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CONTAMINATION PREDICTIONS OF CAPE HAKE FILLETS DURING DISPLAY AND STORAGE BY ARTIFICIAL NEURAL NETWORK MODELING OF HEXADECANOIC ACID

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ABSTRACT

This study aimed to design an artificial neural network (ANN) that could distinguish between Cape hake fillets displayed and stored on ice that have been exposed to excessive contamination and those that were not. The selected variable was a biochemical indicator, hexadecanoic acid, a fatty acid. Cape hake fillets with and without excessive contamination was kept on ice and analyzed every 48 h over a period of 10 days. A novel ANN was designed and applied, which provided an acceptable prediction on the contaminated fillets based only on the hexadecanoic acid changes during day 8 (T4) and day 10 (T5). The ANN consisted of a multilayered network with supervised training arranged into an ordered hierarchy of layers, in which connections were allowed only between nodes in immediately adjacent layers. The network consists of two inputs, T4 and T5 connected to two neurones that are connected to one output neuron that indicates a prediction on contamination of the fillets. These two neurons are connected to one output neuron that indicates a prediction on contamination of the fillets.

PRACTICAL APPLICATIONS

The model sets the stage for the development of alternative quality control measures for retailers and buyers of fish and other foods that contain fatty acids such as hexadecanoic acid to provide saver food.

INTRODUCTION

Artificial neural networks (ANNs) have recently seen an explosion of interest and application in numerous fields, including piscimetrics and food analysis to model complex real-world problems. Piscimetrics were initiated when neural networks and other chemometrics were applied in fisheries research. This entails the life cycle of fish, fish identification, fish stock and factors affecting it. However, there is still scope to apply ANN on final products made available to retail shops with specific focus on shelf life prediction (Suryanarayana *et al.* 2008; Matera *et al.* 2014). ANNs' attractiveness to food science is its ability to model the kinds of data encountered in food science. A limited number of "clean" variables are qualified on a suitable number of samples with a basic linear or at least mildly nonlinear model to those where many variables (possibly noisy or highly correlated) are qualified on a small number of samples and the functional relation is heavily nonlinear (Basheer and Hajmeer 2000; Marini 2009). In ANN, the development of a small database size is a concern because of the inability to partition the database into manageable sized subsets for training, testing and validation. To expand the size of the database, due to difficulty or because it might be expensive to obtain new data in a conventional manner, is to interject random noise in the available examples to generate new ones (Swingler 2001). This addition of noise enhances the ANN's robustness against measurement error (e.g., noise = ± instrument sensitivity) and is called data enrichment (Basheer and Hajmeer 2000).

ANNs, classified as artificial intelligence, is a family of mathematical models where the main algorithmic features are inspired by the functioning of the human brain, simulating human intelligence. However, currently, a neural network is predominantly a mathematical rather than a biological model (Callan 2003; Marini 2009). ANN is based on

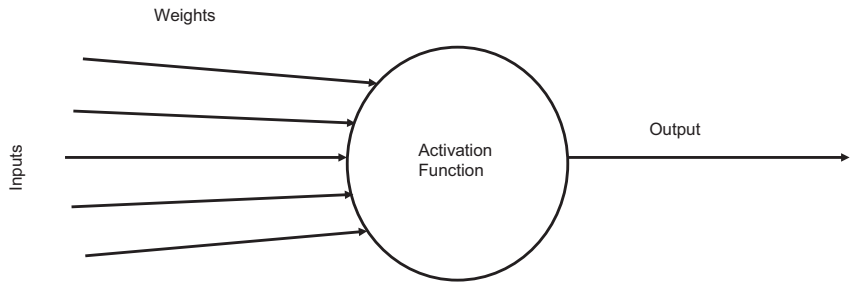

FIG. 1. LAYOUT OF A SINGLE NETWORK NODE (ADAPTED FROM CALLAN 2003)

collections of neurons or nodes that are connected in a tree model to permit communication (Callan 2003). A single node computes by combining the input signals with an activation function to produce an output signal (Fig. 1; Callan 2003).

