TITLE: Shelf-life prediction of gluten-free rice-buckwheat cookies


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Keywords: gluten-free cookies, shelf-life, aldehydes, sensory properties

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Abstract: The objective of this work was to predict 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 by measuring off-flavour volatile compounds (aldehydes), antioxidant capacity, total phenolic and rutin content and evaluating sensory properties. Analysis of variance and Tukey's HSD test at 95% confidence limit showed significant differences between the observed samples. Principal component analysis was used for assessing the effect of storage time, temperature and packaging condition on all investigated cookie parameters. Antioxidant capacity measured using DPPH test showed a decreasing tendency during storage in all investigated cookie samples. The obtained results correlated with a decrease in total phenolics and rutin content and an increase in total aldehydes content in cookies during storage. From the sensory evaluation, it could be concluded that the greatest loss of sensory quality resulted from hardness increase, fracturability decrease, and the raise of uncharacteristic odours and flavours. The end-point of cookie shelf-life obtained from sensory evaluation was lower compared to that obtained measuring total aldehydes content. Therefore, sensory properties might be the markers for gluten-free cookie shelf-life prediction rather than aldehydes content.
Shelf-life prediction of gluten-free rice-buckwheat cookies

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Abstract

The objective of this work was to predict 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 by measuring off-flavour volatile compounds (aldehydes), antioxidant capacity, total phenolic and rutin content and evaluating sensory properties. Analysis of variance and Tukey’s HSD test at 95% confidence limit showed significant differences between the observed samples. Principal component analysis was used for assessing the effect of storage time, temperature and packaging condition on all investigated cookie parameters. Antioxidant capacity measured using DPPH test showed a decreasing tendency during storage in all investigated cookie samples. The obtained results correlated with a decrease in total phenolics and rutin content and an increase in total aldehydes content in cookies during storage. From the sensory evaluation, it could be concluded that the greatest loss of sensory quality resulted from hardness increase, fracturability decrease, and the raise of uncharacteristic odours and flavours. The end-point of cookie shelf-life obtained from sensory evaluation was lower compared to that obtained measuring total aldehydes content. Therefore, sensory properties might be the markers for gluten-free cookie shelf-life prediction rather than aldehydes content.

Keywords: gluten-free cookies, shelf-life, aldehydes, sensory properties, antioxidants, antioxidant capacity
1. Introduction

Celiac disease is a permanent intolerance to gluten proteins of many common cereals such as wheat, rye, barley and oat. Therefore, celiac disease patients are recommended to be on a strict long-life gluten-free diet, which usually lacks in certain essential nutrients (Thompson et al., 2005) and contains lipids of low quality level from the nutritional point of view, as indicated by the high contents of triacylglycerol oligopolymers and oxidized triacylglycerols, as well as, in some cases, high levels of oleic acid trans isomers (Caponio et al., 2008). Due to the limitation of some nutrients, the fortification of gluten-free products is required to obtain a balanced diet for celiac patients. There are several papers about gluten-free added value products (Alvarez-Jubete et al., 2010) some of them focusing on sweet bakery products, such as cookies (Sakač et al., 2015), muffins (Matos et al., 2014) and biscuits (Schoenlechner et al., 2006).

Shelf-life of foods is of great interest since it reflects their nutritional, functional, sensory and safety profile (Jensen et al., 2011; Zieliński et al., 2012). The most remarkable changes in foods during processing, storage and handling result from lipid oxidation and microbiological spoilage leading to quality deterioration of foods, which, furthermore, could have harmful effects on health (Laguerre et al., 2007).

The lipid oxidation leads to the rancidity of high fat/oil containing products, which may affect their shelf-life. Rancidity is related to the development of unpleasant odours and flavours, which contribute to an unacceptable sensory profile of the product. The progress of lipid oxidation can be followed by measuring the content of marker compounds, among which some are volatile compounds, such as aldehydes. These secondary lipid oxidation products are generated from a wide range of hydroperoxides formed during the initiation stage of the reaction.
(Laguerre et al., 2007) and strongly contribute to the aroma at trace amounts due to their low
odour threshold values (Sun et al., 2010).

Cookies are known for their long shelf-life because they are characterized by lower water
activity ($a_w$) values than those that permit the growth of microorganisms ($a_w > 0.6$) (Chieh,
2006). However, they possess a high amount of vegetable fat (20–30% on flour weight basis),
which makes them susceptible to oxidative changes (Zieliński et al., 2012). Hexanal, as the
major volatile oxidation product of linoleic acid or further oxidation of 2,4-decadienal (Pastorelli
et al., 2007), was used as an indicator for oxidation of crackers (Berenzon and Saguy, 1998). The
oxidative deterioration of cookies was also monitored in shortcake biscuits by measuring 2,4-
decadienal and 2,4-heptadienal, which contributed to their rancidity (Yang et al., 2013). Viscidi
et al. (2004) used heptanal as a marker of lipid deterioration, while Mandić et al. (2013)
quantified five aldehydes for the same purpose.

Consumer tests are the most suitable tool for shelf-life determination of food products,
but they are not easy to handle. Instead, the most widely used technique for shelf-life
determination is based on a trained or expert panel, which is usually available at a food
producer’s facility. The panel performs descriptive sensory method based on measuring the
intensity of sensory attribute correlated with the product deterioration.

In our previous work (Sakač et al., 2015), gluten-free cookies based on a mixture (80:20)
of rice flour (RF) and light buckwheat flour (LBF), respectively were chosen as optimal
regarding their enhanced mineral content, increased antioxidant capacity and the most acceptable
sensory properties in comparison with the control cookies (rice cookies) and others based on
rice-light buckwheat flour mixture (RF:LBF – 90:10 and 70:30). The enrichment of the
mentioned cookies was achieved using buckwheat flour which is rich in minerals and rutin
(Sedej et al., 2011). The increased antioxidant capacity of cookies resulted from the presence of rutin as a potent antioxidant (Jiang et al., 2007), which suggests the potential of this compound to extent cookie shelf-life.

Having in mind the susceptibility of cookies to lipid oxidation, the objective of this work was to predict the shelf-life of unpacked and packed (polypropylene bags) gluten-free rice-buckwheat cookies (RF/LBF – 80:20) kept at ambient (23 ± 1°C) and elevated (40 ± 1°C) temperature during storage, measuring off-flavour volatile compounds (aldehydes), antioxidant capacity (1,1-diphenyl-2-picrylhydrazyl radicals (DPPH•) scavenging activity), total phenolic content (TPC), rutin content, and evaluating their sensory properties.

