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THE APPLICATION OF ARTIFICIAL NEURAL NETWORKS (ANN) FOR THE DENATURATION OF MEAT PROTEINS – THE KINETIC ANALYSIS METHOD

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ABSTRACT

Background. Artificial neural networks (ANN) are a common mathematical tool widely used in many research fields. Since they are applicable to non-linear relationships and do not require preliminary assumptions, they are a particularly promising tool in relation to meat processing. Thermal denaturation contains a lot of information concerning the quality of meats. The aim was to create a methodology of kinetic analysis to obtain a quick and accurate tool for meat protein denaturation in non-isothermal conditions based on The Coats-Redfern equation with the use of ANN.

Materials and methods. The analyses were carried out on samples of minced samples of Longissimus dorsi (pork). Thermal properties were determined using the differential scanning calorimetry (DSC) method with a Q100 TA Instruments apparatus. The data obtained was processed using the artificial neural network module in Statistica 13.0 software.

Results. The following models fit well with experimental data: F1 and F2 ($r = 0.99$, F Snedecor's F statistics 836943.20 and 971947.41 respectively). Deviations from experimental conversion degrees were higher for model F2, while for F1, good conformity was obtained across the whole range of $\alpha(T)$.

Conclusions. This preliminary study confirmed that methods of traditional kinetics of processes in non-isothermal conditions based on the Coats-Redfern equation can be successfully applied to meat protein denaturation. The method of kinetic analysis allows a high level of accuracy to be achieved and meets the requirements of an efficient engineering tool.

Keywords: artificial neural networks, DSC, meat, thermal denaturation, pork

INTRODUCTION

The global increase in food production and processing has stimulated the sector to seek less time-consuming and more energy-efficient processes which depend mostly on heat transfer. Considering the wide variation in processed materials, and processing conditions themselves, an idea consistently put forward has been

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the use of artificial neural networks (ANN) to optimize those processes on the basis of prediction models. One of the major advantages of ANN is that it allows the efficient handling of highly non-linear relationships between data. Resulting models are simple approximations of complex processes which occur during heat transfer (Hussain and Rahman, 1999). Additionally, ANN are cost-effective and promise optimization algorithms due to their ability to model functions with high accuracy when training and validation procedures are properly carried out (Mazutti et al., 2010). According to Rahman et al. (2012) the use of artificial neural network algorithms helps to overcome calculation difficulties related to the thermal conductivity of foods which *de facto* depend on food composition, structure and processing conditions. Neural networks function as a universal approximating system with the ability to learn from, adapt to and generalise about the knowledge acquired. The ANN method is especially applicable to multivariate data sets with nonlinear dependencies, and it does not require variables to fit any theoretical distribution (Carling, 1992; Fausett, 1994; Tadeusiewicz, 2001). Therefore, ANN modelling has been widely used in the prediction of the heat transfer of various foods (Rahman et al., 2012). However, in the case of analysis of heat transfer in meat, including characteristics of protein denaturation, no ANN studies have been performed before now.

