Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *82* (12) 1343–1355 (2017)
JSCS–5045
UDC 664.681:547.461.3+543.544.3+543.544.5.068.7:519.87
Original Scientific paper

Application of the SHS-GC-FID method and HPLC-DAD method in the prediction of the shelf-life of gluten-free cookies

MARIJANA SAKAČ[1]*, LATO PEZO[2], PAVLE JOVANOV[1], NATAŠA NEDELJKOVIĆ[1], ANAMARIJA MANDIĆ[1], MLADENKA PESTORIĆ[1] and ALEKSANDRA MIŠAN[1]

[1]*Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, Novi Sad, Serbia and* [2]*Institute of General and Physical Chemistry, University of Belgrade, Studentski trg 12/V, Belgrade, Serbia*

(Received 21 April, revised 8 June, accepted 9 June 2017)

Abstract: The aim of this study was to compare the sensitivity of two analytical methods for the prediction of the shelf-life of unpacked and packed gluten-free rice–buckwheat cookies kept at ambient (23±1 °C) and elevated (40±1 °C) temperature during storage, namely the static headspace gas chromatographic method with flame ionisation detection (SHS-GC-FID) for volatile saturated aldehydes (propanal (C3), pentanal (C5), hexanal (C6), heptanal (C7) and octanal (C8)) and the HPLC method for malondialdehyde (MDA) determination. Both methods resulted in obtaining the same end-points of cookie shelf-life, *i.e.*, 3 and 5 months for unpacked and packed cookies kept at elevated temperature, respectively, and 11 and 14 months for unpacked and packed cookies kept at ambient temperature, respectively. Two computational approaches, *i.e.*, the second order polynomial (SOP) and artificial neural network (ANN) models, were used accordingly. The calculations of the contents of aldehydes and MDA could be predicted with an overall coefficient of determination of 0.722 using the ANN model compared to 0.312–0.773 for SOP models. According to sensitivity analysis, it might be suggested that the relevant parameter for the prediction of the end-point of cookie shelf-life is the MDA rather than the C3, C5, C6, C7 and C8 content.

Keywords: cookies; shelf-life; aldehydes; malondialdehyde; mathematical modelling.

INTRODUCTION

Due to a permanent intolerance to the gluten proteins in many common cereals, celiac patients must be on a strict life-long gluten-free diet, which is usually poor in some essential nutrients. Therefore, one of the main goals in the creation of gluten-free products is their fortification to obtain added value pro-

* Corresponding author. E-mail: marijana.sakac@fins.uns.ac.rs
https://doi.org/10.2298/JSC170421074S

ducts. Many attempts have been made concerning sweet bakery products, such as cookies.[1–3]

Cookies are ready-to-eat food with a long shelf-life, and available at affordable prices, which make them popular among consumers. However, they are often considered as unhealthy food due to their high content of fat (20–30 % based on the flour weight) of questionable quality[4,5] and sugar. Besides the health aspect, the high amount of fat in cookie formulations could lead to oxidative changes during cookie storage.[6]

The lipid oxidation leads to the development of hydroperoxides from which more stable secondary products are derived.[7] These products could be used as markers of lipid deterioration. Some of them, such as hexanal, heptanal, octanal, and nonanal from the group of aldehydes, as well as the products from the group of alkanes, acids, ketones, and alcohols, are responsible for off-flavours.[8]

Although hexanal is usually measured as the marker of lipid oxidation in cookies,[9] their oxidative changes were also determined by quantifying nonanal and 2-nonenal in biscuits.[10] The generation of a particular aldehyde depends on the fatty acid composition of food. Octanal and nonanal represent the main oxidation products of oleic acid,[11] while linoleic acid oxidation leads to the range of compounds, such as hexanal, 2-heptenal, 2-octenal, 2-nonenal and 2,4-decadienal.[7] These volatile aldehydes are measured by static headspace (SHS) or dynamic headspace (DHS) techniques in combination with solid phase microextraction (SPME) or headspace sorptive extraction (HSSE) coupled to gas chromatography/mass spectrometry (GC/MS) or gas chromatography/flame ionization detection (GC/FID).[12] Pasqualone *et al.*[10] used SPME GC/MS to quantify the volatile compounds of biscuits obtained from wholemeal flour of purple and conventional wheat, while Mandić *et al.*[13] measured off-flavour volatile compounds (5 aldehydes) in gluten-free crackers by the SHS-GC-FID technique. The same method was applied to gluten-free cookies to determine changes in the total content of aldehydes in terms of predicting the cookie shelf-life.[14]

Besides the mentioned volatile aldehydes, one of the possibilities to follow lipid oxidation is using malondialdehyde (MDA) as a marker of lipid deterioration. MDA is known to be produced in the decomposition of hydroperoxides derived from the oxidation of both omega-3 and omega-6 polyunsaturated fatty acids.[12] The most frequent analytical approach is to measure the thiobarbituric acid (TBA) value, which corresponds with the content of MDA–TBA complex, but this method lacks specificity as other carbonyl compounds present or formed in the food during the process may also react with TBA.[15] Therefore, HPLC techniques are more advisable for measuring MDA as they give more accurate results.[16]

In a previous work, the total aldehydes were monitored to predict the end-point of the shelf-life of gluten-free rice–buckwheat cookies, but it was concluded that sensory parameters are more relevant for this purpose.[14] Unfortun-

ately, sensory evaluation is time-consuming, because an experienced and trained panel is required to assess a range of previously chosen and defined parameters for a particular type of food. Therefore, in this work two analytical methods (SHS-GC-FID method for volatile aldehydes and HPLC method for MDA) for predicting the end-point of cookie shelf-life were compared in terms of their sensitivities. For this purpose, mathematical modelling was applied. Second order polynomial (SOP) models and an artificial neural network (ANN) model were used for modelling and controlling the storage process.

