

A Nondestructive Method for Fish Freshness Determination with Electronic Tongue Combined with Linear and Non-linear Multivariate Algorithms

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Abstract

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Electronic tongue coupled with linear and non-linear multivariate algorithms was attempted to address the drawbacks of fish freshness detection. *Parabramis pekinensis* fish samples stored at 4°C were used. Total volatile basic nitrogen (TVB-N) and total viable count (TVC) of the samples were measured. Fisher liner discriminant analysis (Fisher LDA) and support vector machine (SVM) were applied comparatively to classify the samples stored at different days. The results revealed that SVM model was better than Fisher LDA model with a higher identification rate of 97.22% in the prediction set. Partial least square (PLS) and support vector regression (SVR) were applied comparatively to predict the TVB-N and TVC values. The quantitative models were evaluated by the root mean square error of prediction (RMSEP) and the correlation coefficient in the prediction set (R_{pre}). The results revealed that SVR model was superior to PLS model with RMSEP = 5.65 mg/100 g, R_{pre} = 0.9491 for TVB-N prediction and RMSEP = 0.73 log CFU/g, R_{pre} = 0.904 for TVC prediction. This study demonstrated that the electronic tongue together with SVM and SVR has a great potential for a convenient and nondestructive detection of fish freshness.

Keywords: fish quality; taste sensors; nondestructive detection; support vector machine; support vector regression; chemical and microbiological analyses

List of symbols and abbreviations: TVB-N – total volatile basic nitrogen; TVC – total viable counts; Fisher LDA – Fisher liner discriminant analysis; SVM – support vector machine; PLS – Partial least square; SVR – support vector machine regression; RMSEP – root mean square error of prediction; R_{pre} – correlation coefficient in the prediction set; E-tongue – electronic tongue; DFs – discriminate functions; LOO-CV – leave-one-out cross-validation; RMSECV – root mean squares error of cross-validation

Fish and fish products cover an important part of the protein demand for human nutrition. The total amount of fish consumption in the world has increased dramatically in recent decades (DOWLATI *et al.* 2012). It is therefore vital to guarantee the safety and quality of fish for the consumers. However, the main problem with fish is that it is highly perishable

after harvesting and *post-mortem* which results from the mechanisms of enzymatic autolysis, oxidation, and microbial growth (GHALY *et al.* 2010). Due to its rapid deterioration, freshness has become one of the most important quality parameters of fish and fish products for the consumers, traders, and processors (HUANG *et al.* 2011).

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The conventional methods for fish freshness determination are usually based on principles of physical, chemical, microbiological, and sensory evaluation (OLAFSDOTTIR *et al.* 2004), these techniques however, although reliable and accurate, are destructive, tedious, involve chemicals use, and above all require highly skilled personnel (ZARAGOZÁ *et al.* 2012). In this regard, there is therefore the need to develop and evaluate a convenient, accurate, reliable, and environmentally friendly analytical method for fish freshness determination.

Actually, up till now, massive sensor technologies have been developed to address the drawbacks of the conventional methods for fish freshness evaluation, using equipment such as spectrophotometers (CHENG *et al.* 2013a; NI *et al.* 2013), image analysers (DOWLATI *et al.* 2012), colorimeters (DINI *et al.* 2010; HUANG *et al.* 2011), devices to test surface electrical properties (GIL *et al.* 2008b; ZHANG *et al.* 2011), electronic noses (TIAN *et al.* 2011; GUOHUA *et al.* 2012), etc. An excellent introduction to sensor technologies for fish freshness detection can be found in refs. (OLAFSDOTTIR *et al.* 2004; CHENG *et al.* 2013b). However, all these sensor technologies have their own advantages and disadvantages (OLAFSDOTTIR *et al.* 2004; CHENG *et al.* 2013b). All these methods are still in the stage of laboratory research and need further exploration and researches for practical application. Actually, the development of convenient and nondestructive methods for fish freshness evaluation in practical application is still an unresolved goal.

