Action of microbial lipases on fatty acids and aldehydes in raw-dried sausages

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Abstract

Studies were made of the changes in the contents of free fatty acids and monocarbonyl compounds in raw-dried sausages, which changes had been caused by an additionally introduced yeast culture. The lipases produced by it bring about an increase in the amount of free fatty acids and changes in the ratio of their acyl chains. The reduction in unsaturated fatty acids correlates with higher values of saturated and unsaturated aldehydes. Free fatty acids were isolated from the lipid fraction by thin-layer chromatography, and were determined quantitatively by gas chromatography. Aldehydes were isolated by column chromatography as 2,4-DNP-hydrazones and were determined quantitatively by absorbance in the UV-region.

The application of starter cultures in the manufacture of raw-dried sausages opens possibilities to intensify technologies, widen product range and regulate ripening processes. In that type of sausages, micrococci are predominating microorganisms, producers of lipases (Cantoni, 1967). Coretti (1965), Stoychev (1972 a,b) reported of lipolytically active lactobacilli in raw-dried sausages. Sczepaniak (1974) and Buyanov (1981) consider that lipases-producing yeasts have a positive effect on the flavour of raw-dried sausages. Long-chain fatty acids resulting from the action of microbial lipases are sources for the formation of saturated and unsaturated carbonyl compounds. The latter are formed through ß-oxidation of unsaturated fatty acids (Demeyer, 1973). On drying the sausages, the level of unsaturated carbonyls decreases faster than the one of saturated carbonyls (Debevar, 1976). Under the influence of strongly lipolytical microorganisms, free fatty acids can also be oxidized to methyl ketones by way of ß-oxidation (Hanke, 1966).

The role of microorganisms in the oxidation of fatty acids to peroxides and carbonyl compounds was elucidated by Alford (1971) on model systems, while importance was attached to the microorganisms producing lipases and lipoxidases. Some of the microorganisms under investigation cause a great rise in the concentration of 2,4-dienals and 2-enals, and others raise the concentration of methyl ketones, a fraction which is absent in freshly rendered pork fat.

The objective of the present work was to determine, in dynamics, changes in the ratios of free

fatty acids and the ratio of unsaturated and saturated aldehydes in raw-dried sausages under the influence of a lipolytically active yeast starter culture.

Materials and Methods

Experiments were conducted on two loukanka-type raw-dried sausage products: smoked and non-smoked. In them, a 72-hour yeast broth culture, a producer of lipolytic enzymes had been introduced while observing adopted technological instructions (Sbornik tehnologichni instrukstii, 1981).
The amount of cell culture, of a titre of 10^9-10^{10} per ml, was 3 l for 100 kg of meat mass. Starter addition was accomplished during the cutting of meat mass with the spices, to assure the uniform distribution of the culture introduced. Simultaneously with experimental lots, controls without a starter were manufactured, from the same raw materials and under the same conditions.
The extraction of the lipid fraction was made with chloroform and methanol in a ratio of 2:1 by the method of Bligh and Dayer (1959). The isolation of the fraction of free fatty acids and the quantification of the individual acids was accomplished by the combined application of thin-layer and gas chromatography by the method of Christie (1970, 1972) and Metcalfe (1966). The individual acids were expressed in terms of % of the total quantity of the fraction of free fatty acids. Carbonyl compounds were isolated from the loukanka sausages as 2,4-dinitrophenylhydrazones by the method of Langner (1971). The separation and the quantification of saturated and unsaturated aldehydes was made by the method of Schwartz (1963), using column chromatography and absorbance in the UV-region, with a molar extinction coefficient of $E = 22500\ M^{-1}C^{-1}$ (Johnes, 1956). Aldehydes were expressed in $\mu M/g$ of product.

Results and Discussion

The ratio of unsaturated and saturated fatty acids in the free fatty acids fraction of the non-smoked product was greater in the experimental lot, compared to the control, during the period of product ripening, with the exception of day 1 (Fig. 1). It is worth noting that, in experimental lots, that ratio grew till day 18, whereupon it diminished. These data are confirmed further by the data in Fig. 2 and Fig. 3 showing the dynamics of the major fatty acids in a non-smoked product. The levels of oleic and linoleic acids in the experimental lot were considerably higher than in the control lot. Unsaturated fatty acids increased in the first days of drying, after which they decreased rapidly, while saturated fatty acids, after an initial decrease, showed a trend of increasing.
In the smoked product, the ratio of the amounts of unsaturated and saturated fatty acids in the fraction of free fatty acids was higher in the lots manufactured using yeast culture throughout the drying period (Fig. 1). A maximum increase of unsaturated acids was noted between days 8 and 11 in experimental samples, and in the controls that process was delayed