2. Material and methods

2.1. Materials

Rice flour – RF (moisture 10.9%, protein (N×5.7) 7.52%, fat 0.29%, ash 0.27%, reducing sugars 1.50%, and starch 87.2%) and light buckwheat flour – LBF (moisture 10.1%, protein (N×5.7) 8.96%, fat 1.39%, ash 1.11%, reducing sugars 1.91%, and starch 84.9%) were obtained from Hemiža Komerc, Novi Sad, Serbia. Vegetable fat originating from refined palm and sunflower oil was (fatty acid composition: C16:0 – 43.2%; C18:1n9c – 42.5%; C18:2n6c – 9.5%; C18:0 – 4.7%; C14:0 – 0.96%; C20:0 – 0.42%) obtained from Puratos NV, Groot-Bijgaarden, Belgium. Sodium hydrogen carbonate (≥ 99.5%, p.a.) was purchased from Carl Roth GmbH, Karlsruhe, Germany, carboxymethyl cellulose sodium salt (CMC) from Alfa Aesar GmbH, Karlsruhe, Germany, diacetyl tartaric acid esters of monoglycerides (DATEM) from InCoPa
GmbH, Munich, Germany, while the other ingredients (salt, sugar and honey) were purchased at
the local market.

2.2. Preparation of cookies

The formulation of gluten-free rice-buckwheat cookies (RF:LBF – 80:20) was made
according to Sakač et al. (2015). Dough mixing, processing and baking were performed on
laboratory-scale equipment as described by the mentioned authors. The ingredients were
weighed as follows: flour mixture (240.0 g of RF + 60.0 g of LBF), deionized water 75.0 g,
vegetable fat 85.0 g, granulated sugar 70.0 g, honey 45.0 g, NaHCO₃ 9.0 g, DATEM 9.0 g, CMC
4.5 g, and salt 2.1 g. Rice/buckwheat flour mixture was transferred into Farinograph mixing
bowl (Brabender GmbH, Duisburg, Germany), which was previously tempered at 30 °C.
Afterwards, the rest of the dry ingredients and vegetable fat were added and mixed for 2 min.
Finally, 45 g of honey which was previously dissolved in water was poured into the mixer bowl
and the dough mass was mixed for 25 min at 30 °C. The obtained cookie dough was let to rest at
8 °C for 24 h in order to allow the hydration of the added CMC. After the resting period, the
dough was tempered at ambient temperature for 30 min and then sheeted to a thickness of 4 mm
using a pilot scale dough sheeter (Mignon, Mestrino, Italy). The dough was cut using a stainless
mould (60 mm × 55 mm) and finally baked at 170 °C for 12 min in a laboratory oven (MIWE

2.3. Packaging and storage of cookies

The gluten-free rice-buckwheat cookies were packed into 40 μm
polypropylene/polypropylene (OPP/OPP) bags, which gas permeability was 3858.9 mL/m² 24 h,
1 bar for CO₂, 1236.3 mL/m² 24 h, 1 bar for N₂, and 418.9 mL/m² 24 h, 1 bar for air. Cookies were packed under atmospheric conditions using a laboratory vacuum sealer (AudionElektro, Swissvac (GB) Ltd, Slough, Great Britain) with teflonized heating areas (vacuum pump was not used). Each cookie was packed separately. Packed cookies were investigated in comparison with those in a bulk form (unpacked cookies).

Both packed and unpacked cookies were stored in a climate chamber (Binder, Tuttlingen, Germany) at ambient and elevated temperature (23 ± 1°C and 40 ± 1°C). The relative humidity was set at a constant value of 40%. The storage period was 9 months for cookies kept at elevated temperature, while those kept at ambient temperature were stored during 16 months. The cookies were analysed monthly for all examined parameters, except sensory parameters, which were evaluated every 15 days during storage.

2.4. Proximate composition

Proximate composition of cookies including protein (Official Method No. 950.36), fat (Official Method No. 935.38), reducing sugar (Official Method No. 975.14), total dietary fiber (Official Method No. 958.29), ash (Official Method No. 930.22) and water contents (Official Method No. 926.5) were determined by standard methods of analysis (AOAC, 2000). Starch content was determined by hydrochloric acid dissolution according to the ICC Standard (ICC Standard No. 123/1, 1994). Fatty acid composition of vegetable fat was determined by the method described by Milovanović et al. (2012).

2.5. Preparation of ethanolic extracts
Cookie powder (5 g) was mixed with 50 mL of ethanol/water (80/20, v/v). Extraction was carried out by shaking the mixture at room temperature (23 ± 1°C) for 1 h. After 1-h shaking, the suspension was left overnight at room temperature. The procedure was repeated twice with 50 mL of solvent, and combined extracts were dried using a vacuum-evaporator. The dried extract was dissolved in ethanol/water (80/20, v/v) to 10 mL volume and used for further investigation of antioxidant activity.

2.6. Total phenolic content

Total phenolic content (TPC) of gluten-free rice-buckwheat cookie extracts was determined spectrophotometrically at 750 nm (6405 UV/VIS, Jenway, Stone, Staffordshire, UK) using Folin-Ciocalteu reagent (Singleton et al., 1999). Gallic acid was used as a standard and results were expressed as gallic acid equivalents (GAE) (μg GAE/g of sample on a dry mass basis).

2.7. DPPH radical scavenging activity

The effect of the examined extracts on the content of 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH•) was estimated according to the modified method of Hatano et al. (1988). The extract at various concentrations (0.05–0.30 g of sample/mL) was added to the reaction mixture, and its absorbance was measured at 517 nm (6405 UV/VIS, Jenway) against the blank (mixture without extract).

The IC₅₀ value (g/mL) was defined as the concentration of an extract which was required to quench 50% of the initial amount of DPPH• under the experimental conditions given.

2.8. HPLC determination of rutin
A mass of 5 g of cookie powder was extracted with 20 mL of boiling methanol/water (80/20, v/v) for 10 min, ultrasonicated for 10 min and filtered through 0.45 μm pore size nylon filter (Agilent Technologies, Santa Clara, CA, USA) before injection into the HPLC system. 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 rutin in cookie extracts. A single rapid resolution HPLC method reported by Mišan at al. (2011) was used.

