INFLUENCE OF AGING TECHNIQUE AND MUSCLE ON PHYSICOCHEMICAL STABILITY AND ANTIOXIDANT CAPACITY OF HIGH-OXYGEN ATMOSPHERE PACKED BEEF

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Abstract – The aim of this study was to compare the effect of aging technique (wet-aging *vs.* carcass-aging), muscle (*longissimus dorsi vs. rectus femoris*) and previous vacuum storage time on color and lipid stability of beef packaged in high-oxygen atmosphere. After a seven-day wet- or carcass-aging step, *longissimus dorsi* and *rectus femoris muscle* cuts from 4 Belgian Blue cows were vacuum packaged and stored at −1 °C for up to 28 days. At different times, part of these samples was repackaged under modified atmosphere – 70 % O_2:30 % CO_2 –, and stored during 7 days at +4 °C. The following parameters were evaluated at different intervals: color (CIE L*a*b*), metmyoglobin %, lipid oxidation (TBARS), antioxidant enzyme activities (catalase, glutathione peroxidase and superoxide dismutase), α-tocopherol and fat content. The sensitivity of modified atmosphere repacked meat cuts to oxidation was influenced by the conditions of the previous aging period (wet > carcass conditions), muscle (*rectus femoris* > *longissimus dorsi*) and length of the vacuum storage. Oxidation stability could be associated with catalase activity, and no association could be established with the α-tocopherol content.

I. INTRODUCTION

Two common approaches for beef aging are wet-aging and carcass-aging. Wet-aging refers to meat aged in a sealed barrier package at refrigerated temperatures (1), while carcass aged at controlled temperatures and humidity is defined as carcass-aging. Carcass-aging is an ancient process used nowadays to produce beef characterized by its unique flavor and superior quality, and mainly destined to high-end consumers (2). Nevertheless, carcass-aging is a costly procedure that requires hygiene and ventilation in the chilling room (3).

The shelf life of fresh meat is mainly limited by the development of pathogenic or spoilage microorganisms, which may result in off-odors, gas and slime formation, and it is also influenced by oxidation of lipids and pigments, causing rancid flavors and surface discoloration. Meat contains endogenous antioxidants and prooxidants, and can use several cellular mechanisms of protection against oxidative processes, including antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) (4).

The Belgian meat sector often complains of a sensitivity of beef to oxidation processes, in particular the discoloration of high-oxygen modified atmosphere packaged (MAP) meat previously aged in vacuum conditions. At retail level, high-oxygen modified atmospheres are often applied to give fresh meat its bright red appealing color.

In this context, the present experiment was conducted to evaluate the potential effect of aging technique (wet-aging *vs.* carcass-aging), muscle (*longissimus dorsi vs. rectus femoris*) and previous storage time in vacuum conditions on the physicochemical stability of meat packaged in high-oxygen atmosphere.

II. MATERIALS AND METHODS

Samples: Three days after slaughter, four *longissimus dorsi* (LD) and four *rectus femoris* (RF) muscles were cut from four half carcasses of Belgian Blue cows (7.9 ± 1.4 yr), vacuum packaged (VP) – vacuum bags were 60 µm thick and oxygen permeability was 13 cm³/m² · 24 h at +23 °C and 0 % RH –, and wet aged at an average temperature of +1.5 °C for 7 days. In parallel, the other four half carcasses from the same animals were carcass aged at the same temperature at 99 % RH and for the same period of time before deboning and cutting. After the seven days of aging, 3 cm thick steaks were cut, vacuum packaged, and stored at −1 °C for up to 28 days. Analyses were performed before aging (d_3) and each 14 days after aging (d_{10}, d_{24} and d_{38}). Moreover, each 14 days a part of the samples was repackaged in polypropylene trays sealed with a polypropylene film (52 µm thick, oxygen permeability of 110 cm³/m² · 24 h at +23 °C and 0 % RH) containing a modified atmosphere – 70 % O_2:30 % CO_2 –, stored during 7 days at +4 °C, and analyzed (d_{10+7}, d_{24+7} and d_{38+7}).

*Color measurement (C.I.E. L*a*b* space)*: instrumental color of samples was evaluated 1.5 h after removal from package using a Minolta CM-600d spectrophotometer (11 mm aperture, D_{65} illuminant, 10° observation angle).

