Red grape skin extract and oat β -glucan in shortbread cookies: technological and nutritional evaluation

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Abstract

Consumers are increasingly interested in innovative products enriched with nutrients that counteract the development of civilisation-associated diseases. Thus, the development of new functional nutrient-enriched food products that meet consumer needs is therefore necessary. The aims of this study were to develop a novel shortbread cookie product by addition of red grape skin extract and oat β -glucan and to evaluate the influence of these modifications on physico-chemical properties and consumer's acceptance. The red grape skin extract used in this product is a source of polyphenols which are beneficial for human health and extend product shelf life. β -glucan is a soluble fraction of dietary fibre that helps to lower reduce blood cholesterol and glycemic levels. The influence of grape skin extract, β -glucan and a combination of both on the β -glucan content, colour and texture parameters, thermal properties, total antioxidant activity, total phenolic content, volatile compounds profile and sensory properties of shortbread cookies was analysed. Product with modified formulations had higher total antioxidant activity, total phenolic content and were more thermally stable than control samples. The obtained innovative functional products were characterized by an increased content of oat β -glucan, which is desirable from a nutritional point of view. The DSC measurements showed higher oxidative stability of cookies enriched with β -glucan and red grape skin extract. The additives caused significant changes in the colour and profile of volatile compounds of the finished final product but did not affect the sensory acceptance among consumers. The use of these additives represents a development in food technology that may be valuable in the food industry to increase the nutritional value and extend the shelf shelf-life of bakery products.

Keywords Functional shortbread cookies · Differential scanning calorimetry · Natural antioxidants · Oat β -glucan concentrate · Bioactive compounds

Abbreviations

Biological, chemical and microbiological

GSE	Red grape skin extract
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
LDL	Low-density lipoprotein
HDL	High-density lipoprotein
DPPH	2,2-Diphenyl-1-picrylhydrazyl

Instrumental techniques

DSC	Differential scanning calorimetry
E_a	Effective activation energy

Z	Pre-exponential factor
K	Constant rate
B	Heating rate
T_{ON}	Onset oxidation temperature
TTA	Total antioxidant activity
TPC	Total phenolic content
HS-GC	Gas chromatography with headspace
MAP	Modified atmosphere packaging

Institution

EFSA	European Food Safety Authority
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Introduction

Consumers often buy convenient or easily accessible bakery products as snacks. These types of products are characterised by high-fat levels [1]. In addition, consumers are increasingly interested in innovative products enriched with

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nutrients that counteract the development of civilisation-associated diseases such as excessive body weight, obesity, type 2 diabetes and atherosclerosis. A compound that possesses the ability to combat these issues is the soluble fibre fraction, β -glucan. It is composed of unbranched linear chains connected by β -(1,3-), β -(1,4-)-D-glycosidic bonds [2]. Sources of β -glucan include cereals such as oats and barley, and consuming foods rich in β -glucan beneficially reduces the blood LDL cholesterol fraction and increases the HDL fraction [3]. Additionally, the European Food Safety Authority (EFSA) has developed two health claims for β -glucan: “consuming oat or barley beta-glucans with a meal helps to reduce the rise in blood glucose after that meal” and “beta-glucans help maintain normal cholesterol levels in the blood” [4–6].

The quality of food products with a high added fat can be ensured throughout their shelf-life by increased oxidative stability of the fat, commonly achieved through the use of antioxidants. Nowadays, consumers are becoming increasingly aware of the health impacts of their dietary intake. They try to avoid products with the addition of synthetic antioxidants such as butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA), which have been used widely for many years to slow lipid oxidation, but current concerns about their safety, together with increasing consumer's preference for natural products, has resulted in an increased demand for natural antioxidants [7, 8]. Alternatives to synthetic antioxidants are natural compounds found in raw plant materials. Natural antioxidant substances include polyphenolic compounds and tannins. They are included among others in: spices, herbs, and berries. The addition of substances containing polyphenols to food products inhibits the oxidation of fats. Consequently, it extends the shelf-life of the product. Red grapes are one such fruit rich in polyphenol compounds. Their skin is rich in bioactive substances, including compounds with antioxidant activity [such as: tannins, anthocyanins, flavonoids, stilbens (resveratrol)] [9, 10]. In addition, they exhibit antibacterial properties and inhibit the growth of cancer cells [11, 12]. Stilbenes (resveratrol and viniferins) are the primary antioxidant compounds in grapes [13, 14]. Phenolic compounds, which are plant-derived secondary metabolites and the antioxidant compounds in grapes, can be divided into two groups, being phenolic acids (and related compounds) and flavonoids. There is a wide degree of variation among phenolic compounds with regards to their effectiveness as antioxidants [15]. They have health-promoting benefits such as antiviral, anti-inflammatory, anticarcinogenic, and antimicrobial activities [16–18].

The quality of confectionery products is determined by their sensory, technological and nutritional properties [19]. The fat contained in the products plays a significant role in shaping the formation of all these features. Also, lipids play a very important role in human nutrition and food

technology. The type and amount of these in food have an influence on the sensory, physicochemical and nutritional characteristics of products [20]. From a nutritional perspective, fats contained in food play an important role in human health and body functions [21]. Fat content in food, beyond supplying calories, is a source of fatty acids, including beneficial unsaturated fatty acids such as α -linoleic and α -linolenic acid. Lipids also play a role as vehicles for fat-soluble vitamins A, D, E and K [22–24]. However, in terms of food technology, they are very susceptible to hydrolysis, oxidation processes and thermal polymerisation [25], which are unfavourable transformations leading to the formation of compounds harmful to health, including primary and secondary oxidation products [26]. Baking temperature, storage time and oxygen availability are factors accelerating negative changes in fat integrity, including oxidation, polymerisation and cyclisation of unsaturated fatty acids [27]. Susceptibility to fat oxidation depends on the chemical composition of the fat type, primarily the fatty acid profile [28]. Oxidation reactions are one of the major sources of deterioration occurring during manufacturing, storage, distribution and final preparation of food products. Furthermore, thermal treatment of food, such as grilling, baking, frying and incorrect storage and transport conditions accelerate the oxidation [29]. Lipid oxidation process have has a negative impact on nutritional value, quality properties (unpleasant flavour and taste) and the formation of toxic compounds in food products, which consequently has a negative influence on shelf-life, consumer acceptability and demand in the food market [30].

Fat oxidation products consumed with food can accelerate oxidative processes in the human body and thereby lead to faster formation of atherosclerotic and neoplastic changes [31, 32]. Consequently, minimising the degree of degradation of the fat in a food product is crucial in ensuring food safety. This is particularly important in the case of products with high-fat content, such as shortbread, where it constitutes between 20 and 30% of the formulation [33].

The quality of food products with high added fat can be ensured throughout their shelf-life by increased oxidative stability of the fat, commonly achieved through the use of antioxidants. Nowadays, consumers are becoming increasingly aware of the health impacts of their dietary intake and try to avoid products with the addition of synthetic antioxidants such as butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA), which have been used widely for many years to slow lipid oxidation, but current concerns about their safety, together with increasing consumer preference for natural products, has resulted in an increased demand for natural antioxidants [22, 23]. Alternatives to synthetic antioxidants are natural compounds found in raw plant materials. Sources of antioxidant substances include spices, herbs and berries [18, 19]. The properties of which are conditioned by the presence of polyphenolic compounds

and natural tannins. These substances prolong the durability of fat by inhibiting oxidation processes. One such raw plant product is grape, the skin of which is rich in bioactive compounds with antioxidant properties. In addition, they exhibit antibacterial and antiprotozoal properties and inhibit the growth of cancer cells [26, 27]. Phenolic compounds, which are plant-derived secondary metabolites and the primary antioxidant compounds in grapes, can be divided into two groups, being phenolic acids (and related compounds) and flavonoids. There is a wide degree of variation among phenolic compounds with regards to their effectiveness as antioxidants [30]. They have health-promoting benefits such as antiviral, anti-inflammatory, anticarcinogenic, and antimicrobial activities [31–33]. In addition, red grape skin or seeds, which are a by-product of processing grapes, have a positive influence on oxidative status [28, 29].