These nodes are interconnected with weighted connections – weight being a multiplying constant for the connection's input. In isolation, these nodes are limited in operation, but interconnection in a multilayered network gives them the ability to perform complex tasks in food analysis and food safety. These include food authentication, prediction whether a foodstuff is contaminated or not, as well as the identification of the kind of microbial contamination and ultimately to determine the freshness, quality and or shelf life of food products (Bertone *et al.* 1996; Siripatrawan and Jantawat 2008; Limbo *et al.* 2009; Marini 2009; Siripatrawan *et al.* 2009; Venter *et al.* 2013).

Freshness of fish during storage depends on various factors, including storage temperature, the fish species and its physiological condition, initial microbial load, contamination and physical handling (Huss 1995; Raatikainen *et al.* 2005). Deterioration of fish lipids are primarily caused by two distinct reactions, namely hydrolysis and oxidation – generally from endogenous enzymes and contaminant bacteria. These will influence among others, the organoleptic properties and therefore the shelf life of the final product (Ackman 1989; Huss 1995; Aubourg 1999; Baixas-Nogueras *et al.* 2002). Sensory scoring methods, such as the quality index method have been developed for the evaluation of fish freshness and chemical and biochemical parameters have been used in numerous studies. These parameters include pH, trimethylamine, K value, peroxide index and free fatty acids (Pacheco-Aguilar *et al.* 2000; Baixas-Nogueras *et al.* 2003; Herrero *et al.* 2003). Free fatty acid levels in hake muscle correlated well with sensory scoring methods from previous studies and a conclusion was formed that the free fatty acid level could be used instead of sensory scoring methods to determine hake freshness (Barassi *et al.* 1987).

Prediction of the remaining shelf life of the whole and filleted fish has been investigated using numerous chemometric applications (Barassi *et al.* 1987; Limbo *et al.* 2009). However, free fatty acids have not been used in combination with ANN to predict if Cape hake fillets have been exposed to excessive contamination. The aim of this study was to apply a custom-designed ANN to a basic variable, hexadecanoic acid, to be applied in producing and validating a recognition pattern that may be used to predict whether fish fillets were exposed to excessive microbial contamination originating from, among others, improper handling or storage.

MATERIALS AND METHODS

Cape hake (*Merluccius capensis* and *Merluccius paradoxus)* samples were harvested by a leading South African fishing industry during the month of February from the South African shoreline close to the city of Cape Town (Méndez and Gonzalez 1997). The Cape hake were mechanically scaled, headed, gutted and kept on ice (average muscle temperature 7 ± 0.5C) for 24 h prior to laboratory analysis. For analyses, 10 fishes, which weighed 166 ± 25 g and had an average length of 21 ± 2 cm (beheaded) were selected. The first five fishes were filleted and one fillet of each fish was used for the shelf life study at 8C on ice – hereafter referred to as "C8" or control. The other set of five fillets were kept at the same temperature, but the fillets were inoculated with an increased load of autochthonous microbiota found on hake (5.84×10^8 cfu/mL) – hereafter referred to as "I8" or inoculated. Both C8 and I8 simulate fillets which are displayed on ice corresponding with retail stores using display refrigerators with the outside temperature monitored and kept at 8C. The second five Cape hakes were filleted and used for the shelf life study at ambient temperature (25C) on ice – hereafter referred to as "C25" and "I25." This simulated fillets displayed on ice in an unmonitored environment corresponded with retail stores where fish are displayed in the open. Fish fillets and whole fish are usually displayed on ice in shops during the day and refrigerated during night time. The standard duration of fish displayed on ice by major retail stores in South Africa is 7 days. According to the study carried out on Mediterranean hake, hake should be rejected after 10 days (Baixas-Nogueras *et al.* 2003).

Fatty Acid Extraction

Of the homogenate, 0.7 g was subjected to total lipid extraction as described by Folsch *et al.* using chloroform : methanol 2:1 (v/v) (Folsch *et al.* 1957). All reagents, solvents and standards were of analytical grade (Merck, Midrand, South Africa and Separations, Randburg, South Africa) and stored in dark bottles.

