

## Review

# Intrinsic and Extrinsic Factors Affecting the Color of Fresh Beef Meat—Comprehensive Review

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**Abstract:** Meat color research from the last two decades suggests that a combination of different intrinsic (ultimate pH, age of the animals, muscle position, breed, slaughter weight, and sex) and extrinsic factors (production systems and feeding, pre-mortem stress, slaughter season, and chilling rates) might have a deep impact in the color of beef muscle and influence consumers' acceptance of fresh meat. Ultimate pH and muscle position were perceived as the most determinant intrinsic factors, whereas production systems, feeding, and ante-mortem stress were the extrinsic factors that more strongly influenced beef color attributes. From an industrial perspective, the extrinsic factors can be improved through the technological process at a higher ratio than the intrinsic ones. This review aims to evaluate the effect of each of those factors on myoglobin oxidation and beef color traits from a comprehensive standpoint. All the information discussed in this manuscript focuses on an industrial environment and offers possible solutions and recommendations for the global meat industry.

**Keywords:** ultimate pH; age of the animals; muscle position; slaughter weight; production systems; feeding; pre-mortem stress; slaughtering season; chilling rates



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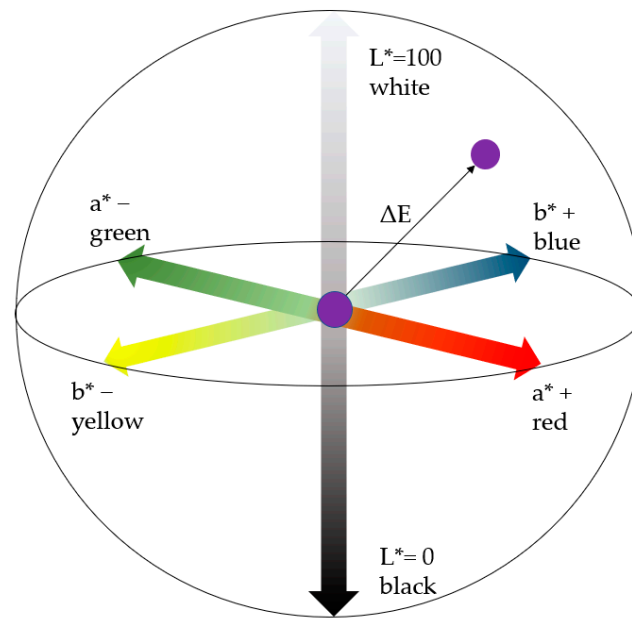
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## 1. Introduction

Visual stimuli have a major impact on consumers' opinions about meat because this first perception might be the difference between buying or rejecting a particular meat product. Since myoglobin content is higher in post-mortem bovine muscle than in pork or chicken, this is much more critical in determining consumers' criteria about color attributes in beef than in muscles from other species. Due to the central role of color in beef commercialization, the intention of this review is to explore the influence of intrinsic factors such as ultimate pH, age of the animals, muscle position, breed, slaughter weight, and sex, and of the extrinsic factors such as production systems and feeding, pre-mortem stress, slaughter season, and chilling rates. Systematic research is included in the methodology section so that other researchers in the field can easily access the information presented in this review.

Color is considered the most important quality property affecting the consumer's judgment of meat because red is highly preferred over purple or brown colors [1]. From all color parameters, redness is considered the single most robust value for predicting meat color acceptability [2,3]. Myoglobin's primary role in living tissues is to transport oxygen to the mitochondria, which are the cellular organelles responsible for respiration and ATP synthesis [4,5]. However, after animal slaughter, myoglobin is the main heme protein that determines the color of beef muscle [6]. Even if myoglobin oxidation in vivo occurs regularly, inherent reducing mechanisms can rapidly transform metmyoglobin to its original form [5,7]. As animal respiration ceases after slaughtering, post-mortem muscle switches from aerobic to anaerobic [8]. These physiological changes result in structural alterations in

myoglobin, which can eventually be converted into one of its three oxidation states [5,9]. Depending on the ligand bound to the sixth coordinate of myoglobin and on the redox status of the heme iron, three different forms of myoglobin can be produced: deoxymyoglobin (purplish-red color, ferrous iron, and no ligand attached), oxymyoglobin (bright cherry-red color, ferrous iron, and oxygen attached), and metmyoglobin (dull-brown color, ferric iron, and water attached) [10]. Myoglobin oxidation/reduction is dictated by oxygen amounts, antioxidant availability, and enzymatic activity [11]. Metmyoglobin reduction can be achieved either from enzymatic, non-enzymatic, or mitochondrial-mediated pathways [12]. All these three processes require electrons produced from NADH regeneration via lactate dehydrogenase (LDH) or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) activity [9,13]. Metmyoglobin enzymatic reduction that happens in the mitochondrial membrane through cytochrome b5 reductase is considered the most important cellular enzymatic process involved in meat color development because of its critical importance in cellular electron transport [14,15]. The metmyoglobin formed during the oxidation phase is the compound that more strongly affects the meat's color and appearance, and it can ultimately determine the consumer's purchase willingness [16]. Oxidative changes that affect meat color have been traditionally quantified using three linear color coordinates, as detailed in Figure 1: L\* (lightness), which ranges from 0 value for black and 100 for white; a\* (redness), which ranges from green if negative to red if positive; and b\* (yellowness), which ranges from blue if negative to yellow if positive. Other color scores such as  $\Delta E$  (total color difference), C\* (chroma), and h\* (hue angle) might also be included in some publications to describe differences of color between two stimuli, three-dimensional perception of color and the degree of vividness, respectively [16–18]. Meat color measurements can be performed using a spectrometer, which determines different myoglobin fractions at specific depressions in the light spectrum, or a colorimeter, which quantifies meat color by a mean calculation of different points scanned on the meat surface [16,17]. Recently, a CVS (computer vision system) was implemented as a more accurate technique for color determination [18,19] due to less light deviation through the meat matrix and a higher scope area of the sample [17,19]. It was reported that the color intensity of fresh meat is influenced by an interaction of multiple intrinsic and extrinsic factors during muscle-to-meat conversion [20]. Intrinsic factors are those characteristics mostly defined by the animals' genotype, which are difficult or impossible to change. On the contrary, extrinsic factors are circumstances related to human handling of the animals during feeding, transportation, slaughtering, and chilling that might be more flexible to adjust through the technological process, and that can be improved to a greater stage than the intrinsic ones. This review paper aims to evaluate the influence of both type of factors on meat color and to establish the interaction of those parameters during muscle-to-meat conversion. It also seeks to find the best strategies that might be helpful for the producers and the meat industry in preventing or reducing these color defects in fresh beef meat.



