

Effect of spices on the growth and the acid production of *Lactobacillus plantarum*

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SUMMARY: The effect of some natural spices and their extracts on the growth and the acid production of the starter culture of *L. plantarum* strain K₆ was studied. It was found that in liquid medium the natural spices savory, red pepper, cummin and coriander had a stimulatory effect while the black pepper and the nutmeg had a small inhibitory effect on the growth of strain K₆. The spices extracts had the similar effect when used as 50% concentration (v/v). It was also found that the natural spices and extracts (as 50% concentration) stimulated the acid production of *L. plantarum* strain K₆. The stimulation of the acid production by savory, red pepper, cummin, coriander and by the same spices extracts was the highest.

INTRODUCTION: The role of the starter cultures of lactobacilli is connected with their primary function of lactic acid production, which inhibits the growth of undesirable microorganisms on one hand (Bartolomew and Blumer, 1980; Katsaras, 1980; Bacus and Brown, 1980) and on the other hand has a positive effect on the flavor and consistency of sausages (Coretti, 1977; Petaja, 1977; Bacus, 1985). The growth of starter cultures is influenced by many factors. One of them is the spices used in the production of dry sausages. Besides their main role to the development of desirable flavor and odor in the sausages spices possess inhibitory or stimulatory effect on the growth of starter cultures (Zaika et al., 1978; Gerhardt and Quang, 1979; Zaika and Kissinger, 1981; Ingolf and Skjelkvale, 1982). Among the starter cultures studied the growth and the acid production of *L. plantarum* was reported to be fairly stimulated by different natural spices (Zaika et al., 1978; Ingolf and Skjelkvale, 1982). In the production of typical bulgarian raw-dried sausages are used mainly natural spices as savory, cummin, red pepper, black pepper. The extracts of these spices has been also used. There is little data on their effect on the growth of starter cultures in raw-dried sausages. The aim of the present study was to investigate the effect of some natural spices and their extracts, which are usually applied in the manufacturing of bulgarian raw-dried sausages, on the growth and the acid production in liquid medium of starter culture of *L. plantarum* strain K₆.

MATERIALS and METHODS: Starter culture: For the investigations the strain K₆ of *L. plantarum* was used. This strain was isolated from bulgarian raw-dried sausage "Panagyurska loukanka" and was applied as starter culture for the manufacturing of the fermented sausages.

Liquid medium: The tested strain was cultured in a medium composed of 0,3% meat extract, 0,5% peptone, 1% NaCl and 2% glucose (Ingolf and Skjelkvale, 1982). The pH value of the broth was 6,0.

Spices: The following natural dry ground spices were used: savory, cummin, red pepper, black pepper, nutmeg, coriander and mixtures of savory + black pepper and cummin + black pepper (1:1). The spices were added in the liquid medium as follows: 3, 5 and 7 g/L. The spices extracts were prepared by decoction method. 300 g of each spices were extracted for 8 hours in a water bath (40-42°C). After filtering extracts were added in the liquid medium as 5, 20 and 50% concentrations (v/v). Determination of the effect of the spices and the spices extracts

on the acid production of *L.plantarum* strain K_6 was done in liquid medium without spices (control) and with the above mentioned natural spices and spices extracts. Liquid medium was inoculated with 24 h old broth culture of *L.plantarum* strain K_6 ($2,5 - 5,0 \cdot 10^6$ cells per ml). The growth of inoculated culture after 24, 48 and 96 hours incubation at 30°C was estimated by counting on blood agar plates. Acid production of strain K_6 in liquid medium was determined by titration with $0,1\text{ N NaOH}$. The amount of the acid produced was calculated as μmol lactic acid/ml (Kuusela et al., 1978).

RESULTS and DISCUSSION: The results of the effect of natural spices and spices extracts on the growth of *L.plantarum* strain K_6 are presented in Figures 1 and 2. The values shown are mean values of 7 experiments. As evident in Fig. 1, the natural spices savory, red pepper, cummin and coriander had a stimulatory effect on the growth of strain K_6 . During the incubation period the number of cells increased significantly in the presence of above mentioned spices. The count of *L.plantarum* strain K_6 in the medium with 5 g/L spices (savory, red pepper, cummin and coriander) had increased $2,0$, $1,8$, $1,5$ and $1,2$ log cycles respectively as compared to the control after 96 h incubation. The results showed that the stimulatory effect of these spices increased as the concentration from 5 g/L to 7 g/L increased, but no significant differences in the cells number between 5 g/L and 7 g/L was recorded. The growth of the tested strain was slightly inhibited in the presence of black pepper and nutmeg (Fig. 1). The use of 5 g/L of black pepper and nutmeg caused a decrease of *L.plantarum*

