The change in the color of heat treated vacuum packed broccoli stem and floret during storage: effects of process conditions and modeling by ANN

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Running Title

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ABSTRACT

BACKGROUND: Vacuum packed broccoli stems and florets were subjected to heat treatment (60 – 99 °C) for various time intervals. The activity of peroxidase was measured after processing. Thermally processed samples were then stored at 4 °C for 35 days and the color of the samples were measured every 7 days. Effects of parameters (heating temperature and duration, storage time) on the color of broccoli were modeled and simulated by artificial neural network.

RESULTS: Simulations confirmed that stems were predicted to be more prone to changes than florets. More color loss was observed at longer processing or storage combinations. The simulations also confirmed that higher temperatures during heat processing could retard color changes during storage. For stems treated at 80 ºC for short durations, color loss was more predominant than both 65 and 99 ºC probably due to the incomplete inactivation of enzymes besides more tissue damage, with increased enzyme access to the substrate.

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CONCLUSION: The greenness of both stems and florets during storage can be better preserved at higher temperatures (99 °C) and short times. The simulation results revealed that ANN method could be used as an effective tool for predicting and analyzing the color values of heat treated broccoli.

Key words: artificial neural network (ANN), broccoli, color, thermal processing, optimization.

INTRODUCTION

Appearance is the first attribute that attracts the attention of consumers in choosing a product. One of the most important aspects of appearance, especially for fruits and vegetables, is the color of the product which should be within an acceptable range. If this is not the case, other quality factors such as flavor and texture will not come into consideration by consumers, thus leading to product rejection [1]. In many situations, it is needed to process vegetables before their consumption i.e. heat treatment. The main purpose of heat treatment of vegetables is tenderizing the texture for consumption or inactivation of enzymes and removing air prior to freezing or canning [2]. However, heat treatment could impose adverse effects on the color forming compounds in the product, exerting undesired changes in the appearance of the products.

Compared to fruits which are consumed in their raw form, vegetables are processed prior to consumption [3]. Broccoli has been considered as one of the main vegetables consumed all around the world [4]. There have been research regarding the influence of different thermal processing on the nutritional quality of broccoli [5, 6]. Among different thermal processing of vegetables, sous-vide processing has gained a special attention due to its advantages over other methods. In this process also known as vacuum cooking, the product is vacuum sealed in a heat-stable plastic bag and is subjected to conventional heating [7]. Vacuum sealing has several advantages such as increased shelf life due to the elimination of the possible risk of contamination during storage, inhibition of the oxidation, prevention of the moisture loss, leakage of solutes into the heating medium, and evaporation of flavor volatile [8]. The green color of plants is mainly caused by the presence of Chlorophyll (a and b) pigments [9]. One of the factors causing chlorophyll degradation is heat treatment [10], therefore, in designing thermal processes, this issue should be taken into account. Hence the aim of the thermal processes is usually to combine the maximum reduction in the activity of enzymes with minimum thermal degradation of chlorophyll. Inactivation of the most heat stable enzyme is used as an indicator of blanching adequacy in food

products. Peroxidase has been considered as the most stable enzyme during thermal processes in vegetables, therefore it has been used as a blanching indicator [11].

It is important to predict the color changes of products during storage for different purposes including shelf life estimation and storage conditions optimization. One of the widely used methods for modeling color changes during thermal processes is the kinetic approach [2, 12, 13]. In this approach, experimental data are fitted to a model (usually fractional first order kinetic model in the case of color change) and the constants (usually rate constant) are calculated. The relationship between the rate constant with the studied variables (usually temperature) is investigated and a package could be introduced for predicting the variables during processing. This method is only applicable when the kinetic rate constant is a function of one variable. In the case of multiple variables this method would not be sufficient to predict the color change behavior. Artificial neural networks (ANN) is a modeling and prediction technique that can be used to study the effects of processing parameters on the properties of food products [14]. This technique has gained its importance in the food industry due to its ability to model complex relationships between processing variables. Furthermore, this technique has been successfully applied to model the kinetics of color changes during processes such as drying and dehydration [15-17]. The main difference between this method and other modeling techniques is that ANN method learns from a set of given data and finds the complex and non-linear relationship between the inputs (independent variables) and outputs (dependent variables). Then an appropriate network of mathematical functions is given to predict the outputs for the input values. This modeling technique could be valuable to predict complex relationships between food quality and factors affecting the properties of the product that cannot be usually analyzed effectively by other methods [18].

The kinetics of broccoli color changes during thermal processing has been studied previously by Tijskens, et al. (2001). However, there is no information in the literature regarding the color changes of thermally processed broccoli stem and floret during storage. Moreover, in most studies the broccoli samples were subjected to the heating medium directly, which may cause oxidation (for hot air treatment) or leakage of coloring substances (in the case of water bath treatment). Therefore, the purpose of this research was to study the behavior of color changes during cold storage of vacuum packed broccoli stem and floret processed at various temperatures and durations, as well as, to evaluate the possibility of predicting the color of foregoing samples by the application of artificial neural networks with a more practical and industrial point of view. The overall outcome of this research can be used in industrial

applications for controlling the process conditions in order to maintain the quality of processed broccoli stem and florets.

EXPERIMENTAL

Materials

Broccoli (*Brassica oleraceae L. cv. Italica*) was supplied by Bama Storkjøkken Stavanger, Ålgård (One day after harvest they were processed). Dipotassium phosphate (K_2HPO_4), monopotassium phosphate (KH_2PO_4), hydrogen peroxide (30%), and guaiacol (99.5%) were purchased from Merck (Germany).

Sample preparation

Florets and stems were separated and cut into pieces with the diameter of 4-5 cm and length of 3-5 cm, respectively. Then, all samples were vacuum-packed at 95% vacuum (38 mmHg) by FH-S Webomatic Vacuum packaging machine (Bochum, Germany) in Sous-Vide plastic pouches (20 µm polyamide inside layer and 70 µm polyethylene outside layer, O_2 permeability: 45 cm^3 $(m^2$ d bar$)^{-1}$) with the size of 15×15 cm. The mass of sample in each package was in the range of 25-30 g. Samples were kept cold (4 °C) throughout the preparation stage until they were processed.