**Figure 1.** Graphic diagram of the quantitative coordinates used for meat color determination.

## 2. Materials and Methods

### 2.1. Data Collection and Eligibility Criteria

This systematic review manuscript followed the research steps cited in the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [21]. For this purpose, the authors followed a manual pre-selection and evaluation of papers published between 2000 and 2022, focusing primarily on the topics and abstracts of those articles. English was preferred as the language for paper selection, and only research articles were considered to ensure the information quality for this investigation. Other reviews, correspondence letters, dissertations, expert opinions, lectures, books, or chapters of books were excluded. Duplicate articles were only considered once. The study was considered relevant when: (i) it included information about the color change of fresh meat; (ii) it had information about instrumental color measurements; (iii) evaluated color changes during retail display time; (iv) no freezing, cooking, curing, or thawing processes in fresh meat were involved; (v) the article was conducted as a unique research manuscript.

### 2.2. Focus Questions

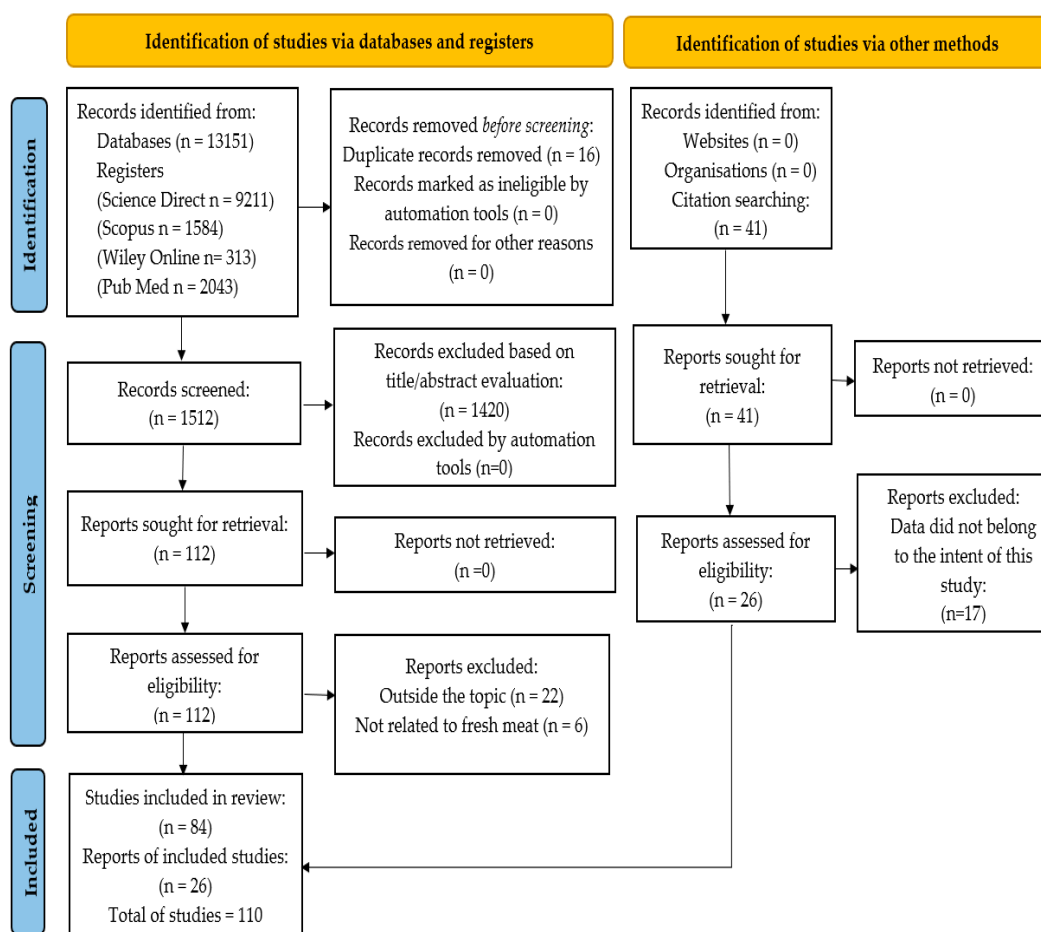
The search for papers was performed similarly in all databases following the PICO (Patient, Intervention, Comparison, and Outcome) strategy [22] to articulate the research questions considering population (P): fresh beef meat; intervention (I): color; comparison (C): intrinsic and extrinsic factors; and outcome (O): color change. The focus questions were: (i) how do intrinsic and extrinsic factors influence the color change of fresh beef meat? (ii) which strategy can be implemented to reach the best factor combination that favors the color change in beef meat?