Metmyoglobin %: Metmyoglobin (MMb) proportion was obtained using the method of Francis and Clydesdale (5) based on the reflectance measurement to calculate *K/S* ratios at isobestic wavelengths for each myoglobin redox form.

Fat content: The fat content was determined by Soxhlet method (ISO 1444:1996) (6).

Lipid oxidation measurement: To assess the lipid oxidation, the TBARS content was measured by spectrophotometric quantification of a complex formed with malondialdehyde (MDA) as described by Raharjo *et al.* (7).

Antioxidant enzyme activities: Five g of muscle were homogenized in 25 mL of phosphate buffer (0.05 M, pH 7) and centrifuged at +4 °C for 2 min at 7000×*g*. The supernatant fraction was filtered and used to determine CAT, GSH-Px and SOD. CAT activity was measured according to Aebi (8). One unit (U) of CAT will decompose 1.0 µmole of hydrogen peroxide to oxygen and water per minute. GSH-Px activity assay was performed as described by DeVore & Greene (9). One unit (U) of GSH-Px was defined as the amount of extract required to oxidize 1 µmole of NADPH per minute. SOD activity was determined following the method of Paoletti & Mocali (10). One unit of SOD activity was defined as the amount of enzyme required to inhibit the rate of NAD(P)H oxidation of the control (blank) by 50 %.

α-tocopherol content: A protocol adapted from Liu *et al*. (11) was used to extract and quantify α-tocopherol. HPLC analysis was carried out using a Model 600 E solvent delivery system, equipped with a Model 717 automatic injector, a Mistral™ oven and both 996 PDA and 2475 Fluorescence detectors (all from Waters).

HPLC conditions: stationary phase: Waters Resolve 5 µm spherical silica column (3.9 × 150 mm), column temperature: +15 °C, mobile phase: isooctane:THF 96 %:4 % (v/v), flow rate: 1.0 mL/min, injection volume: 30 µL, detection: fluorescence (excitation wavelength 296 nm and emission wavelength 325 nm), calculation: external standard method based on peak area.

Statistical analysis: Experimental data for each response variable was analyzed by ANOVA using the GLM procedure. Whenever a *post-hoc* test was suitable, Tukey test was performed.

III. RESULTS AND DISCUSSION

Color: Initial a* values before aging were 20.48 ± 1.89 and 22.43 ± 1.68 for LD and RF respectively, and no significant loss of redness was observed for VP samples during the whole storage. An effect of previous storage time under vacuum conditions was observed for modified atmosphere repackaged (MAP)

samples, and an effect of the aging technique was observed for LD samples after 14 days of previous storage under vacuum conditions (Table 1).

Table 1 Chromaticity a* in MAP samples

Muscle	d	Wet-aged	Carcass-aged
LD	10+7	22.36 ± 1.54^{a}	23.46 ± 0.89^{a}
	24+7	$14.96 \pm 5.31^{b\,A}$	$22.61 \pm 1.95^{a\,B}$
	38+7	$9.73 \pm 3.07^{b\,A}$	$17.88 \pm 4.48^{b\,B}$
RF	10+7	18.48 ± 1.28^{a}	18.88 ± 0.93^{a}
	24+7	13.96 ± 4.56^{ab}	17.74 ± 1.75^{a}
	38+7	8.63 ± 1.10^{b}	10.72 ± 1.72^{b}

d = days. Means and standard deviation are indicated (n = 4). Different small letters within the same column (time effect) or capital letters within the same line (aging technique effect) indicate significant differences ($P < 0.05$).

Metmyoglobin %: Initial oxidized myoglobin proportion before aging in LD samples was 0.15 ± 3.35 %, and it remained stable after 7 d of aging and 28 d of subsequent storage in vacuum conditions. The initial MMb proportion in RF samples was 0.67 ± 1.09 %. After aging and 28 d of vacuum storage, this value increased to 9.30 ± 2.36 % (wet) and 14.12 ± 8.91 % (carcass). Once samples were repackaged under modified atmosphere, an effect of the previous storage duration was observed. LD carcass-aged samples presented higher pigment stability. For both muscles, wet-aging favored pigment oxidation (Table 2).