2.9. Head space analysis of aldehydes

The static headspace gas chromatography (SHS-GC) with flame ionisation detection (FID) was applied for measuring the content of 5 aldehydes (propanal, pentanal, hexanal, heptanal, and octanal) in gluten-free cookies (Mandić et al., 2013).

2.10. Sensory evaluation

Sensory evaluation was conducted 24 h after baking, and in 15-day intervals, for cookies stored at elevated and ambient temperature, during 3 and 6 months of storage, respectively. The trained panel consisted of 8 expert assessors (7 females and 1 male, at the age of 30 to 45) with the necessary knowledge and experience in sensory quantitative descriptive analysis, which included techniques and practice in attribute identification and terminology development. For the purpose of this study, the panellists received further training on gluten-free cookies according to ISO 8586 (2012). Initially, the panel was provided with the terms used in previously published paper (Pestorić et al., 2014). The assessors participated in 2-hour training sessions, which were designed to familiarize them with extremely changed and defective sensory attributes of the examined gluten-free cookies. After each training session the assessors received feedback on
their performance, with the aim of improving and standardizing the panel’s discriminative power. The feedback also focused on consensus in the definition of each attribute and its extent of intensity. Furthermore, panellists generally were aware of the treatments being studied, giving them the information necessary for an adequate evaluation of the examined samples with respect to expected cookie deterioration during storage. However, the panellists were not aware of the treatments to which individual samples belong. To minimize a bias, fresh cookies, as the control, were given to the assessors in each sensory session (Hough, 2010).

Samples were evaluated on a seven-point (0–6) category scale (in word categories from Not at all to Strong) used by the assessors to rate the intensity of a particular stimulus by assigning the value on the scale.

All samples were coded with three random digits and served in closed odourless plastic containers at ambient temperature. A balanced complete-block design was carried out for duplicates of cookie samples. Distilled water was provided to cleanse the palate between the samples during evaluation.

### 2.11. Statistical analysis

Analysis of variance (ANOVA) and Tukey's HSD test for comparison of sample means were used to assess the effect of storage time, temperature and packaging condition.

Second order polynomial (SOP) models in the following form were developed to relate responses (Y) and thirteen process variables (X):

\[
Y_k = \beta_{k0} + \sum_{i=1}^{3} \beta_{ki} \cdot X_i + \sum_{i=1}^{3} \beta_{ki} \cdot X_i^2 + \sum_{j=i+1}^{3} \beta_{kj} \cdot X_i \cdot X_j, \quad k=1-13,
\]  

(1)
where: $\beta_{k0}$, $\beta_{k1}$, $\beta_{kii}$, $\beta_{k12}$ were constant regression coefficients; phenolic ($Y_1$), aldehydes ($Y_2$), rutin content ($Y_3$) and DPPH radical scavenging activity ($Y_4$), as well as sensory properties of the unpacked and packed gluten-free rice-buckwheat cookies ($Y_5$-$Y_{13}$). $X_1$ is storage temperature, $X_2$ is logical constant regarding the packing state of cookies (packed or unpacked) and $X_3$ is time. In this article, ANOVA was conducted to show the significant effects of independent variables to the responses, and to show which of responses were significantly affected by the varying treatment combinations.

Principal component analysis (PCA) was used to discover the possible correlations among measured variables.

3. Results and discussion

3.1. Proximate composition of cookies

The proximate composition of gluten-free rice-buckwheat cookies (RF/LBF) was: protein 4.42%, fat 20.2%, starch 51.0%, reducing sugars 14.4%, ash 1.88% and total dietary fibre 2.21% on a dry mass basis and it was similar to the cookie composition presented in our previous paper (Sakač et al., 2015). The high amount of fat in RF/LBF cookies, common for this type of product, is responsible for their deterioration during storage, leading to the development of off-flavour products and causing their unacceptable nutritional and sensory quality.

3.2. Antioxidants, antioxidant capacity and total aldehydes

3.2.1. Total phenolic and rutin content
Antioxidants undergo changes during storage, including those resulting from lipid oxidation (Jensen et al., 2011). This fact explains the remarkable reduction in TPC (740 μg GAE/g d.m. in freshly baked RF/LBF cookies) of unpacked and packed RF/LBF cookies kept at ambient and elevated temperatures (23 ± 1°C and 40 ± 1°C) during storage (Table 1). The decrease in TPC of packed and unpacked RF/LBF cookies kept at elevated temperature during 9 months was 47% and 49%, respectively, while the percentage of reduction in packed and unpacked cookies kept at ambient temperature during the same period was 48% and 45%, respectively. The decreasing trend in TPC during long-term storage of rye ginger cakes was previously noted by Zieliński et al. (2012), but the mentioned authors found lower TPC reduction rates (2–23%), which could be explained by the fact that phenolics presented in rye ginger cakes probably possess antioxidant capacity which differs from that of RF/LBF cookies.

Rutin content (17.4 mg/kg d.m. in freshly baked RF/LBF cookies) was also determined during cookie storage (Table 1), because it is a dominant polyphenol in LBF (Sedej et al., 2011) and consequently in RF/LBF cookies (Sakač et al., 2015). The decrease in rutin content in both packed and unpacked RF/LBF cookies kept at elevated temperature during 9 month was 43%, while the percentage of reduction in packed and unpacked cookies kept at ambient temperature during the same period was 37% and 28%, respectively.

3.2.2. Antioxidant capacity

The freshly baked RF/LBF cookies (24 h after their production) showed the highest antioxidant capacity measured using the DPPH test (0.11 g of sample/mL) with decreasing tendency during storage in all cookie samples indicating the depletion of antioxidants for suppression of lipid oxidation reactions (Table 1). The decrease in antioxidant capacity (i.e. the
increase in IC50 value) of cookies kept at elevated temperature during 9 months of storage was not as pronounced as in cookies kept at ambient temperature during the same period (Table 1). This finding could be attributed to the development of Maillard reaction products (MRPs) which was more favoured at higher temperature (40 ± 1°C). MRPs are known to possess the antioxidant activity (Michalska et al., 2008), which could contribute to the overall antioxidant capacity of RF/LBF cookies kept at elevated temperature. Cookies stored at ambient temperature are assumed to be characterized by lower MRPs than those kept at elevated temperature and therefore possessed lower antioxidant capacity (Table 1). The observed decrease in antioxidant capacity of cookies during storage is not consistent with the statement of Zieliński et al. (2012), who found the significant increase in antioxidant capacity of long-term stored rye ginger cakes.

