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CHEMICAL AND MICROBIOLOGICAL INVESTIGATIONS

ON THE

CURING OF HERRING

BY

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I. PRELIMINARY COMMUNICATION

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Table of Contents.

		Page
	Introduction.	5
1.	The Norwegian manner of Curing Herring	9
2.	The Composition of Cured Herring	11
3.	The General Properties of the Pickle	12
4.	The Nitrogenous Compounds of the Pickle	15
5.	The Pickle-Sediment	18
6.	Microbiological Investigations	19
7.	Concerning the Methods Employed	21
8.	Bibliography	25

Introduction.

The curing of meat is a process that has long been known, being employed especially for preserving.

But in addition to this kind of preserving, there are a number of processes which have an influence upon the nutritive value, taste, smell, etc.

Scientific investigations of these processes have only been made in recent times, and, strange to say, to a small extent.

Max Rubner's work in 1877 (1), *Ervin Voit's* in 1879 (2), *Polenske's* in 1891 (3), and *Nothwang's* in 1893 (4), are the best known.

These works treat exclusively of the flesh of mammals.

As in the case of meat, so also with regard to fish-curing, several analyses have been made of salted fish-products, as they are found in the general market.

In 1877, *Aug. Almén* (5) published a work on the composition of certain kinds of fish in fresh, salted and dried condition. The salted fish during the process of salting had given off water, and absorbed a comparatively smaller quantity of salt, so that it contained a greater percentage of nutritive substances. Part of the soluble proteid was lost with the water in the pickle.

Investigations based upon the salting process itself, and regarding the nature and course of this process, do not exist. There is little doubt that we here have chiefly to do with osmotic diffusion such as the above-named authors have found in the case of the flesh of mammals.

But neither is there any doubt that in several ways fish-curing presents essential differences, a fact which is proved by the knowledge we have long possessed as to certain peculiarities in the herring-pickle.

As early as 1851, *Wertheim* (6) found a base in this, which he believed to be the propylamine discovered by him.

Hofman and *Winkles* (7), in their examinations in 1855, did not find propylamine, but on the other hand, they found its isomer, trimethylamine. In 1866, *Tollens* (8) demonstrated the presence of methylamine.

The foremost among subsequent investigators who have examined the basic substances of herring-pickle, are *Brieger* and his assistant, *Bocklisch*.

These authors (9) succeeded in demonstrating the presence of choline, trimethylamine, dimethylamine and methylamime, on the whole non-poisonous ptomaines, which were taken to be substances produced by bacteria. The first to follow up the idea that the pickle contained micro-organisms, was *Wehmer*, who stated in 1897 (10), that in samples of a Dutch herring-pickle, he had found an abundant vegetation of micro-organisms, especially of fermentative fungi.

That micro-organisms play an important part in the preparation of certain kinds of mild-cured fish, is well known from the Swedish «surfisk», and probably also the Norwegian «rakørret», products which no doubt undergo partial putrefaction.

«Surfisk», which is prepared from the Strømling (Clupea harengus v. membras), has been examined by *Carl Th. Mørner* (11). In the pickle and the abundance of gas formed by fermentation, he demonstrated typical putrefactive products.

Whether similar metabolisms play any part in any of the stages of herring-curing has not yet been inquired into. Nor have we, as already mentioned, any clear knowledge either of what takes place, or on what it depends. An investigation is therefore desirable, the more so as herring-curing plays an important part in the fish-economy of our country.

At the instigation of Dr. *Johan Hjort,* and supported by the fisheries investigations directed by him, and also to some extent by «*Trondhjems Fiskeriselskab*», the present writer has made some microbiological and physiological-chemical investigations of herring-curing.

The investigations have been especially directed towards the pickle, to ascertain what and how much is lost, and also the «metabolisms» that are conditions of the curing-process.

In connection with this, a few salting-experiments have been made and also analyses of the cured herring itself.

The results thus found naturally only give a provisional and purely orientating account of the peculiar nature of herring-curing in general, without touching upon the questions of gutting, packing, kind of salt, quantity of salt, etc., questions which in practice are very actual, and certainly both interesting and well worth any attention paid to them.

