

Machine-vision requires fewer technical replicates for colour measurement of seafood than Minolta colorimeter

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Objective: There is currently no recommendation for the amount of replicate measurements required per sample of seafood when measuring its colour. The objective of this study was to determine the number of replicate measurements required for each machine to attain a precise and representative colour of two seafood species.

Materials and Methods: Raw Atlantic salmon (*Salmo salar*, n=8) and banana prawn (*Fenneropenaeus merguensis*, n=7) colour was measured. Colour was analysed using a Minolta CR400 colorimeter (2° angle; D65 illuminant) for L*, a*, and b* and a Canon EOS 7D camera to capture images for colour analysis using machine-vision. The salmon was cut to 1 cm sections and stacked 1, 2, 3, 4 or 5 cm thick whereas the prawns were measured as a single layer (~1 cm). The samples were placed on either black or white tiles in a light box (Ortery Photobench 120), under D65 illuminant. Fifteen technical replicate measurements were taken on each sample, for each thickness, and each tile. For the Minolta, the measurements were made across the sample surface. For the digital images, an image of the whole surface was taken. Digital images from the camera were processed using Adobe software to correct for camera and lighting inaccuracy, and to isolate the sample from the background. Average RGB colour for the image data was calculated and converted into L*a*b* colour space.

Statistical analysis was performed to determine the number of technical replicate measurements (r) using method of Holman et al. (2021) as follows. Genstat was used to run a Linear Mixed Model separately for the data from each machine and each species. Thickness, tile colour and their interaction were included in the Fixed Model and Replicate/Block was included in the Random Model. Block is a unique number per combination of thickness and tile colour. Genstat calculated other variance (variance due to changes in thickness, background colour and between sample replicates) and residual variance (variance between technical replicate measurements). The standard error of each replicate sample was calculated as follows (Holman et al. 2021);

Standard error = $\sqrt{(\text{Other Variance} + (\text{Residual Variance})/r)}$

The standard error for each colour variable (L*, a* and b*) for each machine and each species was calculated for integers of r between 1 to 60. The lowest value of r required to reduce the change in standard error between r and r+1 to lower than 1% was determined for each colour variable. The highest r amongst the colour variables was then determined to be the required replicate measurement.

Results and Discussion: For salmon the average machine-vision L*a*b* values (\pm standard deviation) were 32.95 (± 2.75), 32.02 (± 3.10), 38.51 (± 4.08) whereas for Minolta they were 41.10 (± 2.72), 11.38 (± 1.44), 15.14 (± 2.05).

For prawns, the mean machine-vision L*a*b* values (\pm standard deviation) were 47.67 (± 6.88), 1.10 (± 1.96), 11.24 (± 5.73) whereas for the Minolta they were

45.54 (± 1.99), -1.14 (± 0.66), -4.93 (± 2.55). The variability represented by the standard deviation is composed of the residual variance and other variance. The average residual variance value of machine-vision (0.11) was smaller than Minolta (2.02), whilst the average other variance value of machine-vision (2.93) was higher than Minolta (1.71). Machine-vision was expected to have lower residual variance than Minolta because variability in colour within the samples was expected to result in variable L*a*b* Minolta values within technical replicates. The higher other variance however was not expected, and indicates that machine-vision was more affected by altering the factors of thickness and tile colour, and differences between sample replicates. The high residual variance and low other variance of the Minolta translated into requiring more technical replicates than machine-vision to reduce change in standard error below 1%. For salmon, the technical replication required for L*a*b* for machine-vision was 2, and for the Minolta the value was 6. For prawns, machine-vision required 2 whereas Minolta required 23. If not all colour traits are of interest, the number of technical replicates required can vary. For Minolta measuring prawns if only a* and b* were of interest the required technical replicates was much lower: 8 and 10 respectively. In contrast, the required r for Minolta measuring salmon was not very different between L*, a* and b*: 5, 6 and 6 respectively. Similarly, machine-vision for prawns differed little between colour variables -1, 2 and 1 respectively - as for machine-vision with salmon too -1, 2 and 2 respectively. In summary, machine-vision required many fewer technical replicates than Minolta to achieve a suitably precise measurement which aids statistically significant discrimination between treatment groups.

References:

Holman, Benjamin W. B., Simon M. Diffey, Bridgette G. Logan, Suzanne I. Mortimer, and David L. Hopkins. 2021. 'Nix Pro Color Sensor Comparison to HunterLab MiniScan for Measuring Lamb Meat Colour and Investigation of Repeat Measures, Illuminant and Standard Observer Effects', *Food Analytical Methods*, 14: 697-705.

Key words: Colour measurement, Seafood, Machine-vision, Technical replicates, Colorimeter