EXPERIMENTAL

Cookie formulation, production, packaging and storage

Cookie formulation and production were presented by Sakač *et al.*,[2] while packaging and storage conditions were performed according to the procedure described by Sakač *et al.*[14] All relevant details are presented in Fig. 1.

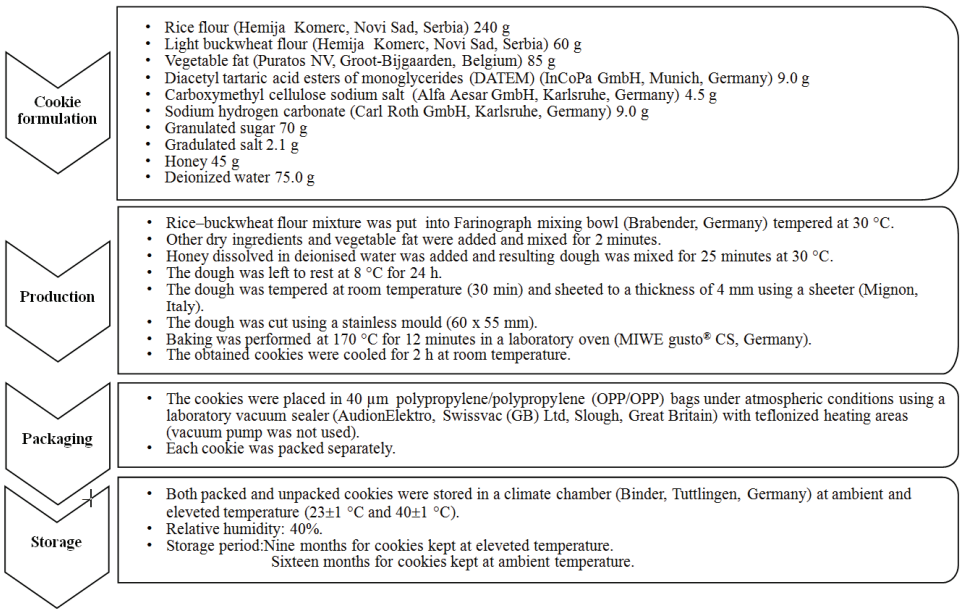

Fig. 1. Cookie formulation, production, packaging and storage.

Headspace analysis of aldehydes

Static headspace gas chromatography with flame ionisation detection (SHS-GC-FID) was applied to quantify the content of 5 aldehydes (C3, C5, C6, C7 and C8) in gluten-free cookies.[13]

Malondialdehyde (MDA) analysis

Preparation of MDA standard curve. A 10-mL volume of 1,1,3,3-tetraethoxypropane (TEP) was diluted to 10 mL with 0.1 mol L^{-1} HCl in a screw-capped test tube, placed in a boiling water bath for 5 min and rapidly cooled under tap water to produce hydrolyzed acetal. A

stock solution of MDA was prepared by pippeting 1 mL of the hydrolyzed acetal into a 100 mL flask followed by dilution with distilled water to achieve 2.92 μg MDA mL^{-1}. Standard solutions were prepared daily by diluting the stock solution, which were used to construct a calibration curve.

Sample preparation. The sample preparation was performed following the procedure described by Tsaknis *et al.*[17] Two grams of cookie powder were measured into a 250 mL flask, 90 mL of distilled water was added and the pH was adjusted to 1.5–1.8 with 2 mol L^{-1} of HCl. The flask was connected to a standard micro-Kjeldahl unit and the content was distilled. The distillation was conducted as rapidly as possible and terminated when 250 mL of distillate had been collected in a 250 mL calibrated flask. The sample was then ready for HPLC-DAD analysis.

HPLC-DAD analysis. The chromatographic separation and quantification of MDA was performed using the HPLC method described by Karatas *et al.*[18] with some modifications.

A liquid chromatograph (Agilent 1200 series, Agilent Technologies, Santa Clara, CA, USA), equipped with a DAD detector and an Agilent Eclipse XDB-C18, 1.8 μm, 4.6 mm×50 mm column was used for the quantification of MDA in the obtained distillates. Separation of the analyte was achieved at a column temperature of 30 °C and using a sample injection volume of 20 μL. The mobile phase consisted of two eluents, 30 mmol L^{-1} KH_2PO_4 (A) and methanol (B), delivered at a flow rate of 0.6 mL min^{-1}. The isocratic elution was performed with the ratio A:B (90:10 volume ratio). The DAD wavelength was set at 260 nm. The total run time of the analysis was 8 min.

Statistical analysis

The comparison of sample means according to the experimental results was studied using the post hoc Tukey's HSD test. Principal component analysis (PCA) was used to separate the observed samples into distinctive groups and to determine possible correlations among the variables.

Six mathematical models for the prediction of C3, C5, C6, C7, C8 and MDA content in the form of SOP models were developed, while the effects of the independent variables (t – storage time, T – storage temperature and P – packaging condition) on the shelf-life of gluten-free cookies were tested using analysis of variance (ANOVA).