Fatty Acid Analysis

Transesterification of the fish lipids was carried out by addition of trimethylsulfonium hydroxide (Merck) (Butte 1983; Gómez-Brandón *et al.* 2008; Trobović *et al.* 2013). Extracts were stored in glass vials and frozen at −18C until chromatographic readings were performed. The fatty acid methyl esters were analyzed and separated on a Finnegan Focus (Thermo Finnegan, San Jose, CA, USA) Gas chromatogram (GC) equipped with a 30 m × 0.25 mm ZB-1 (Separations) glass capillary column. The column contained 100% dimethyl polysiloxane (0.25 μm) with helium as carrier gas (constant flow – 3.0 mL/min) and functioned in a splitless mode of injection. The temperature program is summarized as follows: 40C to 90C at a rate of 8C/min, followed by a ramp from 90C to 280C at 10C/min. The column was attached to a Finnegan Focus DSQ mass spectrometer (MS) for mass detection of fragments with *m/z* smaller than 1,000. Mass analysis was performed at eV with an ion source temperature of 200C. Integration of the peaks was performed on the total ion chromatogram using Xcalibur software version 1.4 SR1 (Finnegan). Fatty acid methyl esters were identified by comparison of their mass spectra and retention time using the internal library of GC-MS equipment and standard. From all the fatty acids originally detected, only hexadecanoic acid was selected to be used in this study. The selection of this acid was carried out as it has been reported to be the most abundant fatty acid in fish, as well as being used often as a reference component in quantitative and qualitative assessments (Baixas-Nogueras *et al.* 2002, 2003; Tocher 2003; Trobović *et al.* 2013).

Data Analysis

Total signal results of all the fatty acids were used to determine the final data used in the ANN. The raw data underwent several preprocessing techniques, e.g., reducing input dimensionality and data transformation, treatment of nonnormally distributed data, data inspection and deletion of outliers to accelerate convergence before it could be used for training in an ANN. Every individual fatty acid's total signal was divided by the total of all the fatty acids to render the data between 0 and 1. All calculations were carried out using Microsoft Office Excel 2003 (Microsoft, Redmond, WA, USA).

It was complex to create an ANN with the available hexadecanoic acid results to determine both the difference between the temperature as well as excessively contaminated samples. In order to increase the number of hexadecanoic acid results, an ANN was created to differentiate only between excessively contaminated samples or noncontaminated samples. With two examples of contaminated and two of noncontaminated in a training set, more data were required to effectively teach the neural network. Random noise of up to ±20% was, therefore, added to the data set at each learning cycle, artificially extending the data set. This provided a larger training set as well as a model with more robust generalization properties (Swingler 2001; Venter *et al.* 2013).

It is important to balance the data in classification problems. Training data were distributed as evenly as possible between the various classes to prevent the network from being biased to the overrepresented classes. Some of the overrepresented classes may, therefore, be removed or extra examples may be added, pertaining to the underrepresented class. Alternatively, the underrepresented input/output examples may be duplicated and random noise could be added to their input data while keeping the output class unchanged (Basheer and Hajmeer 2000; Venter *et al.* 2013).

Calculations

By calculating the error at each net or node followed by the adjustment of weights accordingly to produce all the required outputs, a multilayered network with supervised training was designed to be able to learn a required function. This process can be mathematically simulated with the formula of the neuron as follows (Callan 2003; Venter *et al.* 2013)

$$net_j = \sum_{i=1}^{N} x_{i,j} w_{i,j} \tag{1}$$

where N is the number of inputs, i is the node number for a specific input, j is the number of the net, x is the input value and w is the weights or constants.

This is commonly put through a sigmoid function as follows (Callan 2003; Venter *et al.* 2013)

$$f_j = \frac{1}{1+\left[e^{(-net_j)}\right]} \tag{2}$$

where *net* is the output of the net and j is the number of the net.