Thermal processing

Vacuum-packed samples were subjected to heat treatment in a water bath (Lauda E200, Lauda-Königshofen, Germany) at various combinations of time-temperature. Then, they were taken out from the water bath at each time step and immediately submerged in the ice and water mixture. After cooling, samples were stored at 4 °C and were analyzed for color at different periods. Three replicates for each combinations of time-temperatures were carried out.

The surface temperature of the samples was measured by placing a mini logger (TrackSense Pro Mini-Wireless Data Loggers, Ellab AS, Hilleroed, Denmark) on the surface of vacuum packed samples. The time (lag phase) needed for the surface temperature of a sample to reach to the processing temperature was excluded form processing time calculations.

Color Measurement

Hunter parameters of a^* and b^* were measured for broccoli sample by a digital photo imaging color-measuring system (DigiEye full system, VeriVide Ltd., Leicester, UK). The samples were placed in a standardized light-box with daylight (6500 K) and photographed with a calibrated digital camera (Nikon D80, 35mm lens, Nikon Corp., Japan). The pictures were analyzed with DigiPix software (VeriVide Ltd., Leicester, UK) and the color was quantified. L* describes the products lightness (L* = 100 = white, and L* = 0 = black), a* describes the intensity of color on the red–green axis (a*> 0 = red, and a* < 0 = green) and b* the intensity of color on the yellow–blue axis (b* > 0 = yellow, and b* < 0 = blue). Some researchers have reported the ratio of *a to b* (*a / b*) for monitoring the changes in chlorophyll content for canned green peas [19] and can green beans [20]. This method has also been employed for reporting the greenness of broccoli [2, 21, 22] which is an indicator of visual color in green vegetables. Therefore, in this study, the greenness of broccoli samples was reported by the normalized ratio of *a* to *b* as follow:

$$\frac{a^*}{b^*} = \frac{(\frac{a}{b})}{(\frac{a_0}{b_0})} \qquad (1)$$

where, a*/b* is the normalized ratio of a to b. a_0 and b_0 represent the initial color values of broccoli before processing.

Peroxidase activity analysis

Buffer solution was prepared according to Morales et al. (2002). Briefly, for 0.1 M phosphate buffer with a pH value of 6.5, the solutions of 1M dipotassium phosphate (K_2HPO_4) and monopotassium phosphate (KH_2PO_4) were prepared separately by mixing 174.18 g K_2HPO_4 and 136.09 g KH_2PO_4 with distilled water, respectively, until the volume reached to 1 L for each solution. Then, 32.95 and 67.05 mL of 1M K_2HPO_4 and KH_2PO_4 solutions were combined, respectively and diluted to 1 L by distilled water.

Substrate solution was prepared daily by mixing 0.1 mL H_2O_2 (30%), 0.1 mL guaiacol (99.5%), and 99.8 mL potassium phosphate buffer (0.1 M, pH=6.5).

Broccoli samples were cut into small pieces. Distilled water was added (1:4 w/w) and the mixture was homogenized to obtain the juice. The juice was filtered to remove solid particles, and subsequently centrifuged (18000 g, Rotina 420 R, Hettich centrifugen, Germany) for 30 min at 4 °C to eliminate the remaining turbidity. For measuring the initial activity of enzyme extract, the resulting supernatants were

mixed with 0.1M potassium phosphate buffer (4°C) with the ratio of 1.5:25 (v/v). The final ratio of broccoli to phosphate buffer was 1.5:100 as was proposed by Morales et al. (2002).

Peroxidase activity of broccoli juice was measured according to the method described by Morales et al. (2002), and Hermeda and Klein (1990). In a glass tube, 0.12 mL of enzymatic extract was added to 3.48 mL of substrate solution and mixed with a vortex. Then, the absorbance was measured at 470 nm for 2 min, using a spectrophotometer (UVmini-1240 -UV-VIS Spectrophotometer, Shimadzu, Japan) by blank (mixture of 0.12 mL distilled water with 3.48 mL substrate solution). The activity of enzyme was recorded based on the ΔA/min (A = absorbance at 470 nm). The ratio of enzyme activity (A/A_0, A = after heat treatment, A_0 = before heat treatment) was expressed for the effect of thermal process on the inactivation of enzyme.

For inactivation study of enzyme, 0.6 mL of the supernatants was poured into a glass reagent tube with a diameter of 4 mm before mixing with phosphate buffer solution. The tubes were immersed into the water bath at various combinations of time-temperature. For each time-temperature point, at least two replicates were carried out. Immediately after heat treatment, tubes were immersed into a mixture of ice and water. The treated extracts were mixed with potassium phosphate buffer with the mentioned proportion before measuring the activity.

Artificial neural networks (ANNs) modeling

The data of 108 experimental runs were used for developing the ANN. The independent variables were process temperature (°C), process duration (min) and storage time (day) and the output variable was broccoli color (a*/b*). The combination of process temperature-duration and storage time is shown in table 1. For each combination, 3 replications were investigated. 70, 15, and 15 % of the data were randomly selected for training, cross-validation, and testing, respectively. Multilayer perceptron (MLP) ANNs (Fig. 1) were used for modeling broccoli color. Various number of neurons (1 to 30) in the hidden layer were investigated in order to find the best ANN for predicting the color of stem and floret. Levenberg-Marquardt (LV) back propagation (BP) was selected as the training algorithm of the ANNs. The applied transfer functions in the hidden and output layers were Tangent-Sigmoid (tansig) and linear, respectively. To evaluate the goodness of fit for each topology of ANN, two statistical error criteria namely coefficient of determination (R^2) and root mean square error (RMSE) were taken into account:

$$R^2 = 1 - \frac{\sum_{i=1}^{N}\left(y_{pre}^{i} - y_{exp}^{i}\right)^2}{\sum_{i=1}^{N}\left(y_{pre}^{i} - \bar{y}\right)^2} \quad (2)$$

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(y_{pre}^{i} - y_{exp}^{i}\right)^2} \quad (3)$$

where, y_{pre} and y_{exp} represent the predicted and experimental outputs, respectively. $\bar{y}$ indicates the average of experimental output. N is the total number of experimental runs. ANN modeling was carried out by MATLAB (version 2013a, MathWorks, Massachusetts, United States) software.