A multi-layer perception model (MLP) generally used for approximation of nonlinear functions was used for ANN modelling.[19]

Before the calculation both input and output data were normalized. Broyden–Fletcher––Goldfarb–Shanno algorithm was used. The experimental database was randomly divided into three data sets: training, cross-validation and testing data set (with 60, 20 and 20 % of experimental data, respectively). The learning cycle was repeated several times to obtain the best fit to experimental data.[20]

ANN model can perform an approximation to a partially noisy and imprecise data, and, therefore, sensitivity analysis is necessary to check if the ANN could behave erroneously.[18] Sensitivity analysis was also used to define the influence of input variables on the observed outputs evaluated at specific centile points for each input variable.[21]

All data were processed using Statistica 10.0.[22]

RESULTS AND DISCUSSION

Aldehyde contents

Secondary lipid oxidation products, some of which are aldehydes, are responsible for the appearance of off-flavours during food storage. To monitor the progress of lipid oxidation during cookie storage, volatile saturated aldehydes (C3, C5, C6, C7, and C8) in unpacked and packed cookies stored at ambient and elevated temperature (23±1 and 40±1 °C) were detected. For the same purpose, the content of MDA was also measured. The obtained results are shown in Tables I–IV.

TABLE I. Propanal (C3), pentanal (C5), hexanal (C6), heptanal (C7), octanal (C8) and malondialdehyde (MDA) content (mg kg^{-1} dry matter (d.m.)) of unpacked (P = 0) gluten-free rice–buckwheat cookies during storage at T = 23±1 °C; values are means of three determinations ± standard deviation; values in the same column with the different superscript lowercase letters are statistically different ($p < 0.05$)

t / month	Compound					
	C3	C5	C6	C7	C8	MDA
0	0.232±0.048^{ab}	1.788±0.121^{de}	0.011±0.001^{a}	0.387±0.042^{d}	0.772±0.021^{a}	1.425±0.006^{a}
1	0.339±0.019^{b}	2.060±0.232^{ef}	0.010±0.000^{a}	0.250±0.008^{c}	0.804±0.031^{a}	1.528±0.017^{bc}
2	0.241±0.025^{ab}	1.559±0.032^{cd}	0.009±0.002^{a}	0.163±0.006^{abc}	0.571±0.040^{a}	1.514±0.011^{b}
3	0.097±0.004^{a}	1.548±0.022^{cd}	0.012±0.002^{a}	0.209±0.010^{bc}	0.479±0.003^{a}	1.593±0.022^{c}
4	0.110±0.017^{a}	1.324±0.021^{bc}	0.012±0.002^{a}	0.110±0.024^{ab}	0.497±0.013^{a}	1.682±0.013^{d}
5	0.196±0.026^{ab}	1.366±0.186^{bc}	0.053±0.008^{a}	0.068±0.002^{a}	0.610±0.064^{a}	1.866±0.022^{e}
6	0.213±0.015^{ab}	1.363±0.042^{bc}	0.500±0.017^{b}	0.550±0.017^{ef}	0.620±0.020^{a}	1.892±0.028^{ef}
7	0.303±0.025^{b}	1.470±0.026^{cd}	0.513±0.015^{b}	0.537±0.023^{ef}	0.597±0.035^{a}	1.901±0.023^{ef}
8	0.567±0.006^{c}	0.447±0.015^{a}	0.490±0.010^{b}	0.720±0.026^{g}	0.810±0.020^{a}	1.910±0.013^{ef}
9	0.660±0.010^{cd}	0.333±0.006^{a}	0.510±0.010^{b}	0.453±0.006d^{c}	0.820±0.050^{a}	1.948±0.009^{f}
10	0.747±0.021^{d}	0.493±0.042^{a}	0.557±0.006^{b}	0.563±0.021^{ef}	0.407±0.051^{a}	2.194±0.031^{g}
11	6.570±0.106^{i}	3.860±0.161^{h}	2.970±0.154^{f}	0.603±0.015^{fg}	1.240±0.221^{a}	2.330±0.036^{h}
12	2.330±0.167^{h}	3.503±0.225^{g}	2.593±0.168^{e}	1.363±0.107^{j}	7.403±1.167^{d}	2.652±0.017^{i}
13	1.433±0.021^{f}	1.780±0.020^{de}	1.377±0.021^{d}	0.883±0.023^{h}	3.627±0.035^{b}	2.754±0.029^{j}
14	1.133±0.057^{e}	1.127±0.031^{b}	0.797±0.006^{c}	1.907±0.031^{k}	3.293±0.379^{b}	2.782±0.025^{j}
15	1.190±0.040^{e}	1.287±0.050^{bc}	0.943±0.021^{c}	1.213±0.103^{i}	5.643±0.675^{c}	3.166±0.023^{k}
16	1.867±0.065^{g}	2.200±0.075^{f}	1.473±0.038^{d}	0.957±0.050^{h}	2.933±0.100^{d}	3.228±0.010^{k}

The quantification of the volatile saturated aldehydes in the examined cookies revealed that the most abundant aldehyde was octanal followed by hexanal and pentanal (Tables I–IV). This finding is related to fatty acid composition of vegetable fat used in the cookie formulation, which consisted of palmitic (43.2 %), oleic (42.5 %), linoleic (9.50 %) and linolenic (1.0 %) acid.[14] Oleic acid oxidation led to the established octanal generation,[11] while linoleic acid oxidation resulted in hexanal, together with some other secondary lipid oxidation products,[7] which we were not be able to detect. Since linoleic acid was found as the dominant representative of PUFAs in the vegetable fat, it could be assumed that MDA was mainly generated from hydroperoxides obtained from

linoleic acid,[12] *i.e.*, *via* the secondary oxidation of primary carbonyl compounds (non-3-enal) obtained from linoleic acid.[23]

The appearance of all volatile saturated aldehydes during cookie storage showed increasing trend to a maximum, followed by a decrease, as noted in our previously published paper.[14] This finding is due to their oxidative changes and interaction with proteins leading to products arising from non-enzymatic browning reactions.[24] The peak of aldehyde content can be addressed to the end-point of cookie shelf-life. The peaks obtained using SHS-GC-FID method developed by Mandić *et al.*[13] for measuring 5 aldehydes in gluten-free rice–buckwheat cookies, expressed as total aldehydes content, corresponded with 3 and 4 months for the unpacked and packed cookies stored at elevated temperature, respectively, as well as with 11 and 14 months for the unpacked and packed cookies stored at ambient temperature, respectively.[14] The same conclusions can be derived based on the generation and decomposition of each of the measured aldehydes with the exception of C7 and C8 in the unpacked cookies kept at ambient temperature, which extremely increased in 12th month (Tables I and II). Also, 5 months might be chosen as the end-point of cookie shelf-life rather than 4 months for the packed cookies kept at elevated temperature (Table IV).

