



Predicting sensory evaluation indices of Cheddar cheese texture by fluorescence fingerprint measurement coupled with an optical fibre

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ABSTRACT

A fluorescence fingerprint (FF) was used to develop a quick and non-contact practical method for predicting the sensory evaluation index of cheese texture (cheese body measurement). A partial least-squares (PLS) model was constructed from FF data and cheese body measurements of Cheddar cheeses. The cheese body measurement was successfully predicted by the PLS model with a coefficient of determination for calibration of 0.800. Notably, the reproducibility of the prediction value and the model accuracy were comparable with those of a conventional FF model despite the non-contact measurement. By exploring the variable importance in projection (VIP) and selectivity ratio (SR) of the PLS model, the fluorescence likely corresponding to oxidised lipids, Maillard reaction products, and compounds of proteins and amino acids with oxidised lipids was found to increase in intensity with the progress of ripening. This suggests that the fluorescence of these compounds contributes to the accuracy of the PLS model.

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1. Introduction

The composition (fat, moisture, and intact casein) and maturity of natural cheese as a raw material for processed cheese has a significant impact on the product quality and stability (Guinee, Caric, & Kalab, 2004; Kapoor & Metzger, 2008; Tamime, 2011). Generally, young cheese is used to produce hard processed cheese, whereas aged cheese is used to produce soft processed cheese. For example, block-type processed cheeses that are easy to slice and are elastic require a young cheese, whereas spread-type processed cheeses are primarily based on cheese with medium maturity. However, as natural cheese starts to mature immediately after manufacturing, the maturity of individual natural cheeses used to make processed cheese will differ. Therefore, to achieve products with sufficient uniformity, the blending ratio must be adjusted daily.

Processed cheese has numerous advantages including: (i) a long shelf-life; (ii) the characteristics of multiple natural cheeses through which, with additional seasoning, variations in the taste can be enhanced; (iii) a mild taste, making it palatable for children

and people who may not be familiar with natural cheese; (iv) an easily modified shape and readily adjustable texture (elasticity, hardness, spreadability, and ease of slicing); and (v) high suitability for cooking (flow, ease of browning, and viscosity).

Assessment of the texture of cheese based on detailed measurements represents an active area of research. In particular, the relationship between physical properties and texture evaluated through instrumental measurements and sensory evaluation (Drake, Gerard, Truong, & Daubert, 1999; Foegeding, Brown, Drake, & Daubert, 2003; Guinee & Kilcawley, 2004; Lee, Imoto, & Rha, 1978; Morita et al., 2015; O'Callaghan & Guinee, 2004; Xiong, Meullenet, Hankins, & Chung, 2002) constitutes a primary area of interest. However, only a few measurement methods are actively used by processed cheese manufacturers to evaluate natural cheese as raw materials [an example of such a method currently on the market is "Caseus Pro™" (Gold Peg International, Inc., Braeside, Australia)]. This is because the pretreatment process for evaluation and instrumental measurement is complex, the measurement results cannot be directly related to traditional sensory evaluation results, and the number of samples required for appropriate evaluation is excessively large. Therefore, in general, sensory evaluation approaches are faster.

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Individual processed cheese manufacturers continue to use traditional sensory evaluation or only employ instrumental measurement data directly, with each company using its own methods. For example, an index termed “cheese body measurement” is used for the sensory evaluation of physical properties. The cheese body measurement is an index of the physical maturity determined by trained experts that is based on the physical sensation of crumbling a piece of cheese between the fingers. This index varies between 1 and 10, where 10 points indicates a strong body (young texture without maturing) whereas 1 point indicates a weak body (matured texture), and is likely to vary significantly among cheese manufacturers (Mizuno & Ichihashi, 2007; Muir, 2010; Nakazawa & Hosono, 1989).

Alternatively, methods have been developed that utilise mid-infrared light, near-infrared light, fluorescence, and Raman spectroscopy to predict the components and physical properties of food, which are used as quick measurement methods (Nawrocka & Lamorska, 2013). As fluorescence and near-infrared spectra can be measured without sample pretreatment, these spectroscopic methods exhibit high potential for industrial use. In particular, fluorescence spectroscopy measures the emission spectrum instead of the absorption spectrum and demonstrates high sensitivity compared with that of near-infrared light (Karoui, Mazerolles, & Dufour, 2003; Kulmyrzaev, Karoui, De Baerdemaeker, & Dufour, 2007). Fluorescent compounds are sensitive to their surrounding environment (e.g., temperature, ionic concentration, pH, and polarity of solution); moreover, fluorescence spectroscopy measurements can be rapidly performed (Dufour, 2011). In addition, some investigations have been performed involving the measurement of cheese using fluorescence spectroscopy (Andersen & Mortensen, 2008; Christensen, Nørgaard, Bro, & Engelsen, 2006; Dufour, 2011; Karoui & Blecker, 2011). Furthermore, with the development of information processing technologies in recent years, fluorescence spectroscopy measurements allow the rapid acquisition of emission spectra from excitation light with a continuous wavelength, such as in the form of an excitation-emission matrix (EEM), also known as a fluorescence fingerprint (FF).

