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Physicochemical Changes of the Gluten-Free Rice-Buckwheat Cookies during Storage – Artificial Neural Network Model

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Abstract
The influence of storage time, temperature, and packaging on some physicochemical characteristics of gluten-free rice-buckwheat cookies was studied. Shelf life markers, such as water activity (a_w), hydroxymethylfurfural (HMF), firmness, and color parameters were modelled in relation to different storage conditions. Principal component analysis was applied to study the similarity among samples according to the observed parameters. The mathematical model in the form of an artificial neural network was developed to predict the physicochemical parameters of cookies during 6-month storage. The most evident differentiation among samples was observed for color coordinate a^*, a_w, and *HMF*. Regarding the methods for determination of the parameters, priority should be given to the instrumental determination of color as the most convenient method. The processing of experimental data allowed the creation of useful mathematical model to be used in predicting the behavior of physicochemical changes of cookies by different factor combinations during storage.

Keywords
gluten-free cookies, shelf life markers, mathematical model

1 Introduction

The best strategy for celiac disease patients is continuous adherence to gluten-free diet, which implies the strict utilization of gluten-free ingredients. Their incorporation in various food products has been studied by many authors [1]. In this regard, pseudocereals received a special attention [2].

The absence of structure forming gluten proteins in gluten-free formulation results in poor rheological and baking properties of gluten-free dough [3], and, therefore, production of high-quality gluten-free products represents a significant technological challenge, largely because of inadequate sensory properties of the final products [4]. Consequently, poor quality of gluten-free products can reflect on their shelf life, which has to be predicted.

Among gluten-free products, cookies represent one of the largest food categories, primarily due to their versatility, convenience, acceptable sensory properties, but especially because of their long shelf life. Even for a shelf-stable product, such as short dough cookies, there is a limitation on its shelf life due to deteriorating chemical reactions, determining its best-before date [5].

Physicochemical stability of cookies is primarily governed by their composition, which is characterized by relatively low water activity (a_w) values and high fat content (20–30 % on the flour weight basis). The water activity of cookies is mainly lower than those that permit the growth of microorganisms ($a_w > 0.6$) [6], but this parameter is also important in terms of cookie sensory profiles. Although the moisture content does not exceed 7 % in cookies, moisture migration affects cookie hardness, which represents a major problem in their shelf life.

Since cookies possess a high amount of vegetable fat, they are susceptible to oxidative changes, especially when they are exposed to conditions which favor lipid oxidation [7]. There are several approaches to predict cookie shelf life measuring lipid oxidation markers during storage

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– hexanal [8], heptanal [9], malonyldialdehyde (*MDA*) [10] or some other secondary lipid oxidation products [11].

During baking of cookies, Maillard reaction and caramelization also occur, resulting in the development of the desirable color and flavor of the final product. However, along with these substances, processing contaminants such as acrylamide and 5-hydroxymethylfurfural (*HMF*) are formed [12]. *HMF*, as a marker of non-enzymatic browning reaction, is directly related to the heat load applied during processing or storage [13]. It is known that *HMF* was used for assessing the shelf life of a wide range of carbohydrate-containing foods such as cookies [12]. In addition, *HMF* can also be presented in some cookie ingredients such as honey or caramel.

In addition, chemical deterioration reactions during storage can lead to increased levels of some degradation products of *HMF* such as furan [14] or furan derivatives – furfuryl aldehyde and 2-methylfurfural [13] meaning that mentioned substances can also serve as markers of food deterioration processes.

Our previous work [15] showed the possibility to predict the shelf life of the unpacked and packed gluten-free rice-buckwheat cookies, kept at ambient (23 ± 1 °C) and elevated (40 ± 1 °C) temperature during storage, measuring off-flavor volatile compounds (aldehydes), antioxidant capacity, total phenolic, rutin content, and evaluating sensory properties. Based on the obtained results, the evaluated sensory attributes were suggested to be the relevant parameters for predicting the endpoint of cookie shelf life. Despite the fact that sensory evaluation was assessed essential for cookie shelf life prediction [6], it is an expensive and long-term tool to conduct. Therefore, physicochemical parameters may play a crucial role in stability testing as they can be used either to predict the results obtained by the analytical sensory test or the endpoint of cookie shelf life.

