

## Bacteriology of the Fish Sausage

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The production of fish sausage has been developed over the past 13 years and is now established as one of the main food industries in Japan. The yield of the product in 1964 reached 119,000 metric tons. This amount, slightly exceeding the yield of ordinary animal hams and sausages, is about one sixth of the total 'kamaboko' products which are traditional sausage like products made from fish in Japan.

Although historically the fish sausage originated as a variety of the kamaboko, it is now recognized as a peculiar product having a more animal sausage-like flavour and texture as well as an extremely high storage life. The product, when properly manufactured, can be preserved for more than one month at 30° C without any sign of spoilage.

The long storage life is attained by the following three factors:-

1. Use of artificial plastic casing which prevents the penetration of bacteria.
  2. Heat processing at the relatively high temperature of 85 - 90° C.
  3. Addition of food preservatives
1. Preservatives used in Fish Sausages.

A wide variety of food preservatives have been tested for their application to the fish sausage. According to the results of a series of experiments by W. Simidu and co-workers, <sup>1) 2)</sup>, a 1:4 mixture of nitrofurazone and nitrofuryl acrylamide

extended the storage life by more than 3 weeks at 37° C at a concentration of 20 ppm, while other preservatives such as sorbic acid (1,000 ppm), dehydroacetic acid (2,000 ppm), butyl ester of p-oxybenzoic acid (330 ppm) and aureomycin (10 ppm) failed to show satisfactory effects.

Of the two nitrofurans, nitrofurazone has shown the more stable effect. Nitrofuryl acrylamide, having the slightly stronger effect than nitrofurazone invitro, has a limited and often unstable effect when applied to the fish sausage.

Recently, other furan derivatives; AF-2-(2-furyl - (5-nitro)-2-furyl acrylamide) and Tylosin have been tested by several workers and the results, on the whole, seem to show that they could be possibly used as preservatives in the fish sausage. Both these compounds appear to be less stable than nitrofurazone during heating in meat and the subsequent storage period of the product. However, the main advantage of the two new preservatives over nitrofurazone is their low toxicity.

## 2. Spoilage Problems of Fish Sausage.

### 1) Sausage without Preservatives.

The experience gained in the earlier period of making the fish sausage without adding any preservatives other than 2 - 3 per cent of salt, showed that the sausage could often bear extremely long storage periods sometimes 1 - 2 years at room temperature (10 - 25° C), provided the materials such as tuna and sword fish were in good quality and the whole process was bacteriologically well controlled.

Under ordinary commercial conditions, however, the materials used are a variety of fish, often of lower quality, and starch, gelatin, lard, spices and other miscellaneous ingredients are also added. Under those circumstances, the product, if being kept at 25° C or higher, will spoil in a few days. Swelling accompanied by a pungent, sour odour is the most typical spoilage encountered.

Ino<sup>3)</sup> showed that several species of the genus Clostridium such as Cl. sporogenes, Cl. butyricum and Cl. welchii were responsible for the swelling, while smaller numbers of aerobic spore formers might develop in the sausage and provide more anaerobic conditions favourable for the growth of the clostridia.

2) Sausage Added Preservatives.

Akamatsu<sup>4)</sup> carried out a bacteriological survey of fish sausage of 49 different commercial brands, before and after storage at 30° C for about 2 weeks. Along with predominant Bacillus strains, such as B. firmus, B. lentus and B. coagulans, Gram-positive cocci were found to occur in about 20 per cent of the samples tested.

The viable bacterial counts were of the order of  $10^0 - 10^3$  in about 85 per cent of the sausages. After 2 weeks' storage, the numbers ranged from  $10^2$  to  $10^7$ . He reported that in most of the sausages, spoiled after the 2 weeks' incubation, large numbers of B. subtilis and similar species of bacilli were observed.

The B. subtilis is one of the most sensitive organisms to nitrofurazone which is almost exclusively used as a preservative of commercial fish sausage. The growth of this species in large numbers apparently shows some defect in the manufacturing processes. Due to rapid improvement in the processing technique in recent years, the spoilage caused by anaerobic spore formers, B. subtilis and other bacilli which are among the most commonly encountered in the materials and which have stronger spoilage activity, has been decreasing.

The main spoilage troubles in recent days are those caused by two different factors, i.e. the resistant strains and the

inactivation of the preservatives.

a) Spoilage by Resistant Strains.

The majority of the bacteria and their spores occurring in the materials and ingredients of the fish sausage are killed by heating and the combined effect of heating and preservatives as shown by W. Simidu and Ueno <sup>5)</sup>, and remaining organisms <sup>MS</sup> some spores of bacilli and clostridia, are inhibited their growth after germination.

Recently, increasing amounts of trouble caused by surface spoilage have been reported especially in the summer under inferior conditions of transportation in which the product is often exposed to a temperature as high as 40° C for a considerable period. There has been observed two types of surface spoilage, i.e. spots of several colours and shapes and gassiness between the casing and surface of the meat. The gassiness is sometimes accompanied by softening of the surface area.

