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Lipolytic Changes of Fats in Dry Sausage

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Introduction

Investigations concerning the changes occurring in dry sausage during its preparation and ripening have been published in very great number. Attention has mainly been paid in them to the changes of the bacterial flora. There are numerous studies on the properties of the bacteria reducing nitrate and promoting acid formation. In addition, the bacteria causing spoilage of dry sausages have been studied.

In recent time also the formation of flavor in dry sausage has attracted increasing attention. Investigation and clarification of this aspect offers good possibilities for a great variety of studies. The prospects of efficient penetration into this subject have essentially improved after the investigations by gas chromatography became possible. It is natural that the question of flavor formation in dry sausages is rather complicated for the reason that it is greatly affected e.g. by the smoking, by salt, nitrate and nitrite and their decomposition products, and by the spices, various bacterial ferments and the enzymes inherent in the meat. TERPLAN (1962) refers to the flavor formation caused by bacterial ferments and by the meat enzymes as fermentative flavor formation. In the first place it involves hydrolysis of proteins, fats, and carbohydrates.

A. Earlier investigations

The contribution of the carbonyl compounds formed in the course of the ripening process of dry sausage has been studied by ESER and NIINIVAARA (1964).

Numerous authorities suggest that the bacteria decomposing fat contribute to the formation of flavor in dry sausages (KELLER 1954, KELLER and MEYER 1954, MEYER 1954, KELLER 1955, LOSEM 1956, LEISTNER 1958, TEN CATE 1960, POHJA and NIINIVAARA 1964, etc.). KELLER and MEYER (1954) observe that *Escherichia* and *Achromobacter* species are essential flavor producers in dry sausages. However, several authorities (LERCHE 1956, NIINIVAARA and POHJA 1956, TEN CATE 1960, etc.) state that the number of Gram-negative bacteria diminishes very considerably during the first few days after commencement of the ripening process. LERCHE (1956) notes that after some days almost the entire bacterial flora consists of lactobacilli, mainly of *L. plantarum* and *L. brevis*, of micrococci and yeasts (mainly *Debaryomyces kloeckeri*). Bacilli of the *Mesentericus subtilis* group can be observed throughout the manufacturing period, but their number is rather low. POHJA and NIINIVAARA (1960) observed that when the sausage mixture was inoculated with bacteria of the genus *Bacillus* they did not produce any changes in dry sausages. It is therefore natural that the greatest interest in the investigation of the flavor of dry sausages and of the contribution of bacteria in this respect is concentrated on lactobacilli, micrococci and possibly on yeasts and moulds, although it is kept in mind that other kinds of bacteria may also contribute with a certain share.

MAILLET and HENRY (1960) found that the fatty acid content of dry sausage increased from its initial value of 0.45 % to 2-4 % and even to 5-7 %. TERPLAN (1962) assumes that in the manufacturing of dry sausage lipolysis may be desirable up to a certain limit. If the product also contains oxidative bacteria, oxidation of fat may occur, which is obviously unfavourable from the viewpoint of

flavor formation.

1. Lipolytic microorganisms

The lipolytic microbes constitute a remarkably heterogeneous group. The following genera of bacteria, among others, have been found to include lipolytic strains: *Micrococcus*, *Bacillus*, *Clostridium*, *Pseudomonas*, *Achromobacter*, *Alcaligenes*, *Serratia*, and *Proteus* (DAVIES 1954, GOLDMAN and RAYMAN 1957, RHODES 1959, WILLIS 1960, ALFORD and BLANKENSHIP 1961, HUGO and BEVERIDGE 1962, POHJA and NIINIVAARA 1964, etc.).

Lipolytic strains may possibly occur also among the bacteria of genus *Lactobacillus* (PETTERSON and JOHNSON 1949, CORETTI 1958). Many yeasts and moulds are lipolytic (MUNKHERJEE 1951, SHIPE 1951, NELSON 1953, MATSUMURA 1962, etc.).