The work accomplished up to the present is exceedingly incomplete, and only the beginning of more rational and systematic investigations, which I hope soon to be able to continue.

As the work is ended for the time being, owing, among other things, to this year's bad supply of suitable material, I have thought it best to give this preliminary account of my work.

Among the gentlemen to whom it is my pleasant duty to offer my sincere thanks for their kind interest and valuable aid in my work, for unlimited use of laboratories, etc., I would especially mention *Ivar Bang*, M. B., Christiania, *Barclay*, interim Inspector of Sea-Fisheries, Hougesund, Dr. *Johan Hjort* and Professor *Axel Holst*, Christiania, Dr. *Erik Solberg*, agricultural chemist, Trondhjem, Professor *S. Torup*, Christiania, *Wleugel* head school-master, Trondhjem, under whose guidance I carried out some investigations on fish-salting in the laboratory of «Trondhjems Tekniske Læreanstalt», during the college-year 1896—97. The results obtained from these investigations, and which have been a valuable guide in the present work, will be communicated in a subsequent paper.

I would furthermore express my gratitude to the herring-merchants, Mr. *Theodor Moe*, Mr. *S. Tvethe* and Mr. *Edv. Wahl* of Trondhjem, and Messrs. *Thos. Schram and Son,* Christiania, for the liberal and obliging manner in which they have at all times placed their warehouses at my disposal.

Christiania. March, 1900.

Laboratory of the Fisheries Investigations, University.

Norwegian Manner of Curing Herring.

Immediately after being caught, the herring is more or less gutted, i. e. more or less of the gills, gullet and stomach removed, whereupon it is immediately packed in layers with salt. As soon as the barrel is full, a brine made of sea-water and the fish-salt employed, is poured over it.

When the barrel is headed, the sea-stick herring-barrel is ready.

After the expiration of from 8 to 14 days*, the herring is generally fully salted, i. e. cured, and is ready for consumption.

The practical indications that the herring is fully cured are that the skin comes off easily, that the flesh is easily separated from the bone, and that it has lost its so-called «raw taste».

It must, however, be pointed out that the whole thing is more a matter of judgement.

Before being sent out as merchandise, the herring, after a shorter or longer time, is repacked.

This is done partly in order to sort the article, but chiefly for the purpose of packing more herring into the barrel, as the herring, which during the salting, has diminished in volume, no longer fills the barrel. During this process, only a little fresh salt is added for the lowest and uppermost layers. Whatever salt may be left unmelted from the original packing, is strained from the pickle before the latter is again poured into the barrel.

Formerly herring was salted quite dry, and the pickle was formed by the water the herring itself yielded. The barrel was not then headed

*) This applies to fat herring. In the cold seasons of the year, it often takes several months.

at once, but was set aside unheaded for a day or so, until the pickle was formed, and the herring had sunk.

In those days also, the barrel was packed with one or two layers above its edge, so that after the formation of the pickle, and the sinking of the fish, it could still be full.

This manner is now less frequently employed, chiefly, it is said, because with this salting, it takes more herring to fill a sea-stick barrel (i. e. the marketable product at first hand).

It is calculated that 1 barrel of salt goes to the salting of 4 barrels of herring.

The herring-salt most frequently employed in this country is Trapani-salt. The fatter the herring and the warmer the weather, the greater is the quantity of salt used.

As regards the keeping-quality of the cured herring, it is naturally dependent on a number of factors, such as the kind and quality of the raw product, the treatment it first received after being caught, the gutting, salting, packing, storing, etc.

It is calculated that ordinarily well prepared goods are still fit for use after the expiration of a couple of years, after which time it generally becomes tough and red, and on the whole less tempting. Instances have been known, however, of its having remained good, when kept in hermetically closed vessels, for more than 10 years; but these are exceptional cases.

As a rule, the herring is consumed in the course of a year, that is to say from season to season. And practically, a two-year-old article is deteriorating.

With regard to the course of the process itself, it must at any rate quantitatively be dependent on the raw material.

There is, for instance, a great difference between the fat summer herring with its abundance of subperitoneal fat, the thin spring herring with its roe and milt, and the still thinner, spent herring.

The investigations here reported treat of Norwegian fat herring, exclusively in the condition of sea-sticks (as originally packed).

