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Effect of the Addition of Apple Pomace and Erythritol on the Antioxidant Capacity and Antidiabetic Properties of Shortbread Cookies

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Apple processing into juice generates vast amounts of a by-product, namely fruit pomace, which poses a serious problem for the processing industry. At the same time, fruit pomace features a high health potential. The aim of the present study was to develop recipes of 8 variants of cookies with wheat flour substituted by apple pomace (0, 10, 30 and 50% of flour weight), sweetened with sucrose and erythritol. The cookies were analyzed for their nutritional value; antioxidant capacity ($ABTS^{\bullet+}$ scavenging activity and oxygen radical absorption capacity – ORAC); the ability to inhibit α-amylase, α-glucosidase and pancreatic lipase; and consumer acceptability. In total, 13 phenolic compounds were identified in the cookies with pomace. Cookies with 50% addition of apple pomace had an approximately 8-fold higher content of dietary fibre than traditional products (without the apple pomace) and simultaneously reduced energy value (by 32.6 and 40.5 kcal/100 g of cookies sweetened with sucrose and erythritol, respectively). The antioxidant capacity of cookies was 0.032–0.316 mmol TE/100 g in the ABTS assay and 1.153–2.070 mmol TE/100 g when ORAC was determined. The IC_{50} enabling α-amylase and α-glucosidase inhibition ranged from 138.1 to 221.8 mg/mL and from 976.4 to 1374.9 mg/mL, respectively. The anti-lipase activity of cookies with the addition of 50% apple pomace and erythritol was the highest (IC_{50} of 7.3 mg/mL). Both antioxidant capacity and antidiabetic potential increased significantly with the increasing proportion of pomace in cookies. Replacing sucrose with erythritol favorably influenced the consumer assessment. The study results show that the proposed products can be a perfect alternative to traditional sweet snack products, especially for consumers with diet-related diseases. The feasibility of using waste raw materials, which are a challenge to the food industry, has been proven as well.

Keywords: apple pomace, erythritol, shortbread cookies, LC-MS, antidiabetic activity, antioxidant activity

INTRODUCTION

Apples are one of the most commonly grown and consumed kinds of fruit in different parts of the world. The fruit and vegetable industry focused on apple processing generates a significant mass of waste in the form of pomace – about 11 million tonnes worldwide/year [USDA, 2018]. This by-product is most often transported, stored, composted and destined for low-value animal feed. Such processes generate high costs and pose a threat to the environment.

Apple pomace contains, among others, large amounts of dietary fibre, which constitutes about 40 g/100 g of their dry matter (d.m.) [Alongi *et al.*, 2019] and phenolic compounds with anti-tumor [Nile *et al.*, 2021; Sudha *et al.*, 2016], anti-inflammatory [Barreira *et al.*, 2019; Zhang & Ying, 2011], antibacterial [Santos

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et al., 2023; Zhang *et al.*, 2016] and antiviral properties [Suárez *et al.*, 2010]. Particular attention should be paid to the antioxidant and antidiabetic properties of apple pomace. Their antioxidant capacity is associated with a high content of phloretin, chlorogenic acid and quercetin [Gorjanović *et al.*, 2020]. Research showed that including pomace in food formulations increased antioxidant properties of food products [Tańska *et al.*, 2016]. With the increase in the proportion of apple pomace, the total phenolic content and total flavonoid content was reported to increase significantly, causing an increase in antioxidant activity measured as the ability to scavenge the radicals [Mir *et al.*, 2017]. In addition, the high content of phenolic compounds in these by-products makes them able to improve the impaired oxidative state associated with type 2 diabetes [Grindel *et al.*, 2014]. The low glycemic index of products with apple pomace addition normalizes blood glucose levels and influences body weight control, contributing to the improvement of carbohydrate metabolism and preventing type 2 diabetes [Alongi *et al.*, 2019]. Studies have shown that phenolic compounds are found primarily in the skin of apples [Francini & Sebastiani, 2013]. Thus, the apple skin is a valuable but underused product. Currently, the food industry focuses on sustainable production and the use of health-promoting by-products of the fruit and vegetable industry [Kammerer *et al*., 2014]. Due to the relatively low cost of obtaining apple pomace and at the same time the multi-faceted health benefits that can be obtained from it, it is recognized as a valuable material for further processing in the food industry. Studies involving humans have shown that the consumption of fibre-rich foods can promote health and thus prevent a number of chronic diseases, especially those associated with inflammation (for example, type 2 diabetes and cardiovascular diseases) [He *et al.*, 2022]. In addition, fermentation of fibre by the intestinal microflora induces the production of short-chain fatty acids (SCFA), which play, among others, an immunoregulatory role [Yang *et al.*, 2020].

