

M. Longissimus and M. Gluteobiceps of pork meat are given in Figure 5.

CAT activities in both groups M. Longissimus and M. Gluteobiceps were higher but not significantly compared to the M. Subscapularis (mean 10.52 ± 1.32 U/ml, and 11.225 ± 3.09 U/ml vs 10.03 ± 3.41 U/ml, $p > 0.05$). There was not significant difference between CAT activities in M. Longissimus and M. Gluteobiceps ($p > 0.05$).

4. Discussion

Variations in the oxidative stress parameters and the activities of antioxidant enzymes between different muscle types could lead to differences in oxidative stability of the meat, but direct evidence is scarce. In the current study we investigated and compared: 1) some Real time biomarkers of radical formation such as ROS products and Ascorbat radicals by EPR spectroscopy technique, 2) levels of MDA stable products of lipid peroxidation and 3) activities of the antioxidant enzymes SOD and CAT in different muscles (M. Subscapularis, M. Longissimus and M. Gluteobiceps) of pork meat.

At certain stages of the course of lipid peroxidation formed a variety of unstable radical species that can be measured in real time [22]. Short-lived unstable radicals which are formed during peroxidation can be proved only by EPR spectroscopy combined by spin trapping technique. [23]. For the assessment of the oxidative status of pork meat, we used PBN as a spin trapping agent. Despite, PBN does not exhibit specificity towards the different unstable radicals, stability of its spin adducts is rather high and so that, at present it is widely used in the *in vitro* and *in vivo* spin trapping by EPR spectroscopy. In all meat samples was registered EPR spectrum of the PBN spin adduct representing a typical sextet (not shown). Based on the calculated values of the hyperfine splitting constants (a_N and $a_{H\beta}$) of the registered PBN adducts can be determined whether the radicals trapped by PBN are carbon-centered (PBN•R) or oxygen-centered (PBN•OR). Since, our samples were prepared in DMSO solution of PBN at aerobic conditions and a_N and $a_{H\beta}$ values measured were 13.88 G and 2.35 G correspondingly the free radical species were identified as secondary oxygen centered alkoxy radicals, which resulting from the attack of the primary oxygen-centered radicals towards membrane phospholipids. Our results show a statistically significant higher level of ROS products in M. Subscapularis and M. Gluteobiceps compared to the M. Longissimus ($p < 0.01$), which means that the oxidative processes are stronger in favor of the former.

Another confirmation about "a real-time" oxidative stress availability in this study were statistically higher levels of Asc• radicals, found in both M. Subscapularis and M. Gluteobiceps comparing to the M. Longissimus ($p < 0.01$). In the living organisms, endogenous biomolecules such as ascorbic acid act as antioxidants and can be modified by pathologically generated ROS products in stable organic radicals. For the first time Buettner and Jurkiwicz, [24] proposed the intensity of EPR spectrum of Asc• radical to be

used as an indicator of pro-oxidative changes in biological systems. Since then, Asc• radicals are detected in a variety of biological samples [25], [26]. Ascorbate anion AH, is an endogenous soluble antioxidant presenting in biological systems, and its oxidation produces ascorbate radical. Endogenous ascorbic acid can be oxidized by ROS to a stable ascorbate radical and the last can be detected by direct EPR spectroscopy which is the only method does not interfere with the biochemical processes [27]. In the present study the elevated levels of Asc• radicals established in both M. Subscapularis and M. Gluteobiceps were in accordance with the elevated levels of ROS products measured for the same groups of muscles. Since, ascorbate radicals are real time biomarkers for *in vivo* generated toxic reactive radical species, it is obvious that either in M. Subscapularis and M. Gluteobiceps at the time of the study oxidative processes are still in progress.

Lipids are an important component of meat and contribute to several desirable characteristics of meat and meat products. Lipids are important to enhance the flavour and aroma profile of meat and also increase the tenderness and juiciness of meat. However, it is generally accepted that lipid oxidation is the primary process responsible for quality deterioration of meat during storage. Quality characteristics affected in meat by lipid oxidation include flavour, colour, texture and its nutritional value. The development of rancidity in meat by lipid oxidation begins at the time of slaughter and continues during storage [28]. Our results for final products of oxidation of lipids showed that all studied muscle groups have equal level for lipid peroxidation MDA ($p > 0.05$). Not significant higher levels of MDA products were detected in M. Gluteobiceps and M. Longissimus than in M. Subscapularis ($p > 0.05$). However, the antioxidant enzyme activities of SOD and CAT were also high that might be due to increased capacity of antioxidant enzyme system in the fresh pork meat.

Thus, we suggest the presence of increased oxidative stress in M. Subscapularis and M. Gluteobiceps that could possibly be in response to the production of ROS and need SOD and CAT for detoxication. The increased oxidative stress existed was accompanied with relatively sufficient increased of antioxidant enzyme system. These findings indicate a protective role for antioxidant enzymes SOD and CAT against oxidative stress in meat.

Based on the present study, is obvious that in a bigger risk of oxidative injury are both M. Subscapularis and M. Gluteobiceps, due to the high production of ROS in these muscles. The oxidation process in these muscles could reduce meat quality by a number of ways, including off-flavour formation, drip loss, color changes etc. and could strongly enhanced during cooking and storage of the meat.

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