The FF has an advantage over conventional fluorescence spectra because it includes emission spectra excited at many different excitation wavelengths. Thus, FF yields much more information as compared with the study of conventional single or double excitation wavelengths, and has the potential to estimate phenomena with high accuracy. This approach is used as a measurement method by which a large amount of information can be acquired and processed (Airado-Rodríguez, Galeano-Díaz, Durán-Merás, & Wold, 2009). A FF targets not only the peak intensity of the fluorescence signal but also other wavelength ranges in which the fluorescence signal is weak. A model can then be constructed that only extracts necessary information. There are several instruments that can derive predictions based on fluorescence and a FF. Although a few analytical cases have been examined and numerous discussions have been conducted regarding the predictive accuracy obtained using these techniques, to our knowledge no reports are yet available of a detailed prediction model based on the contributing wavelength range or other factors (Lacotte et al., 2015; Liu, Sajith Babu, Coutouly, Allouche, & Amamcharla, 2016). However, there have been some cases of FFs being applied to study food (Sádecká & Tóthová, 2007) including dairy products containing cheese (Andersen & Mortensen, 2008; Boubellouta & Dufour, 2008; Christensen, Povlsen, & Sørensen, 2003).

Although there have been some studies that predicted cheese texture and physical properties using fluorescence spectroscopy (Garimella Purna, Prow, & Metzger, 2005; Karoui & Dufour, 2006; Karoui et al., 2003; Kulmyrzaev et al., 2005; Lebecque, Laguet,

Devaux, & Dufour, 2001; Ozbekova & Kulmyrzaev, 2017), their predictions were based on the fluorescence of a limited number of entities such as tryptophan and vitamin A. Moreover, the emission spectra were measured by using only single or double excitation wavelengths, with the primary purpose being to analyse the correlation between the peak intensity and components or textures. Recently, Kokawa et al. (2015) showed that a FF can predict indices of cheese maturation. They predicted the maturation time and some chemical analysis values, such as the proteolysis index (the ratio of water-soluble nitrogen content to total nitrogen content) and total free amino acid content, from FFs. They also demonstrated that the FF constitutes an effective tool for capturing changes in cheese through maturation. However, their method of measurement still requires the preparation of samples with a shape that fits the cell of the fluorescence spectrophotometer. In addition, there have been no studies where a FF has been used to predict the cheese body measurement, the index of sensory evaluation for cheese texture, for the raw materials of processed cheese.

In this study, our aim was to develop a rapid method for predicting the cheese body measurement using a FF, regardless of the experience level of the user. Then, we attempted to develop a method for non-contact prediction of the cheese body measurement by exploiting the optical fibre unit in a fluorescence spectrophotometer. In consideration of the practicality for industrial use, we have chosen a non-contact measurement using a fibre optics unit to eliminate the pretreatment of the sample. Although some reports are available in areas other than dairy research, no examples yet exist of application of this method to cheese texture. By removing the sample pretreatment step, measurement of the FF was easily achieved. Non-contact measurement of changes in the cheese with maturation will thus be faster than traditional methods, rendering this method useful for processed cheese manufacturers.

2. Materials and methods

2.1. Cheese samples

Ten Australian Cheddar cheese samples were used for measurements. We performed three measurements and evaluations over time and obtained the results for a total of 30 samples. These cheese samples were matured at $-2\text{ }^{\circ}\text{C}$, $5\text{ }^{\circ}\text{C}$, $10\text{ }^{\circ}\text{C}$, and $15\text{ }^{\circ}\text{C}$ to achieve differences in the cheese body measurement. Each cheese sample was divided into blocks for FF measurement and cheese body measurement evaluation. Cheese samples were also analysed for moisture (ISO/IDF, 2004a), fat (ISO/IDF, 2004b), and protein (ISO/IDF, 2002). The pH was measured by insertion of a pH probe (D-54, Horiba, Kyoto, Japan) into the ground cheese sample.

2.2. Evaluation of cheese body measurement

The cheese body measurement was evaluated by five trained experts in consultation after adjusting the temperature of the cheese to $5\text{ }^{\circ}\text{C}$. The maximum score for the cheese body measurement was 10, corresponding to the strongest texture (young texture), with a score of 1 corresponding to the weakest texture (matured texture). The cheese body measurement and FF were measured on the same day or on consecutive days so that there was no difference in maturation.