RESULTS AND DISCUSSION

The results of heating process temperature and time on the color of broccoli stems and florets are shown in Fig.2. As can be seen in Fig. 2, at the beginning of the heat treatment, the color of the samples increased slightly and then a decreasing trend was observed.

Initial increase in the greenness of the samples at the early stage of processing has also been observed and explained by other researchers. Mackinney and Weast (1940) and Woolfe (1979) attributed this behavior of green vegetables to the removal of air from the space between the cells and around the tiny hairs present on the surface. By increasing the temperature (to 99 °C), the initial increase in the greenness became larger compared to lower temperatures (60 °C) (Fig. 2). Similar results were obtained by Tijskens et al. (2001). This could be explained by the higher rate of evaporation at the surface of the broccoli samples at higher temperatures, associated with higher removal rates of air.

After about 39 minutes of processing at 60 °C, the visual greenness of stems and florets dropped to 50% of their initial values. With further increase in the process duration, the green color of the samples was significantly reduced. This observation indicated that processing at 60 °C should be conducted at durations shorter than 40 minutes. After 35 minutes of processing at 80°C, the greenness of broccoli stems and florets reached to 50 % of their initial value and at 99 °C this happened after 15 minutes. The change from 80 °C to 99 °C was sharper, probably because, in the latter, the temperature was close to the boiling point of water.

The change in the color of processed samples during cold storage (4 ºC) is shown in Figure 3. A decreasing trend was observed for all samples. As mentioned before, for every temperature, different samples were obtained at different time steps and each sample was stored at refrigerated temperature and was analyzed during the storage.

Samples processed at 60 °C, lost their greenness rapidly during the first 10 days of storage with a very slow rate afterwards, except for samples processed at a shorter duration (< 2 min) (Fig. 3). This observation might be due to the fact that processing at longer durations would cause the integrity of cell structures to be destroyed. Therefore, the non-inactivated enzymes at 60 °C (table 2), would have a better access to chlorophyll, resulting in its degradation and accelerated loss of color. As shown in Table 2, the activity of the enzymes could not be inhibited at 60 °C even after longer treatment times. Another hypothesis explaining this observation can be the enzymatic browning phenomenon. This reaction occurs with the oxidation of phenolic compounds by polyphenol oxidase (PPO) [27]. Ansorena et al. (2014) reported the browning potential of minimally processed broccoli during storage. They used a hurdle technology (heat, ultrasound, citric acid) to retain the visual (color), nutritional and microbiological quality of broccoli samples during storage. According to their results, heating (50 °C) time had a positive effect on the browning potential of broccoli samples. The wound-inducing effect of thermal process may result in an increase in the phenylalanine ammonia lyase enzyme which is involved in the production of major phenolic compounds [28]. These compounds can be subjected to oxidation process by PPO and subsequently, browning substances are produced.

At higher processing temperatures (80 and 99 °C) the changes in the color of the samples was linear throughout the storage period, probably owing to the absence of enzymatic activity in these samples after processing, as confirmed by the enzymatic activity analysis results (Table 2). According to the residual greenness after storage for 35 days at 4 °C, samples treated at 99 °C for durations shorter than 7 minutes gave a better result than processing at 80 °C for all studied process durations. Therefore, the best process conditions in terms of final residual color can be heating at 99 °C for a duration of maximum 7 minutes depending on the desired goal. However, it should be noted that if in addition to the inactivation of enzymes, the purpose of heating is to reach to a specific texture or nutritional value,

further analysis is necessary to find the optimum condition. However, investigations for finding those optimum conditions is recommended to be carried out at higher temperatures (> 80 °C).

ANN modeling

As stated in the introduction section, the use of kinetic approaches for modeling the color changes in broccoli samples was a challenging issue due to the complexity of the problem. Because, the rate constant of kinetic models for modeling the color change during storage was not only dependent on the processing temperature, but it was also dependent on the processing duration. Preliminary investigations (data are not shown) revealed that it is very difficult to find a suitable relationship between the rate constant and processing temperature and duration by using kinetic methods for both stem and floret and a rough prediction was obtained for all cases. Therefore, because of the capability of artificial neural networks method in modeling the processes in which there are complex relationships between processing variables, this modeling and simulation technique was applied to predict the color of thermally processed broccoli samples at different times during storage. The prediction model was considered to be dependent on both processing temperature and duration.

In order to obtain the best ANN for predicting the color of stems and florets, 108 experimental runs with their corresponding color values were analyzed with various number of neurons in the hidden layer. Starting from 1 to 10 neurons, the investigation was carried out for this purpose. Therefore, 10 different networks, each containing different numbers of neurons in the hidden layer (1 to 10 neurons), were analyzed. The outcome of each ANN was evaluated based on R^2 and RMSE. It should be noted that the result of each specific ANN would not be constant if repeated. For example, if we get a specific result by training a set of data with an ANN containing 10 neurons in the hidden layer, we would not necessarily get the same result if we repeat the training of the same data with the same ANN. This is due to the fact that the initial conditions vary for each software run and the program randomly selects and divides the data for each part of ANN development procedures including training, cross-validation, and testing. Accordingly, for getting more accurate results in the step of finding the best number of neurons in the hidden layer, it is better to repeat the software run several times for each ANN and report the average of replicates. For this reason, the development of ANN with various number of neurons in the hidden layer was repeated 10 times for each ANN and the average R^2 and RMSE was reported (Fig. 4). For both stem and Floret, 6 neurons in the hidden layer gave the best results and shaped the optimal network.

Moreover, for stem, there was no large difference between performance of ANNs containing more than 4 neurons in the hidden layer. This is valid for floret among ANNs containing more than one neuron in the hidden layer. Comparison between experimental and predicted color values by the optimal network is presented in Fig. 5.

