Validation of photographs usage to evaluate meat visual acceptability of young bulls finished in feedlot fed with or without essential oils

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ABSTRACT
Forty ½ Brown Swiss x ½ Nellore crossbred bulls were distributed into three experimental groups: CON – diet without addition of essential oils; CLO – diet with average 5,000 mg/animal/day of clove essential oils and CIN– diet with average 5,000 mg/animal/day of cinnamon essential oils to evaluate three methodologies of visual acceptability: with steaks directly in Trays and Sequential and Random photos. Seventeen consumers evaluated visual appearance of meat using a 9-point structured hedonic scale. CON group presented higher shelf-life than essential oils groups. Trays and Sequential scores were similar in the majority of days; thus digital images could be used to evaluate colour evolution. However, Random photos resulted in lower scores and slower acceptability decrease than Trays and Sequential photos ($P < 0.05$) among the second and fifth day of display. Random photos presented a lower and more constant standard deviation than Trays and Sequential photos ($P < 0.01$) indicating that this methodology promoted a higher standard situation for meat colour evaluation.

Keywords: clove, cinnamon, images, meat colour, methodology,

1. Introduction

Appearance determines how consumers perceive meat quality and influences purchasing decisions (Faustman & Cassens, 1990). In the case of beef, purchasing decisions are influenced by colour more than by any other quality factor, because consumers relate the bright cherry red colour to freshness and wholesomeness, while the brown colour is considered undesirable (Mancini & Hunt, 2005). In fact, the visible colour on meat surface continuously changes during display and storage, influencing consumer acceptance of beef (Prado et al., 2015). The use of antioxidants in animal nutrition are an alternative to improve colour stability because feed is an effective route of inhibition of animal lipid oxidation (Wulf et al., 1995). Essential oils are natural additives extracted from plants which have antioxidant powers. These additives have phenolic compounds which are able to neutralize the free radicals which are responsible for oxidative processes (Hui, 1996).

Visual assessments are a gold standard for estimating consumer perception (Mancini & Hunt, 2005) but are complex, expensive and time-consuming. O'Sullivan et al. (2003) showed that the sensory visual assessment of meat products can be undertaken effectively without training when the product, or rather the colour of that product, is familiar to the assessors. Difficulties of using meat in consumer surveys could be overcame through the use of photographs for the colour evaluation (Brugiapaglia & Destefanis, 2009).

Digital camera images have been used on instrumental assessing of meat colour (O'Sullivan et al., 2003). According to theses authors the images reduced the number of representative samples to explain the variations of meat colour surface. Lu, Tan, Shatadal, and Gerrard (2000) used digital images and trained panellists to predict pork loin visual colour. When digital images were assessed by consumers, they were able to distinguish before and after blooming (Brugiapaglia & Destefanis, 2009). Another advantage of digital images is their repeatability (Ngapo, Martin, & Dransfield, 2004, 2007). It is anticipated that computer vision inspection of food products will be consistent, efficient and cost effective (Lu et al., 2000).

However, in visual analyses consumers have daily contact with the meat, because they must score samples through the display period. This additional information of freshness provided by time (days of display), which in a real situation consumers do not possess, might influence consumers’ perception and discoloration of meat can be overestimated. For that reason, validation of photographs is an important first step to confirm their usefulness in eliminating between-consumer differences in meat perception.

Therefore, the aim of this study was to compare perception of beef colour between viewing meat and their corresponding photographs with days in sequential or random order, taken under controlled and standardised conditions to assess the reliability and accuracy of using meat photographs as an assessment tool.

2. Material and Methods

2.1. Ethic committee and local

This experiment was approved by Department of Animal Production and Research Ethic Committee at the State University of Maringá and followed the guiding principles of biomedical research with animals (CIOMS/OMS, 1985), and was conducted at the Rosa & Pedro Sector, State University of Maringá, Farm Experimental Station at Iguatemi city, Paraná, and Brazil South.

2.2. Animals and housing

A total of 40 (½ Brown Swiss x ½ Nellore) crossbred young bulls (half-brothers) 12 months old and live weight (LW) of 219 ± 11.7 kg were used in a complete Randomized design. At the beginning of the experiment, animals were adapted for two weeks in individual pens (10 m², partially covered, with concrete floors and automatic waterers) and the concentrate was supplied gradually. Animals were weighed at the beginning of experiment and every 28 days in the scale (Beckehauser Cia Paranavaí city, Paraná, Brazil South).

2.3 Diets

The basal diet was the same for all animals, formulated to be isonitrogenous and isoenergetic according to NRC (2000) recommendations for a 1.4 kg/day average daily gain, which consisted 90% of concentrate and 10% of pellets cane sugar (Table 1). The diet was offered *ad libitum* and the feed intake was recorded daily. Bulls were randomly assigned to one of three finishing diets according to the LW: CON (*n* = 8) – diet without addition of essential oils; CLO (*n* = 16) – diet with average 5.000 mg/animal/day of clove essential oil and CIN (*n* = 16) – diet with average 5.000 mg/animal/day of cinnamon essential oil. The clove essential oil contained 84.5%, 13.3 and 1.3% of eugenol, carophylenne and eugenyl acetate, respectively, and cinnamon essential oil contained 78.8%, 4.7% and 3.2% of cynnamaldehyde, carophylenne and alpha-pinene, respectively, as determined by Biondo et al. (2016). The essential oils were purchased from FERQUIMA® (Vargem Grande Paulista, São Paulo, Brazil) and stored at + 4ºC in refrigerator until the beginning of the experiment. Essential oils antioxidant power were analysed by the ORAC method (Oxygen Radical Absorbance Capacity) as reported by Zulueta, Esteve, and Frígola (2009) which remained constant in the diet for up to 30 days of exposure. Essential oils had a liquid texture and were mixed with the feed from concentrate in a commercial mixer every two weeks, where diets were prepared and adjusted according to the intake of dry matter, in order to keep constant the dosage for animal/day.

2.3. Slaughter and meat sampling

At day 187 (443.5 ± 26.2 kg), bulls were transported to a commercial slaughterhouse (Arapongas city, Paraná, south Brazil). Truck stocking density was 0.8 ± 0.2 bulls/m^2, and transport distance was less than 60 km. Animals were slaughtered, following the usual practices of the Brazilian beef industry, and they were stunned using a captive-bolt pistol. Carcasses were not subjected to electrical stimulation.
Twenty-four hours later, after chilling at 4^o C, *longissimus dorsi* samples (6th to 13th rib) were identified and stored in vacuum bags (Polyamide/Polyethylene pouches of 120 μm and 1 cm3 /m2 /24 h 02 permeability, 3 cm3 /m2 /24 h CO2 permeability measured at 5° and 75% relative humidity; water vapor transmission rate (WVTR) was 3 g/m2 /24 h at 38 °C and 100% RH; the vicat softening point of sealing was reached at 97 °C and it had a dart drop strength of 1300 g), then immediately transported to the Laboratory of Technology and Production of Animal Origin of the Animal Science Department at the State University of Maringá. Two-cm thick steaks were cut, vacuum-packaged (99% vacuum, with a Sulpack SVC 620 machine), aged for one day, before being frozen and stored at -18^{o}C for further analysis.

