***Notes for writing the Research Report**
***Type this form except the date and the signature.**
Please prepare your Research Report in English or Japanese within three to ten pages including this page. The contents should include:

7. Background of Research

Hyperspectral imaging (HSI), as an innovative technology, can simultaneously obtain both spatial and spectral information from a subject. It is a novel method to assess meat quality in the modern meat industry without destructing to the meat products (Kamruzzaman et al., 2016a, b). The application can achieve from to classify the cooked, sliced turkey ham (Iqbal et al., 2013); to predict the quality, chemical composition, microbial contaminants of beef (Naganathan et al., 2015; Naganathan et al., 2016), chicken (Jia et al., 2017), pork (Huang et al., 2013), fish (Cheng and Sun, 2015; Xu et al., 2016), and shrimp (Wu et al., 2013); to detect the fresh or stale chicken or fish; to discriminate the adulteration in minced lamb patties. However, there are a few studies addressing on identifying processed meat products using HSI. From the food safety point of view, using the adulterated components for substitution may increase the risk of microorganism contamination. As the morphological structures of meat muscle are totally destroyed during mincing processing, it is easier to adding some cheap components instead of the expensive ones while selling with a high price. It will thus be more interesting and innovative to apply HSI to detect sausages that can be regarded as a comminuted meat which is packaged in a unique permeable membrane.

Sausages, with unique flavour and special "bite", are important gastronomic and nutritional heritage. Sausages are perishable and the quality of the sausages is one of the key factors for making consumer purchase decision. In the summer in Tokyo, the environmental temperature can reach as high as 35 °C. Even cooked sausages with intact package can get spoiled. In order to cut costs down, fraudulent retailers may substitute fresh sausages by sausages placed under room temperature for several days. Therefore, a rapid and non-invasive prediction approach of sausage quality has always been highly demanded by sausage manufacturers to detect defects and face with mounting global competition.

On the other hand, the burst incidence generated during sausage stuffing frustrates rapid, efficient and large-scale sausage production for sausage industry. According to the Sustainable Development Goal of "**Zero Hunger**", sustainable food production systems are highly required and the agricultural productivity and incomes of small-scale food producers are to be doubled by 2030. Therefore, the casings need to be modified to facilitate the stuffing processing with a lower burst incidence to increase the sausage production and increase the economic incomes of the small-scale food produces.

The overall aim of this research is to enlarge the application fields of hyperspectral imaging. To this end, the following targets were established:

1. **Non-destructive and Rapid Prediction of pH in Japanese Big Sausage with Different Storage Conditions using Hyperspectral Imaging.**

This allows to identify the application of hyperspectral imaging to the cooked sausages after different storage conditions to potentially develop a pH prediction model of cooked sausage.

2. **Real-time prediction of pre-cooked Japanese sausages colour with different storage days using hyperspectral imaging.**

In this research, the quantitative relationships between the spectral data and reference colour values will be established using PLSR. The pixel-wise image processing algorithms to generate the distribution maps of colour were developed.

3. **Visible near-infrared hyperspectral imaging in tandem with multivariate analysis and image processing for detection and visualisation of adenosine triphosphate (ATP) content in ready-to-eat pork sausages.**

In this way, the dynamic changes of ATP content in ready-to-eat pork sausages during the long-term storage will be investigated using hyperspectral imaging. This allows for the better interpreting and mapping the microbial growth in interior sausages, developing mathematical model or algorithm to predict the microbial parameters of sausages and ensuring the food safety.

4. **To broaden the application fields of hyperspectral imaging: cooked sausages stuffed in modified casing.**

In this study, the sausages were stuffed in the casing modified by surfactant solutions and lactic acid. The purpose to do this because natural casings are difficult to handle as they can burst during

sausage stuffing or other processing. As a result, the properties of casing need to be improved. The improved characteristics of the natural casing may enable meat processor or manufactures to enhance the sausage production with a low sausage burst incidence. However, how the sausage matrix responds to the modified casing merits to be attention. Optimal wavelengths will be selected, which will benefit for the usage in a multispectral imaging system.