2. The factors influencing the growth and lipase activity of lipolytic microbes

The growth of microbes, their lipase-producing capacity and their lipase activity are dependent on the composition of the substrate, the pH of the environment and the temperature. It is also likely that different microbes have different optimum temperatures for lipase production. NASHIF and NELSON (1953a) mention that the lipases formed by most bacteria investigated are most strongly active in the pH range of 7.0 - 7.2. Some of them have the pH optimum 7.8. The optimum pH for the lipase produced by several yeasts and moulds is slightly below 7.0. The lipase produced by *Pseudomonas fragi* is active in the pH range of 5.7 - 8.2, with its optimum at pH 5.7 - 6.6. Lipase was formed in greatest quantity when the incubation temperature was 15°C. At 30°C very little or no lipase was formed. The optimum temperature for lipase activity was 40°C (NASHIF and NELSON 1953b).

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ALFORD and ELLIOTT observed also that lipase was formed most abundantly at low temperatures. P s e u d o m o n a s f l u o r e s c e n s produced lipase abundantly at 20°C, while at 30°C the lipase formation was very insignificant. The proliferation and growth of cells was stronger at 30°C that at 20°C.

ALFORD and ELLIOTT (1960) also tried different substrates and observed that lipase was formed in greatest quantity in 1 % peptone broth without fat and at pH 7.0. Lipase production still occurred when the pH value was below 6.0. SULZBACHER and ALFORD (1961) and ALFORD and PIERCE (1961) have noted that a substrate rich in carbohydrates inhibits the lipase formation.

ALFORD and PIERCE (1963) found that the substrate has a notable effect on the lipase formation. They also suggested that it is possible to use synthetic nutrient medium in studies concerning the lipolytic properties of bacteria. This facilitates the possible attempts at isolation and purification of the lipase enzyme. The same authors also observed that P s e u d o m o n a s f l u o r e s c e n s probably requires dipeptides and polypeptides for its growth. Also CUTCHIN et al. (1952) and NELSON (1953) noted that the lipase formation is greatly dependent on the composition of the substrate. Likewise, HUGO and BEVERIDGE (1962) demonstrated that the lipase formation of the seven bacterial strains in their studies was dependent on incubation temperature and pH. Another of their observations was that the choice of buffer is highly important. Different microorganisms are affected in different manner. DLUZOWSKI (1963) observed that the growth of lactic acid bacteria together with lipolytic O o s p o r a l a c t i s inhibited the lipolysis of milk fat. Addition of 2 % NaCl did not influence the strength of lipolysis.

NASHIF and NELSON (1953a) have observed that the lipase formation is inhibited when the phosphate concentration of the substrate is higher than 0.05 M. Furthermore, they noted that already 1 % NaCl inhibited the lipase formation by P s . f r a g i , and when the concentration was 3 %, no lipase activity whatsoever was noted. The size of the fat globules in the substrate is also an important factor. GOLDMAN and RAYMAN (1952) demonstrated that the strength of lipolysis is not so much affected by the molecular

composition of the fat as by the fineness of the globules in which it is distributed in the substrate.

Aeration inhibits the lipase formation although it cannot be considered an anaerobic process (ALFORD and ELLIOTT 1961). JONES and RICHARDS (1952), again, found that the presence of oxygen is indispensable in lipolysis. They observed moreover that staphylococci are strongly lipolytic as a rule. This property is not confined to the coagulase enzyme-forming capacity of the staphylococci.

Some bacterial lipases possess positional specificity with respect to glyceride molecules (SULZBACHER and ALFORD 1961, ALFORD and PIERCE 1961, ALFORD and BLANKENSHIP 1963). The lipase produced by *Pseudomonas fragi* liberates oleic acid in great quantity from lard (from the α position), but little stearic and palmitic acid (from the β position). Staphylococcal lipase liberates the said acids roughly in the proportions in which they occur in lard. The specificity of lipases is best observed by using non-symmetric synthetic triglycerides. ALFORD and BLANKENSHIP (1963) observe that the lipase of *Ps. fragi* is similar in its effects to the pancreas lipase, while the lipases of staphylococci differ from it and might be considered to be esterases. PROKS and SINGROSOVA (1962) demonstrated that *Penicillium camemberti* and *P. caseicolum* hydrolyzed the short-chain saturated fatty acids of milk more easily than its unsaturated fatty acids.