2.3. FF measurement

To measure the FF of cheese, we used a fluorescence spectrophotometer (F-7000, Hitachi High-Technologies Corporation,

Tokyo, Japan) and an optical fibre unit (5J0-0114-F-7000, Hitachi High-Technologies) (Mita Mala et al., 2016). The cheese samples subjected to FF measurement were adjusted to 20 °C and cut into blocks of 3 cm × 3 cm × 6 cm. The side of the cheese samples was selected randomly and was cut immediately prior to FF measurement, and measurements were taken on a fresh surface each time. The samples were then placed on a stage with a fixed fibre unit. During measurement, external light was excluded from the sample using an enclosure. The measurement conditions for the FF were an excitation wavelength of 200–500 nm, an emission wavelength of 200–800 nm, and a wavelength interval of 5 nm. The slit width of the monochromator was 10 nm for both excitation and emission wavelengths. A photomultiplier voltage of 500 V was used and the scan speed was 60,000 nm min⁻¹. The distance between the probe tip of the fibre unit and the cheese sample was 5 mm, and the measurement was made without contact. Three replicates were measured for each cheese sample.

2.4. PLS model for prediction of cheese body measurement

To predict the cheese body measurement from the FF measurements, a partial least-squares (PLS) regression model was constructed. For the multivariate analysis, MATLAB (R2016a) software (MathWorks Inc., Natick, MA, USA) and PLS Toolbox version 8.1.1 (Eigenvector Inc., Manson, WA USA) were used. FF data were preprocessed using the method of Yoshimura et al. (2014). First, as fluorescence constitutes an emission with wavelengths longer than the excitation wavelength, all data with emission wavelengths shorter than the excitation wavelengths were removed. Next, the primary light and the secondary and tertiary scattered lights, which comprise lights with the emission wavelengths at twice and three times the excitation wavelength (Fujita, Tsuta, Kokawa, & Sugiyama, 2010), respectively, were removed. Finally, excitation wavelengths shorter than 230 nm were removed because they contained significant noise. The remaining data were a combination of 3869 excitation wavelengths and emission wavelengths.

In total, we performed PLS regression analysis using 90 (three replicates × 10 samples × three evaluation periods) evaluation results for FF data. The cheese body measurement was measured for 30 samples (10 samples × three evaluation periods). First, the data were divided into calibration and validation sets (2:1). Specifically, the samples were aligned in order of measurement and measurements for every third sample were allocated to the validation set and those for the rest were allocated to the calibration set. Next, a cross-validation model using the calibration set was employed to determine the optimum pretreatment and number of latent variables (LVs). Four preprocessing methods were used for the FF data: mean centering, normalisation followed by mean centering, autoscaling, and normalisation followed by autoscaling. The data were normalised so that the area underneath each spectrum was equal to 1. In autoscaling, each wavelength was scaled to 0.0 mean and unit variance. The number of LVs was determined using a PLS Toolbox algorithm that determines the descending point known as the “knee” in a scree plot (Henry, Park, & Spiegelman, 1999). Next, the selected LVs and pretreatment were used to prepare a model based on the calibration set. Furthermore, we applied this model to the validation set data to confirm the accuracy of the prediction. The accuracy of the prediction model was evaluated using the coefficients of determination of cross-validation (R²CV) and prediction (R²P) and the root-mean-square errors of cross-validation (RMSECV) and prediction (RMSEP). In addition, we calculated the variable importance in projection (VIP), the selectivity ratio (SR), and the regression vector for the model and determined the wavelength range that mainly contributes to the model.

3. Results and discussion

3.1. Fluorescence fingerprints and cheese compositions

Fig. 1 shows the mean FF for the cheese samples. An emission peak was observed in the excitation wavelength range of 290–305 nm and the emission wavelength range of 320–350 nm. This peak had a fluorescence intensity of 6131 (arbitrary units, a.u.) and has been determined to correspond to aromatic amino acids including tryptophan (Andersen & Mortensen, 2008; Andersen, Vishart, & Holm, 2005; Mazerolles et al., 2001). In addition, the intensity was high (1201 a.u.) at the excitation wavelength of 320 nm and emission wavelength of 400 nm, which are likely the wavelengths of vitamin A. We focused on the peak at ex320/em400 because it has been examined in many previous reports. Fluorescence measurements for milk (Boubellouta & Dufour, 2008; Dufour & Riaublanc, 1997), soft cheese (Herbert et al., 2000), semi-hard cheese (Karoui, Dufour, & De Baerdemaeker, 2006), and processed cheese (Christensen et al., 2003) have been previously reported. The peak at the excitation wavelength of 300 nm and the emission wavelength of 680 nm represents the second-order light of the excitation wavelength of 300 nm and the emission wavelength of 335 nm, which appears owing to the light dispersion mechanism of the monochromator (i.e., the same mechanism that creates the second-order scattering light). In the FF data, similar peaks were detected to those in Kokawa et al. (2015); thus, we confirmed that a non-contact FF could be obtained for cheese using an optical fibre.

Table 1 shows the age, composition and pH of 10 sample cheeses. It was confirmed that there was little difference in composition and pH between samples.

