論文の内容の要旨

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Visualization of multiple constituents in food by fluorescence fingerprint imaging

（蛍光指紋イメージングによる食品中成分の可視化）

1. Introduction

The distribution of constituents in foods determine their texture, and ultimately their palatability. This can be recognized from the fact that two products made from the same ingredients can differ greatly in palatability, depending on the manufacturing process. Therefore, technologies to visualize the distribution of constituents in food are essential in food science research.

However, existing visualization methods require the sample to be stained or freeze-dried before observation. These processes have a possibility of altering the sample, and are not suited to visualize multiple constituents. Therefore, in this study, we developed an imaging method that can visualize the distributions of constituents without preprocesses such as staining, by combining the fluorescence fingerprint (FF) with spectral imaging. The FF is a set of fluorescence spectra acquired at multiple excitation wavelengths, and shows a specific pattern for each constituent [1]. The FF imaging method was developed and applied to constituents in food.

2. Structure of thesis

This thesis consists of seven chapters. The introduction explains basic studies regarding the topics of fluorescence and imaging, which are very important to this research. The second chapter shows the setup of the imaging system which was used throughout the research. The main chapters (chapters three to six) explain the applications of FF imaging in three steps, going from model samples to real foods, and increasing the number of components visualized. These chapters are based on five original papers. The last chapter summarizes the conclusions and draws future visions.

3. Development of a quantitative visualization technique for gluten and starch in model dough

In the first step of research, the distribution of gluten and starch in a model dough made from a mixture of gluten and starch reagents was visualized. Dough samples were prepared with different ratios of gluten, starch, and water, and fluorescence images were acquired at multiple combinations of excitation and emission wavelengths. This data can be interpreted as the FFs of all the pixels in the image. A partial least-squares regression (PLSR) model was built to predict the gluten ratio from the FF (Figure 1). The importance of each wavelength in the PLSR model was assessed using the selectivity ratio, and optimum wavelengths for accurate prediction of gluten ratio were selected. Finally, the gluten ratio of each pixel was predicted with the PLSR model using the selected wavelengths, and each pixel was colored according to the predicted gluten ratio. The imaging method developed enables the distribution of constituents to be visualized with colors corresponding to their actual quantities or ratios [2].

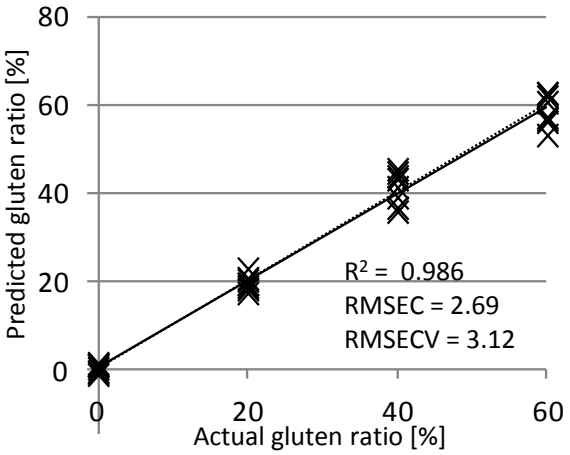

Figure 1 PLSR model predicting gluten ratio from FF

4. Visualization of gluten and starch distributions in wheat flour dough

In the second step, FF imaging was applied to a real food sample, wheat flour dough, and the gluten and starch distributions were visualized. Wheat flour dough was mixed up to three stages, i.e., under-mixing, optimum-mixing, and over-mixing, and thin sections of the dough were prepared with a cryotome. Fluorescence images of the sections were acquired in 63 combinations of excitation and emission wavelengths, thereby constructing the FFs of the constituents at each pixel. Cosine similarity values between the FF of each pixel in the dough and those of gluten and starch were calculated. A pseudo-color image of gluten and starch distribution was created in two ways. For the first method, each pixel was colored by fitting a continuous color scale to the cosine similarity value to gluten and starch. After acquisition of FF data, the dough sample was then fluorescently stained for gluten and starch. The stained image showed patterns similar to the pseudo-color FF image, validating the effectiveness of the FF imaging method (Figure 2) [3]. In the second method,