8. Research methodology

Take one of my previous research studies (i.e. evaluation of redness changes during the long-term storage (up to 57 days) at 8 °C) for example, figure 1 illustrates the main steps for the experiment of real-time monitoring of redness in cooked sausage. A laboratory visible near-infrared (VNIR) hyperspectral imaging (JFE, Techno-Research Corporation, Tokyo, Japan) with the spectral range of 380-1000 nm was used. The focal lengths of a 12-bit charged coupled device (CCD) camera (MC1002PF, Texas Instruments, USA) was 16 mm with 2/3-inch C-mount lens that connected to a spectrograph (ImSpector, V10C, Spectral Imaging Ltd., Oulu, Finland). The flame rate of the camera is 30 fps. The exposure time of the CCD camera was set to 28.8 ms. Acquired images from camera were corrected through images captured using a dark reference with 0% reflectance and a white reference with 100% reflectance. The dark reference image was obtained by completely covering the camera lens with its opaque cap whilst the white reference image was captured by a uniform, stable, and high reflectance white ceramic tile. A computer (Dell Vostro, Intel (R), Dell Inc., Tokyo, Japan) supported with a data acquisition and control software (SpectrumAnalyzer, version 1.8.5, JFE, Techno-Research Corporation, Tokyo, Japan) was used for image acquisition. The total contiguous spectral bands were 125 with 5 nm intervals. A rectangular size of 50 × 50 pixels was manually selected from the center of cooked sausage slice as the region of interest (ROI). The average spectra of ROI for each sausage sample was used for calibration model development. Once the multivariate statistical models were set, the images were segmented automatically. All the captured hyperspectral images were processed and analyzed by using the aforementioned SpectrumAnalyzer software.