### 2.3. Information Sources and Search Strategy

The literature selection was based on a manual validation using several different databases, i.e., Scopus, Science Direct, Wiley Online Library, and PubMed. The exploring and screening for eligible articles were performed from October 2022 until December 2022. The combination of keywords used for this investigation was: “meat” AND “color” AND “beef” OR “fresh” OR “light” AND/OR “scattering” OR “slaughter” AND/OR “season” AND/OR “weight” OR “diet” AND/OR “finishing” OR “chilling” AND/OR “rate.”

## 2.4. Literature Search

A total of 13,151 publications were identified as potential material for this review paper. Of them, 9211 were found in Science Direct, 1584 in Scopus, 313 in Wiley Online, and 2043 in Pub Med. From all the databases, 1512 records were screened, and 112 papers were selected for eligibility. From them, 28 papers were excluded because they were outside the topic or not related to fresh beef. This means that 84 articles were selected from databases, and 26 other papers that matched the eligibility criteria were manually selected from citations to complete a total of 110 articles suitable for this review paper (Figure 2). Detailed information about the main ideas of all papers cited in this manuscript can be downloaded from the Supplementary Materials (Tables S1 and S2).



**Figure 2.** PRISMA flow chart for article selection between the years 2000 and 2022 in the influence of intrinsic and extrinsic factors in beef meat color [21].

## 3. Results and Discussion

### 3.1. Intrinsic Factors

#### 3.1.1. Ultimate pH

Some authors considered that ultimate pH is one of the most significant and discriminating criteria in determining meat color [2,23]. It is commonly agreed that depleted glycogen reserves in animals during stressful pre-mortem events might cause limited lactic acid formation in post-mortem muscle and insufficient pH decline [23,24]. Since high-pH beef muscle fails to reach a bright-red color, this condition is referred to as dark-firm-dry (DFD) meat [13,25]. DFD carcasses had lower glycolytic potential than those with normal pH values, resulting in less glycogen availability for lactic acid formation post-mortem [25]. Previous data also showed that muscle glycogen amounts lower than 50  $\mu\text{mol/g}$  at slaughter could be indicative of DFD meat [26]. The darker color in DFD meat also resulted in

a higher accumulation of myoglobin oxidation products and less light reflectance on the meat surface [23,27]. DFD beef showed a higher percentage of oxidative type I muscle fibers and a lower percentage of glycolytic type II muscle fibers, indicating higher myoglobin and mitochondria contents than normal pH muscles [13]. Previous data showed that higher mitochondrial counts and mitochondrial activity in DFD meat resulted in a darker meat surface color due to higher tissue oxygen consumption, which restricted the capacity for myoglobin oxygenation during blooming [9,13,27]. Greater water-holding capacity in DFD meat resulted in more swollen fibers that increased light scattering from the myoglobin fractions and generated the typical darker superficial appearance of DFD muscle [28,29]. Recently, it was suggested that higher amounts of tricarboxylic acid cycle (TCA) metabolites present in DFD meat could be further used for cellular respiration that increased mitochondrial oxygen consumption and deoxymyoglobin formation [13]. Higher myoglobin concentrations and inefficient mitochondria abundance revealed that animals predisposed to DFD-related issues had an oxidative metabolism that accelerated glycogen depletion as the degree of darkening in DFD meat increased [24,25]. It was also suggested that lower phosphorylation degree of sarcoplasmic proteins in intermediate ( $5.7 < \text{pH} < 6.09$ ) and high pH groups ( $\text{pH} > 6.09$ ) could have influenced the activity of certain metabolic enzymes associated with the development of DFD meat color [30]. Additional proteome studies also showed higher metabolic enzymatic activity in DFD meat with eight overabundant metabolic enzymes compared to normal-pH meat. However, normal-pH meat showed increased LDH-D activity, thus improving meat color [29]. Due to variations in the management systems, handling of the animals, slaughterhouse policies, consumer preferences, and the pH thresholds that the local food authorities may assign to this defect, the incidence of problems involving DFD cattle varies across different countries. However, until now, early detection of animals predisposed to a DFD status has not been substantiated and results still have partial repercussions. The most common strategies have been related to the measurement of some enzymes or hormones in the bloodstream of animals before and during slaughtering, such as cortisol, creatine kinase (CK), and lactate dehydrogenase (LDH). Clear interactions were found between higher production of those substances and animal welfare, but, no relationships were found for ultimate pH or meat color [31–33]. Infrared eye temperature determination also showed contradictory results to predict the incidence of DFD problems in beef [34]. Reliable markers that can determine the relationships between pre-mortem processes and the ultimate pH in meat still need to be validated. DFD carcasses are usually reduced in price due to the meat's undesirable color. This problem results in significant economic losses for the global meat industry. Therefore, any improvement in the color of DFD meat may increase the income for all actors in the supply chain while preventing the waste of valuable environmental resources assigned for meat production.