3.2. PLS prediction model

Table 2 shows the LVs and prediction accuracy of the PLS regression model for each pretreatment. Before performing the PLS regression analysis, four pretreatments were evaluated to optimise the model in terms of R²CV and RMSECV. The present model that used the mean centering and normalisation followed by the mean centering as a pretreatment exhibited a similar level of accuracy with R²CV of 0.787 and RMSECV of 0.456. “Normalise” constitutes the pretreatment whereby the sum of the luminance is normalised to be 1. This pretreatment can reduce the variation caused by differences in overall brightness (although the spectral shapes are the same). In

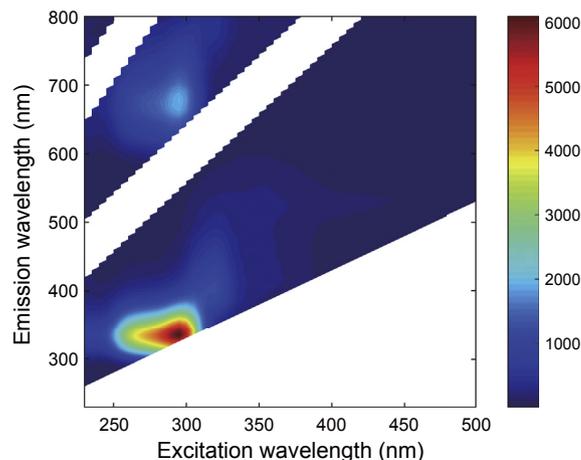


Fig. 1. Examples of fluorescence fingerprints of Cheddar cheese surfaces obtained using optical fibre in the range of normal intensities [0–6000 intensity (a.u.)].

Table 1
Physico-chemical compositions of 10 Cheddar cheeses.

Cheese sample	Age at measurement (days)			Storage temperature (°C)	pH	Dry matter (g 100 g ⁻¹)	Fat (g 100 g ⁻¹)	Protein (g 100 g ⁻¹)	Salt (g 100 g ⁻¹)
	1st	2nd	3rd						
1	231	269	318	-2	5.42	67.4	34.9	27.5	1.9
2	132	170	219	-2	5.40	67.3	34.7	27.4	2.0
3	111	149	198	-2	5.47	67.6	34.6	27.5	2.1
4	231	269	318	5	5.48	67.7	34.9	27.3	1.9
5	132	170	219	5	5.43	67.9	35.3	27.7	2.2
6	231	269	318	10	5.39	67.7	35.5	27.2	1.9
7	203	241	290	10	5.52	67.6	35.5	27.4	1.9
8	231	269	318	15	5.55	68.1	35.6	27.2	2.1
9	203	241	290	15	5.47	68.0	35.1	27.7	2.1
10	132	170	219	15	5.41	67.6	35.3	27.5	2.1

Table 2
Results of PLS regression for four pretreatment methods.^a

Pretreatment	LVs	RMSECV	R ² CV	R ² C	R ² P	RMCEP
Mean centering	3	0.456	0.787	0.800	0.826	0.436
Normalise + mean centering	3	0.456	0.787	0.808	0.747	0.515
Autoscale	3	0.493	0.757	0.822	0.787	0.470
Normalise + autoscale	2	0.482	0.762	0.787	0.739	0.519

^a Abbreviations are: LVs, latent variables; RMSECV, root-mean-square error of cross-validation; R²CV, coefficient of determination of cross-validation; R²C, coefficient of determination of calibration; R²P, coefficient of determination of prediction; RMCEP, root-mean-square error of prediction.

this study, it made no difference in precision whether “normalise” was performed or not; there is thus a possibility that such variation was small. The “autoscale” and “normalise + autoscale” were not adopted because the precision was low.

Fig. 2 (the calibration set) and Fig. 3 (the validation set) show the relationship between the cheese body measurement predicted from the FF using the optical fibre unit and the actually evaluated cheese body measurement. The prediction model was developed with three LVs using the pretreatment of the mean centre. In the validation set, we obtained a strong correlation (R²P = 0.826) and small prediction error (RMSEP = 0.436). Additionally, the relative standard deviation (RSD) of the body value predicted by FFs in the three replicates was 0.21% minimum and 5.4% maximum. The

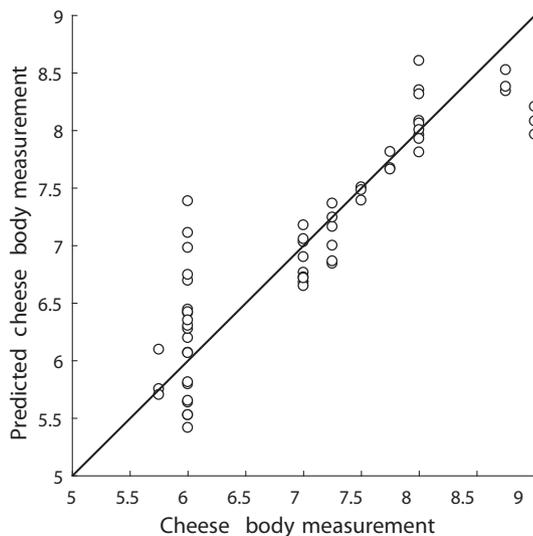


Fig. 2. Predicted versus measured cheese body measurements obtained by PLSR calibration. Bold line shows $y = x$.