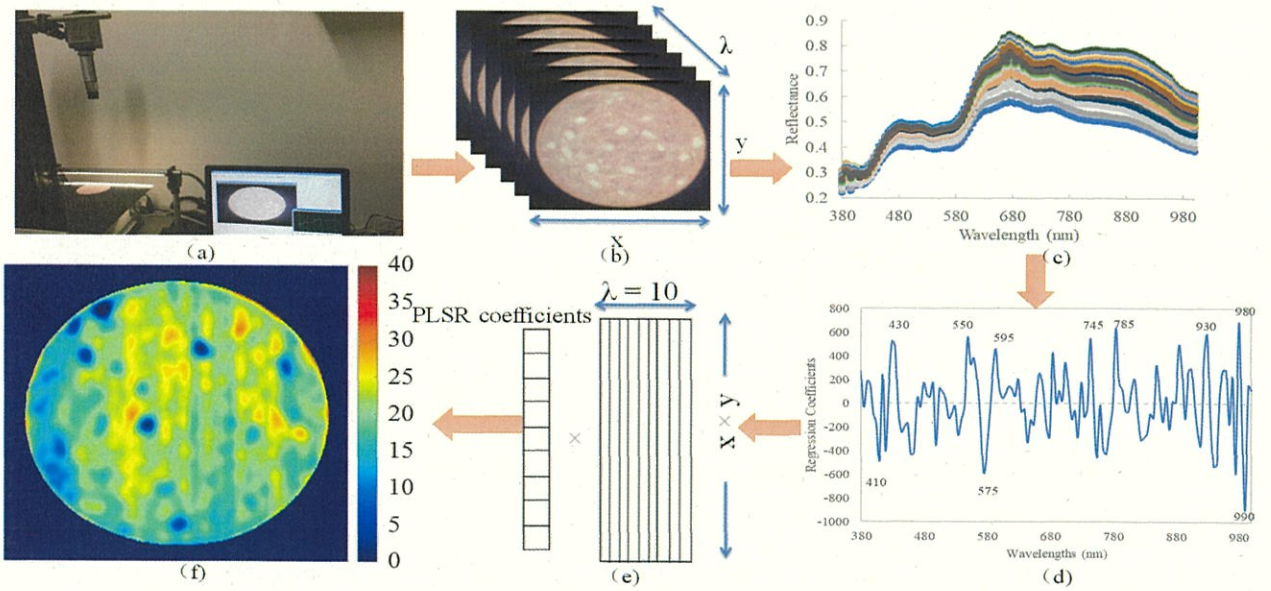


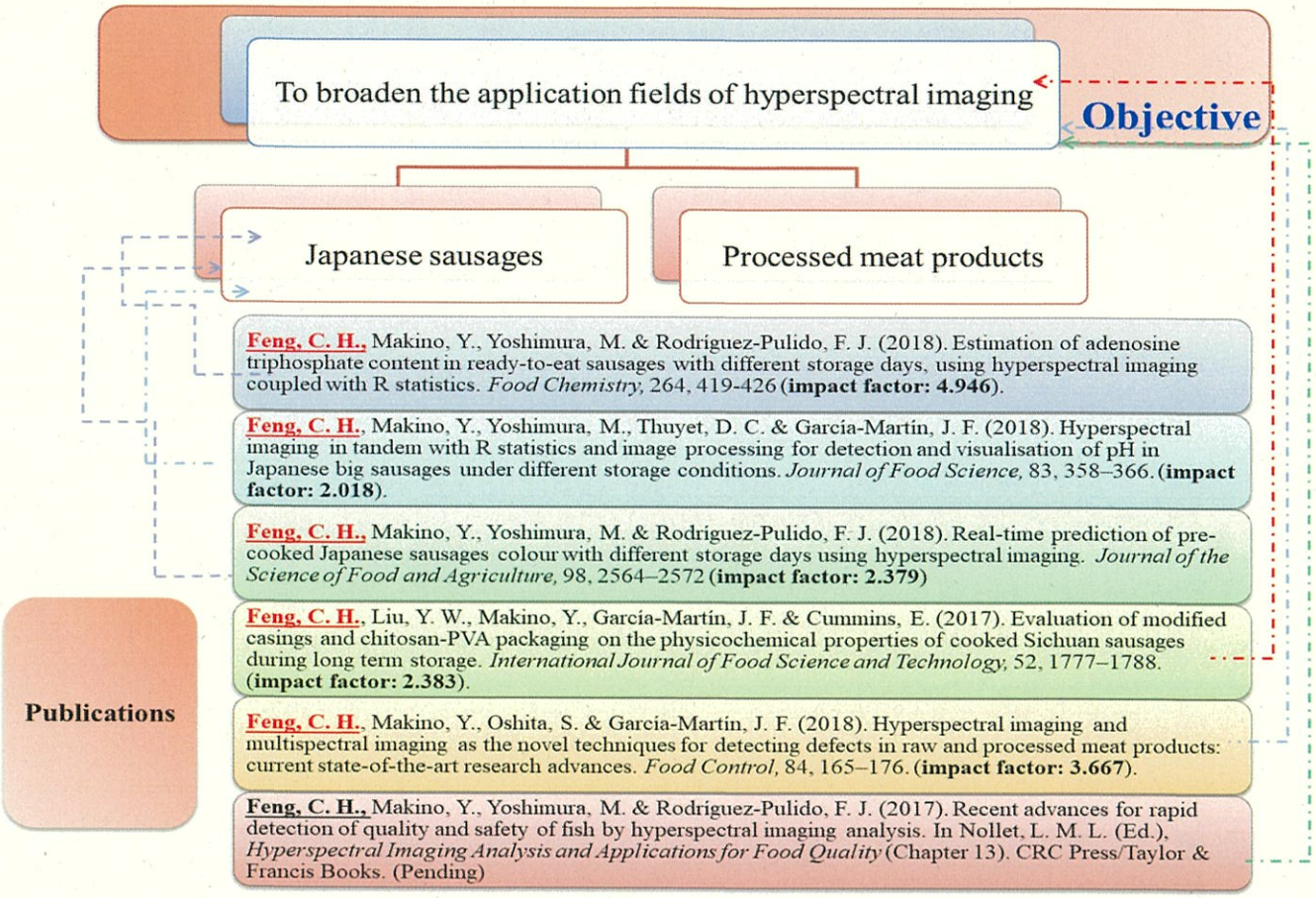
Figure. 1. Flowchart of the main steps to design hyperspectral imaging system for real-time monitoring of redness in cooked sausage. (a) imaging acquisition, (b) hyperspectral image segmentation, (c) spectral extraction, (d) important wavelengths selection, (e) unfoled image to 2D and multiply the regression coefficients of PLSR model using optimal wavelengths, (f) refold the image and built the distribution map

