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1	1 Recent advances in meat color research		
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14	Abstract:		
15 16 17 18 19 20 21 22 23	Types of diets and energy intakes of animals, feeding types and withdrawal times were perceived as most important pre-harvest and the use of vacuum and active packaging in combination with lower temperatures post-harvest factors influencing meat color. The colo remains one of the most important meat quality attributes when consumers are concerned. According to the literature survey of 600 manuscripts, published only in the last two years, more than 40% of them failed to include information necessary to replicate and/or properly interpret instrumental color results. Color measuring systems and devices, that can successfully resolve the problem of meat translucency and its non-uniform refraction index, should be a preferred instrumental choice in the future.		
25	<b>Keywords</b> : feeding, vaccination, packaging, consumers, instrumental evaluation		
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#### Introduction

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28 Although the color of meat is not a reliable forecaster for its safety and quality that does not 29 avert consumers to have certain and specific expectations towards it, often using it as an 30 indicator of meat wholesomeness on which they base their purchasing decisions. For these 31 reasons color of meat remains the most important quality attribute that attracts attention of meat science researchers worldwide. Thus, this manuscript aims to highlight the most 32 33 recent advances, mainly from the last two years, in the area of meat color research. The 34 survey of recent peer-reviewed literature (over 600 manuscripts) suggests that manuscript 35 should focus on applied aspects and following were the major topics/areas of interest 36 identified: pre-harvest and post-harvest factors affecting meat color and visual and 37 instrumental evaluation of meat color.

## Pre-harvest factors affecting meat color

Acevedo-Giraldo et. al. (2020) investigated the effect of feed withdrawal time on meat quality on pigs and found higher L-values for shorter withdrawal times [1]. LH Silva, et al. [2] concluded that increasing time on feed for Nellore cattle improves carcass and meat quality traits in general. They also found out that lower intramuscular fat content from cattle harvested at 0 days on feed, compared to animals harvested after 100 or 200 days on feed, might be related to decreased lightness (L\*) and yellowness (b\*) of steaks. They also explained that lower redness ( $a^*$ ) found for the steaks of lighter and younger cattle might reflect the lower myoglobin content usually found in the muscle of young animals [2]. When it comes to the type of animal feed, Li, et al. [3\*\*] evaluated chicken breasts and how eucalyptus leaf polyphenol extract added to the feed affects their meat color. The authors reported that the  $a^*$  value and the myoglobin content of breast muscle increased, based on higher antioxidant capacity. [3\*\*]. The effect of low-energy diets on the color of chicken meat was also investigated. It was reported that a decrease in dietary energy contributed to an increase in color lightness and a decrease in redness [4]. Costa et. al. 2020 investigated the effect of fat source (sunflower, soybean and linseed oil) in diet in the finishing of BOS indicus steer and found no effect on color of meat [5]. Similar was concluded when the effect of feeding of essential oils to beef cattle was observed [6]. Antonelo et. al. 2020, analyzed meat from Nellore and crossbred male cattle, fed with soybean oil and without it and also found no difference in color [7]. Changing of the fatty acid profile and short periods of feed change cannot change the meat color. This is indirect proven by Langlie et. al. (2020) who used much longer feeding times (>50 days) and used Angus and Angus x Simmental crossed steer and heifer calves in a randomized design. Different feeding types were evaluated: dry lot (bunk fed a high roughage ration consisting of haylage, corn silage, corn, and distillers) perennial pasture and summer annual cover crop. Highest L\*, a\*, and b\* color values for lean meat tissue were reported for dry lot [8]. Most recently, impact of castration and immunocastration on meat quality in general and meat color in particular was frequently reported. Overall, it appears that there is a little [9\*-11] to no [12,13] effect of the castration and immunocastration on the meat color, regardless of the species, and a more pronounce effect on meat color by feeding time and energy intake of investigated animals.