2. The Composition of Cured Herring.

From the analyses found in literature of fresh and salted herring, it will be seen that the salted herring is characterised by the smaller amount of water it contains, the greater amount of nitrogenous substances, and the no inconsiderable quantity of salt.

Nothing, however, is mentioned as to the time the herring has been in the salt.

The present writer has therefore examined a number of different samples of cured herring of different ages.

The results are given in the table below.

No. of Sample	Time in Salt	1000 gr. of Herring Flesh contain			
		Water	Nitrogen	Chlorine (Na Cl)	
F. S. 1	0	638	30.9	1.3	(2.2)
S. 22	3—4 days	506	34.9	34	(56.2)
S. 21	5 „	462	37.4	53	(86)
S. 33	5 „	483	35.9	58	(95)
S. 31	3 weeks	458	37.6	104	(172)
S. 23	1 year	460	33.9	93	(153)
S. 24	2½—3 yrs.*)	523	27.6	108	(179)
S. 34	5 „ *)	555	23.6	98	(161)

König (12) gives as the mean value of 3 analyses by *Payen, König and Forwick*, and *Almén:*

462.3 ‰ water, 30.2 ‰ N., and 88 ‰ Cl. (144 Na Cl).

In the above-mentioned samples, the herring's average weight and percentage of flesh were as given in the table on p. 12.

As the raw material employed must be assumed to have a somewhat different composition, the figures, not including the percentage of chlorine, cannot be placed side by side in order to give the varying composition of the herring during the time of salting.

*) Partly decomposed.

No. of Sample	Average Weight of Herring	No. of gr. Flesh Yielded by 1000 gr. Herring.
F. S. 1	100 gr.	638
S. 22	85 „	531
S. 21	—	505
S. 33	118 „	517
S. 31	69 „	485
S. 23	160 „	541
S. 24	190 „	507
S. 34	82 „	451

That the changes take place quickly will be evident from the rapidly increasing amount of chlorine, and this is still more apparent from the salting experiments mentioned on page 15.

The present writer by experiment has found that the herring requires a minimum of salt in order to be cured. The limit has not yet, however, been fixed. One sample of cured herring, in which the fish was remarkably soft, contained 93 ‰ Cl. (153 ‰ Na Cl.), while another that was quite firm (prepared by pickling in the laboratory) contained 88 ‰ Cl. (144 ‰ Na Cl.).

When about two years old, the flesh begins, as already mentioned, to assume a red colour. At the same time the pickle also acquires a darker colour.

As the pickles contain neither nitric and nitrous acids, nor blood derivatives, while at the same time the formation of peptone is apparent, and the pickle causes colour-reaction of tryptophan, the colour probably arises from a microbiological decomposition of the albuminous molecule.

3. The General Properties of the Pickle.

The colourless brine that was poured into the barrels at the time of salting, has changed its appearance considerably during the curing process.

The pickle, as tapped from the barrels, is a salt, very muddy, more or less dark-coloured liquid of neutral reaction and with a peculiar odour.

The muddiness of the pickle arises from a fine sediment which is gradually deposited. There are also some coarser, detached portions of epithelium and flesh.

In the appearance of the filtered pickles, it is at once noticeable that those that are only a few days old do not become perfectly clear with filtering, but have a grey, milky appearance.

Those that are a little older, and which filter far more easily and become quite clear, are remarkable for a colour that deepens with age — from pale yellow in newer pickles, to a deep port-wine colour in old ones.

In order to see whether this colour could originate from the blood, its spectroscopic conditions were examined into.

A deep-coloured brine — 5 years old — showed, in a layer $5^1/_2$ cm. in thickness, an absorption beginning at about E, and, continuing with rising intensity, extending over the most refrangible part of the spectrum.

Beyond this, no distinct absorption-bands could be demonstrated. There was scarcely any abbreviation of the red end of the spectrum. None of the closest derivatives of blood-colouring matter were present.

On examining newer and comparatively less deeply coloured pickles, of ages down to 2 months, the same spectrum in the main was found, but the absorption is correspondingly less. No further examination into the nature of this colouring matter was made. Presumably it is produced from proteid by bacteria.*)

The specific gravity of the pickle, with very small deviations (± 0.01), has proved to be fairly constant during the entire curing-period to 1.21. This specific gravity, which is equivalent to a saturated fish-salt solution, the pickle has already attained after the expiration of 24 hours.