Taking into account the impact of dietary fibre and phenolic compounds on human health, current trends in food production and the growing consumer awareness and increased demand for so-called "clean label" foods, a study was conducted to evaluate the bioactive potential and nutritional properties of designed low-energy snacks (shortbread cookies) with a functional additive (apple pomace replacing 10, 30 and 50% of wheat flour by weight). The market success of a new food product, in addition to its high nutritional value, also requires acceptance by potential consumers. Therefore, two additional variants of cookies were developed – sweetened with sucrose and its substitute – erythritol. It was assumed that it is feasible to develop a recipe of shortbread cookies that will be characterized by a high bioactive potential and at the same time will be accepted by consumers. Literature data show the beneficial effect of apple pomace addition to bakery/confectionery products on their nutritional value, phenolic content and antioxidant properties [Alongi *et al.*, 2019; Cantero *et al.*, 2022; Ghadam *et al.*, 2023; Kruczek *et al.*, 2023; Sudha *et al.*, 2016; Usman *et al.*, 2020; Valková *et al.*, 2022]. The studies also assessed the organoleptic characteristics of the products obtained [Alongi *et al.*, 2019; Ghadam *et al.*, 2023; Lauková *et al.*, 2016; Rocha Parra *et al.*, 2019; Usman *et al.*, 2020; Valková *et al.*, 2022]. It should be noted, however, that in the studies conducted so far, the addition of apple pomace was most often at the level of 8–30% [Alongi *et al.*, 2019; Cantero *et al.,* 2022; Ghadam *et al.*, 2023; Lauková *et al.*, 2016; Rocha Parra *et al.*, 2019; Sudha *et al.*, 2016; Usman *et al.*, 2020; Valková *et al.*, 2022]. In the present study, the maximum proportion of pomace was as high as 50%. Additionally, studies conducted so far have most often assessed only the impact of adding apple pomace to specific food products. An innovative element of this research is the simultaneous use of various additions of apple pomace and erythritol. Erythritol has been shown to be an excellent substitute for sugar in sweet snacks due to its low energy value (0.2 kcal/g). [Regnat *et al.*, 2018]. It is well tolerated by consumers and has no significant effect on blood glucose and insulin levels, making it suitable for both healthy persons and diabetic patients. Moreover, it has been shown that the consumption of erythritol induces the secretion of intestinal hormones that modulate the feeling of satiety, which promotes weight loss [Mazi & Stanhope, 2023]. It is also considered a beneficial substitute for sucrose due to its anti-caries and endothelium-protective effects [Boesten *et al.*, 2015]. Therefore, composing a recipe for shortbread cookies based on apple pomace and erythritol seems to be an excellent alternative to sweet snacks, both for healthy consumers and for consumers with carbohydrate metabolism disorders.

MATERIALS AND METHODS

Reagents and chemicals

Chemicals for the determination of nutritional value (potassium dichromate, sulfuric acid (VI), sodium thiosulfate, sodium hydroxide, hydrochloric acid, diethyl ether) and dietary fiber (petroleum ether, phosphate buffer, α-amylase, proteases, hydrochloric acid,

Table 1. Recipe of individual variants of shortbread cookies.

Cookie variant (%)*	Composition (g/100 g)				
	Wheat flour type 450	Apple pomace	Butter (82% fat)	Egg yolks	Sugar/erythritol
0	45.4	0.0	30.3	9.1	15.2
10	40.9	4.5	30.3	9.1	15.2
30	31.8	13.6	30.3	9.1	15.2
50	22.7	22.7	30.3	9.1	15.2

*Percentage of replacing wheat flour with apple pomace (by weight).

amyloglucosidase, ethanol, acetone) were purchased from Idalia (Radom, Poland) and Sigma-Aldrich (Steinheim, Germany), respectively. Certified reference material (CRM; the validated reference material BCR-191 was used to confirm the accuracy of the method for determining the mineral content) was purchased from MS Spectrum (Warsaw, Poland). Acetonitrile for chromatography was purchased from Merck (Darmstadt, Germany). Cyanidin 3-*O*-glucoside, quercetin 3-*O*-glucoside, chlorogenic acid, (+)-catechin, and procyanidin B_2 were purchased from Extrasynthese (Lyon, France). 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), formic and acetic acids, phloroglucinol, methanol, 3,5-dinitrosalicylic acid, potassium sodium tartrate tetrahydrate, sodium phosphate monobasic, starch from potato, pancreatic α-amylase from porcine pancreas (type VI-8), dipotassium hydrogen orthophosphate dihydrogen, *p*-nitrophenyl-α-D-glucopyranoside, and intestinal α-glucosidase from *Saccharomyces cerevisiae* (type I) were purchased from Sigma-Aldrich. Ultra-performance liquid chromatography (UPLC) grade water was prepared in the HLP SMART 1000s system (Hydrolab, Gdańsk, Poland), and additionally filtered through a 0.22 µm membrane filter immediately before use.

Materials

Dried apple pomace was purchased from a company GreenHerb-Kuźniar Dariusz (Łańcut, Poland) certified by the Institute of Consumer Research (Poland). The producer dried wet pomace, a by-product of juice production, in the Agromech M829 tumble dryer (Rogoźno, Poland) at 70°C for 3 h, crushed it as needed on Scorpion slicers and grinders (Rozdrażew, Poland) and sieved it using sifters from the same manufacturer. Wheat flour type 450, sucrose, erythritol, butter (82% fat) and eggs, necessary to make the cookies, were purchased from retail outlets (Wroclaw, Poland).

Shortbread cookie preparation

The subject of the research were shortbread cookies with a varied addition of by-product of juicing in the form of dried and powdered apple pomace (10, 30 and 50% of flour weight). Sucrose and its substitute – erythritol were used as sweeteners. A total of 8 variants of shortbread cookies with apple pomace were prepared including 4 variants sweetened with sucrose (SA10, SA30 and SA50, respectively) and 4 sweetened with erythritol (EA10, EA30 and EA50, respectively). Cookies with only wheat flour (without pomace), sweetened with sugar (SA0) or erythritol (EA0) were prepared as control samples. The recipe composition of individual cookie variants is presented in **Table 1**.

