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Article

Relationship between Color and Redox Potential (E_h) in Beef Meat Juice. Validation on Beef Meat

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Abstract: In France, around 3.5 million cattle are slaughtered each year, which represents 1.3 million tons of beef carcasses. However, waste due essentially to organoleptic defects is estimated at 3.4% of the production or 45,000 tons of beef carcasses. Microbiological contamination and color are the two major causes of defect. In order to prevent color defect, a study was performed to develop a new method for measuring rapidly and instantly the redox potential as an indicator of color changes in carcasses without slowing down the slaughter line. This measurement would allow to classify them upstream according to their time of colors changes in order to sort them and to avoid food waste in the future. Meat juice has been shown to be a good mimetic medium for the study of color changes. The effect of different parameters was studied in order to fix experimental conditions. Color change is faster in the juice than in the meat and faster at 20 °C than at 4 °C. Redox potential allows following color changes and a symmetry has been highlighted between this thermodynamic measure and color changes.

Keywords: redox potential; color transfer; beef juice; beef meat

1. Introduction

In France, each year, around 3.5 million cattle are slaughtered for meat production, which represents 1.3 million tons of beef carcasses [1]. Most of this meat is distributed in large and medium-sized stores and alone accounts for 75% of purchases of fresh red meat in France [2]. One in two consumers buy fresh red meat based on color [3]. However, if the red meat does not meet the consumer’s requirements (a beautiful color and a good smell), the latter does not buy it, which leads to food waste. In 2016, waste due to organoleptic defects in beef was estimated at 3.4% of production or 45,000 tons of beef carcasses [4]. Microbiological contamination and color are the two major causes of observed defect in beef meat.

The color of the meat is due to the presence of myoglobin. Myoglobin is a water-soluble intramuscular protein that binds to oxygen and allows cellular respiration in the muscle [5]. It is made up of globin and a prosthetic group called heme. At the center of the heme is an iron atom with its six coordination sites. The sixth coordination site makes it possible to bind a ligand such as dioxygen or even water [6]. Depending on the oxidation state of iron (ferrous Fe^{2+} or ferric Fe^{3+}) and the bound ligand, myoglobin is commonly found in 3 forms: deoxymyoglobin, oxymyoglobin, or metmyoglobin [7]. Each redox form of myoglobin, when it is mainly present in meat, generates different colors. Deoxymyoglobin is purplish in color and is combined with a vacuum product or immediately cut. Oxymyoglobin is bright red in color and is associated with good quality red meat in trays, while metmyoglobin, brown in color on meat, and results in consumer rejection of the

product. Indeed, there is a dynamic of interconversions (redox reactions) between these three forms of myoglobin [7]. Initially, myoglobin or deoxymyoglobin undergoes oxygenation or blooming by binding to oxygen and becomes red-colored oxymyoglobin. Under low partial oxygen pressure such as during cellular respiration by muscle tissue, oxymyoglobin can revert to deoxymyoglobin, which is sensitive to oxidation. This oxidation at the level of the iron atom in the center of the heme causes the passage of the deoxymyoglobin of purplish color in metmyoglobin of brown color.

The aim of this study is to carry out a rapid and instant measurement of the redox potential, E_h , directly on a carcass (without slowing down the slaughter line), at the separation between forequarter and hindquarter in order to evaluate the time necessary for meat color to change from red to brown. To the best of the authors' knowledge, there is no technology to date to measure color and/or redox potential directly on slaughter lines. This would allow carcasses to be classified upstream in order to sort them. Indeed, a carcass whose E_h value would indicate an early transfer would then be sent to a line of processed products, while a carcass whose E_h value would indicate a later transfer would be distributed in large and medium-sized stores and offered in cut and packaged meat. However, since meat is a complex medium and redox probes for semi-solid media do not exist, the use of a mimetic medium such as meat juice (i.e., meat exsudate) has been proposed as a first approach.

The strategy applied in this work consisted in simultaneously measuring the color of a beef meat juice and the E_h in order to search for a relationship between these two variables. The main sources of variability have been identified in order to limit their effect. A validation of the relationship on beef meat was also performed.

2. Materials and Methods

2.1. Biological Products

Rib steaks from young cattle (Puigrenier slaughterhouse, Montluçon, France) were semi-dressed, vacuum-packed, and labeled for all the experiments. After reception, the meat is stored in the refrigerator at $(4 \pm 2) ^\circ\text{C}$. Rib steak was selected because it is easily and quickly accessible during the cutting of the carcass in the forequarter and hindquarter.

2.1.1. Meat Juice

The meat juice preparation is based on the study by Kim and Jeong [8]. After reception, muscles were cut into pieces 3 cm thick with a weight of about 80 g and packaged in Polyamide/Polyethylene (PA/PE) storage bags impermeable to gases (3 welds, 200×300 mm, Sovapack, Cuiseaux, France). The bags were hermetically sealed either under half vacuum (500 mbar) or under vacuum with a double-chamber machine (Multivac, Lagny Sur Marne, France) for packaging in bags. The samples were then frozen at $(-20 \pm 2) ^\circ\text{C}$ for 24 h and then thawed at $(4 \pm 2) ^\circ\text{C}$ for 72 to 96 h depending on the size of the samples. The obtained juices after freezing and thawing of the samples were collected in a sterile manner under a microbiological safety station. The juices recovered have undergone different preparatory stages or not depending on the objectives of the study:

1. centrifugation at $4 ^\circ\text{C}$ at 10,000 g for 5 min;
2. filtration through a filter with a pore diameter of $0.22 \mu\text{m}$;
3. dilution according to the desired volume and rate;
4. use of milliQ water or physiological water for dilution.

2.1.2. Meat Decontamination

Rib steaks were decontaminated with peracetic acid following the study of Lebert et al. [9]. Then, they are cut into slices about 2 to 3 cm thick for a weight of 350 to 400 g. The slices are then placed in sealable trays in PS/EVOH/PE (Form'plast, Chantrans, France). They were finally placed in a modified

atmosphere using a T200 semi-automatic sealer (Multivac, Lagny Sur Marne, France). OPP/T504 film (Soussanna, Thiais, France) was used to seal the trays.