### 3.1.2. Age of the Animals

Higher myoglobin and iron levels were detected in the meat of older animals and resulted in darker muscle colors, probably caused by more oxidative muscle fibers [27,35]. Increasing the slaughter age in cattle also resulted in a higher proportion of red oxidative fibers that grew at a faster pace than the glycolytic ones in the muscles of older animals and generated an overall negative effect on meat color [36]. The meat of older animals from discontinuous growth systems (24 months of age) showed lower  $L^*$ ,  $a^*$ ,  $b^*$ , and pH values in muscle than younger animals (18 months of age) from continuous growth systems [37]. It was also documented that extending the slaughter age in cattle from 14 to 18 months produced similar  $L^*$  and slightly higher pH and  $a^*$  values in the meat of older animals [38]. Another study reported that extending the average slaughter age for three additional months produced beef muscles with lower  $L^*$  and higher  $a^*$  and  $b^*$  values, probably due to higher pH found in the meat of older animals [39]. Interestingly, it was observed that higher myoglobin amounts in the muscles of older animals resulted in significantly lower  $a^*$  and higher  $C^*$  values [40]. Significantly higher  $L^*$  but almost steady  $a^*$  values were reported

when bulls were slaughtered at 17 instead of 12 months of age, probably due to similar meat pH values [41]. It was also reported that steers slaughtered at 30 months of age produced higher pH and  $a^*$  but lower  $L^*$  values than animals slaughtered at 18 months [42]. Increasing the slaughter age in cows (up to 11 years) resulted in lower  $L^*$ ,  $a^*$ , and  $b^*$  values in fresh meat due to higher inherent lipid oxidation and radical production in the muscles of old cattle, even though there was higher production of antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase [35,43]. This manuscript agrees that higher quality of feed provided to animals with increased growth rates could consistently shorten the time to achieve commercial slaughter weight, thus increasing  $L^*$  values in meat. Most of the references also claimed that myoglobin amounts in meat proportionally increased with higher biological age of the animals, thus showing higher  $a^*$  values in the meat of older animals. Lower myoglobin contents in the muscles of younger animals might positively influence in producing brighter and less red fresh beef muscle.

### 3.1.3. Muscle Position

High color-stability muscles were defined to have a lower oxygen consumption rate, lower oxidative rancidity, and lower myoglobin content in comparison with muscles classified as color-unstable [44]. Different authors have recorded higher  $L^*$ ,  $a^*$ , and  $b^*$  readings for fresh color-stable m. longissimus lumborum (LL) in comparison with color-unstable m. psoas major (PM), therefore validating the color difference between these two groups of muscles [15,45]; however, pH differences between color-stable and color-unstable muscles were not discriminant for meat color, as shown in Table 1. It was stated that LL muscle contained more antioxidant proteins ( $\beta$ -enolase and creatine kinase) than PM muscle, significantly reducing myoglobin and lipid oxidation [46]. Previous data also suggested that higher LDH activity was representative of glycolytic muscle fibers, while higher ICDH (isocitrate dehydrogenase) activity was mostly found in oxidative muscle fibers [36]. It was also reported that higher lipid contents in color-unstable muscles proportionally increased the probability of lipid oxidation to occur [45,46]. It was proposed that lower oxidation-reduction potential rates (ORP) could be related to less cytochrome c release, less oxidative stress, and lower oxygen consumption in LL muscle when compared to PM [8]. Higher mitochondrial integrity in ovine LL muscle resulted in lower metmyoglobin accumulation compared to PM [9]. This is consistent with other studies that found that higher metmyoglobin-reducing activity (MRA) and greater amounts of sarcoplasmic NADH in LL were associated with less accumulation of myoglobin oxidation products on meat surfaces than PM [7,9,47]. Lower myoglobin, iron content, and mitochondrial amounts were related to predominant type II white fibers in color-stable muscles; in contrast, a predominant presence of oxidative-type I red fibers in color-unstable muscles made them appear comparably darker [27,48]. Lower levels of taurine and coenzyme Q10 and higher levels of carnosine, creatine, and creatinine were recorded in color-stable muscles, which were related to a higher amount of type II white fibers in comparison with color-unstable muscles [49]. Overall, variations in inherent histological functionality between different bovine muscles played a key role in meat color characterization. These differences between muscle's color stimuli can be calculated using the total color difference ( $\Delta E$ ) Equation [16,18].

$$\Delta E = \sqrt{(L^*_1 - L^*_2)^2 + (a^*_1 - a^*_2)^2 + (b^*_1 - b^*_2)^2} \quad (1)$$

It was stated that when  $\Delta E$  values are higher than 1, color differences can be discernable by the human eye [50]. The  $\Delta E$  values were calculated from several publications found in the literature, as described in Table 1. This information suggested that, overall, consumers can visually identify color variations between different groups of muscles. Thus, the meat industry needs to focus on designing innovative muscle-specific technological solutions that might prolong meat color shelf-life for a more prolonged time. Additionally, consumer-based marketing tactics should be prioritized in promoting faster consumption of those muscles described as less color-stable.

**Table 1.** Color and pH differentiation between color-stable and color-unstable muscles in fresh beef.

Muscle	pH	Parameter	Value	$\Delta E$ Difference	Publication
LL	5.5	L*	36.77	4.31	[46]
		a*	29.5		
		b*	21.84		
PM	5.59	L*	40.63		
		a*	30.5		
		b*	23.48		
LL	5.73	L*	45	4.53	[15]
		a*	32.1		
		b*	24.3		
PM	5.77	L*	47.04		
		a*	29		
		b*	21.7		
LL	6.65	L*	43.63	5.76	[45]
		a*	27.4		
		b*	20.6		
PM	6.87	L*	41.78		
		a*	24.3		
		b*	16.11		
LL	5.5	L*	41.3	5.28	[51]
		a*	34.4		
		b*	27.1		
PM	5.7	L*	46.3		
		a*	33.4		
		b*	25.7		
LL	5.73	L*	-	-	[25]
		a*	17.06		
		b*	-		
PM	5.77	L*	-		
		a*	17.67		
		b*	-		
LL	5.57	L*	-	-	[8]
		a*	30.6		
		b*	-		
PM	5.61	L*	-		
		a*	29.5		
		b*	-		

Table 1. Cont.