Most studies focused on the conformational changes of the proteins in meat have so far focused on a determination of denaturation temperatures using differential scanning calorimetry (DSC), a method used to characterise mainly the thermodynamics of the denaturation of a protein. Since denaturation induces complex physico-chemical and structural changes in meat properties, DSC, with all its advantages, has now become commonplace in the assessment of the thermal stability of proteins (Durowoju et al., 2017; and references therein). However, the results of temperature-induced structural changes in meat are scattered throughout scientific papers and mostly concern a specific food material, only partially revealing the mechanisms and effects of those changes. Generally, available results for the denaturation of pork, beef (Pospiech et al., 2002) or chicken proteins (Murphy et al., 1998) provide only temperature ranges for a given transformation and no thermo-gravimetry (TG) or DSC analyses (TG-DSC) have been performed with regard to ANN-supported kinetics of temperature-induced processes. Kinetic analysis often poses difficulties since calculations are based on theoretical assumptions and results may not be entirely applicable to real processes, owing to heat balance or gas phase mass transfer resistance. Linear or non-linear estimations sometimes ambiguously indicate the best fit. Therefore, the combination of TG-DSC supported by calculations from ANN methods may significantly improve the accuracy of interpretations of DSC-thermograms obtained for meat samples. Meat is mostly the muscle tissue which consists of approximately 20% protein, which can be divided into three groups, i.e. myofibrillar (50–55%), sarcoplasmic (30–34%) and connective tissue proteins (10–15%). As well as proteins, 75% water, 3% fat and 2% non-protein substances (e.g. non-protein nitrogen-containing substances, carbohydrates, inorganic compounds) make up the rest of meat (Tornberg, 2005). The complex structure of meat means that conformational changes of the proteins occurring on heating depend not only on the main protein component but also on other muscle elements and intrinsic interactions between them. Hence, the use of artificial neural networks to assess the kinetic parameters of temperature-induced denaturation of proteins seems justified. In particular, the profiling of meat denaturation will help to determine heating conditions (e.g. time and rate) and lead to the optimization of the thermal processing of pork meat, whose volume of production is increasing rapidly and is projected to follow this trend in view of the constantly growing demand (Marquer et al., 2015). Therefore, the objective of this work was to quantify the kinetics of protein denaturation during the thermal processing of pork loin meat using DSC. Furthermore, it was to develop and assess thermal conductivity prediction models for the denaturation of proteins in analysed samples based on an artificial neural network modelling.

MATERIALS AND METHODS

Raw material and sample preparation

Fresh pork specimens (*Longissimus dorsi*; 24 h post mortem) were bought in local butcher's stores and

transported to the laboratory, where they were held at about 4°C for 1–2 h. The muscles were trimmed of external fat and connective tissues and minced through 4 mm diameter perforations. After mixing, five individual meat samples were immediately collected for testing using DSC.

Differential Scanning Calorimetry

Thermal properties were determined using the DSC method with a Q100 (TA Instruments) apparatus. The instrument was temperature-calibrated using water and indium. Enthalpy was calibrated with indium. Empty pans were used as reference. The samples, which weighed between 10 and 25 mg (accuracy of ±0.01 mg), were placed in pans, hermetically sealed and submitted to DSC analysis. The denaturation enthalpy (ΔH) of the peaks was estimated by using a heating rate of 5°C min^{-1} from 30°C to 90°C.

Kinetics

In kinetics, the reaction rate of a process (r) proceeding with the participation of solid reacting substances can be determined using the following equation:

$$r = \frac{d\alpha}{dt} \tag{1}$$

where:

α – conversion degree,

t – time.

The rate depends on temperature (T) and conversion degree:

$$r = h(T, \alpha) \tag{2}$$

After separation of variables, equation (2) has the form:

$$r = k(T)f(\alpha) \tag{3}$$

where:

$k(T)$ – reaction rate constant,

$f(\alpha)$ – conversion function dependent on mechanism of reaction.

In the theory of non-isothermal processes, the dependence of the reaction rate on the temperature is commonly described by the Arrhenius equation. Thus, formula (3) for isothermal conditions (in a developed form) can be written as follows:

$$\frac{d\alpha}{dt} = A\exp\left\{-\frac{E}{RT}\right\}f(\alpha) \tag{4}$$

where:

A – pre-exponential Arrhenius factor,

E – apparent activation energy,

R – gas constant.

Assuming a linear increase in temperature and constant heating rate, we obtain (Coats and Redfern, 1964; Ortega, 1996; Straszko et al., 2005; Vyazovkin and Width, 1999):

$$\frac{d\alpha}{dT} = \frac{A}{\beta}\exp\left\{-\frac{E}{RT}\right\}f(\alpha) \tag{5}$$

where:

β – heating rate.

After integration formula (5) has the following form:

$$g(\alpha) = \frac{A}{\beta}\int_{T_0}^{T}\exp\left\{-\frac{E}{RT}\right\}dT \tag{6}$$

where:

$g(\alpha)$ – integral form of the kinetic model.

