PE4.03 Study on the mechanism of the lipid peroxidation initiation in dry-fermented sausages 18.00

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Abstract - A study is carried out to determine which of the mechanisms for lipid peroxidation initiation in dry-fermented sausages is dominating. It is estimated that the prooxidative effect of the lipoxygenase complex is 0.21, and that of the metal complex is 0.13 respectively. The results obtained allowed the conclusion to be made that, the prooxidative effect of the lipoxygenase complex is 1.6 times stronger than the one of the concomitant metal complex. A conclusion is made that the lipid peroxidation processes in the dry-fermented sausages are catalyzed mainly enzyme way – most of all by peroxidases. The catalytic action of the "free" metal ions is more limited but passes in parallel with the enzyme systems.

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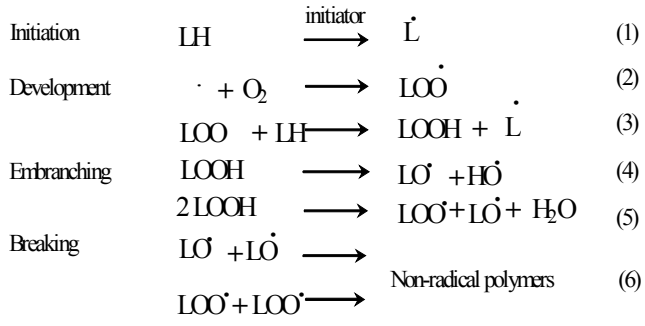
Index Terms: dry-fermented sausages, lipid peroxidation, lipoxygenases, free metal ions.

I. INTRODUCTION

HE dry-fermented sausages are predisposed to lipid peroxidation initiation and development during ripening and drying [19]. The ripening of the dry-fermented sausages is based on biochemical processes. They are connected with the meat proteolytic and lipolytic enzymes action, with the lactic bacteria number increase and their vital activity. The latter is expressed by lactic acid accumulation and medium acidification. During the dry-fermented sausages ripening a row of chemical-physic occurrences is passing as: moisture evaporation, water diffusion from the center to the surface, congestion and welding together of the filling mass, etc. [6]. The characteristic taste and distinctive flavour of the dry-fermented sausages is due to a bouquet of volatile and non-volatile low molecule chemical compounds, derived: 1) during the fermentation processes, caused by lactic micro flora development [16]; 2) as a result of proteolysis [5]; 3) due to lipolysis and lipids oxidation [2], and 4) from the spices and salting materials [8].

Reactive oxygen species is formed enzymatically, chemically, photochemically in food. It is also formed by the decomposition and the inter-reactions of reactive oxygen species [4].

The whole lipid peroxidation mechanism is chain radical. It can be described by processes of initiation, development, embranching and breaking of the chain reaction [12]. The initiation starts with hydrogen reduction and alkyl radical ($L^{\bullet}$) formation (reaction 1). This radical reacts with oxygen and peroxyl radical is formed ($LOO^{\bullet}$) (reaction 2). The latter takes away hydrogen from the fatty acids and forms hydroperoxide (LOOH), which is basic primary product of the auto oxidation (reaction 3).



where: LH – unsaturated fatty acid; $HO^{\bullet}$- hydroxyl radical;
$L^{\bullet}$ - alkyl radical; $LO^{\bullet}$ - oxyl radical; $LOO^{\bullet}$ - peroxyl radical and LOOH-hydroperoxid

Great number of potential initiators and distributors of lipid peroxidation in meat products are known, including hydroxyl radical ($HO^{\bullet}$), perferril and ferril radical, Fe^{2+}-O_2-linked radical and porphirine kation-radical ($P\text{-}Fe^{4+}=O^{\bullet}$) [12] or enzyme systems as lipoxigenases, cyclogenases, dependent on

Nicotineamide-Adenine-Dinucleotide-Phosphate NADPH), Adenosine-Di-Phosphate (ADP)-Fe^{3+} and O_2 enzymes [13, 18].

It is not completely clarified which of the various ferric forms: free or linked, chemic or non-chemic, oxidized or reduced has the ability to oxidize the poly unsaturated fatty acids in the meat and meat products [11]. One of the most important questions connected with the lipid peroxidation, concerns the primary catalysts source, which initiate and develop the process and its cycle character. Several mechanisms for free radicals initiation from the muscle tissue lipids are known (fig1). Main initiators for the poly unsaturated fatty acids oxidation on the three described reaction routes appear the oxygen species [4] and the activated catalysts [10, 20].

