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APPLICATION OF ARTIFICIAL NEURAL NETWORKS FOR MODELLING PEA PROTEIN HYDROLYSIS BY TRYPSIN

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Key words: artificial neural network, pea protein, trypsin hydrolysis

The experimentally-determined degrees of hydrolysis (DH) for different enzyme/substrate (E/S) ratios were used for training the artificial neural network (ANN) to predict the trypsin hydrolysis reaction of pea protein for E/S=15, 25 and 35 mAU/g based on measurements carried out for E/S=5 mAU/g only. The analysis was performed on a personal computer using a Statistica Neural Networks v. 6.0 PL (StatSoft, Tulsa, OK, USA). The input data were randomized into three sets: learning, validating and testing. Good agreement between the calculated DH values with the use of the ANN designed and the experimentally obtained values was achieved with all three sets of data. The correlation coefficient R^2 ranged from 0.978 to 0.999. The differences between the experimental and the calculated data of DH% were very low and ranged from 0.07% to 0.26%. The results obtained show that the ANN method can be useful for the design of the enzymatic processes.

INTRODUCTION

The use of artificial neural network (ANN) is a method of data analysis which emulates the brain's way of working. Neural networks exhibit the way in which arrays of neurons probably function in biological learning and memory. ANNs differ from classical computer programs in that they "learn" from a set of examples rather than are programmed to get the right answer. The information is encoded in the strength of the network's "synaptic" connections [Tadeusiewicz, 1993, 1998; Zupan & Gasteiger, 1993; Kaliszan, 1997]. In chemistry and related fields of research, an increasing interest in neural-network computing has been noted since 1986 [Zupan & Gasteiger, 1991]. Recently, several attempts have also been made to use ANN to modelling retention time in chromatography [Metting & Coenegracht, 1996; Jalali-Heravi & Parastar, 2000; Loukas, 2000; Buciński & Bączek, 2002]. In food science, artificial neural networks were used for modelling technological processes, *e.g.* kinetics of enzymes inactivation [Geeraerd *et al.*, 1998a, 1998b]. The ANN have also been used to predict thermal conductivity of food as a function of moisture content, temperature and apparent porosity [Sablani & Rahman, 2003], to model thermal/pressure food processing [Torrecilla *et al.*, 2004; Chen & Ramaswamy, 2002], and to predict antioxidant capacity of cruciferous sprouts [Buciński *et al.*, 2004]. Artificial neural network combined with gas sensor array technology has been demonstrated to have promising potential for rapid nondestructive analysis of odour and flavour in food [Haugen & Kvaal, 1998].

The goal of the work was to evaluate the application of artificial neural networks in the modelling of the hydrolysis reactions in pea proteins in relation to the enzyme/substrate ratio. The results are discussed in respect to the practical value of time reduction during the development of the technological basis of the enzymatic processes.

MATERIALS AND METHODS

Material. Pea protein isolate *Pisane* (Cosucra s.a., Momalle, Belgium) was used for hydrolysis.

Enzymatic hydrolysis. Hydrolysis of pea protein isolate was carried out using trypsin (Sigma Cat. No T-7409) in a thermostated 1 L vessel equipped with a stirrer and a pH-meter. Pea isolate (60 g) was mixed with distilled water (600 mL). During hydrolysis, pH 8.0 was maintained by the constant addition of 1 mol/L NaOH from a burette. The amount of the base added was recorded as follows: the first time point was set after 5 min and subsequent points in 10 min intervals for a total of 120 min. Incubation temperature was 50°C; the enzyme-to-substrate (E/S) ratios were: 5, 15, 25, 35 mAU/g. The enzymatic hydrolysis was repeated three times.

Degree of hydrolysis (DH). The degree of hydrolysis (DH) was calculated according to the following formula [Adler-Nissen, 1984]:

$$\%DH = B \times N_B \times \frac{1}{\alpha} \times \frac{1}{MP} \times \frac{1}{h_{tot}} \times 100$$

where: B – base consumption (mL); N_B – normality of the base; α – average degree of dissociation of α-NH_2; MP – mass of protein (g); h_{tot} – total number of peptide bonds in the protein substrate (meqv Leu- NH_2/g protein).