The preparation of the shortbread cookies consisted of the following steps: mixing the dry ingredients – wheat flour with apple pomace and ground sucrose/erythritol; adding the butter and egg yolks and kneading the dough for 3 min (KitchenAid model 5KPM5 mixer; Springfield, OH, USA); forming a ball and chilling the dough for 1 h at 4°C; rolling out the dough and cutting out circles (thickness 0.5 cm, diameter 5 cm); and baking for 8 min at 180°C (convection-steam furnace Rational; Landsberg am Lech, Munich, Germany). Three batches of each cookie variant were baked (100 cookies of each type).

Proximate analysis

The methods of the Association of Official Analytical Chemists (AOAC) were used to determine contents of dry matter (AOAC 925.49-1925), ash (AOAC 940.26), proteins (AOAC 920.152), dietary fibre (AOAC 985.29), and fat (AOAC 996.06) [AOAC, 2005] in apple pomace and shortbread cookies. The amount of total carbohydrates was calculated by subtracting the content of dietary fibre, fat, protein and ash from the dry matter content. The energy value was determined using the Rosenthal method [Gronowska-Senger, 2018].

Determination of sugar content

Sugar content in apple pomace and shortbread cookies was determined using the high-performance liquid chromatography (HPLC) system (Merck-Hitachi L-7455; Merck KGaA, Darmstadt, Germany) with an evaporative light scattering detector (ELSD 1000, Polymer Laboratories Inc., Amherst, MA, USA) as of the procedure described by Wojdyło *et al.* [2018]. Weighed samples of the shortbread cookies or apple pomace (4-5 g) were suspended in distilled water (100 mL), vortexed and subjected to sonication for 15 min (Sonic 6D; Polsonic, Warsaw, Poland). Then, the temperature of the suspensions was raised to 90°C, and heating was continued for 30 min with occasional stirring. After this step, the mixtures were centrifuged for 10 min at 19,000×*g* (MPW-55 centrifuge; Warsaw, Poland), and supernatants were subjected to purification on Sep-Pak C-18 columns. Finally, before injection into the HPLC system, the samples were filtered using hydrophilic membrane filter type PTFE (0.20 mm; Millex Samplicity; Merck). Chromatography separation was carried out using the Prevail™ Carbohydrate ES HPLC column (250×4.6 mm, 5 µm; Imtakt, Kyoto, Japan) with an injection volume of 20 µL, a flow rate of 1 mL/min and temperature analysis of 30°C. Isocratic elution based on the mobile phase consisting of acetonitrile and water (75:25, *v/v*) was used. Flow of nitrogen gas was 1.2 mL/min and temperature of nebuliser and evaporation was 80°C. Quantification of sugars was carried out based on standard curves obtained after the injection of standard solutions of sucrose, glucose, fructose and erythritol with known concentrations ranging from 0.05 to 0.10 mg/mL (r^2=0.999–0.997). The results of quantitative analysis were reported as the mean (n=3) and expressed in g/100 g of the cookie variant or apple pomace.

Determination of mineral content

Determination of the content of selected minerals (Cu, Mg, Mn, Fe, Zn, Ca, Na, K) in apple pomace and shortbread cookies was carried out in the certified Laboratory of Food Research of the Department of Human Nutrition of the Wrocław University of Environmental and Life Sciences, Poland. The atomic emission spectrometry (FEAS) method – potassium, sodium, calcium – and the atomic absorption spectrometry (FAAS) method – copper, zinc, iron, manganese, magnesium – were

used to determine the content of minerals. The Varian AA240FS atomic absorption spectrometer (Mulgrave, VIC, Australia) was used for the analyses. For the determination of mineral content, approximately 0.5 g of each variant of cookies or apple pomace was weighed out. Subsequently, 5 mL of 65% nitric acid and 1 mL of hydrogen peroxide were added to each sample. The MARS 6 closed microwave system was used for sample mineralization (CEM, Matthews, NC, USA). Mineralization temperature and time were 210°C and 15 min, respectively. In the next step, samples were quantitatively transferred using double distilled water into 10-mL tubes. Validated reference material BCR-191 was used to confirm the accuracy of the method. The measurement uncertainty was 5% [CEN, 2009, Food and Nutrition Institute, 2013]. The results of the mineral content were reported as the mean (n=3) and expressed in mg/100 g of the specified cookie variant or apple pomace.

