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Studies on discoloration of Norwegian salami sausage

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An important factor in the manufacture of salami-type dry sausage is the development of a red color. The use of color additives in these products is in Norway and likewise in Denmark permitted. In these countries the color of the final product results from both the color additive and the so-called natural color which is due to the formation of nitrosomyoglobin.

In Norway experiments have been made in recent years with the use of lactobacilli starter cultures in the manufacture of salami sausage. Pilot plant experiments gave very promising results since the sausages rather rapidly attained a satisfactory consistency and their flavor was improved. The experiments carried out in sausage plant conditions did not yield as good results. One reason for this seems to be the fact that lactobacilli grow slowly in the sausage mass when the pork fat content rises exceeding 50 %. When lactobacilli starter cultures were used, there developed in some sausage series a color defect, which appeared as a grey center of the sliced surface.

Several investigators (Niven *et al.* 1949, Coretti 1958, Skovgaard 1963, and Nurmi 1965 and 1966) have observed, that lactobacilli cause color defects in dry sausage. At first this could not be encountered in the pilot plant experiments in Norway. Therefore, also in the sausage plant experiments only lactobacilli were used. This resulted, however, in color defects whereas the color due to color additive disappeared and the development of the natural color also was incomplete.

Because of the results of these experiments in sausage plants, efforts were made to investigate the stability of the color additives and the reasons why there occurred no formation of nitrosomyoglobin or why it was transformed into another pigment with a grey color.

MATERIALS AND METHODS

A. Effect of bacterial cultures on the stability of color additives.

In Norway the use of the following four red color additives in food products is permissible: Erythrosine 45430, C. I. 16255 (Nykockin), Ponceau 6 R16290, and Skarlagenrödt 14815. Nykockin and Ponceau 6 R are most commonly used.

The following color additives were employed in the present experiments:

1. C. I. 16255 (Nykockin)
2. Ponceau RR 70 8019
3. Cochineal R 70 13 1229/1
4. 43157 Red meat color, consisting of Ponceau 4 R and Sunset Yellow F C F
5. 78961 Cochineal R, consisting of Carmine naccarat
6. Erythrosine S.

The investigations have mainly been concentrated upon the two first mentioned color additives on the basis of the preliminary experiments in which Nykockin (C. I. 16256) proved to be the least stabile one and Ponceau RR 70 the most stabile one. All these color additives 78961 Cochineal R excluded, are synthetic. The color additives are known defined chemical compounds. The natural color additive Carmine (Cochineal R), on the other hand, consists of dried bodies of a female insect (Coccus cacti). This insect lives on a certain cactus species in Mexico.

The experiments were carried out using test tubes. The nutrient medium consisted of MRS-broth (de Man *et al.* 1960), into which the color additives to be examined were added in recommended concentrations (50, 100, and 150 mg/kg). MRS -medium is very suitable for lactobacilli but micrococci are also growing rather well in it. Based on the salt and nitrate content and initial pH of dry sausage, a modified MRS-broth was prepared in which the NaCl content was 4 %, nitrate content 0.04 %, glucose contents 0.1 %, 0.4 %, and 1 % and the pH value 5.8—6.0.

In some series 30 mg of $NaNO_2$ was added per kg of nutrient broth. The part catalase is playing in the development of color defects was studied by adding 1 mg bovine liver catalase (SIGMA) per kg. Furthermore, the effect of ascorbic acid on the stability of the color additive was examined. The ascorbic acid concentration used was 200 mg/kg.

The influence of the following bacteria on the stability of the color additives was examined:

1. Lactobacillus casei var. OSB/Ia
2. Lactobacillus plantarum 4669/6
3. Lactobacillus casei var. alactosus 3737/3

5. Micrococcus sp., isolated from Baktofermente (commercial starter culture)
6. Vibrio sp.
7. Esherichia coli

There is certain incoherence existing in respect to the catalase-positivity of lactobacilli. This group has generally been defined as catalase-negative. More recent investigations (Sharpe 1956, Dacre & Vancova 1957, Johnston & Delwiche 1962, Nurmi 1966, Tjaberg & Hildrum 1968) have shown that homofermentative lactobacilli exhibit catalase activity. The catalase possessed by them is also called pseudocatalase. Among the strains employed, strain 4669/6 is strongly catalase-positive, strains OSB/Ia and 3737/3 slightly catalase-positive, and strain 76/1 J catalase-negative (Tjaberg & Hildrum 1968).

