Der Einfluss von Additiven auf reine und vereinigten Kulturen für Wurstherstellung

MARIJA ŠUTIĆ

Universität in Belgrad, Landwirtschaftliche Fakultät, Jugoslawien

In diesen Versuchen ist der Einfluss von Additiven und Salzingrediezionen auf die Aktivität der Milchsäubakterien (Streptococcus lactis und Lactobacillus casei) und Micrococcus M-104 untersucht. Die Additiven und Salzingrediezionen sind dem peptonisierten Fleischbouillon zugegeben, der mit genannten reinen und vereinigten Kulturen inokuliert war. Der Einfluss von Additiven auf die Entwicklung der Mikroorganismen war mit der Intensität der Trübung (mit Nephelometer gemessen) und mit dem pH-Werte bestimmt.

Die Ergebnisse zeigen, dass die Additiven und Salzingredienzionen sehr verschieden und wichtig auf die Entwicklung der reinen und vereinigten untersuchten Bakterien beeinflussen. Zum Beispiel, in der Anwesenheit von dem Glucono-Delta-Lacton die Trübung im Nährboden war bei Streptococcus lactis 4.5 und pH-Wert 4.9; bei Micrococcus M-104 die Trübung war 16.6 und pH-Wert 5.0; bei den vereinigten Kulturen Streptococcus lactis + Micrococcus M-104 die Trübung war 30.0 und pH-Wert 4.35. In der Anwesenheit von dem Nitritpökelsalz die Milchsäurbakterien und Mikrokokken zeigen bessere Entwicklung und niedriger pH-Wert als die Streptokokken und Mikrokokken. Bei den Bakterien Lactobacillus casei die Trübung war 36.3 und pH-Wert 4.15; bei Micrococcus M-104 die Trübung war 41.0 und pH-Wert 5.15, während bei den vereinigten Kulturen Lactobacillus casei + Micrococcus M-104 die Trübung war 65.3 und pH-Wert 4.0.

Diese Beispiele zeigen sehr klar die bestimmte Beziehung zwischen Additiven und Mikroorganismen, welche eine spezielle Interesse für die Wurstherstellung haben könnte.

The effect of additives on pure and conjoint cultures for sausages

MARIJA ŠUTIĆ

University of Belgrade - Faculty of Agriculture, Yugoslavia

In these experiments the effect of additives and ingredients of brine on the activity of lactic acid bacteria (Streptococcus lactis and Lactobacillus casei) and Micrococcus M-104 has been investigated.

The additives and ingredients of brine were added in broth media inoculated with above mentioned single and conjoint cultures. The additives effect on microorganisms development was estimated my measuring of the degree of turbidity (Nephelometer readings) and pH value.

The results of investigations show that brine additives and ingredients very differently and significantly influence the development of investigated single and conjoint cultures.

For example, in the presence of gluconodeltalacton turbidity of media caused by Streptococcus lactis was 4.5 and pH value 4.9; with Micrococcus M-104 the turbidity was 16.6 and pH 5.0, whereas with the conjoint cultures Streptococcus lactis + Micrococcus M-104 was 30.0 (the turbidity) and pH - 4.3.

The presence of sodium nitrit lactobacilli and micrococci show better development and lower pH, than streptococci and micrococci. Thus, with Lactobacillus casei the turbidity was 36.3 and pH - 4.15; with Micrococcus M-104 turbidity was 41.0 and pH - 5.15, whereas with conjointcultures Lactobacillus casei + Micrococcus M-104 the turbidity was 65.3 and pH - 4.0.

These examples evidently show the determinated relations between additives, ingredients of brine and microorganisms, which may be of special interest in the manufacture of sausages.

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Influence des additifs sur les cultures pures et associées pour la production des saucisses

MARIJA ŠUTIĆ

Université de Belgrade, Faculté des sciences agronomiques, Yugoslavia

Dans ces expériments nous avons étudié l'influence des additifs et de la saline sur l'activité des bactéries acidolactiques /Streptococcus lactis et Lactobacillus casei) et Micrococcus M-104. Nous avons ajouté les additifs et la saline au bouillion de viande en l'inoculant ultérieurement avec les cultures pures et associées dites. L'influence des ces additifs sur le development des microorganismes est évalué en mésurant l'intensité de trouble (avec Nephelomètre) et la valeur de pH dans le milieu.