Of the inorganic components the most important are sodium chloride and the smaller, varying amounts of magnesium, calcium, sulphuric acid and potash found in the fish-salt employed.

The total quantity of these salts shows practically a saturated solution, and is equivalent to between 150 and 160 $^0/_{00}$ of chlorine (250—270 Na Cl.)

Whether the various components of the fish-salt manifest different capabilities of diffusion in the osmotic interaction with the meat, has not been examined into.

In one sample that was a fortnight old, the amount of potash found was 4 $^0/_{00}$ K_2O, which practically corresponds to the amount of that substance contained in a concentrated solution of solid sea-water.

*) Older liquefied cultures of pickle-bacteria are remarkable for their dark brown colour.

Of the inorganic substances that have passed out of the herring, a comparatively large quantity of phosphoric acid is noticeable, presumably in organic combination.

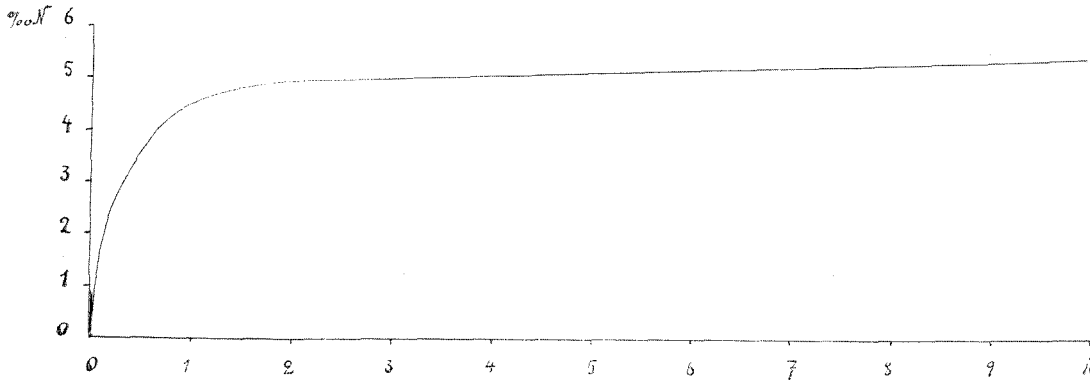
There was found:

In a pickle	14	days old . .	1.6 ‰	P_2O_5
—»—	1	month old .	1.6 ‰	—
—»—	$2_1/_2$	years old .	1.9 ‰	—
—»—	5	years old .	2.1 ‰	—

Neither new, old, fresh, nor tainted pickles have proved to contain nitric or nitrous acid.

The practical standard of measurement to be employed for the series of organic substances that pass into, and are dissolved in the pickle, is the total amount of nitrogen*.

This amount at various times of the curing-period will appear from the graphical table below, which gives the course of the extraction according to average values found by the analysis of 25 different sea-stick fat-herring barrels.



Amount of Nitrogen in Proportion to the Curing-Period.

It will be seen that the quantity of organic matter drawn out of the herring is not insignificant.

The principal exchanges take place at the beginning of the curing-period.

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*) This is nowhere re-calculated for «raw proteid», as the usual factor for this (6.25), as my investigations of nitrogenous compounds show, cannot possibly be employed.

In the course of the first 24 hours, the pickle acquires a nitrogenous value of more than 1 ‰. The increase of nitrogen per day becomes successively less, until, after the expiration of about 2 months, it has practically ceased. For a long time, however, it is still apparent to a remarkable extent, a $2^1/_2$-year-old sample, for instance, containing 9 ‰ N, and another one, 5 years old, as much as 12 ‰ N.

The amount of nitrogen is dependent upon several factors, among which, as is only natural, the temperature plays an important part. The lower the temperature, the less the amount extracted per unit of time.