In order to improve the performance of the regression models, several pre-treatments of spectra, e.g. normalization, standard normal variate (SNV), multiplicative scatter correction (MSC), first derivative, second derivative and so on were usually used to enhance the accuracy of the regression models. Following this, the important wavelengths were usually selected to simplify the model and those wavelengths were involved to the program written either in a R statistical or Matlab for establishing the distribution map that can show where and how much concentration of the attributes were located in the foodstuffs. This is the advantageous over the conventional

spectroscopy analysis.

9. Results/impacts

Thanks to great support of Professor Makino in the University of Tokyo, I achieved a satisfied research outcome in Japan: **5 SCI papers and 8 conference abstracts/papers published, with one book chapter and one patent pending for the 2-year JSPS fellowship**. The total publication I achieve now is increasing to 22 journal papers, including **18 SCI** papers in peer reviewed journals (**newest total impact factor: 61.948; including 15 as a first author SCI papers**) and **15 papers or abstracts** in International and National conferences as a first author. h index = 8, 153 citations (based on Scopus data source). This is very important for developing my early-stage research career. Some achievements during the JSPS fellowship are highlighted below:



Publications in Peer-Reviewed Journals:

1. **Estimation of adenosine triphosphate content in ready-to-eat sausages with different storage days using hyperspectral imaging coupled with R statistics**

ATP is widely present in all living organisms, including in a range of foodstuffs in the form of non-microbial ATP. It was reported that there was a linear relationship between ATP amount and plate count and so the measurement of ATP can be related to the amount of microorganisms, which reduced approximately 99.83% microbial analysis time. However, this method can only know the average ATP content of the foodstuff. It is impossible to know every spot of the measured sample. The prediction (or distribution map) generated by hyperspectral imaging system can achieve this issue. In this study, hyperspectral imaging used for the first time to detect adenosine triphosphate (ATP) content of cooked sausage. **Distribution map of ATP content was innovatively developed by R statistics.** PLSR model developed using optimal wavelengths achieved a high R_p^2 of 0.8606 with a low root mean square error of prediction of 0.014. Hyperspectral imaging, as a promising tool in developing rapid and non-destructive ATP content measurement, is capable to quantify and visualize the ATP evolution in sausage during storage. This research fulfilled the aforementioned target of "visible near-infrared hyperspectral imaging in tandem with multivariate analysis and image processing for detection and visualisation of adenosine triphosphate (ATP) content in ready-to-eat pork sausages" This study has been accepted to be published in *Food Chemistry* with a high impact factor of 4.946.

2. **Hyperspectral imaging in tandem with R statistics and image processing for detection and visualisation of pH in Japanese big sausages under different storage conditions.**

In this research work, hyperspectral imaging is for the first time to detect pH in the cooked sausages using R statistics, which provides another useful information for the researchers who do not have the access to Matlab. 11 optimal wavelengths were successfully selected, which were used for simplifying PLSR model established based on the full wavelengths. This simplified model can achieve a high R_p^2 of 0.909 and a low root mean square error of prediction 0.035, which can be useful for the design of multispectral imaging systems. This research work fulfilled the aforementioned target of "non-destructive and rapid prediction of pH in Japanese big sausage with different storage conditions using hyperspectral imaging". This study has been accepted to be published in *Journal of Food Science* with the impact factor of 2.018.