2.2. Color Monitoring

2.2.1. Meat Juice

A climatic chamber (Binder KMF 240, VWR, Fontenay-sous-Bois, France) was used to reproduce the conditions of an industrial storage fridge for meat. Indeed, it allows temperature between 2 and 20 °C and relative humidity between 95 and 99%. In order to light up the meat and follow the color changes inside the climatic chamber, two strips with fluorescent tubes (T5, cool white color, 27.7 cm, 6 W, Diall, Paris, France) spaced apart 30 cm have been installed. The lighting delivers about 360 lux on the samples (dual-display traceable luxmeter, VWR Collection, France). In addition, a balance (Kern SXS-6K-3M, Timber Production, Esmans, France) was used in situ to monitor the weight over time to ensure that the samples did not dry out during the experiment. A camera (Logitech C270 720p, Logitech, Paris, France) was installed vertically approximately 20 cm above the samples to follow meat juice color.

2.2.2. Meat

An industrial system (ADIV, Clermont-Ferrand, France) was used to follow the color changes of red meat. In a cold room (Dagard, Boussac, France) supplied by a cold group (Arcos, Gorrevod, France), strips with fluorescent tubes (T8, cool white color, 60 cm, 18 W, Sylvania, Saint-Etienne, France) were fixed on both sides others of the device with a distance of 45 cm between each strip. In addition, they were placed 30 cm above the meat trays just like the cameras (Logitech C270 720p, Logitech, Paris, France). Lighting at this facility delivers approximately 1700 lux on meat samples.

2.3. Development of a Redox Probe for Solid Media

Since commercial redox probes for semi-solid media do not exist, a built in-house redox electrode has been developed. It is composed of two “working” electrodes of 1 mm in diameter, one of which consists of a platinum rod (99.95% purity, Surepure Chemetals, New-Jersey, United States) and the other of an oxidized iron rod. Each rod is connected by electric cables in order to recover the electrical signals (measured voltages) at the level of the computer (the whole is molded in a resin).

2.4. Physico-Chemical Measurements

For meat juices, pH probes (HI11310, Hanna Instruments, Lingolsheim, France) and E_h probes (HI36180, Hanna Instruments, Lingolsheim, France) were used. The measurements were taken and recorded automatically every 15 min using tablets (pH-/mV-meter Edge HI2002-02, Hanna Instruments, Lingolsheim, France). For meat, the built in-house probes were used. The data was measured and recorded using our internally developed software. The probes for liquid and semi-solid media were sterilized with 70° alcohol before being used.

Since at 4 °C, the time of color transfer is quite long (up to 400 h), and based on the fact that all the studied reactions are physico-chemical reactions, it has been decided to speed up these reactions by performing the experiment at 20 °C.

2.5. Color Monitoring and Images Analysis

Images of the samples (juice and meat) were taken every 15 min by using a software developed with LabVIEW 2018 and the Vision Development module (National Instruments Corporation, USA). These images in bmp format were saved on a computer before being processed.

An image processing process was performed for each saved image. Using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA), the values of Lightness (L^*), Red/Green index (a^*), and Blue/Yellow index (b^*) were calculated automatically on the selected area (use of macros

developed in-house). The target values were calculated to verify that the camera calibration did not drift over time. In this case, the difference between the initial target value and the target value after derivation has been subtracted or added to the measured value of the sample. This procedure gave the kinetics of a^* .

2.6. Data Normalization

The normalization allows adjusting of a series of values according to a transformation function in order to make them comparable with a few specific reference points. It is necessary when the results and their interpretations can be affected by the incompatibility of the units or scales of measurement between variables. This data normalization method was used for the comparison of the red index (a^*) and the redox potential (E_h) variation curves. The transformation function (Equation (1)) used is:

$$f(V_i) = (V_i - V_{\max}) / (V_{\max} - V_{\min}) \quad (1)$$

where V_i = value to normalize, V_{\max} = maximum value, V_{\min} = minimum value. This function allows to normalize (and then compare) the red index a^* and E_h between 0 and -1 .

3. Results

3.1. Operating Conditions

In the next subsections, different operating conditions applied on the juices were tested: volume (20 mL, 35 mL and 50 mL), dilution (1/5, 1/10 and 1/15), water used for the dilution (milliQ or physiological), packaging (air or vacuum), and centrifugation (or not). Even if it should be interesting *per se*, it is worth mentioning that juice composition and visible spectrophotometry were not used in this study. Firstly, concerning the juice composition, we have considered the composition of the juice as a pre-slaughter factor. It is part of the initial conditions and is dependent on the animal such as the type of animal diet, the stress of animal experienced before slaughter, or also the type of muscle as mentioned by Bekhit et al. [10]. So, it was not relevant to master it for all the different experiments since each animal is different and it is impossible to perform this analysis on the slaughter line. Secondly, monitoring the evolution of visible spectrophotometry peaks of the meat juice in order to quantify the relative percentages of deoxymyoglobin, oxymyoglobin, or metmyoglobin over time using empirical equations as proposed by Tang et al. [11], were not undertaken because these analyzes are not there either feasible on the slaughter line. Lastly, one has to note that the pH evolution of the different juices was also studied, but in all cases, its evolution was stable (between 5.7 and 5.5). These slight variations are in the uncertainties of measurement of the probe (± 0.1 pH unit, corresponding to two times the standard deviation) and do not explain color changes. Consequently, it has been decided to not represent it in the following parts.

3.1.1. Influence of the Samples Volumes and Dilutions

The purpose of this experiment is to show the importance of standardizing samples when measuring color, since the volume and the dilution have an impact on the initial and final color (after transfer) of a meat juice. Samples with different volumes (20 mL, 35 mL, and 50 mL) and different dilutions (1/5, 1/10, and 1/15) were made in order to obtain the best possible compromise (Figure 1).