The rapidity with which the osmose takes place may be further seen from a

Salting Experiment with saturated Salt Solution *)

Qualities of the pickle:

	Spec. Grav.	‰ Cl.	‰ N.
Newly made .	1.200	158	
After 4 hours	1.158	circ. 127	
« 24 «	1.129	100.5	1.37
« 2 days .	1.122	96.7	1.74
« 3 « .	1.120	93.5	2.17
« 4 « .	1.120	93.5	2.38
« 6 « .	1.120	91.4	2.77
« 8 « .	1.121	91.0	3.01
« 13 « .	1.121	90.9	3.58

This and similar experiments confirm the results found in the sea-stick barrels with regard to the great rapidity with which the osmotic interchange takes place.

4. The Nitrogenous Compounds of the Pickle.

In order to discover whence the nitrogen in the pickle was extracted (the flesh or the blood), and whether it might possibly undergo a derivation

*) In the sea-stick barrels, the diffusion of the salt is only apparent from the saltness of the fish. The pickle contains almost a constant amount of salt all the time, as the salt sprinkled on immediately replaces that which has been consumed.

in the pickle, determinations were made in typical samples, of the genuine proteid, of xanthine bases, and as far as possible of amid and amidic compounds. The methods employed, and certain experiences concerning them, are described on pages 21—24.

The results will be found in the table below:

Age of the Pickle	1000 grams of the pickle contain							
	Total dissolved N.	N. as coagulable proteid	N. as proteid precipitated by 2% acet. acid salt-solution	N. as proteid by cuprie hydroxide	N. given off by nitrous acid	N. given off by alkaline hypobromite	N. as xanthine bases	Undissolved N. (sediment)
14 days	3.0[1]							[2]
About 1 month . .	3.4[1]	0.6	0.7	0.9				[2]
About 1 month . .	3.7[1]		0.9	1.2	0.7	0.7	0.6	[2]
1 year	5.3			1.8	1.2			0.3
$2^1/_2$ years	8.8				3.7			1.2
5 years	12.0	0.5	0.9	1.6	5.7	1.6	1.3	0.9

It will immediately cause surprise to see that only a comparatively trifling proportion of the total amount of nitrogen is found as genuine proteid, while the greater part is found in the form of those peculiar nitrogenous compounds that in food-analyses are included under the general name of amido-nitrogen.

Proteid. In the newer pickles, genuine proteid is exclusively found, while its closely hydrolytic derivatives, albumoses and peptones, are not present.

These make their appearance, however, in the older pickles from about a year old onwards, simultaneously with a continuously increasing colour-reaction of tryptophane. In a 5-year-old pickle which is in a complete state of decomposition, genuine proteid, as will be seen, is still found.

With regard to its nature, only a very small proportion of it is composed of globulines.

It does not contain nucleo-proteids, nor is histon to be found.

It consists of at least two different bodies, which in aqueous solutions had the following general properties:

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[1] Samples from winter time.

[2] For young pickles usually 0.2 or less.

Is not precipitated by sodium chloride or magnesium sulphate in substance, or by half saturation with ammonium sulphate. Is not precipitated by saturation with ammonium sulphate.

In saltless liquids, the greater part is precipitated by 2 $^0/_{00}$ acetic acid.

In salt-saturated (Na. Cl.) solutions, the precipitation is quantitative by an amount of 20 $^0/_{00}$ acetic acid. If, on the other hand, the sediment be treated with a 20 $^0/_{00}$ acetic saltless solution, it is dissolved.

The temperature of heat-coagulation for one of these bodies in a neutral solution containing from 5 to 10 $^0/_0$ sodium chloride is from 53^0—65^0. For the other one, which represents the larger part of the proteid, the temperature of heat-coagulation, under the same conditions, is 75^0.

The coagulated sediment is partly dissolved again on being washed with boiling water, and will then scarcely again be precipitated if the heat-coagulation experiment is repeated.

I intend subsequently to return to the more detailed classification of these bodies.

Amido-Nitrogen. Under this comprehensive name are included, in food-analyses, all the heterogeneous nitrogenous compounds that are not proteid.

In addition to the true nitrogenous extractives, or as they are called, xanthin or alloxuric bases, a multiplicity of various amines, amides, amido-acids, and amides of amido-acids are included.

No direct determination of the total amount of these substances, or of each of them separately is made in the general food-analysis, first because their amount is comparatively small, and secondly, because they are all considered to be equally valueless as regards nutrition.