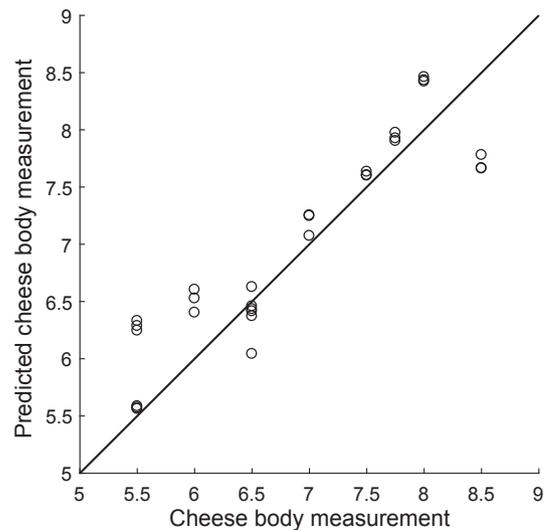


Fig. 3. Predicted versus measured cheese body measurements obtained by PLSR validation. Bold lines shows $y = x$.

average of RSD with three replicates in all 30 samples was 1.6%. These values show that this method can be applied repeatedly with just 1–2% variation and has high reproducibility. The results shown in Figs. 2 and 3 indicate that (i) the FF can predict the cheese body measurement although attention is required for cheese body measurements of around six, and (ii) using the optical fibre unit, the cheese body measurement can be predicted through non-contact measurement.

The evaluation of cheese body measurements by the experts in this study often resulted in cheese body measurements of around six; thus, the distribution of the evaluation data was unequal. This is likely to have reduced the prediction accuracy. As the predicted cheese body measurements of around six have a higher variation than that of other cheese body measurements, the handling of results close to this value requires attention. To further improve the accuracy, the number of samples can be increased, or samples should be selected so that the distribution of cheese body measurements across the scoring range is even.

3.3. VIP and SR scores of the PLSR model plotted using FF contour representation

Fig. 4 shows the VIP and SR scores as a contour map to identify the range of wavelengths that contributes to the PLS regression model. Two wavelength ranges with high VIP scores were

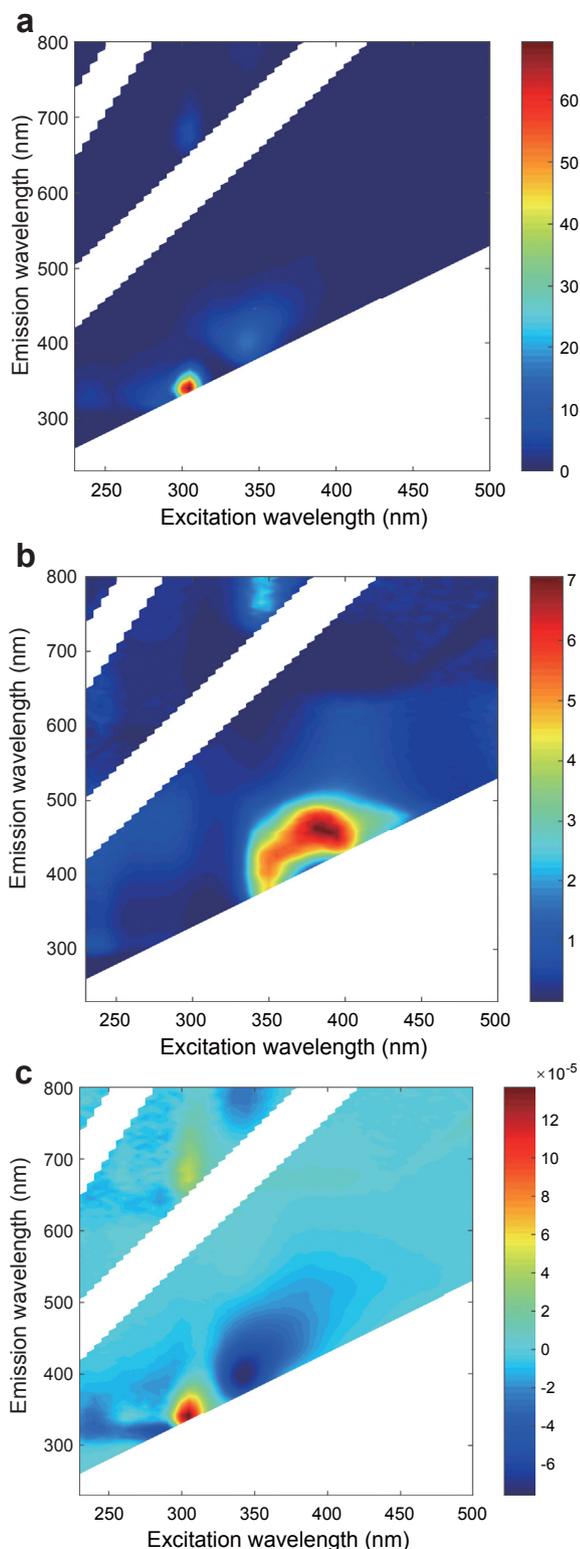


Fig. 4. Contour plot of each score in the PLS model: (a) VIP, (b) SR, (c) regression vector.