The low integration limit results from the fact that within a temperature range from 0 to T_0 the reaction does not proceed. T_0 denotes the initial temperature of a given stage. The integral on the right side of equation (6) does not have an analytical solution. The Coats-Redfern approximation is most often applied (Coats and Redfern, 1964). As a result, the Coats-Redfern equation is obtained:

$$\ln\left(\frac{g(\alpha)}{T^2}\right) = \ln\left[\frac{AR}{\beta E}\left(1-\frac{2RT}{E}\right)\right] - \frac{E}{RT} \tag{7}$$

The foregoing formula is a theoretical linear model and may not be sufficiently fulfilled for real processes due to different particle size and geometry, heat balance or gas phase mass transfer resistance. If the influence of these factors is not big and does not

alter the kinetic model, they can be treated as internal randomness for series at the same, and at different heating rates. In this case, linearization of non-linear equations, as is widely used to treat various problems, can be applied. That means that measurements should be treated as a stochastic process with a deterministic equation, but random errors associated with the coefficients. Linear equation parameters should be estimated so that they best approximate the non-linear model. Calculations are based on minimization of the error, which is treated as a stochastic process. This procedure can be applied for small deviations from linearity.

During the kinetic analysis of multi-stage processes, for each stage, the appropriate form of the $g(\alpha)$ function (kinetic model) is determined and the parameters of the Arrhenius equation (A, E) are calculated. Usually, identification of kinetic models is performed using statistical assessment. However, in many cases models cannot be distinguished in this way and additional criteria are needed, as described below.

A quantitative description of the process studied was based on the DSC curve, specifically on heat flow – W/g. For each transformation peak, integration was performed using a linear baseline and then the heat of transition (enthalpy H, J/g) was calculated. Based on the integral, the conversion degree was estimated as follows:

$$ln\left(\frac{g(\alpha)}{T^2}\right) = \ln\left[\frac{AR}{\beta E}\left(1-\frac{2RT}{E}\right)\right] - \frac{E}{RT} \qquad (8)$$

where:

H_c – current enthalpy,

H_s – the enthalpy of the transition start,

H_e – the enthalpy at the transition end.

The applied method of kinetic analysis consisted of several steps (Biedunkiewicz et al., 2007; 2008; Coats and Redfern, 1964; Hopfield, 1982; Mc Culloch and Pitts, 1943; Ortega, 1996; Sobczyk, 1996; Straszko et al., 2005; Tadeusiewicz, 1993; Widrow and Hoff, 1960; Vyazovkin and Width, 1999). Calculated α(T) functions were assessed by artificial neural networks (ANN) using StatSoft software STATISTICA 13 to check whether, in accordance with theory, the temperature was sufficient for a functional description of the conversion degree. At the first attempt, both multi-layer perceptrons (MLP) and radial basis function networks (RBF) were tested. Better preliminary results were obtained for MLP, therefore, they were used in further analyses. The performance MLP model was assessed by Pearson's correlation coefficient between experimental and predicted data, and also by the SD Ratio (the ratio of prediction error standard deviation to standard deviation of experimental data). These parameters were calculated separately for training (Tr), verification (Ve) and testing (Te) subsets. The subsets were chosen randomly – 70% of cases formed the training subset, 15% the verification subset and the same percentage of cases fell into the testing subset. Consecutive neural networks were designed and trained using back propagation (Haykin, 1994; Fausett, 1994; Patterson, 1996) and conjugate gradient algorithms (Bishop, 1995).

In a subsequent analysis, the following kinetic models were tested: D1, D2, D3, D4, F1, F2, F3, A2, A3, R1, R2, R3 (Table 1). The reason that 12 models were tested was to indicate the best mathematical description of each protein denaturation phase and to test if the models commonly used so far have not excluded other models which would better fit the data. Preliminary selection was based on the assumption of fulfilling linearity in the co-ordinate system. The quality of fit was assessed by linear regression. Additionally, the fulfilment of the Coats-Redfern equation was evaluated with linear neural network models. Parameters of the Arrhenius equation calculated from a given model by means of linear regression were adjusted by stochastic linearization, i.e. correction of the E value so that the mean percentage error in a series approached zero.