In a herring-pickle where «amido-nitrogen» constitutes more than three fourths of the total amount of nitrogen, they are of far greater interest.

It would therefore be most desirable to resolve the amido-nitrogen into its separate factors. As, however, we here come upon uncertain and almost unexplored ground, this has proved for the present to be impracticable.

It has, it is true, been thought that in the treatment with nitrous acid and alkaline hypobromite, we had general reactions for amido-acids and amides. The statements of the various writers, however, are

decidedly opposed to one another as regards the nitrogen groups that are decomposed.

The figures given in the table for the amount of nitrogen yielded by nitrous acid and hypobromite, have therefore little value except in so far as they show, when compared with the very much more certain figures for the amount of xanthine bases, that the greater part of the so-called amido-nitrogen is derived from true amides and amido compounds.*

I have not yet concluded my qualitative investigations of these, and shall therefore reserve further details for the present.

I must, however, even now point out that it appears as if the amido-nitrogen were chiefly extracted directly from the herring, and were not due to changes in the pickle. The flesh of the herring has shown itself to be comparatively rich in compounds of this kind, when in a fresh state.

A purely approximate minimum value was found by finely crushing 500 gr. fresh herring and boiling it with 500 c.cm. of alcohol. In the filtrate, which amounted to 500 c.cm., 3.2 ‰ N. was found, of which 0.8 ‰ was immediately given off with nitrous acid. The flesh of the herring should thus contain at least 0.8 ‰ N. as amide and amido-nitrogen — an amount which is sufficient to explain the relatively large quantity, in the pickle, of these substances.

5. The Pickle-Sediment.

The pickle-sediment contains those constituents, insoluble in strong salt solution, precipitated from the herring during the curing process.

It consists mainly of a very fine precipitate, and a smaller proportion of small, glittering crystal needles.

The amorphous sediment, which is only slightly soluble in water, has proved to contain a little globuline. It is almost completely digested by pepsine in muriatic acid liquid. Beyond this, its nature has not been examined into.

The crystal needles were optically inactive, and easily dissolve in ether.

In new pickles, the sediment increases the amount of nitrogen in the pickle with 0.2 to 0.3 ‰ N., in older pickles with about 1 ‰.

*) In this connection it must be mentioned that but little of the nitrogen of the pickle is given off by distillation with magnesia (a sample containing 3.7 ‰ N gave off 0.1 ‰).

6. Microbiological Investigations.

By direct microscopical examination of some pickle-samples, the only micro-organisms found were bacteria.

The number of the bacteria was ascertained in several sea-stick barrels, in order to find out the proportion of living germs at the various stages, from the commencement of the salting* until after the expiration of some years, when the article must be considered to be unfit for food.

It appeared that the number of bacteria was greatest shortly after the salting, and that they diminished as the pickle became older.

Thus while during the first few days after the salting, there are from some 100,000 to upwards of 1,000,000 living germs per cubic centimetre, in a pickle that is two or more months old, there is found a smaller, and generally decreasing number of bacteria, usually from about some thousands down to a minimum of a few hundred germs per cubic centimetre.

It must be remarked that even very old pickles, for instance, from a 5-year-old sea-stick barrel, still contained living bacteria to the number of two or three hundred per cubic centimetre.

This phenomenon seems to present an analogy to what has elsewhere been observed, with regard to the propagation of bacteria in organic substances, e. g. in cultures, namely, that they multiply rapidly at first, while they increase less rapidly, and gradually die off, as the culture grows older.

This circumstance may seem to be opposed to another observation, namely, that while the number of bacteria that appear in the culture decreases, the number of bacteria that can be observed by direct microscopic examination increases.

Thus a direct microscopic examination of new pickles containing a, relatively large amount of germs, showed only a small number of bacteria while in old pickles, on the contrary, where the amount of germs found by plate-cultures appears to be small, direct microscopic examination reveals a perfect swarm of them. Although not yet venturing to express a more decided opinion, I may say that I believe this phenomenon may have its explanation in a continuous accumulation of dead bacteria. On the other hand, it is a well-known fact from experiments in bacteria-culture,

*) Practically speaking, the pickle is saturated with salt all the time.

that it is generally possible to make only a small number of the living bacteria actually present thrive.

