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Could colour and volatile compounds be measurements of oxidation in horse meat?

Lucrezia Forte[1], Pasquale De Palo[1], Alessandra Aloia[1], Aristide Maggiolino[1]

[1] *Department of Veterinary Medicine, University of Bari A. Moro, Bari, Italy*

ABSTRACT
Predicting and, consequently, controlling oxidative phenomena is a big challenge for meat industry. Since color modifications and volatile compounds synthesis are processes closely linked to the oxidative reactions occurring in meat, oxidation could be measured through colorimetric parameters or some volatile compounds. A Spearman's correlation analysis of oxidative parameters and enzymatic activity with colorimetric parameters and volatile profile was performed on horse steaks subjected to short (14 days) and long aging time (56 days). In long aging, TBARS were negatively correlated with redness (rs = -0.76, p < 0.001). Some volatile compound families, such as thiols, carboxylic acids, lactones, hydrocarbons and sulphur compounds, were well correlated with enzymatic activity (p < 0.001), contrary to aldehydes, alcohols and ketones. Higher correlation indexes were recorded in short aging, if compared to those found in long aging.

Section: RESEARCH PAPER

Keywords: Oxidation; colour; volatile compounds; horse meat, aging

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Corresponding author: Lucrezia Forte, e-mail: lucrezia.forte@uniba.it

1. INTRODUCTION

Despite the consumption of horse meat is still lower than that of other meat, it has increased in recent years, due to the greater knowledge of its nutritional properties [1]. The low intramuscular fat and cholesterol content, but, above all, the high content of unsaturated fatty acids, compared to the saturated ones, make horse meat a healthy alternative to other red meats [2]. However, unsaturated fatty acids are notoriously susceptible to oxidative processes. It is well known that oxidative processes, in particular lipid oxidation during cooking, represent an important pathway for taste and odour compound production in meat. Nevertheless, the main cause of quality decay in meat and meat products, if not considering microbial spoilage, is lipid oxidation [3]. Oxidative processes in general induce sensory modifications in meat that affect consumer acceptance [4]. These modifications include discoloration, deterioration of texture attributes, nutritional losses, and the synthesis of compounds responsible for off-flavours and off-odours [5], [6]. Moreover, oxidation in meat has toxicological implications for human health, due to the production of free radicals, toxic compounds, and primary and secondary by-products of reaction, many of which are known as carcinogenic potentials [7], [8]. As a consequence, in order to avoid economic losses for the meat market, the approach of industry and research is focused on deepening knowledge of oxidation mechanisms and their modulation [9], [10]. Oxidation is a complex process, which includes multiple mechanisms and pathways that interact with each other. Despite the many studies performed, the knowledge of some reactions and interactions is not totally clear. Oxidation and discoloration of meat are closely related processes [11], since the oxidation of heme-proteins determines changes in colour attributes of meat during storage, due to the gradual formation of metmyoglobin [12]. Likewise, most of the major volatile aroma-active compounds are generated via lipid oxidation [13]. Degradation processes start from the slaughter of the animal and continue during the subsequent technological processes [14]. Dry aging is a widely used treatment, which implies the presence of oxygen, and this significantly expedites oxidative reactions [15]. Consumers can identify the decay of meat shelf life through the appearance of rancid flavours and odours and through changes in colour [16]. Thus, colour and volatile compounds could be used as measurements of oxidation in meat during aging. In this regard, the purpose of this study was to assess the potential correlations

of oxidation with colorimetric parameters and volatile profile of horse steaks, subjected to a short and a longer dry aging.

2. MATERIALS & METHODS

2.1. Slaughtering and experimental design

The study involved 44 horses, slaughtered in abattoirs approved by the European Community (1099/2009CE; CE 2009). Following the slaughter process, their carcasses were cooled to 4 °C for 48 hours, and the right rib section was removed from each carcass and transported to the laboratory under refrigerated conditions. The loins (Longissimus thoracis et lumborum muscle) underwent a dry-aging process for 52 days in a controlled aging chamber at 2 °C, with 82 % humidity and 0.4 m/s of ventilation. Analyses were conducted prior to the commencement of aging (T 0), after 14 days (short-term aging), and after 56 days (long-term aging) of aging duration. At each considered time point, a 1.5 cm thick steak was extracted from each loin for analysis.