B. Experiments related to the formation of color in Norwegian salami sausage

To elucidate the development of the color defect, 37 experimental dry sausage series were prepared at 7 different times.

The basic composition of the sausage mixture was as follows:

11	kg	beef
7	»	pork fat
475	g	sodium chloride
100	»	brine salt (containing nitrite)
5	»	potassium nitrate
4	»	garlic salt
54	»	white pepper
144	»	glucose

In all series an addition of 100 ppm Nykockin (C. I. 16255) was made. The composition of the mixture was alternated in respect of e.g. the pork fat content and glucose content. In some series brine salt was only used and in others nitrate was employed. The mixture was stuffed into cellophane fiber casings having a diameter of 85 mm. The weight of each sausage was about 1 kg.

The ripening conditions were as follows:

3 days at 22° C and 92.5 % RH
4 days at 20° C and 85 % RH
Thereafter, at 15° C and 80 % RH

The smoking was performed after 3 days.

The sampling was usually done after 0, 3, 7, 14, and 21 days. The bacteria were mainly added as broth cultures but in some series also as lyophilized preparations.

Table 1 illustrates the bacterial additions used and likewise possible alterations of the composition. When changes were made as regards the salt

employed, the denotation + signifies the use of salt, and the notation — means that the salt in question was omitted. In case none of these denotations is used, both potassium nitrite and potassium nitrate have been included in the ingredients. Each batch comprised 5 or 6 different series of sausages. In Table 1 the different batches have been separated with a line.

The denotation CA refers to a color additive. The figures signifying the bacterial cultures have previously been explained.

The sausages of different series were bacteriologically examined and the pH value of the samples was measured. At the same time the weight loss and the consistency were determined. The color, flavor, odor, and consistency were organoleptically evaluated. Emphasis was put on results relating to factors which influenced the formation and stability of the color and furthermore the flavor and aroma.

RESULTS

A. Effect of bacterial cultures on the stability of color additives

1. Ascorbic acid. Ascorbic acid distinctly intensified the discoloration of the color additive, due to lactobacilli. When the redox potentials were measured, ascorbic acid was found to have a lowering effect on the redox potential

2. Micrococci. The addition of micrococci did generally not prevent the disappearance of color, due to lactobacilli.

3. Catalase and haemoglobin. Neither catalase nor haemoglobin additions were capable of preventing the disappearance of color additives.

4. Escherichia coli and Vibrio sp. These were not either capable of preventing the disappearance of the color in test tubes into which lactobacilli had been added.

5. Sugar content. When the sugar content was increased from 0.1 % to 1 %, it forwarded to some extent the disappearance of the color. In the case of Lactobacillus plantarum 4669/6 it was observed in two different instances that the color of Nykockin (C. I. 16255) disappeared when the sugar content was 0.1 % and 1.0 % but was retained when the sugar content was 0.4 %.

6. Redox potential and pH value. The redox potential generally was lower in those test tubes wherefrom the color disappeared than it was in the tubes where the color was retained. No statistically distinct differences can be found in respect to the data obtained. Simultaneously with the redox potential, the pH value of the broths was measured. The decrease of the pH value did not seem to have any direct influence on the stability of the color additive.

7. Effect of different lactobacilli strains on the stability of the color ad-

ditive. Table 2 shows that strains OSB/Ia (L. casei var.) and 4669/6 (L. plantarum) considerably more often caused the disappearance of Nykockin (C. I. 16255) than did strains 3737/3 (L. casei var. alactosus) and 76/1 J (heterofermentative Lactobacillus sp.). The results obtained in regard to Ponceau RR 70 color additive were parallel.

8. The stability of different color additives. Ponceau RR 70 proved to be considerably more stabile (Table 2) than Nykockin (C. I. 16255). This was distinctly shown when the intensity of the color was exmined several separate times during the 10-day test period and likewise when complete disappearances of the color were registered. Erythrosine S (C. I. 45430) was even better retained than did Ponceau RR 70.

B. Examination of the formation of color in Norwegian salami sausage

Series 1—5.