The identification of the final kinetic model was supported by additional criteria. It was required that linearity in all the series of measurements was fulfilled for the same $g(a)$ function. Plots of $k(T)$ dependencies had to be convergent in spite of differences in A and E values between series. Finally, consistency was necessary between $\alpha(T)$ values calculated from the Coats-Redfern equation and those determined from measurements over a wide range.

Table 1. List of tested kinetic models

Mechanism	Symbol	$f(\alpha)$	$g(\alpha)$
One-dimensional diffusion	D1	α	α^2
Two-dimensional diffusion, cylindrical symmetry	D2	$[-\ln(1-\alpha)]^{-1}$	$(1-\alpha)\ln(1-\alpha)+\alpha$
Three-dimensional diffusion, spherical symmetry, Jander equation	D3		
Three-dimensional diffusion, spherical symmetry, Ginstling-Brounshtein equation	D4		
1st order reaction	F1	$(1-\alpha)$	$[-\ln(1-\alpha)]$
2nd order reaction	F2	$(1-\alpha)^2$	$(1-\alpha)^{-1}-1$
3rd order reaction	F3	$(1-\alpha)^3$	$(1-\alpha)^{-2}-1$
Random nucleation, Avrami I equation	A2		
Random nucleation, Avramiego II equation	A3		
Phase-boundary reaction, zero-order reaction	R1	1	α
Phase-boundary reaction, cylindrical symmetry	R2		
Phase-boundary reaction, spherical symmetry	R3		

RESULTS AND DISCUSSION

Conversion degrees calculated for consecutive stages are shown in Figure 1. Analysis of the transition curves obtained for each sample of *l. dorsi* showed the presence of four peaks related to protein denaturation. In most cases, typical DSC thermograms obtained for pork meat show three peaks which are characteristic for thermal denaturation of myosin (about 56°C), sarcoplasmic proteins (about 65°C), and actin (about 79°C) (Fernandez-Martin et al., 2000; Zhu et al., 2004). These results of the DSC analysis revealed an additional peak (about 42–45°C), which, according to the author, show a denaturation of the least heat-stable myosin and myosin sub-fragments, i.e. helical tail, hinge-region and globular heads (Wright and Wilding, 1984). Additionally, the presence of the 1st myosin peak might have resulted from the influence of the sample treatment (mincing), since this multi-domain protein largely depends on external factors, such as sample temperature, pH and ionic strength (Thorarinsdottir et al., 2002; and references therein). It is interesting to note that the 1st peak also had the lowest enthalpy compared to the 2nd peak, which had the highest enthalpy, and the 3rd and 4th, which had intermediate values (Table 2). Moreover, in comparison to the three other peaks, the size of the 1st peak was the lowest (a broad and very small endothermic peak) indicated on the heterogenic and limited content of the proteins which underwent denaturation. Analysis of the kinetics of the thermal denaturation of meat protein revealed that the 1st, 2nd and 4th peaks followed a first-order reaction (model F1), where the thermal denaturation rate of meat protein is assumed to be proportional to the concentration of non-denatured protein. In contrast, the 3rd peak followed a second-order reaction (model F2), which was manifested by a change in heat capacity (Kajitani et al., 2011).

