	odour is mouldy and bad
S. 40-75	17.5	38	8.0	+)	Appearance shows very little
<b>U.</b> 40–75	17.5	40	10.6	<u> </u>	change except for small
S. 80–75	15.0	40	10.6	+ }	patches of brown and white
<b>U</b> , 80–75	17.5	46	10.0	+1	mould: usual fish-meal odour
8. 40-70	17.5	32	3.4	+)	
<b>U.</b> 40–70	14.5	24	4.6	+	Very little change except for a
S. 80-70	14.5	24	4.6	+ }	little white and brown mould
U, 80-70	14.5	24	4.6	÷)	
S. 40-60	10.0	19.2	2.4	-)	
U. 40-60	9.5	20.0	$\frac{2}{3}\cdot 2$	_	
S. 80–60	9.5	20.0	2.4		
<b>U.</b> 80–60	9.5	16.4	$\overline{2 \cdot 0}$	_]	
S, 40–50	9.0	Not es	timated	_	Mould-free with little change in
U, 40-50	9.0	Not es	timated	_ {	appearance. Samples dried at
S, 80–50	$9 \cdot 5$	Not es	timated	- (	80° C. are perhaps darker than
U, 80–50	9.0	Not es	timated	-	the 40° C. samples
S, 40-40	8.0	Not es	timated		*
U, 40–40	8.0	Not es	timated	-1	
S, 80-40	8.0	Not es	timated	-	
<b>U</b> , 80–40	6.0	Not es	timated	_)	

\* U or S, unsprayed or sprayed with a suspension of S. aureus before drying. † '40' or '80', dried at 40 or  $80^{\circ}$  C.

‡ '95', '90', etc., в.н. values.

S, 40, S, 80, U, 40 and U, 80 were original samples.

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	Moisture	TVB (mg.	(CH <sub>3</sub> ) <sub>3</sub> N (mg.	Presence	~ .
а I	content	$N_2$ per 100 g.	$N_2$ per 100 g.	or absence	Remarks on appearance,
Sample	(%)	meal)	meal)	of mould	odour, etc.
S*, 40†	6.0	34.0	7.0		
S, 80†	6.0	40.6	21.7		
U*, 40	7.0	29.0	9.0		
U, 80	$5 \cdot 0$	29.4	12.6		
					Colour dark brown with red
S 40 054	69.0	400	159.0	. )	mould patches, odour strongly
11 40 05	65.0	400	102.0	+	ammoniacal with wet sticky
C, 40-95	51.0	140	40.0	+}	appearance, some patches are
D, 80-95	59.0	110.3	42.0	+	fairly dry, mostly semi-fluid
0, 00-95	58.0	140	72.0	+)	in consistency, some faecal
					odour
G 40 00	24 7		<b>F</b> O 0		Samples wet looking: patches
S, 40-90	24.5	92.0	58.0	+)	of red, brown and white
U, 40-90	36.5	188	78-0	+ [	mould: odour is mainly
S, 80-90	22.0	164	76.0	+ (	mouldy and not so ammoni-
U, 80–90	<b>44·</b> 5	172	70-0	+ J	acal as the B.H. 95 samples
					All your similar, damp and
S 40-85	20.5	77	28.0	т)	caled looking: small patches
II. 40-85	200	67	20.0		of mould—mostly red and
S 80-85	25.0	50	22.0	+ }	brown mould, adour not
II 80-85	22.5	117	52.0		ammoniacal but musty and
0,00-00	20 0	117	52.0	÷,	ahmomacai but musty and
G 40 00	<b>01</b> 7	0.0			Cheesy
S, 40-80	21.9	30	4.0	+	Colour practically unaltered
0,40-80	21.0	160	40.0	+ }	but some patches of red and
5, 80-80	18.9	38	30.0	+	white mould; odour spicy and
0,80-80	19.9	88	20.0	+)	faintly ammoniacal
S. 40–75	16.0	80	40.0	+ )	Appearance practically un-
U. 40-75	17.5	78	38.0	+1	changed; no marked odour;
S. 80-75	14.5	76	36.0	+ }	only slight traces of mould
Ú, 80–75	16.0	78	38.0	÷!	which have not penetrated
				. ,	the surface
8. 40-70	14.5	50.4	18.4	+ )	Appearance unaltered; practi-
U 40-70	14.5	25.2	9.4		cally free of mould; only
S. 80-70	14.0	25.2	2.4	} }	small patches of very fine
II. 80-70	14.0	20.2	1.6		brown mould; practically
0,00 10	110	210	10	Τ)	normal fish meal odour
S, 40-60	10.0	19.2	3.6	+ )	
U, 40–60	9.5	19.4	3.6		
S, 80–60	9.5	19.4	3.6	-	
U, 80–60	9.5	18.4	3.4	1	All samples free of mould,
S, 40–50	10.5	Not es	timated	_	showing practically no change
<b>U, 40–50</b>	10.0	Not es	timated	_	in appearance except that the
S, 80–50	8.5	Not es	timated	- (	meals dried at 80° C. are
<b>U</b> , 80–50	9.5	Not es	timated	-1	perhaps slightly darker in
S, 40-40	7.5	Not es	timated	- 1	colour
U, 40-40	8.0	Not es	timated	- 1	
S, 80-40	8.0	Not es	timated	- !	
<b>U, 80–4</b> 0	7.0	Not es	timated	_ )	

Table 5. The appearance, moisture contents, total volatile bases, and trimethylamine values in cod meal after storage for 549 days at 20° C. and at various relative humidities over  $H_2SO_4$  solutions

\* U or S, unsprayed or sprayed with a suspension of S. aureus before drying.