Recently, mathematical modelling has been increasingly used for the study of shelf life. Developed empirical models show a reasonable fit to experimental data and successfully predict the shelf life of products [16]. Nonlinear models are found to be more suitable for real process simulation. Artificial neural network (ANN) models have gained momentum for modelling and control of processes [17].

ANN models are recognized as a good modelling tool since they provide the empirical solution to the problems from a set of experimental data, and are capable of handling complex systems with non-linearities and interactions between decision variables [17, 18].

The objective of this work was to study the physicochemical changes of gluten-free rice-buckwheat cookies as a function of temperature, storage time and packaging conditions. The investigation focused on the effectiveness of storage conditions on water activity, *HMF*, color, and firmness as the shelf life markers. Mathematical models, in the form of artificial neural network (ANN) were used for modelling and prediction of cookie storage process.

2 Material and methods

In our previous papers [15, 19], the used ingredients, preparation of cookies, as well as packaging and storage conditions of cookies were described in details. Therefore, in this paper they are shown in Fig. 1.

According to experimental plan, cookies were investigated monthly for changes in physicochemical parameters (during a six month period). Cookies were kept at (23 ± 1 °C) or (40 ± 1 °C), packed or unpacked, which makes 7 x 2 x 2 = 28 samples used in this investigation.

2.1 Water activity value

Approximately 2.5 g of ground cookie sample were placed into the sample holder of an a_w-meter (TESTO 650, Testo AG, Lenzkirch, Germany) at 25 °C. Water activity (a_w) values of three replicates were recorded after equilibration.

2.2 Hydroxymethylfurfural (*HMF*) analysis

2.2.1 Sample preparation

The extraction procedure was performed according to Rufián-Henares et al. [20], with the modifications which were done by Petisca et al. [21]. Ten grams of sample were suspended in 5 mL water:methanol (70:30). The mixture was thoroughly stirred during 1 min and then 2.0 mL of Carrez I and Carrez II solutions were added and centrifuged at 5000 rpm (4 °C) during 15 min, recovering the supernatant to a 15 mL flask. Two more consecutive extractions were made with 2 mL of water:methanol (70:30) until collecting 10 mL of supernatant. Two milliliters of this solution were centrifuged at 8000 rpm for 15 min before being analyzed.

2.3 HPLC-DAD analysis

The chromatographic separation and quantification of *HMF* was performed using the HPLC method described by Ariffin et al. [22], and Tomasini et al. [23], with some modifications. The extracts were filtered through 0.45 µm pore size nylon filter (Agilent Technologies, Santa Clara, CA, USA) before injection into the HPLC system.