POHJA and NIINIVAARA (1964) observed that dry sausage contains lipolytic strains in great abundance, the majority consisting of micrococci. However, the high salt content of dry sausage and the rapid decrease of pH reduce the formation of lipases and their efficiency. Accordingly, they assume that the contribution of lipolytic bacteria to flavor formation is rather insignificant in sausages having merely a random bacterial flora. As is known micrococci may be added to dry sausages (NIINIVAARA 1955). If these micrococci are able to hydrolyze pork fat in conditions prevailing in dry sausage during ripening, this has a considerable effect on the quality of the products. On the strength of the experience gained so far there is no definite knowing whether

this microbe-produced hydrolysis of fat is favorable or detrimental from the viewpoint of flavor formation.

3. Demonstration and isolation of lipolytic bacteria

The lipolytic properties of microbes have been studied by using solid as well as semiliquid and liquid substrates. Numerous different natural fats and synthetic triglycerides have been used in the substrates, the lipolytic colonies becoming surrounded either by a clear zone or by a precipitation. The liberated fatty acids have moreover been demonstrated by means of various indicators, such as Nile blue sulphate, Victoria blue, copper sulphate, etc.

Many of the nutrients that have been employed have been found to possess considerable drawbacks. Tributyrine was tried out by numerous investigators (e.g. ANDERSON 1934, RHODES 1959, and POHJA and NIINIVAARA 1964). They found that the results obtained with this substrate are inaccurate because tributyrine is easily hydrolyzed. TAMMISTO (1933) claimed that strongly alkaline bacteria hydrolyze tributyrine, although they have not been observed to produce lipases.

TURNER (1929) and COLLINS and HAMMER (1934) and EISENBERG (1939) used Nile blue sulphate added to the agar for the demonstration of lipolysis. They found that the substrate was good, except that Nile blue sulphate inhibits the growth of some microbes. LONG and HAMMER (1937) endeavoured to eliminate this drawback by pouring the indicator upon the colonies after completed incubation.

In likeness with Nile blue sulphate, also Victoria blue may be used (RHODES 1952, ALIFAX 1958). It has the same drawbacks in that it usually inhibits the growth of Gram-positive bacteria.

RATH (1961) carried out experiments with several methods. He found none of those used previously to be reliable enough. For this reason he elaborated his own, modified Victoria blue method for quantitative demonstration of lipolytic bacteria.

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WILLIS (1960) in his studies of the capacity of C l o s t r i - d i u m bacteria to produce lecithinase and to hydrolyze fat, used common egg yolk agar. For indicator, he used copper sulphate (BERRY 1933).

SIERRA (1957) employed Tween 80 (polysorbitan monooleate) for the demonstration of lipolytic organisms. The lipolytic colonies are surrounded by a precipitation zone when this method is used.

POHJA and NIINIVAARA (1964) have studied the capacity of strains isolated from dry sausages and from salt brines (particularly micrococci) to hydrolyze animal fats. They also prepared plates containing pork fat, in which the pH value was adjusted with the aid of 0.05 M phosphate buffer to values characteristic of dry sausage, i.e. in the range of pH 5.9 - 4.9. The substrates were moreover given sodium chloride additions at 0 - 5.0 %.

In the study presented in the following, an attempt has been made to penetrate further the question of the capacity of various bacterial strains to hydrolyze pork fat in conditions prevailing in dry sausages during ripening, with the aim of throwing light on the subsequent effect of the decomposition products upon the flavor of the dry sausage. As before, attention has mostly been paid in this study to the investigation of the lipolytic properties of micrococci.

B. Own investigations

1. The bacterial strains employed in the study

In total 90 bacterial strains have been isolated from eight dry sausages and from one cured ham. The isolation was performed from surface cultures on general blood agar plates and from agar plates containing fat (pork fat and glycerol tributyrine). The isolated bacteria were identified on the basis of their growth characteristics on blood agar plates, microscopically by Gram staining and on the basis of their most essential biochemical properties. Of the bacteria, 45 were found to be micrococci, while 41 were bacilli and four were streptococci.

Correspondingly, 33 bacterial strains were isolated from milk and milk products. They were identified as follows: 13 micrococci, 11 strains of *Staphylococcus aureus*, 3 streptococci, 2 *Pseudomonas* species, 2 bacteria of the *Coli* group, 1 *Serratia marcescens*, and 1 *Bacillus* species.

Furthermore, 58 micrococci, 4 *Pseudomonas* species and 1 *Salmonella typhimurium* from strain collections were included in the studies. All 58 micrococci have previously been isolated from meat products, mostly from dry sausages.