The aim of this study was to develop a formulation and production technology for an innovative confectionery product that meets the expectations of customers who wish to prioritise healthy snacks. This included the analysis of physicochemical characteristics and the assessment of consumers' acceptance of shortbread cookies with increased antioxidant properties as a result of due to the addition of red grape skin extract and β -glucan concentrate.

Materials and methods

Preparation of shortbread cookies

Shortbread cookies were prepared according to four different treatment formulations (determined during pilot experiments—Control, G—the addition of red grape skin extract, B—the addition of 30% oat β -glucan concentrate, G + B—the addition of red grape skin extract and 30% oat β -glucan concentrate) according to recipes presented in Table 1. Three different variations of red grape skin extract in combination with oat β -glucan concentrate were used in place of normal flour. The red grape skin extract was prepared from not vinified full-blown *St. Laurent* grape berries using the pressurised fluid extraction (PFE). Ethanol has been used as a solvent for extraction. The red grape skin extract was standardised to 95% (w/w) (Chempol, Poland) and β -glucan concentrate extracted from dehulled oat grain *Avena Sativa* to 30% (w/w) with a composition of 19.04% protein, 10.01% carbohydrates, 8.09% fat, 28.09% insoluble fibre and 31.90% soluble fibre, including β -glucan (Microstructure Inc., Poland). Control samples were prepared without β -glucan concentrate and grape skin extract and any additives. All dry ingredients (wheat flour type 500—the protein content 10.1 g/100 g, sugar powder, red grapes skin extract, oat β -glucan concentrate – 30 g oat β -glucan/100 g concentrate and salt) were mixed and sifted. Butter was chopped and

Table 1 The proportion of ingredients used in the preparation of shortbread cookies with different flour replacement levels with red grape skin extract

Component (g)	Control	G	B	G + B
Wheat flour (type 500)	1000	978	978	956
Red grapes skin extract 95%	0	22	0	22
Oat β -glucan concentrate 30%	0	0	22	22
Butter (milk fat content > 85%)	517	517	517	517
Icing sugar/Sugar powder	464	464	464	464
Whole egg	107	107	107	107
Sour cream	107	107	107	107
Salt	0.4	0.4	0.4	0.4

Treatments: Control, G—flour replacement by red grape skin extract, B—flour replacement by oat β -glucan concentrate, G + B—flour replacement by red grape skin extract and oat β -glucan concentrate

added to sifted ingredients. Ingredients were minced mixed at 200 rpm for 4 min using a TRQ-42, RM Spiral mixer (Gastro, Poland). Whole eggs and sour cream were added and minced mixed for a next 6 min. The dough was formed into 10 × 15 × 50 mm cuboids using a Cornucopia KN 100 automatic encrusting machine (Rheon, Japan). Cookies were placed on a tray and baked at 180 °C for 10.5 min in a CPE 110 convection oven (Küppersbuch, Germany) and cooled at room temperature to 19 °C. Each cookie weighed 15 g.

Cooled cookies were packed under modified atmosphere (MAP) at 100% N₂ into plastic packaging containers using an M3 packaging machine (Sealpack, Germany) and were stored at 20 °C without access to light for 9 weeks. Sampling was carried out immediately after packing (Week 0), for which the cookies which had been packed were immediately unpacked and analysed on the same day as the samples were prepared, as well as after 3, 6 and 9 weeks.

β -glucan content

The content of oat β -glucan in the shortbread cookies was measured using an enzymatic procedure evaluated by AOAC International (Method 995.16) using the β -glucan Assay Kit (Mixed Linkage Megazyme International, Ireland). Absorbance was measured at 510 nm using a Spark™ 10 M spectrophotometric plate reader (Tecan Spark™ 10 M, Switzerland). β -glucan concentration was calculated according to Eq. (1)

$$\begin{aligned}
 &\beta\text{-glucan} \left(\frac{\%w}{w} \right) (\text{"as is"}) \\
 &= \Delta A \times F \times \frac{FV}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{182} \times D \\
 &= \Delta A \times \frac{F}{W} \times FV \times D \times 0.9
 \end{aligned} \quad (1)$$

where ΔA = absorbance after β – glucosidase treatment (reaction) minus reaction blank absorbance, F = factor for the conversion of absorbance values to μg of glucose $= \frac{100(\mu\text{g of D-glucose})}{\text{absorbance of } 100\mu\text{g of D-glucose}}$, FV = final volume (6.4 mL), 0.1 = volume of sample analysed, $\frac{1}{1000}$ = conversion from μg to mg, $\frac{100}{W}$ = factor to express β – glucan content as a percentage of sample weight, W = the weight in mg (as is basis) of the sample analysed, $\frac{162}{180}$ = factor to convert from free D – glucose, as determined, to anhydro – D – glucose, as occurs in β – glucan, D = further dilution prior to incubation with β – glucosidase.

The oat β -glucan content was evaluated in four replicates for each sample at weeks 0, 3, 6 and 9 of storage.

Measurement of colour

The colour of the cookies was measured using a CR-400 chromatometer instrumental method in the CIE $L^*a^*b^*$ system (Konica Minolta Inc., Japan) and analysed according to the CIE $L^*a^*b^*$ colour system as previously described [34]. A standard observer 2° with an 8 mm diameter measuring head and D65 daylight illuminant (colour temperature – 6500 K) illuminant were used. A standard white Minolta calibration tile ($L^* = 98.45\%$, $a^* = -0.10$ [–], $b^* = -0.13$ [–]) was used to calibrate the chromatometer. Colour measurements were performed at 15 points on the surface of each sample at weeks 0, 3, 6 and 9 of storage.

Measurement of texture and measurement of hardness

Instrumental texture analysis, defined as the maximum force required to break each shortbread cookie, was carried out in the form of a three-point bending test as described by Zucco et al. [35]. The analysis was performed using the Universal Testing Machine Instron 5965 (Instron, USA) with a cell capacity of 500 N, equipped with a three-point bending jig. The span between the supports was 40 mm and the test speed was 0.2 mm s^{-1} . The maximum force in Newtons was recorded and data analysed using the Bluehill 2 Software. The texture measurements were performed in six replicates for each sample at weeks 0, 3, 6 and 9 of storage.

Differential scanning calorimetry (DSC) measurement

The DSC measurements were made on the fat samples extracted from cookies. The fat was extracted from ground cookies samples as previously described by Floch et al. [36] with modifications. An ether:acetone (2:1 v/v) mixture was used to extract the fat and this solvent was subsequently removed using a Rotavapor R-3 rotary vacuum

evaporator (BÜCHI, Switzerland) under reduced pressure. Prepared samples were immediately subjected to oxidative stability analysis by differential scanning calorimetry (DSC). The oxidative stability of cookie-derived fat samples was measured using a DSC 1 calorimeter (Mettler Toledo, Switzerland) in an oxygen atmosphere. The instrument was calibrated with pure indium and zinc. The fat samples ($5.0 \pm 0.1 \text{ mg}$) were placed into standard $40 \mu\text{L}$ aluminium sample pans (ME-51119870) closed with piercing lids (ME-51119873) by Mettler Toledo Crucible Sealing Press. Holes were pierced automatically in the centre of the lids just before the measurement by the needle of the autosampler, to allow contact of the sample with the oxygen stream. The aluminium reference pan (also with a hole in the lid) was left empty on the position. The sample and reference pans were heated at rates (β , $^\circ\text{C min}^{-1}$) of 4, 7.5, 10, 12.5 and $15 \text{ }^\circ\text{C min}^{-1}$, with an oxygen flow of 90 ml min^{-1} . When the measurement was completed, the onset oxidation temperature (T_{ON} , $^\circ\text{C}$) was determined as the intersection of the extrapolated baseline and the tangent line (leading edge) of the recorded exotherm. Kinetic triplet parameters (effective activation energy, E_a ; pre-exponential factor, Z and constant rate, k) for each sample were calculated according to the Kissinger method [37, 38] (2) and Arrhenius Eq. (3).

$$\ln\left(\frac{\beta}{T_{\text{ON}}^2}\right) = \ln\left(\frac{ZR}{E_a}\right) - \frac{E_a}{RT_{\text{ON}}}, \quad (2)$$

$$k = Z \exp\left(\frac{-E_a}{RT_{\text{ON}}}\right), \quad (3)$$

where β is the heating rate, T_{ON} is the onset oxidation temperature, R is the gas constant, E_a is the effective activation energy, Z is the pre-exponential factor, k is the constant rate.