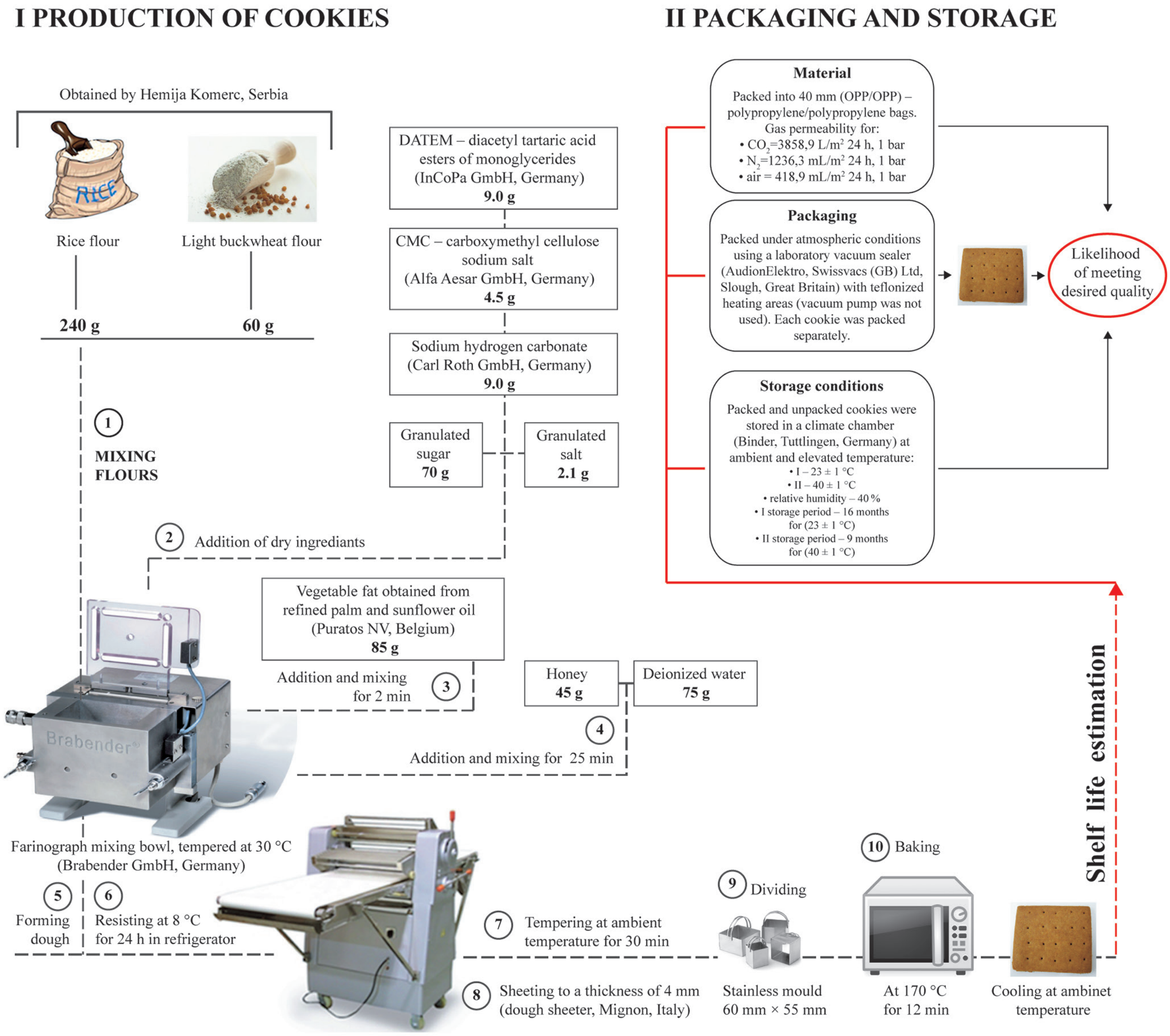

Fig. 1 Cookie manufacturing and storage

Liquid chromatograph (Agilent 1200 series), equipped with a DAD detector and an Eclipse XDB-C18, 1.8 µm, 4.6 × 50 mm column (Agilent) was used for quantification of *HMF* in the obtained extracts. Separation of the analyte was achieved with a column temperature of 30 °C and sample injection volume of 2 µL. The mobile phase consisted of two eluents, H_2O (0.1% HCOOH) (A) and methanol (B), delivered at a flow rate of 0.75 mL/min. The isocratic elution was employed with the ratio A:B (90:10, v/v). The DAD wavelength was set at 284 nm. The total run time of the analysis was 5 min.

2.4 Surface color determination

Color was determined by a chromameter Minolta, type CR 400 (Minolta Co., Ltd., Osaka, Japan) on the top surface of the cookies. Due to the typical lean particle dispersion, color measurements were taken in five areas of cookie (at the center and corners) with a minimum of ten readings per sample and the results were averaged. Color characteristics were presented in the CIE $L^*a^*b^*$ system which defines color by the parameters such as lightness, L^* (L^*=0 (black) and L^* = 100 (white)), a^* (a measure of greenness (–) to redness (+)), and b^* (a measure of blueness (–) to yellowness (+)). The hue value, $h^*\left(h^* = \arctan b^*/a^*\right)$, and degree of color saturation or intensity, C^* $(C^* = ((a^*)^2 + (b^*)^2)^{0.5})$, were also calculated. The color change during storage was expressed as the total color difference, ΔE^*, $(E^* = (L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{0.5}$, where $\Delta L^* = L^* - L^*_0$, $\Delta a^* = a^* - a^*_0$, and $\Delta b^* = b^* - b^*_0$. In this investigation ΔE^* was used instead of browning index (*BI*) [24].