Les résultats des recherches montrent que les additifs et la saline influencent trés différement et important sur le development des cultures pures et associées. Par exemple, dans la présence de Glucono-delta-lacton le trouble du milieu était pour Streptococcus lactis 4.5 et pH-4.9; pour Micrococcus M-104 le trouble était 16.6 et pH-5.0, tandis que pour les cultures associées Str. lactis + Micrococcus M-104 le trouble était 30.0 et pH-4.35.

Dans la présence de nitrite les bactéries acidolactique et Micrococcus montrent meilleur development et la valeur de pH plus basse qui les Streptococcus et Micrococcus. Dans le cas pour Lactobacillus casei le trouble était 36.3 et pH-4.5; pour Micrococcus M-104 le trouble était 41.0 et pH - 5.15, tandis que pour les cultures associées Lactobacillus + Micrococcus le trouble était 65.3 et pH - 4.0.

Ces exemple montrent trés clairement la relation déterminée entre les additifs et microorganismes, qui pourrait avoir un intéret spécial pour la production des saucisses.

The effect of additives on pure and conjoint cultures for sausages

MARIJA ŠUTIĆ

University of Belgrade - Faculty of Agriculture, Yugoslavia

Introduction

It is known that the nitrites manifest the bactericide effect in the brine and in the different meat products on many of bacteria organisms. Until now the effect of the nitrite and some other brine ingredients on the single bacteria cultures has been investigated, mainly. In these kind of investigations more the spore-forming bacteria(Bulman and Ayres, 1952; Johnston et al., 1969; Pivnick et al., 1970 and others) than non spore-forming ones (Castellani and Niven, 1955; Chank et al., 1962) have been included.

However, in the sausage manufacturing the better effect is achieved by using the conjoint cultures of lactic acid bacteria and micrococci than the single cultures of these bacteria (Nurmi, 1966; Šutić and Joksimović, 1973). For that reason it was usefull to investigate the effect of the brine ingredients and additives, which are usually applied in the sausage manufacturing, on the conjoint cultures of lactic acid bacteria and micrococci. Some results in this kind of investigations are presented in this report.

Material and Methods

For these investigations single and conjoint cultures of lactic acid bacteria: Lactobacillus casei 17š, Streptococcus lactis Ak-60 and Micrococcus M-104 were used, which were applied for the manufacturing of the fermented sausages.

As a main medium for these investigations nutrient broth was used in which the other ingredients individually or in combinations were added as follows: 3 and 3.5% NaCl, 0.5% gluconodeltalacton (GDL), 0.05% $NaNO_3$, 0.010, 0.015 and 0.018% $NaNO_2$, 2.5% spices($NaCl+NaNO_3$ + $NaNO_2$ + pepper + garlic), 0.5 and 1.5% sucrose and glucose, 1.5% rodurite + ascorbic acid.
The pH value of the applied broth was 7.2 and during the addition of the individual ingredients the pH value has not been adjusted, and for that reason it was different after the sterilization (Tab. 1). In this work it was possible to compare the results only with the control of each investigated combination.

The tubes with 10 ml of media were inoculated with 0.2 ml of 24^h old broth single cultures and with 0.1 ml of each of conjoint cultures.

The growth intensity of inoculated cultures was measured by Nephelometer readings 24, 48 and 72 hours after incubation on 30^oC. At the same time the pH value, measured by pH-meter, was determined. For each measuring three tubes was used, and the noninoculated media served as a control. In the results, the obtained data, concerning the growth during the 72 hours incubation, were presented in detail in aim of better completeness.

Results and Discussion

The investigations of the effect of individual brine ingredients and additives on lactic acid bacteria and micrococci show the differences in the development intensity and medium pH value with the tested cultures. In the most cases the development is better in the conjoint cultures streptococci and micrococci, respectively lactobacilli and micrococci, than in their single cultures. In the Table 1. the effect of sugars and $NaNO_2$ on broth pH values in single and conjoint cultures of the investigated bacteria are presented.

It is evident from the data in this table that the glucose in higher concentration increases the medium acidity (pH 6.5) and in this conditions the conjoint cultures of lactic acid bacteria and micrococci better grow tnan in the single ones.

The obtained results show that the development and acidity increasing of Str. lactis in media with 0.010% $NaNO_2$ and 0.5% glucose are less than with Micrococcus and Lactobacillus casei. In this conditions Lb. casei and Micrococcus growth better and increase the acidity already after 24 hours (Fig. 1 and 2). In the same $NaNO_2$ concentrations and higher glucose concentrations (1.5%) the growth rates of lactic acid bacteria are very slight and also slightly produce acidity, while Micrococcus better develop and decrease pH value for 0.65 in the same medium.