The juice samples were obtained following the freezing/thawing of a rib steak cut into small pieces (3 cm thick with a weight of about 80 g) packed in air; the juices were centrifuged (at 4 °C, 10,000 g for 5 min), filtered (through a filter with a porosity of 0.22 μm) and diluted with milliQ water. Each sample was transferred to a crystallizer of the same format and monitoring of the color change was carried out for 24 h at 20 °C in the climatic chamber, the choice of 20 °C being motivated by the acceleration of the reaction kinetics. At $t = 0$ h, the juices of 20 mL, 35 mL, and 50 mL diluted 1/5, 1/10, and 1/15, respectively, showed an intermediate red color. After 24 h, all the juices were found to have turned

from a red to brown color where the smaller the volume and dilution, the darker the color of the juice, rendering the color change difficult to analyze. Moreover, in order to measure E_h reliably, the largest volume (50 mL) is better to properly immerse the redox probes in these crystallizers. Finally, in order to modify the properties of the juice initially recovered as little as possible, the smallest dilution (1/5) was used. Following this analysis, a volume of 50 mL and a 1/5 dilution were used in identical crystallizers for all the experiments.

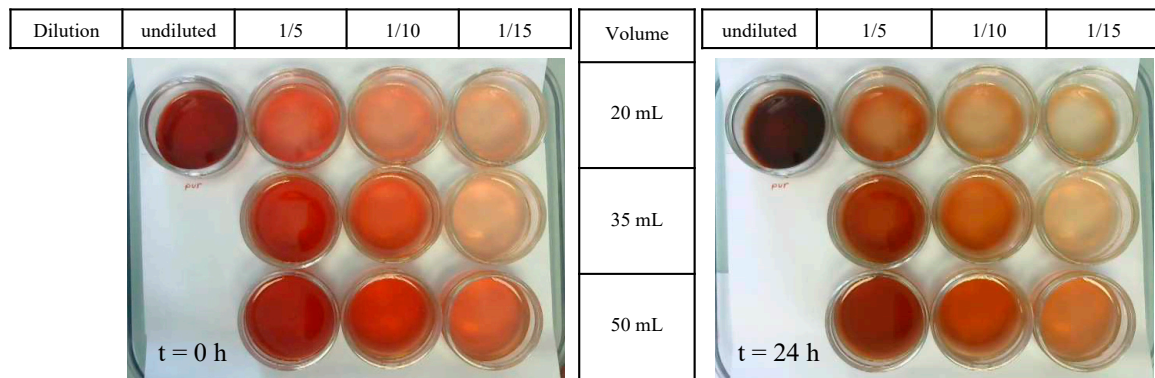


Figure 1. Visualization of the color of the same rib steak juice with different volumes (20 mL, 35 mL, and 50 mL) and different dilutions (1/5, 1/10, and 1/15) at $t = 0$ h and $t = 24$ h, at 20 °C. Pure meat juice (undiluted) is also presented for comparison to diluted juices.

3.1.2. Influence of the Water Type Used for Dilution

In this experiment, two types of water were tested, milliQ water and physiological water. These two waters were chosen because they are the most used in the world of the food industry (except running water). Each meat juice from the same whole rib steak, vacuum-packed before freezing and thawing, was centrifuged, filtered, and then diluted 1/5 in 50 mL milliQ water or 50 mL physiological water previously sterilized. A monitoring of the transfer of duplicate juices was implemented at 20 °C in the climatic chamber. The results showed that the two diluted meat juices had the same initial a^* value (Figure 2a). After the normalization of the data, it was found that the meat juice diluted with water milliQ turned faster than meat juice diluted with physiological water. The juice diluted in milliQ turned completely yellow after 24 h while the juice diluted in physiological water changed completely in color (also yellow) after 48 h (Figure 2b). In fact, in order to not add ionic species in the juices, it was decided to use milliQ water for all the other experiments.

3.1.3. Influence of the Packaging and Centrifugation Conditions

It is known that the oxygen has a role on the oxidation of myoglobin. Thus, it is necessary to determine whether the packaging conditions of the meat, in air or under vacuum, before freezing/thawing, have an influence on the color change of the juice from which it comes. Two rib steak muscles were packed whole under two conditions, one in air (21% of O_2) and the other in vacuum (0% of O_2) before being frozen/thawed. The juices obtained were diluted 1/5 in 50 mL of milliQ water, centrifuged, filtered, and then placed at 20 °C in the climatic chamber. It was found that the meat packaged in air gives more volume of juice (Factor 2) than that packed in vacuum. Figure 3a shows that the normalized values of a^* are identical.

When the juice has been collected after the freeze/thaw step, it may contain residues of connective tissue and adipose tissue. In order not to affect the color change due to the presence of residual, easily oxidizable fats, and so that the juice passes more easily through the filter during the filtration stage, the juice has been centrifuged and therefore clarified. The aim of this experiment was to verify the possible impact of centrifugation on the color change of the meat juice. The juice samples from a vacuum-packed whole rib steak were centrifuged or not centrifuged then filtered (through a 0.22 μ m

porosity filter) and diluted 1/5 in 50 mL with milliQ water. Monitoring of the color change of duplicate juices was implemented at 4 °C in the climatic chamber. This temperature of 4 °C was chosen compared to previous experiments at 20 °C in order to have a first approximation of the transfer time of a juice at 4 °C. After normalization, it was found that the curves of the a^* values of the centrifuged and non-centrifuged juices are identical (Figure 3b).

