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QUALITY DETERMINATION OF VEGETABLE OILS USED AS AN ADDITION TO FERMENTED MEAT PRODUCTS WITH DIFFERENT STARTER CULTURES

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ABSTRACT

There were developed samples of fermented meat products of „Mettwurst“ with an addition of a starter culture *pentosacceus* AS-3/100 or probiotic culture *Lactobacillus casei* Lc-01 and its combinations for this thesis. A part of animal fat was replaced with vegetable oils – sunflower oil and rapeseed oil. For comparison, there was also used a sample without an addition of vegetable oil. There were determined the characteristics of fats in samples: saponification value, acidity value, esteric, iodine and peroxide value. The samples were determined on the day of production and always once a week in a period of three following weeks. Every single sample was hereby determined 3 times. According to the results, it is more advantageous to use the samples with sunflower oil with an addition of specific cultures *Lactobacillus casei* Lc-01 and *Pediococcus pentosaceus* AS-3/100. The saponification value when adding sunflower oil detects that the quality of fat remains stable till the 14th day of storage (p <0.05). The comparison of acid value detects that a sample with sunflower oil and culture *Pediococcus pentosaceus* AS-3/100 is more advantageous due to fast acidification in the first half of storage period. Good results of iodine and peroxide value had the variation of a sample with sunflower oil and a combination of both cultures. The variation of peroxide value maintained the lowest values. By using the samples with sunflower oil and unispecific cultures *L. casei* Lc-01 and *P. pentosaceus* AS-3/100, the culture *P. pentosaceus* AS-3/100, which remained stable till the 14th day of production, reached the best values of peroxide value. The sunflower oil is in spite of high content of PUFA more stable to which also contributes the increased content of vitamin E that works as an antioxidant here. The disadvantage of rapeseed oil is its higher susceptibility to oxidation. For reasons of faster decomposition of vegetable oils would be essential to cut down on the minimum durability. From the 14th day of storage, the content of free FA increases and the fat is still considerably quickly oxidized. The content of unsaturated FA, of which the vegetable fat is a source, quickly decreases. The sensory quality simultaneously decreases, too.

Keywords: Mettwurst; lactic acid bacteria; probiotics; human health; animal fat

INTRODUCTION

The medical organizations all over the world suggest that the total intake of fats should be lower than 30% **(Arhiara, 2006; Fernández-Ginés et al., 2005)**. They also recommend the reduction of intake of SFA (saturated fatty acids) and cholesterol which means the cardiovascular diseases prevention.

Fermented meat products as a „biologically preserved food“ have indispensable place in human nutrition **(Campos et al., 2013; Vandendriessche, 2008)**. There is a complex of biochemical and physical reactions during the fermentation that results in significant changes in the properties of final products **(Casaburi et al., 2007)**. Fermented sausages represent a special group of fermented products **(Romero, 2013; Evans et al., 2004)**. They mature less than 14 days and their spreadability is given by a high fat content. By fermentation the pH drops to 5.6 or 5.4, products do not dry they are only smoked by cold smoke. The products are not very resistant to microbial development, especially when the chain of low temperature is violated **(Buckenhuskes and Fischer, 2001; Rodel and Scheuer, 2001)**. The main raw materials for fermented meat products are pork and beef and pork lard. Selecting raw materials and processing technology can affect the amount of fat in meat products **(Del Nobile et al., 2009)**. Opinions of the need to reduce the amount of fat in the diet associated with promotion of low-fat and also often high-calorie products still remain. Health is not only reduction of fat intake, but choosing the right fats. By changing the lipid content and profile could be improved nutritional quality of so-called Western diet **(Arihara, 2006)**, because some studies have made efforts to replace animal fat with vegetable oils **(Cáceres et al., 2008)**. Vegetable oils are liquid non-hydrogenated oil, and therefore do not contain significant amounts of trans fatty