3. **Real-time prediction of pre-cooked Japanese sausages colour with different storage days using hyperspectral imaging.**

Redness of cooked sausages stored up to 57 d was predicted using hyperspectral imaging in tandem with multivariate data analysis. Partial least squares regression (PLSR) and forward stepwise multiple regression (FSMR) models were used to develop the relevant spectral profiles with the redness of the cooked sausages. The prediction maps for exemplifying redness evolution in sausages were for the first time developed by both R statistics and Matlab. In this work, the comparison displays the distribution maps generated by both softwares with the same samples, which may provide the useful information for the future research work. This research work achieved the aforementioned target of "real-time prediction of pre-cooked Japanese sausages colour with different storage days using hyperspectral imaging". This study has been accepted to be published in *Journal of the Science of Food and Agriculture* with a high impact factor of 2.379.

4. **Evaluation of modified casings and chitosan-PVA packaging on the physicochemical properties of cooked Sichuan sausages during long term storage.**

Evaluation of modified casings and chitosan-PVA packaging (CP package) on the physicochemical properties of cooked Sichuan sausages during long term storage. The improved characteristics of the natural casing will enable meat processors or manufactures to enhance sausage production with a low sausage burst incidence, while the CP packaging will inhibit the intensive microbial growth. This research work provides the information of how the modified casing sausages response to the CP package, which will be the next topic to broaden the application fields of hyperspectral imaging: cooked sausages stuffed in modified casing. This research work potentially achieves the target of "broaden the application fields of hyperspectral imaging" as mentioned above. This study has been accepted to be published in *International Journal of Food Science and Technology* with a high impact factor of 2.383.

5. **Hyperspectral imaging and multispectral imaging as the novel techniques for detecting defects in raw and processed meat products: current state-of-the-art research advances.**

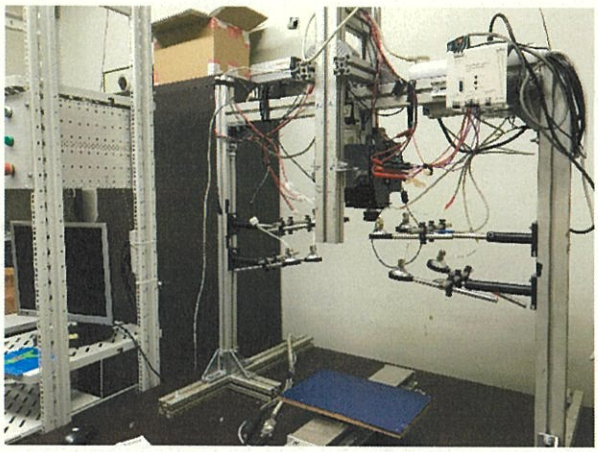
This review provides a detailed overview of the recent efforts devoted to hyperspectral imaging and multispectral imaging technologies for evaluating the quality and safety of different meat products and the probability of its widespread application. Hyperspectral imaging, as a promising tool in developing rapid and non-invasive, is capable to detect defects in raw and processed meat products. This study has been accepted to be published in *Food Control* with a high impact factor of 3.667.

Book Chapter and Patent applications have been made or other tangible outcomes:

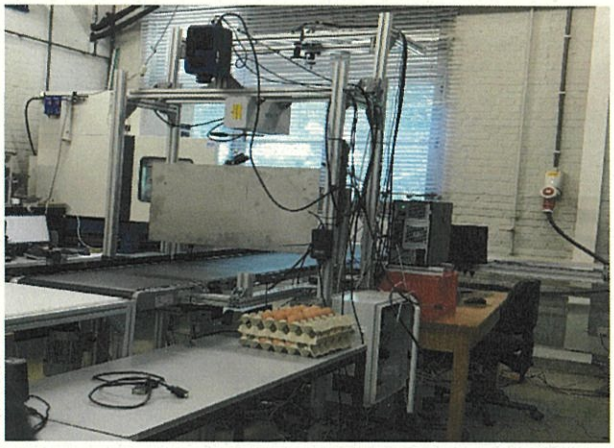
In 2017, I was invited to write a book chapter for hyperspectral imaging: Recent advances for rapid detection of quality and safety of fish by hyperspectral imaging analysis. In Nollet, L. M. L. (Ed.), Hyperspectral Imaging Analysis and Applications for Food Quality (Chapter 13). CRC Press/Taylor & Francis Books. That chapter discussed the fish freshness detected by HSI and introduced the physical properties and chemical compositions of fish evaluated by HSI. The microbial spoilage of the fish inspected by HSI is also elaborated. The book chapter will be published very soon.

At this moment, I have submitted a Chinese patent application about "combination of

hyperspectral imaging with R statistics to evaluate adenosine triphosphate content in ready-to-eat sausage". The application is now under review.
Due to our publications to the world, I was invited to be the peer-reviewers for several journals: Food Chemistry (impact factor: 4.946), Journal of Food Processing and Preservation (impact factor: 1.51), Journal of Food Engineering (impact factor: 3.197), Infrared Physics and Technology (impact factor: 1.851), International Journal of Food Science and Technology (impact factor: 2.383) and so on. Those are very important experience and access for a researcher to learn the new technology and research work.
As a result of our publications to the world, I was invited to have academic exchange at KU Leuven (In the Times Higher Education ranking KU Leuven is ranked as the 21st European university, while in the Reuters Top 100 of the World's most innovative institutions, KU Leuven is listed as the first European university.) in June, 2018 to have a potential cooperation in the future. During the academic communication, I gave the presentation about the research work I have done at the University of Tokyo, rendering them to better know about the research topic in Japan. The host professor in KU Leuven also guided me to visit to their labs, insect rearing facility, HSI equipment for laboratory use, and HSI prototype design for current European industrial utilization. They also hope to have a future cooperation and to bring their new technology and novel food to Japan.



HSI equipment for laboratory use at KU Leuven



HSI prototype for Belgian industrial utilization at KU Leuven

For another, thanks to the great support of host professor for attending international conference, I was also invited to visit Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Melbourne, Australia. This is very renowned research institute in Australia and they boost Australia's potential through excellent science that provides positive economic, environmental and social impacts. During the visit, the host research group leader told me that he would really like to cooperate with the first-class Japanese University, the University of Tokyo in the future. I will also be willing to be this ambassador not only to reinforce the prestige and reputation of Lab of Bioprocess Engineering, the University of Tokyo, but also to bring more cooperation opportunity.

In 2017, thanks to the great introduction from Professor Makino, I participated the academic communication with the Laboratory of Bio-Sensing Engineering, Graduate School of Agriculture, Kyoto University, to disseminate our research work at the University of Tokyo. During this visit, it not only broadened my research network to my research field, but also learned the current state-of-the-art research advances of robotics, automation, instrumentation, sensors, and intelligent system, especially the studies on robotics for food, agricultural and biological productions and applications in Japanese industry or agriculture.

After this fellowship, there are now still some papers for publication, which are relevant to evaluate the quality and microbial attributes for sausages stuffed in modified casing. Due to the wonderful support from Professor Makino and JSPS financial support, some research outcomes at the University of Tokyo were achieved and it benefits me for my future fellowship application. In October, 2018, I was awarded by University of Seville, Grants for the international mobility of

research staff (Reference: VIPPIT-2018-I.3). This is the first year for elaborating our research outcomes to University of Seville, which probably reinforces the future deeper cooperation. Among the 15 winners for this competitive fellowship, we are the only Asian group for this visiting fellowship and I believe this will be the good beginning and am also pleased to introduce to other researchers who is interested in this fellowship.

References:

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Kamruzzaman, M., Makino, Y. & Oshita, S. (2016a). Online monitoring of red meat color using hyperspectral imaging. *Meat Science,* 116, 110–117.