Table 1. EFFECT OF SUGAR AND SODIUM NITRITE ON BROTH pH-VALUE IN SINGLE AND CONJOINT CULTURES OF LACTIC ACID BACTERIA AND MICROCOCCI AFTER 72 HOURS

STARTERS	Glucose					Sucrose			Sugar[1]		
	0.5%	1.5%	0.5% + 0.010% $NaNO_2$	1.5% + 0.010% $NaNO_2$	1.5% + 2.5% spices[2]	1.5%	1.5% + 0.018% $NaNO_2$	1.5% + 2.5% spices	1.5% + 0.010% $NaNO_2$	1.5% + 0.018% $NaNO_2$	1.5% + 2.5% spices
Str. lactis	4.3	4.9	5.8	6.5	6.40	4.3	4.5	7.05	5.45	6.0	5.10
Streptococcus + Micrococcus	4.9	4.05	5.2	6.35	4.85	4.6	5.70	5.25	5.40	6.05	4.85
Micrococcus	5.0	4.65	5.o	5.90	5.00	4.6	5.45	5.40	5.40	6.05	4.90
Lb. casei	4.o	3.75	4.0	6.4	4.05	6.7	6.8	7.05	5.20	6.05	3.95
Lactobacillus + Micrococcus	3.9	3.7	3.9	5.95	4.40	4.5	5.45	5.35	5.35	6.05	4.10
Control broth after sterilization	6.8	6.5	6.8	6.55	6.45	7.4	7.5	7.1	5.50	6.15	5.15

[1] Sugar = rodurit + ascorbic acid

[2] Spices = NaCl + $NaNO_3$ + $NaNO_2$ + pepper + garlic

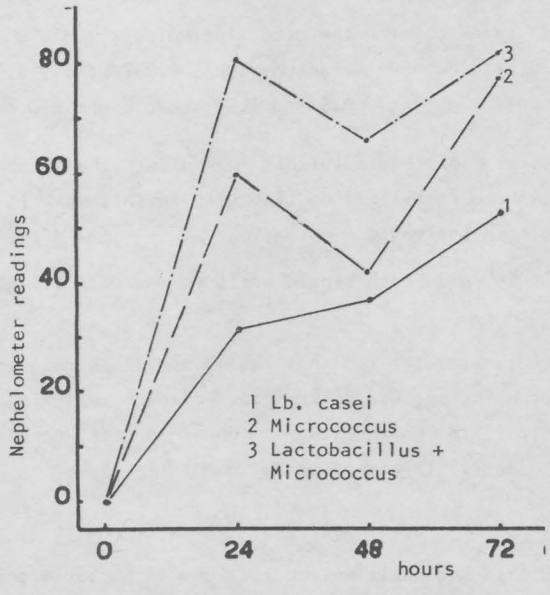

Fig. 1 Effect of 0.010% $NaNO_2$ on growth of bacteria

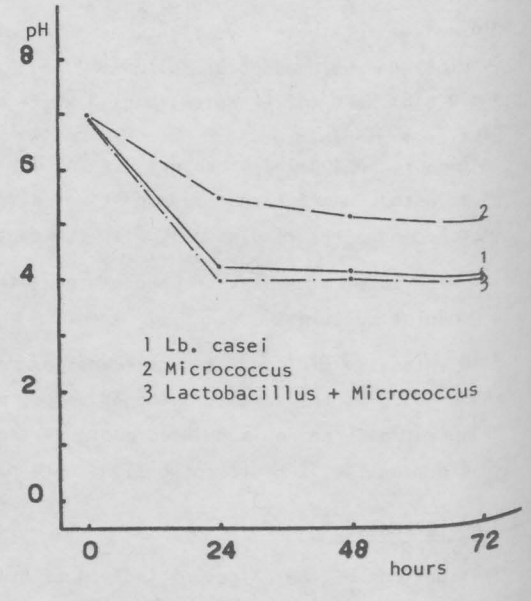

Fig. 2 Effect of $NaNO_2$ on the pH in broth

It is evident that in the presence of higher glucose concentrations (1.5%) the inhibitory nitrite effect is higher, what is probably the consequence of acidity increasing. Shank at al.(1962) reported tha it is the highest bactericide effect of nitrite in acid media on the development of Clostridium 3679 spores. On the other hand Mirna and Coretti (1977) pointed out that the sugar aldehyde may appear in the media, influencing also on the microorganisms growth.