3.2.3. Total aldehydes content

The generation of off-volatiles responsible for unpleasant odour of stored foods is a result of the formation of secondary lipid oxidation products, some of which are aldehydes. While the C6 alcohols and aldehydes are characterized by pleasant odour notes, heptanal, 2-heptenal, octanal, and nonanal are responsible for off-flavors (Majcher and Jelén, 2009; Morales et al., 1997). It is known that fatty acid composition of a lipid phase highly influences the composition of aldehydes generated by the process of lipid oxidation. Regarding the fatty acid composition of the vegetable fat used in this experiment, oleic and linoleic acid degradation products were expected. Oleic acid oxidation results in octanal and nonanal generation (Fullana et al., 2004), while hexanal, as the widely used marker of lipid oxidation (Grosso and Resurreccion, 2002; Purcaro et al., 2007), derives, together with 2-heptenal, 2-octenal, 2-nonenal and 2,4-decadienal, from the oxidation of linoleic acid (Laguerre et al., 2007). Nonanal and 2-nonenal were detected
at significant levels in biscuits (Pasqualone et al., 2015) and in other cereal-based products such as semolina and pasta (Pasqualone et al., 2014). Using SHS GC FID, volatile aldehydes in unpacked and packed RF/LBF cookies were monitored to assess lipid oxidation during storage. Limited by the number of available standards, only five aldehydes were determined. The most abundant aldehyde were octanal, hexanal and pentanal whose ratio varied during the storage period in no consistent manner, and for that reason the results were expressed as total aldehydes (Table 1). Total aldehydes content in freshly baked RF/LBF cookies (24 h after their production) was 3.19 mg/kg and it increased with storage time to a maximum. Thereafter, noticeable decrease in total aldehydes content occurred in all cookies (Table 1), caused by their further oxidative changes and probably a reaction with proteins forming nonenzymatic browning reaction products (Dittrich et al., 2003).

The total aldehydes content of the investigated cookies was in the range of 2.54–4.43 mg/kg during month 1 and month 2 of storage, but there was a remarkable increase in the unpacked cookies kept at elevated temperature after month 3 of storage (12.8 mg/kg). The oxidation processes did not occur in the packed cookies kept at elevated temperature until the end of month 3, but a significant difference ($p < 0.05$) was observed in month 4 (5.07 mg/kg), followed by a tremendous increase in month 5 (65.3 mg/kg). The significant changes in total aldehydes content determined in month 3 and 4 can be addressed to the end-point of shelf-life of unpacked and packed cookies stored at elevated temperature, respectively.

The difference between susceptibility of unpacked and packed cookies kept at 40 ± 1°C to lipid oxidation should be contributed to the packaging, since the unpacked cookies were directly exposed to oxygen from the air, while the packed cookies were in contact with limited amount of oxygen inside the polypropylene bags.
Storage of RF/LBF cookies kept at ambient temperature during 16 months resulted in significant lipid oxidation in month 11 for unpacked (15.2 mg/kg), and in month 14 for packed cookies (47.7 mg/kg) (Table 1). The obtained results point out the positive effect of packaging in extending cookie shelf-life.

The declared shelf-life of commercially available bakery products such as cakes, cookies and biscuits is one year under the adequate storage conditions and our results are consistent with this labelling. The same length of biscuit shelf-life was obtained by measuring the hexanal content during 12 months (Purcaro et al., 2007). In the mentioned experiment, no correlation between hexanal content and panel test, i.e. rancidity scored concerning taste and odour, was established. However, in an experiment in which hexanal was used as a parameter for assessing the applicability of oxygen absorbers for extending shelf-life of crackers during 52 weeks of storage, increased hexanal content (5.39 mg/kg) was measured and addressed to a sensory unacceptable product (Berenzon and Saguy, 1998). The mentioned hexanal content could be comparable with our results, since total aldehydes content of 5.07 mg/kg determined during month 4 (Table 1) could be assigned to the remarkable lipid deterioration in RF/LBF cookies.

3.2.4. Principal component analysis of total phenolic content (TPC), rutin content, DPPH radical scavenging activity (DPPH) and total aldehydes content

The PCA of the presented data explained that the first two principal components accounted for 88.59% of the total variance (63.12% and 25.47%, respectively) in four variables (total phenolic content (TPC), rutin content, DPPH radical scavenging activity (DPPH) and total aldehydes content (aldehydes)) (Fig. 1). Considering the map of the PCA performed on the data, DPPH (which contributed for 27.4% of the total variance) exhibited positive scores according to
the first principal component (PC1), whereas TPC (34.4%) and rutin content (34.7%) showed negative score values according to the PC1 (Fig. 1). The negative contribution to the second principal component (PC2) was observed for total aldehydes content (86.6% of the total variance).

The ANOVA analysis (Table 2) revealed that the linear and the quadratic terms of storage time was the most important for TPC calculation, statistically significant at $p < 0.01$ level. Rutin content was mostly influenced by the linear and the quadratic term of storage time, and also by the linear term of packaging condition ($p < 0.01$). DPPH was mostly influenced by the linear terms of temperature and storage time, as well as by nonlinear terms $T \times t$ and $P \times t$, statistically significant at $p < 0.01$ level. Total aldehydes content was influenced by the linear terms ($T$, $P$ and $t$), and also by all the interchange terms ($T \times P$, $T \times t$ and $P \times t$), statistically significant at $p < 0.01$.

3.3. Sensory evaluation of cookies

3.3.1. Sensory properties of cookies

There are no standards which define sensory acceptability of gluten-free rice-buckwheat cookies, and therefore, the failure criteria are not uniform in sensory evaluation. For the purpose of this study score 3 ('equal to the control') was considered as satisfying for all positive cookie attributes such as colour development (CD), crumbliness (CR), hardness (HR), fracturability (FR), and fattiness (FAT), while values above 4 ('a lot more than the control') and below 2 ('a lot less than the control') were considered inadequate. For odour (O) and flavour (FL), the satisfying upper and lower limits were 3.5 and 2.5, respectively, while for the unpleasant
attributes such as uncharacteristic odours (UO) and flavours (UF), the satisfying upper limit was 2.