2.2. Colorimetric analysis

Colorimetric parameters (L*, a*, and b*) were assessed following the methodology outlined by Gálvez et al. [17], employing a Minolta CR-300 colorimeter (Minolta Camera Co., Osaka, Japan) configured with a Light Source D65. Measurements were obtained under a 0 ° viewing angle using an A-pulsed xenon arc lamp, with an 8-mm diameter measurement area. These assessments were conducted at three distinct locations on each specimen. At each location, a set of three measurements were captured, involving a 90 ° rotation of the detection system compared to the previous reading. This cumulative process yielded a total of nine measurements for each individual sample, and subsequent statistical analysis was performed using the computed mean values.

2.3. Volatile Compounds (VOC) Analysis

For the analysis of volatile compounds, five grams of meat samples were employed (Delonghi, Model CG660, Treviso, Italy), grilled at 130-150 °C until an internal temperature of 70 °C at the core was reached. Temperature monitoring was conducted using a fine constantan wire thermocouple affixed at the geometric centre of the sample (model 5SCTT-T-30-36; Omega Engineering Inc., Norwalk, CT, USA) as described by Maggiolino et al. [18]. Post-cooking, the samples were ground using a commercial grinder (Moulinex/Swan Holding Ltd., Birmingham, United Kingdom). For the extraction of volatile compounds, solid-phase microextraction (SPME) was employed as described by Natrella et al. [19]. The samples were weighed (1±0.05 g) into 20 mL vials, and sealed with a rubber septum and aluminium cap. An internal standard (82 ng 2-octanol) was added to all samples for semi-quantification and loaded into a Triplus RSH automatic sampler (Thermo Fisher Scientific, Rodano, Italy). Subsequently, the fibres were injected into a Trace1300 gas chromatograph (Thermo Fisher Scientific, Rodano, Italy) equipped with an ISQ Series 3.2 SP1 mass spectrometer (Thermo Fisher Scientific, Rodano, Italy), operating in splitless mode. The compounds were separated using a VF-WAX MS capillary column (60 m, 0.25 mm i.d., 0.25 µm film thickness; Agilent, Santa Clara, CA, USA) under the following conditions: injection port temperature, 250 °C; oven temperatures, 35 °C for 5 minutes, then ramped at 1.5 °C/min to 45 °C, followed by 4 °C/min to 160 °C, and a final increase to 210 °C at 20 °C/min; the final temperature was held for 7 minutes. The mass detector was set under the following conditions: detector voltage, 1700 V; source temperature, 250 °C; ionization energy, 70 eV; scan range, 40-300 amu. Quantification of volatile compounds was achieved using peak area integration, while compound identification was carried out by comparing mass spectra of peaks with those from the NIST 2.0 library (National Institute of Standards and Technology, Gaithersburg, USA).

2.4. Oxidative parameters

For the assessment of TBARS and hydroperoxides, 5 g and 2 g of raw meat, respectively, were initially minced and placed in a 50-mL tube. Subsequently, homogenization was performed with 15 mL of deionized distilled water (DDW). The determination of TBARS followed the procedure outlined by Tateo et al. [20], whereas the evaluation of hydroperoxides was executed in accordance with the methodology proposed by De Palo et al. [21]. Analysis of protein carbonyls was conducted following the protocol detailed by De Palo et al. [22]. Concisely, a 2 g meat specimen was homogenized earlier in 20 mL of 0.15 M KCl for a duration of 2 minutes. Subsequently, two aliquots of the homogenate (50 µL each) were mixed with 1 mL of 10 % trichloroacetic acid (TCA), followed by centrifugation at 1200 × g for 3 minutes at 4 °C for the quantification of protein oxidation. One aliquot was utilized as a standard and treated with 1 mL of 2 M hydrochloric acid (HCl) solution. The second aliquot was combined with 1 mL of 2 M HCl containing 10 mM 2,4-dinitrophenyl hydrazine (DNPH).

