論文の内容の要旨

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論文題目 Quantitative Evaluation of Microbial Contamination of Meat with Fluorescence Spectroscopy

(蛍光分光分析による食肉加工プロセスにおける清浄度評価)

1. Introduction

Meat production in the world continues to increase (FAO, 2014). When inspecting meat for bacteriological safety, the degree of contamination is checked by swabbing method for aerobic plate count or ATP content. ATP exists in all living organisms and bacteria also contain ATP; therefore, ATP content is known to be an indicator of microbial contamination. However, the swabbing technique, which is common to aerobic plate count and ATP content, is laborious and time-consuming. Additionally, swabbing technique is spot check; thus, the hygiene monitoring of entire meat processing is not possible. Therefore, nondestructive and rapid hygiene monitoring technique at meat processing plants has been desired. Of several kinds of spectroscopic technique, fluorescence spectroscopy, which has a distinguished sensitivity and selectivity, was used. In particular, excitation-emission matrix (EEM), which provides tremendous amount of data, was used. Therefore, the objective of this study is to quantitatively evaluate microbial contamination of meat with fluorescence spectroscopy. This study consists of two part: quantitative evaluation of microbial contamination of meat surface with excitation-emission matrices and mathematical modeling of bacterial penetration into meat. For the former, previous research developed prediction models for aerobic plate count and ATP content which reflected changes in tryptophan and NAD(P)H of pork surface (Oto et al., 2013). However, the prediction accuracies (r_p = 0.94 and RMSEP = 0.97 $\log_{10}$ (CFU cm^{-2}) were not sufficient. In this study, in order to achieve higher accuracies, ATP fluorescence signal detection was focused because ATP fluorescence directly reflects microorganisms. However, ATP fluorescence peak is usually masked by tryptophan's

fluorescence peak. Then two-dimensional Savitzky–Golay second-order differentiation, which is an extended derivative technique for EEMs, was proposed and ATP fluorescence signal was intended to be separated and detected with this preprocessing (Shirai et al., 2014). The first goal is to develop prediction models with higher prediction accuracies by including ATP fluorescence signal changes as well as tryptophan and NAD(P)H. On the other hand, for the latter, bacteria penetrate into meat. Understanding the degree of bacterial penetration into meat allows to estimate the safe region of interior of meat. Therefore, the mathematical modeling of bacterial penetration into meat is aimed. Of several kinds of pathogenic bacteria such as *Escherichia Coli, Salmonella* etc., *Pseudomonas,* which is highly aerobic, is chosen and penetration of aerobic bacteria into meat was mathematically modeled for mechanistic understanding. This study hypothesized that bacterial transport can be explained by considering several important factors simultaneously: motility, chemotaxis, growth, and proteolysis. The second goal is to understand how each mechanism or factor (and in combination) contributes to bacterial penetration. Penetration depth of aerobic bacteria was quantitatively evaluated with mathematical modeling using the surface bacteria concentration (Shirai et al., 2016).

2. 2. Quantitative evaluation of microbial contamination of meat surface with excitation-emission matrices

Pork loin meat were used and stored at 15°C for 72 h, and EEMs, aerobic plate count, and ATP content were measured every 12 h. Two-dimensional Savitzky–Golay second-order differentiation, which is an extended Savitzk–Golay derivative technique for three-dimensional data, was proposed as a preprocessing of EEMs. EEM of ATP standard solution showed that ATP has fluorescence peaks at excitation wavelength (Ex) 284 nm and emission wavelength (Em) 388, 395, and 410 nm. EEMs of pork surface showed fluorescence peaks of tryptophan, NAD(P)H, flavins, Vitamin B_6, and porphyrins (Shirai et al. 2016). However, the ATP fluorescence peak was masked by tryptophan's peak and was not detected. But fourth-derivative EEMs of pork surface showed a positive peak at Ex 302 nm and Em 410 nm, which was separated from tryptophan's peak and probably due to ATP fluorescence; therefore, ATP fluorescence signal was successfully detected on pork surface with fourth-derivative procedure. Also, this fourth-derivative fluorescence intensity increased with aerobic plate count, which justified that this peak is probably due to ATP. Then prediction models of aerobic plate count and ATP content of pork surface were developed from raw EEMs, second-derivative EEMs, and fourth-derivative EEMs. The highest prediction accuracies were obtained when predicting with fourth-derivative EEMs

with correlation coefficient of 0.95 and RMSEP of 0.68 $\log_{10}$ (CFU cm^{-2}) for aerobic plate count. LV 1 loading showed that decrease in tryptophan fluorescence contributed to the prediction models. Also, LV 2 loading showed that increase in ATP fluorescence signal contributed to the prediction models. In the previous model, the RMSEP was 0.97 $\log_{10}$ (CFU cm^{-2}), but on the other hand, in this study, the RMSEP was about two-third of the previous one. Therefore, it can be concluded that ATP fluorescence as well as tryptophan and NAD(P)H contributed the prediction models and these three compounds to the models offered better prediction accuracies with the two-third prediction errors.

3. Mathematical modeling of penetration of aerobic bacteria into meat

Penetration of aerobic bacteria into meat was mathematically modeled using diffusion-reaction equation which includes bacterial motility, chemotaxis driven by oxygen, growth, and proteolysis. The gaps between muscle fiber endomysium, a pathway of bacterial migration into meat, are filled with sarcoplasmic protein; thus, the effect of sarcoplasmic protein viscosity on motility and oxygen transport was included. Effect of oxygen concentration on bacterial motility was also considered because aerobic bacteria lose motility under critical oxygen concentration. Proteolysis kinetics parameters of *Pseudomonas fluorescens* on sarcoplasmic protein from chicken breast muscle were obtained by the experiment. Deeper penetration into meat due to motility was counteracted by chemotaxis toward the surface where oxygen concentration is higher and by a reduction in motility at deeper locations due to oxygen starvation. Predicted penetration rates compared reasonably well for both non-proteolytic and proteolytic bacteria. More rapid penetration rate during proteolysis is due to high motility and increased oxygen diffusion (reduced chemotaxis and starvation) in a reduced viscosity fluid caused by the degradation of sarcoplasmic protein. From this mechanistic understanding of penetration of aerobic bacteria into meat, the penetration depth was almost independent of the duration of penetration even though the duration of the exposure to air was constant, because bacterial migration into meat is mainly regulated oxygen transport (oxygen starvation in deeper locations in the gaps). Therefore, penetration depth of aerobic bacteria into meat was quantitatively evaluated with surface bacterial concentration (Shirai et al., 2017).

4. Conclusions

To be concluded, simultaneous quantitative evaluation technique of surface aerobic plate count and penetration depth of aerobic bacteria into meat was developed with fluorescence spectroscopy coupled with two-dimensional Savcitzky–Golay second-order differentiation and mathematical modeling.

References

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