U. Simidu and Aiso <sup>6)</sup> and Yokoseki and Okawa <sup>7)</sup> isolated two species of *Bacillus* as causative agents of the surface spoilage. One of them is *B. coagulans* which is associated with different types of dark red, brown, or white spots. The cores of the spots were found to consist of masses of the bacilli, often having an elongated shape, which, after subcultivation on the usual media at 30 - 37°C, gave the typical morphological characteristics of *B. coagulans*.

The other species which causes gassiness or, as a result of located growth, colourless or brown spots is a denitrifying *Bacillus* similar to *B. firmus* but differs in its anaerobic production of gas from nitrate, and in its inability to hydrolyze starch and non-inhibitory growth on glucose media. The strains are also different from denitrifying bacteria isolated by Verhoevan <sup>8)</sup>, or by Eddy and Ingram <sup>9)</sup> from tinned bacon. The organism, when its spores were inoculated into fish sausage material

with sodium nitrate, was shown to reproduce the gassiness and spots.

All strains of B. coagulans and the denitrifying bacilli isolated from spoiled sausage showed a high resistance against nitrofurans and some other preservatives such as dehydroacetic acid and sorbic acid. Tylosin inhibited the growth of both strains at a concentration of 0.5 ppm.

Other than the above two species, some strains of other Bacilli and a Sarcina have been reported as the causative bacteria of surface spoilage, although the actual significance of these strains seems to be restricted.

Some authors claimed that a variety of strains of genus Bacillus such as B. subtilis, B. circulans, B. coagulans, B. cereus var. thermophilus, B. megatherium var. thermophilus cause the surface spots. If strains of many species become resistant to heat and preservatives thus causing the spoilage of fish sausage, it will be a significant problem in sausage manufacture. However, most of their strains tested were found to belong to B. coagulans.

The origin of these bacteria is being investigated by several workers, but the results so far obtained are not decisive. It seems that, rather than one particular material, some materials of inferior quality and also general sanitary conditions give rise to the source of these resistant strains.

#### b) Inactivation of the Preservatives

When the nitrofurans preservatives are applied to sausage containing mammalian meat such as whale and mutton, the effect of the preservatives is often reduced remarkably. U. Simidu et al.<sup>10)</sup> showed that the phenomenon was caused by the reduction of nitrofurans during the heat process by reducing groups or products in the meat

and that the reduction is accelerated by the presence of ferrous ion in the myoglobin of the meat.

The rate of the reduction is dependent on the hydrogen ion concentration of the substrate, as well as on the types of nitrofurans. Thus, nitrofurazone is more stable than AF-2; and nitrofuryl acrylamide, being the most unstable compound, is reduced quickly in the absence of ferrous ion. The reduction of the nitrofurans can be eliminated considerably by reducing the pH of the meat by adding lactic acid, and, in cases of nitrofurazone and AF-2, by pretreatment of the meat by nitrite.

Yokoseki <sup>11)</sup> reported that inactivation was also observed with Tylosin when the antibiotic was added into fish sausage. He suggested that Tylosin loses part of its activity as a result of being absorbed by meat proteins.

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#### SUMMARY

Owing to the pasteurization at the relatively high temperature of 85 - 90° C and to the addition of preservatives, the fish sausage has an extremely high storage life.

Among many food preservatives so far tested for the fish sausage, nitrofurazone has been shown to have highest preserving effect. Tylosin and a new nitrofurane derivative (AF-2) now being tested also seem to be effective.

The main spoilage problems encountered are related to the resistant strains and inactivation of the preservatives. Strains of Bacillus coagulans and a denitrifying Bacillus which have high resistance for nitrofurans cause the formation of spots and gassiness in the surface area of the sausage especially during the summer season.

Inactivation of nitrofurans may occur when mammalian meats are used as materials. The phenomenon was shown to be related to the reduction of nitrofurans by reducing groups in the meat during the heat process. The factors affecting the reduction as well as methods which prevent the reduction have been investigated.

#### ZUSAMMENFASSUNG

Pasteurisierung bei der verhältnismässig hohen Temperatur von 85 - 90° C und die Zugabe von Konservierungsmitteln verleihen der Fischwurst eine sehr hohe Lagerzeit.

Nitrofurazon weist die grösste Konservierungswirkung von den vielen geprüften Konservierungsmitteln auf. Tylosin und ein neues Nitrofuranderivat (AF-2), die jetzt geprüft werden, scheinen auch

wirksam zu sein.

Die hauptsächlichsten Probleme des Verderbs sind mit den widerstandsfähigen Stämmen und der Inaktivierung der Konservierungsmitteln verknüpft. Stämme von Bacillus coagulans und einem denitrifizierenden Bazillus, die eine hohe Widerstandsfähigkeit gegen Nitrofurane aufweisen, veranlassen Fleckenbildung und Gasentwicklung an der Oberfläche der Wurst, besonders während des Sommers.

Inaktivierung des Nitrofurans kann auftreten, wenn Fleisch von Säugetieren als Material benutzt wird. Es wurde nachgewiesen, dass dieser Vorgang mit der Reduktion des Nitrofurans durch Reduktionsgruppen im Fleisch während des Erhitzungsprozesses verbunden ist. Die Faktoren, welche die Reduktion beeinflussen, sowie die Methoden, welche die Reduktion verhindern, wurden untersucht.