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# Oxidative/Antioxidant Status of Different Muscles of Fresh Pork Meat

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**Abstract:** ***The main aim of the work is to investigate and compare oxidative/antioxidant status in different muscles (M. Subscapularis, M. Longissimus and M. Gluteobiceps) of fresh pork meat through some Real time biomarkers of radical formation, levels of stable products of lipid peroxidation and activities of antioxidant enzymes superoxide dismutase and catalase. Portions from fresh pork meat 2–3 h post mortem were washed thoroughly under running water, deboned, minced and used for analysis. Based on the received results 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 and could strongly enhanced during cooking and storage of the meat.***

**Keywords:** ***lipid peroxidation, free radicals, antioxidants***

## 1. Introduction

Reactive oxygen species (ROS), such as $O_2^{\cdot -}$, $H_2O_2$, $OH^{\cdot}$, are highly reactive species generated by the biochemical redox reactions as a part of the normal cell metabolism. Exposure to environmental factors, such as UV light, cigarette smoke, environmental pollutants and gamma radiation, accelerates the generation of ROS [1]. Some exogenic compounds including drugs can result into increased production of free radicals [2]. In addition, it is found excessive generation of ROS by monocytes during phagocytosis of foreign particles such as bacteria, old cells and foreign particles [1]. Low levels of ROS are indispensable as mediators in many cell processes, including differentiation, cell cycle progression or the growth arrest, apoptosis and immunity [3], [4]. In contrast, high doses and/or inadequate removal of ROS result in oxidative stress which may cause severe metabolic malfunctions and damage of biological macromolecules [5], [6]. To prevent the damages caused by the ROS, multiple defense systems, collectively called antioxidants, are present in human serum, erythrocytes as well as in the tissues [7], [8]. Oxidative processes in meat lead to quality deterioration. Flavor is an important quality attribute of muscle foods. The flavor of meat is subject to variability due to both intrinsic and extrinsic factors and it affects the overall acceptability of foods. These factors are of utmost importance because they influence the judgement of the consumer, even before the food is consumed [9]. The shelf life and acceptability of processed, ready-to-eat uncured meats is limited because of the rapid onset of rancidity, denoted as WOF.

To avoid or delay the autoxidation process in the meat, synthetic and natural antioxidants have been successfully utilized [10], [11]. Meat has endogenous antioxidants and prooxidants and living cells have several mechanisms of protection against oxidative processes, including antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). SOD plays an important role in protecting against damage by the superoxide anion radical [12]. Catalase and glutathione peroxidase (GSH-Px) are considered the major peroxide-removing enzymes [12], [13]. Information on factors influencing the activity of antioxidant enzymes in meat is limited. Antioxidant enzyme activities differ between meat of different species [14] and muscle type [15]. Antioxidant enzyme activity could also vary between animals of a single species. Therefore, variations in the activity of these enzymes between different muscle types could lead to differences in oxidative stability of the meat. Muscle lipases and phospholipases contribute to the hydrolysis of the lipid fraction releasing free fatty acids and related free radicals. Differences in the activity of these enzymes could result in different concentration of flavor precursors and, consequently, differences in flavor development [16].

In the current study we investigated and compared to our knowledge for the first time oxidative/antioxidant status in different muscles (M. Subscapularis, M. Longissimus and M. Gluteobiceps) of fresh pork meat through some Real time biomarkers of radical formation; levels of stable products of lipid peroxidation and activities of the antioxidant enzymes superoxide dismutase and catalase.

## 2. Materials and methods

### 2.1. Meat Samples

Portions from fresh pork meat 2–3 h post mortem were washed thoroughly under running water, deboned, minced and used for analysis.

### 2.2. Preparation of Tissue Homogenates

The samples were first washed with saline and dry carefully on filter paper and then 0.5 g tissue was homogenized with 5 ml PBS. The tissue homogenates were centrifuged at 14 000 rpm for 10 minutes at 4˚C and the final supernatants were obtained. They were used for determination of lipid peroxidation, and the activities of superoxide dismutase and catalase.