To calculate the error, the network applies a generalization of the delta rule by starting at the last layer with (Chauvin and Rumelhart 1995; Callan 2003; Venter *et al.* 2013)

$$\delta_j = (t_j - o_j) o_j (1 - o_j) \tag{3}$$

where t is the required output, o is the net output and j is the number of the net.

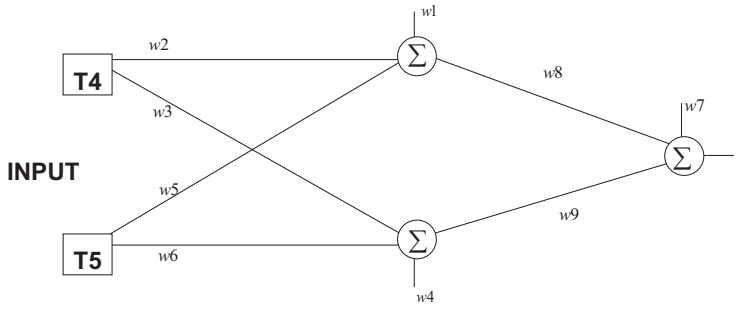

FIG. 2. MULTILAYERED NETWORK FOR THE TWO ANALOGUE INPUTS TO TWO NODES TO ONE NODE TO THE ONE OUTPUT

Subsequently, the error at the hidden layers is calculated as follows (Callan 2003)

$$\delta_j = o_j(1-o_j)\sum_k \delta_k w_{j,k} \tag{4}$$

where o is the net output, j is the number of the net, k is the number of the net from where the error originates, δ_k is the error from the previous layer and l is the number of that specific path.

The weight change for each node is then calculated with (Callan 2003)

$$\Delta w_{i,j} = \eta(x_{i,j}\delta_j) \tag{5}$$

where η is the learning rate, i is the node number for a specific input, j is the number of the net, x is the input value and δ is the error from each layer.

Thereafter, the weights are adjusted as follows (Callan 2003)

$$W_{i,j} = w_{i,j} + \Delta w_{i,j} \tag{6}$$

where Δw is the weight change and W is the old weight.

A training data set was mapped that simulates a real-world problem. This training data set consisted of inputs with the corresponding outputs that were fed to the neural network for weight adaptation. It is advantageous to randomize the order of the presentation for each training sample (Callan 2003; Gurney 2003; Marini 2009; Venter *et al.* 2013).

RESULTS AND DISCUSSION

A multilayered network with supervised training was arranged into an ordered hierarchy of layers, in which connections are allowed only between nodes in immediately adjacent layers, which coded for evaluations. Figure 2 illustrates the multilayered network developed. The network consists of two inputs T4 (data from day 8) and T5 (data from day 10) connected to two neurons. These two neurons are connected to one output neuron. The output neuron produces one output that indicates a prediction on contamination. This is a network with two layers of weights capable of approximating any continuous functional mapping. The inputs of T4 and T5 of hexadecanoic acid, methyl ester, were used to train the network. The output would be a percentage of probability of contamination at 8C.

The sigmoid's best resolution for an output is between 0.9 and 0.1. The input values are between this and need no further processing. The output is converted from yes and no to a probability of yes (contaminated). The output range would be from 0.1 of 0% probability and 0.9 for a 100% probability. To ease interpretations, the output is stepped through a function that would give the probability as a percentage. This function is as follows

$$y = \frac{(x-0.1)}{0.8} \times 100 \tag{7}$$

where y is the output probability in percentage and x is the output of the network in the range of 0.1 for 0% to 0.9 for a 100%.

The yes prediction is converted to 0.9 and a no prediction to 0.1 and the data divided up into training and evaluation sets in Table 1.