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       Once meat is obtained from carcasses, meat color can be influenced by many factors that
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       are interrelated and can lead to important visual changes and ultimately, influence
       consumers' perception of quality and freshness [14,15**]. In this regard, the main factors
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       influencing the color of fresh meat are the temperature, packaging conditions, and
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       lipid oxidation during aging and exposure to consumers. Temperature is an important factor
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       by influencing the stability of myoglobin structure. Low temperatures (below 4°C) are
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       important to reduce the modification in characteristic color of fresh meat [14]. However,
       frozen temperatures can influence the stability of color and blooming capacity of meat
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       during refrigerated storage and can lead to discoloration of meat, especially in the intensity
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       of redness [16*,17]. Moreover, temperature fluctuations during frozen period (-18 \pm 2^{\circ}C)
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       can alter the color of meat and reduce the intensity of redness, especially after 60 days of
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       frozen storage [18].
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       On the other hand, packaging and atmosphere composition can also influence the color of
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       meat, especially by changing the redox status of myoglobin: deoxymyoglobin (purple color),
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       oxymyoglobin (bright cherry-red color), and metmyoglobin (brownish) [19]. Controlling the
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       exposure of meat to oxygen is the key to achieve the appealing bright color (from
       deoxymyoglobin to oxymyoglobin state), but the excessive exposure (longer periods and
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       high partial pressure inside the package) causes the oxidation (from oxymyoglobin to
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       metmyoglobin) and eventual reduced perception of freshness and quality [20]. In this
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       regard, the use of films with partial permeability to oxygen can maintain the levels of
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       oxymyoglobin and the appealing color during storage, especially in meat with myoglobin in
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       deoxymyoglobin state [21]. Modifying the gas composition inside the package can also
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       influence the color. Reducing the partial pressure of oxygen and increasing the partial
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       pressure of carbon monoxide (CO) can improve the preservation of color during storage
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       [22,23]. In this case, carboxymyoglobin is formed from the exposure of myoglobin to CO. It
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       is also relevant to mention that vacuum packaging is a relevant approach to preserve
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       myoglobin and improve the stability of color in fresh meat during storage [22].
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       The progression of lipid oxidation and eventual accumulation of products could favor heme-
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       protein oxidation. Aldehydes alter heme-protein redox stability, resulting in the promoted
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       oxidation of oxy heme-protein, decreasing the met heme-protein reduction and enhancing
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       its pro-oxidant activity [24]. Myoglobin is oxidized by intermediary products of lipid
       oxidation, which induce a change from the bright cherry-red color to brownish tones [19].
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       Moreover, lipid oxidation is affected by many factors (exposure to UV radiation, exposure to
       atmospheric oxygen, unsaturated fatty acids, and endogenous antioxidants, for instance)
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       that can eventually lead to oxidation of myoglobin and discoloration of meat [24]. It also
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       worth mentioning that delaying the formation of lipid oxidation products can be achieved
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       by different strategies: vacuum packaging [22], active packaging containing antioxidants
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       [24], and reducing temperature [25].
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       Finally, pH is another important factor that influences the color of meat. Once the ultimate
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       pH of meat does not fit in the expected range of values color changes can occur. Stress is
       the most frequently identified factor in the pre-slaughter handling of animals. It negatively
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       affects meat quality, which results in economic losses. Stress and energy expenditures in
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       pre-slaughter period cause the depletion of muscle glycogen reserves and, consequently,
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       the insufficient post-mortem production of lactic acid. Low acidity in the ageing period
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       results in the change of color, structure, taste and tenderness of meat [26]. In the condition
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       of ultimate pH higher than expected, meat becomes darker while in pH lower than
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expected, the meat becomes pale. These are related to myoglobin and myofibrils

modifications [27,28].

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#### Visual evaluation of meat color

- 120 Color sensory evaluation can be performed at two levels, by means of trained and qualified
- assessors and by means of consumer studies. Trained panel evaluation is objective,
- reproducible and comparable to a laboratory equipment. Various scoring scales have been
- utilized for panel evaluations of fresh, ground, cooked, cured and other types of meat and
- most of these have been reported in detail in Hunt et al. [29]. Consumer studies, on the
- other hand, provide a subjective hedonic score for color. This section will be centered in
- 126 consumer studies because meat sensory characteristics are related to consumer enjoyment
- of meat and it is crucial to satisfy consumer demands.
- 128 Color is one of the most influential visual appearance traits [30, 31, 32] because it is usually
- the decision-making parameter for consumers when selecting meat at the point of purchase
- 130 [33]. Furthermore, freshness, uniform and red color are the characteristics of the ideal foal
- meat [34\*]. Freshness and color were presented as the most valued intrinsic quality cues in
- lamb [32]. Since dark brick red lamb and beef color has been associated to freshness in
- contrast to pale color, and freshness is an important parameter in meat acceptability by
- some consumers [35], color can be a barrier to consumer meat acceptance.
- 135 Consumers' opinion is affected by psychological and marketing factors [36], thus varied
- between and within countries, and depends on cultural and demographic characteristics
- 137 [32,35]. When consumer studies are carried out it is important to find out segments of
- 138 consumers with similar preference patterns to help to establish marketing strategies that
- can satisfy consumer demands. For instance, beef color is an important trait by consumers
- concerned about the territorial nature of the product and the health, and those that buy
- beef in large retail sales [37]. Lamb and beef color is an indicator of quality by Asian
- consumers who are also more influenced by color than Australian ones [35].
- 143 Consumers evaluate color acceptability or preference using a hedonic scale, either
- 144 continuous or stratified. It is important to ensure an optimum design and to carry out the
- evaluation in good conditions (e.g. lightness, temperature). Consumers can evaluate the
- color both by directly looking at the meat or using photographs. In both cases similar results
- are obtained [38\*\*]. Shelf life of meat is highly affected by color acceptability [20]. Thus, the
- use of photographs might facilitate the evaluation of the color over time, avoiding the need
- to ask consumers to participate more than once in the evaluation, avoiding the creation of
- expectations due to the time between evaluations. This might also allow the evaluation at
- random of the meat, which is more realistic [19]. Evolution of color at aging is also perceived
- by consumers [39] and its study would allow to find out the best aging time to satisfy their
- 153 preferences.