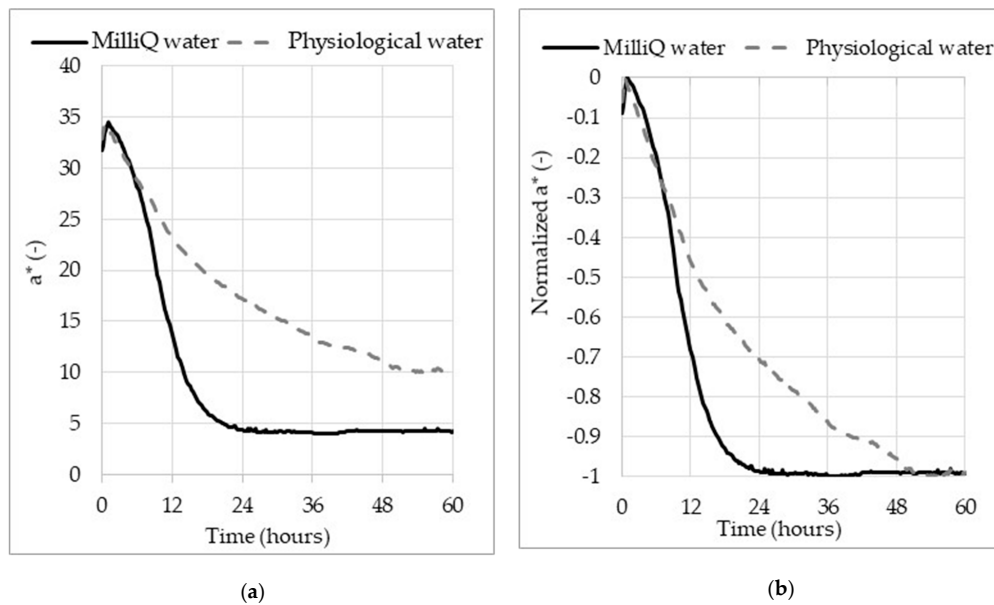


Figure 2. Monitoring of the color change (a^*) as a function of time of (a) a rib steak juice (50 mL of centrifuged juice, filtered and 1/5 diluted) at 20 °C according to the type of water (milliQ water, black solid line, or physiological water, grey dashed line) used to dilute the meat juice as a function of time and (b) the same data in normalized values, using Equation (1).

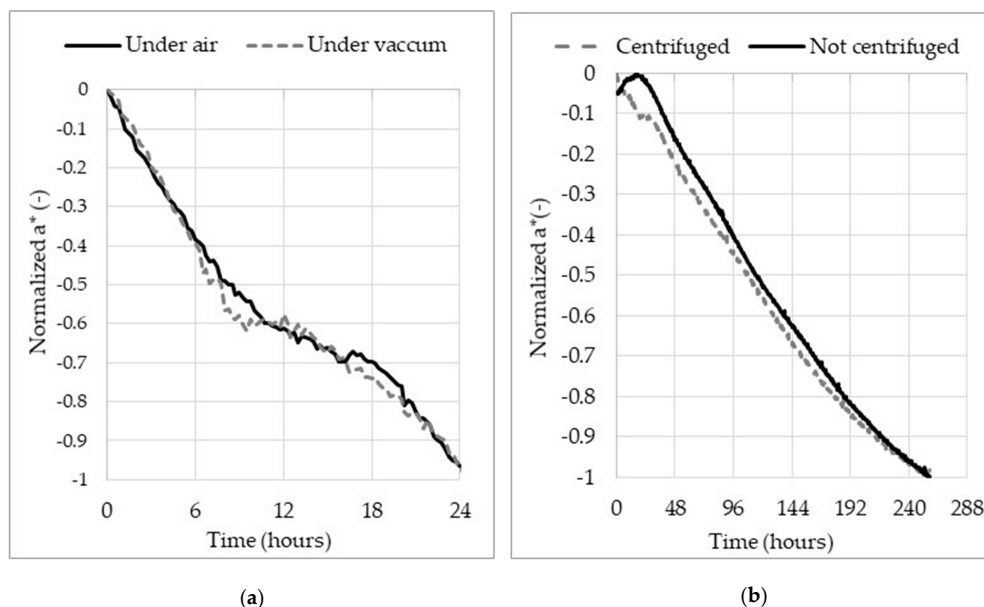


Figure 3. Monitoring of the color change (normalized values of a^*) as a function of time for (a) a rib steak juice (50 mL of centrifuged juice, filtered and 1/5 diluted) at 20 °C depending on the type of packaging (air, in black solid line), or under vacuum, in grey dashed line) and (b) a rib steak juice (50 mL of filtered juice and diluted 1/5) at 4 °C with, in grey dashed line, or without, black solid line, the step of centrifugation (centrifuged or not centrifuged).

3.2. Correlation between Color Transfers and E_h in the Juice

In order to be as close as possible to industrial conditions, it was decided to carry out this experiment with no filtering of the juice. Indeed, in the meat industry, carcasses, muscles, and meat do not undergo any decontamination step between the start and the end of the process. The goal is to check if E_h is really a good indicator of the color change in this case. The parameters of the protocol for obtaining the juice were as follows: freezing/thawing of a whole rib steak muscle under vacuum, centrifugation, and 1/5 dilution of the juice in 50 mL of sterile milliQ water. The same juice was separated in four crystallizers (two duplicates without probes and two duplicates with probes), which were then placed at 4 °C in the climatic chamber. The results of the normalized values of a^* and of E_h are represented in Figure 4. The first observation which can be made is that a certain “symmetry” is observed between a^* and E_h (when a^* decreases, E_h increases). First, the values of a^* start with a phase of slight decrease, up to 105 h where the juices are still red. Then, for the next 45 h, a sudden decrease phase is observed; at this end point, the juices have turned and are yellow/greenish in color. Finally, a new plateau phase is observed. Concerning the E_h evolution, a slight increase is observed during the first 96 h, then, an exponential increase is measured before reaching a plateau after 140 h. One has to note that a crossover is observed at 125 h, when the color transfer begins to be well established.