According to the results of PCA analysis, ΔE^* could be used only for prediction of shelf life, while the $L^*a^*b^*$ color system could be used for more detailed shelf life prediction which include the prediction of possibility of microbial spoilage and the increase of *HMF* amount. Both ΔE^* and *BI* show the changes in color of cookies during the shelf life period (which are also described by the dominant color changes in L^* and b^* color coordinates), while the changes in storage conditions (packaging and temperature) influence a^* color coordinate. The subscript ‘0’ indicates the initial color of the cookie sample.

2.5 Textural measurement

Textural analysis was conducted using a TA.XTPlus Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK), equipped with a 3-point bending rig (HDP/3PB), and a 5 kg load cell. Texture analyzer settings were: mode-measure force in compression; pre-test speed 1.0 mm/s; test speed 3.0 mm/s; post-test speed 10.0 mm/s; distance 5.0 mm; trigger force 50 g. Cookie hardness/firmness expressed as a peak force (F) at the time of interruption (the point of break) was determined. Ten measurements per each sample were performed.

2.6 Statistical analysis

The analysis of variance (ANOVA) was applied for the comparison of means of the collected data, and significant differences were determined according to post-hoc Tukey's HSD test at $p < 0.05$ significance level. Principal component analysis (PCA) was enforced to the experimental data to characterize the internal relations between the variables and to differentiate the investigated samples. All data were processed statistically using the software package STATISTICA 10.0 (StatSoft Inc., Tulsa, OK, USA).

2.6.1 Artificial neural network (ANN) modelling

The ANN model in the form of multilayer perceptron model (MLP), (with input, hidden and output layers) was developed for prediction of the changes in gluten-free rice-buckwheat cookies during storage. This form of model has been proven in approximating nonlinear functions [25]. Input and output data were normalized prior to ANN calculation in order to improve the behavior of the ANN. Broyden-Fletcher-Goldfarb-Shanno (BFGS) algorithm was used for network optimization [26]. The experimental results used for ANN modelling were randomly divided into: training (60 %), cross-validation (20 %) and testing data (20 %). The training data set was used for the learning cycle of ANN, assuming that the successful training was achieved when learning and cross-validation curves approached zero. The coefficients associated with the hidden layer and the output of the ANN model was determined during the training cycle, in which the BFGS algorithm was used as an optimization procedure to minimize the error between network and experimental outputs [26].

2.6.2 Sensitivity analysis

Sensitivity analysis was used to investigate the influence of input variables on the observed outputs, evaluated at specific centile points for each input variable [27]. This analysis is also necessary to check if the ANN could behave erroneously [28]. The infinitesimal amount has been added to each input variable in 10 equally spaced individual points determined by the minimum and maximum of the training data to check the influence of input variables on the observed outputs [27].

2.6.3 The accuracy of the models

The adequacy of the developed models was tested using coefficient of determination (r^2), reduced chi-square (χ^2), mean bias error (*MBE*), root mean square error (*RMSE*) and mean percentage error (*MPE*).

3 Results and discussion

The unpacked and packed gluten-free rice-buckwheat cookies were studied during storage at ambient (23 ± 1 °C) and elevated (40 ± 1 °C) temperature, in terms of chemical parameters (a_w and *HMF*), color values (L^*, a^*, b^*, h^*, C^*, ΔE^*), and texture firmness (F).

The impact of ingredients, manufacturing process, packaging, and storage conditions on the cookie quality during the time was described in the previous paper [15].