The Kissinger plots $\ln(\beta/T_{\text{ON}}^2)$ vs. T_{ON}^{-1} were generated to develop a straight line. The gradient of the line which is represented by $-E_a/R$ was used to determine E_a by multiplication with gas constant, R . The results were compiled using STARE Evaluation Software Version 9.30. The DSC measurements were performed in three replicates for each sample at weeks 0, 3, 6 and 9 of storage.

Preparation of ethanolic extracts

Cookie powder (2.5 g) was mixed with 7.5 ml of ethanol and homogenised for 2 min at 12,000 rpm using a T18 Ultra Turrax basic high-speed homogeniser (IKA, Germany). Samples were extracted at room temperature for 10 min on a MyLab SLRM-3 rotary shaker (NanoEnTek Inc., Korea) and then centrifuged for 10 min at 18,000 rpm

in a 251 MPW centrifuge (MPW Med. Instruments, Poland). The supernatant liquid was carefully poured off from the solid pellet and analysed for total antioxidant activity (TTA) and total phenolic content (TPC).

Total antioxidant activity (TTA)

Anti-radical activity of the analysed samples was measured as reductive capacity relative to 2,2-diphenyl-1-picrylhydrazyl (DPPH) [39]. Briefly, 3.5 ml of DPPH ethanolic solution (0.1 mM) was added to 0.5 ml of an ethanolic extract of the sample, mixed for 30 s and the mixture placed in darkness for 20 min. Absorbance was measured at $\lambda = 517$ nm wavelength using a Spark™ 10 M (Tecan, Switzerland). Ethanol at 80 g l⁻¹ concentration was used as a reference solution and a control sample was prepared without extract addition. TAA measurements were performed in triplicate for each sample at weeks 0, 3, 6 and 9 of storage. TAA was calculated according to the Eq. 4.

$$\text{TAA}(\%) = \left(\frac{\text{Abs}_{\text{Sample}} - \text{Abs}_{\text{Control}}}{\text{Abs}_{\text{Sample}}} \right) \times 100, \quad (4)$$

where TAA is the total antioxidant activity, Abs_{Sample} is the absorbance of sample, Abs_{Control} is the absorbance of control sample.

Total phenolic content (TPC)

Total phenolic content in the samples was determined by the Folin–Ciocalteu method [40] with modifications. For this purpose, a 0.1 ml ethanol extract was diluted with 6.0 ml of distilled water and then 0.5 ml of Folin–Ciocalteu reagent was added. After 3 min, 1.5 ml of sodium carbonate (7.5% w/v) was added and topped up with distilled water to 10 ml. The reaction mixture was stored for 30 min in a WNB7 water bath (Mettler, Germany) at 40 °C. Absorbance was measured at $\lambda = 765$ nm. The sum of phenolic compounds was expressed as an equivalent gallic acid (GAE) based on the previously prepared calibration curve.

Preparation of the standard curve

On an analytical balance, 25 ± 0.1 mg of gallic acid was weighed. The gallic acid was quantitatively transferred to a 5 cm³ volumetric flask and made up to the graduation mark with doubly distilled water (concentration 5 mg ml⁻¹). Five 5 cm³ volumetric flasks were prepared to which the standard solution was added by automatic pipette, taking 0.5, 0.4, 0.3, 0.2, and 0.1 cm³ of the solution, respectively. The contents of each flask were made up to 5 cm³ with distilled water. To each of the five prepared 10 cm³ volumetric flasks, 6 cm³ of distilled water, 0.1 cm³ of the appropriate gallic

acid solution, and 0.5 cm³ of the Folin–Ciocalteu reagent were added. The contents of the flasks were mixed. After 3 min, 1.5 cm³ of sodium carbonate solution was added to each flask. The flasks were made up to the graduation mark with distilled water. The flasks were mixed and left tightly closed for 30 min. in a dark place. At the same time, a blank sample, without the addition of gallic acid, was prepared in the same way as above. After this time, the absorbance was measured at a wavelength of 765 nm. The instrument was zeroed with a blank sample. Thus, the absorbance results of samples containing 0.1; 0.2; 0.3; 0.4; and 0.5 mg of gallic acid in 1 cm³ of the solution. The standard curve of the dependence of the absorbance value on the content of polyphenolic compounds in the standard samples was plotted using MS Excel ($R^2 = 0.9965$).

The results are presented as an average from three replicates repeats in mg GA equivalent per kg 100 g of sample (mg of GAE/100 g). TPC measurements were performed in triplicate for each sample at weeks 0, 3, 6 and 9 of storage.

β -glucan content

The content of oat β -glucan in the shortbread cookies was measured using an enzymatic procedure evaluated by AOAC International (Method 995.16) using the β -glucan Assay Kit (Mixed Linkage Megazyme International, Ireland). Absorbance was measured at 510 nm using a Spark™ 10 M spectrophotometric plate reader (Tecan Spark™ 10 M, Switzerland). β -glucan concentration was measured on a dry weight basis. The oat β -glucan content was evaluated in four replicates for each sample at weeks 0, 3, 6 and 9 of storage.

Volatile compounds evaluation

The profile of volatile compounds was analysed in raw and baked samples using Electronic Nose Heracles II (Alpha M.O.S., France). Analysis was based on ultrafast gas chromatography with headspace (HS-GC) according to the method described by Wojtasik-Kalinowska et al. [41]. Included with the device were an automatic sampling system and a system of detection containing non-polar MXT-5 column and slightly polar MXT-1701 (length 10 m, diameter 180 mm) connected to a FID. The device was operated using AlphaSoft software (Alpha Software Corporation, USA). The 3 g samples were placed in 20 ml headspace vials. The samples were capped with a Teflon-faced silicon rubber cap and placed in the automatic sampler of the headspace system. Each sample was incubated at 55 °C for 15 min under 8.33 Hz agitation. Hydrogen (the carried gas) was circulated at 1 ml min⁻¹. The accumulated gas was injected by autosampler from the headspace into GC at an injection speed of 125 ml s⁻¹. The injected volume was 3500 μ l and the injector temperature was 200 °C. The analytes were accumulated in

a trap at 15 °C (Tenax). The temperature program included the following steps: 60 °C for 2 s; 3 °C s⁻¹ ramp to 270 °C and hold for 20 s. The FID was at 280 °C. The method was calibrated by an alkane solution, *n*-butane to *n*-hexadecane (Restek). To convert retention time into Kovats indices [42]. The volatile compounds were evaluated in six repetitions in three independent biological replicates.