† '40' or '80', dried at 40 or 80° С. ‡ '95', '90', etc., R.H. values.

S, 40, S, 80, U, 40 and U, 80 were original samples.

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Dried cod, prepared as for Exp. 23, was finely ground and placed in shallow layers in Petri dishes over  $H_2SO_4$  at R.H. 0.0–75%. The desiccators containing the dishes were stored at 20° C. in the dark, and at intervals of 1, 3, 6 and 12 months were examined for the presence of moulds, after which viable counts were performed at 20 and 37° C. on representative samples at each R.H.



Fig. 3. Equilibrium moisture contents of dehydrated cod and ling cod at various RH. ●—●—● (cod); ○—○—○ (ling cod, after Tarr).

Table 6.	Viable	counts	(at 20	and	$37^{\circ}$	C.) and	appeara	nce of	moulds	in
deh	ydrated	cod sta	ored at	$20^{\circ}$	C. at	various	relative	humid	lities	

					$\operatorname{Stor}$	age tin	ne (we	eks)			
		0		0 6		12		25		62	
Sample		20° C.	37° C.	20° C. 3	37° C.	20° C. 3	37° C.	20° C.	37° C.	20° C.	37° C.
Initial sample		6·19	<b>4·80</b>								
Stored at R.H.:	0%			6.40	5.28	5.48	6.42	5.77	$5 \cdot 10$	5.36	4.05
	10%			6.89	5.30	4.77	6.04	5.48	<b>4</b> ·89	5.30	$4 \cdot 42$
	15%	-		6.57	5.14	5.51	6.29	5.38	<b>4</b> ·73	5.22	4.28
	25%	_		6.49	4.72	5.43	6.06	5.35	<b>4</b> ·83	4.56	2.91
	35%	<u> </u>		6.02	4.51	5.37	5.96	5.00	4.32	4.50	3.01
	50%			6.27	4.59	5.13	5.89	4.45	3.51	2.88	1.48
	65%			5.90	4.53	3.88	5.22	3.95	3.53	1.85*	1.00*
	75%			6.14*	<b>4·</b> 43*	<b>4</b> ·19*	<b>4·4</b> 9*	<sup>∗</sup> 3·08*	2.79*	1.60*	0*
			*	Visible	moul	ding.					

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The results are set out in Table 6. Visible moulding had occurred even after 6 weeks at R.H. 75% and after 62 weeks at R.H. 65%. This latter finding is of interest in view of Snow's (1945) results, in which mould growth was reported to have occurred in feeding stuffs, including fish meal, at R.H. as low as 65%. This means that in order to prevent mould growth in dehydrated fish over long periods, R.H. lower than 65% must be employed, corresponding to a moisture content below 10.1% (see Fig. 3). This latter figure agrees well with that of 9.9% suggested by Snow, Crichton & Wright (1944) as the safe level for the storage of fish meal.

If a maximum moisture content of 10% were fixed for dehydrated fish, then not only would mould growth be prevented but food-poisoning pathogens could not grow (von Loesecke, Gunderson, Hucker, Jones, Kruse, Remington & Sherwood, 1946; Segalove & Dack, 1951; Burcik, 1950).

Table 6 also shows that the bacterial counts at both 20 and  $37^{\circ}$  C. fell with time, the decreases being greater the higher the R.H. In the counts at  $37^{\circ}$  C. and R.H. 0.0-25%, there was a suggestion of higher survivals at 10 and 15\% than at 0.0%. At 20° C., on the other hand, higher survivals occurred at 0.0, 10 and 15\% than at 25%. It is concluded, therefore, that in the mixed population present in dehydrated fish, whilst higher survival counts at R.H. of 10 and 15\% than at 0.0% might occur with those strains having a growth optimum at  $37^{\circ}$  C., as found by Watts (1945) with *Streptococcus agalactiae*, such relationships do not hold for those strains growing best at  $20^{\circ}$  C.

#### CONCLUSIONS

1. The viable bacterial counts at 20 and  $37^{\circ}$  C. in dehydrated fish stored at temperatures of 20 and  $37^{\circ}$  C., showed a marked fall after 65 weeks, the fall being greatest at  $37^{\circ}$  C. At 0 and  $-10^{\circ}$  C., on the other hand, the counts were approximately the same at the end of storage as at the beginning.

2. Before storage 53 % of the flora growing at 20° C. consisted of Gram-positive cocci, and 43 % of Gram-negative cocci, the corresponding figures for the flora at 37° C. being 95 and 2% respectively. After storage for 65 weeks 54% of the flora growing at 20° C. now consisted of asporogenous rods, 43% of Gram-positive cocci and no Gram-negative cocci, the corresponding figures for flora at 37° C. being 7.5, 31.5 and 59% respectively.

3. The lowest R.H. at which visible moulding occurred was 65 % after 62 weeks' storage, corresponding to a moisture content of 10.1 %.

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