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EFFECT OF MICROCOCCAL MONOCULTURE ON CERTAIN QUALITY-INDICATING CHARACTERISTICS OF RAW-DRIED SAUSAGE

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INTRODUCTION: Micrococci are extensively used in 'European-style' sausage production as a component of mixed starter cultures alongwith lactic acid bacteria mainly due to their ability to improve the colour characteristics of the product by the action of their nitrate-reductase system(6,10,11) and also due to their desirable proteolytic and lipolytic activities(3,4,13). Although many researchers have demonstrated the beneficial effect of micrococci as a component of mixed cultures, Liepe(9) advocated the use of monoculture as there are reports of antagonistic actions among the different microorganisms in a mixed culture. Hence, this study was undertaken to study the influence of micrococcal monoculture on certain quality-indicating characteristics of Bulgarian raw-dried sausage.

MATERIALS AND METHODS:

Micrococcal cultures: Micrococcal strains Micrococcus varians M160 and M483 were isolated from 'lukanka' - a typical Bulgarian raw-dried sausage. Details of the isolation and characterization procedures are reported elsewhere(1). These two strains were studied for their different biochemical characteristics such as, nitrate-reductase activity(2), proteolytic (3), lipolytic activity(4) and other important physiological characteristics.

Preparation of bulk starter culture and processing conditions of the sausage: Active broth cultures(22-24h old) of the two strains were prepared separately by inoculating in Chapman broth(5) without indicator at 30°C.The formulation and procesing conditions of the sausage are described in our earlier report(1). Starter cultures of the two strains were added as monoculture @ 10^7cfu/g sausage mix(concentration found to be most appropriate through a separate experiment). After inoculation, sausage mix were filled in natural casings and were ripened as per standard method followed in the meat plant- M&M '90 Ltd., town Pajarzik, Bulgaria. Control sample was prepared under similar experimental conditions without application of starter cultures.

pH, water activity(a_w) and colour characteristics were determined during different stages of the production process of the sausage. Organoleptic evaluation and the quantity of residual nitrite and nitrate were determined in the ready products.

pH was determined by a pH-meter equipped with combination electrode and thermometer (model MS2994, Microsyst, Bulgaria).

Water activity was determined by an automatic a_w-meter(model EEJ3, Novasina Ltd., Zurich Switzerland).

Colour characteristics (red colour component, a*) was determined spectrophotometrically (model PYE Unicam PU8800 UV/VIS, Philips) as per the CIELab method(7).

Residual nitrite and nitrate was determined as per the HPLC method(14) under the following analytical conditions: HPLC Series 4- Perkin Elmer; columns - PE Analytical(C_{18}), length-25cm, Ø-4.6mm; mobile phase - 0.02M K_2HPO_4(pH 3.2) with H_3PO_4; flow-rate - 1cm^3/min; detector-spectrophotometric, λ- 214mm.

Organoleptic evaluation was done by an 11-membered panelists through the 9-point hedonic scale.

RESULTS AND DISCUSSION: pH values of both the treated and control samples dropped from their initial values upto the 13th d of the production process (Table1). After this period, the treated samples showed a tendency of slight increase in their pH values in contrast to the control samples which showed a slight downward trend towards the ready products. However, the differences in the pH values were not significant between the treated and control samples. This may be due to the fact that micrococci are poor producers of acids. Other researchers working on starter cultures of Micrococcaceae also reported similar findings(8).

Table 1. Changes in pH values of raw-dried sausage at different stages of production*

Sample	Stages of ripening					
	Sausage mix	24h	3d	6d	13d	Ready prod.
	Mean ± SE	Mean ±SE	Mean ±SE	Mean± SE	Mean± SE	Mean± SE
M160	5.90±0.04	5.83±0.03	5.60±0.09	5.46±0.07	5.32±0.05	5.39±0.05
M483	5.89±0.05	5.82±0.03	5.63±0.03	5.41±0.05	5.29±0.02	5.38±0.05
Control samples	5.90±0.03	5.81±0.03	5.77±0.03	5.58±0.06	5.40±0.08	5.34±0.01

* n=9

Studies on the changes of a_w values for both the treated and control samples showed only a slight change upto 3d, after which the a_w values declined somewhat sharply(Table2). And in the ready product, the a_w values of both the treated samples were lower than the controls, the differences, however, being non significant. In the ready products, the a_w values of the samples prepared with the strain M160 were comparatively lower than those prepared with strain M483 indicating acceleration of the drying process more effectively by the strain M160.

