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A new multivariate approach to the problem of fish quality estimation

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Abstract

Use of the interaction of electromagnetic fields with moist materials has typically meant the use of dielectric properties for measurement of water content. Recent work has widened the scope of such dielectric measurements by using a combination of dielectric spectroscopy and multivariate analysis to determine, among other things, some loosely defined qualities of foods. This has led to the work described here on the determination of the quality of chilled Baltic cod (*Gadus morhua*).

The fish obtained had known history and were stored in ice for up to 24 days. At intervals of two days, the dielectric properties of individual fish were measured in the time domain in a range from .01 to 1.0 ns, using an open-ended coaxial sensor and time domain reflectometer. In addition an 'electronic nose' sensor array was used to gather data on various spoilage volatiles. The data so obtained were subjected to principal component analysis and regression to produce a calibration equation for the prediction of number of days on ice and quality index method (QIM).

Keywords: Fish quality; Electronic nose; Dielectric spectroscopy

1. Introduction

1.1. Background

That there exists no entirely satisfactory physical method to determine the quality of fish products was the conclusion of a concerted action on the evaluation of fish freshness undertaken in the 3rd Framework AIR programme of the European Community (AIR3 CT94 2283) (Olafsdottir et al. (1998)). This conclusion was reached after examining, in detail, the various methods available for quality or freshness estimation. Multivariate analysis, for combining results from several measurement techniques, had been discussed as a potentially useful approach and a further project was begun under the 4th Framework FAIR programme (CT98 4076). This, however, was concerned with optimising the use of existing methods (mainly chemical and sensory) in a multivariate approach to the problem of determining fish freshness. In contrast, the work described here relates to the development of a completely new technique, which is a combination of two physical methods: measurement of microwave dielectric properties and use of various gas sensors in a so-called electronic nose.

Recently, as a spin-off from another FAIR project in the 4th Framework programme (CT97 3020), a preliminary test of microwave dielectric spectroscopy has been carried out in an entirely new approach to electrical measurement of quality. Although that project was principally concerned with the measurement of added water in foods, the technique used was able to distinguish different aspects of quality in seafood products, e.g., whether raw materials had been frozen and how well they had been stored (Kent, Mackenzie, Berger, Knöchel, & Daschner, 2000). Also, different 'qualities' of fish (fresh haddock, saithe, cod and frozen cod), defined in this case as suitability for processing into a particular product, were categorised by the method of

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discriminant analysis with a high success rate. Furthermore, mixtures of fish and other materials, such as potato flour and milk protein were also successfully allocated to categories defined as containing fresh or previously frozen fish.

The methodology used relied on measurement of the complex dielectric properties of the samples as a function of frequency. These spectra were transformed using the method of principal component analysis (PCA). This applies a linear transformation to a multidimensional set of correlated variables to produce a new set of uncorrelated, standardised and orthogonal variables called 'principal components' or scores, each of which, explains a fraction of the variance of the data. The PC scores were then used in discriminant functions to allocate samples to particular groups. The original variables, in this case, were the complex spectral values and the temperature, but they could have included any other variable believed to have an effect on the spectra or to be related to the target variables, e.g., proximate composition and quality. This approach allows the addition of variables from other methodologies, physical or otherwise, and has the potential to be independent of prior treatment, e.g., mechanical handling or other interfering factors.

Electrochemical gas sensors have been used by many workers in the study of fish odours (Grigor, Theaker, Alasalvar, O'Hare, & Ali, 2002). An array of such sensors can be used to estimate the freshness of fish, either by simple regression analysis or more robust multivariate methods such as PCA and PC regression or partial least squares regression (PLSR). The combination of the dielectric data with this gas sensor data can give better results than the use of one method alone, as this paper will show.

Thus the approach is multivariate in the sense that the data are analysed by standard chemometric multivariate techniques, as used in many other fields, and also in the sense that it is using more than one kind of methodology.

1.2. Dielectric properties and their use

PCA effectively reconstructs the information in the input data into a new form in which the sources of variation are 'modelled' rather than some attempt at a physical model of the system. To achieve the objective of a hand-held dielectric instrument a more rapid and simple method than that used in the earlier work was needed. This involves measurements in the time domain rather than the frequency domain: to measure the time-dependence of the dielectric response of the material under investigation by an input electromagnetic pulse (time domain reflectometry, TDR). These domains are linked by a family of several mathematical transformations, the most well-known being of course the Fourier transform and its inverse.

