J Sci Food Agric 1998, **78**, 329–336

Prediction of Chemical, Physical and Sensory Data from Process Parameters for Frozen Cod using Multivariate Analysis

Iben Ellegaard Bechmann,*[1] Helle Skov Jensen,[2] Niels Bøknæs,[2] Karin Warm[2] and Jette Nielsen[2]

[1] Department of Chemistry, Technical University of Denmark, Building 207, 2800 Lyngby, Denmark
[2] Danish Institute for Fisheries Research, Department of Seafood Research, Technical University of Denmark, 2800 Lyngby, Denmark

(Received 24 February 1997; revised version received 12 November 1997; accepted 10 March 1998)

Abstract: Physical, chemical and sensory quality parameters were determined for 115 cod (*Gadus morhua*) samples stored under varying frozen storage conditions. Five different process parameters (period of frozen storage, frozen storage temperature, place of catch, season for catching and state of rigor) were varied systematically at two levels. The data obtained were evaluated using the multivariate methods, principal component analysis (PCA) and partial least squares (PLS) regression. The PCA models were used to identify which process parameters were actually most important for the quality of the frozen cod. PLS models that were able to predict the physical, chemical and sensory quality parameters from the process parameters of the frozen raw material were generated. The prediction abilities of the PLS models were good enough to give reasonable results even when the process parameters were characterised by ones and zeroes only. These results illustrate the application of multivariate analysis as an effective strategy for improving the quality of frozen fish products. © 1998 Society of Chemical Industry.

J Sci Food Agric **78**, 329–336 (1998)

Key words: fish; quality; principal component analysis; partial least squares regression

INTRODUCTION

Restricted quotas, leading to smaller amounts of fresh fish landed, have forced the industry to use frozen raw material to be able to produce all the year round. In order to improve the quality of the frozen fish products, it is necessary to identify those parameters in the process of freezing, storing and thawing which have the greatest influence on the properties of the raw material and the quality of the products. During frozen storage, fish muscle undergo protein denaturation and lipid oxidation due to a variety of causes (Shenouda 1980; Kozima 1983). For the gadoid fish species (eg cod), deterioration in texture of the thawed product is a serious problem. The presence of formaldehyde in gadoid fish due to enzymatic degradation of trimethylamine oxide (TMAO), which is present in the fish naturally, into dimethylamine (DMA) and formaldehyde is an increasing factor of the denaturation of proteins, since formaldehyde interacts with the proteins of the fish tissue (Haard 1990; Sotelo *et al* 1995). The amount of formaldehyde and DMA formed is strongly dependent on the frozen storage temperature and the time of frozen storage, but it does not seem to be influenced significantly by freezing rate and thawing rate (Sotelo *et al* 1995). The season of catch determines the spawn status of the fish, which can affect the water holding properties of the thawed product and the stability of the proteins (Love 1988). The place of catch might cause biological variations due to differences in the strains of cod and due to varying water temperatures (Haard 1990). The state of rigor prior to freezing the raw material also affects the water holding properties of the thawed product; if the fish is frozen before or during rigor mortis, a thawing rigor might develop, leading to shrinkage of fillets, high liquid loss and gaping (Love 1988).

* To whom correspondence should be addressed

 J Sci Food Agric 0022–5142/98/$17.50. Printed in Great Britain

The number of methods currently used for the assessment of frozen fish quality is extremely large. Quality parameters that relate directly to the production of formaldehyde (ie formaldehyde, DMA and water holding capacity) are obvious for the assessment of quality for gadoid frozen fish products, but in the industry the quality is traditionally evaluated by both physical, chemical and sensory analysis (LeBlanc *et al* 1988). The quality is thus, in general, registered as a multivariate response depending on variations in several process variables. Due to the multivariate nature of such data, it is of great importance to study the different steps in the freezing process together under well-controlled and defined conditions. The combination of experimental design and multivariate analysis is a powerful tool for assuring the quality of the final product. In this work, a systematic experimental design of five important factors affecting the changes during frozen storage was used. Cod samples (115) with different periods of frozen storage, frozen storage temperatures, places of catch, seasons for catching and state of rigor were used in the experiment. The quality of each sample was evaluated by determination of chemical quality parameters (ie DMA, formaldehyde, total volatile basic nitrogen and protein content) and physical quality parameters (water-holding capacity and dry matter) and by sensory evaluation of whole fish and fillet, respectively, using the quality index method (Bremner *et al* 1987; Nielsen and Jessen 1997). To obtain an overview of the quality of the different cod samples, the data were analysed using principal component analysis (PCA). PCA provides an exploratory data analysis based on a multivariate projection method that helps in visualising all the information contained in a data table. The relations between the different quality parameters and the influence of the different process parameters are expected to be revealed by this analysis. Multivariate models based on partial least squares (PLS) regression were established in order to describe the relationship between the treatments of the frozen raw material. The models would be able to predict the physical, chemical and sensory quality parameters from the five process parameters.