Muscle	pH	Parameter	Value	$\Delta E$ Difference	Publication
OSM	-	L*	39.1	10.41	[52]
		a*	29.13		
		b*	21.21		
ISM	-	L*	47.65		
		a*	32.75		
		b*	25.92		
OSM	6.52	L*	40.8	13.01	[53]
		a*	30.8		
		b*	23.4		
ISM	6.47	L*	52.8		
		a*	34		
		b*	27.3		

PM: Psoas major; LL: longissimus lumborum; OSM: Outside semimembranosus; ISM: Inside semimembranosus.

### 3.1.4. Breed

Commercial meat cattle breeds are divided into two big groups: *Bos taurus*, which are cattle of European ancestry, and *Bos indicus*, which are cattle typically designed for production under tropical weather conditions. Pure *Bos taurus* crosses (Hereford x Angus) exhibited higher carcass weight, improved fat deposition, and intramuscular marbling than *Bos indicus* x *Bos taurus* animals (Sahiwal or Brahman x Hereford or Angus) [54]. Due to hybrid vigor, beef producers also noted that crosses between the two cattle groups resulted in higher resistance and increased potential for muscle accumulation [55]. However, more aggressive temperament from pure *Bos indicus* (Brahman) or *Bos indicus* crosses resulted in darker muscle color and higher pH values in meat compared to Angus (*Bos taurus*) cattle, especially when animals were pasture-fed [56,57]. Significantly lower L\*, a\*, and b\* scores and higher pH values were also found in the meat from feedlot-finished Nellore x Aberdeen Angus crosses in comparison to meat from the pure Nellore breed [58]. It was described that although a more excitable temperament of Braford (*Bos indicus* x *Bos taurus*) crosses than pure Hereford (*Bos taurus*) animals, no effects in meat color were found [55]. A study using only pure Hereford (*Bos taurus*) steers showed that calmer animal temperament had no major influence on muscle glycogen concentration or meat color [59]. On the other hand, a study performed in three *Bos indicus* African races suggested that aggressive behavior seen in long-horn Red Bororo cattle was also correlated with higher injury frequency, especially during unloading operations [60]. Indeed, more bruises in *Bos indicus* x *Bos taurus* cattle were correlated to high pH values and DFD-related problems in meat [61,62]. It was also found that *Bos indicus* carcasses showed similar differences between the so-called color-stable and non-color-stable muscles as the *Bos taurus* counterparts [45]. In our view, pure *Bos taurus* breeds or crosses with a high percentage of *Bos taurus* blood offer the best temperament profiles for enhancing meat color. Also, due to higher fat deposition in the meat of *Bos taurus* animals, muscles had a brighter color compared with the meat of *Bos indicus* cattle [56]. Breed selection should also take into consideration individual nutritional requirements and management systems to obtain brighter beef muscles.

### 3.1.5. Slaughter Weight

Feed intake and production systems might considerably influence the final carcass weight and consequently affect the ultimate pH and color of beef [36,63]. Meat industries worldwide have struggled to cool down beef carcasses at a constant rate due to higher animal-weight variation in the last few years. This inconsistency in heavy-carcass temperature and pH decline might increase the risk of heat shortening and myoglobin autoxidation

processes to occur [64]. It was also evidenced that when animals were slaughtered at the same age, grain-based systems produced heavier animals than pasture-based, due to an average higher calorie diet from the former [49,63]. Improved  $L^*$ ,  $a^*$ , and  $b^*$  measurements from heavier cattle were also a result of higher-energy diets that were offered to the animals before leaving for the abattoir, which promoted slower glycogen degradation until slaughtering [65]. Different authors reported either higher  $L^*$  [64], higher  $L^*$  and  $a^*$  [41], or overall higher  $L^*$ ,  $a^*$ , and  $b^*$  values [37,63] in the meat of heavier than lighter cattle. Other publications reported that heavier Charolais cattle showed improved  $a^*$ ,  $b^*$ , and  $C^*$  values but lower  $L^*$  scores. This experiment concluded that the preference for heavier animals paradoxically created darker lean muscles [20]. Significantly lower  $L^*$  values were also reported in bulls' meat compared to young bulls' meat, even if bulls weighed 90 kg more when slaughtered [66]. Heavier cattle tend to produce meat with increased  $L^*$ ,  $a^*$ , and  $b^*$  attributes than lighter cattle. This effect was associated with a high-caloric diet that promoted better fat deposition, and because slower glycogen decline could be achieved from heavier cattle, which also benefited the formation of lactic acid in the muscles of those animals. The meat industry needs to find the means to standardize the weight of the animals to prevent major differences in meat color.