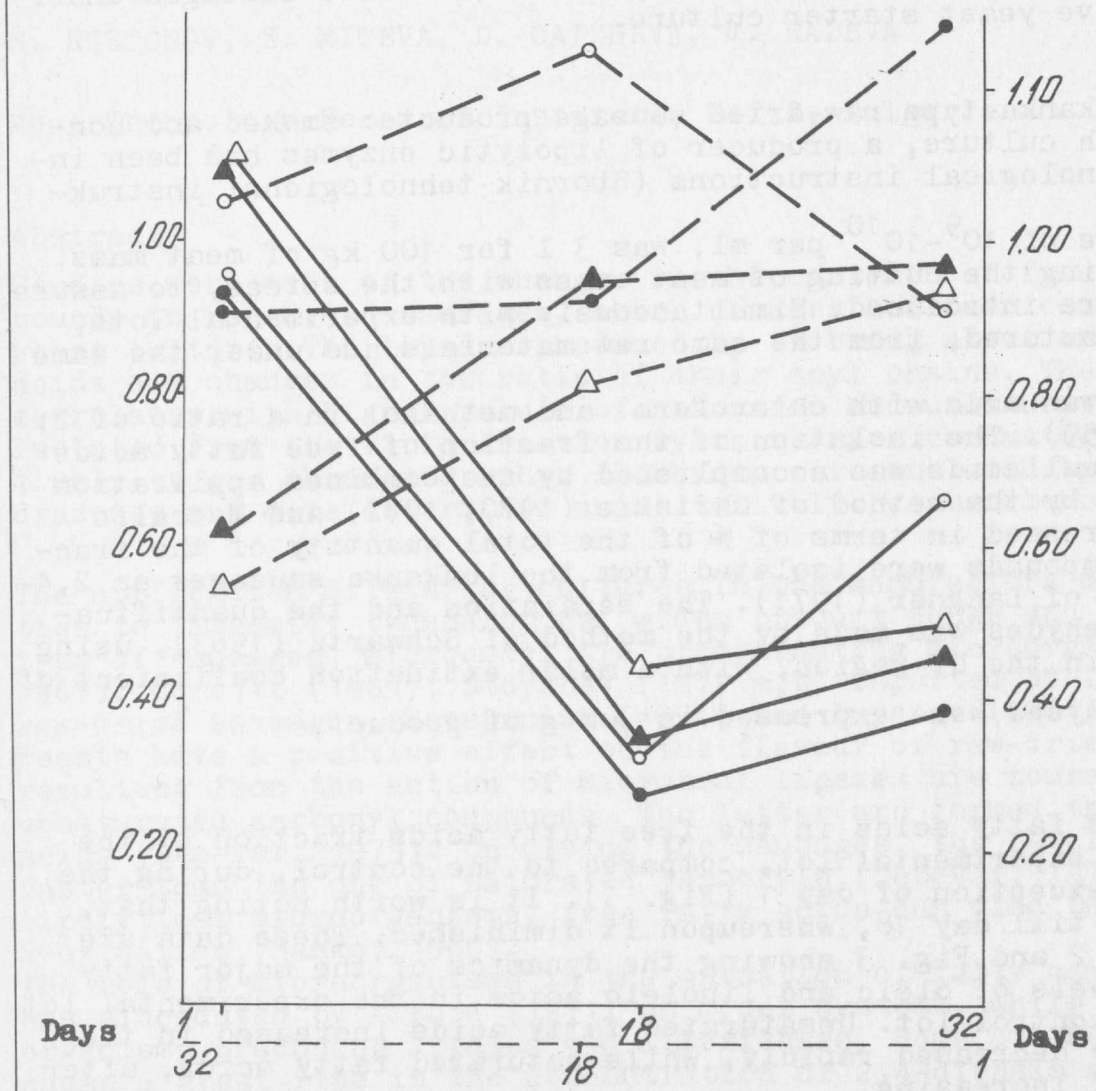

Fig. 1. Ratio of unsaturated and saturated aldehydes (—) from 1,1 to 0,28 and ratio of unsaturated and saturated fatty acids (---) from 1,29 to 0,57
● - non-smoked loukanka, without a yeast culture
○ - non-smoked loukanka, with a yeast culture
▲ - smoked loukanka, without a yeast culture
△ - smoked loukanka, with a yeast culture

till day 18. That is why the ratio of the levels of unsaturated and saturated acids estimated for day 18 in experimental lots did not show any substantial changes compared to day 1. Saturated fatty acids showed a tendency to decrease in the first days of product ripening, and to increase in the last days (Fig.4 and 5).