For the time-varying data, which are acquired in this instrument, the inverse Fourier transform, in its most general form, can be written as in Eq. (1):

$$h(t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} g(f) \mathrm{e}^{-2\pi \mathrm{i} ft} \mathrm{d}f, \quad (1)$$

where $h(t)$ is a time-dependent function, the Fourier transform of which, is a frequency-dependent spectrum $g(f)$. Examination of this equation shows that, at any instant t, every component part of the spectrum contributes to the value of h. Because the subsequent principal component analysis is concerned only with the variations in g, then transformation of $h(t)$ to the frequency domain for PCA is not required, since the same variations are present in $h(t)$. Several problems arise with time domain measurements if the frequency-dependent properties are desired. First, the truncation of the pulse after a finite measurement interval introduces undesirable distortions in the integral. Second, to accurately reconstruct the reflection coefficients in the frequency domain, exact time referencing of the pulse is required, otherwise phase errors cause large inaccuracies at the high frequency end of the spectrum. In the method described here, we are not transforming to the frequency domain and so can truncate the measurements wherever we wish: the same information on variation is still contained in the collected data. Neither is time referencing important since this just introduces another source of variance which is isolated by the PCA.

1.3. Electronic nose

Artificial or electronic noses operate on principles that are similar to the human olfactory system (Gardner & Bartlett, 1999). The main components of these systems are the sensors. There are different groups on the market, but none of them fully meets the requirements of particular applications for precision, reproducibility, sensitivity and stability. Therefore, a careful choice of the sensor array has to be made. To obtain a unique overall pattern or "fingerprint", different sensors with overlapping responses to the range of compounds within the sample are used. Metal oxide semiconductor sensors (MOS) represent the most widely used technique and the signal is measured as a relative change in the resistance of the device. The signal from a field effect sensor (FE) is measured as a shift in the voltage required for keeping a constant current through the device. MOS and FE complement each other, giving an increased selectivity.

Sensors can be problematic due to a change of response pattern with time. To ensure constant results over time, these drifts must be minimised by measuring dummy samples before the actual samples (condition of the sensors) and by including a stable calibration substance (for multiplicative drift correction).

Multivariate data processing techniques (PCR, PLSR) are commonly employed to analyse data from electronic nose instrumentation. In the case of the instrumentation described here, 15 sensors are in the array. A selection of the most relevant sensors can improve the discrimination characteristics of the aroma profiles.

2. Materials and methods

2.1. Cod samples

Fresh Baltic cod (Gadus morhua) with known history (the cod were believed to have been five days on ice at the start of the experiment) were obtained from fish suppliers. These fish were then gutted and stored whole on ice. At 0, 1, 3, 6, 8, 10 and 13 days, cod were removed for similar analysis.

The length and weight were measured and a quality value (QIM) was determined. After QIM, every fish was filleted without removing the skin and prepared for dielectric measurement.

2.2. QIM (quality index method)

The QIM scheme for whole cod, (Luten & Martinsdottir, 1997) suggest ten quality parameters, to be assessed by a panel of eight to ten experts. In this application, only nine of these were evaluated (appearance of skin, stiffness of the fish, form of the eyes, state of the cornea, colour of the pupils, gill colour, its smell and its mucus and colour of the blood). Between zero and three well-defined demerit points are attributed to the score depending on the parameter. The quality index has possible values for the cod, from 0 to 21.

2.3. Dielectric measurements

The reflected pulses from the surface of samples of individual fish, due to an incident step waveform, were measured at 10 ps intervals over a time span of 1 ns, using an open-ended coaxial sensor and TDR. The input waveform had a rise-time of 100 ps. Three strategies were used for the measurements:

1. fillet, probe directly onto the skin,
2. fillet, probe directly onto the flesh,
3. probe on the minced and blended flesh.

In all strategies, five replicate measurements were made on each of five fish at each take-out. A selected section of the pulse (see Fig. 1) comprising 10 time intervals (11 data points) was then subjected to principal component analysis and regression to produce calibration equations for the prediction of number of days on

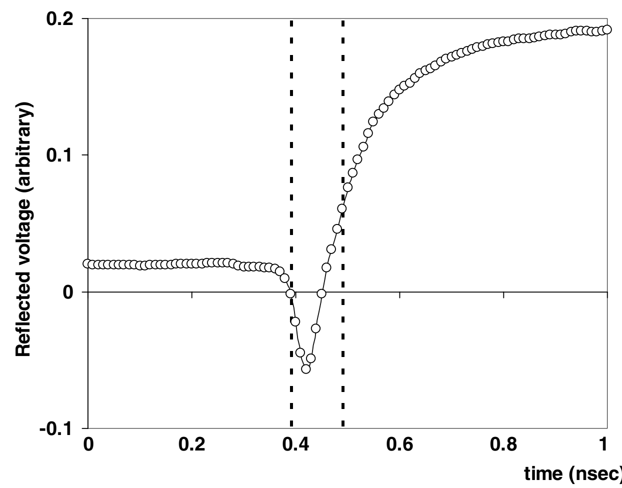

Fig. 1. A typical reflected pulse. The vertical broken lines show the time window used in the PCA. This comprises 11 data points only.

ice or QIM. From the regression equations, the coefficients of determination, adjusted for degrees of freedom R^2_{adj}, and the standard error of calibration (SEC), were calculated. The standard error of prediction (SEP) was calculated by a process of internal cross-validation (ICV) where each sample is removed sequentially from the calibration and its value predicted.