### 3.1.6. Sex

It was reported that male cattle exhibited higher physiological stress, increasing the probability of obtaining DFD meat, particularly when kept in lairage at high stocking densities [66,67]. Lower  $L^*$ , higher  $a^*$  and  $b^*$ , and pH values were reported in the meat of male cattle compared to the meat of female cattle [66]. Similarly, lower carcass fat deposition in bulls resulted in lower  $L^*$  and  $C^*$  values when compared to cows' carcasses [20,68]. It was also found that bulls' meat reached lower ante-mortem glycogen levels and higher pH values due to more aggressive behavior compared to immunocastrated animals, steers, and heifers [59,69]. It was determined that steers were 47% more likely to have DFD meat than heifers due to lower glycogen pre-mortem levels exacerbated by mounting [70]. It was also reported that even if heifers were more susceptible to pre-mortem stress, this was not related to pH decline in meat; however, higher meat pH and lower color scores were promoted due to agonistic bull behavior [71]. A different study reported that male cattle showed more stressed behavior, resulting in lower  $L^*$ ,  $a^*$ , and  $b^*$  meat values for feedlot-finished bulls compared to heifers in the same production system [69]. It was also stated that steers produced meat with significantly higher  $L^*$ ,  $a^*$ , and  $b^*$  values than bulls and cows; however, between the last two, cows showed higher  $L^*$  and similar  $a^*$  and  $b^*$  values than bulls [72]. Conversely, some authors reported that heifers might tend to produce meat with higher pH values if estrus activity is not controlled prior-slaughtering by hormone supplementation [65]. Others determined that bulls produced meat with slightly higher  $L^*$  and  $a^*$  values, despite higher intramuscular fat content in heifers [38]. Most publications reported lower color values from bulls' carcasses due to less fat deposition and more aggressive behavior, especially during close social contact. Therefore, improved beef color might be obtained if animals are kept separated during transport and lairage and if hormone supplementation is controlled for female cattle before slaughter.

## 3.2. Extrinsic Factors

### 3.2.1. Production Systems and Feeding

Management systems and diet compositions might variably influence the color of fresh beef muscle, mainly due to predominant muscle fiber type, number of calories fed to the animals, fatty acid profile, and inter- and intra-muscular fat deposition. Production systems greatly influence lipid oxidation processes, which are strongly associated with myoglobin oxidation and the generation of off-odors and off-flavors in beef [73,74]. Higher total fatty acid content was recorded in the meat of fed concentrate-based animals rather than grass- or silage-based cattle [75,76]. It was also described that higher amounts of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and n-6 polyunsaturated fatty acids

(PUFA) portions were likely to be found in concentrate-finished animals than in pasture- and silage-fed animals [75,77]. In addition, most publications agreed that higher PUFA and n-3 PUFA fractions were obtained from pasture-based diets than from concentrate-based diets [78,79]. Precisely, meat from pasture-fed animals exhibited higher amounts of PUFAs, which could have resulted in higher oxidative phosphorylation than meat from grain-fed animals because it was more accessible for the action of lipases [43,73]. Higher thiobarbituric acid reactive substances (TBARS) values were reported in the meat of grain-fed rather than in pasture-fed cattle, mostly due to an antioxidant defensive mechanism against lipid oxidation in the latter [80]. Animal diet might have a large influence on the generation of lipid oxidation products such as HNE, which decreases both electron transport-mediated and NADH-dependent metmyoglobin reduction processes, thus increasing oxymyoglobin oxidation [52,81]. Moreover, it is generally regarded that intensive-managed (grain, concentrate, feedlot) produced brighter muscles (higher  $L^*$  values) than extensive-managed animals (silage, pasture, grass) [63,75]. This effect is associated with a higher amount of calories fed to the animals in intensive systems, promoting a glycolytic metabolism and increasing total and intramuscular fat compared to the extensive counterparts [63,82]. The quality, the amount, and the duration of concentrate feeding in different management systems might have a variable influence on  $a^*$  values in meat [82,83]. At constant slaughter weights, similar  $a^*$  values were obtained for concentrate and grass-based animals [76,83]. It was also described that grazing periods up to 98 days during the finishing phase did not produce any significant effects in  $a^*$  values [84]. Increasing the feeding intensity on bulls during the growing and the finishing phase also did not show any differences in pH or meat color between treatments, probably because all animals received a high-calorie diet, which also led to similar slaughter weights [85]. Another publication reported that grain-based diets resulted in meat with lower pH and higher  $a^*$  values than grass-based animals [86]. It was also found that when gradually increasing the level of concentrate supplementation in a pasture-based diet, the highest  $a^*$  values were attained at 0.8% of live-weight concentrate replacement, regardless of marbling or myoglobin concentrations in muscle [82]. It was also observed that the higher pigment concentration in the meat of grass-fed animals resulted in higher  $a^*$  values when compared to grass-based exemplars [87,88]. This was probably a consequence of higher physical activity due to more time spent walking, standing, or feeding, which increased the proportion of oxidative muscle fibers [89,90]. It was shown that the oxidative metabolism of cattle-fed pasture-based diets led to higher pH values and darker muscle appearance than their grain counterparts, even if no differences were found in lactate or glycogen concentration (typical for pre-mortem stress) [86]. Production systems can largely affect the color of fresh beef muscle.  $L^*$  values were consistently higher in intensive compared to extensive systems. Almost similar  $a^*$  scores were documented in different production systems as a function of neglectable meat pH variations, as shown in Table 2. Previous studies also suggested that consumers tend to reject the meat once it has reached  $a^*$  value below 14.5 [3]. Those critical values were not reached for most of the data researched in this review (Table 2). Also, despite having a higher proportion of PUFA, meat from pasture-fed animals is less likely to experience lipid oxidation due to a protective antioxidant effect gained from higher  $\alpha$ -tocopherol concentration diets. However, meat color or pH values were not affected by  $\alpha$ -tocopherol amounts found in fresh beef meat [91].

**Table 2.** Consumer’s acceptability related to meat color and pH differentiation between color-stable and color-unstable muscles in fresh beef.