In the finished product, non-smoked loukanka, alkanals, 2-enals and 2,4-dienals were in larger amounts in experimental samples, compared to controls, unlike the situation in smoked loukanka (Fig. 6). In the latter, the levels of saturated and unsaturated aldehydes in the lots with a starter were lower than the relevant levels in smoked loukanka without a starter.

On analysing the results obtain-

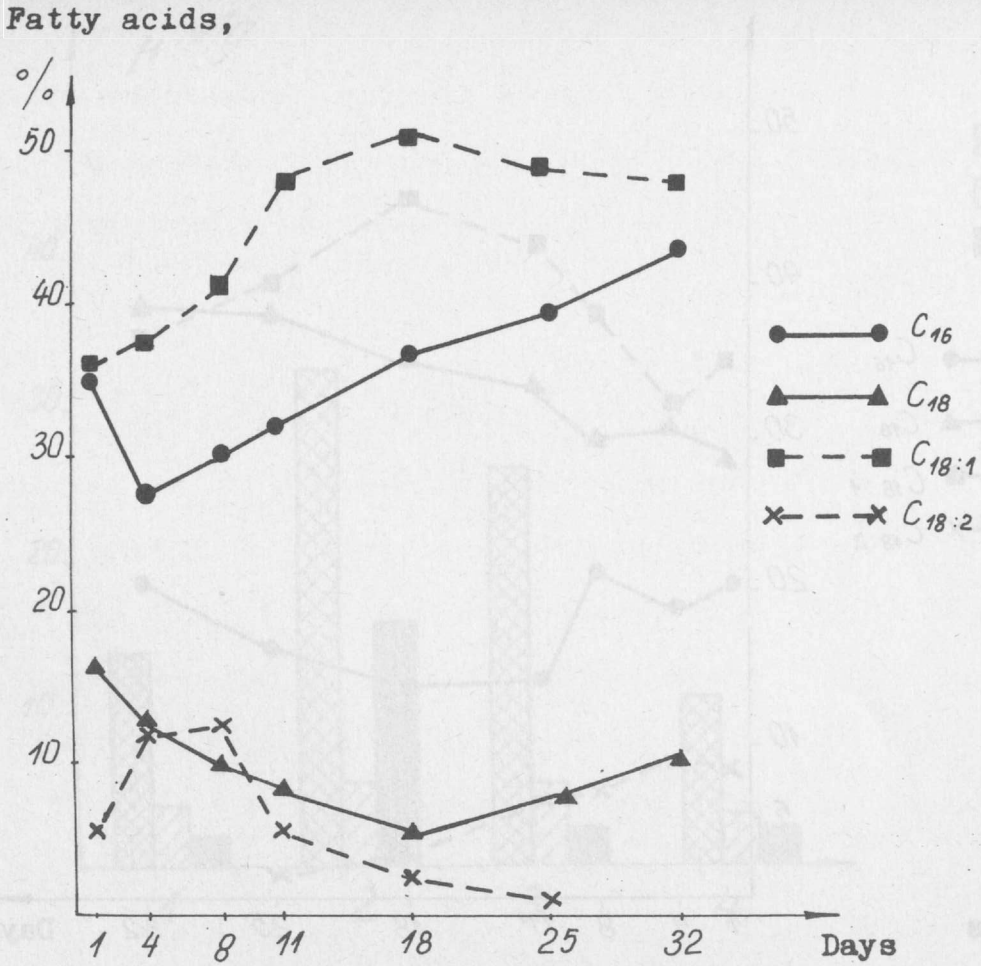

Fig. 2.