### 2.3. Electron Paramagnetic Resonance (EPR) Studies

All EPR measurements were performed at room temperature on an X-band EMXmicro, spectrometer Bruker, Germany, equipped with standard Resonator. All EPR experiments were carried out in triplicate and repeated thrice. Spectral processing was performed using Bruker WIN-EPR and Simfonia software.

### 2.4. EPR *ex vivo* evaluation the levels of ROS products

The levels of ROS were determined according [17] with modifications. To investigate in "real time" formation of reactive oxygen species (ROS) in the tissue samples, EPR spectroscopy combined with *ex vivo* PBN spin trapping was used. The spin trap PBN, upon reaction with unstable radical's forms a relatively stable spin adduct that can be subsequently detected by EPR spectroscopy.

Briefly, to 100µl meat sample was added 900µl 50 mM PBN dissolved in DMSO and after centrifugation at 4000 rpm for 10 min at 4ºC, EPR spectrum of the supernatant was recorded. The levels of ROS products were calculated as double integrated plots of EPR spectra and results were expressed in arbitrary units. EPR setting were as follows: 3503.73 G center field, 20.00 mW microwave power, 5.00 G modulation amplitude, 50.00 G sweep width, 1 x $10^5$ gain, 81.92 ms time constant, 125.95 s sweep time, 5 scans per sample.

### 2.5. EPR *ex vivo* evaluation the levels of ascorbate radicals

The levels of Asc• were studied according to Malanga et al., [18] with some modification. Briefly, meat samples were prepared in DMSO in a ratio of 1:3. After centrifugation at 4000 rpm for 10 min at 4ºC the supernatants were collected and immediately transferred into a quartz tubes and placed in EPR cavity. The levels of Asc• were calculated as double integrated plots of EPR spectra and result was expressed in arbitrary units. EPR settings were as follows: 3505.00 G center field, 20.00 mW microwave power, 1.00 G modulation amplitude, 15 G sweep width, 1x$10^5$ gain, 40.96 ms time constant, 60.42 s sweep time, 10 scans per sample.

### 2.6. Analysis of Lipid Peroxidation

Basal levels of lipid peroxidation as indicated by thiobarbituric acid-reactive substances (TBARS) were determined using the thiobarbituiric acid (TBA) method, which measures the malondialdehyde (MDA) reactive products according to Placer et al., [19]. In the TBARS assay 1 ml of the supernatant, 1 ml of normal saline and 1 ml of 25% trichloroacetic acid (TCA) were mixed and centrifuged at 3000 rpm for 10 minutes.

One ml of protein free supernatant was taken, mixed with 0.25 ml of 1% TBA and boiled 1 h at 95˚C. After cooling the absorbance of the pink color of the obtained fraction product was read at 532 nm.

### 2.7. Measurement of Antioxidant Enzymes Activities

Total SOD activity was determined by the xanthine/xanthine-oxidase/nitroblue tetrazolium (NBT) method according to Sun et al., [20] with minor modification. Superoxide anion radical ($^{\cdot}O_2^-$) produced by xanthine/ xanthine-oxidase system reduced NBT to formazan, which can be assessed spectrophotometrically at 560 nm. SOD competes with NBT for the dismutation of $^{\cdot}O_2^-$ and inhibits its reduction. The level of this reduction is used as a measure of SOD activity. The total SOD activity is expressed in units/mg of protein, where one unit was equal to SOD activity that cause 50% inhibition of the reaction rate without SOD.

The assay of CAT activity was according to Beers and Sizer, [21]. Briefly, hydrogen peroxide (30 mM) was used as a substrate and the decrease in $H_2O_2$ concentration at 22˚C in a phosphate buffer (50 mM, pH 7.0) was followed spectroscopically at 240 nm for 1 min. The activity of the enzyme was expressed in units per mg of protein and 1 unit was equal to the amount of an enzyme that degrades 1 mM $H_2O_2$ per minute. The results are reported as means SE. Statistical analysis was performed with Student's *t*-test and Multiple regression analysis. $P<0.05$ was considered statistically significant.