acids (FA) **(Scollan et al., 2006)**. Type of vegetable oil used in meat products influenced the composition of fatty acids. Vegetable oils are rich sources of MUFA (mono-saturated fatty acids) and PUFA (poly-unsaturated fatty acids) and cholesterol-free. In addition to increasing the fatty acids in the products, vegetable oils contain other bioactive ingredients such as antioxidants **(Pelser et al., 2007; Chasco et al., 1993)**. Important microorganisms in fermented sausages ripening quickly are mainly lactic acid bacteria of the genus *Lactobacillus* and *Pediococcus*. At the beginning of ripening the number is low however, it multiplies rapidly and pushes against competing microflora. Lactic acid bacteria play an important role in the production of fermented meat products **(Mati et al., 2015; Tripathi and Giri, 2014)**. They participate in the creation of sensory active substances involved in the development of texture, color, smell and taste **(Leroy and De Vuyst, 2004)**. Application of starter and probiotic cultures in fermented products may provide additional opportunity to prevention by food pathogens in salami **(Santos et al., 2017; Gioia et al, 2016; Mainar et al., 2016; Corbière Morot-Bizot et al., 2006)**. The aim of an addition of suitable probiotics is to increase the frequency and thus the competitiveness of such microorganisms, which has positive effects on human health **(Ruiz-Movano et al., 2008)**.

The aim of this work was to determine quality and stability of fat in fermented meat products "Mettwurst" with an addition of different starter and probiotic culture for 21 days.

MATERIAL AND METHODOLOGY

Samples of spreadable fermented meat sausage „Mettwurst" were used to determine the qualitative characteristics of fat. The basic composition of "Mettwurst" was pork and back fat (50%), beef (45%),

Figure 1 Emulsion rapeseed (left) and sunflower (right) oil.

Figure 2 Fermented „Mettwurst" before heat treated.

Table 1 Combination of oils and microbial cultures in the samples of fermented meat product „Mettwurst".

Samples	Sampling (days)				Used vegetable oils and microbial cultures
R/L.	0	7	14	21	rapeseed oil / *Lactobacillus casei* Lc-01
R/P.	0	7	14	21	rapeseed oil / *Pediococcus pentosaceus* AS-3/100
R/P.+L.	0	7	14	21	rapeseed oil / *Pediococcus pentosaceus* AS-3/100 + *Lactobacillus casei* Lc-01
S/L.	0	7	14	21	sunflower oil / *Lactobacillus casei* Lc-01
S/P.	0	7	14	21	sunflower oil / *Pediococcus pentosaceus* AS-3/100
S/P.+L.	0	7	14	21	sunflower oil / *Pediococcus pentosaceus* AS-3/100 + *Lactobacillus casei* Lc-01
control	0	7	14	21	control sample

sucrose (0.1%), nitrite salt (2.46%), 0.43% of ground black pepper, capsicum and garlic, starter culture *Pediococcus pentosaceus* AS-3/100 (Almi, Austria) (0.25%) and probiotics *Lactobacillus. casei* (Sacco, Italy) (0.02%). The starter culture and probiotic culture were added to achieve 10^6 at minimum cfu.g^{-1} in sausage mixture. Our samples were made of pork (side, leg, shoulder, in fat up to 5%), oil emulsions, nitrite salt (2.46%), food ingredients - Teewurst fein 12, BIO-ELEMNETA 161, BGP LYOFAST 93 (5 Dosi), starter culture *Pediococcus pentosacceus* AS-3/100 (Almi, Austria) in the amount of 0.25% and probiotic culture *Lactobacillus casei* Lc-01 (Sacco, Italy) in the amount of 0.02%. The samples were produced in combination with an addition of vegetable oils - sunflower oil Lukana (Setuza, JSC) and rapeseed oil Vita, cold pressed (P. Brandl GmbH) (Figure 1 and Figure 2). For comparison, a sample without an addition of vegetable oils was used as well (Table 1). The work was subsequently thrust into the guts with a diameter of 43 mm, type N43 Nalo Krans, each in quantities of 1 kg. The products were smoked by cold smoke in a temperature of 20 ±0.5 °C intermittently for 2 days and stored in a temperature of 6 ±0.5 °C. For each experimental and control group was made 20 kg of meat products in 1 kg pieces. Random sampling were selected 10 pieces from samples and control group and analysis were carried out in three replications after the production and after 7, 14 and 21 days of production.