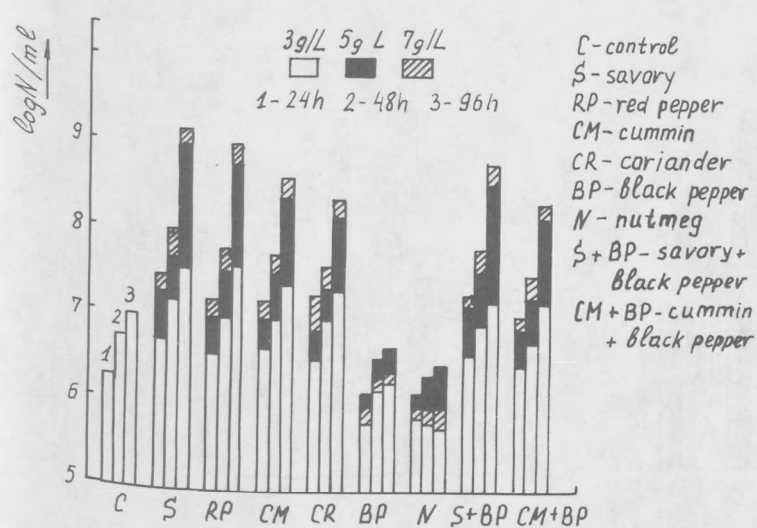


Fig. 1. Effect of natural spices on the growth of *L.plantarum* strain K_6

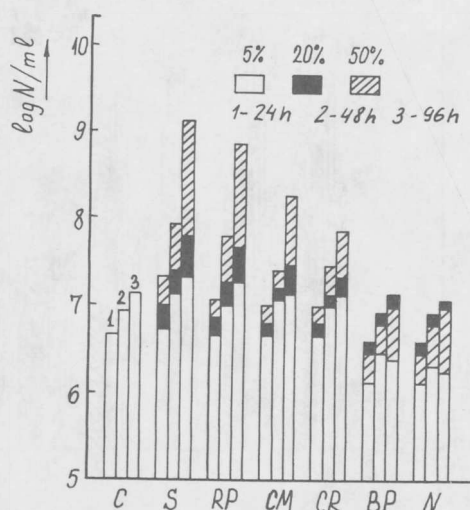


Fig. 2. Effect of spices extracts on the growth of *L.plantarum* strain K_6

count by $0,8$ and $1,0$ log cycles respectively if it was compared to the control after 96 h incubation. Stronger inhibitory effect was observed with the increase of the concentration of the spices and the time of incubation. As seen in Fig. 1 the spices mixtures of savory + black pepper and cummin + black pepper in used concentrations stimulated the growth of *L.plantarum* strain K_6 . Possibly, it was due to the stimulatory effect of the savory and cummin, which they possessed as individual spices. The results in Fig. 2 indicated that the use of

5% spices extracts (v/v) caused no change in the count of strain K_6 during the incubation period if it was compared to the control. When the spices extracts used as 20% concentration only the extracts of savory, red pepper, cummin and coriander had a small stimulatory effect on the growth of tested strain. The count of *L. plantarum* increased by 0.7, 0.6, 0.4 and 0.5 log cycles after 96 h when the above mentioned spices extracts were used. No noticeable change in the count was observed when black pepper and nutmeg extracts were used as 20% concentration. The spices extracts added as 50% concentration had the similar effect (stimulatory or inhibitory) on the growth of strain K_6 as the individual natural spices. At this concentration the number of bacterial cells was close to this, determined when 5 g/L of the different natural spices were added in liquid medium (Fig. 1 and Fig. 2). The results on the effect of the natural spices and spices extracts on the acid production of *L. plantarum* are shown in Figures 3 and 4. As seen in the Figures, all studied spices and their extracts (as 20% and 50% concentration) caused a stimulatory effect on the acid production of strain K_6 during incubation period. Acid production activity of the starter culture, expressed as μmol lactic acid/ml was the highest (58.8, 56.2, 52.0, 49.7 μmol lactic acid/ml) after 96 h incubation when 7 g/L of the natural spices of savory, red pepper, cummin and coriander were used. At the same experimental conditions (7 g/L and 96 h incubation) the black pepper and nutmeg showed the weaker acid production - 39.0 and 37.2 μmol lactic acid/ml. According to our results the amount of the acid produced by the starter culture increased with the in-