The objective of these experimental series was to examine the effect of different amounts of bacteria (Table 1) on the formation of the color. The amount of the bacteria did not affect the color formation. The color of the salami sausage which contained lactobacilli added as a broth culture, was lightest of all. In the organoleptic examination no differences in the odor of flavor could be observed after three weeks.

Series 6—10.

Lactobacilli produce H_2O_2 which changes the red pigments of meat. To find out the role of H_2O_2 produced by lactobacilli, 0.2, 0.02, and 0.002 % of H_2O_2 was added to the sausage mixture. The sausages of series7containing 0.2 % of added H_2O_2 were from the beginning lighter in color. This evidently was due to the fact that the H_2O_2 concentration was too high. The color was, however, uniform and good. Already after two days there appeared discoloration in the center of the sausages of series 8 (0.02 % H_2O_2 addition). This defective grey color stayed throughout the entire ripening period.

Series 11—15.

In the preparation of this batch the meat used partially consisted of meat with a high bacterial content. The bacterial flora mainly comprised psychrophilic water and soil bacteria. In these series corresponding distinct color defects were obtained as was done in earlier sausage plant experiments. The color of series 11 and 12, which both were controls, was satisfactory. The same applied to the sausages of series 13 which were prepared using meat of good quality and which contained lactobacilli added as a lyophilized preparation. In the sausages of series 14, which were prepared using meat of poor quality and which contained added lactobacilli, both the artificial and natural color already disappeared after three days. On the sliced surface only an about two centimeter wide ring next to the edge had a normal red color. In the

Table 1. *Additions used in the preparation of the experimental sausage series*

Series No				
1	Control without bacterial addition			
2	Broth culture OSB/Ia, 1 mill. bacteria/g mass			
3	Lyophilized culture OSB/Ia, 0.1 mill. bacteria/g mass			
4	» » » 1 » » »			
5	» » » 10 » » »			
6	Control without bacterial addition			
7	Lyophilized culture OSB/Ia, 1 mill. + 40 ml 30 % H_2O_2 = 0.2 %			
8	Lyophilized culture OSB/Ia, 1 mill. + 4 ml 30 % H_2O_2 = 0,02 %			
9	Lyophilized culture OSB/Ia, 1 mill. + 0.4 ml 30 % H_2O_2 = 0.002 %			
10	Lyophilized culture OSB/Ia, 1 mill. + 60 mg catalase/ca. 5 kg mass			
11	Control. Good raw material			
12	» Poor » »			
13	Lyophilized culture OSB/Ia, ca. 1 mill. Good raw material			
14	» » » ca. 1 mill. Poor » »			
15	Lactobacilli + E. coli. Poor raw material			
16	Control. Formula including 55 % fat. Good raw material			
17	» » » 55 % » Poor » »			
18	Lyophilized lactobacilli. Formula including 55 % fat. Good raw material.			
19	Lyophilized lactobacilli. Formula indlucing 55 % fat. Poor raw material			
20	Micrococci + lactobacilli. Poor raw material			
21	Control	CA+	NO_3+	NO_2−
22	Lyophilized culture 4669/6	CA+	NO_3+	NO_2+
23	» » 4669/6+micrococci	CA+	NO_3+	NO_2+
24	» » 4669/6	CA+	NO_3+	NO_2−
25	» » 4669/6+micrococci	CA+	NO_3+	NO_2−
26	Control	CA+	NO_3+	NO_2−
27	Broth culture OSB/Ia 18 mill./g mass	CA+	NO_3+	NO_2−
28	» » 4669/6 12 » »	CA+	NO_3+	NO_2−
29	» » 3737/3 10 » »	CA+	NO_3+	NO_2−
30	» » 76/1J 4 » »	CA+	NO_3+	NO_2−
31	Lyophilized 4669/6 + micrococci	CA+	NO_3+	NO_2−
32	Control	CA+	NO_3+	NO_2+
33	Broth culture 4669/6 + 1.2 % sugar	CA+	NO_3+	NO_2+
34	» » 4669/6 + 0.8 % »	CA+	NO_3+	NO_2+
35	» » 4669/6 + 0.4 % »	CA+	NO_3+	NO_2+
36	» » micrococci+4669/6+0.8 % sugar	CA+	NO_3+	NO_2+
37	» » micrococci+4669/6+0.4 % sugar	CA+	NO_3+	NO_2+