The overall purpose of this study was, however, to identify an effective and goal-oriented general strategy for improving the quality of the final frozen fish product. The current work is to form the basis of future work that would enable systematic sorting of the raw material to be carried out, prior to production.

MATERIALS AND METHODS

Experimental design

The experimental design used in this study was a combination of a reduced five factor, two level factorial design and a random design. Different qualities of frozen cod were achieved by varying the following five process parameters:

X1	period of frozen storage
X2	frozen storage temperature
X3	place of catch
X4	season for catching
X5	state of rigor

For each of the five process parameters, a high level (represented by '1' in the design data) and a low level (represented by '0' in the design data) were chosen in order to make the two levels as different as possible.

The low level for the period of frozen storage was chosen to be 0–5 months of frozen storage ('0') and the high level was chosen to be 5–12 months of frozen storage ('1'), since the changes that occur after the first few months of frozen storage are expected to influence the quality of the frozen cod significantly.

The levels of frozen storage temperature were chosen to be constant storage temperatures between -20 and -24°C ('0') and fluctuating frozen storage temperatures between -9 and -24°C ('1') in order to create a difference in the rate of protein denaturation. Fluctuating storage temperature is expected to significantly increase the rate of formaldehyde production (Sotelo *et al* 1995).

The global market for frozen fish products makes it important to represent different strains of cod in the experimental design. Two different places of catch (0, catch in Danish waters; 1, catch in the Barents Sea (northern part of Norway)) were included in this work. The cod caught in Danish waters were transported live to the laboratory, where they were quick frozen in a blast-freezer (-35°C) for 2 h. The cod caught in the Barents Sea were quick frozen on-board in blocks in a plate freezer at -30°C for 2 h. For both places of catch the freezing rate of the cod samples is thus very fast, and the different freezing methods are not expected to influence the quality of the frozen sample.

The two seasons for catching were chosen to vary the spawn status of the cod (0, Spring (ie January to June, corresponding to the spawn period); 1, Autumn (ie July to December)). The levels of the state of rigor (0, fast-freezing pre rigor; 1, fast-freezing in rigor or post-rigor) were chosen partly to represent biochemical differences and partly to represent the time from catch to freezing. The state of rigor was assessed by following the processing of the cod closely from catch to freezing.

By combining these five process parameters, 32 different combinations exist. Due to limited availability of the biological raw material used in this work, it was only possible to include 18 of these in the experiment. (Table 1).

As it appears from Table 1, the distribution of the 115 samples into the 18 factorial combinations is not absolutely even and the 18 factor combinations do not correspond to a traditional fractional factorial design. However, the main point of the experimental design for