PCA was applied to characterize and differentiate between the investigated samples, according to observed parameters. The PCA of the presented data explained that the first two principal components accounted for 70.73 % of the total variance (52.74 % and 17.99 %, respectively), in the nine variables factor space (a_w, *HMF*, L^*, a^*, b^*, C^*, h^*, ΔE^* and F). Considering the map of the PCA performed on the data, the observed variables, with the contribution of the total variance of 17.3 % for L^*, 16.4 % for b^*, 15.9 % for ΔE^*, 14.1 % for C^*, and 19.95 % for h^*, exhibited negative scores according to first principal component (PC1) (Fig. 2). The negative contribution to the second principal component calculation was observed for a_w with a 20.7 % contribution to the total variance, a^* with

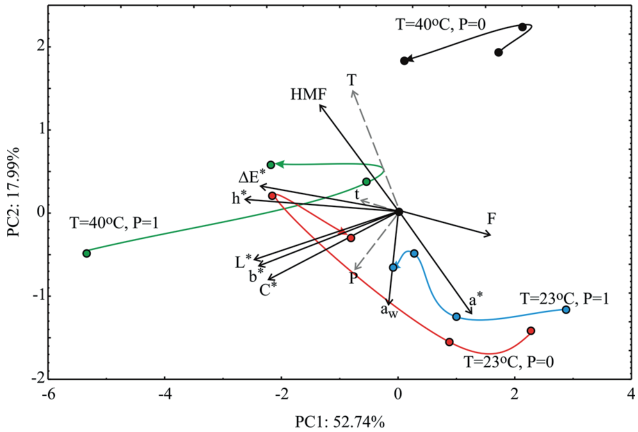

Fig. 2 Principal component analysis (PCA) ordination of all observed variables for gluten-free rice-buckwheat cookies based on component correlations (unpacked (P=0) and packed (P=1) gluten-free rice-buckwheat cookies during 6-month storage (t) at two different temperatures (T) (23 ± 1 °C and 40 ± 1 °C)

a 24.9 % contribution to the total variance, and C^* with a 10.5 % contribution to the total variance. The most positive influence was observed for *HMF* with a 28.8 % contribution to the total variance.

According to the orientation of the vector describing *HMF* in the factor space, the content of *HMF* is more pronounced for samples kept at a higher temperature (upper part of the PCA graphic). These samples had lower values of a^* color coordinate (more greenish samples) and lower a_w values, indicating the reduced potential for microbiological spoilage. *HMF* and a^* color coordinate are opposizionally oriented on the graph, explaining the negative correlation between these variables. The similar direction of L^* and b^* color coordinates indicates that packed samples kept at 40 °C at the beginning of the storage period were more yellowish and lighter than other samples. The angle between L^* and b^* color coordinate vectors indicates the high degree of correlation, as well as the high correlation to color coordinates h^*, C^*, and ΔE^*. Color coordinates L^* and b^* were negatively correlated to firmness (F). According to the obtained results, the observed samples became less acceptable during the storage, regardless of packaging condition.

3.1 Artificial neural network model

The optimization procedures for minimizing the error function between network and experimental outputs were used during the ANN learning cycle [26, 28]. The optimum number of hidden neurons was chosen upon minimizing the difference between predicted ANN values and experimental results. According to ANN performance, it was noticed that the optimal number of neurons in the hidden layer for a_w, F, L^*, a^*, b^*, ΔE^*, C^*, h^*, and *HMF* calculation was 6 (network MLP 3-6-9) to obtain high values of r^2 (overall 0.898 for ANN during the considering period), and low values of the sum of squares (SOS) (Table 1).

Furthermore, ANN models were used to predict experimental variables (a_w, F, L^*, a^*, b^*, ΔE^*, C^*, h^*, and *HMF*) (Fig. 3).

The predicted values were very close to the desired values in most cases in terms of r^2 value of ANN models. SOS obtained with ANN models are of the same order of magnitude as experimental errors for a_w, F, L^*, a^*, b^*, ΔE^*, C^*, h^*, and *HMF*, reported in the literature earlier [26, 29].

3.2 Sensitivity analysis

The influence of the input over the output variables, i.e. calculated changes of the output variables for infinitesimal changes in the input variables are shown in Fig. 4.

As it can be seen in Fig. 4, L^*, b^*, ΔE^*, C^* and h^* color coordinates were most affected by the infinitesimal changes in temperature at the minimum values of the input space. The content of *HMF* was most affected by temperature changes at the maximum values of the input space, i.e. the contents of *HMF* increased throughout the storage time at the higher temperatures. a_w and F were most affected by temperature changes at the lower end of the input space. The influence of storage time was more expressed at the center of the input space for the calculation of a_w, F, L^*, b^*, ΔE^*, C^* color coordinates, and *HMF* content, while the influence of infinitesimal changes in the storage time was more observable for lower values in the input space for a^* and h^* color coordinates.

