

SVENSKA INSTITUTET FÖR KONSERVERINGSFORSKNING, GÖTEBORG

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1964

SIK - Rapport

Nr 147

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A preliminary report

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In Scandinavia whole sprats (Clupea sprattus) are used for the preparation of anchovies. The packing season lasts from October to the end of December or the beginning of January. During this time the fat content of the sprats is highest, namely 15 to 20 per cent and sometimes even higher. The sprats are packed either directly into small 450 g cans or into 115 kg barrels. The first type is allowed to ripen in the cans in which they are later sold. After ripening the barrel anchovies are cut into fillets and repacked into smaller boxes as skinless and boneless anchovies. A new sauce or brine is added before sealing the boxes.

In both cases the sprats are packed with a mixture of dry salt, sugar, and spices. Some preservative is also added. On an average 12 kg of salt, 6 kg of sugar, and 1 kg of a mixture of about 15 different spices are used per 100 kg of sprats. The sprats are thoroughly mixed with this salt-sugar-spice mixture, care being taken to powder the surface of every fish. The cans or barrels are allowed to stand at room temperature for 2 or 3 days, during which the osmotic action of salt and sugar extracts fluid from the fish with the formation of a brine. Any cans or barrels not full to the brim are topped up with some extra brine and then sealed. All packages must be void of air, otherwise the contents will become rancid.

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The ripening is allowed to proceed at a temperature of 10-15°C. During the first few days the sprats are hard and dry owing to loss of water. At the same time the proteins of the fish muscle undergo denaturation, i.e. the solubility of the proteins decreases considerably from about 95 per cent to about 25 per cent at ionic strength 1. After a couple of weeks the solubility of nitrogenic compounds gradually increases again and when the preserves are ripe, the nitrogen compounds have nearly the same solubility as that of the fresh fish. The fish flesh is now smooth and juicy and has got its particular flavour. The degree of ripeness is judged organoleptically by the expert. So far no other methods are available.

#### Changes of the proteins during the ripening process

The increase in solubility of the nitrogen compounds during the ripening process is due to degradation of the proteins. The salt-denatured proteins are gradually decomposed into smaller peptides and free amino acids. The increase in solubility is due mainly to changes of the actomyosin of the muscle.

Many attempts have been made by us to study the products of decomposition in greater detail. It is, however, difficult to get any information about the molecular size of the different peptides. In the still unripe anchovies the polypeptides are more or less insoluble and even when the products are fully ripe there are some insoluble nitrogen compounds. Electrophoresis in free solution is therefore not possible. (Paper electrophoresis has proved non-informative because even soluble peptides adhere strongly to the paper.) Filtration on dextran gels (Sephadex), however, has given promising results. We found that most of the nitrogenic compounds of the anchovies go into solution in a phosphate-borate buffer containing 8 mols of urea per litre. This solution can be run on a Sephadex gel provided that the same urea buffer is used for elution. These experiments have shown that the protein peak of the fresh fish gradually becomes rather small during the ripening period. As ripening proceeds a second peak appears indicating the formation of rather large peptides, but smaller than the original proteins.

This second peak disappears rather soon as ripening proceeds. A third peak soon appears, which gradually increases as ripening goes on indicating the formation of small peptides and free amino acids. Using paper chromatography we were able to establish the whole spectrum of amino acids in fully ripened anchovy.

During the ripening process also a fourth peak appears. The substance responsible for this one does not contain nitrogen but shows maximum absorption at 223 and 260  $\mu$ . It is not present in fresh fish but appears later on and gradually increases during the ripening process. It has not yet been possible to identify this substance, but much suggests that it is some deaminated product of tyrosine.