Kamruzzaman, M., Makino, Y. & Oshita, S. (2016b). Rapid and non-destructive detection of chicken adulteration in minced beef using visible near-infrared hyperspectral imaging and machine learning. *Journal of Food Engineering,* 170, 8–15.

Naganathan, G. K., Cluff, K., Samal, A., Calkins, C. R., Jones, D. D., Lorenzen, C. L. & Subbiah, J. (2015). Hyperspectral imaging of ribeye muscle on hanging beef carcasses for tenderness assessment. *Computer and Electronics in Agriculture,* 116, 55–64.

Naganathan, G. K., Cluff, K., Samal, A., Calkins, C. R., Jones, D. D., Meyer, G. E. & Subbiah, J. (2016). Three dimensional chemometric analyses of hyperspectral images for beef tenderness forecasting. *Journal of Food Engineering,* 169, 309–320.

Wu, D., Shi, H., He, Y., Yu, X. J. & Bao, Y. D. (2013). Potential of hyperspectral imaging and multivariate analysis for rapid and non-invasive detection of gelantin adulteration in prawn. *Journal of Food Engineering,* 119, 680–686.

Xu, J. L., Riccioli, C. & Sun, D.-W. (2016). Development of an alternative technique for rapid and accurate determination of fish caloric density based hyperspectral imaging. *Journal of Food Engineering,* 190, 185–194.

Note: As much as possible, describe the contents and results of your research in a manner that is easily understandable to a non-specialist in your field. Provide a concrete description if (1) papers related to your work have been published in major academic journals, (2) particularly outstanding research results were achieved, or (3) patent applications have been made or other tangible outcomes achieved through the research.

Presentations during the period of the fellowship (Name of the conference, title, place, date)

Thanks for the financial support from JSPS, I participated three national conferences and five international conferences to disseminate our research work. I also obtained **BEST ORAL presentation twice** in the international conference.

① **Feng, C. H.,** Makino, Y., Yoshimura, M. (2018). Non-destructive and rapid prediction of redness in Japanese big sausage with different storage conditions using hyperspectral imaging, in Proceedings of the 65th Annual Meeting of Japanese Society for Food Science and Technology, 22 – 24th August, Sendai, Japan.

② **Feng, C. H.,** Makino, Y., Yoshimura, M. (2018). Combination of hyperspectral imaging and R statistics for monitoring moisture in Japanese big sausages under different storage conditions, in Proceedings of 64th International Congress of Meat Science Technology (ICoMST), 12-17th August, Melbourne, Australia.

③ **Feng, C. H.**, Makino, Y., Yoshimura, M. (2018). Combination of multivariate analysis and image processing for detection of protein in cooked pork sausages by using visible near-infrared hyperspectral imaging. in Proceedings of 3rd Global Food Security and Sustainability Conference, 21-22th May, New York, USA.

④ **Feng, C. H.**, Makino, Y., Yoshimura, M. (2018). Visible near-infrared hyperspectral imaging coupled with multivariate analysis and image processing for detection of yellowness in ready-to-eat pork sausages, in Proceedings of 2nd International Conference on Food and Agriculture Technologies (ICFAT 2018), 18-20 January, Jeju, South Korea. (Obtained the **best oral presentation**)

⑤ **Feng, C. H.**, Makino, Y., Yoshimura, M. (2017). Real-time prediction of pre-cooked Japanese sausages pH with different storage days using hyperspectral imaging, in Proceedings of the 76th Annual Meeting of the Japanese Society of Agricultural Machinery and Food Engineers, 7-9 September, Tokyo, Japan.

⑥ **Feng, C. H.**, Makino, Y., Yoshimura, M. (2017). An alternative and innovative technique for rapid and accurate prediction of lightness of Japanese cooked pork sausages, in Proceedings of 2nd International Conference on Food Chemistry & Nutrition, 24-26 July, 2017, Vancouver, British Columbia, Canada.