Sensory profiles of RF/LBF cookies kept at 23 ± 1 °C were evaluated during six months, while those kept at 40 ± 1 °C were tested during three months of storage (Table 3).

Colour is considered as a very important attribute for consumer acceptability. It remained at the level of acceptability or equal to the control during the whole experiment for all RF/LBF cookie samples (Table 3). Consequently, this attribute could not be considered as an indicator of sensory changes of the investigated cookies during storage. The absence of colour changes in RF/LBF cookies was not in accordance with the results obtained by Zielinski et al. (2012). The mentioned authors found the development of Maillard reaction products (MRPs) during long-term storage in rye ginger cakes, which could contribute to the colour development of the final product during storage (Chevallier et al., 2000). It is assumed that our sensory evaluation was too short to observe colour changes compared to the experiment conducted by Zielinski et al. (2012), which lasted for 5 years.

From Table 3, it could be seen that defined limits for uncharacteristic odours (UO) and flavours (UF) of cookies kept at elevated temperature were obtained within 3 months of storage (in month 1 for the unpacked and at the end of month 2 for the packed cookies), while storage of cookies kept at ambient temperature was extended until the end of month 6, when the mentioned attributes reached the defined limits in both packed (at the end of month 5.5) and unpacked cookies (at the end of month 3) (Table 3). UO and UF evaluated by the panel discriminated RF/LBF cookies much earlier than total aldehydes content as a marker of lipid oxidation suggesting that there is no correlation between those results. The same finding was noted by Purcaro et al. (2007), who did not establish correlation between hexanal content and panel
evaluation in the investigation of biscuits during 12 months of storage. Therefore, sensory
properties might be suggested to be the relevant parameters for predicting the end-point of
RF/LBF cookie shelf-life rather than other parameters determined in our experiment.

In addition, texture attributes also pointed to unacceptable sensory profile of RF/LBF
cookies, especially for their hardness (HR) and fracturability (FR) (Table 3). These properties
discriminated the unpacked cookies kept at elevated temperature at the end of month 1 and 1.5,
respectively, while those kept at ambient temperature were well scored until the end of month
2.5 and 4.5, respectively. The best scores for the mentioned properties were given to the packed
cookies kept at ambient temperature, suggesting that packaging distinctly helped in extending
cookie shelf-life. Measured textural changes probably were the consequence of changes in
cookie water activity during storage (Chieh, 2006).

3.3.1. Principal component analysis of cookie sensory properties

The PCA of the presented sensory data explained that the first two principal components
accounted for 76.31% of the total variance (64.26% and 12.04%, respectively) in the nine
variables (CD, O, UO, CR, HR, FR, FAT, FL and UF) (Fig. 2). Considering the map of the PCA
performed on the sensory data, it can be noticed that the contribution of the total variance
accounted for 15.3% of UO, 15.2% of UF, and 15.6% of HR. These variables exhibited positive
scores according to the PC1, whereas FAT (14.6%), CR (15.9%) and FL (15.3%) showed
negative score values according to the PC1. The positive contribution to the PC2 was observed
for O (64.3%) and FR (10.7%).

The ANOVA analysis (Table 4) revealed that the linear term of storage time was the
most important for CD calculation, statistically significant at $p < 0.01$ level. The linear terms of
storage temperature, time, and storage condition as well as the non-linear terms $T \times t$ and $P \times t$ were the most influential variables for UO, CR, HR, FL and UF calculation. Besides these terms, $T \times P$ term was also important for FAT calculation, statistically significant at $p < 0.01$ level. Most of the SOP models had an insignificant lack of fit tests, which means that all the models represented the data satisfactorily. A high coefficient of determination ($r^2$) reveals that the variation was accounted and that the data fitted satisfactorily to the proposed model. The $r^2$ values for the observed responses were found very satisfactory and showed good fit of the models to experimental results.

4. Conclusions

Unpacked and packed RF/LBF cookies kept at ambient and elevated temperature exhibited different susceptibility to lipid oxidation, which should be contributed to the packaging.

Antioxidant capacity measured using the DPPH test was the highest in freshly baked RF/LBF cookies with decreasing tendency during storage in all investigated cookie samples, but at different rates indicating the depletion of antioxidants for suppression of lipid oxidation reactions. This conclusion was confirmed by the obtained decrease in TPC and rutin content in RF/LBF cookies during storage.

The significant changes in total aldehydes content of unpacked and packed cookies stored at elevated temperature were determined in month 3 and 4, respectively, while those of the unpacked and packed cookies stored at ambient temperature were determined in month 11 and 14, respectively, and they can be addressed to the end-points of cookie shelf-life.
From the sensory evaluation, it was concluded that the greatest loss of sensory quality resulted from HR, FR, UO, and UF properties. Colour development (CD) could not be considered as an indicator of sensory changes in RF/LBF cookies during examined storage period.

The increased UO and UF caused discrimination of the unpacked and packed cookies stored at elevated temperature in month 1 and at the end of month 2, respectively. The unpacked and packed cookies stored at ambient temperature were well scored concerning the mentioned properties until the end of month 3 and 5.5, respectively.

Texture attributes (HR and FR) also contributed to the unacceptable sensory profile of unpacked cookies kept at elevated temperature at the end of month 1 and 1.5, respectively, while those kept at ambient temperature were well scored until the end of month 2.5 and 3, respectively.

In performed PCA procedure, principal components were built to identify and combine variables that were not truly independent and also to maximize the variance of experimental data. The first two principle components accounted for 84.04% of the total variance, and that could be very well explained by the four grouped variables, TPC, rutin content, DPPH, and total aldehydes content, for both unpacked and packed gluten-free rice-buckwheat cookies stored at different temperatures. In the case of the sensory data, the performed PCA revealed that the first two principal components accounted for the 76.31% of the total variance, with an equal positive (HR, UO, and UF) or negative (CR, FAT, and FL) contribution in relation to the observed variables and the PC1. In the case of the PC2, the largest positive contribution was recorded in relation to the characteristic odour (64.3%).
The coefficients of determination for the developed second order polynomial models generally showed good fit of the models to experimental results, and can be used for the prediction of the observed responses during the storage of unpacked and packed gluten-free rice-buckwheat cookies at different temperatures.

Based on the obtained results, sensory properties might be suggested to be the relevant parameters for predicting the end-point of RF/LBF cookie shelf-life rather than total aldehydes content.