The concentration of the red colour component(a*) increased from sausage mix to the 6d of ripening, after which there was a relative stabilization of the process of a* development (Fig.1). This increase in the concentration of a* was more intense in case of the treated

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Table2. Changes in a_w value of raw-dried sausage at different stages of ripening*

Sample	Stages of ripening					
	Sausage mix	24h	3d	6d	13d	Ready prod.
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ±SE	Mean ± SE
M160	0.998±0.001	0.996±0.001	0.987±0.001	0.943±0.002	0.900±0.001	0.859±0.001
M483	0.997±0.001	0.995±0.001	0.986±0.001	0.939±0.001	0.904±0.003	0.863±0.002
Control samples	0.996±0.001	0.994±0.001	0.986±0.001	0.948±0.001	0.911±0.003	0.877±0.002

* n=9

samples. The maximal concentration of a* attained by the control samples at 6d was achieved by the treated samples even on the 2d of ripening. The concentration of a* in both the treated samples was significantly higher than the control samples during the entire process of ripening. Samples prepared with M483 had slightly better colour development than those prepared with M160. This may be explained by the well-defined nitrate reductase activity of the strains.

Analysis of the data on the residual nitrite and nitrate in the ready products showed that the control samples contained significantly higher quantities of residual nitrite and nitrate(33.73±3.83, 21.33±4.67mg/kg, respectively) than both the treated samples (Table3). Samples prepared with the strain M483 contained the lowest levels of residual nitrite and nitrate (28.17±4.15, 8.93±2.05mg/kg, respectively).This is in agreement with the well-defined nitrate reductase activity of the strains(2). Other workers also reported comparatively lesser quantity of residual nitrite in Frankfurt type

Table 3. Residual nitrite and nitrate contents of raw-dried sausage*

Sample	NO_2^- (mg/kg) Mean±SE	NO_3^- (mg/kg) Mean±SE
M160	29.03±3.47[a]	11.73±1.95[a]
M483	28.17±4.15[a]	8.93±2.05[b]
Control sample	33.73±3.83[b]	21.33±4.67[c]

*n=9; Figures with different superscripts row-wise differ significantly($P < 0.05$).

of sausage by the use of micrococcal starter cultures(12).

Organoleptic evaluation of the ready products in terms of external appearance,colour of the cut surface, aroma, taste, consistency and overall acceptability revealed that the treated samples were evidently better than the controls(Fig.2). The panel scores, however, did not differ significantly between the 2 treated samples for all the characteristics evaluated.

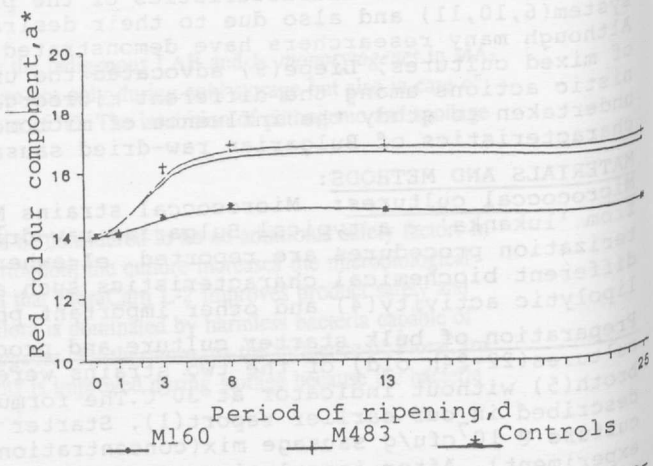

Figure 1.Changes in the red colour component,a* of the cut surface of raw-dried sausage prepared with selected micrococcal monocultures

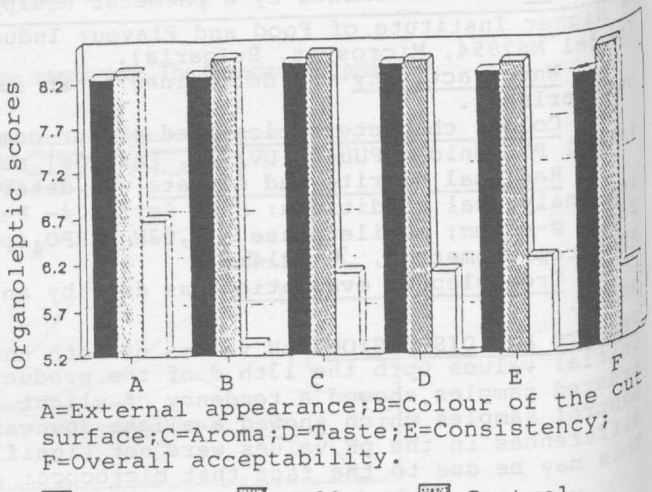

Figure 2. Organoleptic scores of raw-dried sausage prepared with selected micrococcal monocultures

CONCLUSIONS: From the study it may be concluded that micrococci can be successfully used as monocultures in the production of raw-dried sausage. Out of the 2 strains studied, M.varians M483 possessed comparatively superior qualities over M160. Hence, this strain is recommended for commercial use as micrococcal monoculture in the production of Bulgarian raw-dried sausage.

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