Management System	L*	a*	pH	Consumer’s Acceptability	Publication
Concentrate-finished	39.4	17.36	5.56	Accepted	[83]
Grass-finished	37.6	17.3	5.55	Accepted	
Concentrate-finished	35.56	20.42	5.7	Accepted	[75]
Pasture-finished	33.8	20.45	5.7	Accepted	
Concentrate-finished	36.85	15.66	5.63	Accepted	[82]
Pasture-finished	36.37	15.16	5.62	Accepted	
Concentrate-finished	33.18	22.59	5.55	Accepted	[84]
Grazing + concentrate finishing	32.1	22.62	5.55	Accepted	
Concentrate-finished	36.4	11.3	-	Rejected	[76]
Pasture-finished	35	11.1	-	Rejected	
Concentrate-finished	37.9	19.1	-	Accepted	[88]
Pasture-finished	29.4	23.2	-	Accepted	
Feedlot-finished	38.6	15.15	5.56	Accepted	[92]
Pasture-finished	37.8	15	5.66	Accepted	
Grain-finished	40.43	22.66	5.77	Accepted	[87]
Pasture-finished	40.94	22.95	5.77	Accepted	
Concentrate-fed + straw	38	15.5	5.52	Accepted	[93]
Silage-fed + concentrate	37.4	15.9	5.53	Accepted	

### 3.2.2. Pre-Mortem Stress

Accumulative ante-mortem stressors imposed on animals during loading, transport, unloading, lairage, and slaughtering might consistently deplete muscles’ glycogen stores and increase the likelihood of producing DFD meat. It was hypothesized that pre-mortem stress conditions in cattle might increase involuntary movements and mitochondrial biogenesis in DFD muscle, which can result in higher mitochondrial oxygen consumption of fresh meat [13]. The sum of all stress stimuli has an overall negative effect on the psychological and physical status of the animals, which could adversely affect the welfare and meat color [31]. For instance, when long transportation times were combined with only a few hours for cattle to recover after the journey, it drastically increased the probability of producing DFD carcasses [94]. Long processing times also meant that animals typically had access only to water, which can lead to starvation and reduce the glycogen levels in the blood and produce meat with lower L\*, a\*, and b\* values [31]. Conversely, reducing the processing times and good animal handling might increase slaughterhouses’ efficiency, lead to better conditions for the animals, and improve the color values in the meat.

Strange environments could also be detrimental to meat color since some animals might be especially sensitive to sounds and movements made by workers or other animals during routine transportation, lairage, and slaughtering operations [31]. It has been claimed that the incidence of DFD-related problems can be reduced when animals are managed in the same group from the previous weeks before slaughtering [95]. Lower meat pH values were also recorded when bulls were not mixed during the finishing phase due to the calming effect of social interaction between animals of the same group [31]. The risk of obtaining DFD carcasses also decreased when animals came directly from the producing farms instead of when animals were bought from markets or auctions [62]. The separation of cattle into different compartments inside the same truck increased the meat color values, even if animals had the same sex or were collected from different farms [67]. It was also

suggested that mixing the animals right after loading could increment aggressivity and mounting behavior [96].

Extended lairage times in poor conditions (temperature  $> 18^{\circ}\text{C}$  and RH  $> 70\%$ ) increased the risk of producing DFD carcasses from less than 1% to around 40% when animals had to spend more than 19 h before slaughtering, instead of only 7 h [97]. Similarly, lairage times higher than 16 h doubled the probability of obtaining DFD meat when compared to 8 h of lairage [67]. Another study reported that animals staying in lairage for more than 72 h without extra feeding had an 85% probability of producing DFD carcasses [23]. It was also determined that for every extra hour animals spent in lairage, the ultimate pH in meat increased by around 0.013 points [98]. It was also documented that regardless of transportation times, animals kept long holding periods were more susceptible to producing bruises and lesions due to more frequent aggressive reactions [62]. On the other hand, it was suggested that short lairage times (3 h) produced more stressed animals and higher pH values in meat when compared with animals kept overnight (15 h) due to recovery of glycogen stores after a quiet night of rest [55]. It was also observed that after long transportation distances (approximately 1800 km), the incidence of obtaining DFD meat was 40% when cattle spent 72 h in lairage, instead of 90% when lairage time was 24 h or 60% for 48 h resting time [94]. Another study determined that 48 h of lairage was the best treatment for cattle to replenish their glycogen stores after a long trip of approximately 18 h [99]. It was also suggested that lairage time higher than 17 h contributed to glycogen restoration in muscle and reduced the risk of obtaining DFD meat [31]. Another manuscript claimed that reducing lairage time from 18 h to 3 h in cattle that traveled less than 6 h and with no signs of exhaustion had no effect on the meat's ultimate pH [100].

It has been reported that there were no significant differences in meat pH values when cattle were not stunned, electrically stunned, or percussively stunned, even if glycogen concentrations were different between treatments [101]. Another publication mentioned that animals stunned more than once had higher cortisol levels resulting in lower lactic acid production and DFD-related problems [102]. Similarly, it was determined that when incorrect desensitizing of the animals occurs, the probability of obtaining dark-cutting meat increases by 10% [97].

Random mixing of the animals during transportation and lairage might increase the probability of obtaining DFD carcasses, whereas maintaining the animals in the same social groups in which they were grown might enhance the color of their meat. Long transportation and extended lairage times might drastically reduce the glycogen reserves of cattle and generate lower  $L^*$ ,  $a^*$ , and  $b^*$  values in the meat of those animals. However, long and quiet lairage with enough food is recommended if animals need to be transported for long distances so that glycogen reserves can be replenished before slaughtering and the incidence of DFD problems can be reduced to a minimum. A simple and continuous training program about animal handling should also be provided to the workers with direct contact with cattle to optimize slaughterhouse operations, animal welfare, and meat color. Additionally, stunning needs to be precise and performed only by trained personnel to reduce the risk of DFD carcasses.