Chromatography analysis of phenolic compounds

The qualitative and quantitative analysis of phenolic compounds of apple pomace and shortbread cookies was carried out using a UPLC system with a photo diode array (PDA) detector connected to a quadrupole time-of-flight–mass spectrometer (Q/TOF-MS) controlled by Empower 3 software and MassLynx 4.0 ChromaLynx software (Waters Corporation, Milford, MA, USA), as previously described by Tkacz *et al.* [2019]. The sample was grounded in a laboratory mill for extraction, weighed (cookie: 2.0 g and apple pomace: 0.5 g), suspended in 5–6 mL of a mixture of methanol, water, ascorbic acid and acetic acid (3:7:2:1, *v/v/w/v*), placed in a sonicated water bath (Sonic-6D; Polsonic, Warsaw, Poland) at 20°C for 15 min and then extracted for 24 h at 4°C. Next, the mixture was centrifuged (10 min at 19,000×*g*; MPW-350; Warsaw, Poland), and supernatant was separated. Finally, before analysis, all obtained extracts were filtered through a hydrophilic membrane (PTFE, 0.20 µm; Millex Samplicity Filter; Merck). The same chromatographic parameters were used as in our previous study [Wojdyło *et al.,* 2018], *i.e.*, UPLC BEH C18 column (2.1×100 mm, 1.7 µm; Waters Corporation), injection volume 5 µL, column temperature 30°C, elution at a flow rate 0.42 mL/min in a gradient mobile phase system consisting of (A) 2% formic acid and (B) 100% acetonitrile. PDA spectra were reordered, and absorbance at 520 nm for anthocyanins, at 360 nm for flavonols, at 320 nm for phenolic acids, and at 280 nm for flavan-3-ols and dihydrochalcones was measured. Quantification of phenolic compounds was carried out by injecting solutions of chlorogenic acid, procyanidin B_1, quercetin 3-*O*-glucoside and quercetin 3-*O*-galactoside with known concentrations ranging between 0.05 and 0.50 mg/mL and preparing standard curves ($r^2 \leq 0.9998$). The optimized MS parameters included: dissolution and source temperatures of 300 and 100°C, respectively, dissolution and cone gas flow of 300 and 40 L/h, respectively, cone and capillary voltage of 30 and 2500 V, respectively. The mass range from 100 to 1,000 *m/z* in positive (for anthocyanins) and negative ionization (for phenolic acids, flavonols, flavanols, and dihydrochalcone) was used for MS analysis. The results were presented as the mean of 3 replicates (n=3) and expressed in mg *per* kg of apple pomace powder or shortbread cookies.

Determination of antioxidant capacity and ability to inhibit activity of α-amylase, α-glucosidase and pancreatic lipase

To determine bioactivities of shortbread cookies and apple pomaces, first the extracts were prepared. The products were grounded, then weighed (about 0.5 g of apple pomace and 2.5 g of cookies), suspended in 5 mL of a mixture of 80% (*v/v*) methanol with 1% (*v/v*) HCl in the proportion of 8:1 (*w/v*), and sonicated (Sonic 6D water bath; Polsonic) for 15 min. After extraction, suspensions were centrifuged (5 min; 1,000×*g*), and supernatants were collected.

Determination of antioxidant capacity with the $ABTS^{\bullet+}$ was performed according to Re *et al.* [1999]. The $ABTS^{\bullet+}$ was obtained by mixing a solution of 7 mM ABTS with 140 mM potassium persulfate for about 16–18 h prior to analysis in darkness at the room temperature. The working solution was made by mixing $ABTS^{\bullet+}$ diluted with distilled water until its absorbance was 0.70±0.02 at 732 nm. Then, 0.1 mL of the extract was mixing with 2 mL of the working $ABTS^{\bullet+}$ solution in a cuvette, and reaction absorbance was measured after 6 min. Trolox was used as a standard, and the results were expressed as mmol Trolox equivalent (TE) *per* 100 g of apple pomace powder or shortbread cookies.

Oxygen radical absorption capacity (ORAC) assay was performed according to Ou *et al.* [2001]. The mixture of fluorescin (40 nM) with the extract was placed in a cuvette, pre-incubated at 37°C for 15 min and then mixed with 2,2′-azo-bis(2-amidinopropane) dihydrochloride (18 mM). The fluorescence was reordered every 1 min at 493 and 515 nm for excitation and emission, respectively, during 45 min. The results were calculated based on a standard curve plotted for Trolox and presented as mmol Trolox equivalent (TE) *per* 100 g of apple pomace powder or shortbread cookies.

The ability of apple pomace and cookies to inhibit activity of α-amylase, α-glucosidase and pancreatic lipase was determined according the to the procedures described previously in detail by Podsędek *et al.* [2014]. The starches from potato, *p*-nitrophenyl-α-D-glucopyranoside and *p*-nitrophenyl acetate were used as substrates in α-amylase inhibition, α-glucosidase inhibition and pancreatic lipase inhibition assays. The solutions of substrates were mixed with phosphate buffer saline (pH 6.9) and apple pomace or cookie extracts with different concentrations. After pre-incubating the samples at 37°C in a water bath for 5 min, the reactions were started by adding the enzyme solutions. The reactions were carried out at 37°C for 10, 15, 10 min in α-amylase inhibition, α-glucosidase inhibition and pancreatic lipase inhibition assays, respectively, and the absorbance was recorded at 540, 405, and 400 nm, respectively, using a spectrophotometer type UV-2401 PC (Shimadzu, Kyoto, Japan). The half maximal inhibitory concentration (IC_{50}) defined as the amount of inhibitor that is able to reduce the activity of a given enzyme by 50% was calculated and expressed as mg of apple pomace or cookies *per* mL of the reaction mixture under assay conditions.