Consumer evaluation

Consumer evaluation of shortbread cookies was performed by 120 untrained panelists recruited among students and faculty members from the Warsaw University of Life Sciences campus. Panelists ranged in age from 19 to 55 years (60 male and 60 female). Consumer evaluation sessions were conducted in isolated rooms lit with white light and at 23 °C. One link sample and four samples of cookies were delivered randomly in plastic containers identified by three-digit codes. Latin square design (5 × 5) was used to allocate samples to consumers. In effect, completely balanced effects of carryover and position per consumer was achieved. All information regarding degree of likeness for the samples were recorded in a questionnaire. The panellists were asked to express their opinion on a 100 mm unstructured hedonic scale in marked borders and score the attributes. Colour, external appearance, taste, texture, aroma, crunchiness and overall acceptability of samples were evaluated. Between servings, consumers were given 30 s breaks to rinse their mouths with water. The results from the unstructured hedonic scale were converted to numerical values (from 0 to 10 units) where 0 point meant means the lowest level of acceptance and 10 points—the highest one. Variance due to

session number and panellist was considered for the sensory data.

Statistics

Data are presented as the mean value ± standard error. The experiment was designed as a full randomised block with three independent biological replicates. In each replicate, a different batch of ingredients was used). Statistical analysis based on the variance analysis for a two-factor experiment (1st factor: recipe, 2nd factor: storage period). was carried out using Statistica 13.3 (StatSoft Inc., Tulsa, USA). The influence of the treatment group, the time of storage and the interaction between these two factors were analysed by the general linear model procedure (GLM) with Tukey's test at $\alpha=0.05$ significance level to analyse the influence of the recipe on consumer evaluation [43]. The principal component analysis (PCA) of consumer evaluation was carried out using the XLStat 2020 software (Addinsoft).

Results and discussion

β -glucan content

The β -glucan concentration in shortbread cookies at the beginning of the experiment and after 3, 6 and 9 week of storage is presented in Fig. 1. All controls and samples with GSE (G samples) during all weeks of storage contained about 0.01% w/w β -glucan, which can be attributed to small amounts of β -glucan present in wheat grains and wheat products, including wheat flour [44, 45]. In contrast, the two

Fig. 1 Influence of different treatment and storage time on β -glucan content in shortbread cookies measured on the 0th, 3rd, 6th and 9th week of storage (respectively: W0, W3, W6, W9) (mean ± SE). Treatments: Control, G—the addition of red grape skin extract, B—the addition of 30% oat β -glucan concentrate, G + B—the addition of red

grape skin extract and 30% oat β -glucan concentrate. (A, B, C, D)—mean values with different letters showing a significant effect of treatment group in the same day of storage; (a, b, c, d)—mean values with different letters showing a significant effect of storage time in each treatment group; * $P \leq 0.05$

groups of the samples enriched with oat fibre preparation (samples B and G + B) contained above 1% w/w β -glucan at the beginning of the experiment (W0). Consequently, one 15 g shortbread cookie (groups B and G + B) contains about 0.15 g of pure cereal β -glucan. Consequently, to meet the requirement of the EFSA nutrition claim the consumption of innovative cookies can help achieve EFSA's claim 'Beta-glucans contribute to the maintenance of normal blood cholesterol levels', it would be necessary to consume at least seven fortified cookies, delivering approximately 1.05 g of pure β -glucan [5, 6]. A significant decrease in β -glucan content in shortbread cookies during storage was observed only in sample group B ($P < 0.05$), which was attributed to oxidative changes [46, 47]. These decrease also come as a result of enzymatic activity (β -glucanase) in wheat flour and oat β -glucan concentrate [48, 49]. The decrease in β -glucan content during storage was also previously reported by Džafić et al. [50] in biscuits with barley flour addition. The β -glucan content in the control sample, group G and group G + B did not change significantly during storage ($P > 0.05$). Nakov et al. [51] reported similar findings concerning β -glucan content in biscuits with enriched barley flour. Kinner et al. [52] also previously developed a recipe for barley bread with rich in β -glucan to meet the EFSA health claims requirements.

Measurement of colour

The colour parameters of shortbread cookies at the beginning of the experiment and after 3, 6 and 9 weeks of storage are presented in Table 2. The β -glucan concentrate parameter values were: $L^* = 80.98 \pm 0.00$ [%], $a^* = 2.26 \pm 0.00$ [–], $b^* = 15.25 \pm 0.00$ [–] and red grape skin extract $L^* = 46.82 \pm 0.00$ [%], $a^* = 15.71 \pm 0.01$ [–], $b^* = 16.76 \pm 0.01$ [–]. The modification of the recipe composition had a statistically significant effect on the value of the L^* colour parameter ($P < 0.05$). The low level of the L^* colour parameter (46.82%) of the red grape skin extract caused significant colour changes in the final product. The addition of red grape skin extract (G group) or β -glucan and red grape skin extract (G + B group) resulted in a statistically significant decrease in the L^* colour parameter value at all time points of storage ($P < 0.05$). The inverse was observed in samples enriched only with β -glucan (B group). All β -glucan-enriched cookies (group B samples) had a statistically significantly higher value of the L^* colour parameter than the control sample ($P < 0.05$). It is associated with the high L^* colour parameter value of β -glucan concentrate (80.98%). In addition, no changes in the value of the L^* colour parameter caused by storage were noted ($P > 0.05$) in these samples. However, the value of the L^* colour parameter of the remaining samples (control sample and G and G + B groups) increased with statistical significance during storage. The value of the a^* colour parameter increased

statistically significantly only with the addition of grape skin extract (group G samples) and red skin extract and β -glucan (groups G + B) ($P < 0.05$). The samples with β -glucan alone (group B) did not differ significantly in the a^* colour parameter value from the control samples ($P > 0.05$). During storage, the value of the a^* colour parameter changed statistically significantly in all samples subjected to modification of the recipe composition (groups B, G and G + B). During storage, the a^* colour parameter value of samples in groups B and G increased, while in G + B samples it decreased ($P < 0.05$). The a^* colour parameter value of control samples did not change during storage ($P > 0.05$). In all samples with the recipe modification (groups B, G and G + B), a statistically significant decrease in the b^* colour parameter was noted ($P < 0.05$) compared with the control sample.

The colour change of innovative products is primarily related to the high content of polyphenols, including flavonoids. Flavonols, tannins, anthocyanins are major flavonoids determining the colour of red grapes and, consequently, the visual features of products enriched with extracts from these fruits [53, 54]. Kuchtová et al. [55] noticed a similar change in the colour of confectionery products with the addition of an extract of grape peel and grape seeds. On the other hand, the changes caused by the addition of β -glucan were not noted in the work of Lee et al. [56] who analysed changes in the physicochemical characteristics of β -glucan-enriched sponge cake.

Measurement of texture

Results of the three-point bending test are presented in the Table 2. Measurements showed that cookies containing the grape skin extract were the hardest (G group) on all time points of the experiment (W0, W3, W6, W9). In contrast, cookies made using β -glucan concentrate were characterised by significantly lower hardness than control samples and samples with added β -glucan and grape skin extract (G + B group) ($P < 0.05$). Storage time had a significant effect on cookie texture ($P < 0.05$). All samples except G + B group became harder during the storage time ($P < 0.05$). The texture of samples enriched with red grape skin extract and β -glucan (G + B group) did not change statistically significantly during storage ($P > 0.05$). These results may be related to the high oxidative stability of the final product. Previous studies have linked lipid oxidation to food texture. Oxidized lipids can react with proteins through cross-linking reactions that modify the texture and functionality of the product [57]. In addition, Toyosaki [58] investigated the effect of hydroperoxides on dough fermentation and found that oxidized linoleic acid can interact with gluten proteins, inducing gluten denaturation. The consequence of this reaction is the tightening of the network [58]. The increase in hardness of samples with

Table 2 Evaluation of CIE parameters (L^* , a^* and b^*), hardness, total antioxidant activity (TTA) and total phenolic content (TPC) of shortbread cookies during storage in the modified atmosphere at refrigerated conditions on the 0th, 3rd, 6th and 9th weeks of storage (respectively: W0, W3, W6, W9) (mean $\pm$ SE)