2.4. Evaluators

Consumer-based sensory panels were conducted with semi-trained evaluators (n = 17) who consumed beef on a regular basis (at least twice a week) were recruited from the Animal Science Department of the State University of Maringá (Table 2). Each evaluator attended two evaluation sessions separated by a two-week interval. In the first session, evaluators evaluated samples directly in a commercial expositor (Trays) and in the second session evaluators evaluated correspondent photos of samples in Sequential (Sequential) and Randomized (Random) orders.

2.5. Session 1 (Trays)

Samples were thawed for 24 h at 4°C. They were packaged individually in polystyrene trays (Darnel Embalagens LTDA, Curitiba, Paraná, Brazil, 14 x 21 cm) over wrapped with a retractile films (Goodyear ® , Americana, São Paulo, Brazil, with oxygen permeability of 8.200 cm 3 /m 2 /d, and rates (RH) of 262 cm 3 /m 2 /d) and displayed at 4 ± 1°C under simulated retail conditions using fluorescent light (1200 lx, 12 h) in a commercial expositor (Klima Expositor Practice, Model 05B0500.1, Venâncio Aires – Rio Grande do Sul, Brazil). Every two days, the individual codes of the samples and the positions of the trays inside the expositor were randomly changed to avoid sample recognition. In the visual analyses Trays were evaluated daily using a 9-point structured hedonic scale (one = dislike extremely to nine = like extremely) (Prado et al., 2015). The shelf-life of the meat was limited by the number of days at which samples were assigned with scores equal or higher than 5.

2.6. Session 2 (Sequential and Random photos)

In order to produce images in standardized conditions, photographs were taken according to Chan, Moss, Farmer, Gordon, and Cuskelly (2013). The over-wrapped steaks were photographed every day through the eleventh days of evaluation, using a NIKON D3100 digital camera mounted on a photographic stand containing two D65 fluorescent light tubes as the standard illuminant. An additional grey-colour cardboard was used to cover the entrance of the cabinet to provide lighting evenly distributed across the sample and also to avoid exposure to external light. The camera was fixed perpendicularly 45 cm to the surface of the meat sample. Following preliminary experiments to fix meat samples for appearing entirely in photos, the camera parameters were chosen: manual mode; shutter speed, 1/20; aperture size, F5.3; ISO, 1600; focal distance 40 mm. Images were stored and transferred to computer as JPEG file . A GretagMacbeth mini Colour-Checker (Colour-confidence, Birmingham, UK) which contains 24 coloured patches was photographed with each meat sample for checking the colour reproduction capability.

Training was done with evaluators prior to the visual analyse and it was determined that 5 seconds was sufficient time to evaluate one photograph and after every 40 evaluation, a 1 minute break was taken to avoid fatigue. The analyses occurred in a comfortable place, the conference room of the Animal Science Department of State University of Maringá, where evaluators were distributed on individual chairs in such a way they could not have direct contact with each other. At the beginning of the analyses evaluators were instructed to evaluate the acceptance of meat colour, disregarding other aspects as size, purge, marbling and subcutaneous fat. Photographs were projected individually in the room by a multimedia device (Epson Powerlite S27 2700 - UHE lamps 200 watts) in two methods: Sequential and Random. Because we want to evaluate the effect of the additional information of meat freshness, first photographs were sequentially shown from the first to the last day (Sequential) and individuals were asked the same question on acceptability. After completing the evaluations and a 30-minute break, the recorded photographs were randomly shown (Random) as new samples to the consumers.

2.7. Statistical analyses

One way ANOVA for the scores assigned and its respectively standard deviations were analyzed by the IBM Statistical Package for the Social Science (SPSS version 19). Days of display, diets and methodologies were considered as fixed factors. Interactions were observed between days of display and diets and between days of display and methodologies, thus these effects were evaluated in a GLM analyses for each day and differences among means were assessed by using the Tukey Test ($P > 0.05$). To analyze the evolution of scores and their standard deviation among the display period, a simple regression for the effect of days was performed.

3. Results

3.1. Diet and methodology effects on visual scores of meat

In the first five days, meat from bulls fed the three diets presented similar ($P > 0.05$) scores of evaluation (Table 3). In the sixth, seventh and eighth days the meat from bulls fed essential oils in the diets presented lower scores ($P < 0.01$) than meat from bulls from control diet. From the ninth to the eleventh day the scores were similar ($P > 0.05$), as they were observed in the first five days. All the diets presented a decrease ($P < 0.01$) throughout the time in the scores of visual acceptability, which started being higher than 7.6, and finished with scores lower than 2.4 in the eleventh day.

Scores from the first to the fourth days were higher ($P < 0.01$) for samples evaluated in Trays and by Sequential photos than in samples evaluated by Random photos (Table 3). In the fifth day the scores were similar ($P > 0.05$) among methodologies. From the sixth day onwards the scores from Sequential were similar to the scores from Random method, which were higher ($P < 0.01$) when compared with the scores from Trays method.

3.2. Day effect on visual scores

In Table 4 the equations of effects of the days of display are presented. For the three diets, scores of visual acceptability presented a strong relationship with the days of evaluation in the form of a cubic equation ($R^2 > 0.70$). However, the shelf-life was different among the treatments and evaluation methods. For the CON diets Random photos presented the highest shelf-life (8.14 days), Sequential photos the intermediary (7.22 days) and Trays the lowest (6.98 days). Trays and Sequential photos presented a similar curve throughout days of display. However, Random photos presented an enhanced decrease in the curve after the fourth day (Fig. 1).

For the CLO diets, Random photos presented the highest shelf-life (7.19 days), Sequential photos the intermediary (6.60) and Trays the lowest (6.29 days). In this diet group, Sequential photos presented an intermediary curve fit, while the decrease in scores was similar to Trays in the first days, but similar to Random photos in the last days (Fig. 2). For the CIN diets Random photos presented the highest shelf-life (7.66 days), Sequential photos intermediate (7.03 days) and Trays the lowest (6.65 days). In this diet group, as happened in CLO diet group, Sequential photos curve fit was similar to Trays across the first days of display, but similar to Random photos in the last days (Fig. 3).