In order to examine the ability of optimal ANN obtained for stems and florets for predicting new data, or in other words, unseen data, 102 experimental runs were simulated by the optimal ANN. The simulated color values of stems and florets were compared with the data obtained by experiments (Fig. 6) and a relatively high correlation coefficient for both stems and florets was obtained indicating the capability of the model to simulate the color of broccoli samples. Other researchers have also stated the ability of ANN in prediction of food color change during processing [15, 29].

The obtained ANN was employed to simulate a wider range of processing parameters. Simulation results shown in Figure 7 can be used for optimization and process design purposes, as the contours could give practical and useful information in this regard. First of all, Fig. 7 shows that the color changes in florets and stems were different. Stems were predicted to be more prone to changes than florets probably because of smaller surface area of the samples. Secondly, for all temperatures, more color loss was observed at longer processing or storage combinations, as confirmed by the diagonal pattern of the contours. Despite a similar pattern for color change at different temperatures, the extent of color change was totally different and different behaviors could be detected. At lower temperatures, process-storage time lines tend to be concave shaped but by increasing the temperature they were changed to linear or convex patterns. This observation confirmed that at higher temperatures color could be better preserved as the enzymes were inactivated effectively. The contours also confirmed that higher temperatures during heat processing could retard color changes during storage. For stems treated at 80 ºC for short durations, color loss was more predominant than both 65 and 99 ºC at the final stages of storage. This might be due to the incomplete inactivation of enzymes at these conditions (Table 2) beside more tissue damage than the samples treated at 65 ºC, that could increase the enzyme access to the substrate.

CONCLUSION

Temperatures higher than 80 °C gave a better result in preserving the color of broccoli stems and florets during storage. This was mainly due to adequacy of these conditions to inactivate the enzymes. Moreover, the results of enzyme activity revealed that the adequacy of blanching can be obtained easily at temperatures higher that 80°C. Therefore, in thermal processing of broccoli, higher temperatures are preferred in terms of color retention during storage. The result of modeling the changes of color by ANN revealed that this technique can be used as an effective tool in predicting the color of broccoli processed at different temperatures (60 – 99 °C) and durations at any time during storage. This observation can be an important matter, especially for industrial purposes in which the control of process conditions is a crucial task.

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References

1. Francis F. Quality as influenced by color. Food quality and preference. 1995;6(3):149-55.
2. Tijskens L, Schijvens E, Biekman E. Modelling the change in colour of broccoli and green beans during blanching. Innovative Food Science & Emerging Technologies. 2001;2(4):303-13.
3. Kosewski G, Górna I, Bolesławska I, Kowalówka M, Więckowska B, Główka AK, et al. Comparison of antioxidative properties of raw vegetables and thermally processed ones using the conventional and sous-vide methods. Food chemistry. 2018;240:1092-6.
4. FAO. Production quantities of Cauliflowers and broccoli by country 2014. Available from: http://www.fao.org/faostat/en/#data/QC/visualize.
5. Florkiewicz A, Ciska E, Filipiak-Florkiewicz A, Topolska K. Comparison of Sous-vide methods and traditional hydrothermal treatment on GLS content in Brassica vegetables. European Food Research and Technology. 2017:1-11.
6. Florkiewicz A, Berski W. Application of sous vide method as an alternative to traditional vegetable cooking to maximize the retention of minerals. Journal of Food Processing and Preservation. 2017.
7. Baldwin DE. Sous vide cooking: A review. International Journal of Gastronomy and Food Science. 2012;1(1):15-30.
8. Church IJ, Parsons AL. The sensory quality of chicken and potato products prepared using cook–chill and sous vide methods. International journal of food science & technology. 2000;35(2):155-62.

9. Schwartz SJ, Lorenzo TV. Chlorophylls in foods. Critical Reviews in Food Science & Nutrition. 1990;29(1):1-17.
10. Weemaes CA, Ooms V, Van Loey AM, Hendrickx ME. Kinetics of chlorophyll degradation and color loss in heated broccoli juice. Journal of Agricultural and Food Chemistry. 1999;47(6):2404-9.
11. Severini C, Giuliani R, De Filippis A, Derossi A, De Pilli T. Influence of different blanching methods on colour, ascorbic acid and phenolics content of broccoli. Journal of food science and technology. 2016;53(1):501-10.
12. Wawire M, Oey I, Mathooko FM, Njoroge CK, Shitanda D, Hendrickx M. Kinetics of thermal inactivation of peroxidase and color degradation of African cowpea (Vigna unguiculata) leaves. Journal of food science. 2016;81(1):E56-E64.
13. Gonçalves EM, Pinheiro J, Alegria C, Abreu M, Brandão TR, Silva CL. Degradation kinetics of peroxidase enzyme, phenolic content, and physical and sensorial characteristics in broccoli (Brassica oleracea L. ssp. Italica) during blanching. Journal of agricultural and food chemistry. 2009;57(12):5370-5.
14. Zhou W. Artificial Neural Networks in Food Processing. In: Farid M, editor. Mathematical modeling of food processing. United States: CRC Press; 2010. p. 901-19.
15. Fathi M, Mohebbi M, Razavi SMA. Application of Image Analysis and Artificial Neural Network to Predict Mass Transfer Kinetics and Color Changes of Osmotically Dehydrated Kiwifruit. Food and Bioprocess Technology. 2009;4(8):1357-66.
16. Shafafi Zenoozian M, Devahastin S. Application of wavelet transform coupled with artificial neural network for predicting physicochemical properties of osmotically dehydrated pumpkin. Journal of Food Engineering. 2009;90(2):219-27.
17. Nadian MH, Rafiee S, Aghbashlo M, Hosseinpour S, Mohtasebi SS. Continuous real-time monitoring and neural network modeling of apple slices color changes during hot air drying. Food and Bioproducts Processing. 2015;94:263-74.
18. Dolatabadi Z, Elhami Rad AH, Farzaneh V, Akhlaghi Feizabad SH, Estiri SH, Bakhshabadi H. Modeling of the lycopene extraction from tomato pulps. Food Chem. 2016;190:968-73.
19. Gold HJ. Degradation of chlorophyll to pheophytin during sterilization of canned green peas by heat: University of Wisconsin--Madison; 1958.
20. Hayakawa KI, Timbers GE. Influence of heat treatment on the quality of vegetables: changes in visual green color. Journal of Food Science. 1977;42(3):778-81.
21. Gunawan MI, Barringer SA. Green color degradation of blanched broccoli (Brassica oleracea) due to acid and microbial growth. Journal of Food Processing and Preservation. 2000;24(3):253-63.
22. Tijskens L, Barringer S, Biekman E. Modelling the effect of pH on the colour degradation of blanched broccoli. Innovative Food Science & Emerging Technologies. 2001;2(4):315-22.
23. Morales-Blancas E, Chandia V, Cisneros-Zevallos L. Thermal inactivation kinetics of peroxidase and lipoxygenase from broccoli, green asparagus and carrots. Journal of Food Science. 2002;67(1):146-54.
24. Hemeda H, Klein B. Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. Journal of Food Science. 1990;55(1):184-5.
25. Mackinney G, Weast C. Color changes in green vegetables. Industrial & Engineering Chemistry. 1940;32(3):392-5.
26. Woolfe ML. Pigments. In: Priestley R, editor. Effects of heating on foodstuffs: Applied Science Publishers; 1979.
27. Holderbaum DF, Kon T, Kudo T, Guerra MP. Enzymatic browning, polyphenol oxidase activity, and polyphenols in four apple cultivars: dynamics during fruit development. HortScience. 2010;45(8):1150-4.
28. Ansorena MR, Moreira MR, Roura SI. Combined effect of ultrasound, mild heat shock and citric acid to retain greenness, nutritional and microbiological quality of minimally processed broccoli (Brassica oleracea L.): An optimization study. Postharvest Biology and Technology. 2014;94:1-13.