identified (Fig. 4a). Wavelengths with VIP scores of 1 or higher are known to be important variables in the model (Mehmood, Liland, Snipen, & Sæbø, 2012). The highest VIP score (69.9) was observed in the wavelength ranges with an excitation wavelength of 305 nm and an emission wavelength of 340 nm. Subsequently, the wavelength ranges with an excitation wavelength of 340 nm and an

emission wavelength of 400 nm also showed high VIP value (VIP score = 15.9). The VIP value of the wavelength ranges with an excitation wavelength of 300 nm and an emission wavelength of 680 nm was also high, but we did not pursue this range because it represented the second-order light of an excitation wavelength of 300 nm and an emission wavelength of 340 nm. As has been previously reported, the excitation wavelength of 305 nm and emission wavelength of 340 nm were considered to represent tryptophan and aromatic amino acids (Andersen et al., 2005; Andersen & Mortensen, 2008; Mazerolles et al., 2001).

As an alternative, Fig. 4b shows a contour map of the SR scores. SR scores provide a numerical evaluation of the utility of each variable in a regression model. The SR scores can be used to build a model with even higher accuracy by excluding wavelength ranges with low SR scores and selecting wavelengths with only high SR scores (Rajalahti et al., 2009). In the present study, we used the F-test (95%) standard to select wavelength ranges with a large contribution (Farrés, Platikanov, Tsakovski, & Tauler, 2015). The SR score of the F-test (95%) was 1.55. Wavelength ranges with a SR score above this value (SR score > 1.55) included the excitation wavelengths of 335–445 nm and emission wavelengths of 370–520 nm. In these ranges, the excitation wavelength of 385 nm and emission wavelength of 460 nm comprised the peak wavelengths. The peak SR score for these excitation-emission wavelengths was 7.11. The SR score of the excitation wavelength of 350 nm and the emission wavelength of 770 nm was also high, but this wavelength range was also not further pursued because it represented second-order light of the excitation wavelength of 340 nm and the emission wavelength of 400 nm.

As VIP scores only take positive values, we used the regression coefficient (regression vector) shown in Fig. 4c to determine whether the wavelengths correlated positively or negatively with the cheese body measurements. The regression vector for each VIP peak revealed that the value was positive at the excitation wavelength of 305 nm and emission wavelength of 340 nm and negative at the excitation wavelength of 340 nm and emission wavelength of 400 nm. This suggests that the fluorescence intensity of the former wavelengths decreases as the cheese body measurement decreases, whereas that of the latter wavelengths increases.

Fig. 5a shows the emission spectra of all samples at an excitation wavelength of 340 nm. The peak intensity around the emission wavelength of 400 nm increased as the cheese body measurement decreased; the correlation coefficient of the fluorescence at the excitation wavelength of 340 nm and emission wavelength of 400 nm with the cheese body measurement was -0.77 . The fluorescence intensity of each fluorophore reflects how they are metabolised during cheese maturation. In this study, we considered that the wavelength range of excitation wavelength of 340 nm and emission wavelength of 400 nm, which exhibited high correlation with cheese body measurement, was related to substances that increased during cheese maturation.

Numerous investigations have focused on the range near the excitation wavelength of 340 nm and the emission wavelength of 400 nm. Stapelfeldt and Skibsted (1994) reported that in a model system of dairy products and β -lactoglobulin, accumulated secondary lipid oxidation products emit fluorescence at excitation wavelengths of 350 nm and emission wavelength of 410 nm. Morales, Romero, and Jiménez-Pérez (1996) monitored the fluorescence of Maillard reaction products during the thermal processing of a model system using milk and dairy products at an excitation wavelength of 347 nm and emission wavelength of 415 nm. These model system studies demonstrated that oxidised lipid and Maillard reaction products of milk and dairy products could be monitored via their fluorescence in the range near the excitation wavelength of 340 nm and the emission wavelength of

