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Comparison of portable spectral imaging (443–726 nm) and RGB imaging for predicting poultry product “use-by” status through packaging film

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The objective of this study is to compare portable visible spectral imaging (443–726 nm) and conventional RGB imaging for detecting products stored beyond the recommended “use-by” date and predicting the number of days poultry products have been stored. Packages of chicken thighs with skin on were stored at 4°C and imaged daily in pack through plastic lidding film using spectral and RGB imaging over 10 days. K-nearest neighbour (KNN) models were built to detect poultry stored beyond its recommended “use-by” date and partial least squares regression (PLSR) models were built to predict the storage day of samples. Model overfitting in the spectral PLSR model was prevented using a geostatistical approach to estimate the number of latent variables (LV). All models were built at the object level by using mean spectra and colour values per image. The KNN model built using spectral images (acc. = 93%, sen. = 75%, spec. = 100%) was more suitable than the model built using RGB images (acc. = 80%, sen. = 42%, spec. = 96%) for detecting poultry stored beyond its “use-by” date. The PLSR model built using spectral images ($R^2=0.78$, $RMSEC=0.92$, $RMSEV=1.11$, $RMSEP=1.34$ day) was more suitable than the model built using RGB images ($R^2=0.60$, $RMSEC=1.66$, $RMSEV=1.67$, $RMSEP=1.92$ day) for predicting storage day of poultry products.

Keywords: portable spectral imaging, K-nearest neighbour (KNN), partial least squares regression (PLSR), poultry, “use-by”, plastic lidding film

Introduction

For highly perishable foods, such as poultry, reliably determining the amount of time a product has been stored is a matter of both product quality and consumer safety. As the storage time of poultry products increases,

the meat begins to deteriorate and microbial growth occurs.¹ Beyond a certain point, the meat is considered spoiled and no longer fit for human consumption.² In the European Union, the shelf life of poultry products

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is declared by a “use-by” date as a recommendation for consumers to ensure optimal safety and product quality in accordance with Regulation (EU) No. 1169/2011. The “use-by” date is a prediction made based on predictive microbiology, laboratory analysis and sensory analysis.³ Current methods of verifying poultry quality and safety are destructive and occur prior to products reaching the retail level (e.g., bacterial plating, chemical assays, sensory panels). Many extrinsic factors can affect the shelf life of poultry, including temperature, gas atmosphere, relative humidity and manufacturing hygiene practices.⁴ Although the predicted “use-by” dates attempt to take these extrinsic factors into account, the actual shelf-life of products varies.^{1,4} Spectral imaging and RGB imaging have potential to be used as a non-destructive tool to quickly identify poultry products beyond their recommended “use-by” date and predict the number of days poultry products have been stored.

Spectral cameras measure the complete spectral signature within a defined wavelength range, allowing for the identification of a sample's chemical composition. Rather than capturing a complete spectral signature, RGB cameras are limited to capturing the relative intensities of red, green and blue colours (Figure 1). Reduction of the spectral signature to three colours results in the loss of information potentially relevant to sample identification. Although the increased spectral resolution of spectral cameras can result in more robust models than RGB imaging,⁵ recent advances in machine vision technology have allowed for impressive RGB imaging applications in the field of food quality monitoring (e.g. prediction of mango ripening quality,⁶ assessing fish quality⁷ and determining avocado ripeness using smartphone images.⁸ Both imaging techniques are fast and non-destructive. The objective of this study is to compare portable spectral imaging in the visible range and RGB imaging for detecting poultry

products beyond their “use-by” date and predicting the number of days they have been stored without removing products from packaging. Because this study follows the same packages over time, it was not possible to conduct destructive microbiological experiments to confirm the “use-by” status. If consumers could non-destructively identify products that behave like those beyond their recommended “use-by” date, they could avoid consuming products of lesser quality and safety.

Methods and materials

Poultry samples

Packages of chicken thighs with skin on ($n_{\text{packages}} = 12$) from cereal fed chickens were acquired from a local supermarket and stored at 4 °C for the duration of this experiment. The reference “use-by” date is defined by the supermarket label, which corresponds to day 7 of imaging. Packages were removed from 4 °C directly prior to imaging and returned to 4 °C immediately after imaging. Samples were imaged in their original modified atmosphere packaging (MAP) through the polyethylene terephthalate (PET) plastic lidding film sealing the top of the packaging tray. Each package was imaged once daily for a total of 10 days, resulting in a total of 120 images.

