# PREDICTIVE MODELING OF MICROORGANISMS IN PORK USING DRIP METABOLITES AND ELASTIC NET REGRESSION

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## I. INTRODUCTION

In pork, non-destructive prediction of the microbial populations and compositions is important for both economic and safety reasons. Metabolites in drip, which flow from meat due to environmental factors, have the potential to predict the condition of the meat [1]. When modeling to predict the condition of meat using metabolite data, it is crucial to prevent multicollinearity and overfitting due to variable interactions. Elastic Net regression addresses these issues by combining the variable selection capability of Lasso regression with the coefficient size determination of Ridge regression [2]. Therefore, our research aims to use Elastic Net regression to model the metabolites in pork drip for non-destructive predictions of microbial counts and composition, and to adjust it for predicting the specific microbial counts in meat.

## II. MATERIALS AND METHODS

*Longissimus thoracis* muscles from both sides of three sows were purchased. They were cut to approximately 7 cm thickness and vacuum-packaged. A total of 30 loin cuts (5 experimental days × 3 animals × 2 sides) were stored at 4°C for 27 days. The drip was collected using a 10mL syringe from vacuum-packaged samples. Total aerobic bacteria (TAB) count and microbial composition by 16S rRNA were measured in meat. Metabolites in drip were measured by nuclear magnetic resonance. The 16S rRNA was analyzed at 3 points (4, 13, 20 days). The obtained metabolite and microbial composition data were augmented using the Noise method with Python (n=500, N=1,500) [3]. The model was trained using augmented data, and the original data were used to validate the model. Normalization was performed with Python [2]. Elastic net regression was carried out in R [2].

## III. RESULTS AND DISCUSSION

We examined the Net Elastic regression technique as a computational approach to predict metagenomic sequencing outcomes based on metabolite characteristics, integrating biological insights through taxonomic or functional profiles. The derived taxonomic profiles are calculated as the weighted totals of the relative abundances of metabolite features, using the regression coefficients from the Elastic Net model as weights in the prediction algorithm. The microbial profile and metabolites of pork used in the model (Figure 1). During the storage period, 17 types of microbial genes and 64 metabolites were identified in pork, with the TAB count increasing from 3.73 to 7.62 log CFU/g.



Figure 1. Changes in total bacterial count (a), microbial composition (b), and metabolites (c) during storage of pork.

The Percent model applied Elastic Net regression using metabolites from pork drip and the microbial composition. The Count model incorporated pork drip metabolites with data obtained by multiplying the microbial composition by the TAB count (Figure 2a). Spearman values increased for 12 types of microorganisms when the TAB count was applied to predict microbial composition (Count model). After applying unaugmented data to the models, the differences between predicted and actual values were confirmed through multidimensional scaling (MDS; Figure 2b). For comparison between the percent model and the count model, the scale of MDS 1 and MDS 2 was applied identically to the axes of the percent model. Compared to the percent model, the predicted MDS1 value for microorganisms is reduced in the count model, which suggests that the TAB count positively influences the model.



Figure 2. Assessment of microbial predictions by Elastic Net regression models. Spearman rank correlation (a), multidimensional scaling (MSD) of percent model (b) and count model (c).

### IV. CONCLUSION

The Elastic Net regression model was constructed using the metabolites from pork drip and the microbial composition of pork. The counting model was able to predict the microbial characteristics using the metabolites of pork drip. Consequently, the metabolites in drip can be utilized to predict the microbial status of pork without destructive analysis. However, the concentration of metabolites and microbial composition in pork and drip can be influenced by environmental factors, so additional batch studies and considerations of collection conditions like packaging and storage are necessary.

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