2.5. Enzyme Activity and Total Antioxidant Capacity

Two samples (each weighing 400 mg) of raw meat underwent homogenization within a tissue homogenizer with 4 mL of saline at a temperature of 4 °C. After centrifugation at 7000 × g for 20 minutes at 4 °C, the resultant supernatant was isolated and subjected to analysis for the quantification of antioxidant enzyme activity. The activities of superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and glutathione peroxidase (GPx, EC 1.11.1.9) were gauged following the methodology outlined by Maggiolino et al. [23]. Superoxide dismutase activity was assessed through its capacity to inhibit epinephrine autoxidation. A SOD unit was defined as the enzyme quantity required to impede the epinephrine autoxidation rate by 50 % [24]. Catalase activity was determined by monitoring the reduction in absorbance of H_2O_2 at 240 nm (with an extinction coefficient, $\varepsilon = 40\ M^{-1}cm^{-1}$). The enzyme activity was defined as the amount of enzyme necessary to decompose 1 micromole of H_2O_2 within 1 minute. Measurement of glutathione peroxidase activity was conducted by observing the rate of GSH oxidation catalysed by GPx in response to tert-butyl hydroperoxide [25]. The ferric-reducing antioxidant power (FRAP) assay was employed for the assessment of overall antioxidant potential, adhering to the approach described by Dinardo et al. [26]. Three millilitres of freshly prepared FRAP reagent (composed of 1 mL of a 10 mM solution of 2,4,6 tripyridyl-s-triazine in 40 mM HCl, 1 mL of 20 mM $FeCl_3$ in 10 mL of H_2O, and 10 mL of 300 mM acetate buffer at pH 3.6) were incubated at 37 °C for 40 minutes, post the addition of 100 µL of supernatant. The absorbance of the reaction mixture was recorded at 593 nm, and the resultant antioxidant power was expressed in µmol Trolox equivalents/mL. For the evaluation of 2,2'-Azino-bis [3-ethylbenzothiazoline-6-sulphonic acid] (ABTS) radical scavenging activity, the procedure as previously detailed by Dinardo, Maggiolino, Martinello, Liuzzi, Elia, Zizzo, Latronico, Mastrangelo, Dahl, and De Palo [26] was employed.

Briefly, the ABTS radical cation was generated by combining a 7 mM ABTS stock solution with 2.45 mM potassium persulfate, incubating the mixture in darkness at 25 °C for 12 to 16 hours. The solution was then diluted in PBS to achieve an absorbance of 0.70 ± 0.02 at 734 nm. Subsequently, 10 µL of supernatant was introduced to 990 µL of diluted ABTS radical cation solution and allowed to incubate at 30°C for 5 minutes. The reagent blank was prepared by substituting 10 µL of PBS for the sample. The scavenging of the ABTS radical cation was quantified spectrophotometrically at 734 nm. Antioxidant activity was expressed as the percentage inhibition of ABTS radical cation, computed using the following equation:

$$inhibition = 100 \cdot \frac{Absorbance_{734,Control} - Absorbance_{734,Sample}}{Absorbance_{734,Control}}. \quad (1)$$

2.6. Statistical analysis

The data set was tested for normal distribution (Shapiro–Wilk test). Subsequently, Spearman's correlation was performed using the SAS statistical software [27]. Statistical significance was set at p-value < 0.05.