TABLE II. Propanal (C3), pentanal (C5), hexanal (C6), heptanal (C7), octanal (C8) and malondialdehyde (MDA) content (mg kg^{-1} dry matter (d.m.)) of packed (P = 1) gluten-free rice–buckwheat cookies during storage at T = 23±1 °C; values are means of three determinations ± standard deviation. Values in the same column with the different superscript lowercase letters are statistically different (p < 0.05)

t / month	Compound					
	C3	C5	C6	C7	C8	MDA
0	0.232±0.048^{a}	1.788±0.121^{bcd}	0.011±0.001^{a}	0.387±0.042^{e}	0.772±0.021^{abcd}	1.425±0.006^{b}
1	0.325±0.013^{a}	1.970±0.009^{cd}	0.011±0.001^{a}	0.178±0.005^{abcd}	0.870±0.012^{bcde}	1.153±0.019^{a}
2	0.277±0.004^{a}	2.349±0.137^{d}	0.018±0.005^{a}	0.249±0.077^{cde}	1.042±0.153^{de}	1.189±0.017^{a}
3	0.247±0.034^{a}	2.211±0.041^{cd}	0.013±0.003^{a}	0.267±0.025^{de}	0.937±0.012^{cde}	1.364±0.031^{b}
4	0.218±0.005^{a}	2.157±0.031^{cd}	0.017±0.006^{a}	0.075±0.017^{abc}	1.230±0.094^{e}	1.582±0.032^{c}
5	0.245±0.006^{a}	2.553±0.303^{c}	0.043±0.004^{a}	0.043±0.015^{ab}	1.035±0.031^{de}	2.026±0.029^{de}
6	0.643±0.031^{a}	1.207±0.021^{abc}	0.463±0.015^{a}	0.023±0.006^{a}	0.793±0.180^{abcd}	2.107±0.033^{fg}
7	0.627±0.021^{a}	1.223±0.025^{abc}	0.537±0.035^{a}	0.043±0.006^{ab}	0.893±0.070^{cde}	2.069±0.027^{ef}
8	0.650±0.010^{a}	1.160±0.046^{abc}	0.480±0.000^{a}	0.050±0.026^{ab}	0.840±0.053^{bcde}	2.145±0.041^{gh}
9	0.593±0.012^{a}	0.853±0.021^{ab}	0.523±0.021^{a}	0.110±0.010^{abcd}	0.543±0.021^{abc}	2.153±0.027^{gh}
10	0.680±0.010^{a}	0.517±0.015^{a}	0.477±0.012^{a}	0.227±0.025^{bcde}	0.543±0.055^{abc}	2.194±0.011^{h}
11	0.710±0.010^{a}	0.540±0.020^{a}	0.483±0.015^{a}	0.217±0.021^{bcde}	0.403±0.015^{a}	2.170±0.019^{gh}
12	0.710±0.010^{a}	0.550±0.020^{a}	0.490±0.000^{a}	0.223±0.015^{bcde}	0.460±0.010^{ab}	1.959±0.021^{d}
13	0.713±0.038^{a}	0.563±0.047^{a}	0.507±0.021^{a}	0.270±0.026^{de}	0.763±0.031^{abcd}	2.002±0.019^{de}
14	8.173±0.389^{b}	24.977±0.382^{f}	9.300±0.142^{d}	1.610±0.085^{g}	3.673±0.325^{g}	2.198±0.031^{h}
15	14.903±0.765^{c}	25.417±1.242^{f}	7.880±0.789^{c}	1.637±0.212^{g}	4.193±0.370^{h}	2.196±0.028^{h}
16	7.947±0.371^{d}	10.850±0.497^{e}	4.747±0.156^{b}	0.807±0.015^{f}	1.953±0.067^{f}	2.196±0.028^{h}

Contrary to the generation and decomposition of volatile saturated aldehydes, the MDA content tended to increase during the storage period to a maximum after which it became constant (Tables I–IV). The measured content of MDA (1.425–5.017 mg kg^{-1} d.m.) during the investigated storage period conforms with previously determined data obtained by Papastergiadis *et al.*[25] who found that cookie samples contained more than 1.3 mg kg^{-1} MDA (between 1.344 and 4.175 mg kg^{-1} MDA). The obtained pattern of MDA generation was not similar to that observed by Botosoa *et al.*[26] who investigated the TBARS value of sponge cookies stored at 20 °C and found an increasing trend (up to 20 days of storage) followed by further reduction (200 days of storage), which could be explained by the reaction of MDA with a wide range of compounds (amines, amino acids, proteins) or its polymerization resulting in formation of dimers or trimers.[27] The same observation was obtained in the experiment conducted by Papastergiadis *et al.*,[28] in which the MDA content in cookies stored up to 92 days became lower after reaching a maximum, suggesting the mentioned reactivity of MDA with proteins.