Consumer evaluation

Consumer evaluation of shortbread cookies was performed using a 9-point hedonic scale. Each cookie variant was evaluated for five characteristics: colour, taste, odor, crispness, and overall acceptability [Land & Shepherd, 1988]. The study involved 62 participants who were informed about the purpose of the study. Each of them then received a questionnaire to record their own sensory perception. The scoring description was as follows: 1–definitely dislike, 2–very dislike, 3–dislike, 4–slightly dislike, 5–neither like or dislike, 6–slightly like, 7–like, 8–very like, 9–definitely like. The tests were conducted in a sensory laboratory, free of foreign odors, disturbing light and sound. Each participant in the consumer evaluation has given written consent to participate in the study. The study was based on the guidelines of the Declaration of Helsinki [World Medical Association, 2013]. The personal data of the participants of the organoleptic evaluation were coded in accordance with the guidelines of the General Regulation of the European Parliament on the Protection of Personal Data (GDPR 679/2016). Participants gave informed consent *via* the statement: "I am aware that my responses are confidential, and I agree to participate in this survey" where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption. The research was approved by the Research Ethics Committee of the Wrocław University of Environmental and Life Sciences, Poland (no. 28/2023).

Statistical analysis

The data obtained were statistically analyzed using STATISTICA version 13.3 (StatSoft®; Tulsa, OK, USA). A one-way analysis of variance (ANOVA) and Tukey's multiple range test were performed with the significance level set at $p \leq 0.05$. Principal component analysis (PCA) and Spearman's correlation were also used. Data from three replicates were presented as mean and standard deviation.

RESULTS AND DISCUSSION

Nutritional value

Table 2 presents the nutritional value of the apple pomace and shortbread cookies. The energy value of cookies was reduced with increasing pomace addition – for cookies sweetened with sucrose, the decrease was 32.6 kcal/100 g (SA0 *vs.* SA50); for cookies sweetened with erythritol, 40.5 kcal/100 g (EA0 *vs.* EA50). The erythritol-sweetened cookies had a significantly lower energy value than their sucrose-sweetened counterparts. This was due to the difference in the energy value of the sweeteners used – sucrose, 4.0 kcal/g and erythritol, 0.2 kcal/g [Regnat *et al.*, 2018]. According to literature data, the dry matter content of wheat flour and apple pomace ranges from 85.7 to 92.4 g/100 g [Czaja *et al.*, 2020] and 20.0–24.5 g/100 g, respectively [Hang & Woodams, 1987; Villas-Bôas & Esposito, 2000]. In our study, the dry matter content of apple pomace was significantly higher and amounted to 95.54 g/100 g (**Table 2**). The differences are related to the fact that the other authors analyzed the composition of wet pomace, while dry apple pomace powder was used in our study. The fat and protein contents of the apple pomace powder were also different compared to the literature results reported for the dry matter of apple pomace – the fat content was 0.9–4.3 g/100 g d.m., while the protein content was 1.6–4.6 g/100 g d.m. [Hosseini & Pazhouhandeh, 2023; Yadav & Gupta, 2015]. The share of dry matter in apple pomace powder was similar to that of wheat flour; hence, the differences between cookie variants were small (**Table 2**). For fat content, the only source was butter, the use of which was the same in all variants of the cookies (**Table 1**). The slight differences in protein content of the tested cookies were due to differences in protein content between pomace and wheat flour (6.98 g/100 g and 11.00 g/100 g, respectively). The largest differences were observed for dietary fibre and ash contents which increased significantly with the increasing proportion of pomace in the cookies. This applied to cookies with both sweeteners. The dietary fibre content increased an approximately 8-fold from 2.79 (SA0) to 22.54 g/100 g (SA50), while the ash content ranged from 0.44 (EA0) to 0.73 g/100 g (SA50). Studies involving the addition of apple pomace to shortbread cookies were also conducted by Alongi *et al.* [2019]. The share of dietary fibre in apple pomace was lower and amounted to almost 40%; additionally, pomace was only added at levels of 10 and 20%. Despite the lower addition levels of pomace, the glycemic index of the tested cookies decreased by 5 and 10%, respectively, compared with those of products without the addition of pomace.

Erythritol was included in the total carbohydrate content; thus, carbohydrate share in individual cookies variants was similar and ranged from 55.49 (EA10) to 57.73 g/100 g (EA50) (**Table 2**).

Analysis of the nutritional value of cookies with apple pomace showed that they were characterized by a favorable nutritional profile in terms of the increasing content of dietary fibre, as well as a decreasing energy value with an increasing proportion of pomace and replacement of sucrose to erythritol.

Sugar content

The sugar content of the various types of cookies and apple pomace is presented in **Table 2**. Four sugars/polyols were identified: glucose, fructose, sucrose and erythritol. The largest quantities were found for sucrose and erythritol, which was due to their use as sweeteners in the shortbread cookies. Apple pomace and SA50 cookies also contained glucose at 4.96 and 1.03 g/100 g, respectively. The content of fructose in pomace was lower than that of glucose, but fructose was also identified in the cookies with a pomace content of 30% and 50%, sweetened with both sucrose and erythritol. SA50 products, compared to the other cookie variants, were characterized by a significantly higher total sugar content (20.12 g/100 g). The difference in total sugars between SA50 and EA50 was 3.93 g/100 g. The results reported by other authors showed that the content of glucose in apple pomace ranged from 2.5 to 12.4, and that of fructose from 18.0 to 31.0 g/100 g [Queji *et al.*, 2010]. Antonic *et al.* [2020] found that such discrepancies may result from, among other factors, the production process, harvest time and variety of apples.