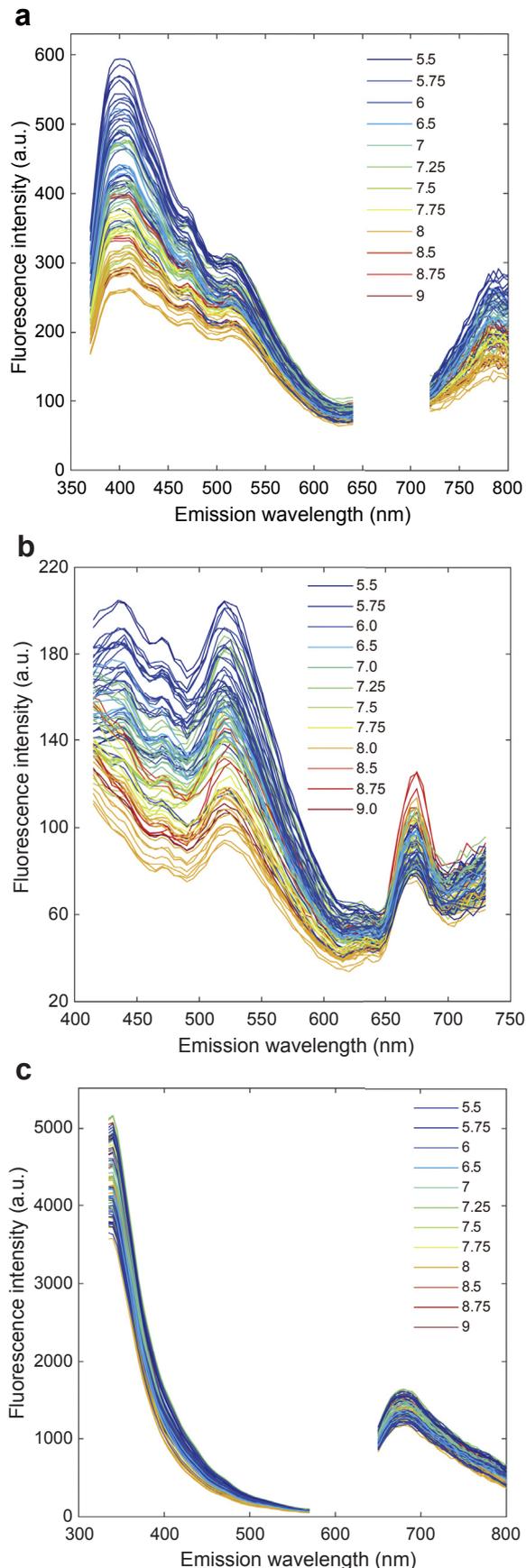


Fig. 5. Fluorescence emission spectra colour-coded by cheese body measurement at excitation wavelengths of (a) 340 nm, (b) 385 nm, and (c) 305 nm; the gaps in the

400 nm. In addition, [Kokawa et al. \(2015\)](#) selected peaks at an excitation wavelength of 345 nm and emission wavelength of 400 nm as the wavelength values with a large contribution to the PLS regression model in the FF of cheese maturation indices, which suggested that oxidised lipids and Maillard reaction products are present in Cheddar cheese. These are thus considered to be the same substances as measured in the present study, as we also focused on these wavelengths based on the VIP values in this study. Notably, although Maillard reactions do not proceed actively in hard and semi hard cheeses, studies using Manchego ([Corzo, Villamiel, Arias, Jiménez-Pérez, & Morales, 2000](#)), Cheddar, Gouda and Emmental ([Schwietzke, Schwarzenbolz, & Henle, 2009](#)), Harzer and Gouda cheeses ([Spanneberg, Salzwedel, & Glomb, 2012](#)) have been reported that the Maillard reaction proceeded along with cheese maturation of these cheeses. These studies measured Maillard reaction products in young and matured cheese using high performance liquid chromatography analysis. As the amount of Maillard reaction products tends to increase during cheese maturation, they are likely to show correlation with the cheese body measurement.

[Fig. 5b](#) shows the emission spectra at an excitation wavelength of 385 nm, where the SR score was the highest. The maximum wavelength of the fluorescence was between 500 and 550 nm, although multiple peaks were also confirmed around 460 nm. The fluorescence peaks around 460 nm increased in intensity as the cheese body measurement decreased, with the correlation coefficient between the cheese body measurement and the fluorescence intensity at an excitation wavelength of 385 nm and emission wavelength of 460 nm being -0.79 .