TABLE III. Propanal (C3), pentanal (C5), hexanal (C6), heptanal (C7), octanal (C8) and malondialdehyde (MDA) contents (mg kg^{-1} dry matter (d.m.)) of unpacked (P = 0) gluten-free rice–buckwheat cookies during storage at T = 40±1 °C; values are means of three determinations ± standard deviation. Values in the same column with the different superscript lowercase letters are statistically different (p < 0.05)

t / month	Compound					
	C3	C5	C6	C7	C8	MDA
0	0.232±0.048^{ab}	1.788±0.121^{b}	0.011±0.001^{a}	0.387±0.042^{b}	0.772±0.021^{a}	1.425±0.006^{b}
1	0.203±0.037^{a}	2.046±0.004^{bc}	0.013±0.003^{a}	0.280±0.009^{ab}	0.823±0.020^{a}	1.496±0.013^{b}
2	0.534±0.044^{b}	2.617±0.204^{d}	0.077±0.009^{a}	0.195±0.036^{a}	1.006±0.026^{a}	1.518±0.065^{b}
3	2.316±0.140^{e}	3.224±0.054^{g}	2.618±0.044^{d}	0.915±0.049^{d}	3.745±0.041^{c}	3.256±0.038^{d}
4	3.602±0.262^{f}	5.642±0.235^{e}	5.032±0.237^{e}	4.184±0.061^{g}	16.976±0.322^{e}	4.689±0.040^{c}
5	3.810±0.062^{f}	5.792±0.264^{e}	5.287±0.015^{f}	4.635±0.051^{h}	19.753±0.332^{f}	4.622±0.029^{c}
6	2.043±0.107^{e}	2.380±0.177^{cd}	1.507±0.068^{c}	2.213±0.137^{f}	9.217±0.850^{d}	5.009±0.029^{a}
7	1.710±0.046^{d}	0.977±0.071^{f}	0.670±0.020^{b}	1.237±0.042^{e}	4.207±0.143^{c}	5.006±0.031^{a}
8	1.600±0.046^{cd}	0.453±0.035^{a}	0.510±0.000^{b}	0.707±0.031^{c}	2.250±0.082^{b}	5.010±0.023^{a}
9	1.353±0.050^{c}	0.463±0.060^{a}	0.453±0.057^{b}	0.800±0.020^{cd}	1.550±0.056^{ab}	5.017±0.043^{a}

According to high MDA content, cookies could be positioned in the food category that significantly contributes to the daily intake of MDA. Consumption of foods characterized by high MDA contents are known to be dangerous for biological systems due to its potential toxicity to humans, which is attributed to its high reactivity with proteins and DNA.[29]

There were significant increases in the MDA content of unpacked and packed gluten-free cookies stored at elevated temperature for 3 and 4 months (Tables I–IV), respectively, and the increases could be used to position the end-

points of cookie shelf-life. Each end-point obtained using MDA estimation corresponds with the one obtained using SHS-GC-FID method. Ke *et al.*[30] cited that TBA values higher than 1.5 mg kg^{-1} refers to food rancidity and unacceptability, but according to the results, MDA contents in cookies lower than 2 mg kg^{-1} may be considered as not rancid, especially if the results of Papastergiadis *et al.*[25] are taken into consideration.

TABLE IV. Propanal (C3), pentanal (C5), hexanal (C6), heptanal (C7), octanal (C8) and malondialdehyde (MDA) content (mg kg^{-1} dry matter (d.m.)) of packed (p = 1) gluten-free rice–buckwheat cookies during storage at T = 40±1 °C; values are means of three determinations ± standard deviation. Values in the same column with the different superscript lowercase letters are statistically different ($p < 0.05$)

t / month	Compound					
	C3	C5	C6	C7	C8	MDA
0	0.232±0.048^{a}	1.788±0.121^{a}	0.011±0.001^{a}	0.387±0.042^{a}	0.772±0.021^{a}	1.425±0.006^{b}
1	0.162±0.017^{a}	1.783±0.027^{a}	0.011±0.001^{a}	0.229±0.014^{a}	1.268±0.018^{a}	1.384±0.085^{b}
2	0.202±0.009^{a}	1.792±0.057^{a}	0.029±0.001^{a}	0.265±0.012^{a}	1.205±0.017^{a}	1.632±0.016^{c}
3	0.048±0.010^{a}	1.770±0.034^{a}	0.172±0.016^{ab}	0.208±0.007^{a}	1.397±0.065^{a}	1.700±0.011^{c}
4	0.134±0.008^{a}	1.751±0.043^{a}	0.489±0.010^{b}	0.425±0.016^{a}	2.269±0.017^{a}	2.359±0.015^{d}
5	6.507±0.105^{d}	25.993±0.399^{b}	21.078±0.241^{f}	2.790±0.037^{c}	8.963±0.043^{d}	2.703±0.042^{e}
6	13.280±0.855^{e}	30.350±1.511^{d}	7.133±0.327^{e}	4.603±0.246^{b}	12.493±0.519^{b}	2.863±0.028^{f}
7	9.213±0.277^{b}	26.253±0.313^{b}	5.890±0.082^{c}	5.223±0.042^{d}	14.843±0.706^{c}	3.039±0.023^{a}
8	8.670±0.062^{b}	25.863±0.366^{b}	5.663±0.118^{c}	4.873±0.021^{b}	13.907±0.263^{bc}	3.063±0.019^{a}
9	3.260±0.035^{c}	10.340±0.203^{c}	1.597±0.047^{d}	5.890±0.183^{e}	13.697±1.610^{bc}	3.073±0.025^{a}

According to the present measurements, the contents of C3, C5, C6, C7 and C8 were positively correlated with each other ($p < 0.01$), while the content of MDA was only positively correlated with the C7 and C8 contents (Table V).