3.3. Methodologies and day effect on variation of visual scores

Standard deviation was used to measure the variation among consumers for the scores assigned for each sample. No interactions were observed between methodologies and diets, through the eleven days, thus results are presented as an overall evaluation . Differences in the standard deviation were observed in days 3, 4, 5, 8, 10 and 11 (Table

5). In the days 3, 4, 5 and 8 the standard deviation was lower in the Random photos when compared to the other methodologies, with exception for the Trays in day 3 which was similar. However in days 10 and 11 the standard deviation from the Trays methodology was lower than those from Sequential and Random photos.

Differences among days in standard deviation were observed for all methodologies ($P < 0.01$). Specifically, no differences in standard deviation were observed in Sequential photos with the exception of the first day. On the other hand, for Random photos, the standard deviation increased after the 9^{th} day. Trays methodology presented a fluctuation in the standard deviation, which increased after the third day and decreased after the ninth day of evaluation.

Since differences in standard deviation among days were observed and an analysis of regression was made to observe the model behaviour (Table 6). Display days presented a cubic effect for the Trays methodology and a linear increase for Sequential and Random photos ($P < 0.05$). The Trays methodology presented a weak coefficient of determination ($R^2 = 0.232$) but no relationships were observed for the Sequential and Random photos ($R^2 = 0.030$ and 0.081 respectively) of the exposition days and the standard deviation. These results indicate that independently of the treatment, the effect of days plays a higher role in the differences observed among the consumers in the Trays methodology (Fig. 4).

4. Discussion

4.1. Diet and methodology effects on visual scores of meat

4.1.1 Diet effect

The three diets presented a decrease in the visual scores, which was expected because meat deteriorates during display, especially for animals fed with high percentages of concentrates (Warren et al., 2008). The visual scores observed were similar to the results of other studies with animals finished in feedlot. (Muramoto, Nakanishi, Shibata, & Aikawa, 2003; Prado et al., 2015).

In the current work, significant differences in the acceptability of meat were observed among the diets during the transition period (6, 7 and 8^{th} days), when average scores of acceptance became lower than 5.0. Similar results were observed by Vitale, Pérez-Juan, Lloret, Arnau, and Realini (2014) when evaluating different ageing times in beef, when differences among treatments occurred after the sixth day of display. However, in the current work the lower scores observed for the essential oils diets were unexpected since essential oils of clove and cinnamon are largely used in human medicine due to their antioxidant properties (Hart, Yáñez-Ruiz, Duval, McEwan, & Newbold, 2008).

According to Kerry, Buckley, Morrissey, O'Sullivan, and Lynch (1998), antioxidant incorporation in cells membranes can delay oxidation of myoglobin. These compounds acts in the capture of free radicals which are formed during lipid oxidation, delaying the conversion of the cherry red pigment (oxymyoglobin) to the brown pigment (metmyoglobin) (Hayes et al., 2009). When essential oils of clove and cinnamon were added directly in the meat surface (Ouattara, Simard, Holley, Piette, & Bégin, 1997) a reduction in the growth of bacteria responsible of meat deterioration was observed.

According to Wulf et al. (1995) antioxidants can be added into diets because feeding is an efficient route of oxidation inhibition of animal lipids. However, in our study animals fed with essential oils presented a lower visual acceptability than Control. In this sense, Rivaroli et al. (2016) when feeding crossbred bulls (Angus x Nelore) with two concentrations of a commercial essential oils mixture (3,500 and 7,0000 mg animal/day), observed that the highest dose increased lipid oxidation. Then, depending on the content, these compounds might penetrate mitochondria and initiate oxidative process in these membranes organelles. Thus in high concentrations essential oils may cease antioxidant activity to start pro-oxidant activity (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). Due to the divergence in literature, other analyses as lipid oxidation, antioxidant capacity or instrumental colour should be carried out to better understand the effect of addition of these compounds in the diets over the parameters of meat quality.

4.1.2. Methodology effect

When diets were evaluated together, the colour evolution of meat was different according to the assessment methodology. A decrease in the scores of acceptance occurred similarly for the Trays and Sequential photos, however no differences were observed from the second to the fifth day for the Random photos (Table 3). The progressive decrease in visual scores acceptance in Trays and Sequential photos might be related more to consumer's perception of deterioration of the meat through display than to a real discolouration of myoglobin.

Differences were observed among the three methodologies for animals fed with or without different essential oils (Table 3). Trays, Sequential photos, and Random photos are different techniques, thus the differences observed in the results were expected. Specially in the Trays, several factors play a role during consumers' evaluation that could be associated to errors. Firstly, consumers have additional information about meat freshness because they know the number of days that meat was displayed. Something similar could happen with Sequential photos. This kind of information might explain why consumers assigned higher scores in the first days and lower scores in the last days when compared to Random photos. In real situations, consumers do not know how many days meat has been displayed for.

Secondly in Trays, samples were presented together and positions were switched every two days. However, inside a displayer there are different locations, as the centre, corner, etc. Thus, in this methodology samples might not be evaluated in standardized conditions of viewing. Thirdly, Trays methodology demands a long duration and consumer's perception or evaluation might change from day to day. Therefore, more accurate and standardized methodologies should be developed in order to minimize these effects.

No differences were observed between Trays and Sequential photos scores in the majority of evaluation days, thus digital images can be utilized to assess the evolution of colour, as in Trays methodology. Corroborating to our findings, Chan et al. (2013), observed no differences in the preferences of doneness of meat when samples were presented physically (purchased in supermarkets) or its respective photographs. Thus it can inferred that the evaluation of meat preferences by photographs could replace the traditional methodologies without compromising consumer perception.

Trays and Sequential photos scores were higher than Random photos in the first days of display. The results observed indicate that consumers are keen to assign higher scores because they knew that samples were displayed for few days and were fresh. However after the seventh day, Sequential and Random photos presented similar scores, but higher than Trays. It can be inferred that after that point, with the increase of the metmyoglobin content (Greene, Hsin, & Zipser, 1971; Muramoto et al., 2003) consumers are induced to assign lower scores in Trays.

4.2. Day effect on visual scores

Throughout the 11-days period, consumer acceptance of the appearance of the meat decreased exponentially. The gradual decline in visual acceptability was expected because oxidative processes cause meat to deteriorate (Renerre, 1990) especially in meat from concentrate-fed animals. Trays and Sequential photos presented similar curve behaviour but different to Random photos in CON group (Fig. 1). On the other hand Sequential photos presented an intermediary curve between Trays and Random photos in the essential oil diet groups (Fig. 2 and 3).

In our evaluation, when samples were assigned scores lower than 5.0 it reflected rejection of the meat. Trays and Sequential photos presented a shelf-life between 6 and 7 days (Table 4), which are similar to those observed by Prado et al. (2015). However, when samples were evaluated by Random photos a longer shelf-life of 8.14 days appeared for CON diets, 7.19 days for CLO diets and 7.66 for CIN diets.