3. RESULTS

Among the results, only correlation indexes higher than ± 0.50 have been reported. Regarding Spearman's correlations between oxidative and colorimetric parameters at 56 days of dry-aging (Figure 1), TBARS were negatively correlated with redness (rs = -0.76, p < 0.001) and positively correlated with lightness (rs = 0.55, p < 0.001). Among VOC families, TBARS were positively correlated with carboxylic acids (rs = 0.57) and ketones (rs = 0.50) (p < 0.001). Hydroperoxides showed a negative correlation with yellowness (rs = -0.52, p < 0.001). Protein carbonyls were shown to be negatively correlated with redness (rs = -0.54, p < 0.001). Regarding the volatile profile, protein carbonyls were positively correlated to sulphur compounds (rs = 0.64), thiols (rs = 0.63), and carboxylic acids (rs = 0.57) (p < 0.001) and negatively correlated to non-aromatic hydrocarbons (rs = -0.52, p < 0.001). Among results concerning enzyme activity, superoxide dismutase showed a negative correlation with non-aromatic hydrocarbons (rs = -0.55, p < 0.001). Catalase values recorded negative correlations with thiols (rs = -0.71), sulphur compounds (rs = -0.70) and carboxylic acids (rs = -0.55) (p < 0.001). Instead, positive correlations were recorded between catalase and non-aromatic hydrocarbons (rs = 0.66) and lactones (rs = 0.54) (p < 0.001). Glutathione peroxidase was negatively correlated with non-aromatic hydrocarbons (rs = -0.82, p < 0.001), while positive correlations were recorded with thiols (rs = 0.76), sulphur compounds (rs = 0.66), aldehydes (rs = 0.63) and VOCs total content (rs = 0.52) (p < 0.001). Lastly, ABTS showed to be positively correlated with non-aromatic hydrocarbons (rs = 0.67, p < 0.001). As regards Spearman's correlations detected in dry-aged meat for 14 days (Figure 2), among oxidative parameters, TBARS showed a negative correlation with redness (rs = -0.57, p < 0.001), and positive correlations with ketones (rs = 0.58), carboxylic acids (rs = 0.57) and sulphur compounds (rs = 0.54) (p < 0.001). Hydroperoxides were positively correlated with ketones (rs = 0.50) (p < 0.01). Protein carbonyls showed positive correlations with carboxylic acids (rs = 0.57), thiols (rs = 0.55), and sulphur compounds (rs = 0.53) (p < 0.001). Regarding enzyme activity, superoxide dismutase was positively correlated with yellowness (rs = 0.54, p < 0.001) and negatively correlated with furans (rs = -0.57, p < 0.001). Catalase was positively correlated with redness (rs = 0.60, p < 0.001) and negatively correlated with carboxylic acids (rs = -0.79), thiols (rs = -0.76), and sulphur compounds (rs = -0.60) (p < 0.001). Moreover, positive correlations were detected with lactones (rs = 0.81) and non-aromatic hydrocarbons (rs = 0.59) (p < 0.001). Glutathione peroxidase was positively correlated with thiols (rs = 0.86) and carboxylic acids (rs = 0.59) (p < 0.001) and negatively correlated with non-aromatic hydrocarbons (rs = -0.81) and lactones (rs = -0.58) (p < 0.001). A positive correlation was found between FRAP and redness (rs = 0.62, p < 0.001). Among VOCs, FRAP was negatively correlated with sulphur compounds (rs = -0.60) and carboxylic acids (rs = -0.57) (p < 0.001). The