TABLE V. Correlation matrix for the contents of aldehyde (propanal (C3), pentanal (C5), hexanal (C6), heptanal (C7) and octanal (C8)) and malondialdehyde (MDA); $^{+}$: correlation significant at the $p < 0.01$ level

	C5	C6	C7	C8	MDA
C3	0.927^{+}	0.722^{+}	0.657^{+}	0.571^{+}	–
C5		0.802^{+}	0.699^{+}	0.597^{+}	–
C6			0.568^{+}	0.552^{+}	–
C7				0.951^{+}	0.395^{+}
C8					0.475^{+}

Principal component analysis of the aldehydes contents

PCA was utilized to describe the differences among the observed cookie samples (Fig. 2). The PCA of the presented data clarified that the first two principal components represented 86.27 % of the total variance (66.15 and 20.12 %, respectively) with the respect to six variables (C3, C5, C6, C7, C8 and MDA).

A good discrimination among samples could be observed in the graphical presentation of the PCA (Fig. 2). The starting sample (0) is positioned at the left side of the plot, while the samples with gradually increasing aldehydes and MDA content are located at the right side of the plot. Based on PCA analysis, it was concluded that the MDA content could be considered as a more accurate indicator of lipid deterioration of cookie samples compared to aldehydes content due to its correlation to temperature (T) and packaging (P), Fig. 2.

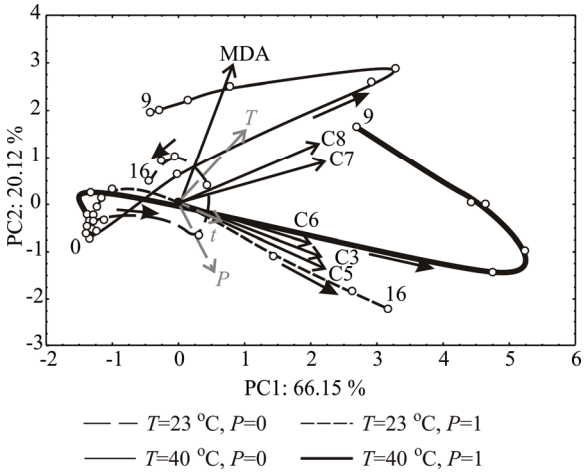

Fig. 2. Principal component analysis (PCA) ordination of the aldehyde (propanal (C3), pentanal (C5), hexanal (C6), heptanal (C7) and octanal (C8)) and malondialdehyde (MDA) content; T – storage temperature; t – storage time; P – logical constant regarding the packaging condition of cookies (packed (P = 1) or unpacked (P = 0)).

Analysis of variance and SOP model

Linear terms of packaging condition and storage time were important for the calculation of the C3 content; statistically significant at $p < 0.01$ level (Table VI). The nonlinear term of $t \times P$ was also influential in the SOP model for the calculation of C3, as well as the linear term of temperature ($p < 0.01$). The contents of C5 and C6 were more influenced by linear terms of packaging condition and temperature, but an influence of storage time was also observed. The influence of non-linear terms ($t \times P$ and $T \times P$) in the SOP model for calculation of C5 was evident ($p < 0.01$). The C7 and C8 contents were mostly dependent on linear terms of temperature and storage time ($p < 0.01$). The nonlinear terms ($t \times P$ and $T \times P$) also influenced the calculation of C7, statistically significant at the $p < 0.01$ level. The linear terms of temperature and packaging condition, as well as the storage time were the most influential in the SOP model for the prediction of the MDA content, while non-linear terms (the quadratic term of storage time and $t \times P$ and $T \times P$ terms) showed a minor impact on MDA calculation ($p < 0.01$ level). The

obtained coefficient of determination for the content of MDA was higher than those for other aldehydes (Table VI) suggesting MDA evaluation being a more appropriate model.

TABLE VI. ANOVA table of the evaluation (sum of squares) of the propanal (C3), pentanal (C5), hexanal (C6), heptanal (C7), octanal (C8) and malondialdehyde (MDA) contents; $^{+}$: significant at the $p < 0.01$ level. *: significant at the $p < 0.05$ level. **: significant at the $p < 0.10$ level Error terms were found statistically not significant. *df* – degrees of freedom; *T* – storage temperature; *t* – storage time; *P* – packaging condition

	df	C3	C5	C6	C7	C8	MDA
t	1	135.076^{+}	596.612^{+}	33.410**	29.974^{+}	174.808^{+}	7.205^{+}
t^2	1	20.447	148.375**	0.588	1.026	5.527	4.279^{+}
T	1	88.338^{+}	613.293^{+}	44.541*	48.599^{+}	388.179^{+}	17.761^{+}
P	1	115.699^{+}	1209.189^{+}	73.649*	4.936*	8.019	10.340^{+}
t×*T*	1	15.732	141.024**	0.378	12.440^{+}	63.310*	3.474^{+}
t×*P*	1	98.173^{+}	588.087^{+}	40.511**	4.631**	14.678	0.669
T×*P*	1	25.590**	370.506^{+}	27.529	7.001^{+}	13.316	5.341^{+}
Error	43	333.749	1835.236	461.239	51.936	667.702	15.234
r^2		0.545	0.568	0.312	0.582	0.456	0.773

ANN model

According to the ANN performance, it was noticed that the optimal number of neurons in the hidden layer for the calculation of the aldehyde contents was 3 (network MLP 3–3–6) to obtain high values of r^2 (0.722 for ANN during the training period, compared to 0.312–0.773 for the SOP models). The results obtained by the ANN model were better than those obtained by the SOP models due to the high nonlinearity of the ANN model.[31]

Sensitivity analysis

The variations of the outputs caused by infinitesimal changes in the input variables at a certain position in the input space are presented in Fig. 3.