Imaging system

Spectral images were acquired using a portable Specim IQ camera system (Specim Ltd, Oulu, Finland) using white LED Venus V29C ring light illumination (Guangdong Nanguang Photo&Video Systems Co., Ltd, Shantou, China) around the lens of the camera. The dimensions of the resulting spectral images were 512 rows $\times$ 512 columns $\times$ 204 spectral bands. The spectral camera was positioned directly above the sample at a height of 46 cm, resulting in a pixel size of approximately 0.49 $\times$ 0.49 mm. Along with capturing the spectral image, the portable Specim IQ camera system contains a 5 MP colour camera, which produces an RGB image.

Software

The software used to acquire spectral images was Specim IQ Studio (Specim Ltd, Oulu, Finland). All data analysis was completed using MATLAB R2020b (MathWorks, Massachusetts, USA) using functions written in house along with functions from the Statistics and Machine Learning Toolbox and the Image Processing Toolbox.

Figure 1. Example of data collected by spectral imaging (a) and RGB imaging (b).

Data pre-treatment

The spectral range of spectral images was cropped to 443–726 nm, to match the spectral range of the LED illumination. Spectra were then pre-treated by standard normal variate (SNV) pre-processing followed by Savitzky–Golay (SG) smoothing (13-point window size, 2nd order, 1st derivative) to reduce variability introduced by scattering effects of polymer films.⁹ RGB images were converted to the CIE L*a*b* colour space. To reduce variation due to shadows, only a* and b* values were used for data analysis. This is in agreement with guidelines for meat colour evaluation published by the American Meat Science Association Committee, which recommends the use of the CIE L*a*b* colour space to evaluate meat, because the a* and b* values are particularly informative about meat discolouration.¹⁰

Image segmentation

Both spectral and RGB images were manually cropped to exclude the background and sticker labels on top of the lidding films. Oversaturated pixels due to glare were masked out using a manual threshold. Spectral images were then masked to segment chicken from the plastic tray by Otsu's method of automatic threshold selection on the score image of the first principal component (PC1).¹¹ RGB images were masked using *Lazy Snapping* graph-based segmentation to mask chicken from the plastic tray.¹²

Data analysis

Detecting poultry products beyond “use-by” date
For both spectral and RGB image sets, images were split by randomly assigning images from eight packages to the calibration set ($n_{\text{images}} = 80$) and from a separate four packages to an independent test set ($n_{\text{images}} = 40$). As Day 7 corresponds to the “use-by” date, images from Days 1 to 7 are classified as before “use-by” and images from Days 8–10 are classified as after “use-by”. KNN models ($K = 5$) using Euclidean distances were applied to the spectral and RGB calibration sets, and evaluated using five-fold cross validation to calculate accuracy. The value of K was chosen based on the number of neighbours that resulted in the highest cross-validation accuracy in the calibration set. Next, the same KNN model parameters were used on the independent test set to validate the accuracy, sensitivity and specificity of the model. The models were built and tested at the object level using mean spectra and a*b* values. Next, the respective models were applied at the pixel level to images in the test set to visually assess model prediction.

Predicting poultry product storage day

The same calibration sets ($n_{\text{images}} = 80$) used to detect poultry products beyond their “use-by” date were retained for both spectral and RGB image sets. However, images from two packages in the former test set were allocated to a validation set ($n_{\text{images}} = 20$) used to pick the number of latent variables (LV) to be included in the models. The remaining two packages were used as the independent test set ($n = 20$). PLSR models were built using the calibration set and evaluated using the test set based on R^2 , $RMSEC$ and $RMSEP$ values. To prevent model overfitting in PLSR models built using spectral data, a geostatistical approach was used to estimate the number of LV to be included in the model using the validation set.¹³ This approach uses the spatial distribution of prediction maps to inform the optimal number of LV to be used in the calibration of the model. All 20 chicken spectral images from the validation set were concatenated for this analysis. The background platform and labels were masked out of the image using the masks obtained in the previous image segmentation step. Prediction maps from 1 to 12 LVs were obtained from this validation concatenated image. Three spatial indexes were extracted from each prediction map: total variance in the image [$\text{Var}(\hat{I})$], spatially structured variance C1 and random unstructured variance, C0. In essence, this method demonstrates that under certain assumptions, C1 is related to the slope or “sensitivity” of the model, while C0 reflects the random noise introduced by the model. In this case, it needs to be assumed that the true concatenated image, this is, the image that would represent the spoilage in chicken samples for each pixel, is expected to be spatially structured. These differences in chicken spoilage are expected to be prompted both by “use-by” date and by differences within each individual chicken thigh. This means that we expect pixels that are close (adjacent) to each other to have similar “decay” characteristics, while differences in “decay” values would manifest as contrast between areas of pixels that are further apart, this is, as spatially structured variance. The higher the variance between the predicted values of pairs of pixels that are spatially close, the higher the noise that is assumed to have been introduced by the model. This high frequency variance is quantified by C0 (unstructured variance of the prediction map). In addition, it is assumed that the greater the spatially structured variance found in the image, the more the model is able to reflect differences in chicken spoilage. In other words, it is assumed that most of the spatially structured differences in the prediction maps can be attributed to differences in chicken spoilage. The spatially structured variance is quantified by C1 as

the difference between the total variance, $\text{Var}(\hat{I})$, and CO . Further explanations and mathematical demonstrations behind this method are detailed by Herrero-Langreo *et al.*¹³

Finally, the respective models were applied pixel-wise to spectral and RGB images to visually assess the differences between the model predictions. Estimating LV was not required for RGB data, as the dataset contains only two predictor variables (i.e. a^* and b^* values).