The $\alpha(T)$ dependencies obtained, with all the samples considered jointly, were assessed using a neural network technique. The architecture and performance of the neural models are given in Table 3. A high level of accuracy, as indicated by SD Ratio and correlation coefficients, was achieved for all the stages. Therefore, any errors occurring in the analyses would be caused by an insufficient fulfilment of the Coats-Redfern equation. The applied method of kinetic analysis will be presented using the example of stage I. In this case,

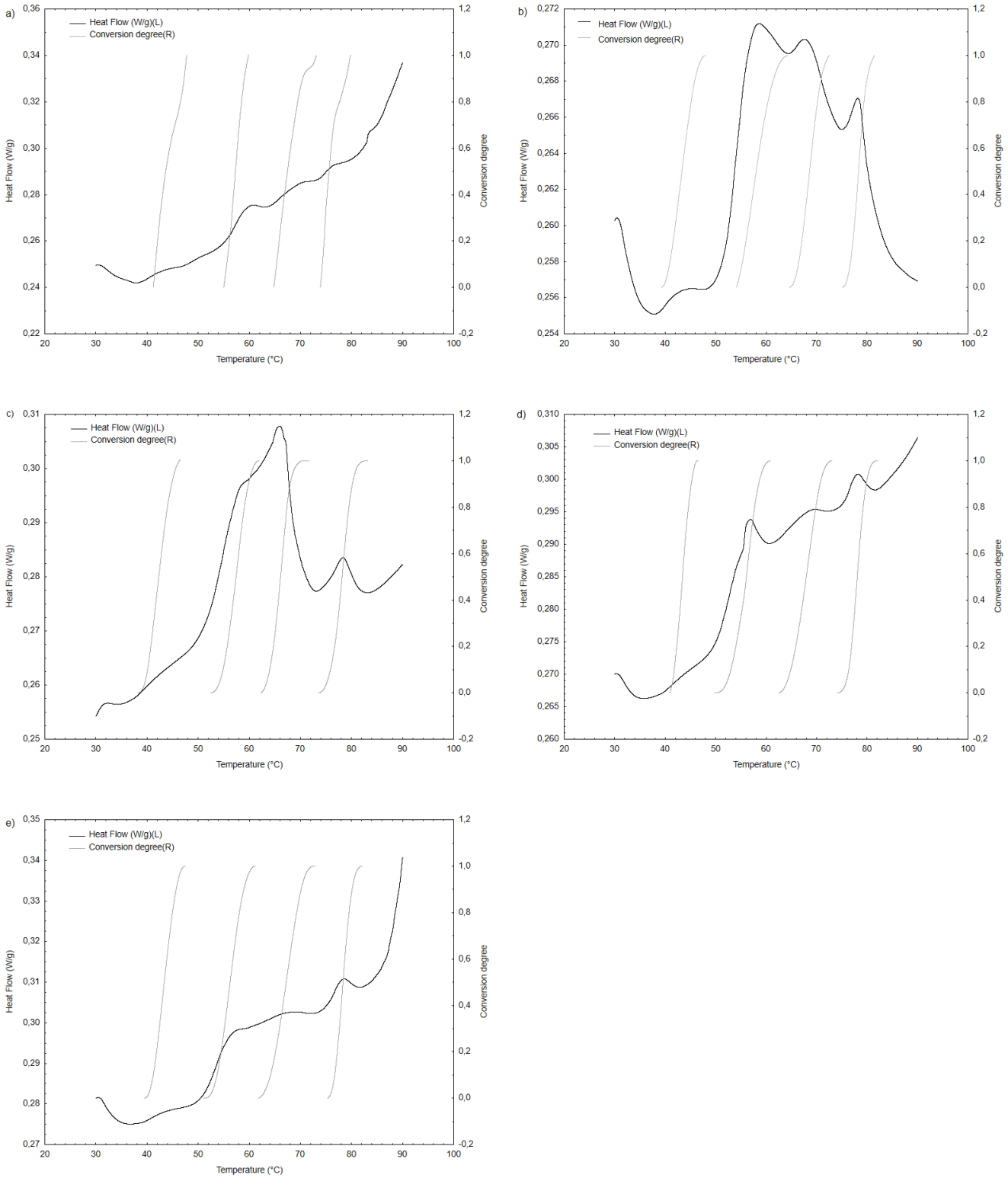

Fig. 1. Conversion degrees calculated for consecutive stages: a–e analysed samples