Treatments	Storage time [weeks]	L^* [%]	a^* [–]	b^* [–]	Hardness [N]	TTA [%]	TPC [mg GAE/100 g] TPC [mg GA eq/kg]
Control	0	74.34 $\pm$ 0.20 ^{Ca} 74.34 ^{Ca}	3.82 $\pm$ 0.14 ^{A3} .82 ^A	35.87 $\pm$ 0.13 ^{Bd} 35.87 ^{Bd}	50.58 $\pm$ 0.06 ^{Ba} 50.58 ^{Ba}	13.41 $\pm$ 0.22 ^{Ad} 13.41 ^{Ad}	50.79 $\pm$ 1.84 ^{Ac} 0.51 ^{Ac}
	3	74.56 $\pm$ 0.21 ^{Ca} 74.56 ^{Ca}	3.82 $\pm$ 0.20 ^A 3.82 ^A	34.90 $\pm$ 0.23 ^{Bd} 34.90 ^{Bd}	50.91 $\pm$ 0.05 ^{Bb} 50.91 ^{Bb}	8.09 $\pm$ 0.23 ^{Ac} 8.09 ^{Ac}	42.34 $\pm$ 2.73 ^{Ab} 0.42 ^{Ab}
	6	76.25 $\pm$ 0.18 ^{Cb} 76.25 ^{Cb}	3.26 $\pm$ 0.36 ^A 3.26 ^A	35.45 $\pm$ 0.30 ^{Bd} 35.45 ^{Bd}	50.91 $\pm$ 0.05 ^{Bb} 50.91 ^{Bb}	6.05 $\pm$ 0.40 ^{Ab} 6.05 ^{Ab}	41.31 $\pm$ 0.31 ^{Ab} 0.41 ^{Ab}
	9	76.93 $\pm$ 0.07 ^{Cc} 76.93 ^{Cc}	3.22 $\pm$ 0.35 ^A 3.22 ^A	32.70 $\pm$ 0.48 ^{Ad} 32.70 ^{Ad}	50.95 $\pm$ 0.09 ^{Bb} 50.95 ^{Bb}	4.85 $\pm$ 0.23 ^{Aa} 4.85 ^{Aa}	23.69 $\pm$ 0.64 ^{Aa} 0.24 ^{Aa}
G	0	43.81 $\pm$ 0.14 ^{Ba} 43.81 ^{Ba}	16.21 $\pm$ 0.03 ^{Ba} 16.21 ^{Ba}	20.75 $\pm$ 0.07 ^{Ab} 20.75 ^{Ab}	56.78 $\pm$ 0.08 ^{Da} 56.78 ^{Da}	85.39 $\pm$ 0.56 ^{Cd} 85.39 ^{Cd}	153.65 $\pm$ 3.10 ^{Cc} 1.54 ^{Cc}
	3	43.85 $\pm$ 0.13 ^{Ba} 43.85 ^{Ba}	16.14 $\pm$ 0.05 ^{Ba} 16.14 ^{Ba}	21.19 $\pm$ 0.08 ^{Bb} 21.19 ^{Bb}	57.07 $\pm$ 0.10 ^{Dab} 57.07 ^{Dab}	74.52 $\pm$ 1.47 ^{Cc} 74.52 ^{Cc}	145.02 $\pm$ 2.71 ^{Bc} 1.45 ^{Bc}
	6	43.27 $\pm$ 0.11 ^{Ba} 43.27 ^{Ba}	16.96 $\pm$ 0.04 ^{Bb} 16.96 ^{Bb}	21.98 $\pm$ 0.07 ^{Bb} 21.98 ^{Bb}	56.92 $\pm$ 0.07 ^{Dab} 56.92 ^{Dab}	54.01 $\pm$ 0.53 ^{Cb} 54.01 ^{Cb}	132.28 $\pm$ 3.04 ^{Bb} 1.32 ^{Bb}
	9	44.99 $\pm$ 0.13 ^{Bb} 44.99 ^{Bb}	16.97 $\pm$ 0.04 ^{Bb} 16.97 ^{Bb}	22.08 $\pm$ 0.07 ^{Cb} 22.08 ^{Cb}	57.25 $\pm$ 0.14 ^{Db} 57.25 ^{Db}	42.59 $\pm$ 0.49 ^{Ca} 42.59 ^{Ca}	110.92 $\pm$ 2.39 ^{Ca} 1.11 ^{Ca}
B	0	79.52 $\pm$ 0.37 ^D 79.52 ^D	3.41 $\pm$ 0.01 ^{Aa} 3.41 ^{Aa}	32.14 $\pm$ 0.12 ^{Bc} 32.14 ^{Bc}	49.06 $\pm$ 0.06 ^{Aa} 49.06 ^{Aa}	17.14 $\pm$ 0.32 ^B 17.14 ^B	58.59 $\pm$ 1.22 ^{Bd} 0.59 ^{Bd}
	3	79.77 $\pm$ 0.40 ^D 79.77 ^D	3.42 $\pm$ 0.01 ^{Aa} 3.42 ^{Aa}	31.88 $\pm$ 0.14 ^{Bc} 31.88 ^{Bc}	49.67 $\pm$ 0.04 ^{Ab} 49.67 ^{Ab}	13.82 $\pm$ 0.58 ^B 13.82 ^B	47.04 $\pm$ 1.05 ^{Ac} 0.47 ^{Ac}
	6	79.40 $\pm$ 0.51 ^D 79.40 ^D	3.41 $\pm$ 0.01 ^{Aa} 3.41 ^{Aa}	32.11 $\pm$ 0.12 ^{Bc} 32.11 ^{Bc}	49.60 $\pm$ 0.03 ^{Ab} 49.60 ^{Ab}	11.74 $\pm$ 0.26 ^B 11.74 ^B	40.78 $\pm$ 1.19 ^{Ab} 0.41 ^{Ab}
	9	78.67 $\pm$ 0.45 ^D 78.67 ^D	3.53 $\pm$ 0.01 ^{Ab} 3.53 ^{Ab}	31.05 $\pm$ 0.08 ^{Ac} 31.05 ^{Ac}	49.73 $\pm$ 0.04 ^{Ab} 49.73 ^{Ab}	10.00 $\pm$ 0.29 ^B 10.00 ^B	34.86 $\pm$ 1.37 ^{Ba} 0.35 ^{Ba}
G + B	0	41.84 $\pm$ 0.13 ^{Aa} 41.84 ^{Aa}	17.88 $\pm$ 0.06 ^{Cb} 17.88 ^{Cb}	18.90 $\pm$ 0.06 ^{Ba} 18.90 ^{Ba}	52.30 $\pm$ 0.07 ^C 52.30 ^C	87.63 $\pm$ 0.29 ^{Dd} 87.63 ^{Dd}	160.92 $\pm$ 1.16 ^{Cb} 1.61 ^{Cb}
	3	41.84 $\pm$ 0.19 ^{Aa} 41.84 ^{Aa}	17.04 $\pm$ 0.04 ^{Ca} 17.04 ^{Ca}	18.74 $\pm$ 0.08 ^{Ba} 18.74 ^{Ba}	52.35 $\pm$ 0.03 ^C 52.35 ^C	84.15 $\pm$ 0.36 ^{Dc} 84.15 ^{Dc}	152.63 $\pm$ 2.81 ^{Bb} 1.53 ^{Bb}
	6	41.85 $\pm$ 0.12 ^{Aa} 41.85 ^{Aa}	17.24 $\pm$ 0.05 ^{Ba} 17.24 ^{Ba}	18.93 $\pm$ 0.06 ^{Ba} 18.93 ^{Ba}	52.44 $\pm$ 0.03 ^C 52.44 ^C	64.64 $\pm$ 0.54 ^{Db} 64.64 ^{Db}	127.42 $\pm$ 2.88 ^{Ba} 1.27 ^{Ba}
	9	43.28 $\pm$ 0.16 ^{Ab} 43.28 ^{Ab}	17.17 $\pm$ 0.07 ^{Ba} 17.17 ^{Ba}	18.28 $\pm$ 0.04 ^{Aa} 18.28 ^{Aa}	52.40 $\pm$ 0.03 ^C 52.40 ^C	56.41 $\pm$ 0.55 ^{Da} 56.41 ^{Da}	128.34 $\pm$ 1.49 ^{Da} 1.28 ^{Da}
SEM effects		0.644	0.254	0.256	0.168	2.612	4.2100.042
Treatment		*	*	*	*	*	*
Storage time		*	*	*	*	*	*
Treatment $\times$ storage time		*	*	*	*	*	*