Electronic tongue (E-tongue) as an artificial intelligence system has received increasing interests from many researchers in recent years. The E-tongue technologies coupled with multivariate algorithms have proved to be a powerful analytical tool widely applied in food quality analysis of such products as wine (CETÓ *et al.* 2012; KIRSANOV *et al.* 2012), tea (GHOSH *et al.* 2012), milk (WEI *et al.* 2013), oil (MEN *et al.* 2013), meat (CAMPOS *et al.* 2010), cocoa (TEYE *et al.* 2014) etc., and have been found to be simple, rapid, convenient, reliable, and accurate methods.

Recently, E-tongue has also found its way into the fish industry for fish quality control. Gil and co-workers used E-tongue to analyse the freshness of cultured sea bream (*Sparus auratus*) fillet (GIL *et al.* 2008a) and other studies can be found in refs. (BARAT *et al.* 2008; GIL *et al.* 2008b). The results of all these studies suggest the feasibility of E-tongue for a real, easy, and effective assessment of fish freshness. However, up till now, the nondestructive detection of the freshness of the whole fish by E-tongue technique combined

with multivariate algorithms has not been studied. This research aims at using E-tongue coupled with linear and non-linear multivariate algorithms to detect conveniently and nondestructively the freshness of whole fish stored at 4°C by predicting the storage time and other main indices (i.e., TVB-N and TVC) associated with the fish freshness.

MATERIAL AND METHODS

Samples. Fish samples of *Parabramis pekinensis* species were purchased alive from a local supermarket in Zhenjiang, China. A total of 144 fish samples with an average weight of 0.6 kg were rinsed and each one was placed into a separate polythene bag before storing them in a cold store at 4°C. This storage condition was selected to mimic the commercial and domestic refrigeration systems. Fish freshness measurements were conducted every 24 h starting from 2 h *post-mortem*. On each day, 12 fish samples were selected at random and each was placed into a container with distilled water for 45 min (fish to water ratio was 2.5:1.0). The water was then collected out and filtered through filter paper (GB/T1914-93). The filtrate was used for E-tongue measurements.

E-tongue data acquisition. An α -Astree™ E-tongue (Alpha M.O.S., Toulouse, France) with a taste sensor array was employed in this study. The taste sensor array consisted of seven sensors based on Chemically Modified Field-Effect-Transistor (CHEMFET) technology and an Ag/AgCl reference electrode that converts the real-time chemical information concerning the presence of the compounds in the complex mixture into electrical signal (LI *et al.* 2007). Taste sensors are partially and cross sensitive and the measurement represents the voltage differences between the sensors and reference electrode (WEI *et al.* 2009). The sensors measure the dissolved chemical compounds in the solution and give the measurement of the voltage difference between the sensors and the reference electrode (Ag/AgCl) (which has a fixed voltage by definition). The voltage difference measured refers to the voltage of the sensor minus that of the reference electrode. The interactions between the dissolved molecules present in the analysed samples and the sensor affect the voltage of the sensor. The voltage difference measured is linked to the variation of the voltage of the sensor which is representative of the dissolved substances in the liquid sample used.

According to the experience gained in the pre-experiment and the requirement for the system stability, each sample was measured 5 times. The sensor

array was rinsed 20 s with distilled water between the measurements. Actually, it could be concluded from the preliminary experimental results that the intensity of each sensor value varied greatly between the first two measurements, but it tended to be stable in the subsequent three measurement, so the next two measurement results were selected for analysis. During the initial 100 s, the response of each sensor tended to remain steady. Although there were some noise signals linked with the sensor intensity, they were finally stabilised after about 120 s, resulting in the relative standard deviation of the response signal of each sensor all being less than 5% after that point. In this research, the intensity value of each sensor at 121–130 s was extracted and analysed. The average intensity value of each sensor at 121th–130th second of the last 2 measurements was used as the original data for further processing. The experiments lasted for 12 days, because after 12 days deterioration it was too high. In all, 144 sets of data with seven variables (seven sensors) were acquired.