However, when the concentration of $NaNO_2$ was increased (0.018%) in the presence of 1.5% sucrose the growth of Str. lactis was very good and decreased pH to 4.5, while Micrococcus in single and conjoint cultures with Str. lactis had the same pH value (5.45), what means that it produced the acid slightly.

Lb. casei produces also the acid less than Str. lactis but in comparison with the control better than in the presence of glucose at lower $NaNO_2$ concentration. It is necessary to point out that sucrose increased the pH value in broth (Tab.1), which certainly influenced on the better Str. lactis development. Chank et al.(1962) reported that the nitrites at pH 7.5 more stimulated the bacteria growth, what could be said for Str. lactis but not for Lb. casei and Micrococcus.

Taking into consideration, that for the sausage production the rodurite mixed with ascorbic acid is applied, the $NaNO_2$ influence in the presence of the above mentioned compounds on lactic acid bacteria and micrococci in the concentrations used in the practical work (1.5%) was investigated. Rodurite with the addition of ascorbic acid decreased the medium pH value and in a combinations with $NaNO_2$ caused the inhibitory effect on single and conjoint cultures of lactic acid bacteria and micrococci.

When to the nutritive broth the mixture of brine ingredients and spices was added, the development of single and conjoint cultures was very poor, as well as in the combination with GDL, while at the same time the decrease of pH value was no significant. However, when glucose or sucrose were added the these ingredients, the growth was better in conjoint cultures than in single ones. The decrease of pH value is better in conjoint cultures Str. lactis and Micrococcus in the presence of sucrose, when the pH decreased for 1.95, while in the glucose presence for 1.60. In the glucose presence Lb. casei stronger decreased the pH value in pure culture (to 4.05), than in the conjoint culture with Micrococcus (4.40). However, it developed slightly in pure culture with sucrose, decreasing pH for 0.15, and for 1.85 in the sace of conjoint cultures with Micrococcus.

Besides the results in Table 1, the effect of NaCl, $NaNO_3$ and GDL on single and conjoint cultures of lactic acid bacteria and Micrococcus was investigated. In the presence of 3.5% NaCl Str. lactis developed slightly, while the growth of Micrococcus in a single culture and in the conjoint culture with Str. lactis was considerably better (Fig. 3). At the decreasing of NaCl concentration to 3% and with the addition of 0.5% glucose Str. lactis developed a little better, but decreased the pH slightly (6.8), while Micrococcus decreased the pH to 4.95 under the same conditions. Lb. casei developed much better in this medium in conjoint cultures with Micrococcus; it

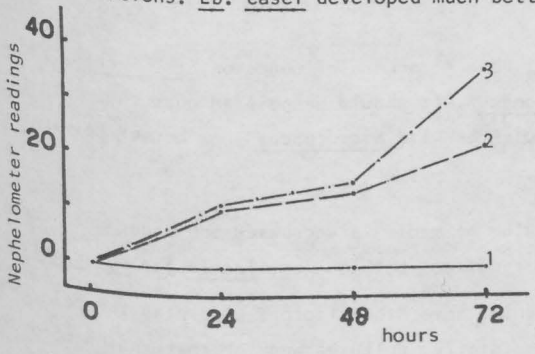

Fig. 3 Effect of 3.5% NaCl on growth on:
1. Str. lactis, 2. Micrococcus,
3. Streptococcus + Micrococcus

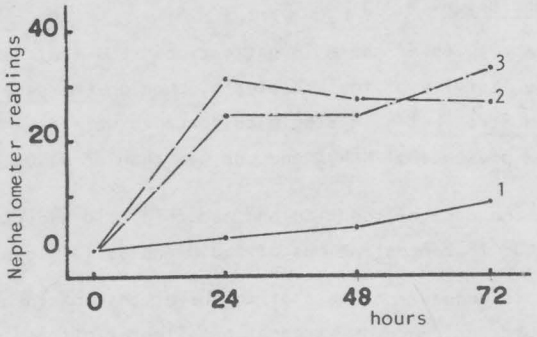

Fig. 4 Effect of $NaNO_3$ on growth on:
1. Str. lactis, 2. Micrococcus,
3. Streptococcus + Micrococcus

decreased stronger the pH value (4.1) than in the single culture (4.45).

$NaNO_3$ had not the particular effect on the Str. lactis development because the turbidity was the same like in the broth without $NaNO_3$ (8.5 Nephelometer readings). However, Micrococcus developed better in the presence of $NaNO_3$ as well as in the conjoint development with Str. lactis (Fig. 4).