Table 1 Artificial neural network ANN performance summary

Network name	**MLP 3-6-9**
Training's performance	0.899
Test performance	0.778
Validation performance	0.556
Training error	0.075
Test error	0.261
Validation error	1.146
Training algorithm	BFGS 24
Error function	SOS
Hidden activation	Tanh
Output activation	Exponential

*Tanh – *Hyperbolic tangent*, SOS – sum of squares

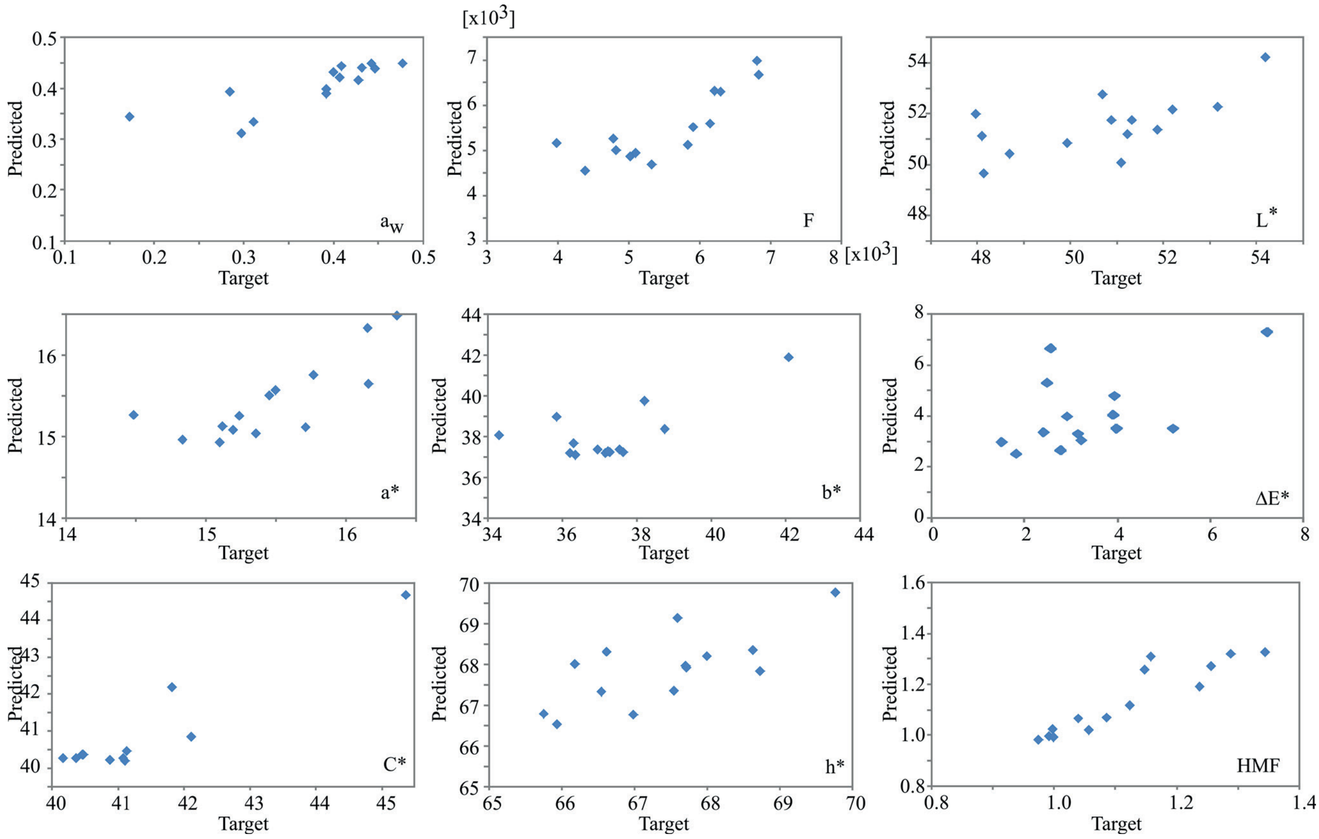

Fig. 3 Experimentally measured and the ANN model predicted values of all observed variables