29. Fazaeli M, Emam-Djomeh Z, Omid M, Kalbasi-Ashtari A. Prediction of the physicochemical properties of spray-dried black mulberry (Morus nigra) juice using artificial neural networks. Food and Bioprocess Technology. 2013;6(2):585-90.

Table 1. The combination of process temperature-duration and storage time which were taken into account in the experimental design.

	Process Temperature (ºC)		
	60	80	99
Process Duration (min)	1.57 - 79.57	0.34 - 54.34	1 - 44
Storage Time (Day)	0 - 35	0 - 35	0 - 35

Table 2. Peroxidase activity in broccoli samples (average and standard error) processed at various temperatures and durations.

60 (°C)		80 (°C)		99 (°C)	
Time (min)	Activity (A/A0)	Time (min)	Activity (A/A0)	Time (min)	Activity (A/A0)
2	0.975 ± 0.000	1	0.384 ± 0.000	1	0.000 ± 0.000
10	0.856 ± 0.000	7	0.000 ± 0.000	3	0.000 ± 0.000
20	0.728 ± 0.002	15	0.000 ± 0.000	7	0.000 ± 0.000
40	0.526 ± 0.001	25	0.000 ± 0.000	14	0.000 ± 0.000
60	0.380 ± 0.000	34	0.000 ± 0.000	24	0.000 ± 0.000
80	0.275 ± 0.001	55	0.000 ± 0.000	44	0.000 ± 0.000

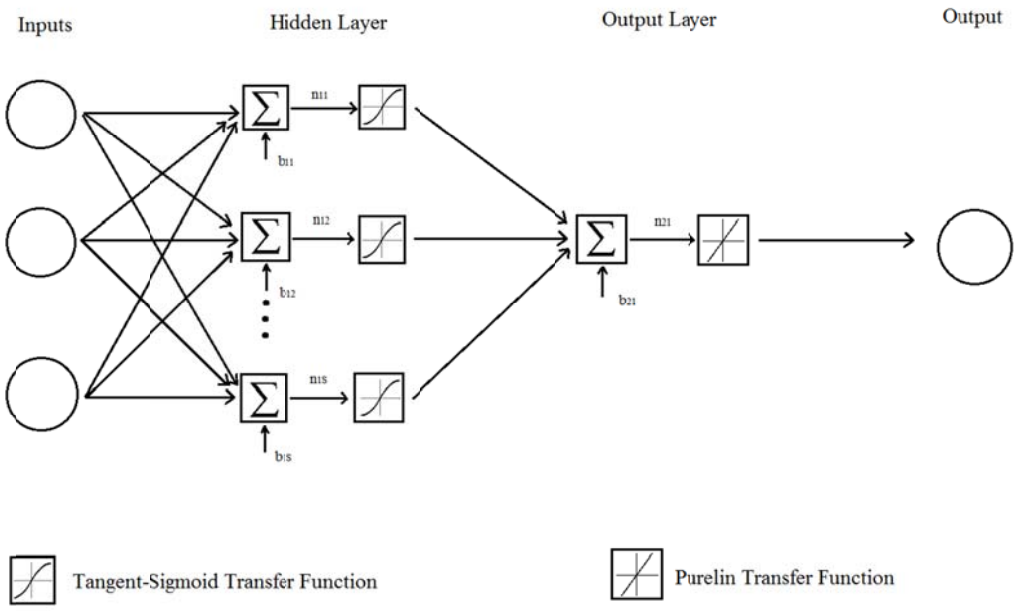

Fig. 1. Structure of MLP ANN for modeling the kinetics of broccoli color. definition of symbols in this figure are as follows: n = neuron, s = number of neurons, b = bias.