TABLE 1
Review of the 18 frozen cod series in the experimental design

Series no.	*Number of samples*	*Variable*				
		X1	*X2*	*X3*	*X4*	*X5*
1	5	0–5 months	Constant	Denmark	Spring	Post-rigor
2	5	0–5 months	Constant	Denmark	Autumn	Pre-rigor
3	5	0–5 months	Constant	Denmark	Autumn	Post-rigor
4	5	0–5 months	Constant	Barents Sea	Spring	Pre-rigor
5	5	0–5 months	Constant	Barents Sea	Spring	Post-rigor
6	5	0–5 months	Fluctuating	Denmark	Spring	Post-rigor
7	5	0–5 months	Fluctuating	Denmark	Autumn	Pre-rigor
8	6	0–5 months	Fluctuating	Denmark	Autumn	Post-rigor
9	10	0–5 months	Fluctuating	Barents Sea	Autumn	Post-rigor
10	6	>5 months	Constant	Denmark	Spring	Pre-rigor
11	5	>5 months	Constant	Denmark	Spring	Post-rigor
12	7	>5 months	Constant	Denmark	Autumn	Pre-rigor
13	5	>5 months	Constant	Denmark	Autumn	Post-rigor
14	5	>5 months	Constant	Barents Sea	Spring	Pre-rigor
15	6	>5 months	Constant	Barents Sea	Spring	Post-rigor
16	5	>5 months	Fluctuating	Denmark	Spring	Pre-rigor
17	15	>5 months	Fluctuating	Denmark	Spring	Pre-rigor
18	10	>5 months	Fluctuating	Barents Sea	Spring	?

explorative data analysis and multivariate calibration is the spanning of all important types of variability in the experimental space. In this case, this means that the design must cover the possible combinations of raw material treatments and, for prediction purposes that the design covers the expected possible variations in the chemical, physical and sensory quality parameters. Likewise, it has not been possible to choose the levels very precisely, and the resulting design is therefore in some sense a random design where the samples are randomly distributed in the experimental space.

Physical, chemical and sensory analyses

The frozen cod were thawed in water with a starting temperature of 18°C and a cod to water ratio of 1 : 2. The thawing time was about 15 h. The thawed cod samples were then analysed by physical, chemical and sensory analyses.

Dry matter (*DM*)
Dry matter (DM) was determined in duplicate on approximately 2 g of minced cod fillet weighed and placed in small glasses. The samples were dried to constant weight at 105°C for 20–24 h in an oven, cooled in a desiccator and weighed. DM is reported as percentage of DM in the samples as the average of the two determinations.

Water-holding capacity (*WHC%*)
The water-holding capacity (WHC%) was determined in quadruple on approximately 2 g of minced cod fillet (Eide *et al* 1982) weighed and placed in plastic tubes with a special filter bottom (pore size 100 mm). The samples were centrifuged (1500 *g*, 10°C, 5 min) and weighed again. WHC% was reported as percentage of water left in the samples (average of the four determinations).

Protein
The protein nitrogen content was determined by a Kjeldahl method, which was a slightly modified version of the AOAC methods No. 937.07 and No. 981.10. The application of a mercury containing catalyst is avoided, and the distillation into boric acid is substituted by distillation into hydrochloric acid.

Total volatile basic nitrogen (*TVB-N*)
The total volatile basic nitrogen (TVB-N) content was determined by the Conway method (Conway and Byrne 1933).

Perchloric acid extracts
The thawed fish samples were cut into small pieces and 30 g samples were homogenised with 60 ml of 6% (w/w) $HClO_4$ by an Ultra-Thurrax at maximum speed for 5 min. The samples were cooled with ice during the extraction procedure. The mixture was filtered though a Whatman No. 1 filter and the filtrate was adjusted to pH 7·0 with a known amount of 30% (w/w) KOH. The neutralised extracts were stored on ice for 1 h to allow $KClO_4$ crystals to precipitate.

Formaldehyde (HCHO)
Assays for free formaldehyde (HCHO) were performed using a modification of an enzymatic flow injection analysis (FIA) method (Bechmann 1996). The analyses were performed on neutralised perchloric acid extracts, and for this reason the FIA system used for the HCHO determination in this work was not furnished with a gel-filtration column.

Dimethylamine (DMA-N)
The determination of dimethylamine (DMA-N) was performed by a gas chromatographic method (Manthey 1988). The analyses were performed on perchloric acid extracts.