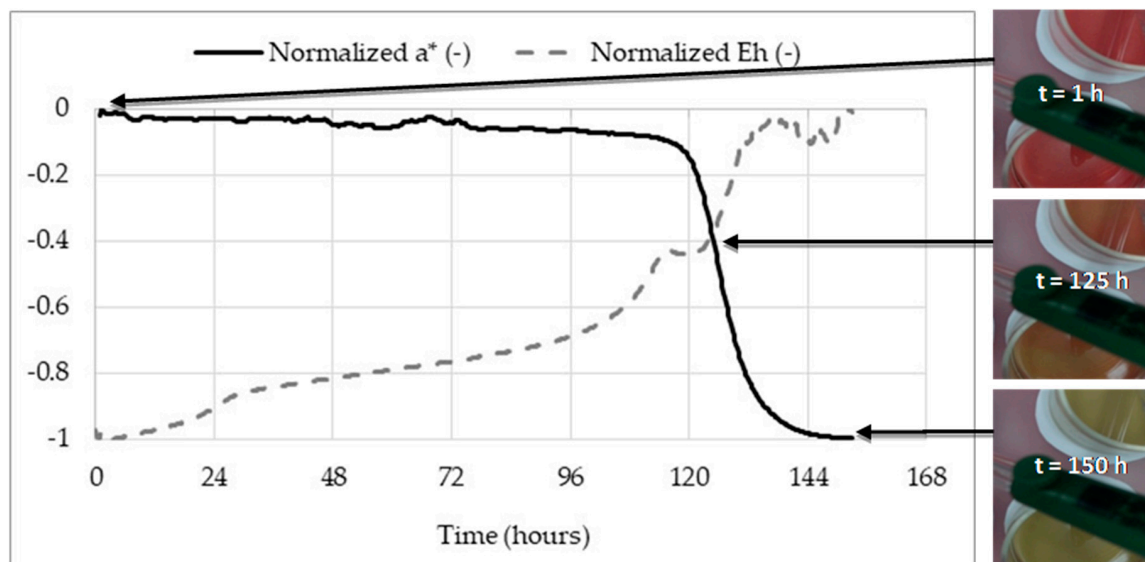


Figure 4. Monitoring of the color transfer (normalized values of a^* , in black solid line) of a non-decontaminated rib steak juice (50 mL of juice diluted 1/5) at 4 °C and of the redox potential (normalized values of E_h , in grey dashed line) as a function of time and associated photos showing the color transfer of non-decontaminated juices (duplicated on the right) at $t = 1$ h, $t = 125$ h, and $t = 150$ h.

3.3. From Beef Juice to Beef Meat Measurements

3.3.1. Detection Limit for Color Changes in Meat

As the color transfers in the juice samples are homogeneous, the image processing process made it possible to detect the color transfers using the whole surface of the juice. However, for meat, a detection limit had to be determined. Indeed, if the values of a^* are determined by taking into account the entire surface of the meat, the tacking time cannot be detected on the curve (only some pixels are concerned by a color transfer) as shown in Figure 5a. To solve this problem, different sizes of meat sample area were used as the area for measuring the values of a^* . These different areas were calculated by dividing the total number of pixels of the area of the meat sample by 1, 2, 4, 16, 32, 64, and 128 (Figure 5a). The results presented in Figure 5b show that from the 1/64 cut, the values of a^* obtained no longer vary for the same sample. Indeed, the precision is such that the transfer is then detectable on the curve of

values of a^* . Therefore for all meat samples, a measurement of the values of L^* , a^* and b^* was carried out on 1/64 of the total area at the place where the tack was observed.

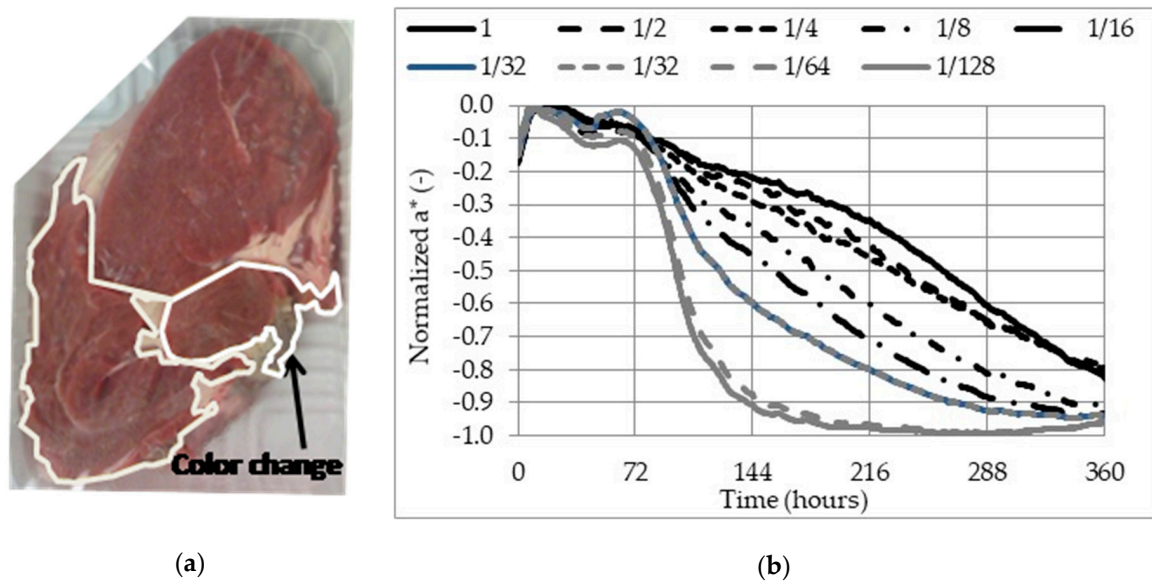


Figure 5. (a) Example of a limit detection of the color transfer, using a surface of 1/2 (white separation); (b) normalized a^* values for different areas of meat measured for the same sample (area divided by 1, 2, 4, 16, 32, 64, and 128) over time.

3.3.2. Comparison between Beef Juice and Beef Meat

The aim of this experiment is to compare the color changes of beef meat and its associated juice in order to know if the juice is really a mimetic medium of the meat. For this experiment, a rib steak and its associated juice were collected and placed at 4 °C in the climatic chamber. The juice underwent the same protocol as for the other experiments and results are shown in Figure 6, with top left, pure juice and top right, 1/5 diluted juice. The results represent the normalized values of a^* of rib steaks treated in 1/64 and its 1/5 diluted juice. With regard to the juice, the values of a^* have the same tendency as previously observed: a slight decrease up to about 96 h, followed by a more pronounced slope up to 240 h. For meat, the measured a^* values follow exactly the same trends as the juice, i.e., first of all a slight decrease ($t < 80$ h) then a more significant fall ($t < 240$ h). A color change from red to greenish was identified. The normalized values shown in Figure 6 are pretty close, even if the fall was slightly more rapid for the a^* values of the juice than for those of the meat. The juice is therefore a good mimetic medium with regard to color transfers although the kinetics of the transfer is faster in the juice than in the meat (which is consistent because the diffusion coefficients are different).