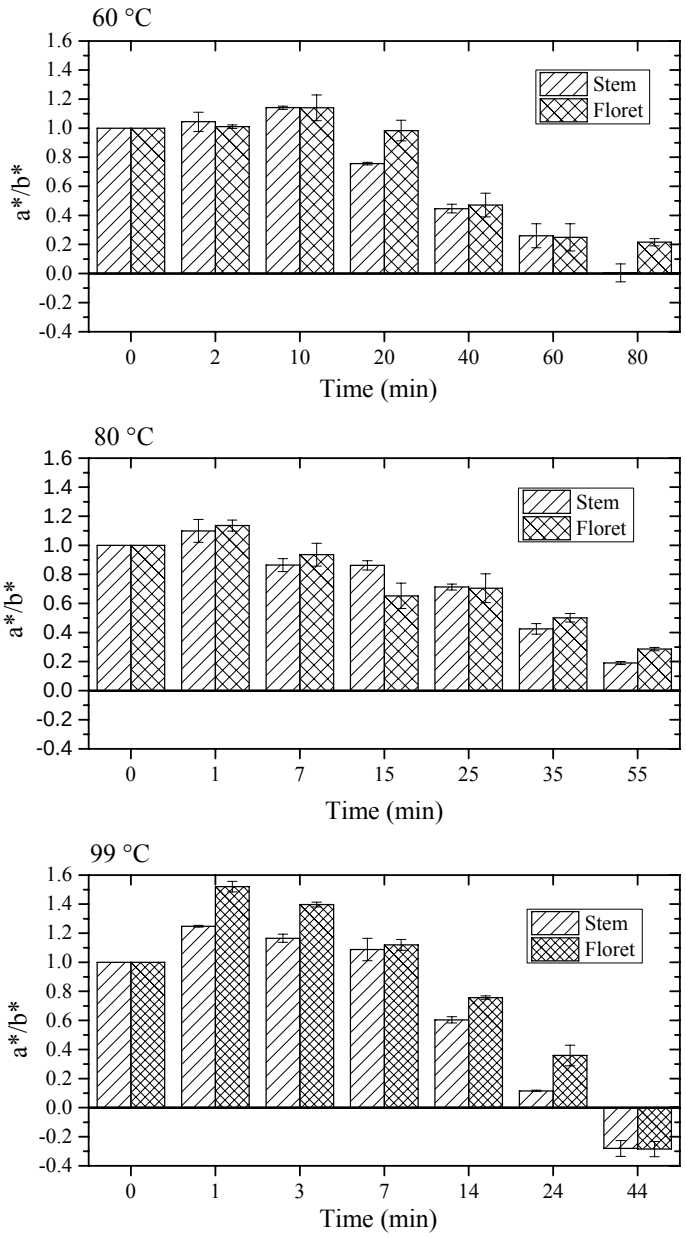

Fig. 2. Effect of processing temperature and time on the color (average a*/b* and standard error) of broccoli stem and floret.

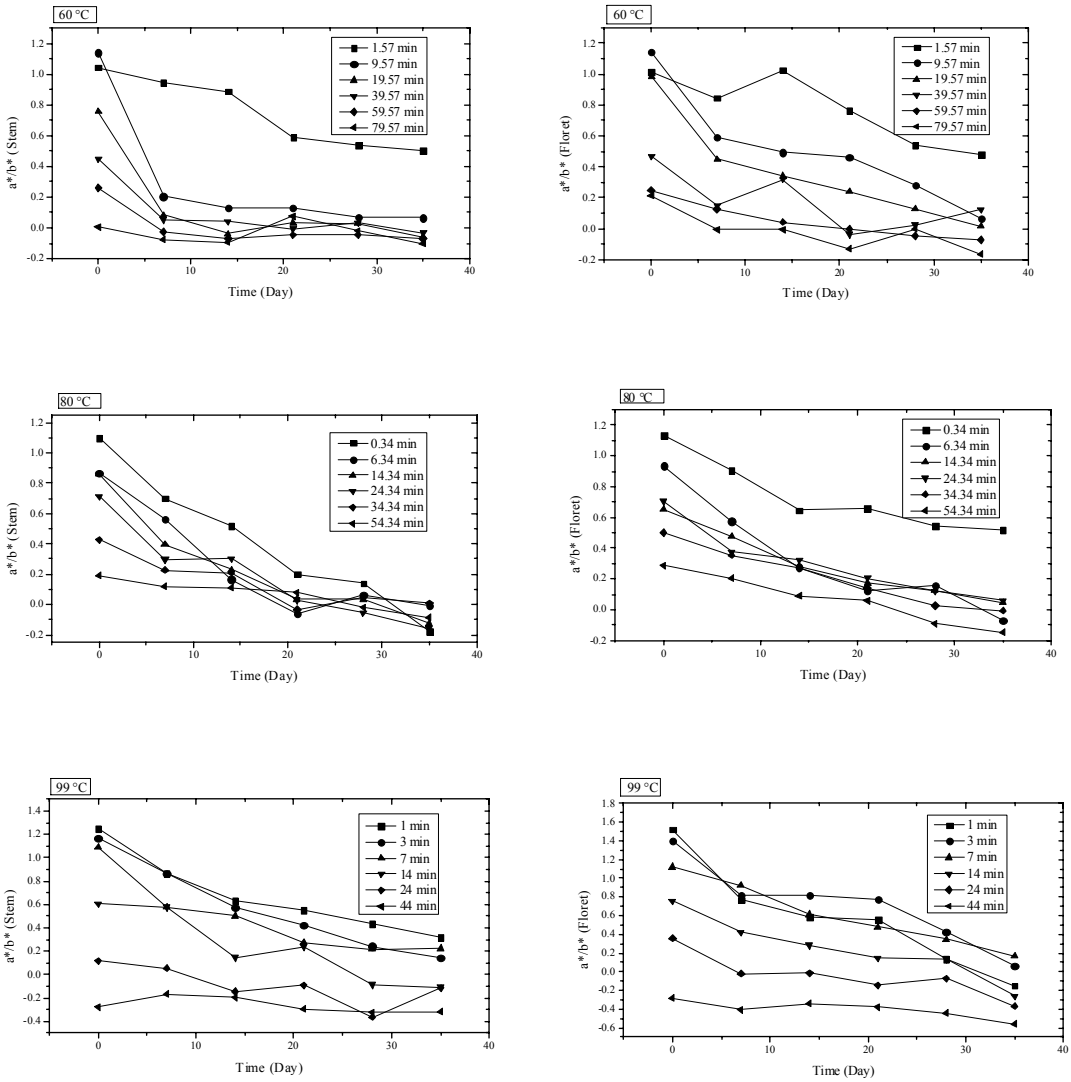

Fig. 3. The change in the greenness of vacuum packed broccoli stem and floret processed at various temperatures (°C) and durations (min) during storage (Day) at 4 °C.