Table 2. *Effect of four Lactobacillus strains on the stability of two color additives.*

Color additive	*Lactobacillus strain*							
	OSB/Ia		*4669/6*		*3737/3*		*76/1 J*	
	I	*II*	*I*	*II*	*I*	*II*	*I*	*II*
C. I. 16255 (Nykockin)	31	51	29	51	16	51	9	51
Ponceau RR 70	8	24	3	24	2	24	1	24

I = number of test tubes showing the disappearance of color.
II = total number of test tubes examined.

sausages of series 15, which were prepared using meat of poor quality and which contained added lactobacilli (L. casei var . OBS/Ia) and also Esherichia coli, a uniform and good red color was retained throughout the entire experimental period. The catalase activity and the ability of E. coli to reduce nitrate presumably resulted in the decomposition of H_2O_2 produced by lactobacilli and effected the formation of nitrosomyoglobin.

Series 16—20.

The objective of these series was to partially reproduce the results of the preceding series with the exception, however, that instead of E. coli, micrococci were employed. Furthermore, in a part of the series the pork fat content was increased from 40 % to 55 %.

When the pork fat content was 55 %, it was observed that the lactobacilli did not have similar possibilities to grow as they did in the sausages with a pork fat content of 40 %. In the controls (series 16 and 17) and in series 18 and 19 there only were slight differences in the numbers of lactobacilli, examined in different phases of the preparation. In the sausages of series 20 with a pork fat content of 40 % the numbers of bacteria were, on the other hand, considerably higher. In those series which were prepared using raw material of poor quality the color of the sliced surface was lighter than in corresponding sausages which were prepared using meat of good quality. In the sausages of series 20, which were prepared using poor raw material but with micrococci addition together with lactobacilli addition, the color was very good and uniformly red throughout the experimental period. The importance of micrococci in the formation of the color is emphasized when series 20 is compared with series 14 which was prepared using the same raw materials but without the addition of micrococci. In the sausages of series 14 a very distinct color defect was observed.

Series 21—25.

In connection with these series it was further attempted to clarify the importance of lactobacilli and micrococci in the formation and prevention of color defects. Furthermore, the objective was to study the effect of nitrite added together with nitrate as compared to the addition of only nitrate. The raw material of these series was good and the composition of the mixture was the original one. Very distinct differences could be observed in the colors of the sliced surfaces in different series. The color of the control sausages, series 21, was not yet sufficiently good after 7 days. A satisfactory color did not develop until after 14 days. In series 24, with lactobacilli and only nitrate added, the color was better but somewhat lighter areas could, however, be seen. When both lactobacilli and micrococci were added, the development of the color was good in the sausages prepared both with nitrate and nitrate-nitrite additions.

Series 26—31.

It was found in the preceding series that lactobacilli distinctly brought about the disappearance of the color in salami sausage. In the series 26—31 it was attempted to discover whether a color defect was also produced by other lactobacilli, especially taking into consideration their different catalase activities. In these series potassium nitrate was only used since in this way it was easier to detect the color defects.

The most distinct discoloration developed in the experimental sausages of series 27—30. On the sliced surface only a 5 cm ring next to the casing had retained the normal color. No differences between different lactobacilli could be observed. Series 31 showed again that micrococci together with lactobacilli and the nitrate-nitrite mixture gave a good permanent color.

Series 32—37.

The addition of lactobacilli and of salt mixture comprising nitrate and nitrite, together with varying amounts of sugar resulted in a color defect of different extent (grey center). The size of the grey center seemed to be in proportion to the amount of sugar added. The larger the amount of sugar added was, the smaller was the defective area. In two series (36 and 37), with both micrococci and lactobacilli added, a normal red color developed rapidly.