The higher similarity between Sequential and Random photos curves in CLO and CIN than in CON, might partially be explained because of their unexpected lower shelf-life. In essential oil diets, as visual scores decreased faster, it resulted in a faster discolouration from red to brown pigment. Light is a physical phenomenon composed of energy particles which move as wave. However, the human eye is sensitive to a very straight band of wave length denominated visual spectre, situated between 380 to 780 nm. (Ramos & Gomide, 2007). The chromaticity diagram of CIE 1931 represents every colour as point and can represent the range of all colours that can be produced (Lammens, 1994). The spectral range of brown colour is situated between 590 to 600 nm which is smaller than red which is situated between 600 to 750 nm, indicating lower discrimination possibilities to the human eye. Corroborating to this idea, Chan et al. (2013) observed that when photographs of different meat doneness were evaluated by consumers, they were less able to distinguish grades of doneness (Maillard reaction) in the external surface than in the internal surface. Thus, it can be inferred that as meat increases in brown pigments others aspects which are not perceived in photographs of meat surface, such as the profundity, could play a role in consumer decision.

Overwrap packaging, vacuum, skin packaging and modified atmosphere packaging (MAP) are packaging systems available for fresh meat in the market (McMillin, 2008), but each of these can influence meat colour (Nassu et al., 2012). Historically, consumers use colour as their primary indicator of freshness and wholesomeness, and want packages in the retail case that promote a cherry red colour (Hood & Riordan, 1973; McMillin, 2008; Morrissey, Buckley, Sheehy, & Monahan, 1994). Digital image reliability for visual evaluation decreases when brown pigments increase in meat surface; however it appears to be a promising tool, since the determination of shelf-life occurred before the reduction of its accuracy. Our results point out that when consumers have additional information of time they are induced to assign lower scores, but when samples were evaluated by Random photos shelf-life was increased in one day. Colour is important to meat shelf-life and when deviations from the cherry-red colour occurs it results in severe retail discrimination (Liu, Lanari, & Schaefer, 1995). Factors as length and temperature of storage play a role in shelf-life duration, which is a major concern to the meat industry (Martin et al., 2013), thus digital images presented in Random sequence could be used as a tool to more accurately evaluate these factors which have direct impact in meat discolouration.

4.3. Methodology and day effects on variation of visual scores

Despite of the results observed in the scores, another objective of our study was to test which methodology presented the lower variation among consumers, reflecting on a methodology with the higher standardized situation for the evaluations. Arnold, Scheller, Arp, Williams, and Schaefer (1992) used standard deviation to measure variations of beef colour stability between visual appraisal and spectrophotometer evaluations. The same authors reported that a regression analysis of standard deviation of visual appraisal should be avoided since the effect of heteroscedasticity occurs in this kind of data and is a major concern in a regression analyse. Our hypothesis was that heteroscedasticity effect would occur in the three methods, and the average standard deviation should remained constant. However differences in the standard deviation among days was observed in the ANOVA analyses (Table 5), and the regression analysis was performed in order to test if this effect really occurred (Table 6).

When diets were evaluated together, Trays and Sequential photos presented equal or higher standard deviation to Random photos in the majority of days. The higher standard deviation at the 3rd, 4th, 5th, 8th can be explained by the lower influence of reference of freshness, which increased the variation among consumer responses. At 10th and 11th days of display standard deviation of Trays was lower than Sequential and Random photos. The lower standard deviation in Trays methodology occurred due to the higher number of lower scores assigned. This result observed indicates a possible effect on consumers evaluations caused by the days of display.

Sequential and Random photos resulted in a low or zero R^2, which means that in these methodologies the differences in scores between consumers were not influenced by the days of display. However, a linear increase in the standard deviation occurred with the increase of display period, probably because of the lower capacity of human eyes to discriminate degrees of brown colour than other colours, as mentioned before. Our results are similar to Arnold et al. (1992), who observed an increase in the standard deviation when metmyoglobin content was measured either by visual appraisal and spectrophotometry.

However, the regression analyses of the standard deviation showed a cubic effect of days of display for the Trays methodology, leading to fluctuation in results through days of display as can be seen on Figure 4. When consumers have a reference of freshness, (high freshness in the first days, and lower freshness in the last days) they are induced to assign more homogeneous scores. However, when the reference of freshness weakens in the intermediary period, standard deviation increased. This fluctuation did not occur in Sequential and Random photos. In these methodologies, consumers evaluated samples at the same day, and photos were presented individually one after another, which promoted better conditions to consumers focusing in evaluating meat appearance. On the other hand, the fluctuation of Trays methodology might be explained due to its form of presentation, which occurred every day with samples placed together in the expositor, allowing comparison between sample to sample and day to day.

Consumers evaluation of meat quality is based on their past experiences and are dependent of a series of psychological and sensorial answers unique for each person (Ramos & Gomide, 2007). As differences between consumers are expected, operational sources of errors should be reduced. Digital images appear to be a promising tool because it is possible to "freeze" the meat colour and guarantee that samples are evaluated in the same conditions.

5. Conclusions

Animals fed with addition of essential oils clove and cinnamon resulted in lower visual scores of meats, suggesting lower shelf-life. However due to the complexity of these compounds, other parameters should be used to measure their effects in meat oxidation.

Digital images can substitute for the display evaluation, since their results were similar to those evaluated directly in Trays in the majority of days. In addition, Random photos appeared to be a promising tool, because it reduced the reference freshness influence and promoted a better standardized condition for evaluations.

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7. References

Arnold, R., Scheller, K., Arp, S., Williams, S., & Schaefer, D. (1992). Visual and spectrophotometric evaluations of beef color stability. *Journal of Food Science, 57*(2), 518-520.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils–a review. *Food and Chemical toxicology, 46*(2), 446-475.

Biondo, P. B. F., Carbonera, F., Zawadzki, F., Chiavellia, L. U. R., Pilau, E. J. P., Prado, I. N., & Visentainera, J. V. (2016). Antioxidant capacity and identification of bioactive compounds by GC-MS of essential oils commercialized in Brazil. *Journal of Essential Oil Research, in press*.

Brugiapaglia, A., & Destefanis, G. (2009). Sensory evaluation of meat colour using photographs. *Italian Journal of Animal Science, 8*(2s), 480-482.

Chan, S. H., Moss, B. W., Farmer, L. J., Gordon, A., & Cuskelly, G. J. (2013). Comparison of consumer perception and acceptability for steaks cooked to different endpoints: Validation of photographic approach. *Food Chemistry, 136*(3–4), 1597-1602. doi: http://dx.doi.org/10.1016/j.foodchem.2012.04.069

CIOMS/OMS. (1985). Council for International Organizations of Medical Services *International Guiding Principles for Biomedical Research Involving Animals*. Geneva, Switzerland: WHO Distribution and sales service.