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### Instrumental evaluation of meat color

- 155 It was already established that (among others) factors that affect instrumental meat color
- readings and their successive evaluation, by definition include the type of device used and
- its proper calibration, illuminant, aperture size and observation angle [40]. Adequate
- reporting of these parameters and other data regarding instrumental meat color protocols
- and procedures used by the authors is a conditio sine qua non for appropriate
- understanding of their results and potential replication of their studies. This is why we have

161 conducted a survey on if and how the aforementioned factors have been reported in journal articles that measured meat color (n = 600) for the last two years (2019–2020). 162 Calibration of any colorimetric device is the only way to maintain consistent and reliable 163 readings. Yet, in 340 manuscripts (56.7%) we have surveyed the authors failed to report if 164 165 and how the device used in their research was calibrate. Among those reported, 10° continues to be the most popular observation angle (35.1%) compared to the similar study 166 167 published by Tapp et al. [40] a decade ago. Most recent investigation suggest that 2° 168 standard observer may be more useful for color measurement especially with regard to color stability [41]. Share of manuscripts not reporting observation angle at all has 169 170 decreased from 65.9% to 48.7%. Unfortunatly, it still remains very high (Table 1). Guidelines 171 that define optimal number of readings per sample for different colorimeters remain 172 inconsistent or unavailable [42]. However, the majority of researchers (36.2%) are still 173 performing them in triplicate. Concerning is the fact that more than a third (214) of 174 manuscript surveyed failed to report on this important matter. Share of articles not 175 reporting illuminants used remained very high and above 40% (Table 1). Despite the fact 176 that illuminant A produces higher correlations with visual meat color scores [43], the 177 researchers are using illuminant D65 (46.9%) even more so than 15 years ago (32.3%) [40]. This is mainly due to the fact that Minolta Colorimeter is the device of choice (67.5%) when 178 179 meat color measurements are concerned, and it does not provide illuminant A as an option. 180 However, it is noticeable that computer vision system (CVS) is gaining popularity among color researchers because of its obvious advantages. CVS is rapid, consistent, objective, non-181 182 invasive, and economic [44]. It is now apparent that CVS method gives a valid 183 measurements more similar to the real color of the pork and beef [45\*\*], chicken, turkey, duck and goose [46] and quail, wild boar, rabbit, deer and pheasant [47] meat 184 185 samples. Although the illuminants are the same (D65) in both devices, because Minolta 186 colorimeter and its light source are placed on the sample surface and CVS lamps and digital 187 camera are 50 cm away from the sample, the light penetration in CVS (5 mm) is 4 times smaller (20 mm) than for Minolta colorimeter [45\*\*]. As a consequence, CVS measurements 188 are less affected by the problem of meat sample translucency and its non-uniform refraction 189 index, generating meat color coordinates that better correspond to the true color of meat 190 191 samples [45\*\*,46,47]. It is generally suggested that the aperture, another important factor that is hugely (57.7%) 192 not reported (Table 1) in the color investigations, should be as large as the color measuring 193 instrument will allow. It seems that this is only true for uniformly colored meat samples. 194 195 When it comes to non-uniformly colored meat samples (highly marbled meat or fermented 196 sausages) the aperture size of Minolta colorimeter (8 mm) and its measuring surface of 197 5cm2 works into its disadvantage. In this case its aperture size is actually too big to provide independent color coordinates of meat and fat parts of non-uniformly colored meat 198 199 samples. Instead, it produces color measurements that does not respond to the true color 200 of neither meat nor fat parts [48\*\*]. Because CVS can take its color readings from a surface of a sample as small as a single pixel of an image (around 0.5 mm), it excels Minolta 201 colorimeters when it comes to the color evaluation of non-uniformly colored meat samples. 202 The one disadvantage of CVS in comparison to most frequently used meat color measuring 203 204 device would be its more complicated and timely consuming calibration procedure that 205 involves specialized pieces of (additional) hardware and software (Figure 1).