Table 2. Results of DSC analysis of pork meat protein denaturation

Sample	Sample weight, mg	Peak	Temperature range, °C	Enthalpy, J/g	Model	*E*, J/mol	*A*, 1/min
1	15.8	1	41.24–47.75	0.05	F1	244.46	1.30E + 16
		2	54.01–59.87	0.22	F1	125.36	1.69E + 08
		3	64.79–73.14	0.15	F2	116.67	3.04E + 07
		4	73.89–79.79	0.07	F1	183.65	3.71E + 11
2	23.6	1	38.38–48.18	0.05	F1	92.53	5.25E + 05
		2	51.49–64.20	0.60	F1	86.43	1.48E + 05
		3	64.80–73.37	0.12	F2	191.51	1.43E + 12
		4	75.24–82.33	0.15	F1	97.11	1.19E + 06
3	10.5	1	38.20–46.50	0.02	F1	90.33	2.93E + 05
		2	52.38–61.88	0.41	F1	223.93	4.13E + 14
		3	62.16–71.66	0.79	F2	115.22	3.04E + 07
		4	73.52–82.22	0.30	F1	106.42	5.23E + 06
4	19.4	1	40.78–46.53	0.01	F1	183.60	3.70E + 11
		2	49.67–60.84	0.58	F1	92.72	5.48E + 05
		3	62.42–73.09	0.13	F2	86.40	1.47E + 05
		4	74.11–82.15	0.16	F1	245.16	1.20E + 16
5	18.5	1	39.37–47.43	0.04	F1	123.38	1.49E + 08
		2	50.81–61.16	0.37	F1	130.65	3.01E + 07
		3	61.63–72.85	0.11	F2	153.64	3.61E + 11
		4	75.23–82.05	0.18	F1	99.52	5.35E + 05

Table 3. Statistical assessment of $\alpha = f(T)$ neural models of samples of consecutive stages of protein denaturation. Neural network architecture: input neurons/hidden neurons

	Parameter					
	Tr	Ve	Te	Tr	Ve	Te
	Stage I, MLP 1/4/1			Stage II, MLP 1/3/1		
SD Ratio	0.0120	0.0127	0.0129	0.0172	0.0183	0.0181
Correlation	0.999	0.999	0.999	0.999	0.999	0.999
	Stage III, MLP 1/3			Stage IV, MLP 1/3		
SD Ratio	0.0240	0.0243	0.0240	0.0190	0.0194	0.0189
Correlation	0.999	0.999	0.999	0.999	0.999	0.999

Tr – training, Ve – verification, Te – testing subsets.
MLP 1/4/1: number of input neurons / number of hidden neurons / number of output neurons.

Table 4. Statistical assessment of $\ln(g(\alpha)/T^2) = f(\Theta)$ neural models for all the samples. Protein denaturation in stage I

	Model F1			Model F2		
	Tr	Ve	Te	Tr	Ve	Te
SD Ratio	0.1516	0.1663	0.1734	0.1649	0.1760	0.1827
Correlation	0.988	0.986	0.985	0.986	0.984	0.983

Tr – training, Ve – verification, Te – testing subsets.

on the basis of the preliminary assessment of linearity in the co-ordinate system, the following models fit well with the experimental data: F1 and F2 (r = 0.99, F Snedecor's F statistics 836943.20 and 971947.41 respectively). Kinetic parameters differed between each series of measurements because of deviations from linearity rather than physical factors. Plots during linear regression calculations had different slopes between series and therefore different A and E values. Statistical parameters were at the same level for all the models and an unambiguous choice of one of them was not possible. Using linear neural network models, which considered all the samples jointly (Table 4), similar results were obtained, and further criteria had to be used. Dependencies $k(T)$ were highly convergent for models F1 and F2 (and on that basis they were chosen for further calculations). Using A and E parameters, values of the $\ln(g(\alpha)/T^2)$ function were calculated. A considerable systematic error was corrected by a slight change in parameter E in order to improve the fit of the linear model. The objective function was a minimal error in a series (close to 0). Using the corrected values of the Arrhenius parameters, dependencies $\alpha(T)$ were calculated for both models and compared with those determined from measurements. Deviations from the experimental conversion degrees were higher for model F2 while, for F1, good conformity was obtained in the whole range of $\alpha(T)$ (Fig. 2).