Treatments: Control, G—the addition of red grape skin extract, B—the addition of 30% oat β -glucan concentrate, G + B—the addition of red grape skin extract and 30% oat β -glucan concentrate

(A, B, C, D)—mean values with different letters in column show a significant effect of the treatment group in the same day of storage; (a, b, c, d)—means with different letters in column show a significant effect of storage time in each treatment group; * $P \leq 0.05$

added red grape skin extract is attributed to the presence of polyphenols and tannic acid. Plant polyphenols have influence on gluten protein functionality, tannic acid in grape skin extract has a negative influence on disulphide bond formation, but interacts with gluten proteins via other covalent bonds which consequently affects the texture and rheological properties of bakery products [59, 60]. The samples enriched with β -glucan (group B) had statistically

significantly lower hardness than the control cookies at all time points of storage ($P < 0.05$). Similar results were obtained by Duta and Culetu [61], who showed a decrease in hardness of the bakery products with the addition of oat bran rich in β -glucan, while Hager et al. [62], showed that the addition of β -glucan to gluten-free bread reduces the hardness of the crust. However, the same author observed

a reverse tendency, an increase in crust hardness, in wheat breads enriched with β -glucan [62].

Differential scanning calorimetry (DSC) measurement

The onset temperature parameter (T_{ON}), which is typically used to describe the susceptibility of oils and fats to oxidation, is presented in Table 3. The stability of the samples is related to the T_{ON} value, which indicates the initiation of lipid oxidation and, consequently, a change in fat quality [63]. Samples with a higher T_{ON} value are more oxidatively stable than a sample with lower T_{ON} values at the same heating rate [64, 65]. Modification of the shortbread cookie recipe and the storage time of the cookies had a statistically significant effect on the T_{ON} parameter value ($P < 0.05$). Even the control sample, tested immediately after baking (W0), was characterised by relatively high oxidative stability. However, higher T_{ON} values were observed for lipid fractions extracted from cookies with grape skin extract (G samples), β -glucan (B samples) or both additives (G + B samples) than in control samples throughout the storage period ($P < 0.05$). The highest T_{ON} value (193.74 °C) for lipid fractions extracted from the shortbread cookies immediately after baking were observed for samples containing grape skin extract (group G). Similar observations were reported by Kozłowska et al. [66], who analysed oxidation stability of lipid fraction of sponge-fat cakes enriched with green tea extracts.

During storage, a large decrease in T_{ON} was observed for the lipid fraction of the control sample, as well as B and G samples ($P < 0.05$). Conversely, during storage of the G + B sample, an increase in the T_{ON} value in W3, W6 and W9 was observed compared to the measurements immediately after baking (W0) ($P < 0.05$).

The presence of natural ingredients natural additives (red grape skin extract and co-passangers extracted together with β -glucan – primarily phytate β -glucan) appeared to improve the oxidative stability of the lipid fraction [67]. The antioxidant effect of red grape skin extract is related to the ability of phenolic compounds to scavenge free radicals [68]. The oxidative stability of G and G + B samples is related to the presence of anthocyanins, flavanols, and resveratrol contained in the GSE. These compounds with antioxidant properties had a significant influence on growth T_{ON} value, thus increase of oxidation stability [69].

The thermo-oxidation kinetic parameters were calculated according to Arrhenius equation (effective activation energy— E_a ; logarithm of pre-exponential factor— $\log Z$; constant rate— k) of samples, measured after 0, 3, 6 and 9 weeks of storage, are presented Table 3. For analysed extracted fat samples, a linear correlation ($0.988 < r^2 < 0.998$)

was found of type $\ln\left(\frac{\beta}{T_{ON}}\right) = a\left(\frac{1}{T_{ON}}\right) + b$, where a and b are adjustable coefficients. Obtained E_a values for the oxidation of shortbread cookies lipid fraction, revealed that the antioxidant ability is associated with addition of grape skin extract, β -glucan concentrates and both additives. The calculated E_a values ranged from 59.65 to 89.93 kJ mol⁻¹ and $\log Z$ value from 6.38 to 9.67 s⁻¹. At the beginning of the experiment (Week 0), E_a values of lipids oxidation were significantly higher in groups with additives (G, B and G + B samples) than in control samples ($P < 0.05$). The highest value of E_a at the beginning of the experiment was observed for the lipid fraction of G + B samples (89.93 kJ mol⁻¹). The kinetics parameters values determined for the analysed fat fractions correlated well with those reported in the literature [70]. Also, Wirkowska-Wojdyla et al. [71] observed E_a values ranging from 91.97 to 199.71 kJ mol⁻¹ for fats extracted from cookies and structured lipids of milk fat, as well as concentrates of fish and rapeseed oils. Additionally, Cord-eiro et al. [72] and Kowalski et al. [73] observed a significant impact of the presence of various antioxidants on lipids stability and kinetics parameters values analysed using DSC. In the present study, the τ values calculated at 160 °C ranged from 0.028 to 0.153 min. The smallest τ value (0.028 min) was obtained for sample G + B after 3 weeks of storage (W3). The τ values of all samples with additives (G, B and G + B samples) were significantly lower ($P < 0.05$) compared to those of the control sample at all time points of storage (W0, W3, W6 and W9).

Total antioxidant activity (TTA)

Total antioxidant activity (TAA) of shortbread cookies before and after storage is presented in Table 2. Significant differences were found between the control group and the treatment samples (G, B and G + B) on the beginning of the experiment (W0) and after storage time ($P < 0.05$). The addition of grape skin extract (G samples), β -glucan concentrate (B samples) and both of these ingredients (G + B samples) caused a significant increase in total antioxidant activity in cookies from 13.41% in control samples to, respectively, 85.39% in G samples, 17.14% in B samples and 87.62% in G + B samples on the first day of the experiment (W0) ($P < 0.05$), which also remained the case after 3, 6 and 9 weeks of storage. However, antioxidant activity decreased gradually during the storage time in all samples except for group B ($P < 0.05$). Samples enriched with red grape extract have high antioxidant activity, thanks to phenolic compounds, including anthocyanins, derivatives of hydroxycinnamic acid, flavonols and stilbenoids, which are mainly stored in seeds and peel. The most common polyphenols found in grape seeds and skins are gallic acid, catechins and epicatechins, while in the skinrind: ellagic acid,

Table 3 Extrapolated DSC thermooxidation onset temperatures [$t_{ON}/^{\circ}\text{C}$] measured at different heating rates (β) and kinetic parameters characterizing the thermooxidation of samples studied measured on the 0th, 3rd, 6th and 9th week of storage (respectively: W0, W3, W6, W9). (mean $\pm$ SE)