Chemical and microbiological analysis. In this study, TVB-N in fish was measured by the semimicro-Kjeldahl method according to Chinese industry standard (SC/T 3032:2007). TVC measurements were performed by the standard plate count method: referring to Chinese standards (GB 4789.2:2010). TVB-N and TVC determinations were performed concurrently after the E-tongue measurements.

Data analysis and software. The aims of this study were to detect accurately and nondestructively the fish freshness by E-tongue technique combined with linear and non-linear multivariate algorithms. It is therefore important to train pattern recognition models to predict fish freshness qualitatively and quantitatively by means of linear and non-linear multivariate algorithms.

Fisher liner discriminant analysis (Fisher LDA) and support vector machine (SVM) were applied to process the E-tongue outcomes for qualitative analysis, while, E-tongue data and the values of TVB-N & TVC were analysed using partial least squares (PLS) regression

and support vector regression (SVR) for quantitative prediction. The performances of Fisher LDA and SVM models were evaluated by the identification rate in the prediction set, while the performances of PLS and SVR models were evaluated by the root mean square error of the prediction (RMSEP) and correlation coefficients in the prediction set (R_{pre}) (CHEN *et al.* 2012). All the multivariate algorithms were performed in Matlab Version 7.14 (Mathworks, Natick, USA) using Windows 7.

RESULTS AND DISCUSSIONS

E-tongue data analysis. Fisher LDA and SVM models were built to classify the fish samples according to their storage times lasting different numbers of days for qualitative analysis of their freshness.

Fisher LDA result. Three quarters of the samples taken on different days were selected at random as the training set and the other samples as the prediction set for establishing Fisher LDA model. The results show that the top three discriminate functions (DFs) scores amounted to 92% and this could represent the greatest volume of information of the original data. E-tongue data were processed by the top three DFs. The results show that 6 samples of the prediction set were judged wrongly and the identification rate was 83.33%.

SVM result. Three quarters of the samples taken on different days were selected at random as the training set, other samples as the prediction set for building the SVM model. RBF kernel function was selected as the kernel function of SVM because it has been applied widely and its theoretical system is also more mature than other kernel functions (ZHAO *et al.* 2006). The performance of the SVM model is particularly vulnerable to the parameter g of RBF kernel function and the regularisation constant c which determines the tradeoff between minimising the training error and minimising the model complexity (CHEN *et al.* 2007). The leave-one-out cross-validation (LOO-CV) (DONG & WANG 2011) was applied for parameters optimisation of c and g

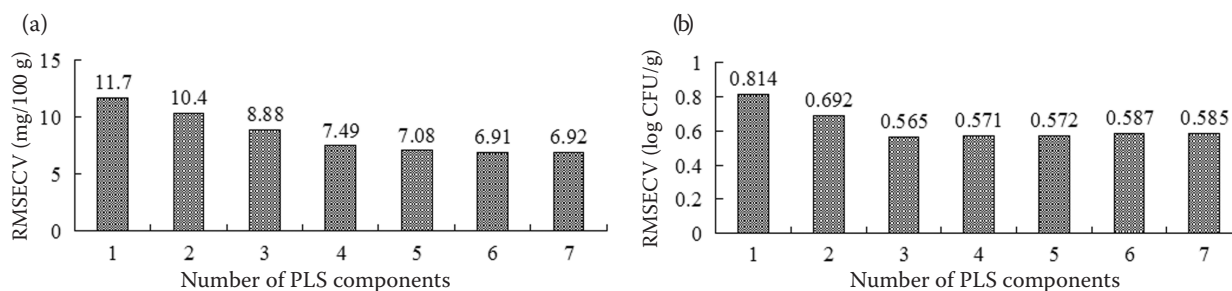


Figure 1. RMSECV values of PLS for (a) TVB-N and (b) TVC predictions under different principal components