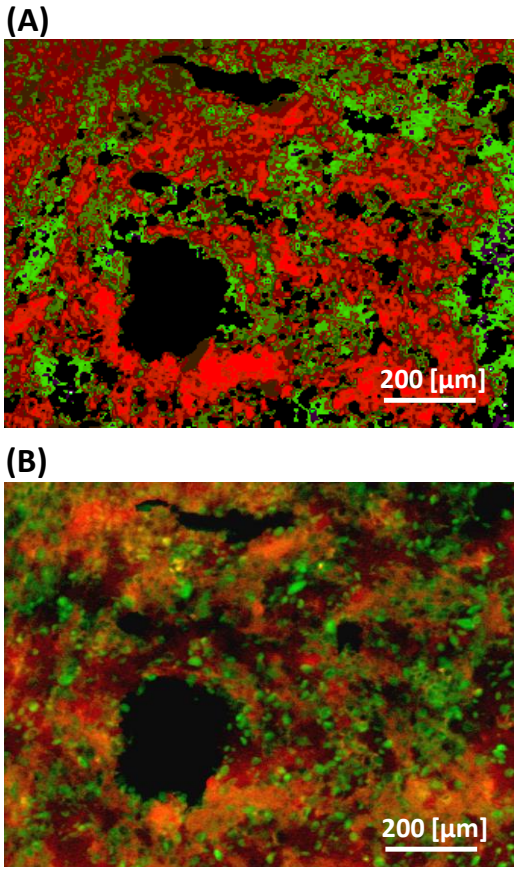

Figure 2 The Distributions of gluten and starch by (A) FF imaging and (B) staining

the pixels were arranged in order of cosine similarity to gluten and pixels with higher values of cosine similarity were categorized as “gluten” and the rest as “non-gluten”. The number of pixels categorized as “gluten” was based on the overall ratio of gluten in the dough. The same process was performed with the FF of starch, and all pixels were divided into “starch” and “non-starch”. Colors were assigned to each division, and the distributions of gluten and starch were visualized. Changes in the distributions of gluten and starch were observed at the over-mixing stage, which suggested the breaking up of gluten and the alteration of gluten and starch [4].

5. Quantification of the distributions of gluten, starch and air bubbles in dough at different mixing stages

In the latter half of step 2, the changes occurring to the distributions of gluten, starch and air bubbles through mixing were quantified. Quantitative parameters concerning gluten and starch distributions and bubble area were extracted from the dough images at each mixing stage (Figure 3). The changes observed were as follows: (1) the distribution of gluten and starch became more even from the under- to the optimum-mixing stage, (2) the total bubble area became larger from the optimum- to the over-mixing stage (this was supported by the increase in specific volume), (3) the mean area of the bubbles became larger in the over-mixing stage, and (4) the shapes of the bubbles were circular in the optimum-mixing stage but were more elongated in the under- and over-mixing stages [5].

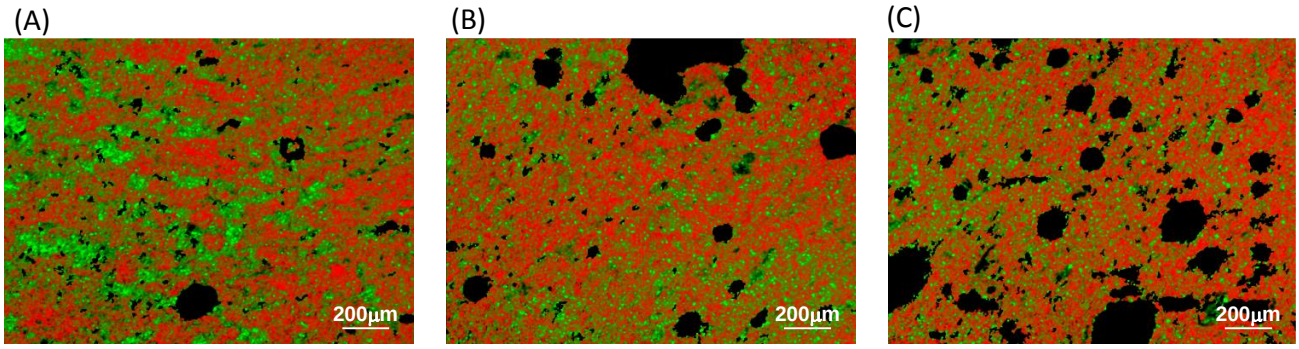

Figure 3 Distributions of gluten, starch and bubbles in dough at the (A) under-mixing, (B) optimum mixing and (C) over mixing stages

