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The effect of technical replicate (repeats) on Nix Pro Color Sensor™ measurement precision for meat: A case-study on aged beef colour stability

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ABSTRACT

The Nix Pro Colour Sensor™ (NIX) can be potentially used to measure meat colour, but procedural guidelines that assure measurement reproducibility and repeatability (precision) must first be established. Technical replicate number (*r*) will minimise response variation, measureable as standard error of predicted mean (SEM), and contribute to improved precision. Consequently, we aimed to explore the effects of *r* on NIX precision when measuring aged beef colour (colorimetrics; L*, a*, b*, hue and chroma values). Each colorimetric SEM declined with increasing *r* to indicate improved precision and followed a diminishing rate of improvement that allowed us to recommend $r = 7$ for meat colour studies using the NIX. This definition was based on practical limitations and a* variability, as additional *r* would be required if other colorimetrics or advanced levels of precision are necessary. Beef ageing and display period, holding temperature, loin and sampled portion were also found to contribute to colorimetric variation, but were incorporated within our definition of *r*.

1. Introduction

Instrumental colour measurements (colorimetrics) are routinely collected in meat science and have been extensively applied to detect relative changes in meat colour (Suman & Joseph, 2014) as well as to quantify consumer perception of meat acceptability and value (Holman, van de Ven, Mao, Coombs, & Hopkins, 2017). Meat colorimetrics have also been associated with pH levels, microbial loads, oxidative and shelf-life stability, fatty acid content, and a plethora of other parameters (Abril et al., 2001; Kannan, Kouakou, & Gelaye, 2001; Li et al., 2015). This is often in an effort to capitalise on the relative ease of colour evaluation so it can be used as a proxy for more labour intensive or expensive analyses. Based on these applications, colorimetrics must be precise in their representation of the meat surface analysed.

Precision describes any variation from a hypothetical mean and doing so reflects measurement repeatability and reproducibility, which are both fundamental to the scientific process (Petrie & Watson, 2013). Precision can be improved by increasing measurement frequency or the number of technical replicates (*r*) that contribute to a measurement or data point (Mason, Gunst, & Hess, 2003). In practice, *r* is limited by experimental resources and therefore an understanding of *r* required, before their improvements to precision become negligible, would be useful in optimising precision and operational costs. This is true for instrumental colour determination and yet, to the best of our knowledge, this information is unavailable for colorimetric evaluation of meat using the Nix Pro Color Sensor™ (NIX). That said, guidelines which define optimal *r* for other colorimeters and spectrophotometers also remain inconsistent or unavailable – an observation based on Tapp, Yancey, and Apple (2011) survey found 52.4% of studies reporting meat colorimetrics failed to include *r* details and those which did employed *r* which ranged between 1 and 30 per sample.

The NIX is a handheld colorimeter that is garnering interest because of its comparative cheapness and user-friendly interface, as a result of its smart device pairing (Hodgen, 2016; Stiglitz, Mikhailova, Post, Schlautman, & Sharp, 2016). However, caution should be applied when exploring its capacity to measure meat colour before first establishing procedural aspects aimed to optimise precision. Here, we aimed to fulfil this paucity and examine the effect of *r* on NIX precision when measuring aged beef colour (CIE values, hue and chroma) and explore its implications when comparing colour stability.

2. Materials and methods

2.1. Experiment design and sampling

On the same day, a total of 20 beef strip loins (LL; *M. longissimus*

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Table 1
Temperature control unit (TCU) mean ± standard deviation ambient temperatures and internal temperatures of their assigned samples.

TCU	Ambient Temp. (°C)	Internal Temp. (°C)
A	3.22 ± 2.33	3.57 ± 0.47
B	4.53 ± 1.22	4.53 ± 0.55
C	6.35 ± 1.52	6.80 ± 0.62
Control1	1.03 ± 2.07	0.59 ± 0.26
Control2	1.63 ± 1.75	1.44 ± 0.54

lumborum) were randomly selected from the boning room of an Australian abattoir. These were divided into eight equal portions (n = 160) that were individually vacuum-packaged and then randomly assigned so that 12 portions representing six different LL were allocated to each of four ageing periods (Aged; 6, 8, 10 and 12 days) x three holding temperatures (Temp; A, B and C). Each Temp was applied within a single temperature control unit (TCU; *CF*-80DZ WAECO™, Dometic Australia Ltd., Burleigh, AUS) and monitored using ambient temperature loggers (Table 1). All remaining LL portions were held for 14 days at control temperatures that represented industry practice and in two TCU (n = 8 per TCU). It should be noted that portions aged 6 days were not measured for colour using the NIX and therefore were excluded from this experiment.