#### The enzymic degradation of the proteins

The main source of the enzymes responsible for the degradation of the proteins seems to be the intestines of the herring. Degutted sprats packed in the same way as ordinary anchovies do not ripen. The product remains dry and stiff and the raw fishy taste does not disappear. On the other hand, if a homogenate of the intestines is added to the newly packed degutted sprats, the latter ripen in a rather normal way. We found that the proteolytic activity of the pyloric caeca of sprats is highest in the autumn, i.e. the time when anchovies are packed. Furthermore, there is a good correlation between the seasonal variation of the fat content and the enzymic activity. It is said that anchovies can not be made from sprats with a fat content below 10 per cent. It would seem more reasonable to assume that this can be explained by the low proteolytic activity of the intestines than by the low fat content of the sprats.

The proteolytic enzymes of the intestines diffuse into the flesh and into the brine of the anchovy with consequent decomposition of the proteins. Salt seems to favour this diffusion by increasing the solubility of the enzymes. On the other hand, salt gradually inactivates the enzymes. The result is that the proteolytic activity in the brine gradually reaches a maximum owing to increasing amounts of enzymes dissolving in the brine. This

maximum is followed by a decrease in the activity of the brine owing to inactivation of the enzymes. Proper ripeness of anchovies seems to depend on a satisfactory balance between the original activity of the enzymes and the rate of consumption of these enzymes during the ripening process. In other words, if the activity is expended before the anchovies are ripe, the latter will remain under-ripe, and if the activity continues after the anchovies are ripe, the product will be over-ripe.

In some respects the enzymes of the brine resemble those of the pyloric caeca from which they presumably originate. Thus, they are both irreversibly and very rapidly inactivated at a pH of 3 or less. This may provide a possibility to stop further enzymic activity in ripened products and thereby prevent over-ripeness, particularly of skinless and boneless anchovy and perhaps also in herring tidbits, both of which are repacked after ripening. The new sauce usually contains some vinegar, and treatment of the fillets directly in strong vinegar before repacking may help to prevent further breakdown of the peptides.

The brine and the intestinal enzymes also have the same pH activity optimum, pH 7 - 9. Both are inhibited, but not completely, by soybean trypsin inhibitor. One difference we have found between the brine enzyme and the intestinal enzyme is, that the former is always associated with some unknown red substance, which we have so far been unable to get rid of without loss of activity.

Experiments with benzoyl-tyrosine-ethyl ester and benzoyl-arginine-ethyl ester have shown that the brine enzyme reacts with these substrates in a similar way as chymotrypsin from bovine pancreas. Casein, on the other hand, is decomposed to a significantly higher degree by the brine enzyme than by chymotrypsin from pancreas. It is not known whether the brine contains a single enzyme or some complex made up of various enzymes.

Some attention was also given to the proteolytic activity of the feed of the sprats. Calanus finmarchicus, which is often found in the stomach of the sprats definitely has a strong proteolytic activity, the enzymes being rather salt-tolerant. These organisms may be responsible for the bursting of the belly of the sprats after death, but they seem to be of little importance in the ripening process. In an experiment with sprats full of Calanus the packed anchovies did not even become properly ripe.

#### The significance of bacteria

Bacteria seem to have little influence on the degradation of the proteins during the ripening process. The number of bacteria growing on salt-containing media under aerobic as well as anaerobic conditions very soon after packing of the sprats drops to low levels, at which it usually persists until the products are ripe. Sometimes, and especially if the temperature is high, the bacterial count increases to high levels towards the end of ripening with fermentation and gas formation as a result. It is noteworthy that gas formation does not start until many weeks after the number of bacteria has begun to increase. The bacteria form lactic acid and they grow slowly in small colonies on salt-containing agar media.

A number of bacteria were isolated and studied for proteolytic activity. None were found to possess such activity. Experiments were also carried out on formaldehyde sterilized fish fillets to which sterile filtered extract of intestinal enzymes was added, on anchovies packed with an addition of chlorotetracycline, and on  $\gamma$ -irradiated anchovies. Non-inoculated samples of these three different products and corresponding samples inoculated with bacteria from ordinary anchovies were compared. No difference in protein decomposition could be observed. Other comparisons were not possible because the taste and consistency had been altered by the sterilizing agents. It is conceivable that bacteria contribute to the flavour by forming aromatic compounds such as acetyl-methyl-carbinol and other substances, but this point has not been investigated by us.