Table 2 The 'goodness of fit' tests of the developed mathematical models

	χ^2	*RMSE*	*MBE*	*MPE*	r^2	Skewww.	Kurt	Mean	SD	Var.
a_w	0.05	0.12	$6.04 \cdot 10^{-16}$	70.64	0.81^*	0.30	3.33	$8.62 \cdot 10^{-17}$	0.04	0.00
HMF	0.13	0.18	$1.11 \cdot 10^{-16}$	34.44	0.84^+	-0.67	1.14	$1.59 \cdot 10^{-17}$	0.07	0.00
L^*	22.76	2.39	$2.84 \cdot 10^{-14}$	10.86	0.95^+	0.23	0.21	$3.79 \cdot 10^{-15}$	0.89	0.78
a^*	1.67	0.65	$-2.00 \cdot 10^{-14}$	8.02	0.97^+	-1.31	3.65	$-2.66 \cdot 10^{-15}$	0.24	0.06
b^*	15.61	1.98	$9.59 \cdot 10^{-14}$	11.88	0.91^+	0.00	-0.03	$1.28 \cdot 10^{-14}$	0.73	0.54
C^*	13.05	1.81	$-7.11 \cdot 10^{-14}$	9.92	0.86^+	0.10	-0.24	$-9.47 \cdot 10^{-15}$	0.67	0.45
h^*	8.13	1.43	$-8.17 \cdot 10^{-14}$	4.70	0.97^+	0.12	-0.11	$-1.09 \cdot 10^{-14}$	0.53	0.28
ΔE	$4.2 \cdot 10^1$	3.3	$2.1 \cdot 10^{-14}$	$4.3 \cdot 10^3$	0.76^*	0.350	2.322	0.000	1.208	1.46
F	$2.85 \cdot 10^6$	843.50	$-4.2 \cdot 10^{-12}$	26.74	0.83^*	-0.57	3.25	$-8.01 \cdot 10^{-13}$	377.23	$1.42 \cdot 10^5$

χ^2 – reduced chi-square; *RMSE* – root mean square error; *MBE* – mean bias error; *MPE* – mean percentage error; Skew. – skewness; Kurt. – Kurtosis; SD – standard deviation; Var. – variance. $^+$ model is statistically significant at $p < 0.01$ level; * model is statistically significant at $p < 0.05$ level.

3.3 'Goodness of fit' tests and residual analysis

The common 'goodness of fit' tests and residual analysis were performed [27] and the obtained results are shown in Table 2.

The obtained r^2 values were high, and χ^2, *MBE*, *RMSE*, and *MPE* were low, indicating that this model generally shows a good fit to experimental data in the experimental domain at which points were not included in the regression, as mentioned earlier by Madamba [30].

4 Conclusions

Assessing the characterization and differentiation patterns based on PCA analysis, it can be concluded that cookies became significantly brighter during storage, i.e. longer storage caused significant changes in the cookie color. At the same time, storage at ambient temperature led to an increase in a_w value, and as time passed, the samples became softer, in contrast to the samples kept at a higher temperature. The content of *HMF* was more pronounced