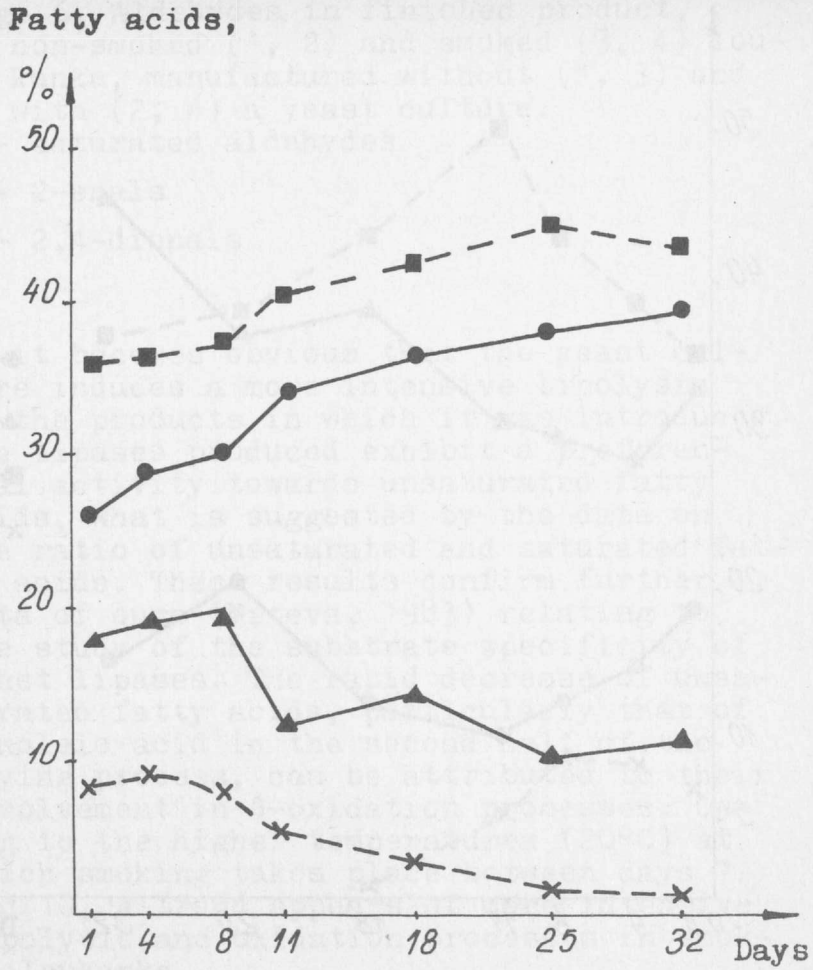

Fig. 3.

Changes in free fatty acids: palmitic (C_{16}), stearic (C_{18}), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) upon the drying of non-smoked loukanka with a yeast culture (Fig. 2) and without a yeast culture (Fig. 3).