With regard further to the factors that affect the number of germs in a given pickle-sample, it must be mentioned that a slight increase in the amount of water, e. g. caused by placing the sample in a damp room, will in a short time result in a rapid increase in the number of germs.

It may moreover be stated that new pickles that have stood in closed bottles without coming into contact with the fish, have proved, after the expiration of from six to twelve months, to be free from living germs. In other words, it appears as if the bacteria, in the unfavorable conditions of vegetation afforded by the pickle, only succeed in retaining life in the presence of the fish itself.

The *bacteria-forms* found indicate a multitude of species, among which there does not seem to be any single prevailing typical form.

Small cocci and very short bacilli were the most conspicuous. Several were pigment bacteria, especially in yellow colours.

Most of the gelatine cultures were liquefied after a longer or shorter period.

The pickle bacteria are facultative putrefactive bacteria, whose mode of action is changed, owing to the large amount of salt. By sterile dilution with less than half its volume of water, the pickle will in a short time be brought into a condition of acrid and offensive-smelling putrefaction. Experiments in animals with this gave contradictory results, and were therefore not brought to a conclusion.

Whether the bacteria, in some way or other, have any decided significance for the curing itself, is not altogether easy to determine.

In some salting-experiments in which small quantities of antiseptics (e. g. salicylic sodium and fluoride of sodium) which hinder a development of bacteria, were added to the salt and pickle, it appeared that the curing of the herring could be accomplished without them. But experiments have hitherto been too few and incomplete to allow me to say with certainty that the curing of the herring is independent of bacteria. Even if it may be, it need not be in practice, nor is it always. Under all circumstances, the bacteria must be taken into consideration.

A clear distinction must moreover be made between the changes that take place during the first few days or weeks, and are a condition of the

curing of the herring, and the slow but sure microbiological changes which in the course of years destroy the herring.

Culture experiments showed, among *other micro-organisms*, the presence of a small number of mould fungi (the common species of Penicillium and Mucor) in nearly all the samples.

Yeast fungi, which *Wehmer* (10) has found to be typical of a Dutch pickle examined by him, could not be demonstrated either directly or by means of cultures in any of 30 different pickle-samples.

7. Concerning the Methods Employed.

The Preparation of the Samples. The pickle-samples are taken directly from the sea-stick barrel, after the barrel has been rolled round two or three times.

The samples for bacteriological examination were tapped into sterilised flasks.

The coarser, detached particles of epithelium and flesh were strained off, whereupon the principal part was filtered through filter-paper.

When not otherwise stated, the analyses refer to the paper-filtered clear pickle, and specify what was always actually dissolved.

It is all expressed as *weight per mille.*

The analyses of the herring refer to the flesh itself, as it is after all the refuse (i. e. head, skin, bones and intestines) is removed. The preparatory drying was executed in a vacuum apparatus, after which the sample, before weighing, was placed for a few days in the air.

Determinations of nitrogen were made according to Kjeldahl's method. Strong sulphuric acid and a drop of quicksilver were employed in the dissolving process.

In distilling the ammonia over by means of sulphidous caustic soda, zinc dust was employed to prevent the otherwise violent shocks. The strength of the sulphuric acid for titration was fixed by weight analyses. Urea was employed for test analyses. *As a measure for the amount of salt,* the amount of chlorine was used. The corresponding amount of sodium chloride is given in parentheses. Owing to the large quantity of organic substances, this cannot be determined directly by titration.

After several experiments, it was found best first to dry the substance carefully, and then carbonise it at a low temperature, after which the residue was pounded up with water.

The titration was then performed in an aliquot part with an $\frac{n}{2}$ solution of nitrate of silver in the usual manner with potassium chromate as indicator. With regard to the pickle, repeated series of experiments showed that the amount of chlorine was followed regularly by the specific gravity at 15 ° C, whereupon this more simple determination was most frequently employed as a standard of measurement for the amount of salt contained in the pickle.

Phosphoric acid was determined in the usual manner, according to the molybdenum method; the destruction of the organic substances was performed with nitric acid.