Faustman, C., & Cassens, R. G. (1990). The biochemical basis for discoloration in fresh meat: a review. *Journal of Muscle Foods, 1*(3), 217-243.

Greene, B. E., Hsin, I., & Zipser, M. Y. W. (1971). Retardation of oxidative color changes in raw ground beef. *Journal of Food Science, 36*(6), 940-942.

Hart, K. J., Yáñez-Ruiz, D. R., Duval, S. M., McEwan, N. R., & Newbold, C. J. (2008). Plant extracts to manipulate rumen fermentation. *Animal Feed Science and Technology, 147*(1–3), 8-35. doi: http://dx.doi.org/10.1016/j.anifeedsci.2007.09.007

Hayes, J., Stepanyan, V., Allen, P., O'Grady, M., O'Brien, N., & Kerry, J. (2009). The effect of lutein, sesamol, ellagic acid and olive leaf extract on lipid oxidation and oxymyoglobin oxidation in bovine and porcine muscle model systems. *Meat Science, 83*(2), 201-208.

Hood, D. E., & Riordan, E. B. (1973). Discolouration in pre-packaged beef: Measurement by reflectance spectrophotometry and shopper discrimination. *International Journal of Food Science & Technology, 8*(3), 333-343.

Hui, Y. H. (1996). Oleoresins and essential oils. *Bailey's industrial oil and fat products. New York: Wiley-Interscience Publication, 6*, 145-153.

Kerry, J., Buckley, D., Morrissey, P., O'Sullivan, K., & Lynch, P. (1998). Endogenous and exogenous α-tocopherol supplementation: effects on lipid stability (TBARS) and warmed-over flavour (WOF) in porcine M. longissimus dorsi roasts held in aerobic and vacuum packs. *Food Research International, 31*(3), 211-216.

Lammens, J. M. G. (1994). *A computational model of color perception and color naming.* PhD, State University of New York, New York.

Liu, Q., Lanari, M., & Schaefer, D. (1995). A review of dietary vitamin E supplementation for improvement of beef quality. *Journal of Animal Science, 73*(10), 3131-3140.

Lu, J., Tan, J., Shatadal, P., & Gerrard, D. E. (2000). Evaluation of pork color by using computer vision. *Meat Science, 56*(1), 57-60.

Mancini, R. A., & Hunt, M. C. (2005). Current research in meat color. *Meat Science, 71*(1), 100-121.

Martin, J. N., Brooks, J. C., Brooks, T. A., Legako, J. F., Starkey, J. D., Jackson, S. P., & Miller, M. F. (2013). Storage length, storage temperature, and lean formulation influence the shelf-life and stability of traditionally packaged ground beef. *Meat Science, 95*(3), 495-502. doi: http://dx.doi.org/10.1016/j.meatsci.2013.05.032

McMillin, K. W. (2008). Where is MAP going? A review and future potential of modified atmosphere packaging for meat. *Meat Science, 80*(1), 43-65.

Morrissey, P. A., Buckley, D. J., Sheehy, P. J. A., & Monahan, F. J. (1994). Vitamin E and meat quality. *Proceedings of the Nutrition Society, 53*(2), 289-295.

Muramoto, T., Nakanishi, N., Shibata, M., & Aikawa, K. (2003). Effect of dietary β-carotene supplementation on beef color stability during display of two muscles from Japanese Black steers. *Meat Science, 63*(1), 39-42.

Nassu, R. T., Uttaro, B., Aalhus, J. L., Zawadski, S., Juárez, M., & Dugan, M. E. R. (2012). Type of packaging affects the colour stability of vitamin E enriched beef. *Food Chemistry, 135*(3), 1868-1872. doi: http://dx.doi.org/10.1016/j.foodchem.2012.06.055

Ngapo, T. M., Martin, J. F., & Dransfield, E. (2004). Consumer choices of pork chops: results from three panels in France. *Food Quality and Preference, 15*(4), 349-359. doi: http://dx.doi.org/10.1016/S0950-3293(03)00082-X

Ngapo, T. M., Martin, J. F., & Dransfield, E. (2007). International preferences for pork appearance: II. Factors influencing consumer choice. *Food Quality and Preference, 18*(1), 139-151. doi: http://dx.doi.org/10.1016/j.foodqual.2005.09.007

NRC. (2000). *Nutrient Requirements of Beef Cattle* (7th rev. ed.). Washington, DC, USA: Natl. Acad. Press.

O'Sullivan, M. G., Byrne, D. V., Martens, H., Gidskehaug, L. H., Andersen, H. J., & Martens, M. (2003). Evaluation of pork colour: prediction of visual sensory quality of meat from instrumental and computer vision methods of colour analysis. *Meat Science, 65*(2), 909-918. doi: http://dx.doi.org/10.1016/S0309-1740(02)00298-X

Ouattara, B., Simard, R. E., Holley, R. A., Piette, G. J. P., & Bégin, A. (1997). Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. *International Journal of Food Microbiology, 37*(2–3), 155-162. doi: http://dx.doi.org/10.1016/S0168-1605(97)00070-6

Prado, I. N., Campo, M. M., Muela, E., Valero, M. V., Catalan, O., Olleta, J. L., & Sañudo, C. (2015). Effects of castration age, protein level and lysine/methionine ratio in the diet on colour, lipid oxidation, and meat cceptability of intensively reared Friesian steers. *Animal, 9*(8), 1423-1430.

Ramos, E. M., & Gomide, L. A. M. (2007). *Avaliação da qualidade de carnes: fundamento e metodologias* (Vol. 1). Viçosa: Universidade Federal de Viçosa.

Renerre, M. T. (1990). Factors involved in the discoloration of beef meat. *International Journal of Food Science & Technology, 25*(6), 613-630.

Rivaroli, D. C., Guerrero, A., Valero, M. M., Zawadzki, F., Eiras, C. E., Campo, M. M., . . . Prado, I. N. (2016). Effect of essential oils on meat and fat qualities of crossbred young bulls finished in feedlot. *Meat Science, in press*.