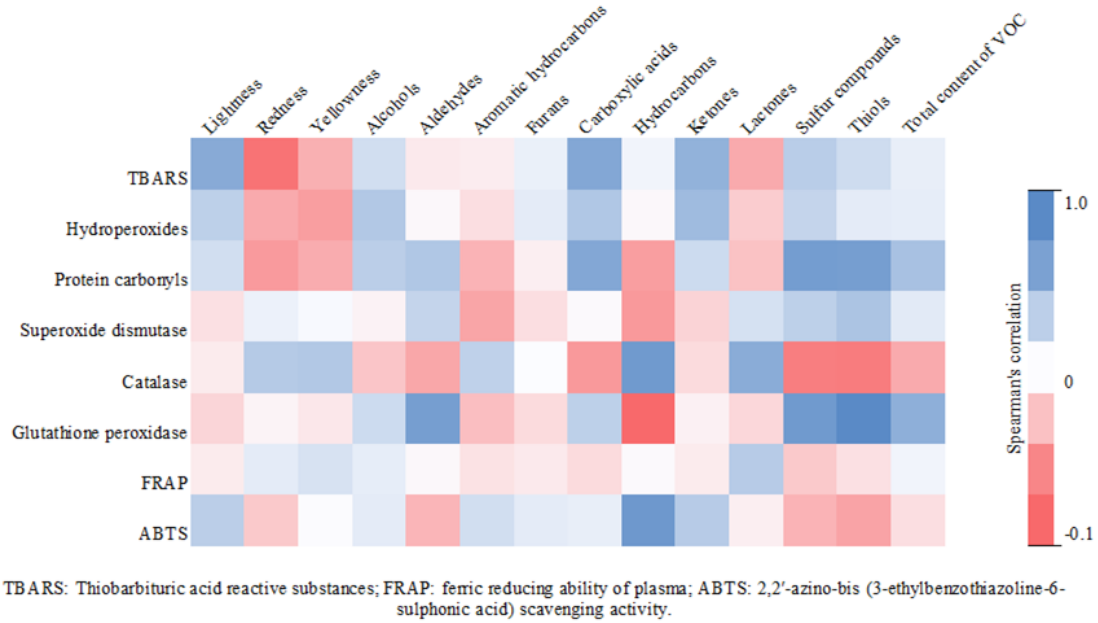

Figure 1. Spearman's correlations of oxidative parameters and enzyme activity with colorimetric parameters and VOC families detected in horse loins (*Longissimus thoracis et lumborum*) dry aged for 56 days (n = 44).

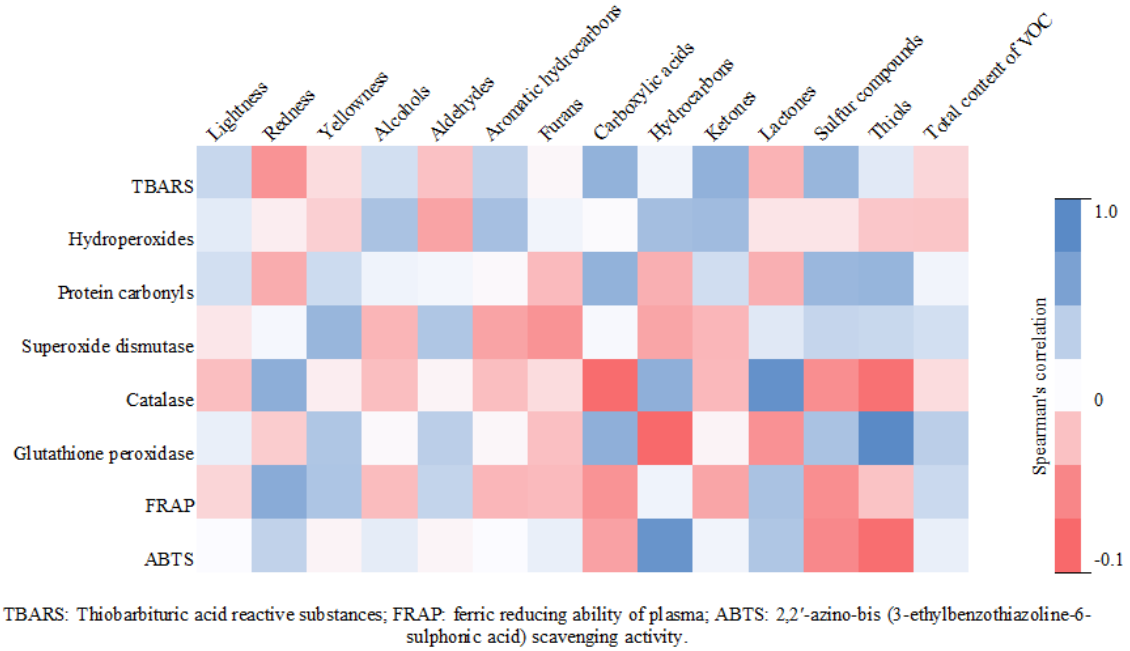

Figure 2. Spearman's correlations of oxidative parameters and enzyme activity with colorimetric parameters and VOC families detected in horse loins (Longissimus thoracis et lumborum) dry aged for 56 days (n = 44).

ABTS was positively correlated with non-aromatic hydrocarbons (rs = 0.79, $p < 0.001$) and negatively correlated with thiols (rs = -0.78) and sulphur compounds (rs = -0.63) ($p < 0.001$).