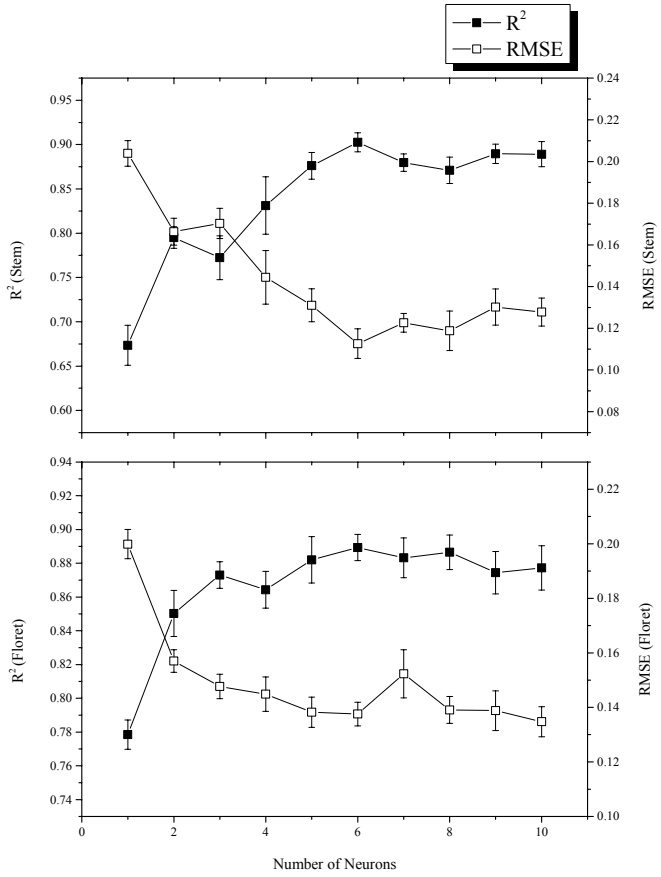

Fig. 4. Average and standard error results of 10 software runs for obtaining the best number of neurons in the hidden layer of feed-forward back propagation ANN with tangent-sigmoid and linear transfer function in hidden and output layer, respectively.

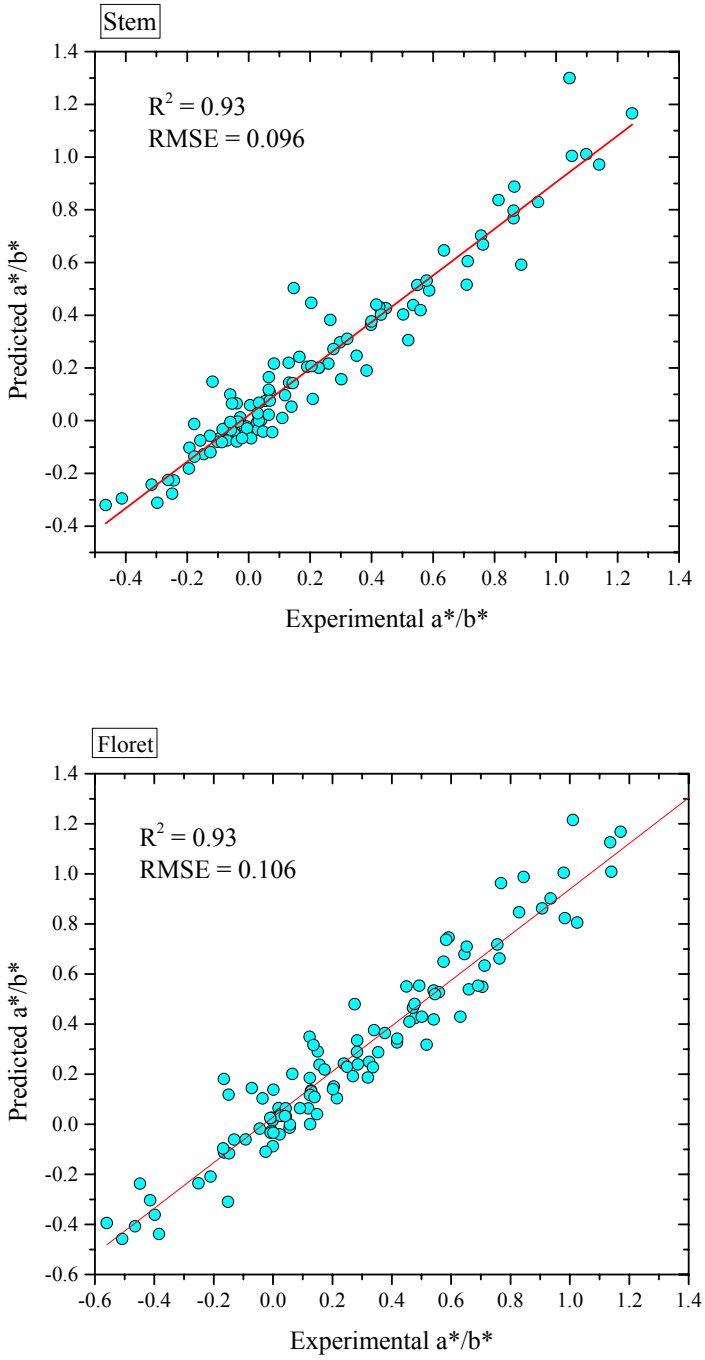

Fig. 5. Comparison between predicted values of a*/b* obtained by the best ANN topology and experimental values used for constructing ANN.