Heating rate. β [$^{\circ}\text{C min}^{-1}$]	Control	G	B	G + B
After baking (W0)				
4	170.94 $\pm$ 0.00 ^{Ad}	193.74 $\pm$ 0.01 ^{Db}	182.66 $\pm$ 0.00 ^{Ca}	180.53 $\pm$ 0.01 ^{Ba}
7.5	183.36 $\pm$ 0.01 ^{Ad}	205.29 $\pm$ 0.02 ^{Db}	196.31 $\pm$ 0.01 ^{Ca}	192.29 $\pm$ 0.01 ^{Ba}
10	189.64 $\pm$ 0.01 ^{Ac}	212.07 $\pm$ 0.02 ^{Cc}	199.75 $\pm$ 0.01 ^{Ba}	198.22 $\pm$ 0.01 ^{Ba}
12.5	194.94 $\pm$ 0.02 ^{Ac}	216.61 $\pm$ 0.02 ^{Db}	206.25 $\pm$ 0.02 ^{Ca}	201.92 $\pm$ 0.02 ^{Ba}
15	199.70 $\pm$ 0.02 ^{Ac}	221.45 $\pm$ 0.02 ^{Db}	211.49 $\pm$ 0.02 ^{Ca}	204.38 $\pm$ 0.02 ^{Ba}
After 3 weeks of storage (W3)				
4	166.75 $\pm$ 0.00 ^{Ac}	183.77 $\pm$ 0.01 ^{Ba}	181.23 $\pm$ 0.01 ^{Ba}	197.67 $\pm$ 0.01 ^{Cb}
7.5	179.92 $\pm$ 0.00 ^{Ac}	196.52 $\pm$ 0.01 ^{Ba}	196.13 $\pm$ 0.01 ^{Ba}	209.59 $\pm$ 0.01 ^{Cc}
10	187.78 $\pm$ 0.01 ^{Ab}	203.00 $\pm$ 0.01 ^{Cb}	200.78 $\pm$ 0.01 ^{Ba}	215.53 $\pm$ 0.01 ^{Dc}
12.5	191.77 $\pm$ 0.01 ^{Ab}	206.98 $\pm$ 0.01 ^{Ca}	204.81 $\pm$ 0.02 ^{Ba}	219.38 $\pm$ 0.02 ^{Db}
15	199.04 $\pm$ 0.01 ^{Ac}	210.10 $\pm$ 0.02 ^{Ba}	210.29 $\pm$ 0.02 ^{Ba}	225.90 $\pm$ 0.02 ^{Cc}
After 6 weeks of storage (W6)				
4	163.18 $\pm$ 0.00 ^{Ab}	182.66 $\pm$ 0.00 ^{Ba}	195.21 $\pm$ 0.00 ^{Cb}	197.67 $\pm$ 0.00 ^{Cb}
7.5	177.11 $\pm$ 0.00 ^{Ab}	197.31 $\pm$ 0.01 ^{Ba}	209.92 $\pm$ 0.01 ^{Cb}	208.59 $\pm$ 0.01 ^{Cc}
10	184.99 $\pm$ 0.01 ^{Aa}	201.29 $\pm$ 0.01 ^{Ba}	217.27 $\pm$ 0.02 ^{Cb}	216.53 $\pm$ 0.02 ^{Cc}
12.5	189.13 $\pm$ 0.01 ^{Aa}	207.05 $\pm$ 0.02 ^{Ba}	222.11 $\pm$ 0.02 ^{Db}	220.38 $\pm$ 0.02 ^{Cc}
15	195.94 $\pm$ 0.02 ^{Ab}	210.79 $\pm$ 0.02 ^{Ba}	225.22 $\pm$ 0.02 ^{Cb}	225.90 $\pm$ 0.02 ^{Cd}
After 9 weeks of storage (W9)				
4	160.54 $\pm$ 0.00 ^{Aa}	182.66 $\pm$ 0.00 ^{Ca}	180.12 $\pm$ 0.00 ^{Ba}	193.22 $\pm$ 0.00 ^{Db}
7.5	173.66 $\pm$ 0.00 ^{Aa}	196.31 $\pm$ 0.01 ^{Ba}	195.37 $\pm$ 0.01 ^{Ba}	205.65 $\pm$ 0.01 ^{Cb}
10	183.67 $\pm$ 0.01 ^{Aa}	200.75 $\pm$ 0.01 ^{Ba}	199.59 $\pm$ 0.01 ^{Ba}	213.41 $\pm$ 0.02 ^{Cb}
12.5	188.92 $\pm$ 0.01 ^{Aa}	206.25 $\pm$ 0.01 ^{Ca}	205.64 $\pm$ 0.02 ^{Ba}	217.74 $\pm$ 0.02 ^{Db}
15	192.33 $\pm$ 0.01 ^{Aa}	211.49 $\pm$ 0.02 ^{Ba}	211.75 $\pm$ 0.02 ^{Ba}	221.90 $\pm$ 0.02 ^{Cb}
<i>Parameters</i>				
After baking (W0)				
r^2	0.998	0.997	0.988	0.996
E_a [kJ mol ⁻¹]	73.01 $\pm$ 0.01 ^{Ad}	83.85 $\pm$ 0.01 ^{Cb}	78.08 $\pm$ 0.01 ^{Bc}	89.93 $\pm$ 0.01 ^{Dc}
log Z [s ⁻¹]	7.85 $\pm$ 0.01 ^{Ac}	8.66 $\pm$ 0.01 ^{Bb}	8.21 $\pm$ 0.01 ^{Bc}	9.67 $\pm$ 0.02 ^{Cd}
τ at 160 $^{\circ}\text{C}$ (min)	0.110 $\pm$ 0.001 ^{Ca}	0.035 $\pm$ 0.001 ^{Aa}	0.060 $\pm$ 0.001 ^{Bb}	0.067 $\pm$ 0.001 ^{Bc}
After 3 weeks of storage (W3)				
r^2	0.992	0.997	0.991	0.991
E_a [kJ mol ⁻¹]	64.40 $\pm$ 0.01 ^{Ac}	82.99 $\pm$ 0.01 ^{Cb}	77.23 $\pm$ 0.01 ^{Bbc}	85.84 $\pm$ 0.01 ^{Db}
log Z [s ⁻¹]	6.86 $\pm$ 0.00 ^{Ab}	8.76 $\pm$ 0.01 ^{Cb}	8.12 $\pm$ 0.01 ^{Bc}	8.80 $\pm$ 0.01 ^{Cc}
τ at 160 $^{\circ}\text{C}$ [min]	0.125 $\pm$ 0.001 ^{Db}	0.057 $\pm$ 0.001 ^{Bb}	0.064 $\pm$ 0.001 ^{Cbc}	0.028 $\pm$ 0.001 ^{Aa}
After 6 weeks of storage (W6)				
r^2	0.995	0.993	0.996	0.991
E_a [kJ mol ⁻¹]	62.21 $\pm$ 0.01 ^{Ab}	78.79 $\pm$ 0.01 ^{Ca}	75.67 $\pm$ 0.01 ^{Bb}	83.31 $\pm$ 0.01 ^{Db}
log Z [s ⁻¹]	6.65 $\pm$ 0.00 ^{Aab}	8.28 $\pm$ 0.01 ^{Ca}	7.65 $\pm$ 0.00 ^{Bb}	8.52 $\pm$ 0.01 ^{Cb}
τ at 160 $^{\circ}\text{C}$ [min]	0.141 $\pm$ 0.001 ^{Cc}	0.060 $\pm$ 0.001 ^{Bb}	0.034 $\pm$ 0.001 ^{Aa}	0.030 $\pm$ 0.001 ^{Aa}
After 9 weeks of storage (W9)				
r^2	0.991	0.995	0.998	0.998
E_a [kJ mol ⁻¹]	59.65 $\pm$ 0.01 ^{Aa}	78.15 $\pm$ 0.01 ^{Ca}	70.55 $\pm$ 0.01 ^{Ba}	79.51 $\pm$ 0.01 ^{Ca}
log Z [s ⁻¹]	6.38 $\pm$ 0.00 ^{Aa}	8.21 $\pm$ 0.01 ^{Ca}	7.35 $\pm$ 0.00 ^{Ba}	8.15 $\pm$ 0.01 ^{Ca}
τ at 160 $^{\circ}\text{C}$ [min]	0.153 $\pm$ 0.001 ^{Cd}	0.062 $\pm$ 0.001 ^{Bb}	0.069 $\pm$ 0.001 ^{Bc}	0.037 $\pm$ 0.001 ^{Ab}

