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A new method for assessing the freshness of fish meat based on metabolome analysis technology

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Introduction

While the freshness of fish meat can be assessed by its appearance, viable bacteria counts, and K values [1], objectively assessing freshness is extremely difficult. The emerging technology of metabolomics, which provides comprehensive analysis of metabolites, has attracted increasing attention as an important tool for food quality assessment [2,3]. As fish meat loses freshness and begins to deteriorate, various metabolites are either formed or changed. We hypothesized that a comprehensive analysis of these metabolites could provide clarify the changes in the freshness of fish meat. NMR-based metabolomic analysis has been reported to show the changes that occur in the metabolite profiles of shellfish during storage [4]. However, no studies have clarified the changes in the metabolites of fish meat during storage, nor have predictive models been developed to assess fish meat freshness. In this study, yellowtail muscle was used as a model for assessing changes in fish meat freshness during storage. The metabolites that changed during storage were analyzed to determine the potential of this method for use as a freshness assessment tool.

Materials and methods

The yellowtails were transported to the laboratory on ice and filleted within 9 h of being killed (*ikejime*) at the market. Fillets were separated into ordinary and dark muscle before being minced using a food processor. The fish meat was stored at 0°C and 5°C for between 0 and 14 days, and the samples were pulverized after freeze-drying and stored at -80°C until analysis. Samples were prepared using a previously reported method with modifications [5]. Briefly, a methanol-chloroform-water (2.5:1:1) solution was used to extract water-soluble, low molecular weight metabolites from the samples. After oxime-trimethylsilyl derivatization, the metabolites were subjected to gas chromatography-mass spectrometry (GC/MS) analysis. The GC/MS Metabolite Component Database (Shimadzu Corporation) was used to annotate peaks, and a list of the peak areas of the annotated metabolites was created and subjected to multivariate analysis using SIMCA 14 software (MKS Umetrics AB, Umeå, Sweden). Orthogonal partial least squares (OPLS) analysis with *Pareto* scaling was performed using storage days as the *Y* variable and the peak areas of each metabolite as the *X* variable.

Results and discussion

As a result of the OPLS analysis, a model was produced that predicted the storage day at different temperatures for each type of fish meat (i.e., ordinary and dark muscle). Figure 1 shows the score plot obtained for ordinary muscle stored at 0°C.

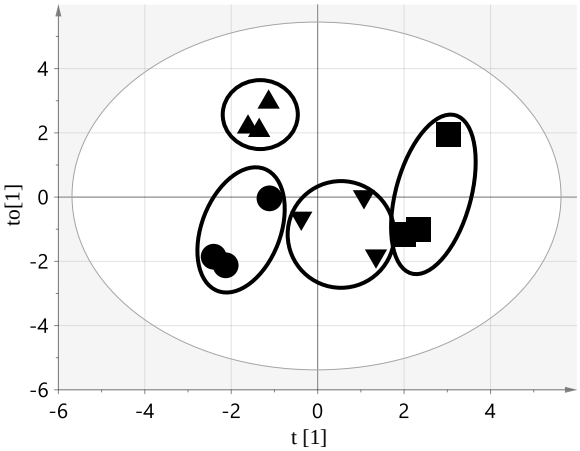

Fig. 1. Score plot obtained by OPLS analysis (ordinary muscle, 0°C storage). ●: not stored (0 days); ▲: stored for 3 days; ▼: stored for 7 days; ■: stored for 14 days.

Differences in the metabolite profiles were observed for each storage period. The derived model (1+1) had an R^2Y of 0.891 and a Q^2Y of 0.692 (Fig. 1). A good prediction model was obtained when $Q^2Y > 0.5$ [6,7].

Next, by expressing the predictive values on the horizontal axis and actual measured values on the vertical axis, a regression equation could be derived to

estimate freshness (Fig. 2). The regression line, *y=0.99x+0.11* and R^2*=0.891*, had an *RMSEE* of 2.00 and an *RMSEcv* of 2.93.