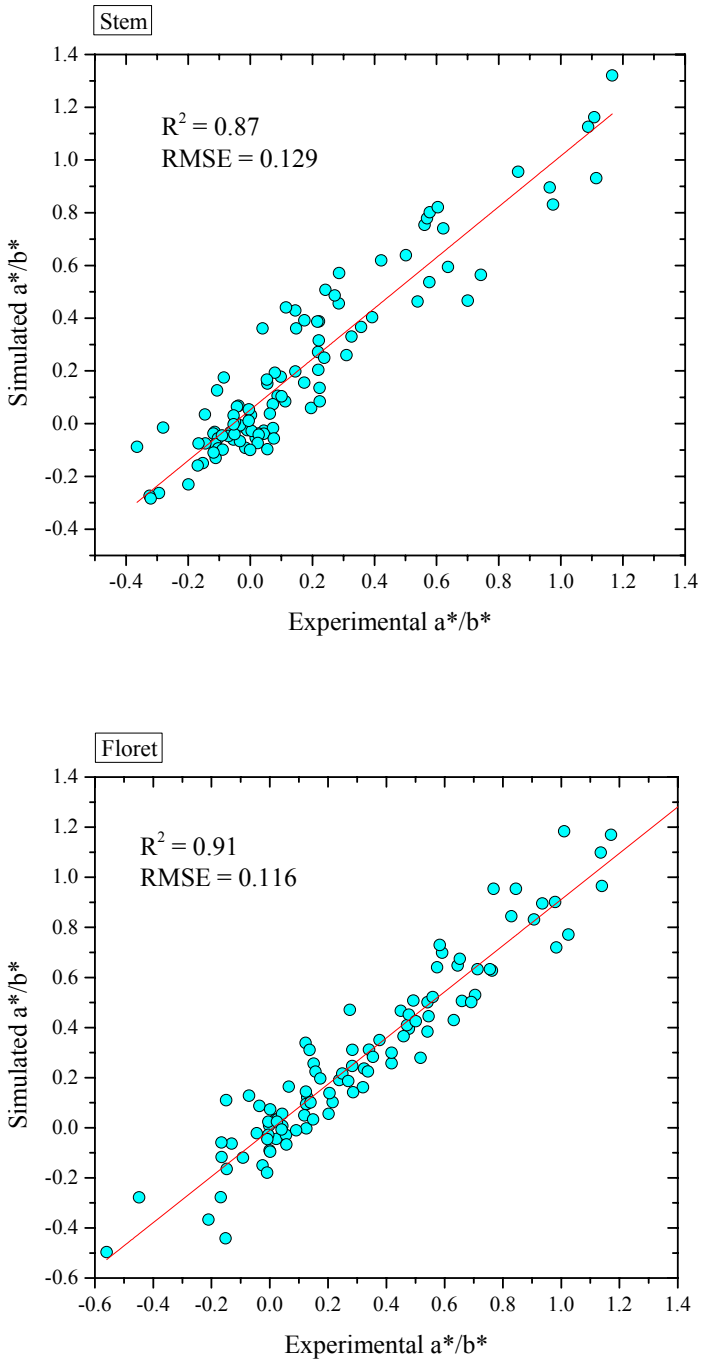

Fig. 6. Comparison between simulated values of a*/b* obtained by the best ANN topology and unseen experimental values during testing step.

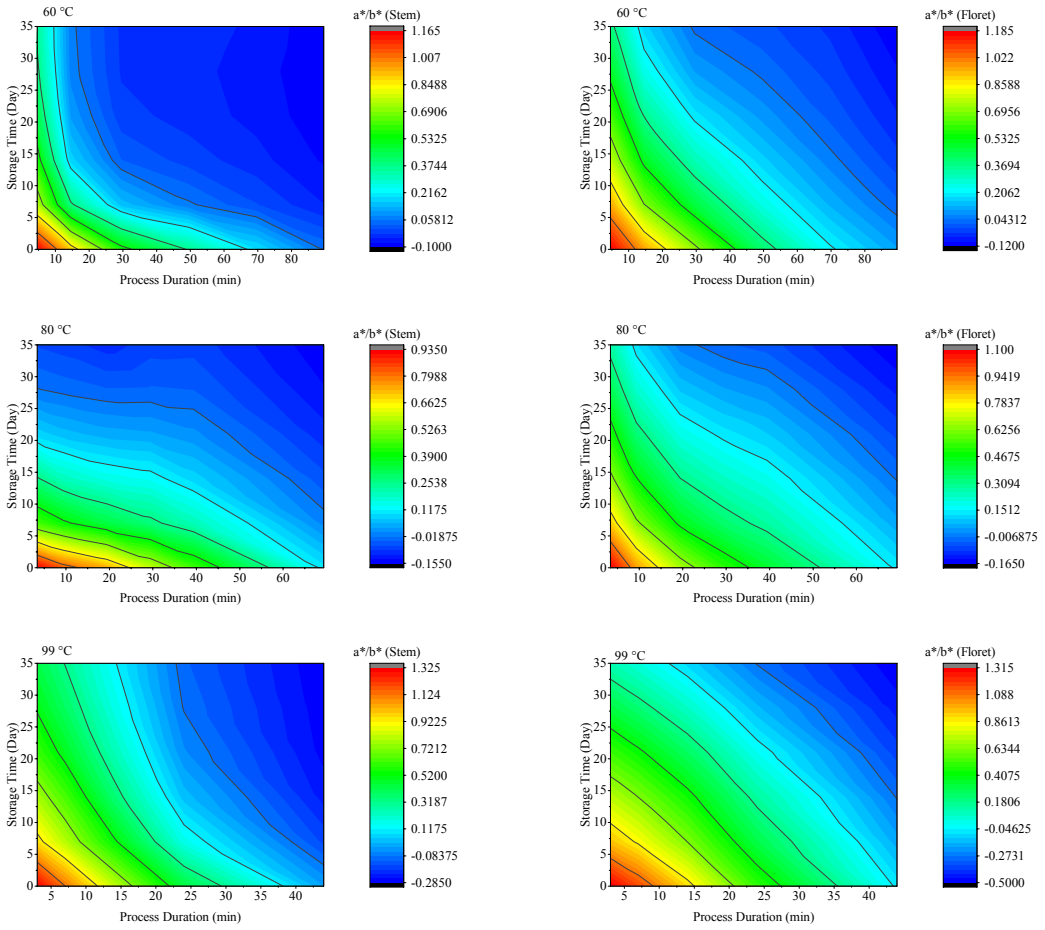

Fig. 7. Simulated color change of broccoli stem and floret during storage which have been processed at various temperatures and heating durations.