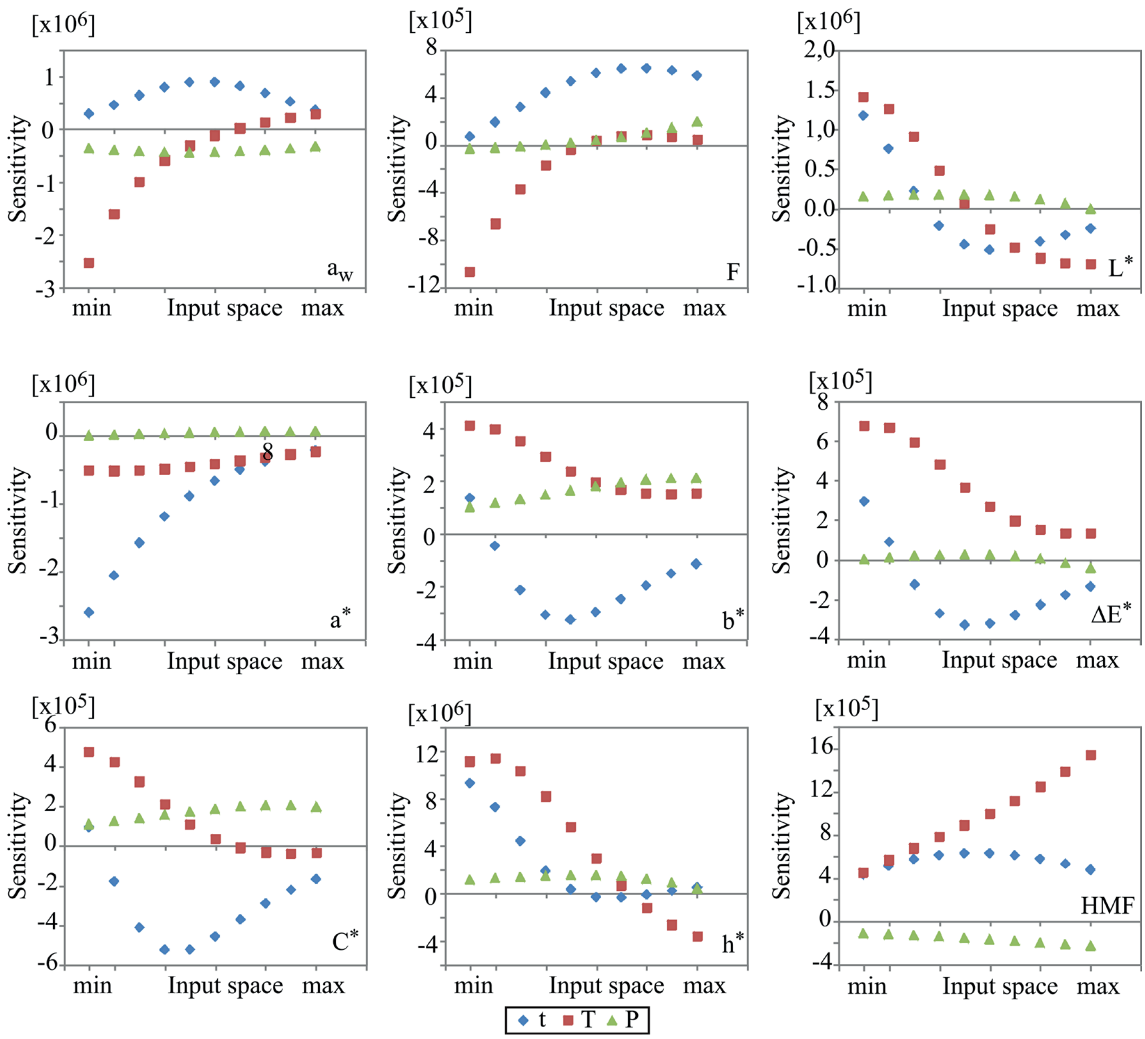

Fig. 4 Sensitivity analysis – the influence of the input over the output variables *T* – storage temperature; *t* – storage time; *P* – logical content regarding the packing state of cookies (packed or unpacked)

for samples kept at a higher temperature. These samples were also characterized by lower a^* and a_w value. L^* and b^* color coordinates were highly correlated for all samples and these variables were negatively correlated to firmness. According to the obtained results, samples became less acceptable during the storage, regardless of the packaging condition. Depending on the storage conditions, the best differentiation among samples was achieved on the basis of parameters a^*, a_w, and *HMF*. Regarding the methods for determination of these parameters, priority should be given to the instrumental determination of color as the easiest way to monitor the changes during the cookie storage. The results of this study showed that the processing of experimental data allowed the creation of useful mathematical model to be used in predicting the behavior of physicochemical changes of cookie samples by different factor combinations during storage. The predicted values fitted well to the experimental ones in most cases, according to the 'goodness of fit' tests for the developed mathematical model, and can be successfully used for prediction in controlling the storage process of this type of product.

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