## 3. Results

The results of ROS products estimated in the muscles subscapularis, latissimus dorsi and biceps glucoris of pork meat are given in **Figure 1.**

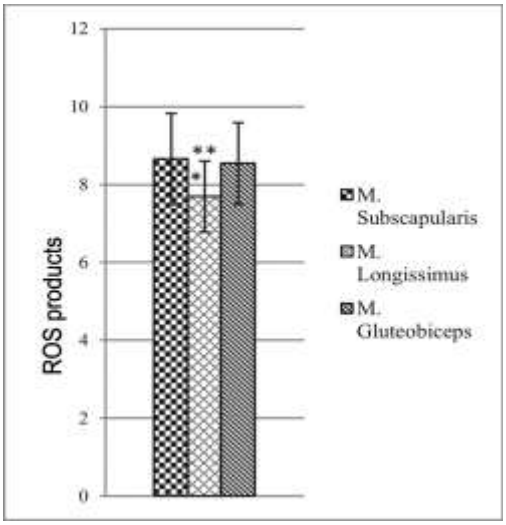


**Figure 1:** Levels of ROS products expressed in arbitrary units estimated in the muscles subscapularis, latissimus dorsi and biceps glucoris of pork meat. The results are expressed as

mean ± s.d.: *P<0.01 compared to M. Subscapularis; **P<0.01 compared to M. Gluteobiceps.

As can be seen from the figure, ROS products in the M. Longissimus were statistical significant lower than those in M. Subscapularis (mean 7.69 ±0.91 vs. mean 8.66 ±1.17, p<0.01, t-test) and also than those of M. Gluteobiceps (mean 8.54 ±1.05, p<0.01, t-test). There was not significant difference between levels of ROS in the M. Subscapularis and M. Gluteobiceps (p>0.05).

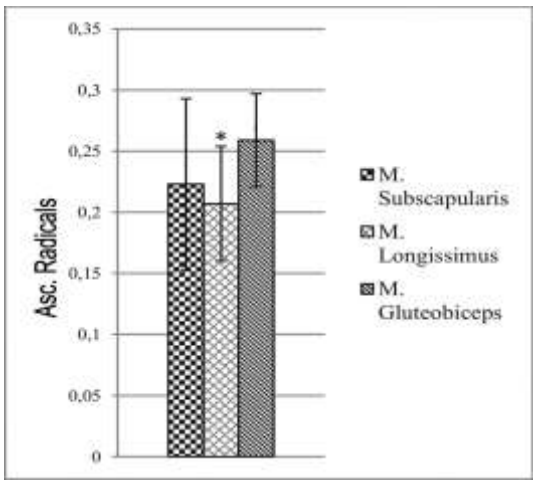


**Figure 2:** Levels of Asc• radicals expressed in arbitrary units estimated in the M. Subscapularis, M. Longissimus and M. Gluteobiceps of pork meat . The results are expressed as mean ± s.d.: *P<0.001 compared to those in the M. Gluteobiceps.

**Figure 2** shows the elevated levels of Asc• estimated in the M. Subscapularis, M. Longissimus and M. Gluteobiceps of pork meat. The levels of Asc• radicals in the M. Longissimus were lower although not statistical significant than those of M. Subscapularis (mean 0.207 ±0.047, vs. 0.223 ±0.068, p>0.05, t-test) and statistical significant lower than those in the M. Gluteobiceps (mean 0.207 ±0.047 vs. 0.259 ±0.038, p<0.001, t-test). There was not significant difference between levels of ROS in the M. Subscapularis and M. Gluteobiceps, (p>0.05).

The results of MDA-reactive products estimated in M. Subscapularis, M. Longissimus and M. Gluteobiceps of pork meat are given in **Figure 3**.