⑦ **Feng, C. H.**, Makino, Y., Yoshimura, M. (2017). Non-invasive and accurate prediction of pH in cooked Japanese big sausage with different storage days using hyperspectral imaging, in Proceedings of the 53rd Annual meeting of Kanto Branch the Japanese Society of Agricultural Machinery and Food Engineers, 9 June, 2017, Nagano, Japan.

⑧ **Feng, C. H.**, Makino, Y., Kamruzzaman, M., Oshita, S. (2017). Rapid and non-destructive prediction of pH in cooked pork sausages using hyperspectral imaging, in Proceedings of 4th International Conference on Food Security and Nutrition (ICFSN 2017), 13-15 March, 2017, Prague, Czech Republic. (Obtained the **best oral presentation**)

11. A list of paper published during or after the period of the fellowship, and the names of the journals in which they appeared (Please fill in the format below). Attach a copy of each article if available.

Thanks to the great support from Professor Makino, I have published 5 SCI papers in the peer-reviewed international journals and there will be several papers to be published in the near future.

Author(s)	Title	Name of Journal	Volume	Page	Date	Note
Feng, C. H., Makino, Y., Yoshimura, M. & Rodríguez-Pulido, F. J.	Estimation of adenosine triphosphate content in ready-to-eat sausages with different storage days, using hyperspectral imaging coupled with R statistics.	Food Chemistry	264	419-426	2018	impact factor: 4.946
Feng, C. H., Makino, Y., Yoshimura, M., Thuyet, D. C. & García-Martín, J. F.	Hyperspectral imaging in tandem with R statistics and image processing for detection and visualisation of pH in Japanese big sausages under different storage conditions.	Journal of Food Science	83	358-366	2018	impact factor: 2.018

Feng, C. H., Makino, Y., Yoshimura, M. & Rodríguez-Pulido, F. J.	Real-time prediction of pre-cooked Japanese sausages colour with different storage days using hyperspectral imaging.	Journal of the Science of Food and Agriculture	98	2564-2572	2018	impact factor: 2.463
Feng, C. H., Makino, Y., Oshita, S. & García-Martín, J. F.	Hyperspectral imaging and multispectral imaging as the novel techniques for detecting defects in raw and processed meat products: current state-of-the-art research advances.	Food Control	84	165-176	2018	impact factor: 3.667
Feng, C. H., Liu, Y. W., Makino, Y., García-Martín, J. F. & Cummins, E.	Evaluation of modified casings and chitosan-PVA packaging on the physicochemical properties of cooked Sichuan sausages during long term storage.	International Journal of Food Science and Technology	52	1777-1788	2017	impact factor: 2.383

12. Awards during the period of the fellowship (Name of the award, Institution, date etc.)

① 2018 **Certification for FY2018 Leading Initiative for Excellent Young Researchers**

② 2018.10-2018.12 Grants for the international mobility of research staff (Reference: VIPPIT-2018-I.3), funded by University of Seville.

③ 2018 **Seal of Excellence for Horizon 2020's Marie Skłodowska-Curie actions**, Certificate delivered by the European Commission, as the institution managing Horizon 2020, the EU Framework Programme for Research and Innovation 2014-2020. following evaluation by an international panel of independent experts WAS SCORED AS A HIGH-QUALITY PROJECT PROPOSAL IN A HIGHLY COMPETITIVE EVALUATION PROCESS.

④ 2018 **Best oral presentation at 2018 2nd International Conference on Food and Agricultural Technologies** in Jeju, South Korea

⑤ 2018 Two **Review certification** for Food Chemistry, awarded by Elsevier.

⑥ 2017 **Review certification** for Journal of Food Processing and Preservation, awarded by Wiley Online Library.

⑦ 2017 **Best oral presentation at the International Conference on Food Security and Nutrition in Czech Republic**

⑧ 2017 Two **Certifications for Japanese Language Study,** awarded by the University of Tokyo