Extraction

For extraction it was used Soxhlet extractor with petrolether (Sigma-Aldrich, USA) at 50 °C. The rest of petrolether in samples was evaporated by nitrogen (Linde, Czech Republic, purity 99.996%) at room temperature.

Saponification value

The saponification value is an amount of potassium hydroxide needed to neutralize the free and bound fatty acids in 1 g of fat. A sample is saponified by boiling with an excess of alcoholic potassium hydroxide solution and an excess of KOH was set by the back titrating with hydrochloric acid to phenolphthalein **(Knothe, 2002)**.

Acid value

The acid value indicates the content of free fatty acids in fat and is expressed as an amount of potassium hydroxide required to neutralize 1 g of fat under the terms of the method. The sample was dissolved in hot ethanol and titrated with standard potassium hydroxide solution to phenolphthalein **(Gunstone et al., 2007)**.

Ester value

The ester value is an amount of potassium hydroxide required to neutralize the ester-bound acids in 1 g fat.

Iodine value

The iodine value indicates an amount of iodine (mg) which is bound to 100 g fat under the terms of the method. It is a measure of the content of double bonds in fat and is used to assess its purity, identification of unknown lipids and the applicability for different purposes. Halogens are requires to double bonds of unsaturated fatty acids. The reaction takes place in chloroform which acts as a fat solvent and acetic acid which provides the necessary environment polarity **(Haryati et al., 1998)**.

Peroxide value

The peroxide value indicates an amount of hydroperoxides in fat which are able to oxidize iodide into iodine under the terms of the method. It is expressed in milligrams of oxygen in 1 kg of fat and is a suitable measure for the degree of oxidation of fats **(Aksu et al., 2007)**.

Statistical analysis

The results were processed by using statistical functions in MS Excel to calculate the arithmetic mean and standard deviation. For the statistical evaluation method was used the method of linear regression (p <0.05) in program Statistic CZ 12.0 by Duncan's test.

RESULTS AND DISCUSSION

The measured values of acid value of „Mettwurst“ with an addition of rapeseed and sunflower oil and cultures *Pediococcus pentosaceus* AS-3/100, *Lactobacillus casei* Lc-01 and their combinations for storage of 21 days from production are shown in Figure 3.

The samples with an addition of rapeseed oil with culture *Lactobacillus casei* Lc-01 and the mixed culture of *Pediococcus pentosaceus* AS-3/100+*Lactobacillus casei* Lc-01 show direct growing dependence of acid value of „Mettwurst“ ($p = 0.0006$; $r^2 = 0.1358$). The content of fatty acids in these two samples grows slowly, for the other combinations in which the content of free fatty acid significantly decreases or increases in steps in relation to the production of other metabolites, possibly due to enzyme degradation of fatty acids, which is reflected in the final stage of maturation (week 3rd) (p <0.05). The content of free fatty acids in the samples with an addition of sunflower oil at a time abruptly decreases, increases and then decreases again, due to biochemical changes caused by enzymes. This trend is evident when using the culture of *Pediococcus pentosaceus* AS-3/100. The most significant changes in free fatty acids between the first and the third week of storage recorded the sample with the addition of sunflower oil and a combination of cultures *Pediococcus pentosaceus* AS-3/100+*Lactobacillus casei* Lc-01 (p <0.05). The control sample used in animal fat has a higher resistance to enzymes ripening cultures. The slight reduction in acid value at the end of shelf life explains **Koutsopoulos et al. (2008)** in fermented sausages probably due to formation of ammonia and amines that arise by the reactions associated with the metabolism of free amino acids during proteolysis as well as enzymatic digestion of fatty acids. The measured values for the determination of saponification value of „Mettwurst“ with added sunflower oil and rapeseed oil and cultures *Pediococcus pentosaceus* AS-3/100, *Lactobacillus casei* Lc-01 and their combination for 21 days from production are shown in Figure 4.