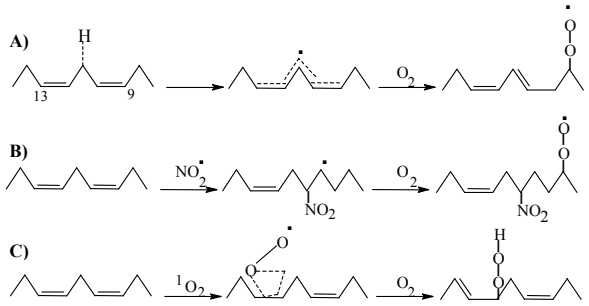


Figure 1. Lipid peroxidation initiation:
A) by reduction of hydrogen;
B) by double linkage attacking of free radicals;
C) by singlet oxygen on so called "en" reaction

As it is seen from the references review, the lipid peroxidation processes in the meat products can be initiated and spread on non-enzyme catalyzed routes, also [7].

The aim of the current study is to determine which one of the two mechanisms dominates and thus to recommend more effective methods for lipid peroxidation inhibition during the production and storage of dry-fermented sausages.

II. MATERIALS AND METHODS

A. Materials

The samples dry-fermented sausage "Manastirska lukanka" type are prepared from: 550 g/kg chilled beef type CL 95 stored 72 h *post mortem* at 0 - 4°C, frozen to -5°C, 6 h prior its grinding; 250 g/kg chilled semi-fat pork - type 50/50, stored 72 h *post mortem* at 0 - 4°C and frozen to -10°C, 6 h prior its cutting; 200 g/kg frozen to -10°C hard dorsal bacon, stored 24 h *post mortem,* prior its use; 22 g/kg salt; 0.4 g/kg potassium nitrate; 3 g/kg black pepper; 2 g/kg cumin, and 2 g/kg sweet red pepper.

To carry out the analyses distilled clear chloroform and methanol were purchased (Fluka Chemie AG, Buchs, Switzerland). The rest of the reagents were AP or GPL quality and were supplied by Aldrich Chemical Co (Steinheim, Germany).

B. Sample preparation

The setting of the experiment for investigation the mechanism for lipid peroxidation initiation in dry-fermented sausages is presented at fig2. To clarify the mechanism for lipid peroxidation initiation, the method for accelerated

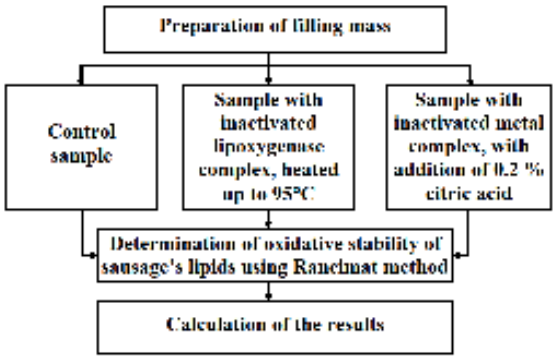


Figure 2. Setting of the experiment for study of lipid peroxidation initiation mechanism in dry-fermented sausages

determination of the pro-oxidative effect of both the lipoxygenase and concomitant metal complex was used. For the purpose two samples for each of the methods were extracted. According the recommendations of Ivanov [9], for lipoxygenase complex inactivation, heating up to 95°C was applied, while for the metal complex inactivation to the samples 0.2 % citric acid solution was added. After the lipid extraction from the sausage samples according Bligh and Dyer [3], the oxidative stability was determined according Rancimat method [17]. The pro-oxidative effect of the lipoxygenase complex was calculated as ratio between the thermally inactivated sample stability and the stability of the unheated sample.

C. Determination of oxidative stability of extracted lipids

The oxidative stability of extracted lipids from samples was presented by induction period. The induction period was determined with the Rancimat model 679 (Metrohm AG, Switzerland) [17] at 100°C and the air flow of 20 L/h. The oxidative stability was

calculated from the measured induction periods of samples.

D. Statistical analysis

Data were analyzed using SPSS for Windows, version 10.0.1 (SPSS Inc., Chicago, IL, USA). All determinations were carried out in three triplicate and data were subjected to analysis of variance. Analysis of variance (ANOVA) was made with the General Linear Models (GLM) with a significant level of $P \leq 0.05$. The Tukey's test with significant difference at $P \leq 0.05$ was used to compare sample means. Significant differences between means less than 0.05 were considered statistically significant.