2.4. Electronic nose

The volatile compounds of the pure minced fish muscle were analysed by an electronic nose (NST 3320 Applied Sensor, Sweden), using a sensor array of nine FE- and six MOS-sensors. Of these, twelve were deemed useful for sensitivity to spoilage. Two strategies were used with these data. First, the response of each sensor was used in a multiple regression against either days-on-ice or QIM, with similar parameters being calculated as for the above dielectric measurements. Second, the raw data were subjected to PCA and PCR also, as above.

2.5. Intellectron Fischtester

As a comparison of existing methods for measuring freshness of fish, readings were taken on each fish, using the Intellectron Fischtester VI. This well-known instrument measures the electrical conductivity through the body of the fish at two different frequencies in the low frequency range. In this way, electrode polarisation can be eliminated and the true conductivity measured. It is found that such measurements relate to freshness of the fish.

2.6. Combination of data

Finally, the artificial nose data and the TDR data were combined and PCA and PCR were applied to yield a further set of performance parameters for prediction of age and QIM.

3. Results

3.1. TDR measurements

All the predictive equations obtained are presented in Table 1. These represent the optimum conditions for the avoidance of overfitting, i.e., the number of PCs used in each calibration has been selected for minimum difference between SEC and SEP, coupled with the highest R^2_{adj} consistent with that. SEC and SEP are in the appropriate units, i.e., QIM or days-on-ice.

For the dielectric measurements alone, it is clear that the results obtained using minced samples are the best both for QIM and days-on-ice. The order is clearly mince>fillet flesh side>fillet skin side. As might be expected from the subjective nature of sensory scores, the results for days-on-ice, which are known precisely, are slightly better. Significance tests of this are not possible because each model not only refers to a different system of units but also differs in the number of PCs used.

3.2. Electronic nose

Although 15 sensors were used in these experiments a multiple regression on QIM or days-on-ice was only possible if three were eliminated, due to a high degree of co-linearity between them and the other sensors. For the remaining twelve, the results are shown in Table 1. PCR of the data from these twelve achieved much the same results, though with fewer components (only 5 PCs as opposed to twelve sensor variables) and with some reduction in overfitting.

Table 1
Summary of the various predictive equations obtained

	Number of PCs	R^2_{adj}	SEC	SEP
QIM				
Mince TDS	6	79.8	1.7	1.9
Fillet TDS	5	75.3	1.9	2
Skin TDS	7	67.4	2.2	2.8
Nose	12 sensors	85.6	1.4	2
PCR nose	5	84.3	1.5	1.8
Nose + mince TDS	4	90.6	1.2	1.3
Nose + skin TDS	9	87.5	1.3	1.7
Nose + fillet TDS	4	85.6	1.4	1.6
Days-on-ice				
Mince TDS	7	86.8	1.6	1.8
Fillet TDS	5	75.3	2.3	2.5
Skin TDS	7	70.6	2.5	3
Nose	12 sensors	95.5	1	1.3
PCR nose	5	93.5	1.2	1.3
Nose + mince TDS	5	96.4	0.9	0.9
Nose + skin TDS	9	96.2	0.9	1
Nose + fillet TDS	5	92.6	1.2	1.4
QIM				
Intellectron	N/A	76.4	2.2	–
Days-on-ice				
Intellectron	N/A	70.0	2.1	–

3.3. Combined artificial nose and TDS

The addition of the two data sets with subsequent PCR shows a marked improvement, in general, over the results for each technique alone. However, for days-on-ice, the improvement over the artificial nose alone when TDS data are added is perhaps marginal, but there is a decrease in the amount of overfitting with only the same number of PCs used (5) in the mince calibration. The SEC and SEP are both below ±1 day or ±1.3 QIM units.

All these results should be compared with the Intellectron data collected at the same time on these samples, for which a regression of days-on-ice against meter reading gave R^2_{adj} of 76.4 and a standard error of ±2.2 days and for QIM, R^2_{adj} of 70.0 and a standard error of ±2.1 units. The fact that these results are comparable to those obtained with the TDR sensor placed directly on the skin may not be coincidence, since both of these techniques use measurements made on the whole fish through contact with the skin.

4. Conclusions

The methods in this study if used independently, could provide reasonable estimates of freshness of fish. However, the combination of the electronic nose and dielectric TDR gives an improved performance that may be difficult to achieve by other methods. However, it must be said that these results are on a batch of fish which although heterogeneous in size, were all obtained under identical conditions of ground, season and handling. How the new methodology will cope with variations introduced by these and other factors will be the subject of further work in this project.

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