Vitale, M., Pérez-Juan, M., Lloret, E., Arnau, J., & Realini, C. E. (2014). Effect of aging time in vacuum on tenderness, and color and lipid stability of beef from mature cows during display in high oxygen atmosphere package. *Meat Science, 96*(1), 270-277. doi: http://dx.doi.org/10.1016/j.meatsci.2013.07.027

Warren, H. E., Scollan, N. D., Nute, G. R., Hughes, S. I., Wood, J. D., & Richardson, R. I. (2008). Effects of breed and a concentrate or grass silage diet on beef quality in cattle of 3 ages. II: Meat stability and flavour. *Meat Science, 78*(3), 270-278. doi: 10.1016/j.meatsci.2007.06.007

Wulf, D., Morgan, J., Sanders, S., Tatum, J., Smith, G., & Williams, S. (1995). Effects of dietary supplementation of vitamin E on storage and caselife properties of lamb retail cuts. *Journal of Animal Science, 73*(2), 399-405.

Zulueta, A., Esteve, M. J., & Frígola, A. (2009). ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. *Food Chemistry, 114*(1), 310-316.

Table 1
Chemical composition of diets fed to young bulls with or without essential oils (% DM).

Ingredient	DM[1]	CP[2]	OM[3]	Ash[4]	EE[5]	NDF[6]	ADF[7]	TDN
Pellet sugar cane[a]	94.7	1.8	98.0	2.0	0.5	78.7	49.2	45.0
Cracked corn[b]	88.9	10.0	99.1	0.9	3.5	17.7	4.4	90.0
Soybean meal[c]	88.6	49.7	93.7	6.2	1.3	13.7	5.9	72.0
Econbeef[d*]	88.0	56.0	94.7	4.3	17.0	12.0	6.0	90.0
Limestone[e]	98.0			98.0				
Salt[f]	98.0			98.0				
Yeast[g]	98.0	30.0	98.0	2.0				
Diet								
CON[I]	89.9	13.3	97.5	2.49	3.99	29.3	12.5	82.5
CLO[II]	89.7	13.3	97.6	2.53	3.98	29.4	12.4	82.5
CIN[III]	89.3	13.3	97.7	2.55	3.96	29.4	12.4	82.4

[1]Dry matter, [2]Crude protein, [3]Organic matter, [4]Ashes,[5]Ether Extract, [6]Neutraldetergent fiber, [7]Acid detergent fiber, [8]Total digestible nutrients.

[a]Pellet sugar cane = 10.31 %DM, [b]Cracked corn = 79.31%DM, [c]Soybean meal = 5,28%DM, [d]Econbeef = 4.22%DM [e]Limestone, [f]Salt 0.42 %DM, [g]Yeast = 0.04%DM

[*]Econbeef = Calcium (50 g/kg), magnesium (57 g/kg), sodium (81 g/kg), sulfur (3.75 g/kg), cobalt (20 mg/kg), copper (500 mg/kg), iodine (25 mg/kg), manganese (1.500 mg/kg), selenium (10 mg/kg), zinc (2.000 mg/kg), vitamin A (400.000 UI/kg), vitamin D3(50.000 UI/kg), vitamin E (750 UI/kg), ether extract (168 g/kg) and urea (200 g/kg)

[I]CON = control (without essential oil); [II]CLO = diet with average 5000mg/d of clove essential oil; [III]CIN = average 5000mg/d of cinnamon essential oil;

Table 2

Profile of semi-trained evaluators from the Animal Science Department of State University of Maringá

Evaluator	Age	Gender	Position
1	40	Male	Professor
2	23	Female	Graduate student
3	22	Female	Graduate student
4	33	Female	Researcher
5	24	Female	Master student
6	26	Male	PhD student
7	27	Male	Master student
8	25	Male	PhD student
9	60	Male	Professor
10	23	Male	Graduate student
11	35	Female	Researcher
12	26	Female	PhD student
13	25	Female	Graduate student
14	26	Female	PhD student
15	26	Male	Master student
16	23	Female	Graduate student
17	25	Male	Graduate student

Table 3

Visual acceptability of meat from bulls fed with or without essential oils evaluated with three evaluation methodologies

	Diets*			Methodologies**				Diets*	Methodologies**	D x M***
Days	CON	CLO	CIN	Trays	Sequential	Random	SEM	p-value	p-value	p-value
1	7.84a	7.69a	7.99a	8.22aA	7.92aA	7.39aB	0.068	0.167	0.001	0.809
2	7.35ab	7.34ab	7.42ab	7.85aA	7.51abA	6.77bB	0.074	0.853	0.001	0.993
3	7.04bc	7.18ab	7.18bc	7.26bA	7.33bcA	6.82bB	0.072	0.657	0.007	0.894
4	6.82bc	6.83bc	6.90bc	7.05bA	6.91cdA	6.61bB	0.071	0.883	0.037	0.837
5	6.57c	6.32c	6.62c	6.36c	6.52d	6.63b	0.067	0.149	0.230	0.921
6	5.90dA	5.41dB	5.77dA	5.41dB	5.73eAB	5.94cA	0.063	0.003	0.003	0.568
7	5.33dA	4.67eB	4.94eB	4.76eB	4.99fAB	5.21dA	0.069	0.001	0.031	0.432
8	4.64eA	4.06fB	4.13fB	3.64fB	4.51fA	4.71deA	0.082	0.002	0.001	0.840
9	3.99f	3.72f	3.78f	3.46fB	3.84gAB	4.19eA	0.080	0.313	0.001	0.031
10	3.10g	2.96g	2.97g	2.40gB	3.20hA	3.44fA	0.079	0.710	0.001	0.524
11	2.37h	2.30h	2.39g	1.65hB	2.69iA	2.72gA	0.078	0.832	0.001	0.349
SEM	0.026	0.023	0.025	0.025	0.022	0.023				
p-value	0.001	0.001	0.001	0.01	0.001	0.001				

* Diets: CON = diet without addition of essential oils; CLO diet with addition of clove essential oil; CIN diet with addition of cinnamon essential oil

** Methodologies: Trays = Samples evaluated in retail condition in a commercial expositor; Sequential = Photos of samples evaluated in sequential order; Random = Photos of samples evaluated in random order.

*** Interaction between Diets and Methodologies.

a-h Different letters in the same column means significant differences (Tukey 0.05).

A-B Different letters in the same line means significant differences (Tukey 0.05).