### 3.2.3. Slaughtering Season

Heat or cold stress might have different effects on the physical and psychological status of the animals, especially shortly before being slaughtered, which might result in meat with high pH and DFD-like conditions. Hot temperatures might impair the body's heat dissipation and increase animal stress, while cold temperatures might increase muscular shivering, which can rapidly deplete pre-mortem glycogen reserves. Slaughtering cattle during wintertime produced meat with significantly lower  $L^*$ ,  $a^*$ , and  $b^*$  scores and higher pH due to harsh average temperatures ( $-3.3^{\circ}\text{C}$ ) and low relative humidity (57.73%) during this season. It was also recommended that when cattle were processed at temperatures higher than  $5^{\circ}\text{C}$ , the  $L^*$ ,  $a^*$ , and  $b^*$  beef values also increased [72]. It was demonstrated that longer showering times (2 h, water at  $10$  to  $15^{\circ}\text{C}$ ) during cold days increased the incidence

of DFD meat due to higher muscle pH and lower  $L^*$  and  $b^*$  values [103]. Another study concluded that winter temperatures (12 °C) slightly produced more stress in the animals than summer temperatures (21 °C), but no significant differences were observed in meat color [33]. It was also reported that increased stress in bulls started at daily temperatures higher than 18 °C during the summer months, resulting in higher DFD-meat incidence than in winter months [31]. Lower  $L^*$ ,  $a^*$ , and  $b^*$ , and higher pH values were also found when animals were slaughtered during summer months with an average temperature of 23 °C [104]. Similarly, it was reported that cattle were more susceptible to reaching DFD conditions during summer months (average pH = 5.92), compared to winter months (average pH = 5.8), probably due to higher stress caused by heat [66]. Another study determined that there was a higher probability of obtaining DFD meat during the summer months (15.7%), compared to spring (13.32%) and winter (12.63%), due to temperatures that reached up to 35 °C [67]. It was also documented that slaughtering animals during the hot season (average temperature = 34 °C) drastically depleted the glycogen amounts pre-mortem and increased the probability of obtaining DFD carcasses up to 59% versus none during the cool season [105]. It was also found that 15.4% of the carcasses were classified as DFD during the hot season, where temperatures in the lairage pens attained values up to 48 °C versus only 8.15% during the cold season [106]. It seemed that cattle were overall more stressed for hot rather than for cold temperatures. Harvesting cattle at temperatures higher than 18 °C might be problematic in increasing animal heat stress, thus increasing the probability to produce DFD meat. Unnecessary stressors like long cold showers during cold days should be strictly avoided to increase beef color values. Pre-mortem conditions should guarantee a comfortable environment for the animals to reduce temperature-induced stress and improve meat color.

### 3.2.4. Chilling Rates

The temperature at which carcasses are processed might influence the uniformity of meat color parameters. Heavier and fatter animals processed under intensive systems were more prone to experience slower chilling rates and faster pH decline post-mortem, which might develop in high-rigor temperatures, also referred to as PSE (pale, exudative, and soft) meat [107,108]. The high-rigor condition occurs when pH decreases at values lower than 6 when the muscle temperature is higher than 35 °C [108]. This defect might also promote shrinkage in muscle fibers, longer sarcomeres, and improved light scattering, which resulted in lighter (higher  $L^*$ ) muscle color [109]. Intramuscular color variations were reported in m. semimembranosus (SM), due to a difference in chilling rates that caused a faster pH decline in the inner part of the muscle when the temperature was higher than 35 °C compared to the outer part [52,53]. Another important quality defect arises when carcasses attain 0 °C in less than 5 h, which might result in the so-called cold shortening [110]. It was reported that cold-shortening defects are more frequent than high-rigor under industrial operations [111]. Carcasses with low fat/meat conformation are more likely to confront this problem and may generate high inconsistency in the meat color under industrial operations [111,112]. In ovine muscle, fast chilling (0 °C in 5 h) resulted in higher pH and superficial metmyoglobin accumulation and lower  $L^*$  values than in the conventional chilling treatment (2.75 °C in 14.5 h). It was reported that cold shortening reduced the visual appearance of ovine carcasses below the consumer's acceptability threshold (metmyoglobin: oxymyoglobin ratio lower than 3.5) [110]. In beef, cold-shortening was related to small carcasses, which showed slower pH decline rates, leading to meat toughness [111]. Cold-shortening might also decelerate carcasses' glycolysis processes which can eventually result in DFD meat [96]. In conclusion, chilling rates post-mortem might play a key role in preventing meat quality problems and in achieving uniform carcass colors. As mentioned before in this paper, the meat industry needs to standardize the animal weight at slaughter, in a way that the carcasses can be evenly cooled under commercial conditions.

#### 4. Conclusions

Intrinsic and extrinsic factors were evaluated in the influence of myoglobin oxidation and its superficial accumulation in fresh beef muscle color. The information provided in this manuscript suggested that the intrinsic factors were mostly related to genetic variations of the animals, which were impossible or very difficult to change during the muscle-to-meat conversion. From all the intrinsic factors, ultimate pH, and muscle position were probably the most important parameters determining meat color. On the other hand, correctly managing extrinsic factors could improve meat color at a greater scale. Production systems and feeding, and pre-mortem stress were considered the most important extrinsic parameters defining meat color.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app13074382/s1>, Table S1: Summary of the papers related to the influence of intrinsic factors on meat color; Table S2: Summary of the papers related to the influence of extrinsic factors on meat color.

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