2. The substrates employed in the study

The following substrate (JONES and RICHARDS 1952) was used as the basic substrate:

Yeast extract (Difco)	3.0 g	Agar	20.0 g
Peptone (Difco)	10.0 g	Tap water ad	1000 ml
NaCl	5.0 g		

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Tributyrine was added to the substrate in the proportion of 1 ml per 100 ml, and 2 ml pork fat per 70 ml substrate. Tributyrine and pork fat were homogenized by using an Ultra-Turrax homogenizer.

The pH of the substrate was adjusted to pH 7.0. Using the same basic substrate, plates containing tributyrine and pork fat in the above-mentioned proportions were prepared and their pH was adjusted with a phosphate or citric acid-citrate buffer to 6.0, 5.5 and 5.0. The substrates had about 0.05-molar phosphate or citrate content and their NaCl concentration was 3.0 or 4.0 %, according to the following schedule:

(a)	pH	6.0,	NaCl	3.0 %
(b)	"	5.5,	"	3.0 "
(c)	"	5.5,	"	4.0 "
(d)	"	5.0,	"	4.0 "

Furthermore, standard egg yolk substrate was used in the studies in order to elicit the lecithinase reaction and the potential properties of hydrolyzing egg fat (WILLIS 1960).

3. Demonstration and interpretation of positive lipolysis reactions

The hydrolysis of tributyrine on the agar plates employed in this study is noticed by the formation of a clear zone in the substrate around the colony. The strength of the reaction was assessed as follows:

-	No change around the colony
(+)	Slight clarification around the colony
+	Distinct clear zone around the colony. In the case of colonies which do not spread out considerably, the diameter of the clear zone is 6-9 mm.
++	Diameter of the clear zone 10-14 mm
+++	" " " " " 15-20 mm
++++	" " " " " over 20 mm.

The hydrolysis of pork fat and of other fats with the substrates employed in this study can be demonstrated by treating the

incubated plates with saturated copper sulphate solution for about 5 minutes. As BERRY (1933) observed, free fatty acids are produced around the colonies by the effect of lipolytic microorganisms and can be demonstrated by treating the plates with copper sulphate. The procedure results in the formation of colored (blue to bluish green) copper soaps.

The strength of the lipolysis of pork fat was assessed as follows:

- \- Negative. No change in color differing from the color of the substrate elsewhere can be seen under or around the colony.
- (+) Slightly positive. A distinct blue to bluish green area is seen under the colony.
- \+ Positive. A distinct blue to bluish green ring is seen around the colony, less than 2 mm in width, measured from the rim of the colony.
- ++ Strongly positive. The width of the colored ring, measured from the rim of the colony, exceeds 2 mm.

4. Effect of the buffer on the lipolysis reaction

Initially, the ability of the bacterial strains isolated and of the bacteria from the strain collections to hydrolyze pork fat in conditions typical of dry sausages was studied on pork fat agar plates containing phosphate buffer. Right from the start the observation was made that a blue zone, frequently rather extensive, was produced around the colonies upon copper sulphate treatment on these substrate plates by numerous such bacterial strains which did not cause hydrolysis of fat on pork fat plates without buffer or only produced a slight positive reaction in such instances. The reaction was very slight on the day of the test, but the color became gradually more intense during a period of several days. This reaction could, as a rule, only be noted with plates containing pork fat and having the pH 5.5 or 5.0. When the pH value was 6.0, the entire plate acquired a more intense blue tinge than a pork fat plate without buffer.

During the initial phase of the study it was believed that the said color reaction was due to copper soaps of the fatty acids. The reactions visible on the plates were not recorded as a rule before the day after the copper sulphate treatment. The result of this procedure was that the chance to observe the positive (bluish green), frequently narrow zones of lipolysis reaction that might have formed around the colonies was entirely missed.

In order to clarify the character of the said blue color reaction, plates without added pork fat were prepared from the same basic substrate. Their pH was adjusted (a) with phosphate buffer and (b) with lactic acid.

When several different bacterial strains were cultivated at 20°C and at 30°C on plates prepared in this manner, the observation was made that a similar blue reaction was also elicited on fat-free plates containing phosphate. Immediately upon the copper sulphate treatment a precipitation began to form on the otherwise clear substrate around the colonies. It was at first very pale blue in color, but the color gradually increased in strength similarly as on the plates with pork fat. Further study of the reaction revealed that lactic acid and the salt concentration of the substrate had no share in this color reaction, which shall be called a pseudo-reaction.