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From various pre-harvest factors, types of diets and energy intakes of animals, feeding types and withdrawal times are the most recent and important ones that have been reported to have a strong effect on the meat color of different species. On the other hand, presence or absence of (immuno)castrations seems to have very little or no effect at all. The use of vacuum and active packaging in combination with lower temperatures remains the most prominent post-harvest strategy to preserve the color of meat during its storage and exposure to consumers. The color of meat remains one of the most important quality attributes when it comes to the consumers and their preferences in this regard. However, distinct variances were observed between clusters of consumers from different countries, cultures and demographics. The 10° observer and larger aperture sizes are still the most commonly used for meat color measurements, but are recommended only when a larger portion of the sample needs to be evaluated. The decision on the best illuminant needs to be based on the type of sample being evaluated. However, when wanting to compare the results of their work with the work of others, investigators should keep in mind the fact that D65 was and still is the most commonly used illuminant in meat color research for the last two decades. The need for standardized set of minimum reportable parameters for instrumental meat color evaluation still remains to be identified and incorporated in peerreviewed journals guidelines for authors, as it was the case a decade ago. We are proposing that all manuscripts containing instrumental color data must report of instrumental detailes that include (at least) the information on: instrument and its calibration, illuminant, aperture size, degree of observer and number of readings per sample. It is to be expected that, in the foreseeable time, the attention of meat researchers (when it comes to the preferred color measuring instruments) will shift towards systems and devices that are less affected by the problem of meat translucency and its non-uniform refraction index. This will ensure that reported color values will better reflect visually perceived color of meat and meat products.

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#### **Conflict of interest statement**

Nothing declared.

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# **CRediT authorship contribution statement**

- 239 Igor Tomasevic: Conceptualization, Methodology, Writing original draft, Visualization. Ilija
- 240 **Djekic:** Investigation, Data curation. **Maria Font-i-Furnols:** Methodology, Writing original
- draft. Nino Terjung: Methodology, Writing original draft. Jose M Lorenzo: Writing original
- 242 draft, Supervision.

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- 248 \*\*of outstanding interest

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    \*\* This study sucssesfuly accentuates that the problem with meat translucency and
    - its non-uniform refraction index, observed when evaluating meat color with Minolta colorimeter, can be sucssesfuly resolved with the use of computer vision system.
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- \*\* The authors provide strong evidence and explain the reasons why computer vision system is a superior instrumental meat color evaluation technique, in particular when non-uniformly colored meat products are concerned, compared to Minolta colorimeter.

Table 1. Differences in reportable parameters reported in articles dealing with instrumental meat color published over a twenty-year period

	Percentage of articles	
	1998 to	2019-2020
	2007*	(n=600)
	(n=1.068)	
Device		
Minolta	60.0	67.5
Hunter	31.6	16.3
CVS	-	3.2
Other	5.8	12.0
Not reported	2.6	1.0
Illuminant		
D65	32.3	46.9
Α	8.6	4.8
С	8.6	4.8
Other	1.5	2.3
Not reported	48.9	41.2
Observation angle		
0	3.8	4.3
2	5.3	11.2
10	24.2	35.1
More than 10	0.9	0.7
Not reported	65.9	48.7
No. of readings per		
sample		
1	0.3	0.2
2	6.6	0.3
3	21.9	36.2
4	5.9	4.2
5	5.2	8.7
6+	7.8	14.7
Not reported	52.4	35.7
Aperture size		
Reported	26.4	42.3
Not reported	73.6	57.7

<sup>\*</sup>Data adopted from: [28]Tapp WN, Yancey JWS, Apple JK: **How is the** instrumental color of meat measured? *Meat Sci.* 2011, **89**:1-5.

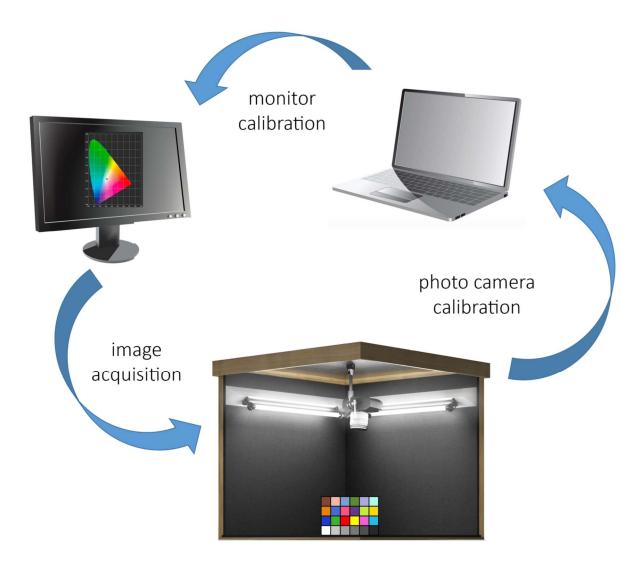


Figure 1. Calibration sequence for computer vision system.