TABLE 1. THE DATA SETS USED IN THE NEURAL NETWORK, DIVIDED INTO THE NECESSARY TRAINING AND EVALUATION SETS

Training data set	1	2	3
T4	0.551429	0.541386	0.551429
T5	0.487019	0.518307	0.487019
Control	0.1	0.1	0.1
T4	0.541386	0.522321	0.522321
T5	0.518307	0.515228	0.515228
Control	0.1	0.1	0.1
T4	0.497288	0.506668	0.497288
T5	0.50756	0.516287	0.50756
Contaminated	0.9	0.9	0.9
T4	0.506668	0.498248	0.498248
T5	0.516287	0.502239	0.502239
Contaminated	0.9	0.9	0.9
Evaluation set	**1**	**2**	**3**
T4	0.522321	0.551429	0.541386
T5	0.515228	0.487019	0.518307
Control	0.1	0.1	0.1
T4	0.498248	0.497288	0.506668
T5	0.502239	0.50756	0.516287
Contaminated	0.9	0.9	0.9

TABLE 2. SIX EXAMPLES OF EACH SAMPLE (T4 AND T5) WITHIN EACH TRAINING DATA SET (1, 2 AND 3) PLUS NOISE AFTER THE NEURAL NETWORK WAS TAUGHT FOR 500,000 CYCLES AT A RATE OF 0.5, INCLUDING THE EVALUATION DATA SET AND THE RESULTS

Training data set 1	First sample	Control	Second sample	Control	Third sample	Contaminated	Fourth sample	Contaminated
T4	0.5514	0.1	0.5414	0.1	0.4973	0.9	0.5067	0.9
T5	0.4870		0.5183		0.5076		0.5163	

Six examples of input values from each set plus random ±20% noise

Input/output	First set	Output	Second set	Output	Third set	Output	Fourth set	Output
T4	0.4741	–11.9581	0.4806	2.2766	0.4330	97.8478	0.4582	97.1088
T5	0.4187		0.4601		0.4419		0.4669	
T4	0.5758	–12.1758	0.4451	4.4470	0.4219	97.4932	0.5741	99.6816
T5	0.5085		0.4261		0.4306		0.5850	
T4	0.6222	–12.2235	0.4529	3.9397	0.4490	98.3333	0.4627	97.2353
T5	0.5495		0.4336		0.4583		0.4715	
T4	0.5132	–12.0681	0.6106	–3.1895	0.4152	97.2726	0.4086	95.5912
T5	0.4533		0.5846		0.4237		0.4163	
T4	0.5801	–12.1811	0.5288	–0.1423	0.4618	98.7015	0.4882	97.9036
T5	0.5123		0.5063		0.4713		0.4975	
T4	0.4730	–11.9545	0.4627	3.3266	0.4470	98.2759	0.5188	98.6195
T5	0.4178		0.4429		0.4563		0.5286	

Evaluation data set 1			Status	Trained net output	Prediction
T4	First set	0.5223	Control	40.3814	✓
T5		0.5152			
T4	Second set	0.4982	Contaminated	84.4114	✓
T5		0.5022			

Training data set 2	First sample	Control	Second sample	Control	Third sample	Contaminated	Fourth sample	Contaminated
T4	0.5414	0.1	0.5223	0.1	0.5067	0.9	0.49825	0.9
T5	0.5183		0.5152		0.5163		0.50224	

Six examples of input values from each set plus random ± 20% noise

Input/output	First set	Output	Second set	Output	Third set	Output	Fourth set	Output
T4	0.5880	–12.4453	0.5880	3.4874	0.4523	109.2513	0.5127	98.9813
T5	0.5630		0.5630		0.4609		0.5168	
T4	0.4518	–12.2487	0.4518	6.3607	0.4286	108.7255	0.5707	101.2974
T5	0.4325		0.4325		0.4367		0.5753	
T4	0.4601	–12.2727	0.4601	3.1107	0.5796	110.9931	0.5700	101.2706
T5	0.4404		0.4404		0.5906		0.5746	
T4	0.6109	–12.4566	0.6109	5.8861	0.5839	111.0299	0.4045	93.7142
T5	0.5848		0.5848		0.5950		0.4078	
T4	0.5103	–12.3735	0.5103	4.4205	0.4228	108.5856	0.5978	102.2628
T5	0.4885		0.4885		0.4309		0.6026	
T4	0.5077	–12.3697	0.5077	8.1403	0.5251	110.4236	0.4535	96.2505
T5	0.4860		0.4860		0.5351		0.4571	