It was inferred from these studies that the use of phosphate buffers on agar plates containing fat may be misleading when the hydrolysis of fat is demonstrated by means of copper sulphate because the phosphates of the buffer probably react with the copper in the indicator. If phosphate buffer is used, the lipolysis reactions have to be recorded immediately upon copper sulphate treatment.

A comparison was made of the effects of phosphate buffer and of citric acid-citrate buffer on the lipolysis reaction on pork fat agar plates. For this study 18 bacterial strains were chosen (8 micrococci, 3 strains of *Staphylococcus aureus*, 1 *Bacillus* species, 4 *Pseudomonas* species, 1 *Aerobacter aerogenes*, and 1 *Serratia marcescens*). Parallel cultures were

made on pork fat plates without buffer (at pH 7.0) and on buffered pork fat plates (pH 5.5 - NaCl 3.0 %, and pH 5.0 - NaCl 4.0 %). The plates were incubated for five days at 30°C. Hydrolysis of pork fat at pH 7.0 was caused by seven of the 18 bacteria investigated. Five bacteria were found to produce hydrolysis of pork fat on plates with pH 5.5 and with 3 % NaCl concentration when citric acid-citrate had been used for buffering. When the phosphate buffer was used, positive hydrolysis of pork fat was established with two bacteria only. Correspondingly, when the pH value was 5.0 and the NaCl concentration 4 %, hydrolysis of pork fat was noted on the plated buffered with citric acid-citrate in the case of five bacteria, while this reaction was seen with one bacterium only on the phosphate-buffered plates.

Comparison of the hydrolysis of tributyrine on plates buffered with phosphate and citric acid-citrate revealed that the hydrolysis zones were roughly equal in size at one and the same pH and NaCl concentration independent of the buffer.

5. The effect of the incubation time on the lipolysis reaction

On pork fat plates without a buffer and with pH 7.0 altogether 24 micrococci and bacilli were studied in parallel. The incubation temperature was 30°C and the incubation time, 3 and 5 days. In both instances twelve strains were found to produce the hydrolysis of pork fat of varying degree. However, the results were different in the respect that 11 of these twelve bacteria were identical. One strain, which caused hydrolysis of pork fat after incubation during three days, did not produce such hydrolysis in the parellel test with 5 days' incubation. On the other hand another bacterial strain produced hydrolysis of pork fat after 5 days' incubation but not after three days.

Simultaneously with the comparisons concerning the effect of the buffers on the lipolysis reaction, also the effect of incubation time on the lipolytic properties of the said 18 strains was studied. The incubation temperature was 30°C and the incubation

periods were 5 and 10 days. Seven of the 18 investigated bacteria were found to produce the hydrolysis of pork fat at pH 7.0 after 5 days' incubation. When the incubation time was increased to ten days, one further slightly lipolytic strain was discovered. In the case of three of the above-mentioned seven strains the lipolysis reaction was considerably strengthened. On plates buffered with citric acid-citrate positive lipolysis reactions were noted after incubation during 5 days as follows: in five instances on plates with pH 5.5 and with 3 % NaCl, and equally in five on plates with pH 5.0 and with 4 % NaCl. When the incubation period was increased to 10 days, the number of positive reactions on plates with pH 5.5 and with 3 % NaCl increased to seven. The lengthening of the incubation time had a considerably greater effect in the case of the plates buffered with phosphate. On the plates with pH 5.5 and with 3 % NaCl the number of positive reactions increased from two to six and on the plates with pH 5.0 and with 4 % NaCl, from one to four.

Examination of the effect of the incubation time on the hydrolysis of tributyrine revealed that most bacteria caused at least a slight hydrolysis reaction, (+), after the incubation at 20°C as well as 30°C during 7-10 days. When the plates were examined after 1-3 days upon the incubation at 30°C and after 3-5 days upon the incubation at 20°C, it was found that several bacteria either had not hydrolyzed tributyrine at all or produced only a slight positive reaction. Some other strains, again, caused a distinct hydrolysis of tributyrine, at least + or ++, after the said time. Only such strains have been considered lipolysis-positive strains in the present study.