Table 4

Regression analysis of three evaluation methodologies of visual acceptability of meat from bulls finished in feedlots fed with or without addition of essential oil

Diets*	Methodology**	Days** *	Equation	R^2	F	p-value
CON	Trays	6.98	$Y=8.579-0.331X-0.019X^2-0.001X^3$	0.836	316.65	0.001
	Sequential	7.22	$Y=7.769+0.116X-0.098X^2+0.004X^3$	0.789	233.34	0.001
	Random	8.14	$Y=7.477-0.346X+0.054X^2-0.006X^3$	0.745	182.31	0.001
CLO	Trays	6.29	$Y=8.178+0.130X-0.145X^2+0.007X^3$	0.857	371.26	0.001
	Sequential	6.60	$Y=7.740+0.074-0.091X^2+0.004X^3$	0.898	545.85	0.001
	Random	7.19	$Y=6.887+0.256X-0.108X^2+0.005X^3$	0.744	180.93	0.001
CIN	Trays	6.65	$Y=8.257+0.014X-0.109X^2+0.005X^3$	0.845	338.91	0.001
	Sequential	7.03	$Y=8.035+0.060X-0.105X^2+0.005X^3$	0.801	250.51	0.001
	Random	7.66	$Y=7.249+0.156X-0.074X^2+0.002X^3$	0.790	234.94	0.001

*Diets: CON = diets without addition of essential oils; CLO = diets with addition of clove essential oil; CIN = diets with addition of cinnamon essential oil
**Methodology: Trays = Samples evaluated in retail condition in a commercial expositor; Sequential = Photos of samples evaluated in sequential order and Random = Photos of samples evaluated in random order
***Days: Number of days which consumers evaluated meat with scores equal or higher than 5.0

Table 5

Overall standard deviation of the visual acceptability evaluated by three methodologies of meat from bulls finished in feedlot fed with or without addition essential oils

Days	Trays*	Sequential*	Random*	SEM	p-value
1	1.071b	0.978a	1.031a	0.023	0.238
2	1.106b	1.187b	1.008abc	0.022	0.193
3	1.156bcAB	1.232bB	1.023abcA	0.018	0.013
4	1.193cbB	1.265bB	1.045abA	0.021	0.001
5	1.225cbdB	1.1676bB	1.040abA	0.020	0.001
6	1.194bc	1.119ab	1.081ab	0.024	0.145
7	1.190bc	1.110ab	1.163abcd	0.022	0.310
8	1.377dB	1.207bA	1.131abcdA	0.022	0.001
9	1.279cd	1.258b	1.235cd	0.019	0.643
10	1.096bA	1.279bB	1.257dB	0.019	0.001
11	0.810aA	1.236bB	1.18bcdB	0.024	0.001
p-value	0.001	0.001	0.001		
SEM	0.012	0.012	0.009		

*Trays = Samples evaluated in retail condition in a commercial expositor; Sequential = Photos of samples evaluated in sequential order and Random = Photos of samples evaluated in random order

a-b Different letters in the same column means significant differences (Tukey 0.05%).

A-B Different letters in the same line means significant differences (Tukey 0.05%).

Table 6

Regression analysis of standard deviation of three evaluation methodologies of visual acceptability of meat from bulls finished in feedlots fed with or without addition of essential oils

Methodology*	Equation	R^2	F	P-valor
Trays	$Y=1.182-0.111X+0.037X^2-0.003X^3$	0.232	45.26	0.001
Sequential	$Y=1.100+0.014X$	0.030	14.38	0.001
Random	$Y=1.012+0.019X$	0.081	2.40	0.001

Methodology*: Trays = Samples evaluated in retail condition in a commercial expositor; Sequential = Photos of samples evaluated in sequential order; Random = Photos of samples evaluated in random order

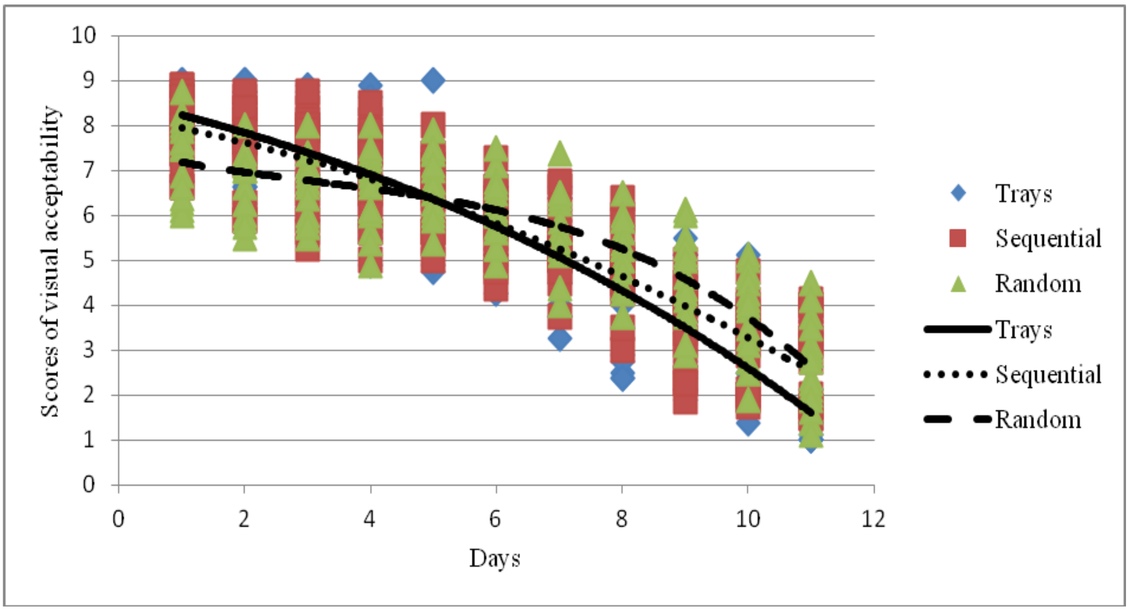

Fig. 1. Visual acceptability (1 = dislike extremely; 9 = like extremely) evaluated by methodologies to meat (*Longissiumus dorsi*) from bulls finished in feedlot fed without addition of essential oils (CON)