Table 2. Nutritional profile, contents of ash, dry matter, sugars and minerals of apple pomace and shortbread cookies containing apple pomace.

	SA0	SA10	SA30	SA50	EA0	EA10	EA30	EA50	AP
Nutritional profile, ash and dry matter (*per* 100 g)									
Energy value (kcal)	519.72±5.17^{a}	496.82±6.08^{ab}	491.67±1.96^{ab}	487.09±9.99^{b}	426.26±11.36^{c}	414.82±8.00^{cd}	405.84±5.68^{cd}	385.78±10.71^{d}	114.81±1.57
Fat (g)	31.16±0.07^{ab}	31.42±0.03^{ab}	32.24±0.08^{a}	32.28±0.13^{a}	29.51±1.68^{b}	30.46±0.10^{ab}	31.49±0.08^{ab}	31.86±0.36^{ab}	nd
Protein (g)	8.41±0.07^{a}	7.50±0.24^{b}	7.25±0.12^{b}	6.81±0.24^{b}	6.81±0.24^{b}	6.81±0.24^{b}	6.81±0.24^{b}	5.26±0.00^{c}	6.98±0.24
Total carbohydrates (g)	57.51±0.05^{a}	57.57±0.56^{a}	56.74±0.25^{a}	57.37±0.42^{a}	56.66±1.48^{a}	55.49±0.13^{a}	57.19±0.12^{a}	57.73±0.38^{a}	87.06±0.29
Dietary fiber (g)	2.79±0.14^{d}	6.83±0.05^{c}	15.23±0.27^{b}	22.54±0.30^{a}	3.17±0.03^{d}	7.03±0.04^{c}	15.40±0.23^{b}	22.73±0.00^{a}	59.04±0.26
Dry matter (g)	97.51±0.03^{a}	97.01±0.02^{c}	96.86±0.04^{d}	97.19±0.03^{b}	93.43±0.03^{g}	93.26±0.00^{h}	96.09±0.03^{e}	95.53±0.00^{f}	95.54±0.03
Ash (g)	0.45±0.02^{d}	0.51±0.01^{c}	0.63±0.00^{b}	0.73±0.01^{a}	0.44±0.01^{d}	0.48±0.03^{c}	0.61±0.01^{b}	0.70±0.03^{a}	1.65±0.07
Sugar content (g/100 g)									
Glucose	nd	nd	nd	1.03±0.19^{a}	nd	nd	nd	nd	4.96±0.81
Fructose	nd	nd	0.86±0.09^{bc}	1.43±0.42^{ab}	nd	nd	0.27±0.00^{c}	1.75±0.16^{a}	2.08±0.61
Sucrose	15.04±1.29^{b}	15.17±0.12^{b}	14.09±0.80^{b}	17.66±0.83^{a}	nd	nd	0.25±0.06^{c}	0.63±0.27^{c}	2.60±0.41
Erythritol	nd	nd	nd	nd	13.59±0.30^{a}	13.98±0.03^{a}	12.87±0.96^{a}	13.80±0.51^{a}	nd
Total sugars	15.04±1.29^{b}	15.17±0.17^{b}	14.95±0.71^{b}	20.12±1.44^{a}	13.59±0.30^{b}	13.98±0.03^{b}	13.40±1.02^{b}	16.19±0.40^{b}	9.97±1.90
Mineral content (mg/100 g)									
Cu	0.19±0.05^{d}	0.24±0.04^{cd}	0.31±0.04^{bc}	0.42±0.04^{a}	0.20±0.03^{d}	0.24±0.04^{cd}	0.39±0.02^{ab}	0.39±0.07^{ab}	0.96±0.02
Mg	19.81±0.28^{d}	22.99±0.21^{c}	30.59±0.36^{b}	36.11±0.84^{a}	20.21±0.05^{d}	23.10±0.38^{c}	31.05±0.7^{b}	36.96±1.86^{a}	92.62±3.89
Mn	0.22±0.01^{d}	0.25±0.01^{d}	0.35±0.02^{c}	0.49±0.07^{a}	0.13±0.02^{e}	0.17±0.01^{e}	0.31±0.00^{cd}	0.43±0.02^{b}	1.43±0.11
Fe	1.70±0.03^{b}	2.37±0.46^{b}	4.04±0.06^{b}	12.95±9.42^{a}	1.28±0.16^{b}	1.92±0.19^{b}	5.76±1.37^{ab}	6.93±3.02^{ab}	19.59±0.89
Zn	0.74±0.03^{abc}	0.68±0.02^{bcd}	0.72±0.01^{abcd}	0.76±0.05^{ab}	0.66±0.07^{cd}	0.63±0.01^{d}	0.77±0.02^{ab}	0.82±0.06^{a}	0.85±0.19
Ca	42.08±2.21^{d}	55.74±0.98^{c}	76.57±1.40^{b}	93.67±2.97^{a}	40.66±0.89^{d}	52.40±0.34^{cd}	74.01±2.40^{b}	105.16±0.12^{a}	170.36±15.01
Na	13.13±0.51^{b}	20.80±7.20^{a}	18.11±0.31^{ab}	18.20±0.68^{ab}	17.63±0.53^{ab}	17.37±0.26^{ab}	17.37±0.17^{ab}	17.57±1.30^{ab}	17.83±0.96
K	68.13±1.63^{d}	81.11±1.80^{c}	108.79±2.83^{b}	128.01±2.01^{a}	70.77±0.84^{d}	81.12±0.48^{c}	110.39±1.03^{b}	127.38±4.27^{a}	348.05±16.17