Generally, the saponification value decreases at the last stages of storage with an increasing number of free fatty acids, which grows faster than in the sample without an

addition of vegetable (p = 0.0014; r^2 = 0.1182). The high saponification value characterizes the quality and no left fat. The reduction of the saponification value agrees with the study in which **Johansson et al. (1994)** states that the content of free MK was in the early ripening of sausages low (0.6%), gradually increased and at the end of storage (after 63 days) reached values of 6.8%. **Beriain et al. (2000)** evaluated the fermented sausages with added cultures *Pediococcus pentosaceus* and *Micrococcus varians* and states that the concentration of free FA has gradually increased since the beginning of measurements (raw materials) to the final product. The rapid increased was recorded during the two weeks of fermentation and indicated a high enzymatic activity in this phase. These results could reflect declining saponification value of „Mettwurst". Statistically, it was demonstrated that the samples with the addition of rapeseed oil were not changed depending on the time (p >0.05). When using sunflower oil, the saponification value statistically significantly decreases (p <0.05) depending on the time only for a sample with a combination of *Pediococcus pentosaceus* AS-3/100+*Lactobacillus casei* Lc-01 cultures.

The ester value as well as the saponification value decreases with an increasing number of free FA. When using vegetable oils in the second half of the shelf life values suddenly decrease. Nevertheless, when using only animal fats, the ester value gradually decreases suggesting a slower breakdown of fat (Figure 5).

When using sunflower oil, the ester value statistically significantly decreases (p <0,05) depending on time only for a sample with a combination of cultures of *P. pentosaceus* AS-3/100 + *L. casei* Lc-01. Statistically, it has been demonstrated that the ester value of samples with the addition of rapeseed oil depending on the time change (p = 0.0002; r^2 = 0.1590). The measured values for iodine value determination of „Mettwurst" samples with an addition of sunflower and rapeseed oils and cultures *P. pentosaceus* AS-3/100, *L. casei* Lc-01 and its combinations for 21 days of production are shown in Figure 6.

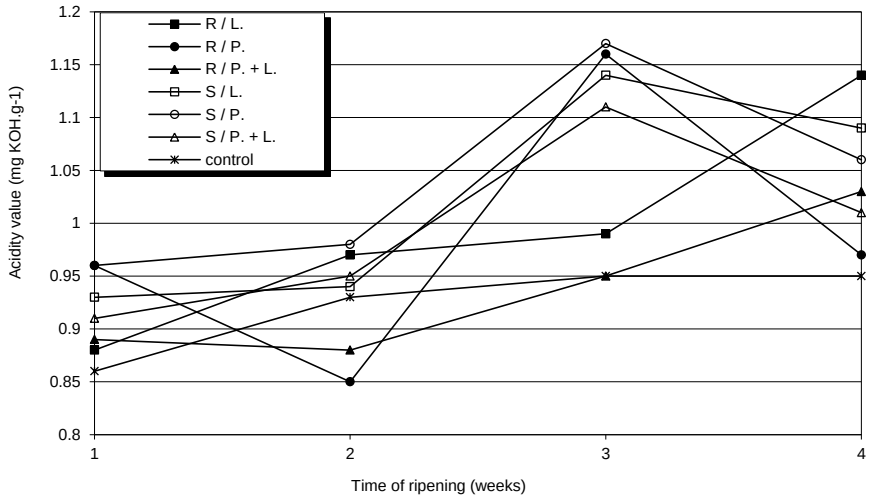

Figure 3 Acid value in mg $KOH.g^{-1}$ at the sample of „Mettwurst" with an addition of sunflower and rapeseed oil depending on the time of ripening.