Results

Detecting poultry products beyond "use-by" date

For both spectral and RGB imaging, changes in mean reflectance and a^*b^* values could be observed over storage time (Figure 2). Mean object spectra were most noticeably different between 443nm and 660nm. Mean spectra of samples stored beyond their "use-by" date lack peaks at 550nm and 650nm. Additionally, the peak at 592nm is lower and shifted to 602nm, lower at 493nm and higher between 443nm and 450nm in comparison with samples before their use-by date. Although less noticeable, a difference between RGB values can be seen before and after the "use-by" date in the red and green channels. The separation can be better seen when images are converted to the CIE $L^*a^*b^*$ colour space.

The KNN model built using spectral images resulted in high accuracy in both the calibration (93.8%) and test (92.5%) sets, indicating reasonably good ability to detect poultry products stored beyond their "use-by" date (Table 1). The KNN model built using RGB images was reasonable, though inferior to the spectral model, with lower accuracy in both the calibration (87.5%) and test (80%) set (Table 1). In the model built using spectral imaging, all cases of misclassification occurred on the first day following the "use-by" date (Day 8). Meanwhile, the RGB model resulted in cases of misclassification on all days following the "use-by" date. For both imaging methods, sensitivity was lower than specificity, indicating samples before their "use-by" date are more likely to be correctly identified than samples after their "use-by" date.

When the KNN models were applied to their respective test sets at the pixel level, spectral imaging resulted in a clearer separation between samples before and after their "use-by" date (Figure 3). More areas were classified incorrectly as after the "use-by" date in the RGB test set than in the spectral test set, which is supported by the confusion matrix results (Figure 4). Visually, these areas tend to be on the edges of individual samples.

Predicting poultry product storage day

When samples are identified by storage day, sample mean spectra and RGB values gradually move from

Table 1. Accuracy (%), sensitivity (%) and specificity (%) of KNN models ($K = 5$) at the object level in the calibration and test sets.

	Calibration set ($n = 80$)	Test set ($n = 40$)		
	Accuracy (%)	Accuracy (%)	Sensitivity (%)	Specificity (%)
Spectral imaging	93.8	92.5	75.0	100
RGB imaging	87.5	80.0	41.7	96.4

behaving like samples before to after their “use-by” date with each increasing storage day (Figure 5). In the spectral data set, this includes a gradual increase of the peak between 443nm and 660nm, decrease of the peak at 592nm and shift to 602nm, and decrease at 650nm with increasing storage day. Several spectra of samples imaged on the recommended “use-by” date behave more like those stored beyond their shelf-life, while others are visibly more similar to spectra of samples before their “use-by” date, indicating heterogeneity in the sample set. This is in agreement with the results of the KNN prediction maps (Figure 3), where pixels on the 2nd and 4th packages continue to be classified as before the “use-by” date on day 8 and many pixels on the 3rd package show mixed prediction between days 6 to 7. For the RGB data set, particularly in the red and green channels, RGB values decrease with increasing storage time. The trend of decreasing values with storage age can be better seen when images are converted to the CIE $L^*a^*b^*$ colour space.

Figure 6 shows $\text{Var}(\hat{l})$, C_0 and C_1 values used to estimate the number of latent variables (LV) to include in the PLSR models. For 1 and 2 LV both the spatially unstructured variance C_0 and the spatially structured variance C_1 are very low ($C_0 < 0.1$ and $C_1 < 6.17$). This indicates that while the random error introduced by the model is very

close to 0, the variance gathered by the model is also very low. This is a typical indication of an under-fitted model (too few LV). From 4 to 7 LV, C_0 is moderately low ($C_0 < 2.7$) while C_1 values stabilise at a local maximum plateau (between 15.2 and 16.2). Thus, for this interval, the structured information gathered by the model is at its highest, while the random error introduced by the model remains low. Within this interval, maximum C_1 is found at 5 LV (16.2) and 7 LV (15.8). From this result and considering that model calibrations with fewer LV are usually preferable to avoid overfitting and promote more robust models, 5 LV could be selected as the optimum LV number. From 8 LV to 12 LV, C_0 increases dramatically while C_1 decreases, indicating that adding more LV to the model is both increasing the random error introduced by the model and decreasing the structured variance gathered by the model.