The contents of C3, C5 and C6 were mostly affected by infinitesimal changes in storage time at the maximum values of the input space, while the influence of temperature was more expressed at the centre of the input space. The contents of C7 and C8 were mainly influenced by the changes in storage time and temperature close to the upper limit of the input range. The influence of temperature was more expressed at the centre of the input space for the calculation of C3, C5 and C6 contents, while the influence of infinitesimal changes in temperature are more observable for higher values in the input space for C7 and C8 contents.

The influences of storage time and of temperature on the evaluation of the MDA content were almost the same at the centre of the input space. The influence of packaging condition was negligible for the calculation of C7 and C8,

while it was very important in the case of the C3, C5 and C6 content (Fig. 3). The MDA content was negatively correlated with packaging condition throughout the input space range.

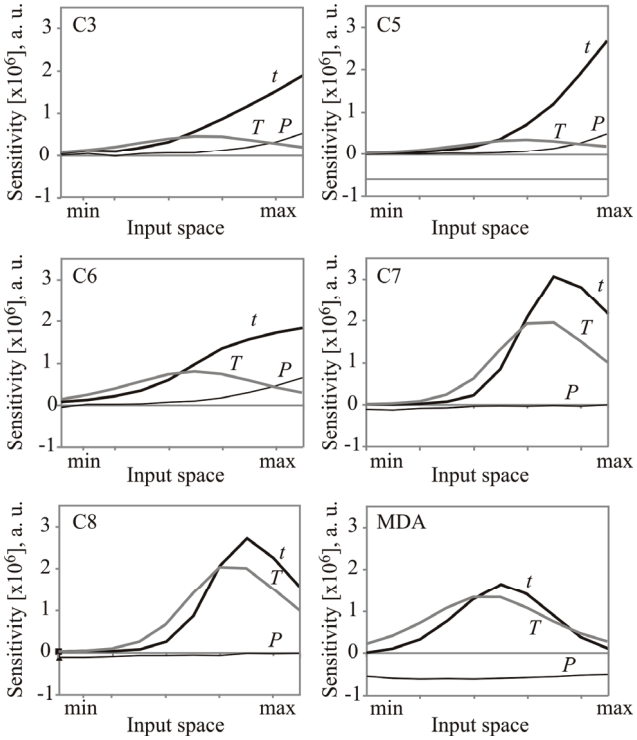

Fig. 3. Sensitivity analysis – the influence of the input over the output variables; C3 – propanal; C5 – pentanal; C6 – hexanal; C7 – heptanal; C8 – octanal; MDA – malondialdehyde; *T* – storage temperature; *t* – storage time; *P* – logical constant regarding the packaging condition of cookies (packed or unpacked).

CONCLUSIONS

Significant changes in the volatile saturated aldehydes content (C3, C5, C6, C7 and C8) of unpacked and packed gluten-free rice–buckwheat cookies stored at ambient and an elevated temperature were determined and the obtained peaks were addressed to the end-points of cookie shelf-life. For the same purpose, the MDA content was monitored. Based on the data obtained using the investigated methods, the end-points of cookie shelf-life were determined – 3 and 5 months for unpacked and packed cookies kept at elevated temperature, respectively, and 11 and 14 months for unpacked and packed cookies kept at ambient temperature, respectively. The PCA indicated a high order of correlation between the aldehyde contents (C3, C5, C6, C7 and C8), statistically significant at the $p < 0.01$ level, while the correlations between MDA and C7 and C8 were also statistically

significant at the $p < 0.01$ level. The aldehydes and MDA contents calculation could be predicted with an overall coefficient of determination of 0.722 using an ANN model compared to 0.312–0.773 for the SOP models. These findings coincide with those of PCA and ANOVA analysis. Based on the results obtained in this study, it might be suggested that the relevant parameter for predicting the end-point of cookie shelf-life is the MDA content rather than C3, C5, C6, C7 and C8 content.

Acknowledgement. This work was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. TR-31029).

ИЗВОД

ПРИМЕНА SHS-GC-FID МЕТОДЕ ЗА ОДРЕЂИВАЊЕ ИСПАРЉИВИХ ЗАСИЋЕНИХ АЛДЕХИДА И HPLC-DAD МЕТОДЕ ЗА ОДРЕЂИВАЊЕ САДРЖАЈА МАЛОНДИАЛДЕХИДА ЗА ПРЕДВИЂАЊЕ РОКА ТРАЈАЊА БЕЗГЛУТЕНСКОГ КЕКСА

МАРИЈАНА САКАЧ[1], ЛАТО ПЕЗО[2], ПАВЛЕ ЈОВАНОВ[1], НАТАША НЕДЕЉКОВИЋ[1], АНАМАРИЈА МАНДИЋ[1], МЛАДЕНКА ПЕСТОРИЋ[1] и АЛЕКСАНДРА МИШАН[1]

[1]*Научни институт за прехрамбене технологије у Новом Саду, Универзитет у Новом Саду, Булевар цара Лазара 1, Нови Сад и* [2]*Институт за општу и физичку хемију, Универзитет у Београду, Студентски трг 12/V, Београд*

У овом раду је вршено поређење осетљивости две аналитичке методе за предвиђање рока трајања неупакованог и упакованог безглутенског кекса од пиринчаног и хељдиног брашна, који је складиштен на собној (23±1 °C) и повишеној температури (40±1 °C) – статичке „headspace“ методе гасне хроматографије са пламено-јонизујућим детектором (SHS-GC-FID) за одређивање испарљивих засићених алдехида (пропанал (C3), пентанал (C5), хексанал (C6), хептанал (C7) и октанал (C8)) и методе течне хроматографије високих перформанси (HPLC) за одређивање малондиалдехида (MDA). Применом обе методе добијени су исти резултати којима су установљени рокови трајања кекса – 3 месеца и 5 месеци за неупаковани и упаковани кекс складиштен на повишеној температури, и 11 месеци, односно 14 месеци за неупаковани и упаковани кекс складиштен на собној температури. Два математичка модела, полином другог реда (SOP) и модел вештачке неуронске мреже (ANN), коришћена су за поређење метода. Коришћењем ANN модела за предвиђање садржаја алдехида и MDA добијена је вредност коефицијента детерминације 0,722, а употребом SOP модела добијени су коефицијенти детерминације 0,312–0,773. Према анализи осетљивости, могло би се закључити да је релевантан параметар за предвиђање рока трајања кекса садржај MDA пре него садржаји C3, C5, C6, C7 и C8.