Sensory analyses by the quality index method (QIM)
Whole thawed cod and raw fillet were evaluated by 2–4 trained assessors using the quality index method (QIM) for frozen cod (Bremner *et al* 1987; Nielsen and Jessen 1997; Warm *et al* 1997). The method is based on a selected number of independent parameters which describe the quality of the thawed cod. The parameters chosen for frozen cod vary considerably with time and condition of frozen storage and catch handling. The maximum score of each parameter depends on the detectable variability of that parameter and thereby determines its relative importance in the total quality index. For whole cod texture, marks from fishing tackles/catch handling, odour and flesh colour in open spaces are scored from 0 to 3 and remain of guts, shape of fish and appearance have scorings from 0 to 2. There are, in this way, no high scores for any parameter that can place undue emphasis on one particular criterion. For all parameters, 0 represents the quality of freshly caught, appropriately handled and frozen cod. The scores for all single parameters are added to give the total quality index. For fillets the parameters are texture, colour, blood stains and gapping, which vary from 0 to 3, and odour and parasites, which vary from 0 to 2. The quality indices for whole cod (QIM_w) and fillet (QIM_f) were used in the multivariate analysis and determined as averages over assessors.

Multivariate analysis

The data obtained from the experiments were analysed using the multivariate methods principal component analysis (PCA) and partial least squares (PLS) regression (Martens and Næs 1989; Esbensen 1994).

PCA, is a method for extracting the systematic variations in a single data set represented as a matrix (X). Each component in a PCA model is characterised by the loadings describing the relations between the variables (columns), the scores describing the properties of the samples (rows) and, finally, the variance (explained variance or residual variance), which describes how much information is (or is not) taken into account by the successive principal components (PCs).

The general purpose of PLS regression (Martens and Næs, 1989) is multivariate calibration, ie to find a mathematical relation between two data sets, X and Y. PLS performs a simultaneous decomposition of the X and Y matrices in such a way that the information in the Y matrix is directly used as a guide for the decomposition of X, and then performs a regression on Y. PLS is a linear method but can be extended by including second-order variables, ie products of pairs of the primary X-variables.

Both PCA and PLS algorithms can handle data matrices in which some data are missing. If there is sufficient redundancy in the data material, a few evenly distributed missing values do not affect the modelling.

All calculations were performed in Unscrambler ver. 6·1, CAMO A/S, Trondheim, Norway.

RESULTS AND DISCUSSION

Data

The data set consisted of a design matrix representing the frozen storage conditions of each of the 115 cod samples and a data table containing the results of the physical, chemical and sensory analysis performed on the samples. Besides the five original storage parameter variables, second-order terms from logical 'AND' operations between each possible variable pair were included in the design matrix, with the result of improving the models considerably. The resulting design matrix was a 115 × 15 matrix containing zeros or ones. For binary data the logical 'AND' operator corresponds to the product of the variables.

The physical, chemical and sensory quality parameters (DM, WHC%, protein content, TVB-N, HCHO, DMA, QIM_f and QIM_w) measured for each of the samples resulted in a 115 × 8 matrix. The mean value, the standard deviation (SD) and the coefficient of variability (CV) of each variable in this 115 × 8 matrix are shown in Table 2.

Analysis of variance using the non-parametric Kruskal–Wallis test (Sokal and Rohlf 1981) were performed on the data for the physical, chemical and sensory quality parameters. The analysis showed that the variation between the different series of cod samples were significantly ($P < 0.01$) larger than the variation within the series.

Principal component analysis

The first step in the multivariate analysis was to achieve an overview of the main variations in the physical, chemical and sensory quality parameters measured on the 115 fish samples. A PCA-model with physical,

TABLE 2
Mean value, standard deviation (SD) and coefficient of variation (CV) of the physical, chemical and sensory parameters[a] for the 115 cod samples included in the experiment

	DM (%)	*WHC%* (%)	*Protein* (%)	*TVB-N* (*mg-N 100 g*$^{-1}$)	*HCHO* (*mg kg*$^{-1}$)	*DMA-N* (*mg-N kg*$^{-1}$)	QIM_f	QIM_w
Mean	17·77	66·57	17·57	12·41	2·13	20·58	5·95	6·27
SD	0·75	10·82	0·89	2·99	1·98	19·18	1·77	2·31
CV (in %)	4%	16%	5%	24%	92%	93%	29%	36%

[a] DM, dry matter; WHC%, water-holding capacity; TVB-N, total volatile basic nitrogen; HCHO, formaldehyde; DMA-N, dimethylamine; QIM_f, QIM_w, quality index method for fillet and whole code, respectively.

chemical and sensory variables was made. It was decided to keep the parameters of DM and protein content out of the calculations, due to the fact that the dry weight and the protein content actually do not vary much in cod under the conditions employed here (Table 2). The remaining variables were weighted with the inverse of the SD of all objects. This was done to compensate for the different scales of the variables. It was found that three PCs explained 76% of the variation in the data set (PC1, 50%; PC2, 20%; and PC3, 6%). The loadings of the first two PCs are shown in Fig 1.