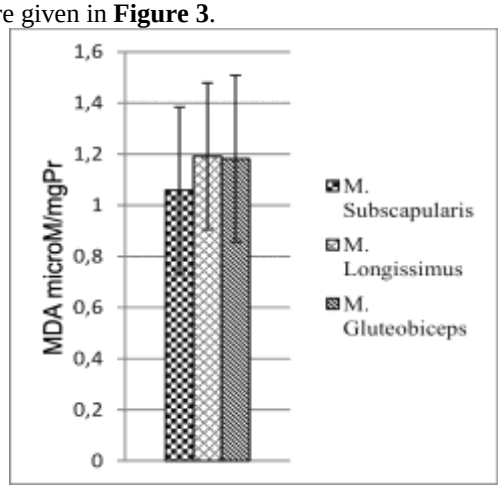


**Figure 3:** Lipid peroxidation (MDA) levels estimated in the M. Subscapularis, M. Longissimus and M. Gluteobiceps of pork meat. The results are expressed as mean ± s.d.

Higher plasma levels of lipid peroxidation products but not significantly were detected in both M. Longissimus and M. Gluteobiceps compared to M. Subscapularis (mean 1.192 ± 0.287 µmol/l and 1.182 ± 0.326 µmol/l, vs 1.059 ± 0.326, respectively, p > 0.05, *t*-test). There was not significant difference between levels of MDA in M. Longissimus and M. Gluteobiceps of pork meat (p > 0.05).

When SOD activity in the M. Subscapularis, M. Longissimus and M. Gluteobiceps of pork meat was studied, it was established higher SOD activity in M. Gluteobiceps compared to the M. Subscapularis and M. Longissimus (mean 1.609 ± 0.436U/ml vs 1.443 ± 0.313 U/ml and 1.473 ± 0.361 U/ml p > 0.05, respectively), **(Figure 4).** There was not significant difference between SOD activities in M. Subscapularis and M. Longissimus of the studied pork meat (p > 0.05).

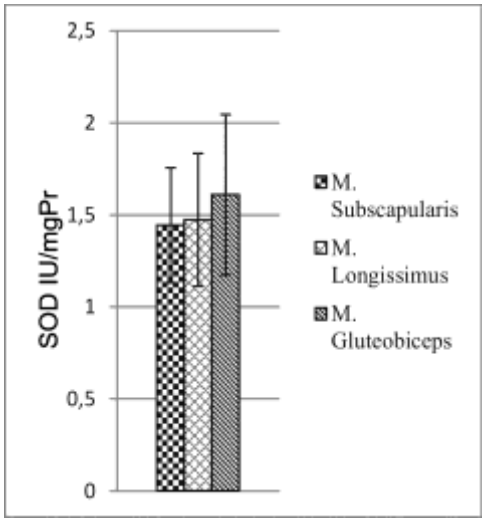


**Figure 4:** Superoxide dismutase (SOD) activity estimated in the M. Subscapularis, M. Longissimus and M. Gluteobiceps of pork meat. The results are expressed as mean ± s.d.

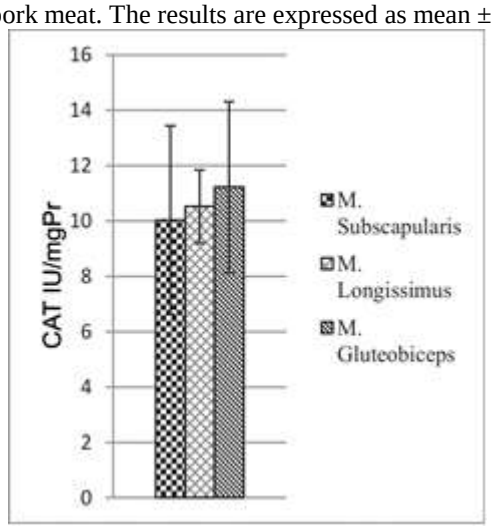


**Figure 5:** Catalase (CAT) activity estimated in the M. Subscapularis, M. Longissimus and M. Gluteobiceps of pork meat. The results are expressed as mean ± s.d.

Results of CAT activity estimated in the M. Subscapularis,

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 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 (aN and $aH_{\beta}$) 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 aN and $aH_{\beta}$ 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. Endogenic 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.

## 5. Acknowledgements

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