Acknowledgement

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References


Figure captions

Fig. 1  Principal component analysis (PCA) ordination of total phenolic content (TPC), rutin content, DPPH radical scavenging activity (DPPH) and total aldehydes content (aldehydes) based on component correlations during the storage time.

Fig. 2  Principal component analysis (PCA) ordination of sensory properties (CD – colour development; O – odour; UO – uncharacteristic odours; CR – crumbliness; HR – hardness; FR – fracturability; FAT – fattiness; FL – flavour; UF – uncharacteristic flavours) based on component correlations during the storage time.
Table 1
Total phenolic content (TPC), rutin content, DPPH radical scavenging activity (DPPH) and total aldehydes content of unpacked (UP) and packed (P) gluten-free rice-buckwheat cookies during storage at 23 ± 1°C and 40 ± 1°C.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>T 23°C</th>
<th>40°C</th>
<th>23°C</th>
<th>40°C</th>
<th>23°C</th>
<th>40°C</th>
<th>23°C</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC (µg GAE/g d.m.)</td>
<td>UP</td>
<td>P</td>
<td>UP</td>
<td>P</td>
<td>UP</td>
<td>P</td>
<td>UP</td>
<td>P</td>
</tr>
<tr>
<td>Rutin content (mg/kg d.m.)</td>
<td>UP</td>
<td>P</td>
<td>UP</td>
<td>P</td>
<td>UP</td>
<td>P</td>
<td>UP</td>
<td>P</td>
</tr>
<tr>
<td>DPPH (IC50 g/mL)</td>
<td>UP</td>
<td>P</td>
<td>UP</td>
<td>P</td>
<td>UP</td>
<td>P</td>
<td>UP</td>
<td>P</td>
</tr>
<tr>
<td>Total aldehydes content (mg/kg d.m.)</td>
<td>UP</td>
<td>P</td>
<td>UP</td>
<td>P</td>
<td>UP</td>
<td>P</td>
<td>UP</td>
<td>P</td>
</tr>
</tbody>
</table>

| Months | 1 | 689<sup>b</sup> | 722<sup>bc</sup> | 704<sup>ab</sup> | 718<sup>cd</sup> | 17.0<sup>a</sup> | 16.5<sup>bc</sup> | 15.1<sup>bc</sup> | 13.8<sup>b</sup> | 0.12<sup>b</sup> | 0.13<sup>b</sup> | 0.13<sup>b</sup> | 0.13<sup>b</sup> | 3.46<sup>a</sup> | 3.35<sup>a</sup> | 3.36<sup>a</sup> | 3.45<sup>a</sup> |
|        | 2 | 668<sup>b</sup> | 702<sup>c</sup> | 699<sup>a</sup> | 707<sup>b</sup> | 17.0<sup>b</sup> | 15.0<sup>b</sup> | 14.4<sup>a</sup> | 13.2<sup>b</sup> | 0.13<sup>b</sup> | 0.14<sup>b</sup> | 0.14<sup>b</sup> | 0.15<sup>a</sup> | 1.4<sup>a</sup> | 1.4<sup>a</sup> | 1.5<sup>a</sup> | 1.4<sup>a</sup> | 2.54<sup>c</sup> | 3.93<sup>a</sup> | 4.43<sup>b</sup> | 3.49<sup>a</sup> |
|        | 3 | 546<sup>a</sup> | 599<sup>b</sup> | 684<sup>d</sup> | 624<sup>c</sup> | 16.4<sup>c</sup> | 13.4<sup>f</sup> | 14.2<sup>d</sup> | 12.7<sup>d</sup> | 0.15<sup>b</sup> | 0.14<sup>d</sup> | 0.14<sup>d</sup> | 0.12<sup>d</sup> | 0.13<sup>b</sup> | 2.4<sup>d</sup> | 2.34<sup>d</sup> | 2.36<sup>d</sup> | 2.12<sup>d</sup> | 3.59<sup>a</sup> |
|        | 4 | 537<sup>a</sup> | 557<sup>bc</sup> | 607<sup>c</sup> | 579<sup>bc</sup> | 16.5<sup>b</sup> | 13.1<sup>d</sup> | 15.7<sup>cd</sup> | 12.8<sup>d</sup> | 0.15<sup>d</sup> | 0.14<sup>d</sup> | 0.15<sup>d</sup> | 0.13<sup>b</sup> | 1.35<sup>a</sup> | 2.05<sup>b</sup> | 2.37<sup>a</sup> | 3.70<sup>a</sup> | 3.54<sup>c</sup> | 5.07<sup>a</sup> |
|        | 5 | 515<sup>a</sup> | 543<sup>d</sup> | 560<sup>b</sup> | 504<sup>a</sup> | 16.0<sup>b</sup> | 12.7<sup>d</sup> | 15.3<sup>bc</sup> | 12.8<sup>d</sup> | 0.13<sup>b</sup> | 0.16<sup>b</sup> | 0.16<sup>b</sup> | 0.12<sup>a</sup> | 0.14<sup>b</sup> | 2.29<sup>c</sup> | 3.92<sup>b</sup> | 39.28<sup>b</sup> | 65.33<sup>d</sup> |
|        | 6 | 510<sup>c</sup> | 483<sup>a</sup> | 501<sup>b</sup> | 490<sup>ab</sup> | 15.8<sup>d</sup> | 12.1<sup>d</sup> | 14.6<sup>bc</sup> | 11.3<sup>a</sup> | 0.15<sup>b</sup> | 0.15<sup>d</sup> | 0.13<sup>a</sup> | 0.14<sup>a</sup> | 3.25<sup>a</sup> | 3.13<sup>a</sup> | 17.36<sup>b</sup> | 67.86<sup>c</sup> |
|        | 7 | 497<sup>d</sup> | 377<sup>b</sup> | 433<sup>d</sup> | 475<sup>c</sup> | 13.8<sup>bc</sup> | 11.8<sup>d</sup> | 13.1<sup>bc</sup> | 11.2<sup>a</sup> | 0.16<sup>b</sup> | 0.17<sup>de</sup> | 0.13<sup>ab</sup> | 0.12<sup>b</sup> | 3.42<sup>a</sup> | 3.32<sup>a</sup> | 8.80<sup>b</sup> | 61.42<sup>c</sup> |
|        | 8 | 455<sup>d</sup> | 374<sup>b</sup> | 402<sup>c</sup> | 423<sup>c</sup> | 13.1<sup>cd</sup> | 11.3<sup>bc</sup> | 13.0<sup>bc</sup> | 12.3<sup>bc</sup> | 0.16<sup>bc</sup> | 0.18<sup>de</sup> | 0.12<sup>a</sup> | 0.13<sup>ab</sup> | 3.03<sup>a</sup> | 3.18<sup>a</sup> | 5.52<sup>b</sup> | 58.98<sup>c</sup> |
|        | 9 | 410<sup>b</sup> | 383<sup>ab</sup> | 375<sup>b</sup> | 391<sup>a</sup> | 12.5<sup>c</sup> | 11.0<sup>bc</sup> | 12.8<sup>bc</sup> | 11.6<sup>bc</sup> | 0.12<sup>b</sup> | 0.16<sup>bc</sup> | 0.12<sup>a</sup> | 0.13<sup>ab</sup> | 2.78<sup>a</sup> | 2.62<sup>a</sup> | 4.62<sup>b</sup> | 34.78<sup>e</sup> |