The loading plot shows that all the variables contributed strongly to the variation described by the first PC. The WHC% was negatively correlated to the rest of the quality parameters. This is in agreement with the fact that a cod sample of poor quality has a low WHC% but a high content of DMA, HCHO and TVB-N and a high score in the QIM analysis (corresponding to poor quality). The sensory variables are located near each other in the loading plot, indicating that these variables were co-linear. Correspondingly, the three chemical variables are grouped in the plot.

When the fish samples are marked in accordance with the storage temperature in the scores plot (Fig 2), a clear grouping is seen along the first PC. The samples with constant low storage temperature are located on the left side of the plot, while the samples with fluctuating storage temperature are mainly placed on the right side. It is not possible to make a clear grouping in the scores plot using any of the other process parameters as markers for the samples. This indicates that, among the five process parameters varied in this experiment, the storage temperature is the most important for the differences in the measured quality parameters. However, the difficulties of making a clear grouping can as well be caused by the experimental design used in this investigation. The two levels of storage temperature chosen are very different, and this parameter cannot be excluded to a certain degree to smear out the influence of the remaining process parameters.

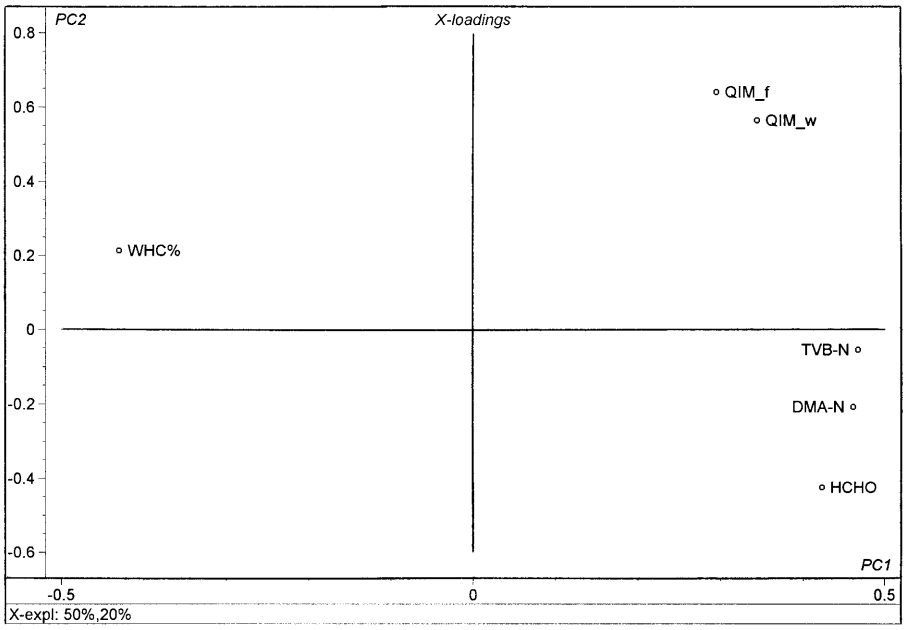

Fig 1. Loading plot for the first two principal components (PCs) of the principal component analysis (PCA) model. The first (PC1) and the second (PC2) explained, respectively, 50 and 20% of the variation in the data set. See footnote to Table 2.