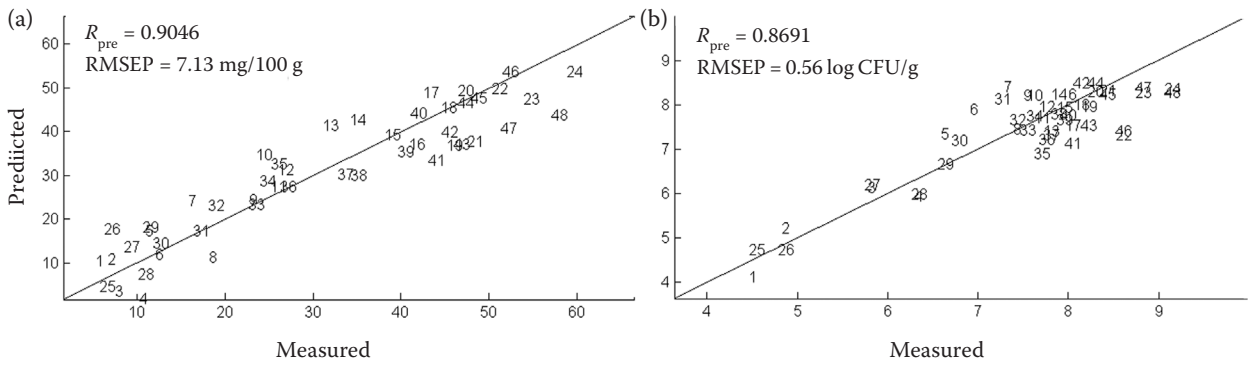


Figure 2. Measured versus E-tongue predicted of (a) TVB-N and (b) TVC by PLS in predictions set

subjects to maximise the identification rate. In this research, $[2^{-8}, 2^8]$ and 0.5 were selected as the range and step of parameters of c and g , respectively. All columns of the E-tongue data were structured to $[0, 1]$ by the normalisation method. The conversion formula of normalisation method was:

$$x \rightarrow y = \frac{x - x_{\min}}{x_{\max} - x_{\min}} \quad x, y \in R^n \quad x_{\min} = \min(x), \quad x_{\max} = \max(x) \quad (1)$$

The results show that the optima of c and g were 16 and 5.66, respectively, and the highest identification rate was 96.3%. SVM model was built under this condition and the identification rate in the prediction set was 97.22%.

E-tongue data and chemical and microbiological analyses. The relationships between the results of the E-tongue measurements together with chemical and microbiological tests were established by using PLS and SVR procedures.

PLS result. Two-thirds of the samples coming from different days were selected at random as the training set and other samples as the prediction set for building the PLS model. The LOO-CV was applied for parameters optimisation of the numbers of PLS components subject to minimising the root mean squares error of cross-validation (RMSECV). The result of parameters optimisation for TVB-N and TVC

predictions is shown in Figure 1. The PLS models for TVB-N and TVC predictions were established with the optimal parameter. The performances of the PLS models for TVB-N and TVC predictions are show in Figure 2, the sample points in the figure having been renumbered sequentially according to their order in the original samples set.

SVR result. SVR is the application of SVM in non-linear multiple regression. In this research, two-thirds of the samples coming from different days were selected at random as the training set and other samples as the prediction set for building the SVR models. Radial basis function was selected as the kernel function of SVR model. $[2^{-10}, 2^{10}]$ was selected as the range of parameters of c and g , 0.5 was selected as the step size of c and g . All columns of the E-tongue data were structured to $[0, 1]$ by the normalisation method (Eq. (1)).