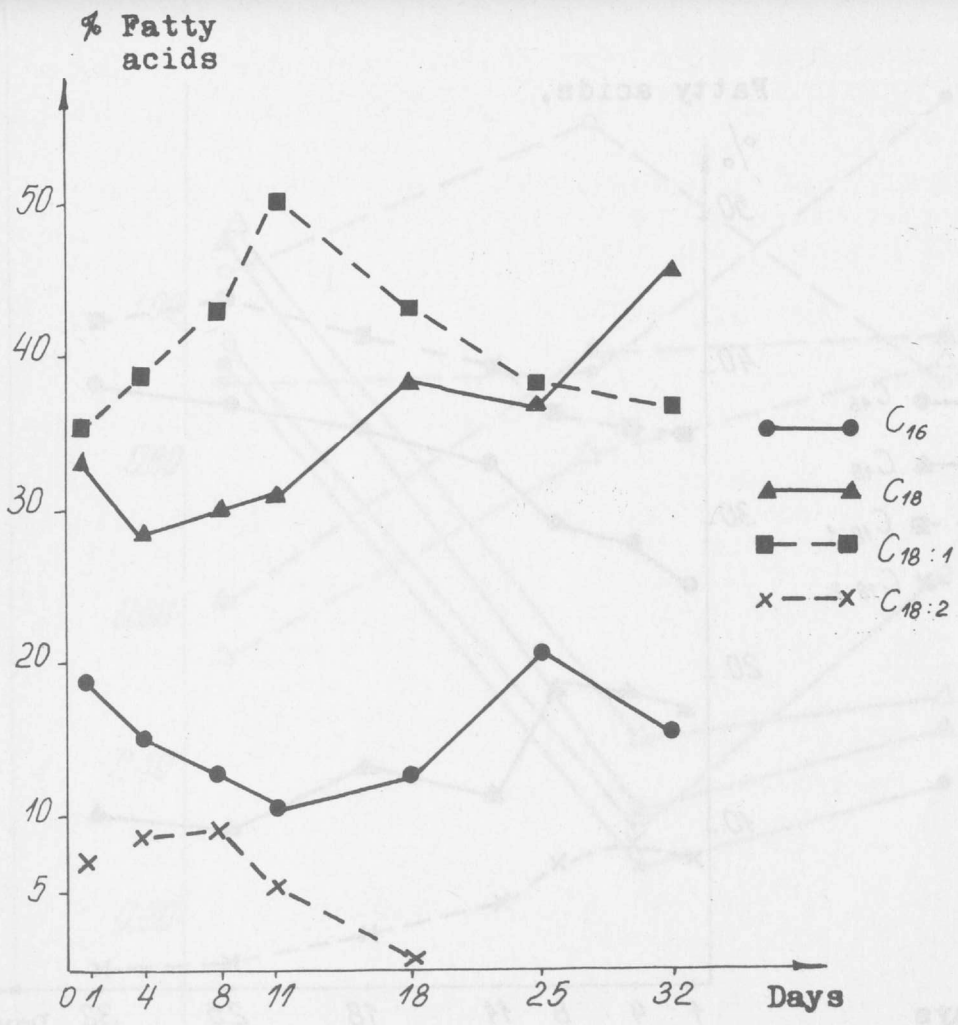

Fig. 4.

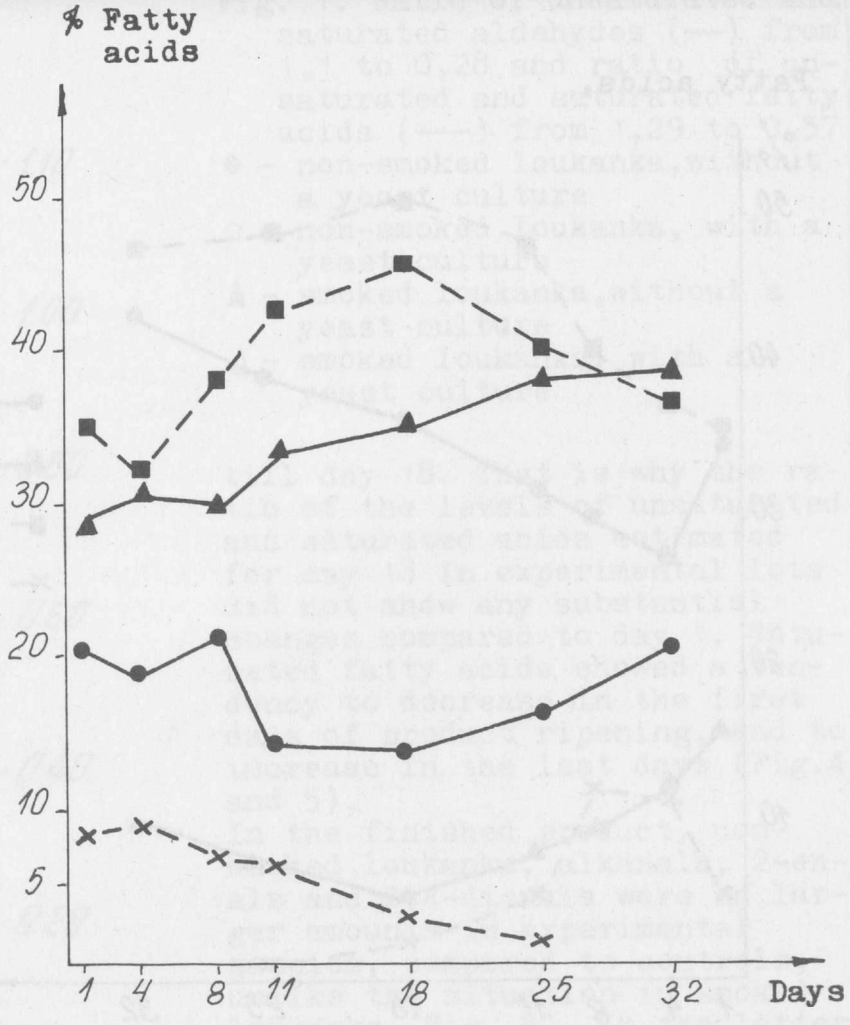

Fig. 5.

Changes in free fatty acids: palmitic (C_{16}), stearic (C_{18}), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) upon the drying of smoked loukanka with a yeast culture (Fig. 4) and without a yeast culture (Fig. 5).