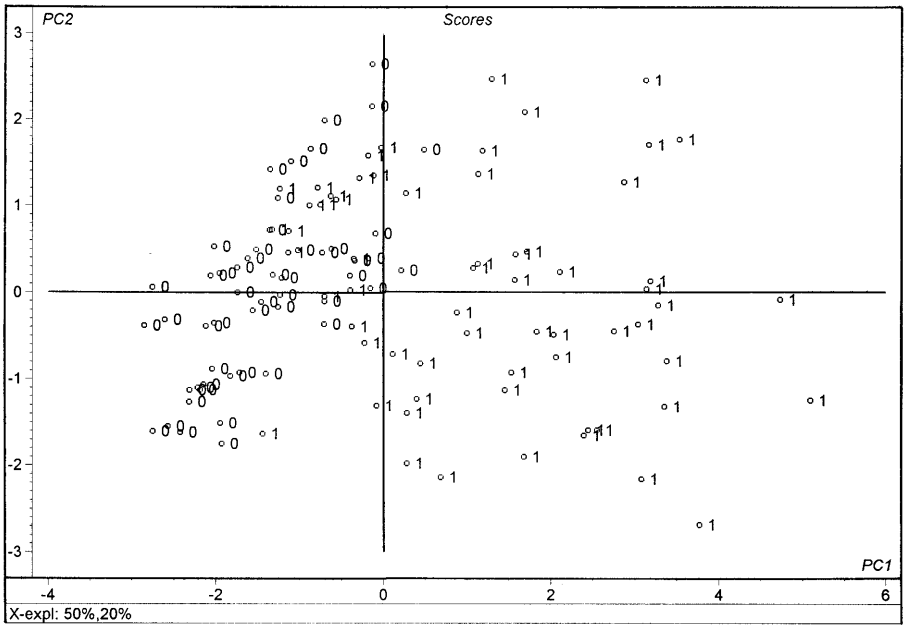

Fig 2. Scores plot for the first two principal components (PCs) of the principal component analysis (PCA) model. The samples with constant low storage temperature (marked with '0') are located on the left side of the plot, and the samples with fluctuating storage temperature (marked with '1') are mainly placed on the right side.

Partial least squares regression

For prediction purposes, eight PLS regression models were made. The X-matrix (115 x 15) contained the process parameters and the second order terms of these, and the Y-matrices (115 × 1) contained each of the eight chemical, physical and sensory values measured. Validation parameters for the eight PLS models are represented in Table 3. The PLS models were evaluated by the root mean square error of prediction (RMSEP), by the correlation between predicted and measured values and by the amount of explained validation variance of Y. The models were validated by a systematic cross validation using five segments, and the objects were sorted according to Y-values before modelling.

It is seen that the prediction ability was best for WHC% and that 82% of the variation in this variable was explained by the use of four PLS components. Relatively good prediction results according to QIM_w and QIM_f were achieved after 4 and 7 components, respectively. Fairly good prediction results for TVB-N, DMA and HCHO were achieved, but it was not pos-

TABLE 3
Validation of partial least squares (PLS) regression models for the prediction of the physical, chemical and sensory variables[a] from process data

Y-value	*Number of PLS components*	*RMSEP*[b]	*Correlation*[c]	*Percentage of Y variance explained*[d]
DM	4	0·66	0·43	26 (22)
WHC%	4	3·88	0·92	82 (66)
Protein	3	0·8	0·46	11 (7)
TVB-N	6	2·22	0·67	44 (23)
HCHO	5	1·11	0·76	46 (33)
DMA-N	3	9·42	0·8	58 (49)
QIM_f	7	1·28	0·69	48 (31)
QIM_w	4	1·3	0·83	68 (54)

[a] See footnote to Table 2.
[b] The root mean square error of prediction (in the same units as the original variables).
[c] The corresponding correlations between predicted and measured values.
[d] The amount of variance in the Y-matrix explained by the models; the numbers in brackets denote the amount of Y-variance explained by the first PLS component.

sible to predict the content of protein and DM in the samples. The reason for the unsatisfactory prediction ability for these two variables is the fact that protein and DM do not vary sufficiently in this experiment. This can be explained by biological conditions or can be expressive of an insufficient experimental design.

The presence of some missing data in the design matrix was found to have only limited influence is the PLS models. If the samples with missing values (series 18) are removed before calibration, the amount of explained Y variance in the PLS models was increased a little, but this was followed by an increase in the RMSEP values, presumably due to the reduction in number of samples included in the models.

The importance of each of the five process parameters and the product variables can be evaluated by inspecting the loadings of each of the eight PLS models. For all models it was found that the first PLS component accounts for the majority of the total amount of variance in the Y-matrix which was explained by the models (Table 3). The loadings for the first PLS component of the eight models are given in Fig 3.