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The degree of dissociation of α-amino groups was computed from the following equation:

$$\frac{1}{\alpha}=1+10^{pK-pH}$$

By comparing the pairs of hydrolysis at different pH (pH_1 and pH_2), for which Leu-NH_2 eqw and B eqv are linearly correlated with the slope b, pK was calculated from the following equation [Adler-Nissen, 1986]:

$$pK=pH_2+\log(b_1-b_2)-\log(10^{pH_2-pH_1}\times b_2-b_1)$$

After acid hydrolysis of the initial material (0.5 g) with 10 mL 6 mol/L HCl at 105°C for 12 h in a flame-sealed glass ampoule [Hajós *et al.*, 1988], the total number of α-amino group was determined using a spectrophotometric method with 2,4,6-trinitrobenzene sulfonic acid (TMBS) [Panasiuk *et al.*, 1998].

Artificial Neural Network simulations. The experimentally-determined values of DH for different enzyme/substrate (E/S) ratios were used for learning the ANN model which would be able to predict the trypsin hydrolysis reaction of pea proteins for E/S=15, 25 and 35 mAU/g based only on measurements carried out for E/S=5 mAU/g.

The analysis was performed on a personal computer using a Statistica Neural Networks v. 6.0 PL (StatSoft, Tulsa, OK, USA). The input data were randomized into three sets: learning, validating and testing one. Artificial neural network was based on the multilayer perceptron consisting of 1 artificial neuron in the input layer, 2 hidden layers and 3 neurons in the output layer. The architecture of the model utilized is depicted in Figure 1. A supervised method of learning with a back-propagation strategy was used. Learning of the ANN was executed over 100 epochs, the learning coefficient was 0.01 and the momentum equaled 0.3. Learning was then continued with the use of a conjugated gradient descent algorithm of up to 144 epochs, which minimised RMS error. Data from the learning set were presented in a randomized manner during the learning process. The changes in RMS error were recorded for the learning- and validating sets during the learning process (Figure 2). The artificial neural network characterized by the smaller RMS error with regard to validating set of data was taken for further consderation.

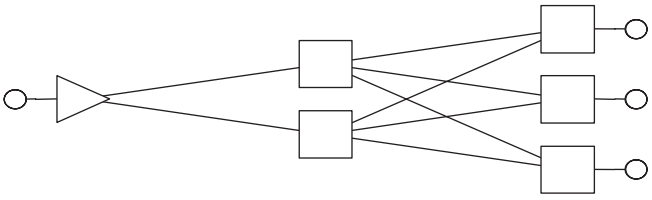


FIGURE 1. The architecture of artificial neural networks used for predictions of DH.

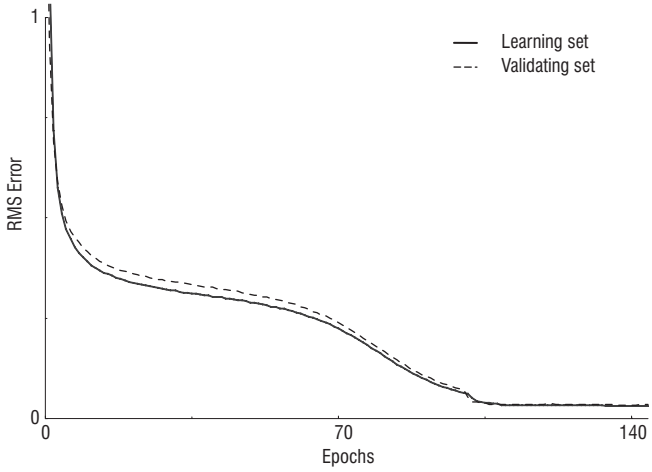


FIGURE 2. Training error graph.