Values (mean of three replications) ± standard deviation followed by the same letter (a, b, c,…), within the same row, are not significantly different ($p>0.05$; Tukey's test); apple pomace (AP) was not subjected to statistical analysis. SA; sucrose-sweetened shortbread cookies; EA, erythritol-sweetened shortbread cookies; 0, 10, 30 and 50 after EA and SA, percentage (by weight) of replacing wheat flour with apple pomace in the cookie recipe; nd, not detected.

In turn, based on multivariate analysis of the spectroscopic profile of the sugar fraction of apple pomace, Gabriel *et al.* [2013] showed a significant impact of apple variety on the sugar profile of apple pomace. It has also been shown that the sugar content of apples and therefore of apple pomace can vary depending on the position of the fruit in the tree canopy, with south-facing fruit having a lower sugar content due to increased respiration and oxidation of carbohydrates [Lazar *et al.*, 2009]. In the case of Golden Delicious apples, the sugar content was also influenced by the harvest date – the percentage of sucrose increased with later harvest dates, while the glucose content decreased [Núñez-Gastélum *et al*., 2015].

Mineral content

The content of selected minerals in the various variants of shortbread cookies and apple pomace is shown in **Table 2**. With the increase in pomace proportion in the recipe, the mineral content of baked goods increased. This relationship applied to all minerals except sodium. The minerals found in the highest content were potassium (from 68.13 mg/100 g for SA0 to 128.01 mg/100 g for SA50), calcium (from 40.66 mg/100 g for EA0 to 105.16 mg/100 g for EA50), magnesium (from 19.81 mg/100 g for SA0 to 36.96 mg/100 g for EA50) and iron (from 1.28 mg/100 g for EA0 to 12.95 mg/100 g for SA50). In most cases, no significant effect of the sweetener on the content of individual minerals was found ($p>0.05$). Similar conclusions were reached by Mir *et al.* [2017], who analyzed brown rice-based apple pomace biscuits and showed that the mineral content of crackers increased with apple pomace ratio increase in the formula. Also, the study of Er & Özcan [2010] reported the high content of Ca, Mg and K in pomace from different apple varieties, which was 8,420.50, 4,707.83 and 929.85 mg/kg, respectively.

The mineral content in the cookies differed from what would be expected based on the mineral content in apple pomace and the share of pomace in the recipe of individual cookie variants (**Table 2**). This may be because the process of baking cookies can affect the mineral content. Bredaliol *et al.* [2020], who examined the impact of baking conditions on the content of minerals in wheat bread, showed that as the baking temperature increased, the content of macro minerals decreased. However, taking into account the longer baking time, the losses of macro minerals were smaller. Baking at a temperature of 220°C and simultaneously extending the baking time from 15 to 20 min resulted in an increase in the content of the macro minerals. The parameters used in the initial baking phase most likely resulted in a more intense reduction of phytate content, which favored greater availability of macro minerals. The optimal temperature for phytase activity is 55°C; therefore, the degradation of phytic acid probably still occurred during baking (especially in the initial stage) due to the activation of phytase contained in the flour. This process promoted the reduction of phytates and thus increased the content of macro minerals. It should be noted, however, that baking parameters had a significant impact on the content of macro minerals, but were not significantly related to the content of micro minerals.