By comparison of the size of the stacked bars, it appears that the period of frozen storage (X1), the frozen storage temperature (X2) and the product term (X1*X2) corresponding to these process variables were generally important for the variance explanation along the first PLS component. However, as discussed in relation to the PCA model, the experimental design used in this investigation cannot be excluded to a certain degree to smear out the influence of the remaining process parameters. Furthermore, it must be emphasised that the remaining process variables (and product terms) are important for some of the eight models and that they might as well be important for the variance explanation along the PLS components of higher order.

CONCLUSION

The use of multivariate analysis has turned out to simplify the interpretation of the relationships between the process parameters and the quality indices measured in this work. The prediction abilities achieved in this work were not sufficient to enable sorting of the raw material. The work has, however, provided a basis of future work.

For further investigation of the influence of frozen storage conditions on the quality of frozen fish products, a complete experimental design of the process parameters should be performed (Carlson 1992). For validation of a final model, an independent test set should be used. The establishment of a final model that would be appropriate for prediction purposes requires, however, a systematic collection of cod samples over at least 2 years.

In future investigations the inclusion of some other process parameters in the experiment should be considered. The biological condition of the fish, the rate of freezing and the rate of thawing are examples of factors which would be interesting to include.

Likewise, the inclusion of additional analytical methods, eg specific measurements of muscle state in

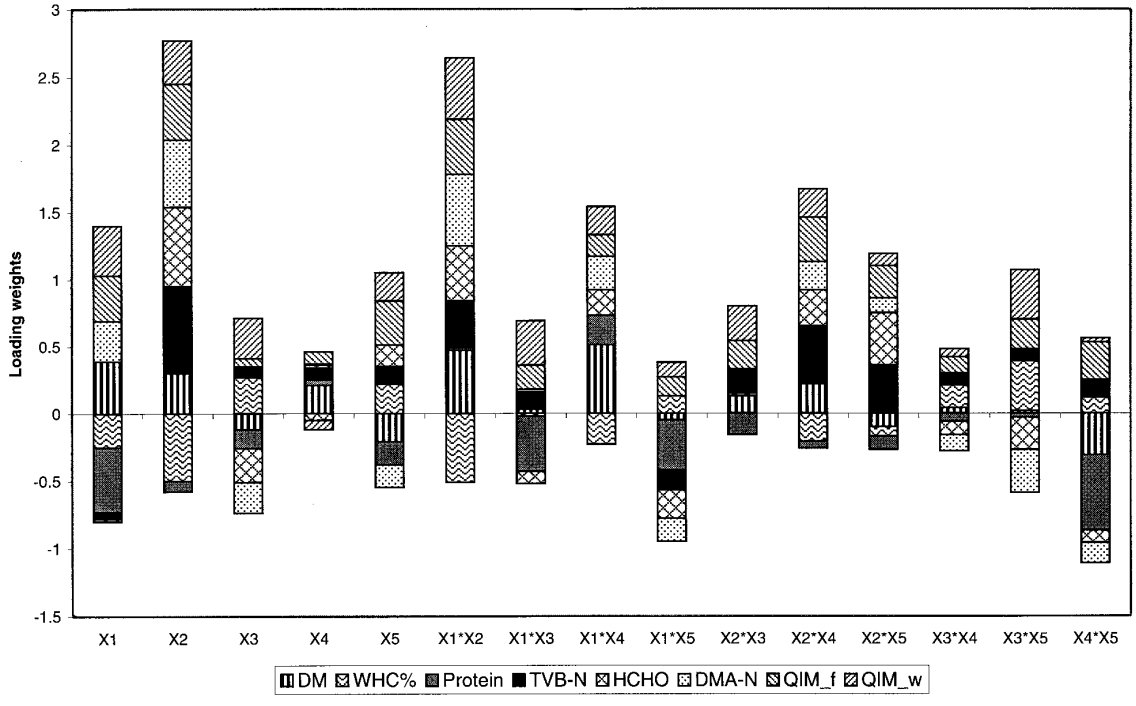

Fig 3. Stacked bar chart depicting the loading weights for the first PLS component for the eight PLS models used for the prediction of the physical, chemical and sensory variables from process data: X1, period of frozen storage; X2, frozen storage temperature; X3, place of catch; X4, season for catching; X5, state of rigor. See footnote to Table 2.