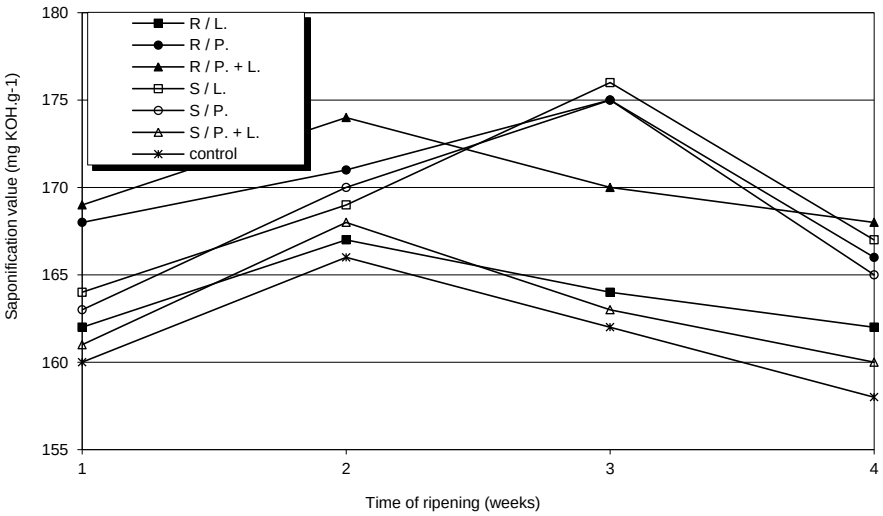

Figure 4 Saponification value KOH $mg.g^{-1}$ in the samples „Mettwurst" with an addition sunflower and rapeseed oil depending on the time of ripening.

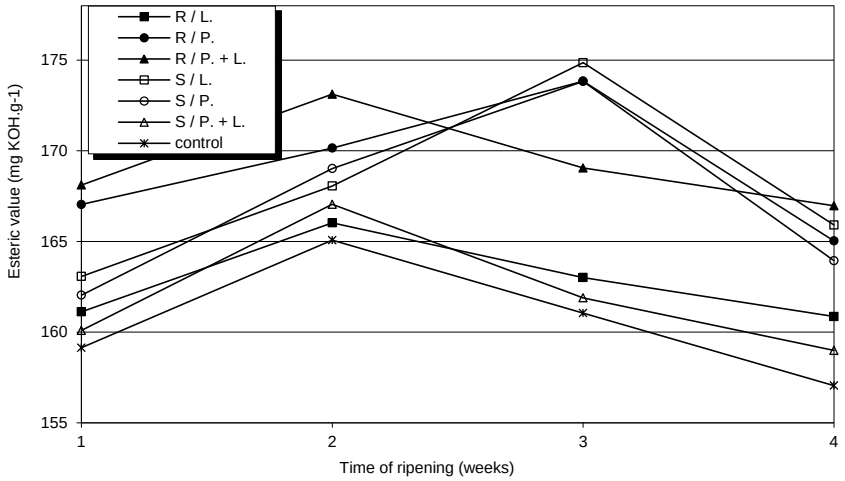

Figure 5 Esteric value mg KOH.g^{-1} in the samples „Mettwurst“ with an addition sunflower and rapeseed oil depending on the time of ripening.