3.3.3. Calibration of the Built in-House Redox Probes

Meat juice being a good mimetic medium for meat to study color changes, it was decided to use the built in-house redox probe in the juice to perform their calibration. Indeed, the probes were built with two “working” electrodes, as shown in Figure 7a (see Material and Methods section for details), so that there is no longer a reference electrode and the values cannot be compared with values of commercial redox electrode. To verify the validity of our built in-house electrode, it was introduced into an unfiltered juice, diluted to 1/5 with a commercial redox probe (Hanna Instrument). The juice recovery protocol is the same as for the previous experiment. The results of this experiment, in normalized values, are shown in Figure 7b. Even if there is no reference electrode on our built in-house electrode, the normalized E_h curves follow the trends. Following these results in the juice, the built in-house electrode developed for semi-solid media seems to be very promising for tests on meat.

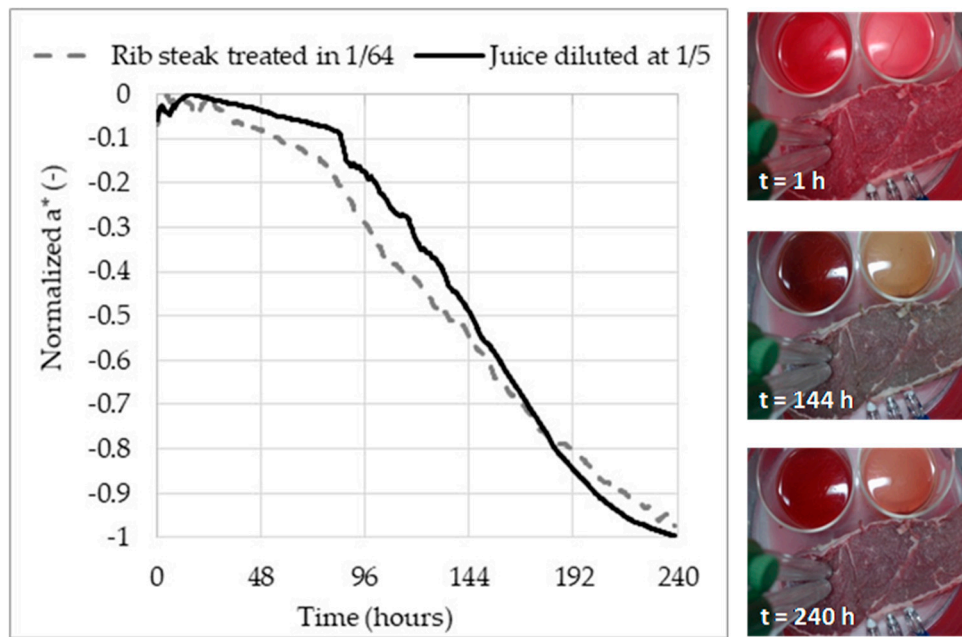


Figure 6. Monitoring of the color transfer (normalized values of a^*) of a rib steak, in grey dashed line, and its associated juice (50 mL pure on the left and 50 mL diluted 1/5 on the right), in black solid line, at 4 °C as a function of time, and the associated photos showing their color transfers at $t = 1$ h, $t = 144$ h, $t = 240$ h.

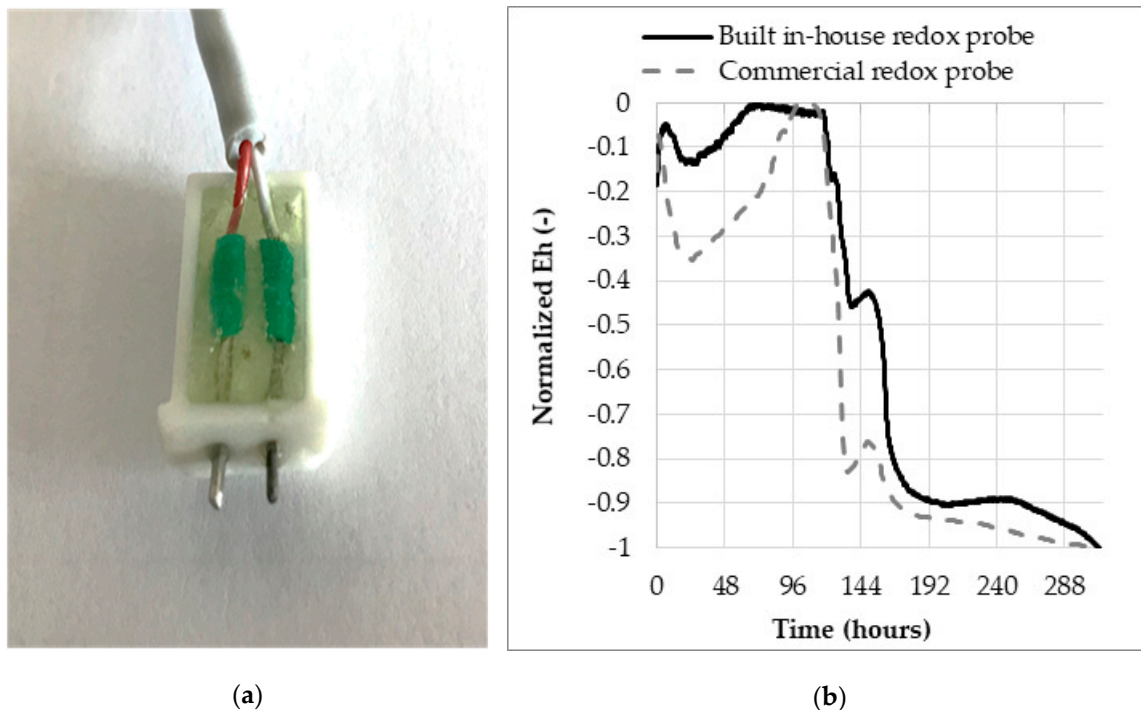


Figure 7. (a) Built in-house redox electrode composed of two “working” electrodes of 1 mm in diameter, one of which consists of a platinum rod (left) and the other of an oxidized iron rod (right) molded in a resin; (b) Monitoring of normalized E_h measured with a commercial redox probe (Hanna Instrument, in grey dashed line) and our built in-house redox probe (in black solid line) in a steak juice diluted 1/5 unfiltered as a function of time.