RESULTS AND DISCUSSION

The trypsin hydrolysis of the pea proteins over 120 min affected the hydrolysis degree by 7.1% (E/S 5 mAU/g), 9.5% (E/S 15 mAU/g), 10.4% (E/S 25 mAU/g), and 11.5% (E/S 35 mAU/g). These results comply with the literature

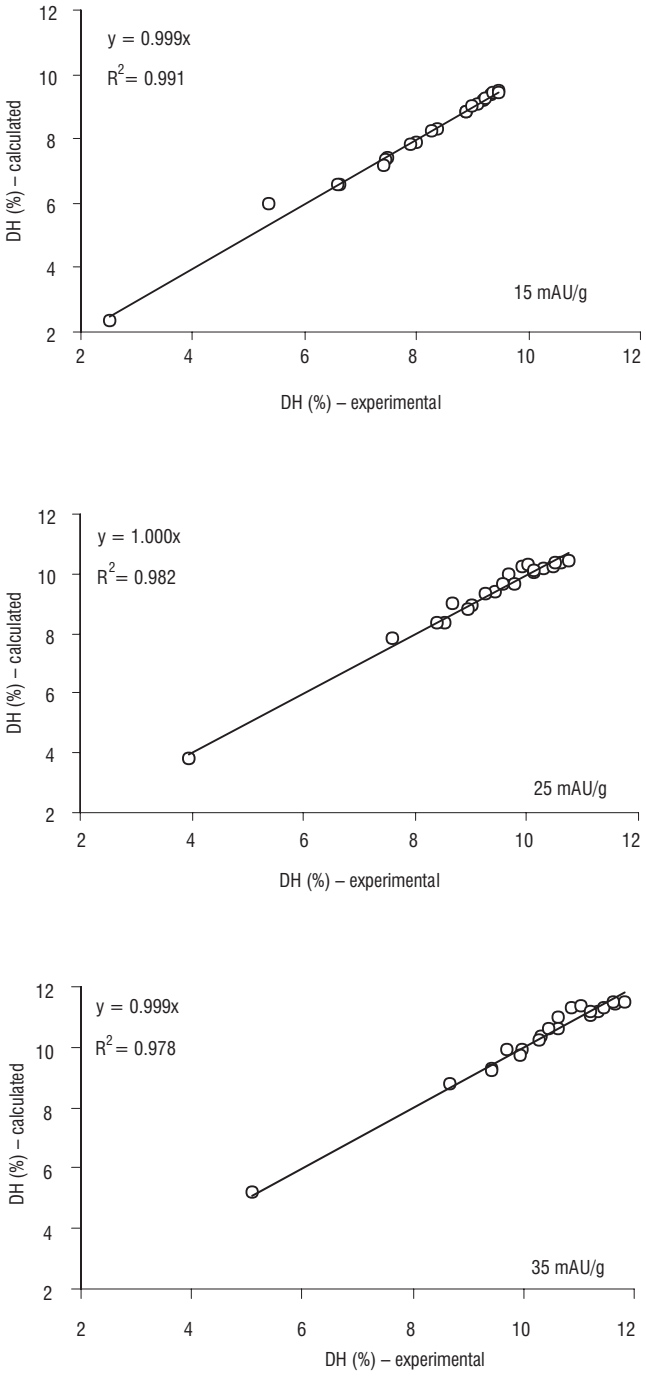
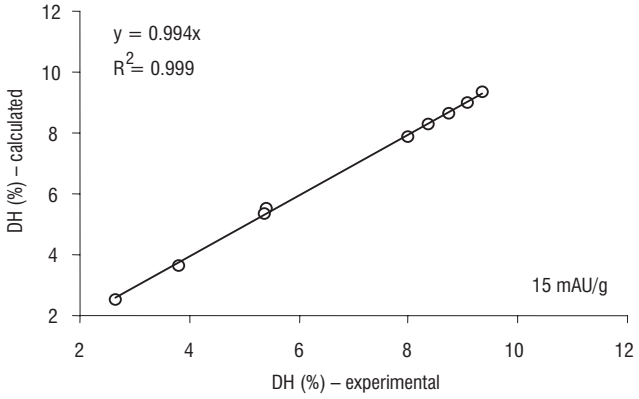
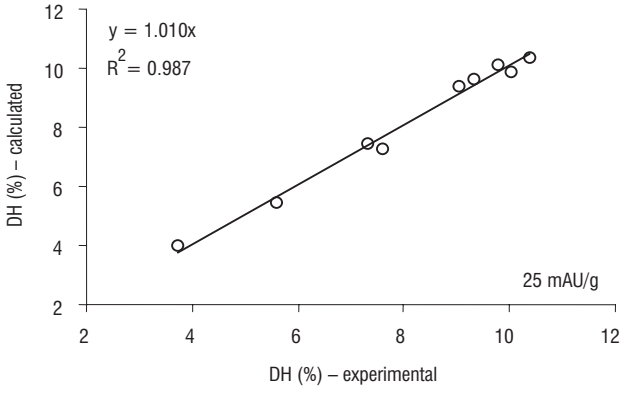