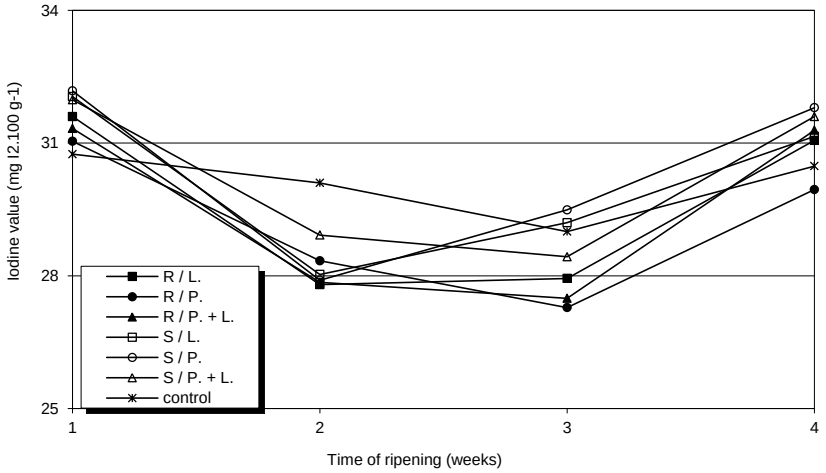

Figure 6 Iodine value mg I_2.100g^{-1} in the samples „Mettwurst“ with an addition sunflower and rapeseed oil depending on the time of ripening.

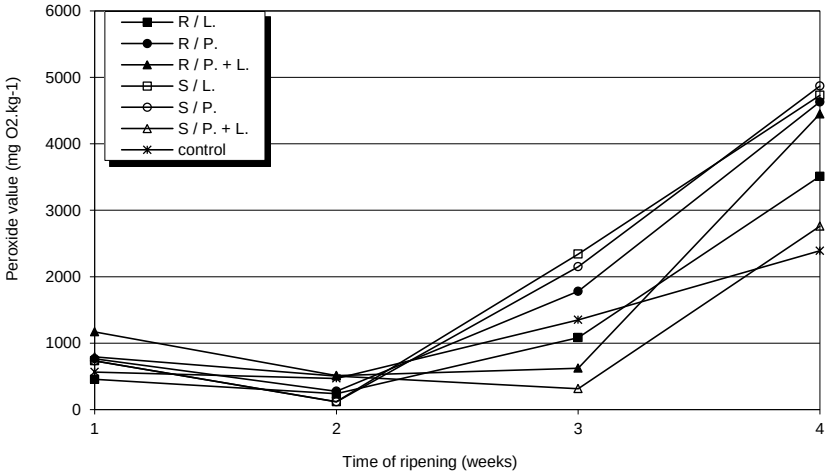

Figure 7 Peroxide value mg O_2.kg^{-1} in the samples „Mettwurst“ with an addition sunflower and rapeseed oil depending on the time of ripening.

Iodine value during the first week of storage has plummeted, at the end of storage time again slightly increased (p = 0.0033; r^2 = 0.1006). This can be explained by the increase of oxylabile substances during the storage, resulting starter and probiotic cultures. **Chasco et al. (1993)** in his work indicates that the initial fermentation is responsible for the increase of unsaturation, however, it varies later in the maturation of meat products. There is a reduction of MUFA and simultaneously an increase of the content of SFA. Oxidation processes particularly affect the unsaturated linoleic and oleic acid. These changes in unsaturation of lipid are reflected in the iodine value which shows the same behavior, increases with the development of microbial fermentation and decreases with maturation. Statistically, it has been shown that the iodine value for all samples depending on the time does not change (p >0.05). The best values are achieved with a combination of sunflower oil with cultures *P. pentosaceus* AS-3/100+*L. casei* Lc-01 or the use of of *L. casei* Lc-01 cultures. The most apparent change of samples was in the peroxide value during the storage of samples „Mettwurst“, (Figure 7).