3.4. Validation on Beef Meat

This experiment was done under semi-industrial conditions. The rib steaks were placed in a heat-sealed tray and the built in-house probes were placed on the meat before air sealing (21% of oxygen). The experiment was carried out with 6 samples (6 successive slices) from the same muscle. As for the location of the probe on the meat, it was positioned in the muscle making up the rib steak which turned most often according to other experiments carried out during this study. At this stage, the important point is to test the probe on the meat medium to see if it works. The values of a^* were measured as close as possible to the probe (a few mm) and represent 1/64 of the slice of meat. The results of this experiment are shown in Figure 8. At the start of the experiment, the value of a^* is 20 and the meat is red. A sharp drop was measured after about 5 h. A color transfer was detected at the redox probe after 20 h of experience. A greenish color can then be observed at the level of the redox probe. After 96 h, the values of a^* reach a plateau corresponding to a marked green color on the beef meat. For E_h , the trends are the same as what has just been presented, the values ranging from 460 mV to 520 mV during the increase of the redox potential, then a plateau of a few hours was measured and finally a start of fall occurs, ending at 485 mV after 144 h. Normalized values are shown in Figure 8 on the right. This standardization highlights that E_h follows the color change (change from red to green) and that there is a crossover after 20 h which corresponds to the start of the color change. The built in-house redox probe therefore makes it possible to follow the color change in the semi-solid medium that is beef meat.

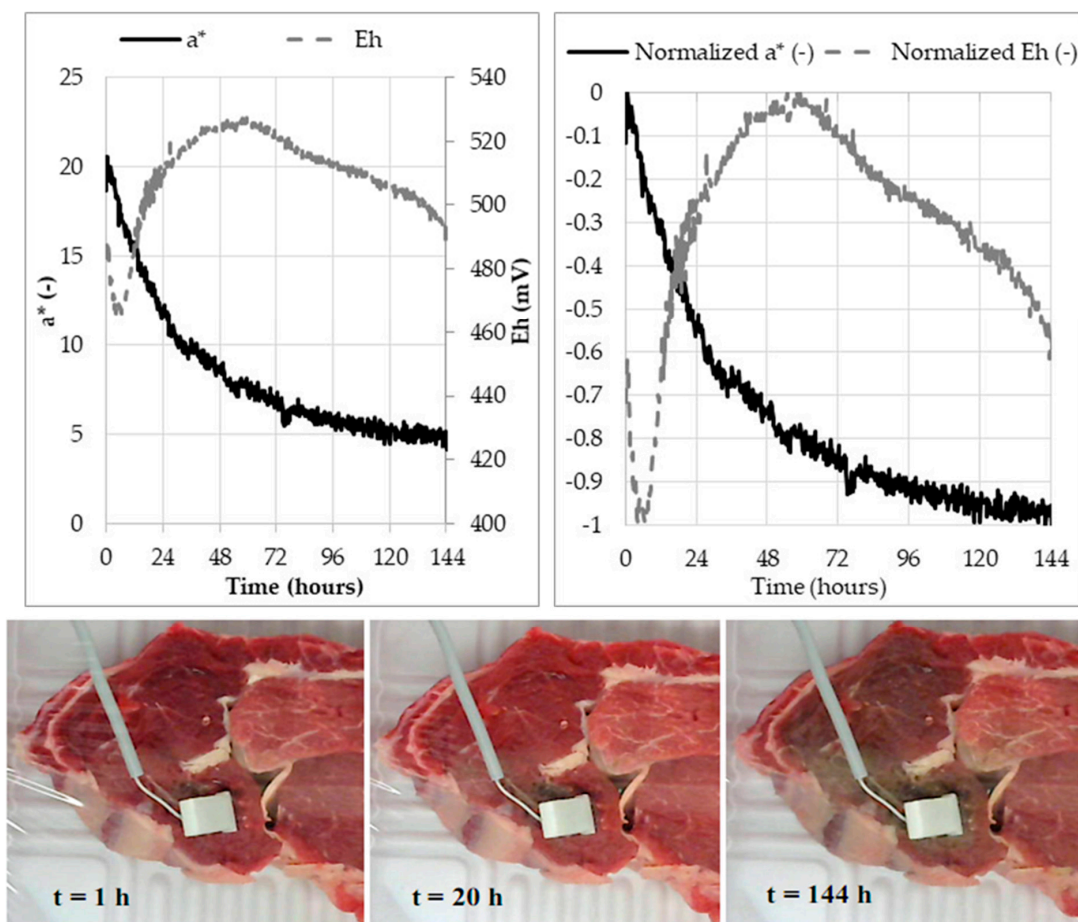


Figure 8. Monitoring the color transfer a^* of a rib steak in a heat-sealed tray in air and the redox potential, E_h , measured with a built in-house redox probe at 4 °C as a function of time (left), the values normalized as a function of time (at right) and the associated photos showing their color transfers at $t = 1$ h, $t = 20$ h, and $t = 144$ h.