III. RESULTS AND DISCUSSION

On fig3 the results from the experiments with "Manastirska" luanka samples for determination of the pro-oxidative effect of both lipoxygenase and metal complex are presented. It is estimated that after inhibition and deactivation

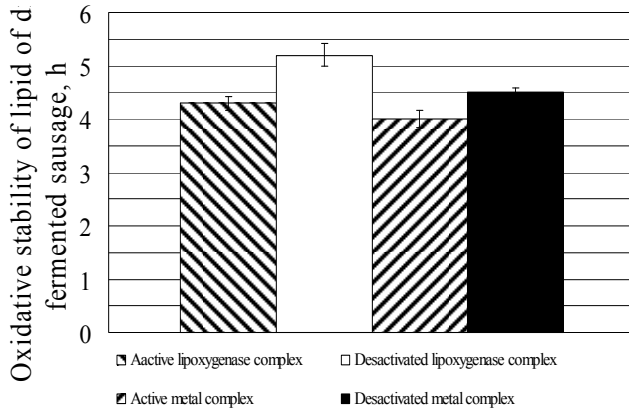


Figure 3. Oxidative stability of the lipids in dry-fermented "Manastirska" lukanka sausage samples with active and inhibited lipoxygenase and metal complexes, h

of the lipoxygenase complex, the oxidative stability of the lipids, extracted from the "Manastirska" lukanka samples, increases with 20.9 %, or the lipoxygenase complex pro-oxidative effect is 0.21 (table.1). In comparison with these results, after the metal complex inhibition, the oxidative stability of the lipids, extracted from the "Manastirska" luanka samples is increased only with 12.5 %. The pro-oxidative effect of the metal complex is estimated as 0.13, respectively. (table1).

Table 1. Pro-oxidative effect of the lipoxygenase and metal complexes in dry-fermented sausage "Manastirska" lukanka

Pro-oxidative effect of the lipoxygenase complex	**0.21**
Pro-oxidative effect of the metal complex	**0.13**

The results obtained in this way allowed to make the conclusion that the pro-oxidative effect of the lipoxygenase complex is 1.6 times stronger than the concomitant metal complex. Therefore, in the dry-fermented sausages the lipid peroxidation processes are catalyzed mainly on enzyme route. As we know, the dry-fermented sausages are not treated thermally, but are dried and ripened [7]. Due to this reason the enzyme complexes are not desactivated and continue their action during the sausage technological treatment [5]. The dry-fermented lukanka type sausages are produced from beef and pork. According Turubatović et al. [20] an imbalance between oxidative stress and the cell's anti-oxidant defense system may have effects on cell membranes through the indiscriminate oxidation of susceptible molecules such as polyunsaturated fatty acids, the main substrates for lipid peroxidation. Those investigators have suggested that the alteration in the CuZn-superoxide dismutase/ selenium dependant glutathione peroxidase plus citosolyc antioxidative defense enzymes – catalase ratio correlate well with increases in lipid damage.

The catalytic action of the "free" metal ions is more limited, but it occurs in parallel with the enzyme systems hemoprotein and non-heme iron components are active catalysts of lipid peroxidation. In raw meat, lipid oxidation is inhibited at high pH because of removal of oxygen by enzymatic reducing systems. Both heme and non-heme iron were active at lower pH values [14] such as in the beginning of the ripening of dry-fermented sausages. In raw meat systems heme pigments catalyze oxidation of tissue lipids causing a stale or rancid odor and flavor. Free radicals from lipid oxidation can oxidaze and decompose the red ferrous hemes [10]. In order the enzyme complex to be desactivated and as far as possible, the "free Fe^{2+}" ions disintegration processes to be inhibited, it is necessary the natural anti oxidative factors of muscle tissue to be preserved as active as possible [20]. That is why, the lipid peroxidation inhibition in the dry-fermented sausages must start with the pre-slaughtering treatment of the animals and to proceed during their technological processing and storage [13].

During the ripening and drying of the dry-fermented sausages, as during their consequent storage, it is recommended, as far as possible, the access of oxygen and light to be limited, and the temperature to be the lowest possible for the technology applied [19].

The above mentioned methods for the lipid peroxidation inhibition in the dry-fermented sausages production are practically inapplicable, because the contact of the raw materials and the finished dried product with the air oxygen can not be avoided. Insurmountable obstacle also appears the product to be isolated from the light. Thus the technological effects applied are least of all ineffective to inhibit the initiation, development and the spread of the lipid peroxidation. Therefore it is proposed the scientists and technologists' attention to be directed towards easily applicable in the practice methods for oxidative processes inhibition [7]. Such method is the addition of proper antioxidants to the dry-fermented sausages filling mass, which are effective regarding both enzyme and non-enzyme catalytic pro-oxidative factors.

IV. Conclusions

Practical conclusions for initiating factors inhibition are made. In order to deactivate the enzyme complex and as far as possible to inhibit the processes of Fe^{2+} and Cu^{2+} ions dissociation, it is recommended the natural anti oxidative factors of the muscle tissue to be preserved as more active as possible.

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