The results show that the best c and g were 128 and 1.41, respectively, and the lowest RMSECV was 5.75 mg/100 g of SVR model for TVB-N prediction. While the best c and g were 5.66 and 2, respectively, and the lowest RMSECV was 0.41 log CFU/g of SVR model for TVC prediction. SVR models for TVB-N and TVC predictions were established under the parameters optimal conditions. The performances of the SVR models for TVB-N and TVC predictions are show in Figure 3, and the samples points in the

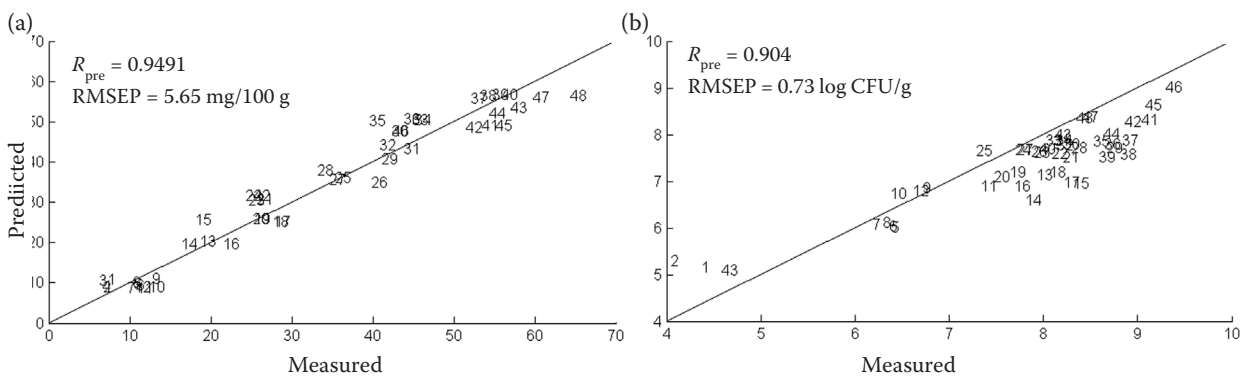


Figure 3. Measured versus E-tongue predicted of (a) TVB-N and (b) TVC by SVR in predictions set

figure having been renumbered sequentially according to their order in the original samples set.

DISCUSSIONS

During fish spoilage, a breakdown of various components and the formation of new compounds occur, mainly due to the microbial actions (FRASER & SUMAR 1998; GHALY *et al.* 2010). Small molecules of amines, ketones, and aldehydes, ammonia, organic acids, alcohols, etc. produced during fish spoilage have very good water solubility. Therefore, distilled water can dissolve most of the products generated from the fish tissue during storage. Varieties and concentrations of these products in the fish-distilled water solution were related in view of the fish freshness. Also, the amount of microorganisms in fish was relevant to the concentration of these small molecules (GRAM & HUSS 1996). The taste sensors of the E-tongue are sensitive to both inorganic and organic chemical compounds in liquid samples as ionic species and neutral species (CAMPBELL *et al.* 2012). Therefore, the E-tongue technique has a great potential to qualitative and quantitative analysis for the fish freshness in this study.

As can be seen from the Fisher LDA and SVM models results, SVM was better than Fisher LDA in the processing of E-tongue data for qualitative analysis. The results of PLS and SVR models for TVB-N and TVC predictions show that SVR was better than PLS for quantitative analysis of these two quality indices by E-tongue technique because SVR models show higher correlation coefficients of all the training sets and prediction sets are those that of PLS models. This is mainly owing to the fact that the relationships between the data matrices were more complex than linear as a result of the complexity composition of the test samples and the basic principles of the taste sensors which are partially and cross sensitive. SVM and SVR have a great advantage in processing nonlinear problems (CHEN *et al.* 2012) in comparison with Fisher LDA and PLS, respectively. This indicates that the effects of prediction by E-tongue data analysis from the nonlinear multivariate algorithms are better than the linear multivariate algorithms because of a better ability of nonlinear methods of self-learning and self-adjusting.

CONCLUSIONS

The E-tongue technique coupled with multivariate algorithms to detect non-destructively the fish freshness was studied. The SVM was superior to Fisher LDA for the classification of the fish samples

stored at 4°C for different numbers of days. The SVR was better than PLS for the prediction of the TVB-N and TVC values of fish samples. All the findings reveal that E-tongue technique together with SVM and SVR has a great potential for a convenient and nondestructive detection of fish freshness.

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