2.2. Colour measurement

At the completion of each treatment combination, the corresponding portions were removed from their TCU and their internal temperatures were recorded (Table 1) using a HACCP infrared thermometer (Model 8838, AZ Instrument Corp., Tiachung City, TAI). These were then prepared into slices (thickness: 3.0–4.0 cm), placed onto individual black Styrofoam trays (area: 13.5 cm^2), and overwrapped with PVC food film (thickness: 15.0 μm) so that muscle fibres were perpendicular to the exposed surface. Slices were kept under continuous fluorescence lighting (NEC Tubes delivering 789 lx to portion surfaces, measuring using a handheld lux and foot-candles light meter) in a chiller (temperature: 3–4 °C) where they were bloomed for 45–60 min before being first measured *in situ* and still overwrapped, using a NIX (Nix Pro Color Sensor™, Nix Sensor Ltd., Ontario, CAN). A total of 40 measurements were recorded for each sample, taken periodically over the display intervals (Display; 0, 1, 2 and 3 days) so that ten readings (or r) were made in succession per interval. The NIX were positioned to avoid connective and fatty tissue deposits, and lifted and repositioned between each r. Two NIXs were used to measure half the available samples at each display interval, their assignment being randomised, with both having a 15.0 mm aperture and 45/0° measuring geometry, and using Illuminant D65 and 10° standard observer settings. All CIE (1978) colour coordinates (L*, a* and b* colorimetrics) were collated and then used to calculate hue (h*) and chroma (C*) (AMSA, 2012).

2.3. Statistical analysis

The analysis was based on Holman, Alvarenga, van de Ven, and Hopkins (2015). The key idea was to estimate the relative magnitude of the variation between readings compared to other sources of variation, such as TCU and LL and portion within LL effects, and thus infer how the standard error (SE) at the LL by portion level varied with r from this. The colorimetric data was modelled using a mixed model with fixed effects of Aged, Display and their interactions; and random effects of Temp, LL, portion within LL, and all associated interactions as well as interactions with Days Aged and Display. This model was fitted using asreml software package (Butler, 2009) within R (R Core Team, 2016).

From this analysis we calculated: SE (LL by portion) = √ (otherVar + resVar/r), where otherVar refers to the sum of the other sources of variation listed in Table 2.

3. Results and discussion

Fig. 1 shows that NIX precision was improved by increasing r for each colorimetric, evident as the decline to SE for predicted means, but the rate of these improvements was found to decrease with increased r. As suggested in the introduction, this outcome was not unexpected as SE is a measure of variation between independent observations made of the same sample and can therefore be reduced when sample size is increased through increasing r. From this we could advocate the use of infinite r, but this would be farcical, because of the constraints to experimental labour, time and monetary resources, amongst many other practical limitations. Instead we can develop a sensible definition of r to provide satisfactory precision – definable in terms of a sufficiently small SE for a measured variable (Mason et al., 2003). Using this and an arbitrary level where a reduction in SE of < 5% when compared to r equal to 20, allows us to suggest seven as the ideal r when using a NIX to measure meat colour. This recommendation is based not only on the diminishing improvements to a* precision with increased r, but also a* interpretation of changes to relative redness (CIE, 1978) and the availability of a threshold for consumer acceptability of beef colour using this colorimetric (Holman et al., 2017).

Additional r were found to be necessary to achieve this same arbitrary level of precision for L* (r = 10), b* (9), h* (10) and C* (8) (Fig. 1), and this must be considered when these are the focus of research or advanced precision is desirable to limit type II error – for example, to avoid false negatives when comparing the NIX to other existing colorimeters or using a NIX to differentiate between closely aligned experimental treatments. Consequently, NIX r is much higher than that prescribed for the HunterLab MiniScan (n = 4) (Anonymous, 2012) and all (n = 3) (Honikel, 1998) colorimeters – an observation thought to result from aperture size differences (Hodgen, 2016). The NIX aperture size allows only a small fraction of the complex and

Table 2
The percentage of residual variation contributed by the modelled factors to the estimation of colorimetric means, determined using a Nix Pro Color Sensor™ to measure beef colour. The recorded colorimetric ranges were included.[a]

Experimental Factors	L*	a*	b*	Hue	Chroma
Range	(26.3–73.8)	(5.8–31.2)	(0.1–20.8)	(0.01–0.98)	(7.5–37.5)
Temp	0.0	0.0	0.0	0.0	0.0
Temp × Aged	4.0	7.0	6.0	4.0	7.0
Temp × Display	0.0	2.0	0.0	0.0	1.0
LL	9.0	10.0	4.0	7.0	7.0
Temp × Aged × Display	1.0	3.0	3.0	3.0	3.0
LL × Portion	26.0	42.0	28.0	10.0	40.0
LL × Portion × Display	5.0	20.0	5.0	16.0	13.0
Residual variance	14.3	6.5	4.3	< 0.01	9.3

[a] Holding temperature (Temp); ageing period in weeks (Aged); display period in d (Display); individual strip loins or *M. longissimus lumborum* (LL); individual LL portion (Portion). Interactions between these factors are indicated using ×.