6. The lipolytic properties of the bacteria investigated on different kinds of plates containing fat

The results concerning the lipolytic properties of the bacteria investigated are presented in the table.

As can be seen from this table, the attention has mainly been centered on the lipolytic properties of micrococci and bacilli.

The following observations emerge from an examination of the results:

(a) Hydrolysis of tributyrine, at least equivalent to the notation +, was caused by 71 of the 108 micrococci investigated and by 26 of the 37 bacilli investigated. All 11 *Staphylococcus aureus* strains produced hydrolysis of tributyrine.

(b) Hydrolysis of pork fat at pH 7.0 was caused by 32 of 91 micrococci investigated, by 8 of 20 bacilli investigated and by 6 of 8 *Staphylococcus aureus* strains investigated.

(c) Hydrolysis of egg yolk fat at pH 7.0 was only elicited in the case of 4 micrococci out of 70 strains investigated. No less than 15 of 20 bacilli investigated and 3 out of 4 *Staphylococcus* strains investigated caused hydrolysis of egg yolk fat. The *Serratia marcescens* included in the studies also caused hydrolysis of egg yolk fat but neither of pork fat nor of tributyrine. The results seem to suggest that different bacteria cause hydrolysis of different fats, or that their optimum for the hydrolysis of different fats is different.

(d) A fairly remarkable part of the micrococci, staphylococci and bacilli investigated hydrolyzed pork fat on plates buffered with citric acid-citrate, with pH 5.5 or 5.0 and with 3 % or 4 % NaCl concentration. Several of the bacteria also caused hydrolysis of tributyrine at the said pH values and NaCl concentrations.

(e) The streptococci (seven strains) did not grow on the lipolysis substrates employed in the study.

(f) None of the enterobacteria investigated (*Escherichia coli*, *Aerobacter aerogenes* and *Salmonella typhimurium*) caused positive lipolysis reaction on the substrates employed in the study.

(g) On comparison of the hydrolysis of tributyrine and of pork fat on substrates with pH 7.0 the observation was made that several strains causing distinct hydrolysis of tributyrine, at least equalling the notation +, also produced hydrolysis of pork

fat. The strength of the hydrolysis of tributyrine was assessed upon incubation at 30°C during three days and that of the hydrolysis of pork fat after incubation during 3-5 days. Further study of the compatibility of these lipolysis reactions revealed that (1) of 56 bacteria investigated causing strong hydrolysis of tributyrine, at least equalling the reaction ++, 48 also produced hydrolysis of pork fat; and (2) those 56 bacteria which caused hydrolysis of pork fat showed the following distribution as regards their effect on tributyrine plates:

Strength of tributyrine hydrolysis		Number of bacteria
-	Negative	0
(+)	Slight	1
+	Distinct	7
++	Strong	25
+++ and ++++	Very strong	23
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It can be stated on the basis of these results that strong hydrolysis of tributyrine is an indication of lipase activity of the bacterium in question. Since plates containing tributyrine are very convenient in quantitative studies of lipolytic organisms or in attempts at their isolation, this is an observation of considerable significance.

Summary

The lipolytic properties of 199 microbial strains have been studied, paying particular attention to the ability of the microbes to hydrolyze pork fat at the pH value and salt content prevailing in dry sausages during the ripening and storage. The main object of study has been the lipolytic behaviour of micrococci isolated from dry sausages and of various B a c i l l u s species.

The pH of the substrates containing fat was adjusted with the aid of phosphate and citric acid-citrate buffers. Comparison of the buffers employed in the study revealed that the use of phosphate buffer in the substrates on which the liberated fatty acids are to be demonstrated by means of copper sulphate may have a misleading effect in that the copper in the indicator probably reacts with the phosphates of the buffer, producing a blue compound. The citric acid-citrate buffer proved more appropriate for the use in the pH adjustment of the substrates containing fat.

It was also found in the course of the studies that the use of tributyrine is possible in the investigations concerning the lipolytic properties of the bacteria. A remarkable percentage (85.7 %) of the strains causing a strong hydrolysis of tributyrine alsc caused the hydrolysis of pork fat.

Fairly many of the examined M i c r o c o c c u s and B a c i l l u s species were found to grow and to cause the hydrolysis of pork fat and of tributyrine at pH 5.5 and 5.0 with a NaCl concentration of 3 and 4 %. The effect of these strains on the hydrolysis of pork fat observable in dry sausages is likely, and the matter is further investigated in the studies in progress.