The fluorescence constituents most commonly reported in this range are the reaction products between amino acids, proteins, and oxidised lipids. [Kikugawa, Takayanagi, and Watanabe \(1985\)](#) reported that the reaction product of malondialdehyde (MDA) and lysine monomer generated by lipid oxidation has a maximum excitation wavelength of 395 nm and maximum emission wavelength of 466–470 nm. In addition, they reported that the reaction product of MDA and a model protein, polylysine, has a maximum excitation wavelength of 398 nm and maximum emission wavelength of 470 nm. The same group ([Kikugawa & Beppu, 1987](#)) reported that with the generation of fluorescent substances through lipid oxidation in tissues and cells, the reaction of MDA and primary amines in the presence of monofunctional aldehydes is promoted, generating fluorescent 1,4-disubstituted 1,4-dihydropyridine-3,5-dicarbaldehydes. The maximum excitation wavelength of the reaction products was 386–403 nm and maximum emission wavelength was 444–465 nm. [Yamaki, Kato, and Kikugawa \(1992\)](#) reported that the reaction product of hexenal and glycine ethyl had a maximum excitation wavelength of 392 nm and maximum emission wavelength of 455 nm. [Veberg, Vogt, and Wold \(2006\)](#) fixed the excitation wavelength at 382 nm and reported the fluorescence of the reaction product of oxidised lipids (aldehydes) and amino acids. They found that the reaction product of lysine and 2-hexenal has a maximum fluorescence at 471 nm, whereas the reaction product of lysine and 2,4-heptadienal has a maximum fluorescence at 472 nm. The reaction product of malondialdehyde and glycine shows maximum fluorescence at 465–469 nm, whereas the reaction with lysine shows maximum fluorescence at 467–469 nm (both with an excitation wavelength of 382 nm). Furthermore, they showed that the fluorescence is generated at a temperature of 4 °C under refrigeration. The same group ([Veberg, Olsen, Nilsen, & Wold, 2007](#)) also reported that the maximum

spectra are due to removal of scattered light. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

emission wavelength for oxidised lipids in butter during light irradiation was 465–470 nm, and indicated that the peak emission of the oxidised lipids occurs around 470 nm regardless of the type of food (e.g., salted cod, turkey, chicken meat, or salmon p ate). In the present experiment, we did not use any irradiation during storage; however, oxidised lipids may be generated during long-term storage.

In comparison, lumichrome, generated by the photo-oxidation of riboflavin, has an excitation-emission peak near the same range; however, the peak wavelengths were an excitation wavelength of 360 nm and emission wavelength of 450 nm in the model system (Fox & Thayer, 1998), but an excitation wavelength of 370 nm and emission wavelength of 430 nm in yoghurt (Christensen, Becker, & Frederiksen, 2005). In addition to this discrepancy, the low contribution of riboflavin fluorescence to the model also suggests that the effect of lumichrome was small. If lumichrome had high relevance with the accuracy of the PLS model, riboflavin should also show high relevance. However, the wavelength area corresponding to riboflavin fluorescence did not show high contribution to the PLS regression model.

Fig. 5c shows the emission spectra at an excitation wavelength of 305 nm, at which there is a peak in VIP. Peaks near the emission wavelength of 340 nm showed high fluorescence intensity; however, the correlation with the cheese body measurement was low, with the correlation coefficient of the fluorescence intensity with the cheese body measurement at an excitation wavelength of 305 nm and emission wavelength of 340 nm being 0.05. Although tryptophan and aromatic amino acids represented by these peak wavelengths have been used to predict the parameters of dairy products including cheese (Andersen & Mortensen, 2008), in the present study, the correlation coefficient was low and the contribution to the PLS regression model was low. This wavelength range has strong fluorescence intensity and captures changes well, but by performing a comprehensive analysis such as on the FF, changes during cheese maturation will have even higher correlation, which will allow the identification of the range of wavelengths contributing to the accuracy of the PLS regression model. We also suggest that in future studies, it should be possible to confirm the substances showing high correlation with the changes in the cheese body measurement by performing chemical analysis (e.g., extraction and high performance liquid chromatography) to quantify the substances, and then comparing the relative quantity of the substance of interest with the cheese body measurement.

4. Conclusion

We showed that non-contact FF measurements using an optical fibre probe can be used to predict the cheese body measurement of Cheddar cheese. Compared with conventional fluorescence measurements in a photometer chamber using cells to hold the sample, it was possible to develop a more practical method. The predictive ability of the model was reliable with a strong correlation ($R^2P = 0.826$) and small prediction error (RMSEP = 0.436). Moreover, the average RSD of the three replicates in all 30 samples was 1.6%. These values show that this method can be applied repeatedly with only 1–2% variation and has high reproducibility. Despite the non-contact FF measurement, it was confirmed that the accuracy of this prediction model is equivalent to that of previous studies. However, as the predicted values for cheese body measurements of around six show large variation, it is necessary to improve the accuracy by using data that reflect a more uniform distribution of cheese body measurements.

The wavelength ranges of oxidised lipids and Maillard reaction products (excitation wavelength of 340 nm and emission

wavelength of 400 nm), which were assumed to make a large contribution to the prediction of indices of cheese maturation (maturation time, proteolysis index, and free amino acid content) in previous reports, were shown to also make a large contribution to the present cheese body measurement prediction model.

By exploring the SR scores, we discovered a new wavelength range with a large contribution to the PLS regression model. These wavelengths were considered to correspond to oxidised lipids or to compounds of proteins and amino acids with oxidised lipids. Additional studies are necessary to confirm whether these products are responsible for these new wavelength changes, such as by comparing the results of quantification of these product contents with the FF measurements. Thus, our results suggest that the rapid and simple non-contact FF method developed herein for predicting the cheese body measurement may be suitable for industrial application to enhance the quality and reliability of processed cheese production.

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