(Примљено 21. априла, ревидирано 8. јуна, прихваћено 9. јуна 2017)

REFERENCES

1. A. Chauhan, D. C. Saxena, S. Singh, *LWT–Food. Sci. Technol.* **63** (2015) 939
2. M. Sakač, M. Pestorić, A. Mišan, N. Nedeljković, D. Jambrec, P. Jovanov, V. Banjac, A. Torbica, M. Hadnađev, A. Mandić, *Food Technol. Biotechnol.* **53** (2015) 38
3. M. Kaur, K. S. Sandhu, A. P. Arora, A. Sharma, *LWT–Food. Sci. Technol.* **62** (2015) 628
4. F. Caponio, C. Summo, M. L. Clodoveo, A. Pasqualone, *Eur. Food Res. Technol.* **227** (2008) 135
5. F. Caponio, C. Summo, D. Delcuratola, A. Pasqualone, *J. Sci. Food Agric.* **86** (2006) 135
6. M. Bialek, J. Rutkowska, A. Bialek, A. Adamska, *Pol. J. Food Nutr. Sci.* **66** (2016) 77

7. M. Laguerre, J. Lecomte, P. Villeneuve, *Prog. Lipid Res*. **46** (2007) 244
8. V. M. Paradiso, C. Summo, A. Pasqualone, F. Caponio, *Food Chem.* **113** (2009) 543
9. S. Pastorelli, L. Torri, A. Rodriguez, S. Valzacchi, S. Limbo, C. Simoneau, *Food Addit. Contam.* **24** (2007) 1219
10. A. Pasqualone, A. M. Bianco, V. M. Paradiso, C. Summo, G. Gambacorta, F. Caponio, A. Blanco, *Food Chem*. **180** (2015) 64
11. A. Fullana, A. A. Carbonell-Barrachina, S. Sidhu, *J. Sci. Food Agric.* **84** (2004) 2015
12. E. N. Frankel, in *Lipid Oxidation*, 2nd edition, E. N. Frankel, Ed., The Oily Press, Bridgewater, 2005, p. 99
13. A. Mandić, I. Sedej, M. Sakač, A. Mišan, *Food Anal. Method*. **6** (2013) 61
14. M. Sakač, M. Pestorić, A. Mandić, A. Mišan, N. Nedeljković, D. Jambrec, P. Jovanov, V. Lazić, L. Pezo, I. Sedej, *J. Cereal Sci.* **69** (2016) 336
15. F. Shahidi, Y. Zhong, in *Bailey's Industrial Oil and Fat Products*, 6th ed., F. Shahidi, Ed., Wiley, Hoboken, NJ, 2005, p. 357
16. Z. Wei, X. Li, D. Thushara, Y. Liu, *J. Food Eng*. **107** (2011) 379
17. J. Tsaknis, S. Lalas, M. Hole, G. Smith, V. Tychopoulos, *Analyst* **123** (1998) 325
18. F. Karatas, M. Karatepe, A. Baysar, *Anal. Biochem*. **311** (2002) 76
19. X. Hu, Q. Weng, *Remote Sens. Environ*. **113** (2009) 2089
20. B. J. Taylor, B. J. Taylor, *Methods and Procedures for the Verification and Validation of Artificial Neural Networks*, Springer, New York, 2006
21. L. L. Pezo, R. R. Čolović, D. V. Palić, A. P. Jovanović, *Food Feed Res*. **42** (2015) 73
22. Statistica, v. 10, StatSoft, Inc, Tulsa, OK, 2010, http://www.statsoft.com
23. J. Fernández, J. A. Perez-Alvarez, J. A. Fernández-Lopez, *Food Chem*. **59** (1997) 345
24. R. Dittrich, F. El-Massry, F. Rinaldi, C. C. Peich, M. W. Beckman, M. Pischetsrieder, *J. Agric. Food Chem*. **51** (2003) 3900
25. A. Papastergiadis, A. Fatouh, L. Jacxsens, C. Lachat, K. Shrestha, J. Daelman, P. Kolsterena, H. Van Langenhove, B. De Meulenaer, *Food Chem. Toxicol*. **73** (2014) 51
26. E. P. Botosoa, C. Chèné, R. Karoui, *Food Chem*. **141** (2013) 1130
27. H. Estebauer, R. J. Schaur, H. Zollner, *Free Radical Biol. Med.* **11** (1991) 81
28. A. Papastergiadis, E. Mubiru, H. Van Langenhove, B. De Meulenaer, *J. Agric. Food Chem.* **60** (2012) 9589
29. M. D. Guillén, E. Goicoechea, *Crit. Rev. Food Sci*. **48** (2008) 119
30. P. J. Ke, E. Cervants, C. Robles-Martinez, *J. Agric. Food Chem*. **35** (1984) 1248
31. P. B. Chattopadhyaya, R. Rangarajana, *Agric. Water Manage*. **133** (2014) 81.