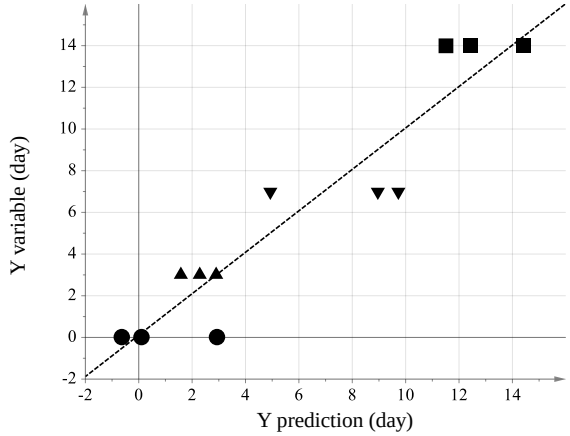

Fig. 2. A freshness prediction model for ordinary muscle stored at 0°C. ●: not stored (0 days); ▲: stored for 3 days; ▼: stored for 7 days; ■: stored for 14 days.

In this way, a robust predictive model was developed for both muscle types and both storage temperatures (i.e. 0°C and 5°C) (Table 1).

Table 1. List of model assessment obtained by OPLS analysis

Models	Ordinary (5°C)	Dark (0°C)	Dark (5°C)
A[a]	2+2	1+1	2+5
R^2Y	0.879	0.734	0.985
Q^2Y	0.767	0.581	0.868
Regression line	*y=1.02x+0.01*	*y=0.95x+0.53*	*y=0.96x+0.002*
R^2 [b]	0.925	0.738	0.984
RMSEE	0.97	3.13	0.63
RMSEcv	1.10	3.49	0.92

[a] Number of models
[b] R^2 was obtained from the regression line

Furthermore, after construction of the OPLS model, the variable importance for projection (VIP) of each explanatory variable was calculated. VIP values, which are greater than 1.0, are important metabolite markers [8]. Metabolites with VIP values ≥ 1.0 are shown in Fig. 3.

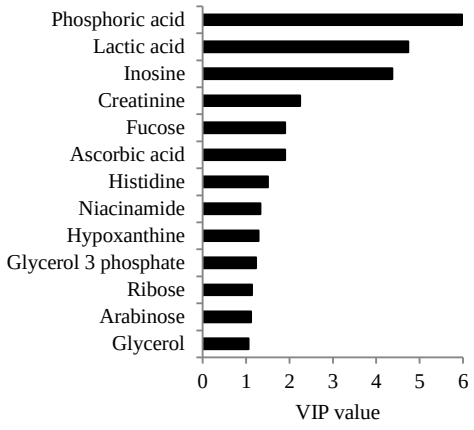

Fig. 3. Metabolic components with high VIP values obtained from the predictive model for ordinary muscle stored at 0°C.

For example, inosine and hypoxanthine, which are ATP-related compounds used to calculate K values, had high VIP values; K values are a model assessment method for fish meat freshness [1]. However, in this study, in addition to ATP-related compounds, metabolites that play a role in various metabolic pathways, such as organic acids, saccharides, and vitamins, were also detected. These findings show that the methodology presented in this study is effective for assessing the freshness of fish meat. Furthermore, the magnitude and ranking of VIP values changed depending on the model (Table 2).

Table 2. List of metabolites with high VIP values obtained using each model (VIP ranking)

VIP ranking	Ordinary (5°C)	Dark (0°C)	Dark (5°C)
	Metabolites name (VIP values)		
1	Phosphoric acid (6.24)	Galactose (4.95)	Taurine (4.52)
2	Lactic acid (4.54)	Taurine (3.75)	Galactose (3.76)
3	Inosine (2.82)	Tagatose (2.74)	Tagatose (3.37)
4	Histidine (2.51)	Mannose (2.51)	Mannose (2.34)
5	Creatinine (1.70)	Phosphoric acid (2.20)	Trehalose (1.80)

Interestingly, metabolites with high VIP values differed between ordinary and dark muscle, suggesting that metabolic pathways may differ as a function of the muscle type of the fish being stored as well as the storage temperature.

Conclusions

This model could potentially be used as a new method for predicting fish meat freshness. In addition, various metabolites, such as nucleic acid-related compounds, amino acids, sugars and organic acids, were found to be important metabolite markers for this predictive model of fish meat freshness.

Acknowledgements

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