relation to place of catch, season and state of rigor, should be considered. The experience achieved by this work can be used in further investigations, not only in order to predict the physical, chemical and sensory quality parameters from process parameters, but generally in work concerning the quality of frozen raw material. Sufficient spanning of the variation in physical, chemical and sensory quality is necessary in the attempts to use near-infrared (NIR) spectroscopy as a quick method for the assessment of frozen fish quality, which is an important area in the authors' future research.

ACKNOWLEDGEMENTS

The authors thank Dr Bo Jørgensen for valuable discussions during the preparation of this manuscript. The Danish Ministry of Food, Agriculture and Fisheries is acknowledged for financial support. Analyses of DMA were performed at the Institute of Biochemistry, Federal Research Centre for Fisheries in Hamburg. The authors thank Dr H Karl for his collaboration.

REFERENCES

Bechmann I E 1996 Determination of formaldehyde in frozen fish with formaldehyde dehydrogenase using a flow injection system with an incorporated gel-filtration chromatography column. *Anal Chim Acta* **320** 155–164.

Bremner H A, Olley J, Vail A M A 1985 Estimating time-temperature effects by a rapid systematic sensory method. In *Dev Food Sci 15: Seafood Quality Determination*, ed Kramer D E & Liston J. Elsevier, Amsterdam, The Netherlands, pp 413–435.

Carlson R 1992 *Design and Optimisation in Organic Synthesis.* Elsevier, Amsterdam, The Netherlands.

Conway E J, Byrne A 1933 An absorption apparatus for the microdetermination of certain substances. The microdetermination of ammonia. *Biochem J* **27** 419–429.

Eide O, Børresen T, Strøm O 1982 Minced fish production from capelin (*Mallotus villosus*). *J Food Sci* **47** 347–354.

Esbensen K 1994 *Multivariate Analysis—In Practice.* CAMO A/S, Trondheim, Norway.

Haard N F 1990 Biochemical reactions in fish muscle during frozen storage. In: *Seafood Science and Technology.* Fishing News Books, Halifax, Canada, pp 176–209.

Kozima T T 1983 Effect of temperature fluctuations on quality of frozen fish. *Refrigeration* **58** 23.

LeBlanc E L, LeBlanc R J, Blum I E 1988 Prediction of quality in frozen cod (*Gadus morhua*) fillets. *J Food Sci* **53** 328–340.

Love R M 1988 *The Food Fishes: Their Intrinsic Variation and Practical Implications.* Farrand Press, London, UK.

Manthey M 1988 Gaschromatographische Bestimmung von Dimethyl- and Trimethylamin in Fisch und Fischerzeugnissen. *Info Fischwirtschaft* **35** 131–135 (in German).

Martens H, Næs T 1989 *Multivariate Calibration.* John Wiley, Chichester, UK.

Nielsen J, Jessen K 1997 New developments in sensory analysis for fish and fishery products. In: *Proceedings International Seafood Conference* 1995, ed Luten J B, Børresen T & Oehlenschläger J. Elsevier, Amsterdam, The Netherlands, pp 537–547.

Shenuoda S Y 1980 Theories of protein denaturation during frozen storage of fish flesh. In: *Advances in Food Research* ed Chichester C O, Mrak E M & Steward G F. Academic Press, New York, USA, pp 275–311.

Sokal R R, Rohlf F J 1981 *Biometry: The Principles and Practice of Statistics in Biological Research*, (2nd edn). W H Freeman & Co, San Fransisco, CA, USA.

Sotelo C G, Piñeiro C, Pérez-Martín R I 1995 Denaturation of fish proteins during frozen storage: role of formaldehyde. *Zeitschr Lebens-Unter Forsch* **200** 14–23

Warm K, Bøknæs N, Nielsen J 1997 Development of quality index methods for evaluation of frozen cod (*Gadus morhua*) and cod fillets. *J Aquat Food Prod Technol* **7** (1) 45–59.