Taking into consideration that the lactic acid bacteria and micrococci developed well in the $NaNO_3$ presence, it could be possible to decrease the nitrite quantity in the sausages production, increasing at the same time the amount of nitrate. Petäjä (1977) determined that in the Frankfurter sausage production with nitrates in pure cultures the satisfactory colour was obtained and flavour could be even improved; at the same time the nitrite amount was lower than in the production on the traditional way. Howeever, Leistner (1973) reported that 50% reduction in the addition of nitrite would have less inhibiting effect on gram-negative microorganisms and on the spore-forming ones.

In broth with 0.5% GDL the development of Str. lactis and Micrococcus is slight, but somewhat better in their conjoint cultures. This indicates that in the course of sausage manufacturing with starter cultures and GDL the optimal results could not be expected. In our experiments the more effect of starter cultures on organoleptic and other sausage properties was achieved without GDL than with its addition to the sausage (Šutić and Joksimović, 1973). The results of Reuter (1972) showed also that the addition of 0.7% GDL caused the reduction of lactobacilli, enterococci and some other micrococci during the ripening of dry sausages, while the number of yeast increased. For that reason in the sausage with GDL the full aroma is not obtained. However, in these investigations when to the medium with GDL 0.3% of yeast extract was added, the development of Str. lactis and Micrococcus was much better, particularly in the conjoint cultures (Fig. 5 and 6).

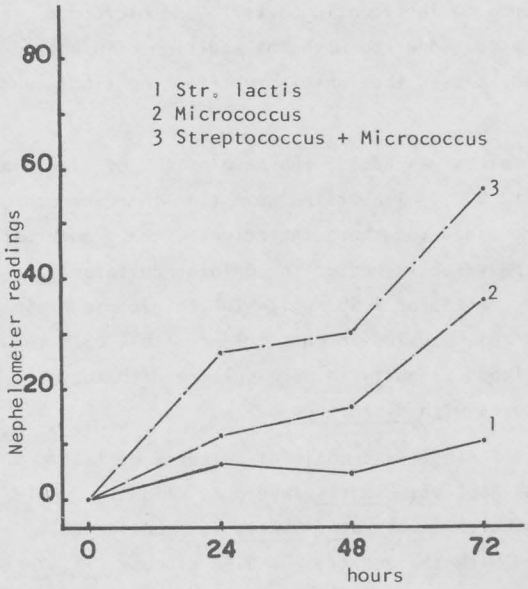

Fig. 5 Effect of GDL on growth on bacteria

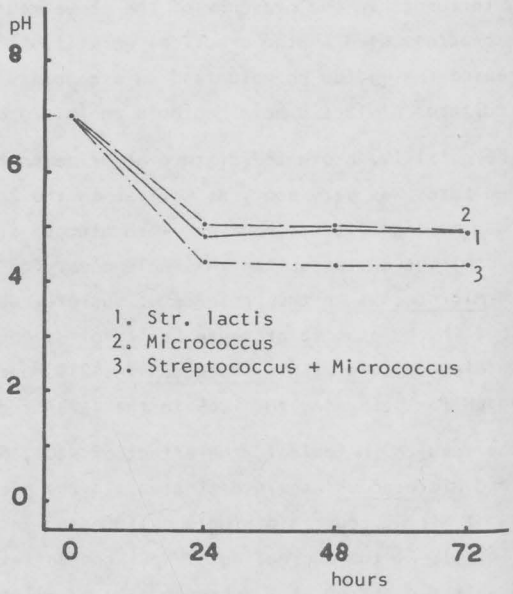

Fig. 6 Effect of GDL on the pH in broth

Conclusions

The results of these investigations show that NaCl and $NaNO_2$ have stronger influence on Str. lactis, reducing the activity of these bacteria, than on Lb. casei and Micrococcus. It should be pointed out, that both Lb. casei and Str. lactis in single cultures as well as in conjoint cultures with Micrococcus show better development in the presence of $NaNO_2$ and sucrose than of $NaNO_2$ and glucose.

In the case of rodurite and ascorbic acid addition the pH value of media is decreased and under such conditions $NaNO_2$ in concentrations of 0.010 and 0.018 % causes inhibitory effect both on Str. lactis and Lb. casei.

It is unquestionable that in the process of the sausage ripening some other factors also play the role, which could influence on stronger or slighter nitrite effect on the starter cultures and for that reason more precise investigations of various groups of products, in aim to recomment the reduction of nitrite amount have to be done in future.

Finally, the obtained results evidently showed the determined relations among additives, ingredients of brine and microorganisms, which may play a particular role in the sausage manufacturing.

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