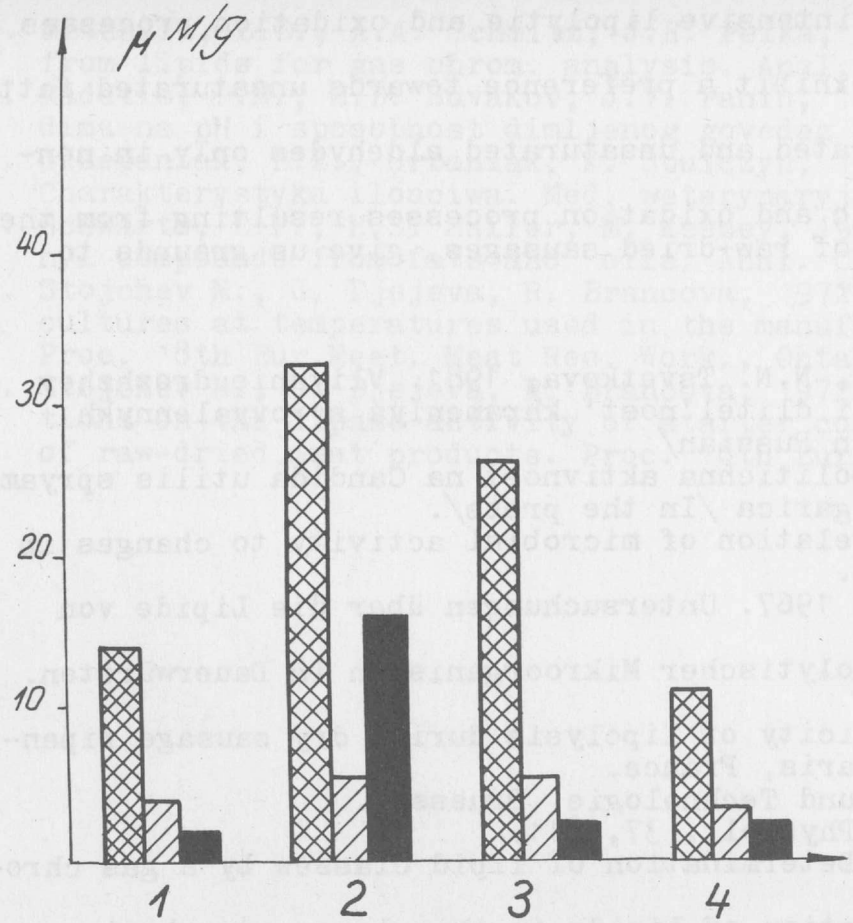

Fig. 6. Aldehydes in finished product, non-smoked (1, 2) and smoked (3, 4) loukanka, manufactured without (1, 3) and with (2, 4) a yeast culture.
▩ - saturated aldehydes
▨ - 2-enals
■ - 2,4-dienals

ed it becomes obvious that the yeast culture induces a more intensive lipolysis in the products in which it was introduced. The lipases produced exhibit a preferential activity towards unsaturated fatty acids, what is suggested by the data on the ratio of unsaturated and saturated fatty acids. These results confirm further data of ours (Miteva, 1983) relating to the study of the substrate specificity of yeast lipases. The rapid decrease of unsaturated fatty acids, particularly that of linoleic acid in the second half of the drying process, can be attributed to their involvement in ß-oxidation processes. Owing to the higher temperatures (20°C) at which smoking takes place between days 7 and 10, a trend appears of more intensive lipolytic and oxidation processes in smoked loukanka.

The higher values of unsaturated fatty acids in the non-smoked raw-dried sausage with a starter are in line with the greater amounts of alkanals, 2-enals and 2,4-dienals. In that our results confirm the findings of Alford (1971) about some species of microorganisms, producers of lipases. The lower values obtained for aldehydes in smoked loukanka, compared to the ones in the non-smoked sausage, can be attributed to the interaction of those strongly reactive compounds with the active components of smoke (Radetic, 1982). The general trend of reduction in the ratio of unsaturated and saturated aldehydes is in confirmation of the data of Debevar (1976) and is obviously due to unsaturated aldehydes being oxidised further.

On the basis of the investigations made and the results obtained, the following conclusions can be drawn:

(1) The yeast starter culture used induces more intensive lipolytic and oxidation processes in the raw-dried sausages manufactured with it.

(2) The lipases produced by the yeast culture exhibit a preference towards unsaturated fatty acids.

(3) The yeast culture induces increases in saturated and unsaturated aldehydes only in non-smoked raw-dried loukanka-type sausages.

The results obtained, of more intensive lipolytic and oxidation processes resulting from the application of yeast starter in the manufacture of raw-dried sausages, give us grounds to expect an improvement in their flavour qualities.

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