Zusammenfassung

In den dargestellten Arbeiten sind die lipolytischen Eigenschaften einer Anzahl (199) von Mikrobenstämmen untersucht worden, und zwar unter besonderer Berücksichtigung des Vermögens dieser Mikroben, Speck bei dem pH-Wert und bei der Salzkonzentration abzubauen, die in Rohwurst während der Herstellung herrschen. Die grösste Aufmerksamkeit wurde den lipolytischen Eigenschaften der aus Rohwürsten isolierten Mikrokokken und verschiedenen Bazillusarten zugewandt.

Der pH-Wert der fetthaltigen Nährböden wurde mittels Phosphat- bzw. Zitronensäure-Zitratpuffer einreguliert. Ein Vergleich zwischen den angewandten Puffern zeigte, dass die Verwendung von Phosphatpuffer bei solchen Nährböden, in denen die freigesetzten Fettsäuren mit Hilfe von Kupfersulphat nachgewiesen werden, irreführen kann, indem das Kupfer des Indikatormittels wahrscheinlich mit den Phosphaten des Puffers reagiert und eine blaue Verbindung erzeugt. Der Zitronensäure-Zitratpuffer wurde als besser geeignet zur Einstellung des pH-Werts bei fetthaltigen Nährböden befunden.

In den ausgeführten Untersuchungen wurde auch festgestellt, dass die Anwendung von Tributyrat in Untersuchungen der lipolytischen Eigenschaften von Bakterien möglich ist. Ein sehr beachtenswerter Teil (87,5 %) der Tributyrat stark hydrolysierenden Stämme führte auch Speckhydrolyse herbei.

Es wurde wahrgenommen, dass recht viele von den untersuchten Mikrokokken und Bazillusarten bei pH 5,5 und 5,0 und bei einer NaCl-Konzentration von 3 und 4 % wachsen und Hydrolyse von Speck sowie von Tributyrat bewirken. Einwirkung dieser Stämme auf die in Rohwurst beobachtete Hydrolyse von Speck ist wahrscheinlich, und dieser Umstand wird in weiteren Untersuchungen verfolgt.

The lipolytic properties of bacteria

Bacterial strain				Micrococcus sp.	Staphylococcus aureus	Bacillus sp.	Pseudomonas sp.	Streptococcus sp.	Coliform bacteria	Serratia sp.	Salmonella sp.
Total number of strains				116	11	42	6	7	2	1	1
Culture medium											
fat	pH	buffer	NaCl								
pork fat	7.0		0	32/91	6/8	8/20	2/6	no growth	0/2	0/1	0/1
egg yolk fat	7.0		0.5	4/70	3/4	15/20	0/5	"	0/2	1/1	0/1
tributyrine	7.0		0.5	71/108	11/11	26/37	4/6	"	0/2	0/1	0/1
pork fat	6.0	phosphate	3.0	26/70	5/6	4/5		"	0/2		0/1
"	5.5	"	3.0	0/8	1/3	1/1	0/4	no lipolysis	0/1	0/1	
"	5.0	"	4.0	0/8	0/4	0/1	0/4	"	0/1	0/1	
"	6.0	citrate	3.0	14/21	2/3	8/19	0/4	"	0/1	0/1	
"	5.5	"	3.0	13/21	2/3	13/19	0/4	"	0/1	0/1	
"	5.0	"	4.0	12/21	2/3	13/19	0/4	"	0/1	0/1	
tributyrine	6.0	phosphate	3.0	24/77	3/4	17/20	1/6	"	0/2	0/1	0/1
"	5.5	"	3.0	21/70	3/4	17/20	1/6	"			
"	5.5	"	4.0	19/77	3/4	13/20	1/4	"	0/2	0/1	0/1
"	5.0	"	4.0	17/74	3/4	3/14	2/6	"	0/2	0/1	0/1
"	6.0	citrate	3.0	11/21	2/3	15/19	1/4	"	0/1	0/1	
"	5.5	"	3.0	6/21	2/3	13/19	0/4	"	0/1	0/1	
"	5.0	"	4.0	3/13		8/18					

The numbers e.g. 32/91 mean that of 91 strains investigated 32 gave a positive lipolysis reaction.

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