4. Discussion

The aim of this study is to carry out a measurement of the E_h on a carcass, at the separation between forequarter and hindquarter in order to evaluate, by the measurement of the color transfer a^* , the time necessary for meat color to change from red to brown. Since meat is a complex media, linked to the fact no commercial redox probes exist for semi-solid media, a preliminary study was the use of meat juice as a meat mimetic medium. For that, the volume which has been retained is 50 mL so that the redox probes used can be completely immersed. Regarding the dilution, it was decided to modify the juice as little as possible to stay as close as possible to the pure juice, the 1/5 dilution in milliQ water was therefore selected. The more the juice is diluted the more the amount of myoglobin (Mb) present decreases, which can influence the results. In fact, the muscles containing high concentrations of Mb appear darker than those containing less. These differences in myoglobin concentrations, oxygen consumption rate and autoxidation rate may explain the variations in color stability between different muscles [12]. The juice preparation steps are just as important and should be monitored to ensure that they have the least impact on color changes. Pure juice is not a good choice to follow color changes because it is too dark. A 1/5 dilution was applied with two different waters. The results showed that the color change was slowed down with the use of physiological water. Indeed, the use of NaCl is well known to act as a preservative and this can explain this slowing down although the study done by Maruitti and Bragagnolo [13] has highlighted the pro-oxidant effect of NaCl on lipid oxidation and so, on color transfers. In contrast, milliQ water has the same behavior as distilled water and according to the experience of Thiem et al. [14] on the preparation of meat juices, distilled water was used and the components in the meat juices were not changed after dilution. This thus validates the method used in this study.

Experience on the packaging conditions of meat before freezing has highlighted the fact that the juice extracted from meat frozen in air has a value of a^* higher than that of meat frozen in vacuum. This can be explained by the fact that the meat packaged in air was able to oxygenate during the freezing and thawing stages, resulting thus in a higher beginning value. However, no difference was observed on the color transfer itself. According to Baran et al. [15], when fresh meat is packaged with films having a high impermeability to oxygen (under vacuum), anaerobic growth of native microorganisms is favored and the growth of aerobic native microorganisms is limited unlike packaging under oxygen (under air). Moreover, Daniloski et al. [16] also showed similar results with these two types of packaging. The different types of packaging can influence the color change due to the presence of microorganisms. However the filtration step done in this study has removed all the microorganisms from the juices. This could explain why no difference was observed in the color transfer between the two studied packaging conditions.

The centrifugation step did not affect the color transfer but only the initial a^* value. Indeed, the non-centrifuged juice has a value of a^* higher than the centrifuged juice. This can be explained by the fact that the centrifuged juice has been clarified and is therefore clearer than the non-centrifuged juice. As a result, only the value of a^* was impacted. The purpose of the centrifugation step is thus to standardize the juice samples by removing any debris recovered from the juice after thawing and to facilitate filtration.

During the experiments on the unfiltered beef juices at 4 °C and on beef meat at 4 °C, it was observed that the redox potential and color changes are linked; it is thus possible to follow the color changes using the redox potential and vice versa. Indeed, a symmetry was highlighted between this thermodynamic quantity (E_h) and the color changes (a^*). During the color transfer, heme iron oxidizes and changes from ferrous to ferric [17]. The increase in the redox potential would therefore be linked to the loss of the red color of the meat which is closely linked to the oxidation of heme iron. In the work of Ke et al. [18], the redox potential of the *Psoas major* muscle (PM) increased significantly between 0 and 7 days and was associated with an increase of the percentage of metmyoglobin and a decrease in color stability (values of a^*). Additionally, the redox potential of the *Longissimus lumborum* (LL) muscle did not show any significant variation over time, and was associated with a stability of the color of the muscle. We thus confirmed the observations of Ke et al. [18] in meat juice at 4 °C by the E_h increase, reflecting the oxidation of the medium during the loss of red color of the juice. Moreover, the experience comparing the color transfer between beef meat and its juice has shown that the juice and the meat have a simultaneous color transfer but that the kinetics of

the transfer are faster in the juice. This latter observation is supported by the fact that the juice being a liquid medium, the physico-chemical reactions has to be faster than in a semi-solid media, where the species diffusion coefficients are greater.

The temperature has also an influence on the kinetics of the color change. The color transfer curves for juice or meat at 4 °C are generally composed of three phases in our study. First, a plateau which corresponds to the latency time, that is to say the time necessary for the oxidation to set up, then a fairly brutal fall corresponding to the oxidation (change from red to yellow/brown) in itself and finally a plateau phase once the transfer is complete. On the other hand, in experiments at 20 °C, this first stage of the plateau is shorter, even sometimes nonexistent and the transfer time is twice as short. Indeed, at 4 °C, transfers started between 96 and 144 h for juice and meat and ended after 144 and 240 h for juice and meat, respectively, while at 20 °C transfers started quickly between 1 and 10 h depending on the juices and ended after 24 h. This is explained by the oxidation of myoglobin which is kinetically favored and faster at high temperatures [17,19]. However, for the experiment on the color transfer of meat with the monitoring of E_h with the built in-house redox probe at 4 °C, the color transfer was earlier than for the experiment of comparison of the color transfer meat and its juice. Two hypotheses can be put forward. The first would be that this meat is more sensitive to the color change than another because of its internal variability. The second would be that the redox probe favored the color change. Indeed, although the built in-house redox probe correctly follows the color change, with an almost constant delta compared to the values measured with the commercial redox probe, the oxidized iron that composes it may have initiated the color change at the location where it was inserted. Moreover, one has to note that this observation was exactly the same for the five other slices of meat under study, indicating that the probes helped trigger the color transfer on the six different samples. For example, Warner et al. [20] have shown that the iron concentration has an impact on the color change in mutton, where the color stability is reduced when the muscle had a high iron content. This argument would explain the systematic faster color transfer observed using the built in-house redox probe since, an electrode is made using an oxidized iron (Fe(III) state) rod. This iron rod was used since the aim was to not introduce other reducing metal species which would have produced metal ions which could have caused complexation reactions, which could themselves influence the color transfer of the meat. It is clear that the use of these probes is ultimately problematic in an industrial point of view, particularly in slaughterhouses. However, the clear demonstrated relationship between the redox potential E_h and the color transfer a^* seems to open the way to color measurement directly in contact with the meat. This would seem all the more interesting as this measurement is faster than that of the redox potential, for which probe development would also have to be carried out. Future work will then to model and predict these color transfers according to different factors, such as oxygen partial pressure, temperature or maturation time; this would allow carcasses to be classified upstream in order to sort them.

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