DISCUSSION

A. Effect of bacterial cultures on the stability of color additives.

In the conditions of the experiments it could be observed that bacteria, lactobacilli in particular, caused fading of red color as regards color additives

used in the manufacture of sausages. Complete disappearance of the color was also seen. Different strains resulted in the disappearance of color to a various extent but this was not in any correlation with the catalase activity of these strains. It has been difficult to detect the reason for such disappearance of color. By adding catalase either in the form of micrococci or as beef catalase, it was aimed at preventing the color defect which was presumed to be due to H_2O_2 produced by lactobacilli. This theory could not be confirmed, however, in the experimental conditions. The disappearance of the color of the additive — greatest when the sugar content was either 0.1 % or 1 % and the color was not prevented by the addition of micrococci, Escherichia coli or Vibrio sp. together with lactobacilli. Haemoglobin was also added to increase the production of catalase by lactobacilli. The hem-group forms an important part of the catalase molecule and lactobacilli possess a so-called procatalase which together with the hem-group obtained from outside forms catalase enzyme. The addition of haemoglobin did not, however, decrease the disappearance of the color additive, brought about by lactobacilli.

Danish experiments have shown that color additives lose their red color in the redox range from −250 to + 300 mV. Our studies did not give similar results. It seems, however, that the redox potential would in this connection be of significance since the redox potential of the test tubes showing the disappearance of the color generally was lower than it was in the other tubes. In our experiments the disappearance of the color often appeared in the redox range from +70 to −100 mV.

The results obtained did not unambiguously explain the reasons for the disappearance of the color of the additives but they gave, however, important information which porved useful when the experiments were carried on by studying he retaining of the color in salami sausages.

1. Different lactobacilli were found to greatly differ in their ability to bring about the fading or disappearance of the color of the additive.

2. Ponceau RR 70 proved to be considerably more stabile than Nykockin (C.I. 16255).

3. The sugar content was found to play a part in the disappearance of the color. With three of the strains (OSB/Ia, 3737/3, and 76/1 J) the tendency of the color to fade was in proportion to the sugar content. On the other hand, in the case of strain 4669/6 the extent of the fading of the color was greatest when the sugar content was either 0,1 % or 1 % and the color was retained when the sugar content was 0.4 %.

4. Ascorbic acid, which is commonly used in the manufacture of dry sausage, proved to be unfavorable. Ascorbic acid had a reducing effect and in the experimental series certain discoloration always was observed, also in the test tubes with no microbes added.

B. Studies on the formation of color in Norwegian salami sausage.

By preparing 37 different experimental sausage series results were obtained which elucidated the development of a color defect in Norwegian salami sausage.

The number of the bacteria added did not seem to be of any importance. Likewise, no differences were observed between the use of either lyophilized lactobacilli or broth cultures. The addition of H_2O_2 resulted in discoloration in one of three series. H_2O_2 possibly had an oxidative effect on nitrosomyoglobin and myoglobin. The experiments with the use of good or poor raw materials resulted in most series in a similar color defect, grey center, as did the previous experiments in sausage plant scale. In these cases lactobacilli only were employed as bacterial additions. When strongly catalase-positive and nitrate-reducing micrococci or E. coli were added, the color defect always could be prevented. The results obtained agree with the results of Nurmi (1966).

When potassium nitrate was used, the results revealed that to prevent the color defect caused by lactobacilli, the use of micrococci always was necessary. By adding nitrite it also was possible to partially prevent the discoloration.

In case a manufacturer of Norweigian salami sausage wants to use bacterial cultures, it is necessary to use combined micrococci and lactobacilli cultures to avoid the occurrence of a color defect. If discoloration appears in normal production without starter cultures, it is possible to prevent its occurrence by adding only micrococci. Furthermore, the development and retaining of a normal color can also be advanced by using nitrite in addition to nitrate. The amount of nitrite can be rather small, in the present experiments it proved to be sufficient when the quantity of brine salt was 1/6 (ca. 6 g/kg sausage mixture) of the total amount of salt. At present their simultaneous use is not permitted in Norway. Consequently, a choice has to be made between nitrite and nitrate.

When the acceleration of the manufacturing process is desirable, combined starter cultures seem necessary. Micrococci aid in the reduction of nitrate and, on the other hand, have a decomposing effect on H_2O_2 and thus the oxidative effect on color additives of H_2O_2 produced by lactobacilli will be prevented. Lactobacilli, moreover, accelerate the ripening by lowering the pH value of the sausage and thus they aid in the rapid development of the desired consistency and, furthermore, have an improving effect on the flavor and aroma.

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