Each value is the mean of three independent measurements.

Values in the same column with the different superscript lowercase letters are statistically different (p < 0.05).

Values in the same row with the different subscript uppercase letters are statistically different (p < 0.05).

T – temperature; d.m. – dry matter; GAE – gallic acid equivalents.
Table 2
ANOVA table of total phenolic content (TPC), rutin content, DPPH radical scavenging activity (DPPH) and total aldehydes content (aldehydes) evaluation (sum of squares)

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>TPC</th>
<th>Rutin</th>
<th>DPPH</th>
<th>Aldehydes</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>1</td>
<td>220</td>
<td>0.00</td>
<td>0.005+</td>
<td>4765.16+</td>
</tr>
<tr>
<td>P</td>
<td>1</td>
<td>13</td>
<td>22.70+</td>
<td>0.001</td>
<td>2329.88+</td>
</tr>
<tr>
<td>t</td>
<td>1</td>
<td>337048+</td>
<td>54.45+</td>
<td>0.005+</td>
<td>3499.61+</td>
</tr>
<tr>
<td>t²</td>
<td>1</td>
<td>47940+</td>
<td>7.68+</td>
<td>0.000</td>
<td>220.85</td>
</tr>
<tr>
<td>T × P</td>
<td>1</td>
<td>242</td>
<td>0.05</td>
<td>0.000</td>
<td>1188.75+</td>
</tr>
<tr>
<td>T × t</td>
<td>1</td>
<td>846</td>
<td>4.21+</td>
<td>0.003+</td>
<td>1133.43+</td>
</tr>
<tr>
<td>P × t</td>
<td>1</td>
<td>64</td>
<td>1.50</td>
<td>0.003+</td>
<td>1233.28+</td>
</tr>
<tr>
<td>Error</td>
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<td>39314</td>
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<td>0.014</td>
<td>8958.24</td>
</tr>
<tr>
<td>$r^2$</td>
<td></td>
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<td>0.904</td>
<td>0.784</td>
<td>0.513</td>
</tr>
</tbody>
</table>

+Significant at $p < 0.01$ level; Error terms have been found statistically insignificant; df – degrees of freedom, T – temperature, P – packaging condition, t – storage time
Table 3
Mean scores for sensory properties of unpacked (UP) and packed (P) gluten-free rice-buckwheat cookies during storage at 23 ± 1 °C and 40 ± 1 °C

<table>
<thead>
<tr>
<th>Property</th>
<th>Colour development (CD)</th>
<th>Odour (O)</th>
<th>Uncharacteristic odours (UO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UP</td>
<td>P</td>
<td>UP</td>
<td>P</td>
</tr>
<tr>
<td>23 °C</td>
<td>40 °C</td>
<td>23 °C</td>
<td>40 °C</td>
</tr>
<tr>
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<tr>
<td>23 °C</td>
<td>40 °C</td>
<td>23 °C</td>
<td>40 °C</td>
</tr>
<tr>
<td>Months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>3.60ₐ</td>
<td>3.60ₐ</td>
<td>3.60ₐ</td>
</tr>
<tr>
<td>0.5</td>
<td>3.17ₐ</td>
<td>3.25ₐ</td>
<td>3.18ₐ</td>
</tr>
<tr>
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<td>3.13ₐ</td>
<td>3.10ₐ</td>
<td>3.17ₐ</td>
</tr>
<tr>
<td>1.5</td>
<td>3.10ₐ</td>
<td>3.05ₐ</td>
<td>3.14ₐ</td>
</tr>
<tr>
<td>2.0</td>
<td>3.07ₐ</td>
<td>3.00ₐ</td>
<td>3.13ₐ</td>
</tr>
<tr>
<td>2.5</td>
<td>3.03ₐ</td>
<td>2.95ₐ</td>
<td>3.09ₐ</td>
</tr>
<tr>
<td>3.0</td>
<td>3.0ₐ</td>
<td>2.9ₐ</td>
<td>3.0ₐ</td>
</tr>
<tr>
<td>Months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>2.7ₐ</td>
<td>2.ₐ</td>
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</tr>
<tr>
<td>4.0</td>
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<td>2.ₐ</td>
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<td>2.ₐ</td>
<td>2.ₐ</td>
</tr>
<tr>
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<td>2.ₐ</td>
<td>2.ₐ</td>
</tr>
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<td>5.ₐ</td>
<td>2.ₐ</td>
<td>2.ₐ</td>
<td>2.ₐ</td>
</tr>
<tr>
<td>Months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.₀</td>
<td>2.ₐ</td>
<td>2.ₐ</td>
<td>2.ₐ</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Property</th>
<th>Crumbliness (CR)</th>
<th>Hardness (HR)</th>
<th>Fracturability (FR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UP</td>
<td>P</td>
<td>UP</td>
<td>P</td>
</tr>
<tr>
<td>23 °C</td>
<td>40 °C</td>
<td>23 °C</td>
<td>40 °C</td>
</tr>
<tr>
<td>23 °C</td>
<td>40 °C</td>
<td>23 °C</td>
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</tr>
<tr>
<td>23 °C</td>
<td>40 °C</td>
<td>23 °C</td>
<td>40 °C</td>
</tr>
<tr>
<td>Months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>3.0₀ₐ</td>
<td>3.0₀ₐ</td>
<td>3.0₀ₐ</td>
</tr>
<tr>
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<td>2.ₐ</td>
<td>2.ₐ</td>
</tr>
<tr>
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</tr>
<tr>
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<td>2.ₐ</td>
<td>2.ₐ</td>
<td>2.ₐ</td>
<td>2.ₐ</td>
</tr>
<tr>
<td>Months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Months</td>
<td>property</td>
<td>Fattiness (FAT)</td>
<td>Flavour (FL)</td>
</tr>
<tr>
<td>--------</td>
<td>----------</td>
<td>----------------</td>
<td>--------------</td>
</tr>
<tr>
<td>0.0</td>
<td>3.60\textsuperscript{a}A</td>
<td>3.60\textsuperscript{a}A</td>
<td>3.60\textsuperscript{a}A</td>
</tr>
<tr>
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<td>3.53\textsuperscript{a}B</td>
<td>3.50\textsuperscript{b}B</td>
<td>2.65\textsuperscript{a}A</td>
</tr>
<tr>
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<td>3.33\textsuperscript{a}B</td>
<td>3.47\textsuperscript{b}B</td>
<td>2.50\textsuperscript{b}B</td>
</tr>
<tr>
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<td>3.25\textsuperscript{b}B</td>
<td>3.25\textsuperscript{b}B</td>
<td>1.98\textsuperscript{a}A</td>
</tr>
<tr>
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<td>2.83\textsuperscript{a}B</td>
<td>3.03\textsuperscript{b}B</td>
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</tr>
<tr>
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<td>1.20\textsuperscript{A}</td>
</tr>
<tr>
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<td>2.24\textsuperscript{e}A</td>
<td>2.78\textsuperscript{d}B</td>
<td>1.92\textsuperscript{d}C</td>
</tr>
<tr>
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<td>2.07\textsuperscript{f}A</td>
<td>2.70\textsuperscript{c}B</td>
<td>2.24\textsuperscript{d}B</td>
</tr>
<tr>
<td>4.5</td>
<td>2.05\textsuperscript{f}B</td>
<td>2.60\textsuperscript{d}B</td>
<td>2.12\textsuperscript{d}A</td>
</tr>
<tr>
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<td>2.56\textsuperscript{d}B</td>
<td>2.05\textsuperscript{d}B</td>
</tr>
<tr>
<td>5.5</td>
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</tr>
<tr>
<td>6.0</td>
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<td>2.30\textsuperscript{f}B</td>
<td>1.60\textsuperscript{d}A</td>
</tr>
</tbody>
</table>