The genuine proteid was determined (1) be heat-coagulation, (2) by precipitation with cupric hydroxide according to Ritthausen's method, and (3) by precipitation with 20 ‰ acetic acid.

(1). *By heat-coagulation.* It proved at first to be an impossibility to obtain easily filterable solutions. The filtration was generally rapid, and the coagulation was complete, and the filtrate perfectly clear by the following process: 50 cubic centimetres of pickle received an addition of double the volume of strong solution of sodium chloride, and was repeatedly boiled rapidly, acetic acid being added for very slight acid reaction. After the last boiling, the liquid was diluted immediately with an equal volume of boiling water. The coagulum then deposits rapidly and easily. Washing with boiling water by decantation was repeated until a chlorine-free filtrate was obtained. The nitrogen of the sediment is determined according to Kjeldahl.

(2). *By Ritthausen's method.* 50 cubic centimetres of pickle were diluted with 150 cubic centimetres of water, and 30 cubic centimetres of a 10 % solution of sulphate of copper, and an equal quantity of caustic soda added.

In order to be sure of having neutral reaction, a few superfluous cubic cm. of solution of copper were added, after which the liquid was heated to about 70 °, and was filtered warm. The precipitate was washed with boiling water to a chlorine-less filtrate, whereupon the amount of nitrogen was determined according to Kjeldahl. The filtrate was employed in the determination of xanthic bases.

(3). *By 2 % acetic acid.* To 50 cubic centimetres of pickle were added 100 cub. cm. of strong salt solution, and then 150 cub. cm. of a

similarly salt-saturated 4 % acetic acid. The nitrogen was determined according to Kjeldahl.

Of these three methods of determination, the acid precipitation is probably the one that in this special case gives the most correct expression for the quantity of genuine proteid present.

In the coagulation experiment, some of the precipitated proteid will be again dissolved in the washing-water (see page 17); in the precipitation with cupric hydroxide the results found will be too high, as some not easily soluble double salts of the amido compounds will doubtless be left behind with the precipitating agent.

The amount of *amido-nitrogen* is apparent as the difference between the total amount of nitrogen and the amount of nitrogen found in the form of genuine proteid.

It comprises the basic nitrogen, the true amide and amido-nitrogen, and ammonia.

Basic nitrogen, i. e. the nitrogen contained in the xanthine bodies, or, as they are now called, alloxur or purin bases, was determined by precipitating the not easily soluble double salts of these substances with cuprous oxide in Ritthausen filtrate, by the aid of Fehling's liquor and a reduction agent. In the sediment, the nitrogen was determined after Kjeldahl's method in the usual way (during the dissolving, the boiling is continued for 6 hours after the liquid has become clear).

Dextrose is the means employed for reduction. The reduction, mainly on account of the large amount of salt, is less easily accomplished.

The reduction given by *Krüger* (13) with sulphate of copper and bisulphite gave no result.

The amount of nitrogen that will be given off *with nitrous acid and hypobromite* was determined by the methods given by *König* (14).

It was proved that these reactions are not quantitative. The greater part indeed, was poured out immediately, but no inconsiderable quantities were further poured out little by little. Even after 24 hours, the reaction had often not altogether ceased.

On the basis of these methods, it is not possible to divide this part of nitrogen between amides and amido acids, for in the first place, some of the bases will also be able to give off nitrogen with nitrous acid — e. g., according to *Hammarsten* (15), guanine and adenine — in the second place, it is impossible from the data in the present literature to lay down

any general rule for the nitrogen groups that can be given off under the given conditions. With regard to the execution of the analyses it must also be remarked that the treatment with nitrous acid was carried out after the proteid was removed by Ritthausen's method; while the treatment with hypobromite might just as well be carried out directly in the pickle.

Determination of *the amount of germs* was made by the aid of plate-culture in Nielsen's rectangular flasks. As nutritive substrate, sea-water fish-gelatine was first employed, but as the same results were obtained by using «Salomonsen's 10 % meat-water pepton-gelatine», this was subsequently exclusively used.

On account of the large number of germs in the new pickles, sterile diluted pickles in the proportion of $^1/_{100}$ or $^1/_{1000}$ were used, of which 1 c.cm. was always used for the culture. The period of incubation was from 4 to 8 days.

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