(A, B, C, D)—mean values with different letters showing significant effect of the treatment group in the same day of storage; (a, b, c, d)—mean values with different letters showing significant effect of storage time in each treatment group; * $P \leq 0.05$

myricetin, quercetin, kaempferol and transresveratrol [74]. Sample groups B and G + B also exhibited high antioxidant properties as a result of the addition of β -glucan concentrate. Kofuji et al. [75] demonstrated that β -(1,3–1,4)-D-glucan, extracted from cereals, has significant antioxidant activity. An inverse tendency (increase in antioxidant activity during storage) was noted by Sakač et al. [76] in cookies supplemented with sugarbeet dietary fibre. The antioxidant activity of β -glucan is influenced by various physiological properties (such as structure and particle size), which differ depending on the source and the extraction method used [75, 77, 78].

Total phenolic content (TPC)

Total phenolic content in control formulation cookies, as well as those with added red grape skin extract, 30% oat β -glucan concentrate or both additives, at weeks 0 (beginning of the experiment) and after weeks 3, 6 and 9 of storage is presented in Table 2. Additives to the recipe and storage time both had significant impacts on the content of phenolic compounds in the samples. Total phenolic content in cookies ranged from 23.69 ± 0.64 to 160.92 ± 1.161 mg of GAE/100 gmg GAE/kg. The highest content of phenolic compounds was detected in the sample with both added grape skin extract and β -glucan (G + B group), at the beginning of the experiment (W0). The loss of TPC during storage is related to the enzymatic and chemical oxidation of phenols over time [79]. There was a significant decrease in the content of phenolic compounds during storage ($P < 0.05$) and a strong correlation

between total phenolic content and total antioxidant activity ($R = 0.985$) was observed. This correlated with the findings of Dordoni et al. [80], who observed an increase in polyphenol content after enrichment of walnut paste in grape skin extract.

Volatile compounds evaluation

E-nose PCA data (Fig. 2) demonstrate the differences in the volatile compounds of various formulations of shortbread cookies, before and after heat treatment. The 82.35% data variance seen in the horizontal axis and 12.19% interpreted by the vertical axis indicate the differences between samples caused by the addition of grape skin extract and β -glucan concentrate. A value of 12.19% variance (on the horizontal axis) highlights the differences between formulation which are induced by baking. A value of 82.35% variance (on the vertical axis) clarified the differences caused by modifications in cookie composition. PCA analysis demonstrated that the addition of grape skin extract, β -glucan concentrates and both of these additives changed the profiles of volatile compounds detected in raw and baked cookies relative to the control samples. Additionally, baking increased the differences in these profiles between the samples, especially for samples with grape skin extract (G group) and samples with both grape skin extract and β -glucan concentrates (G + B group). The changes in the samples enriched with red grape skin extract (G and G + B groups) are due to the presence of various volatile compounds contained in the fruit of the grapes [81]. The grape flavour is synthesised in the fruit by

Fig. 2 PCA analysis of the effect of oat β -glucan, grape skin extract, and both of them addition on the volatile compounds in shortbread cookies. Treatments: Control, G—the addition of red grape skin extract, B—the addition of 30% oat β -glucan concentrate, G + B—the addition of red grape skin extract and 30% oat β -glucan concentrate

various enzymes including terpene synthase, *O*-methyltransferase, cytochrome P450, carotenoid breakdown dioxygenase and many others as yet unidentified enzymes [82, 83]. Among the volatile compounds present in grapes are terpenes, which give wines a distinct floral aroma (Muscat and Gewurztraminer grapes), and methoxypyrazines, compounds evoking the typical capsicum aroma in Sauvignon wines [84, 85]. The samples enriched with β -glucan concentrate (B and G + B groups) had an aroma containing volatile compounds derived from oats. The compounds responsible for the flavour of oats include hexanal, octanal, and trans-2,3-epoxystyrene [86]. In addition, the content of hexanal and hexanoic acid, hexanal and nonanal and hexanal increases during storage of oat bran and oat pomace [87–89]. Pasqualone et al. [90] analysed the effect of grape marc extract addition to biscuits and noticed a statistically significant increase in the intensity of volatile compounds, such as ethanol, phenylethyl alcohol/2-phenylethanol, nonanal, (E)-2-decenal and phenylacetaldehyde/benzeneacetaldehyde. In conclusion, the volatile compounds profile of the cookies (both enriched and unenriched) has clearly changed during heat treatment with the non-enzymatic browning and Maillard reactions [91].

Consumer evaluation

Figure 3 shows the influence of different treatment and storage times on sensory attributes of shortbread cookies

measured at the beginning of the experiment (W0) and after 3, 6 and 9 weeks of storage. PCA plot showed that there are two main factors accounting for 77.84% of the variance. Data analysis of consumer evaluation results showed a good separation of samples, with 57.11% of the variation accounted for PC1 and 20.73% for PC2. The primary factor (57.11%) was related to texture, overall acceptability, aroma and taste. The secondary factor (20.73%) was related to crunchiness, colour and external appearance. Consumers recognized detected differences between control samples and enriched cookies, grouping them into distinctive clusters. Variations in the preference and acceptance of consumers are associated with changes in sensory properties caused by red grape skin extract and 30% oat β -glucan concentrate addition. The external appearance, crunchiness and colour of cookies were dominating descriptors. Furthermore, taste, aroma, texture and overall acceptability were distinguishing variables, which strongly distinguished cookies with additives from cookies without additives. Samples enriched with grape skin extract (G samples) and grape skin extract and β -glucan (G + B samples) were characterised by similar acceptance of most sensory discriminants compared to the control samples. Only the β -glucan-enriched samples (B samples) showed significantly ($P < 0.05$) lower sensory acceptance compared to the control samples. The experiment showed that the addition of either grape skin extract or β -glucan to the recipe formulation had much greater

Fig. 3 Influence of different treatment and storage time on sensory attributes of shortbread cookies measured on the 0th, 3rd, 6th and 9th week of storage (respectively: W0, W3, W6, W9). Treatments: Control, G—the addition of red grape skin extract, B—the addition of 30% oat β -glucan concentrate, G + B—the addition of red grape skin extract and 30% oat β -glucan concentrate

impacts on the sensory properties descriptors acceptance than the storage time. Consumer evaluation showed that the most appreciated cookies with additives were G and G + B samples. Kozłowska et al. [70] reported similar findings with respect to sensory evaluations upon adding different amounts of spice extracts to cookies to inhibit oxidation.

Conclusions

The reported findings showed that the addition of red grape skin extract and oat β -glucan in recipe formulations for shortbread cookies significantly improved the quality and nutritional biological value of the product. Samples with these additives had higher total antioxidant activity, total phenolic content and were more thermally stable than control samples. The additives caused significant changes in the colour, texture parameters and volatile compounds profile of the finished product, but it did not affect the sensory acceptance among consumers. The use of these types of additives represents a development in food technology which may be of value in the food industry to increase the nutritional value and extend the shelf life of bakery products. In conclusion, use of red grape skin extract and 30% oat β -glucan concentrate has a positive effect on the pro-health, technological and storage properties of shortbread cookies.

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Availability of data and material The authors declare the availability of data and material.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval There were no experiments in the study.

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