4. DISCUSSION

The outcomes of the correlation of oxidative parameters and enzyme activity with meat colour gave different results in the two aging time. Some correlations between enzyme activity and colorimetric parameters were higher and more significant in short aging, if compared to those in long aging. The antioxidant activity of these enzymes is a key in vivo defence mechanism of cells against oxidative damage [28]. Intracellular proteinases can denature or hydrolyse these enzymes since post-mortem or during storage Therefore, usually, during aging the antioxidant activity of these enzymes may be reduced or absent [29], [30], explaining the few and weak correlations between enzyme activity and colorimetric parameters in short aging and the absence of correlations in long aging. Otherwise, relationships between oxidative and colorimetric parameters showed an opposite condition. Correlation indexes were higher and more significant in long aging, if compared to those in short aging. With the loss of oxidative and anti-oxidative balance, oxidative processes could lead to an accumulation of metabolites which could presumably increase with the lengthening of the aging time [31]. It is noteworthy the high and negative correlation between redness and TBARS in long aging. The oxidation of ferrous-oxymyoglobin (Fe2+) to ferric-metmyoglobin (Fe3+) leads to discoloration of meat during storage, thereby affecting redness [32]. The relationship between lipid and heme-protein oxidations has been supported by several authors, who suggested that these processes occur simultaneously in meat and appear to enhance each other [32]. Except for the correlation between TBARS and redness, r values referring to the correlation with the other colorimetric parameters, although significant, never exceeded the threshold of ± 0.62. As in the determination of colour, oxidative reactions play a pivotal role also in the synthesis of taste and odour compounds of meat [33], to the point that some volatile compounds are considered markers of lipid oxidation in meat. In our results, in long aging the total amount of volatile compounds, as well as alcohols, aldehydes, aromatic hydrocarbons, furans, and ketones were weakly correlated to oxidative parameters and enzyme activity. Although reducing aging times, some correlations increased, the r values never exceeded the threshold of 0.58 and -0.57. These findings are inconsistent with the previous literature, as most of these compounds, deriving from oleic, linoleic, and linolenic acids, are secondary products of lipid oxidation [34]. Among these, aldehydes are the most released breakdown products and strongly contribute to the volatile flavour of meat [35]. Several authors reported a strong correlation between aldehydes content, particularly saturated aldehydes, and TBARS, and this correlation justifies their use as markers of lipid oxidation [14], [36], [37]. Within aldehydes, hexanal, the main volatile compound isolated in horse meat and in meat of other species [20], has been considered the greatest indicator of lipid oxidation in meat and meat products for years [14]. Nevertheless, in our results, hexanal was weakly correlated with oxidative parameters and enzyme activity. As regards the other families, a different trend was observed. The correlations between VOCs and oxidative parameters did not exceed the threshold of ± 0.64 in long aging and ± 0.58 in short aging. On the other hand, strong correlations were found between VOCs and enzyme activity in long aging and mainly in short aging. Considering a threshold value r = ± 0.70, in long aging catalase showed negative correlations with thiols and sulphur compounds. In short aging, the correlation index with thiols was higher. Moreover, a positive correlation with lactones and a negative one with carboxylic acids were detected. As regards glutathione peroxidase, in long aging, it was negatively correlated with hydrocarbons and positively with thiols ($p < 0.001$). In short aging, the correlation with hydrocarbons was the same, whereas that with thiols was higher. Lastly, in short, aging ABTS was strongly correlated with hydrocarbons and thiols. Since these compounds can also result from lipid oxidation, they could be indicators of oxidation in meat during aging time [38], [39].

5. CONCLUSION

The analysis of the correlations shed light on better understanding oxidative processes during aging of horse meat and their correlation with parameters as important as colour and volatile compounds. Colorimetric parameters were overall weakly related to oxidative parameters and enzyme activity, except for redness which was strongly correlated with TBARS (rs = -0.76, $p < 0.001$). As regards volatile compounds, the chemical families synthetized via lipid oxidation, such as aldehydes, ketones, alcohols, and aromatic hydrocarbons, were weakly or not correlated with oxidative parameters and enzyme activity. In our study, hexanal did not behave as a marker of lipid oxidation. On the other hand, the highest correlation indexes were recorded between enzyme activity and carboxylic acids, hydrocarbons, lactones, and thiols. Aging time presumably affected correlation indexes, since in many cases the correlations found were higher in short aging if compared to those in long aging. This could suggest that the predictability of oxidation through volatile compounds could be higher in shorter aging periods.

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