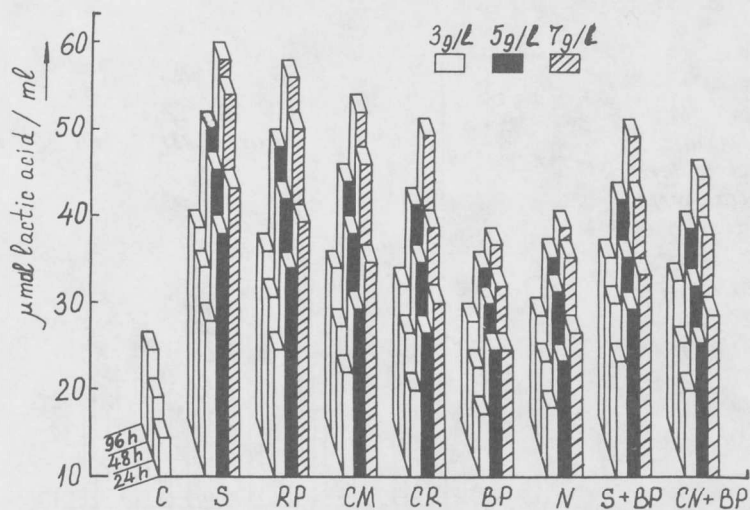


Fig. 3. Effect of natural spices on the acid production of *L. plantarum* strain K_6

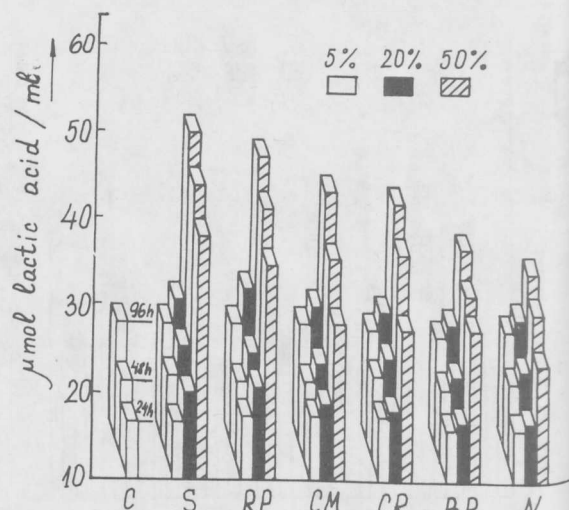


Fig. 4. Effect of spices extracts on the acid production of *L. plantarum* strain K_6

crease of the spices concentrations. The results presented in Fig. 4 also showed that the acid production of *L. plantarum* strain K_6 was significantly influenced by the different concentrations of the spices extracts used in this study. No stimulation on the acid production occurred when the spices extracts were used as 5% concentration. Regarding the results on the effect of 20% concentration of the applied spices extracts it was evident that the amount

produced was the highest when savory and red pepper extracts were used - 30,5 and 31,2 μmol lactic acid/ml after 96 h incubation. The stimulation of the acid production by extracts of savory, red pepper, cummin and coriander when they applied as 50% concentration was the most pronounced. After 96 h the amount of the acid in the medium with these spices extracts was 49,8, 47,9, 44,1 and 42,0 μmol lactic acid/ml respectively, while at the same concentration of black pepper and nutmeg extracts the amount of the acid was 37,3 and 34,2 μmol lactic acid/ml respectively. Based on the obtained results it was evident that the spices extracts when used as 50% concentration had similar effect on the acid production as individual natural spices used as 5 g/L concentration. Our results on the effect of the spices and their extracts on the acid production of *L. plantarum* strain K₆ are in agreement with those reported by Ingolf and Skjelkvåle, 1982.

CONCLUSIONS: The natural spices savory, red pepper, cummin, coriander and spices mixtures of savory+black pepper and cummin+black pepper have a stimulatory effect on the growth of *L. plantarum* strain K₆ in liquid medium while the black pepper and the nutmeg have a slight inhibitory effect. The spices extracts as 50% concentration (v/v) have the similar effect on the growth of strain K₆ as the individual natural spices. The natural spices and spices extracts (as 50% concentration) used in this study have a stimulatory effect on the acid production of *L. plantarum* strain K₆. The stimulation of the acid production by savory, red pepper, cummin, coriander and by their extracts (as 50% concentration) is the highest.

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