Evaluation data set 2			Status	Trained net output	Prediction
T4	First set	0.5514	Control	–12.5000	✓
T5		0.4870			
T4	Second set	0.4973	Contaminated	110.6229	✓
T5		0.5076			

Training data set 3	First sample	Control	Second sample	Control	Third sample	Contaminated	Fourth sample	Contaminated
T4	0.5514	0.1	0.5223	0.1	0.4973	0.9	0.49825	0.9
T5	0.4870		0.5152		0.5076		0.50224	

Six examples of input values from each set plus random ± 20% noise

Input/output	First set	Output	Second set	Output	Third set	Output	Fourth set	Output
T4	0.5056	–12.5000	0.4212	2.9666	0.5103	109.9299	0.5842	97.2802
T5	0.4465		0.4154		0.5208		0.5889	
T4	0.4518	–12.5000	0.5223	–1.1512	0.4797	109.3321	0.4067	86.0592
T5	0.3990		0.5152		0.4896		0.4100	
T4	0.5899	–12.5000	0.5325	–1.4766	0.4601	108.8758	0.4556	89.5618
T5	0.5210		0.5253		0.4696		0.4592	
T4	0.6604	–12.5000	0.5657	–2.4413	0.3999	106.9862	0.4235	87.2979
T5	0.5833		0.5580		0.4082		0.4269	
T4	0.6418	–12.5000	0.5614	–2.3239	0.5718	110.7972	0.4980	92.3508
T5	0.5669		0.5538		0.5837		0.5020	
T4	0.6433	–12.5000	0.4262	2.7193	0.4483	108.5647	0.4052	85.9470
T5	0.5682		0.4204		0.4575		0.4084	

Evaluation data set 3			Status	Trained net output	Prediction
T4	First set	0.5414	Control	–12.4513	✓
T5		0.5183			
T4	Second set	0.5067	Contaminated	109.0538	✓
T5		0.5163			

There are only two examples of contaminated and two examples of not contaminated in a training set. This is not enough to effectively teach the neural network. To expand this, random noise of up to approximately 20% was added to the data set at each learning cycle. Artificially extending the data set would provide a larger training set as well as a model with more robust generalization properties (Swingler 2001).

A training cycle would be as follows

- Read randomly in a sample from the training set (T4, T5 and the corresponding output).
- Add random noise of up to ±20 % to T4 and T5.
- Compare the network output with the required output to calculate the error and adjust the weights.
- Start again

The training cycle is performed until acceptable results are produced. The evaluation is fed in to evaluate (it was never part of the training set) and the neural network is evaluated. The neural network is evaluated on the learning set plus noise including the evaluation set. The network is trained with a training set that will consist of T4, T5 with their corresponding outputs. This is randomized and the noise is added (Table 2).

Because the random noise is added, it is not possible to over-train the network, whereas 500,000 training cycles at a learning rate of 0.5 would be a sufficient training. Training took 232 s on an Intel Pentium (Intel, Santa Clara, CA, USA) 4 Central Processing Unit (CPU) 3.40 GHz, 2.87 GB Ram.

The ANN model was able to predict correctly whether the fillets were contaminated or not (control) after all three training sets during the evaluation set. Even on the first set, where the prediction was high (40%), it was still under the 50% split, suggesting the control fillet, which is correct.

CONCLUSION

This study confirms the possibility to use a selected fatty acid, e.g., hexadecanoic acid in an ANN model to effectively predict whether a fillet has been exposed to contamination. The neural network created, trained and tested during this study ensured an objective and reliable prediction of the Cape hake fillets under given conditions. The model supports the development of an alternative quality control measures for retailers and buyers of fish and other foods that contain fatty acids such as hexadecanoic acid. This would contribute in providing food with a higher level of safety to consumers and to improve the due diligence that a supplier has to prove in their food safety management systems. However, the ANN presented in this paper is less suitable for endpoint sampling, as growth was followed over 10 days. A network designed to incorporate more than one biological indicator in addition to chemical indicators should, therefore, be considered.

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