FIGURE 3. Correlation between the calculated and the experimental data for learning set.

data. Gwiazda *et al.* [1994], using Alcalase and Neutrase for rapeseed proteins, obtained DH values in the range of 8 to 10%, whereas if milk proteins were digested by trypsin and chymotrypsin, the DH values obtained ranged from 6.5% to 7.5% and from 5.5% to 6.0%, respectively [Pouliot *et al.*, 1995]. Much higher DH values (approximately 70%) were noted by Mahmoud *et al.* [1992] who used a porcine pancreatin for the enzymatic hydrolysis of casein. In this study, the α-amino nitrogen was assayed using the formol titration procedure.

The results for learning, validating and testing processes performed during the research of the network model are presented in Figures 3–5. A very high agreement between the calculated DH values with the use of ANN designed and experimentally obtained data was achieved for learning, validating and testing sets of data. For E/S 15 mAU/g, the correlation coefficient R^2 equalled 0.991, 0.999 and 0.999 for learning, validating and testing sets of data, respectively (Figure 3–5). For the same sets of data and for E/S 25 mAU/g, the obtained R^2 values equalled 0.982, 0.987 and 0.996, respectively (Figure 3–5); for E/S 35 mAU/g, equalled 0.978, 0.981 and 0.994, respectively (Figure 3–5).





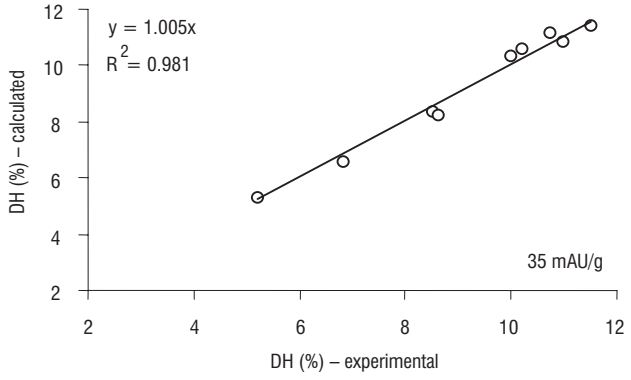
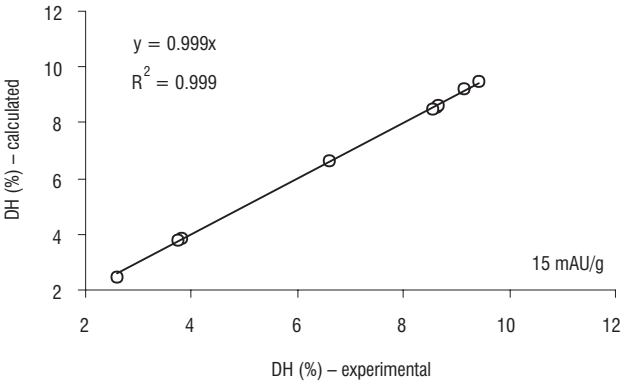
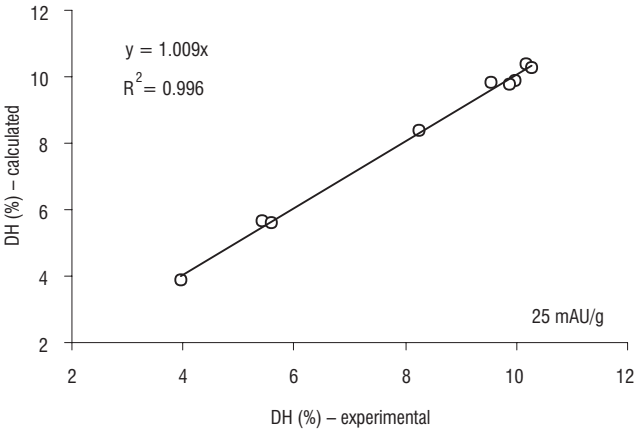


FIGURE 4. Correlation between the calculated and the experimental data for validating set.