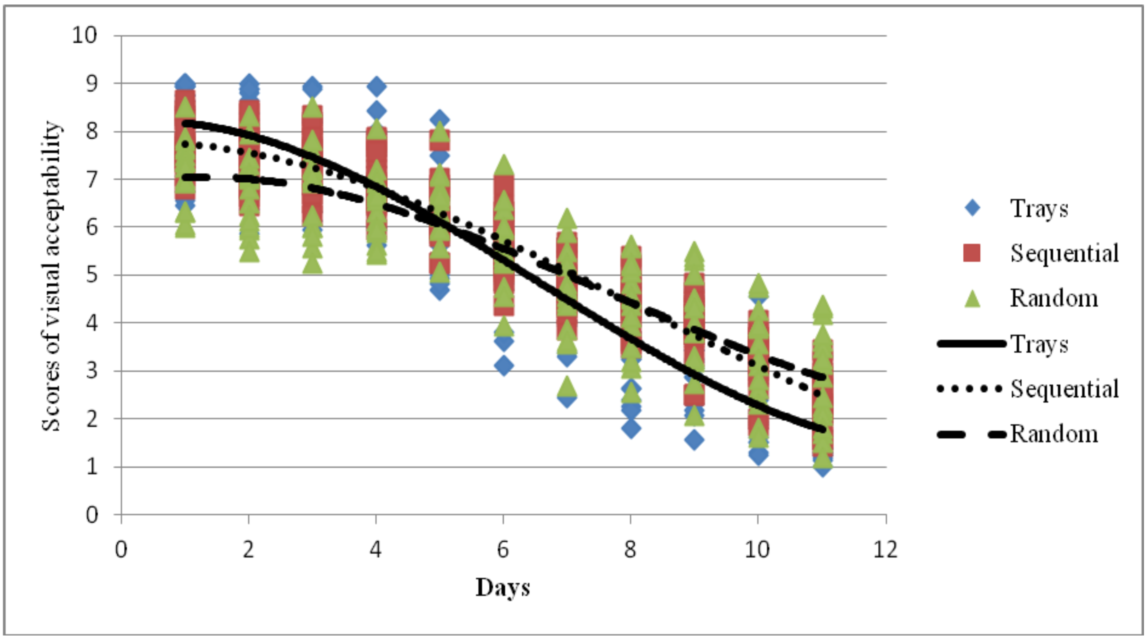

Fig. 2. Visual acceptability (1 = dislike extremely; 9 = like extremely) evaluated by methodologies to meat (*Longissimus dorsi*) from bulls finished in feedlot fed with addition of clove essential oil (CLO)

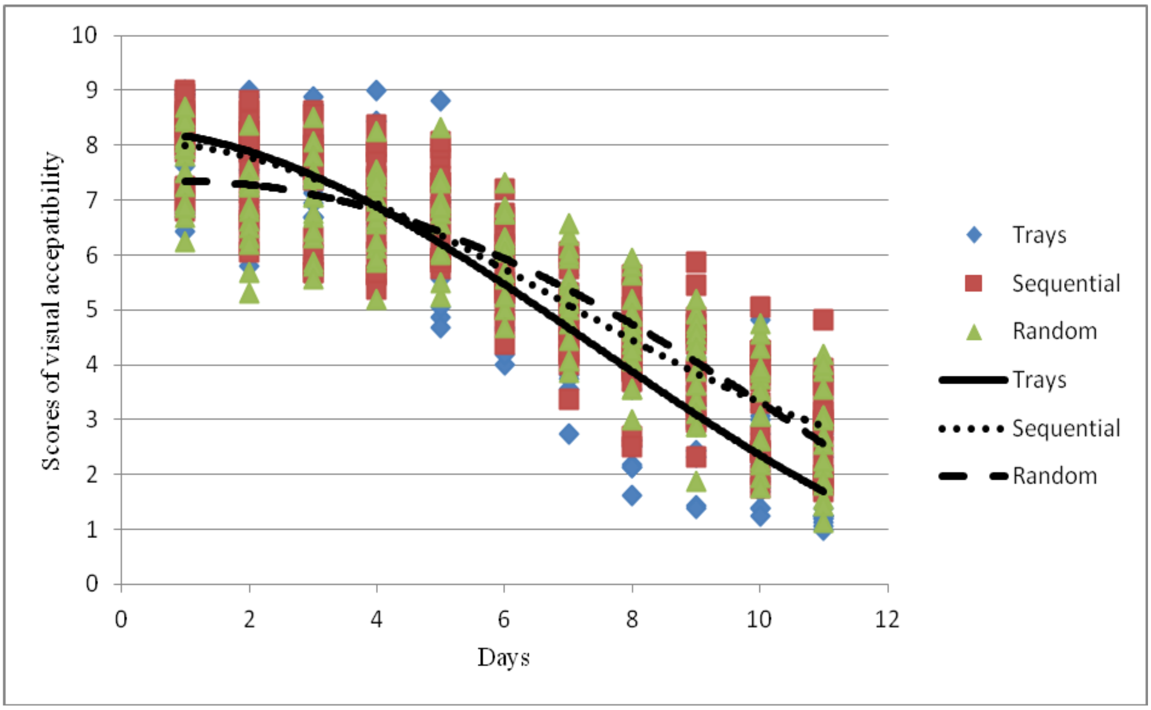

Fig. 3. Visual acceptability (1 = dislike extremely; 9 = like extremely) evaluated by methodologies to meat (*Longissimus dorsi*) from bulls finished in feedlot fed with addition of cinnamon essential oil (CIN)

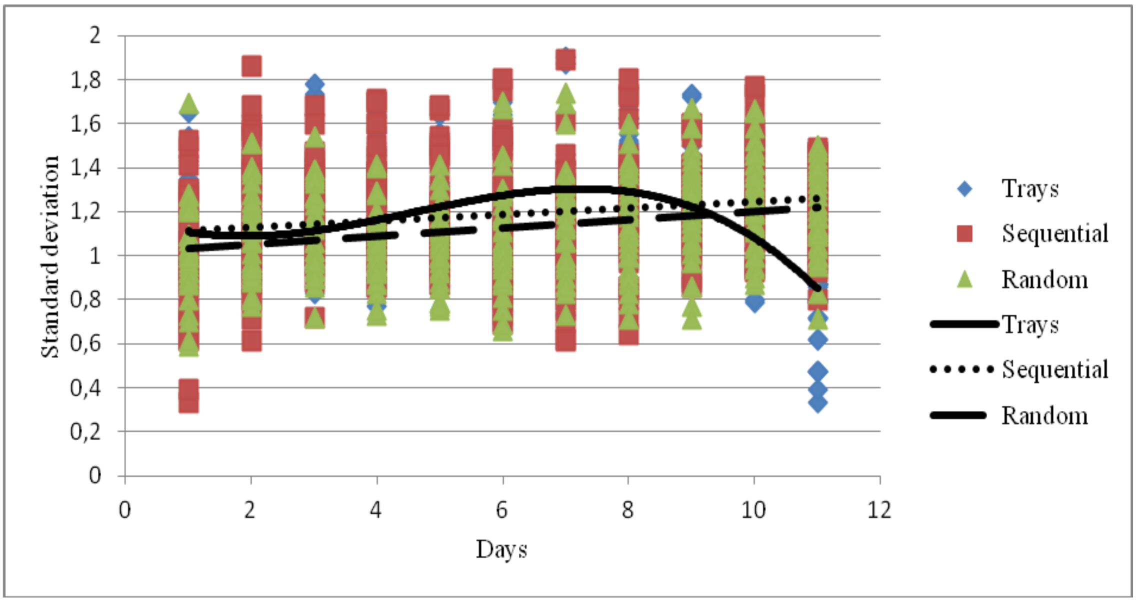

Fig. 4. Overall standard deviation of consumer's visual acceptability evaluated by methodologies to meat (*Longissimus dorsi*) from bulls finished in feedlot fed with or without addition of essential oils.