The 5 LV PLSR model built using spectral images resulted in $R^2 = 0.78$, $RMSEC = 0.92$, $RMSEV = 1.11$ and $RMSEP = 1.34$, indicating there is a trend of changing spectra with increased storage time (Table 2). The PLSR model built using RGB images was inferior to the spectral model, with $R^2 = 0.60$, $RMSEC = 1.66$, $RMSEV = 1.67$ and $RMSEP = 1.92$ (Table 2). Based on the regression vector (β) of the spectral PLSR model, the most important wavelengths in decreasing order of importance for

Figure 5. Mean reflectance spectra (a), RGB (b), L^* values (c) and a^* and b^* values (d) of packaged chicken thighs over 10 days of storage. Day 7* corresponds to the “use-by” date indicated by the store label.

Figure 6. Geostatistical indexes, C0, C1 and total variance $[\text{Var}(\hat{I})]$ of the concatenated prediction maps from 1 to 12 latent variables obtained from the validation dataset concatenated image.

predicting storage day are 487, 628, 631, 640, 637, 484, 490, 634, 446 and 492 nm (Figure 7).

When the PLSR models were applied to their respective test sets at the pixel level, spectral imaging resulted in a clearer trend of increasing storage days than RGB imaging (Figure 8). Individual chicken thighs show a trend of faster aging around the edges of samples in both the spectral and RGB data set.

Discussion

As poultry products age, chemical and microbiological changes occur associated with meat deterioration.^{2,14} Since both the spectral KNN and PLSR models both observed a change in spectra with increased storage time using data collected in the visible light region, this work confirms that the colour change of meat during storage

Figure 7. Vector of coefficients (β) of the PLSR model built using spectral images.

Figure 8. Maps predicting samples imaged before and after "use-by" date (Day 7*) of packages in the test set on a pixel-level using spectral and RGB imaging.

is important for storage day prediction. The colour of poultry tissues is dependent on the redox form of the oxygen supplying protein, myoglobin.^{15,16} Lipid oxidation during storage results in myoglobin oxidation and subsequent colour change.¹⁷⁻¹⁹ When meat is first exposed to air, deoxymyoglobin oxidises to cherry-red oxymyoglobin, then to a brownish-red metmyoglobin within a week of storage.¹⁹ The regression coefficients that contribute the most to the PLSR model are similar to those attributed to deoxymyoglobin and metmyoglobin in the literature.^{16,20} Although these are changes occurring in the visible wavelength region, they are not intuitive for human inspectors to interpret by eye alone.

Table 2. Results of PLSR models (R^2 , RMSEC, RMSEV, RMSEP) and number of latent variables (LV) used for predicting storage day of poultry products using portable visible spectral imaging and RGB imaging.

	R^2	RMSEC	RMSEV	RMSEP	LV
Spectral imaging	0.78	0.92	1.11	1.34	5
RGB imaging	0.60	1.66	1.67	1.92	2

When the KNN and PLSR models were applied at a pixel level to create prediction maps, many pixels are incorrectly predicted. Pixels closest to the midpoints of individual chicken pieces are more likely to be misclassified as having a lower storage day than those on the edges. This could be due to exposed perimeters of individual chicken pieces aging at a faster rate than the centres. When chicken thighs are processed, they are cut at the joints on both sides of the thigh bone creating “cut-muscle” surfaces susceptible to faster spoilage.^{2,19} As more meat is exposed to air, lipid oxidation could occur at a higher rate around the perimeter of samples. If the models are classifying based on the colour changes observed during myoglobin oxidation, this could explain the observed variability in pixel-wise model predictions. Another possible reason for differences in prediction between centres and perimeters of individual chicken thighs could be related to curvature induced effects on spectra. Due to the curved nature of individual chicken thighs in relation to the illumination source, unwanted light scattering occurs and can potentially contribute to misclassification of pixels.

Conclusion

Portable visible spectral imaging was determined to be more suitable than RGB imaging for both predicting samples beyond their recommended “use-by” date and the storage day of poultry products. The KNN model built using spectral images resulted in higher accuracy, sensitivity and specificity than the model built using RGB images. The predictive PLSR model built using spectral images had a higher R^2 and lower RMSE values than the PLSR model built using RGB images. Successful prediction is likely a consequence of colour changes due to myoglobin oxidation during storage. The colour changes were too subtle for RGB imaging to successfully predict day of storage. In future work, microbiological spoilage will be monitored using bacterial counts to serve as a more direct reference method for spoilage. These findings indicate that spectral imaging could potentially be

used as non-destructive, fast alternative to conventional methods for evaluating poultry products and validating “use-by” date expectations set by predictive microbiology.

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