The high and rapid increase of the peroxide value in samples of vegetable oils reflects easier oxidation of vegetable oils in comparison with the control sample made only from raw meat. Increasing peroxide value depending on the time for all samples was statistically significant (p <0.05). Starter culture also affects the pH of the product, which length storage (fermentation) decreases. **Del Nobile et al. (2009)** shows the initial pH about 6.5 and decline to 5.0 after 22 days from production fermented sausages with the addition of extra virgin olive oil. The same changes also states **Bloukas et al. (1997)** in fermented meat products with a fat content of 25%. There were 10% and 20% lard replaced with olive oil in emulsion with the addition of soy protein isolate.

Auto-oxidation of unsaturated free fatty acids is one of the main reactions associated with the formation of volatile compounds. Some of them contribute to the specific flavor of meat products. **Ansorena and Astiasarán (2004)** demonstrated that it is possible to replace a part of the animal fat with vegetable oils containing n-3 fatty acids.

Pelser et al. (2007) in his work reduced the content of SFA by 20% in fermented sausage by adding of linseed oil and canola oil (*Brassica napus* var. *Napus*) compared to the control sample without an addition of vegetable oils. The low amount of palmitic and stearic acid in linseed and rapeseed oil is responsible for this effect. The products with linseed and rapeseed oil have increased the amounts of PUFA, especially sausages with linseed oil. The similar conclusions also stated **Ansorena and Astiasarán (2004)**. Peroxide value is higher in oils containing more n-3 FA due to easier oxidation **(Pelser et al., 2007)**. That would explain larger values of peroxide in rapeseed oil which contains linoleic acid, unlike sunflower oil which contains almost no linoleic acid **(Gunstone et al., 2007)**.

The susceptibility to oxidation of linolenic acid is 2.4 times higher than linoleic acid, linoleic acid is 40 times more reactive than oleic acid. The similar ratio of linolenic and linoleic acid would correspond with the results of the combination of cultures *P. pentosaceus* AS-3/100+*L. casei* Lc-01 between rapeseed and sunflower oil (p <0.05).

When using sunflower oil, it is preferable to use a combination of cultures *P. pentosaceus* AS-3/100+*L. casei* Lc-01 for the lowest peroxide value. The most stable sample was the one with the culture of *L. casei* Lc-01 with an addition of rapeseed oil. From the perspective of a lower susceptibility to oxidation the use of sunflower oil, which suggests a lower value of peroxide of sunflower oil samples against samples with an addition of rapeseed oil, is more advantageous (p = 0.0001; r^2 = 0.5737).

Pelser et al. (2007) decreased the content of SFA by 20% in fermented salami. **Muguerza et al. (2001)** decreased in fermented salami the content of fat by 20% and 10% in the study (unlike the value of 30%) and replaced 20% of animal fat with olive oil. Reduction of the whole fats and the replacement of animal fat is hereby in meat products possible, however, it is essential to still perfect it. **Rubio et al. (2008)** and **Sheard et al. (2000)** reported that a high content of MUFAs and PUFAs causes greater susceptibility to oxidation and hence it is necessary to adjust the length of the shelflife and storage condition. Oxidation of lipids may affect the sensory characteristics of the products and participate in the creation of aroma, flavor, juiciness and texture **Muguerza et al. (2001)**.

CONCLUSION

The aim of this work was to determine quality of fat in fermented meat products “Mettwurst” with an addition of starter or probiotic culture and its combinations. Part of the animal fat has been replaced by sunflower and rapeseed oil. The determined characteristics of fats were saponification, acidity, ester, iodine and peroxide value. The samples were determined in a period of three following weeks. According to the result, using sunflower oil with culture *Pediococcus pentosaceus* AS-3/100 or *Lactobacillus casei* Lc-01 is preferable. Good results of peroxide value were reached also in „Mettwurst“ sausage with added sunflower oil and starter P. pentosaceus AS-3/100. It was fixed till 14th day from producing. This variation acidified the sample enough in the first week of storing. Due to faster decomposition of vegetable oils it would be appropriate to shorten the minimum shelf life. From the 14th day of the storage period, the content of free FA rises and the fat is rather rapidly oxidized (p <0.05).

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