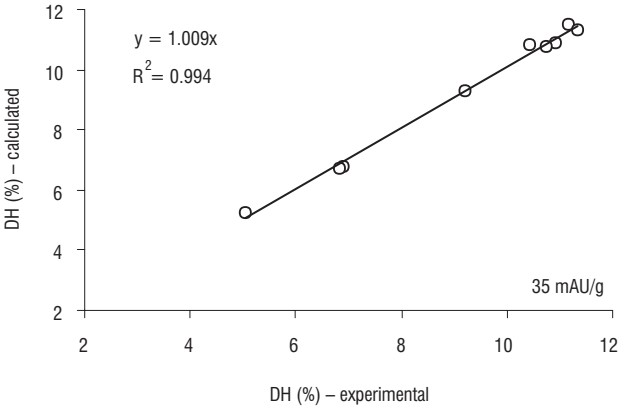


FIGURE 5. Correlation between the calculated and the experimental data for testing set.

The results presented in Table 1 showed that differences between the experimental and the calculated data of DH% were very low and ranged from 0.07% to 0.26%. In general, the learning set was characterized with the lowest differences. The highest differences were noted for the E/S ratio of 25 mAU/g.

Firm agreement between calculated values using the ANN and the experimentally measured ones was noted in several studies dealing with food science. Correlation coefficients for the performance of ANN modeling of variable retort temperature ranged from 0.967 to 0.999 [Chen & Ramaswamy, 2002]. Torrecilla *et al.* [2004] reported accurate predicted times fitting (R^2=0.98) for the thermal/pressure

TABLE 1. Differences between the experimental and calculated data of DH%.

Kind of set	E/S 15 mAU/g	E/S 25 mAU/g	E/S 35 mAU/g
Learning	0.08 ± 0.14	0.09 ± 0.05	0.07 ± 0.04
Validating	0.16 ± 0.11	0.23 ± 0.12	0.13 ± 0.11
Testing	0.17 ± 0.12	0.26 ± 0.14	0.16 ± 0.13

food processing. A very good correlation (R^2>0.95) of experimental *versus* neural network values of the thermal conductivity of food with training and validation data sets was reported by Sablani and Rahman [2003]. A very high agreement was found between the calculated trolox equivalent antioxidant capacity of germinated cruciferous seeds with the use of ANN designed and those obtained experimentally reported by Buciński *et al.* [2004]: the correlation coefficient R^2 equalled 0.906, 0.934 and 0.931 for learning, validating and testing sets of data.

CONCLUSIONS

The use of the artificial neural networks modelled the hydrolysis reactions of pea proteins by trypsin in terms of the enzyme/substrate ratio. The results obtained can be used for the design and development of enzymatic processes.

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ZASTOSOWANIE SZTUCZNYCH SIECI NERONOWYCH DO MODELOWANIA HYDROLIZY BIAŁKA GROCHU TRYPSYNĄ

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Sztuczne sieci neuronowe (ANN) zostały wykorzystane do zbudowania modelu zdolnego przewidywać stopień hydrolizy (DH%) białka grochu trypsyną. Sieć typu perceptronu wielowarstwowego uczono dwufazowo. W pierwszej fazie zastosowano algorytm wstecznej propagacji błędu, następnie kontynuowano uczenie przy użyciu algorytmu gradientów sprzężonych. Analizy przeprowadzono korzystając z oprogramowania Statistica Neural Networks v. 6.0 PL (StatSoft, Tulsa, OK., USA). Uzyskano wysoką zgodność wartości DH% wyliczonych przy użyciu ANN z wartościami wyznaczonymi eksperymentalnie. Współczynnik korelacji R^2 w zależności od zbioru i stosunku E/S wynosił od 0.978 do 0.999. Różnice pomiędzy wartościami DH% wyznaczonymi eksperymentalnie i wyliczonymi przez ANN były bardzo niskie i wahały się od 0.07% do 0.26%. Otrzymane w pracy wyniki wskazują, że metoda sztucznych sieci neuronowych może być pomocna przy opracowaniu podstaw technologicznych procesu enzymatycznego.