Values in the same row with the different superscript lowercase letters are statistically different ($p < 0.05$).
Values in the same column with the different superscript uppercase letters are statistically different ($p < 0.05$).
Underlined values in columns indicate the time in which individual properties were at the failure criteria in accordance with a predefined number of scores.
Table 4
ANOVA table of sensory properties evaluation (sum of squares)

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>CD</th>
<th>8×10^16</th>
<th>O</th>
<th>24.51^t</th>
<th>1.14^t</th>
<th>1.01^t</th>
<th>0.59</th>
<th>0.76^t</th>
<th>7.12^t</th>
<th>28.37^t</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>1</td>
<td>0.07^t</td>
<td>8×10^16</td>
<td></td>
<td>12.57^t</td>
<td>0.44^t</td>
<td>3.37^t</td>
<td>227.18</td>
<td>6.06^t</td>
<td>2.53^t</td>
<td>8.33^t</td>
</tr>
<tr>
<td>P</td>
<td>1</td>
<td>0.04^t</td>
<td>4×10^17</td>
<td></td>
<td>12.80^t</td>
<td>3.55^t</td>
<td>3.56^t</td>
<td>223.85</td>
<td>3.76^t</td>
<td>7.84^t</td>
<td>32.10^t</td>
</tr>
<tr>
<td>t</td>
<td>1</td>
<td>0.46^t</td>
<td>1×10^16</td>
<td></td>
<td>0.21</td>
<td>0.00</td>
<td>0.06</td>
<td>20.67</td>
<td>0.12**</td>
<td>0.00</td>
<td>0.02</td>
</tr>
<tr>
<td>t^2</td>
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<td>0.07^t</td>
<td>4×10^16</td>
<td></td>
<td>0.21</td>
<td>0.00</td>
<td>0.06</td>
<td>20.67</td>
<td>0.12**</td>
<td>0.00</td>
<td>0.02</td>
</tr>
<tr>
<td>T × P</td>
<td>1</td>
<td>0.00</td>
<td>4×10^17</td>
<td></td>
<td>0.54^t</td>
<td>0.00</td>
<td>0.10</td>
<td>281.25</td>
<td>2.19^t</td>
<td>0.15^t</td>
<td>0.08</td>
</tr>
<tr>
<td>T × t</td>
<td>1</td>
<td>0.03***</td>
<td>1×10^16</td>
<td></td>
<td>7.58^t</td>
<td>0.40^t</td>
<td>0.44^t</td>
<td>187.48</td>
<td>0.10**</td>
<td>2.56^t</td>
<td>9.16^t</td>
</tr>
<tr>
<td>P × t</td>
<td>1</td>
<td>0.04^t</td>
<td>8×10^-1</td>
<td></td>
<td>2.63^t</td>
<td>0.08^t</td>
<td>0.40^t</td>
<td>33.97</td>
<td>0.91^t</td>
<td>0.94^t</td>
<td>1.59^t</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>0.30</td>
<td>8×10^18</td>
<td></td>
<td>3.38</td>
<td>0.51</td>
<td>1.20</td>
<td>8141.84</td>
<td>0.90</td>
<td>0.40</td>
<td>1.67</td>
</tr>
<tr>
<td>r^2</td>
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<td>0.910</td>
<td>0.125</td>
<td></td>
<td>0.948</td>
<td>0.935</td>
<td>0.901</td>
<td>0.142</td>
<td>0.948</td>
<td>0.977</td>
<td>0.976</td>
</tr>
</tbody>
</table>

^tSignificant at p < 0.01 level; **Significant at p < 0.05; Error terms have been found statistically insignificant; df – degrees of freedom, T – temperature, P – packaging condition, t – storage time CD – colour development; O – odour; UO – uncharacteristic odours; CR – crumbliness; HR – hardness; FR – fracturability; FAT – fattiness; FL – flavour; UF – uncharacteristic flavours