6. Visualization of gluten, starch and butter in pie pastry

In the last step, three constituents, gluten, starch and butter in pie pastry were visualized by combining FF imaging with spectral unmixing methods. Two types of pie pastry were made: puff pastry, in which wheat flour dough and butter are alternately layered, and short pastry, in which flour and butter are mixed together with water. Samples of 10 μm thickness were made, and fluorescence images were acquired with excitation and emission wavelengths in the range of 270-320 nm and 350-420 nm, respectively, at 10 nm increments. The FFs of each pixel were unmixed into the FFs and abundances of five constituents, gluten, starch, butter, slide glass, and ferulic acid, using two spectral unmixing methods: non-negative matrix factorization (NMF) and constrained least squares method. NMF was only applicable to puff pastry (Figure 4) and was unable to visualize starch and butter in the

short pastry, in which the two were mixed together. Least squares method was coupled with constraints of non-negativity, full additivity (the sum of the constituents in one pixels is unity) and quantum restraint on the abundances of the slide glass (abundances take values of one or zero). With this method, distributions of the constituents in both puff and short pastry were visualized.

(A) (B)

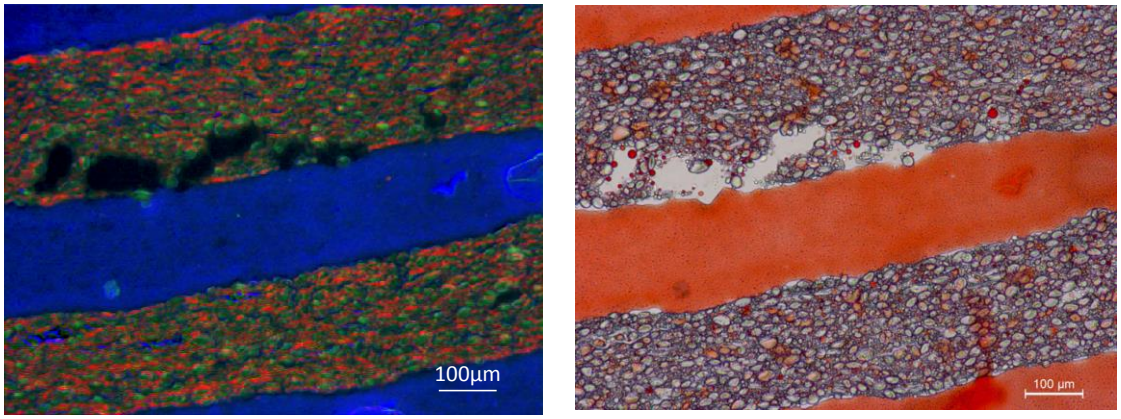

Figure 4 (A) RGB image of gluten (red), starch (green) and butter (blue) in puff pastry visualized with FF imaging and (B) stained image of the same sample.

7. Conclusions

This thesis has described the theories and applications FF imaging, an imaging method which can be categorized as a type of hyperspectral imaging. FF imaging has distinct advantages compared to other hyperspectral imaging methods such as (1) high spatial resolution can be achieved, (2) requires little sample preparation, and (3) measuring equipment is less expensive than most imaging methods. On the other hand, disadvantages such as the time required for data acquisition need to be overcome, if the imaging method is to be applied for practical uses.

References

1. Jiang, J.K., J. Wu, and X.H. Liu, Spectrosc. Spect. Anal., (2010). **30**(6): p. 1525-1529.
2. Kokawa, M., et al., Food Bioprocess Technol., (2013). **6**(11): p. 3113-3123.
3. Kokawa, M., et al., Biosci., Biotechnol., Biochem., (2011). **75**(11): p. 2112-2118.
4. Kokawa, M., et al., Food Sci. Technol. Res., (2013).
5. Kokawa, M., et al., J. Cereal Sci., (2012). **55**(1): p. 15-21.