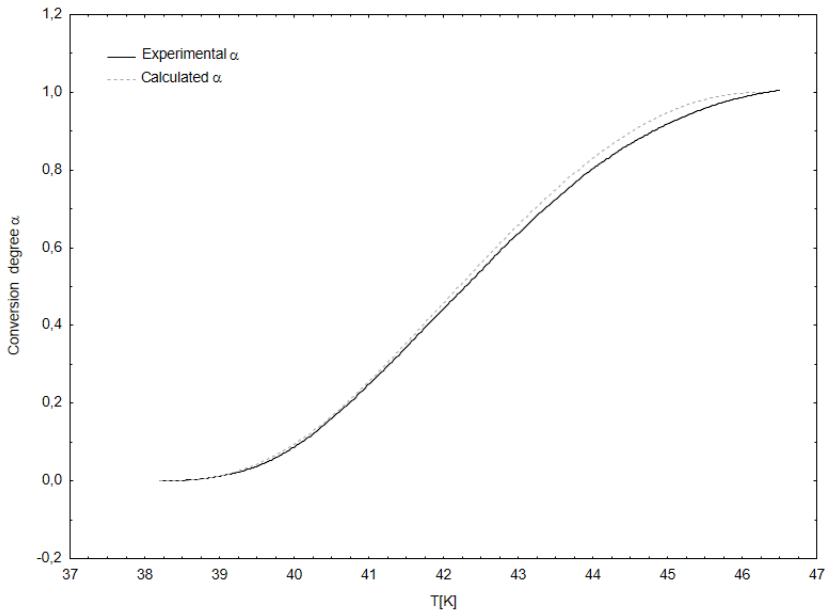

Fig. 2. Comparison of experimental $\alpha(T)$ functions and those calculated from the kinetic model for stage I of protein denaturation

Kinetic parametrization has so far mostly consisted of an evaluation of the reaction order, reaction rate, rate constant, pre-exponential factor and activation energy for a given heating rate using the Arrhenius equation (e.g. Skipnes et al., 2008). Recently, some studies have reported on the application of the Coats-Redfern equation to a kinetic analysis of temperature-induced processes occurring in foodstuffs (e.g. Amorim et al., 2004; Santos et al., 2004), but it has not been applied to meat yet.

This example of a calculation procedure was applied to all the stages of protein denaturation in this study and produced results at the same level of model performance accuracy. This method has so far been used in chemical and process engineering studies (Biedunkiewicz et al., 2008), but for the first time it has given good results in food research. Protein denaturation in meat, as seen in Figure 1, produces highly diverse DSC curves compared to chemical laboratory experiments and is difficult for mathematical description. The changeability of the meat structure and its chemical composition cause problems in unifying the protein denaturation processes. Taking into account that we analysed five different samples and obtained corresponding results, our method seems to provide the chance for extrapolation to other meat samples. Samples differed by the source, i.e. differed by their chemical composition. The use of ANN can overcome that problem in kinetic analysis as we look at the accuracy of experimental and calculated conversion degrees as shown in Figure 2. This was the final assessment of the performance of the analysis method and shows its high level of accuracy, compared to materials synthesised in a laboratory, like nanocomposites (Biedunkiewicz et al., 2008).

CONCLUSIONS

This preliminary study confirmed that methods of the traditional kinetics of processes in non-isothermal conditions based on the Coats-Redfern equation can be successfully applied to meat protein denaturation. The method of kinetic analysis allows a high level of accuracy to be achieved and meets the requirements of an efficient engineering tool. Further application of the proposed methodology applied to a larger number of samples with other distinguishing factors (feeding, age, breed, species, *post mortem* conditions of ageing etc.) is promising in building a universal model of meat protein denaturation.

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