Table 2 Metmyoglobin % in MAP samples

Muscle	d	Wet-aged	Carcass-aged
LD	10+7	7.82 ± 6.62^{a}	3.23 ± 5.75^{a}
	24+7	$57.55 \pm 26.11^{b\,A}$	$6.97 \pm 9.29^{ab\,B}$
	38+7	$73.71 \pm 16.01^{b\,A}$	$34.52 \pm 19.32^{b\,B}$
RF	10+7	25.49 ± 6.95^{a}	26.12 ± 3.31^{a}
	24+7	58.69 ± 26.28^{ab}	34.58 ± 13.10^{a}
	38+7	$85.47 \pm 2.22^{b\,A}$	$77.16 \pm 5.56^{b\,B}$

d = days. Means and standard deviation are indicated (n = 4). Different small letters within the same column (time effect) or capital letters within the same line (aging technique effect) indicate significant differences ($P < 0.05$).

Fat content: Fat content was 2.0 ± 0.8 % for LD and 1.3 ± 0.3 % for RF samples.

Lipid oxidation: The TBARS values for RF samples stayed below the limit of quantification during the whole storage under vacuum conditions. For LD samples, the TBARS values could be quantified after 14 days of storage under vacuum and remained between 0.12 and 0.14 mg/kg MDA-equivalent. After repackaging under modified atmosphere, an effect of previous storage time was observed for RF samples. Despite its higher fat content, LD samples presented higher lipid stability than RF samples (Table 3).

Table 3 MDA-equivalent (mg/kg) in MAP samples

Muscle	d	Wet-aged	Carcass-aged
LD	10+7	0.92 ± 0.75	0.57 ± 0.37
	24+7	1.52 ± 0.69	1.02 ± 0.66
	38+7	1.48 ± 0.86	1.30 ± 1.00
RF	10+7	1.34 ± 0.65^{a}	1.04 ± 0.28^{a}
	24+7	1.95 ± 0.97^{ab}	1.57 ± 0.47^{a}
	38+7	3.00 ± 0.98^{b}	3.35 ± 0.43^{b}

d = days. Means and standard deviation are indicated (n = 4). Different letters within the same column (time effect) indicate significant differences ($P < 0.05$).

Antioxidant enzyme activities: The activities of CAT, GSH-Px and SOD were measured in LD and RF samples after aging. Only catalase activity differed according to muscle and this could partially explain the higher predisposition of RF samples to oxidation (Table 4).

Table 4 Activities of CAT (U/g), GSH-Px (U/mg) and SOD (U/g) in VP samples after aging (d_{10})

Enzyme	Muscle	Wet-aged	Carcass-aged
CAT	LD	245 ± 26^{a}	248 ± 57^{a}
	RF	190 ± 30^{b}	157 ± 48^{b}
GSH-Px	LD	150 ± 83	105 ± 32
	RF	92 ± 24	85 ± 29
SOD	LD	191 ± 76	321 ± 176
	RF	250 ± 81	281 ± 210

Means and standard deviation are indicated (n = 4). Different letters within the same column (muscle effect) indicate significant differences ($P < 0.05$).

α-tocopherol content: α-tocopherol content was measured after aging. Even if the α-tocopherol content was higher in RF samples, it could not

prevent this muscle from being more oxidative (Table 5).

Table 5 α-tocopherol content (μg/g) in VP samples after aging (d_{10})

Muscle	Wet-aged	Carcass-aged
LD	2.22 ± 0.63[a]	2.78 ± 0.33[a]
RF	4.42 ± 1.21[b]	4.11 ± 0.76[b]

Means and standard deviation are indicated (n = 4). Different letters within the same column (muscle effect) indicate significant differences ($P < 0.05$).

Fat content was not directly proportional to α-tocopherol content. As reviewed by Jensen *et al.* (12), this fact may be explained by a higher capillary supply of RF, which increases the availability of vitamin E, and by the fact of the higher mitochondria content of RF, in which the membrane-bound vitamin E accumulates.

IV. CONCLUSIONS

In this experiment, a higher sensitivity to oxidation was observed with wet-aging, and LD showed a higher oxidative stability than RF samples. The length of previous vacuum storage favored oxidation reactions when the samples were repackaged under modified atmosphere.

Oxidation stability could be associated with the catalase activity in samples, but no association could be found regarding the α-tocopherol content.

Further research will be conducted to study the fatty acid profile of the samples in order to better understand the lipid oxidation process.

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