Identification of phenolic compounds

Identification of phenolic compounds was carried out with the UPLC-PDA-Q/TOF-MS method. The results are shown in **Table 3**. Five groups of phenolic compounds were identified in the tested cookie variants (with the addition of pomace): anthocyanins, flavonols, phenolic acids, flavan-3-ols and dihydrochalcones. The first identified fraction in the resulting products was that of anthocyanins, represented by two compounds: cyanidin 3-*O*-glucoside (t_R=4.77 min) and cyanidin 3-*O*-glucoside (t_R=4.28 min), both of them with $[M–H]^+$ at *m/z*=449 with an MS/MS fragment at *m/z*=287. The anthocyanins were previously detected in apples of different varieties with red-colored skin [Wojdyło *et al.*, 2008]. The next group of phenolic compounds identified in the cookies was that of compounds representing flavonols (**Table 3**), including: quercetin 3-*O*-rutinoside ($[M–H]^-$ at *m/z*=609; MS/MS fragment at *m/z*=301, and t_R=6.52 min), quercetin 3-*O*-galactoside ($[M–H]^-$ at *m/z*=463; MS/MS fragment at m/z=301, and t_R=6.58 min), quercetin 3-*O*-glucoside ($[M–H]^-$ at *m/z*=463; MS/MS fragment at *m/z*=301, and t_R=6.73 min), quercetin 3-*O*-xyloside ($[M–H]^-$ at *m/z*=433; MS/MS fragment at *m/z*=301, and t_R=7.00 min), quercetin hexoside ($[M–H]^-$ at m/z=463; MS/MS fragment at *m/z*=301, and t_R=7.34 min), and quercetin 3-*O*-rhamnoside ($[M–H]^-$ at *m/z*=447; MS/MS fragment at m/z=301, and t_R=7.55 min). The flavonols identified in this study were consistent with the flavonol profiles reported by other authors studying apple pomace [Ćetković *et al.*, 2008; Gumul *et al.*, 2021]. Phenolic acids of cookies with apple pomace were represented by chlorogenic acid (t_R=3.96 min) (**Table 3**). Also, two dihydrochalcones – phloretin 2'-*O*-xyloglucose ($[M–H]^-$ at *m/z*=567; MS/MS fragment at *m/z*=273, and t_R=7.66 min) and phloretin 2'-*O*-glucose ($[M–H]^-$ at *m/z*=435; MS/MS fragment at *m/z*=273, and t_R=8.40 min) – and flavan-3-ols were determined. The latter fraction consisted of procyanidins B_1 and B_2 with $[M–H]^-$ at *m/z*=577; MS/MS fragment at *m/z*=289, and t_R=2.62 min, and t_R=1.81 min, respectively. Chlorogenic acid, phloretin glycosides and procyanidins were previously determined in apple pomace and bakery/confectionary products with apple pomace [Gorjanović *et al*., 2020; Gumul *et al.*, 2021; Kruczek *et al*., 2023]. Among the important bioactive compounds, procyanidin B_2 deserves special attention, as it exhibits a significant antioxidant activity and inhibits the oxidation of the low-density lipoprotein (LDL) cholesterol [Xiao *et al.,* 2020]. Chlorogenic acid is another compound worthy of note for its anti-cancer potential [Kasai *et al.*, 2000]. In turn, quercetin elicits a preventive effect in the development of hormone-dependent cancers and cardiovascular diseases [Gormaz *et al.*, 2015; Zand *et al.,* 2002].

Content of phenolic compounds

The content of phenolic compounds in individual variants of cookies and apple pomace is presented in **Table 4**. Flavonols (44.4%) and flavan-3-ols (37.8%) had the highest share of the total content of phenolic compounds of apple pomace. Considerably fewer compared to flavonols and flavan-3-ols were dihydrochalcones (10.0%), phenolic acids (4.8%) and anthocyanins (3.1%). The content of each phenolic compound in cookies increased

Table 3. Phenolic compounds identified with ultra-performance liquid chromatography with a photodiode detector-quadrupole/time-of-flight mass spectrometry (UPLC-PDA-Q/TOF-MS) in shortbread cookies containing apple pomace.

Group of phenolics	Compound	t_R (min)	λ_{max} (nm)	Parent ion* (*m/z*)	MS/MS ion (*m/z*)
Anthocyanin	Cyanidin 3-*O*-glucoside	4.77	520	449+	287+
	Cyanidin 3-*O*-galactoside	4.28	519	449+	287+
Flavonol	Quercetin 3-*O*-rutinoside	6.52	352	609	301
	Quercetin 3-*O*-galactoside	6.58	355	463	301
	Quercetin 3-*O*-glucoside	6.73	350	463	301
	Quercetin 3-*O*-xyloside	7.00	350	433	301
	Quercetin hexoside	7.34	357	463	301
	Quercetin 3-*O*-rhamnoside	7.55	345	447	301
Phenolic acid	Chlorogenic acid	3.96	320	353	191
Flavan-3-ol	Procyanidin B_2	1.81	280	577	289/245
	Procyanidin B_1	2.62	280	577	289/245
Dihydrochalcone	Phloretin 2′-*O*-xyloglucose	7.66	285	567	273
	Phloretin 2′-*O*-glucose	8.40	285	435	273

*$[M–H]^+$ for anthocyanins (positive-ion mode) and $[M–H]^-$ for other phenolic compounds (negative-ion mode). t_R, retention time; λ_{max}, absorption maximum of PDA UV spectrum.

with the increasing share of pomace in their recipe, in the products sweetened with either sucrose or erythritol. The total content of phenolic compounds determined in apple pomace was 2515.35 mg/kg, which was higher compared to the value of 89.4 mg gallic acid/100 g d.m. determined by Gumul *et al.* [2021]. Also, Leyva-Corral *et al.* [2016] reported a different content of these compounds, reaching 324.2 mg gallic acid/100 g d.m. Both literature values were obtained *via* the colorimetric method, which may explain the differences from the values in our studies. Analysis of apple pomace conducted by Oszmiański *et al.* [2011] showed a quite high content of polymeric procyanidins and flavan-3-ols, dihydrochalcones and flavonols. Polymeric procyanidins, dihydrochalcones and flavonols accounted for 57.0%, 5.5%, 3.0% of total phenolics in pomace, respectively.

The share of individual phenolic compounds from the group of flavonols in apple pomace and cookies with their addition was as follows: quercetin 3-*O*-galactoside > quercetin 3-*O*-rhamnoside > quercetin hexoside > quercetin 3-*O*-xyloside > quercetin 3-*O*--glucoside > quercetin 3-*O*-rutinoside (**Table 4**). The most abundant flavonol turned out to be quercetin 3-*O*-galactoside, with its content in cookies ranging from 17.35 (EA10) to 108.66 mg kg (SA50). The cookies with 50% pomace addition had about 6–8 times higher flavonol content compared to the variants with 10% pomace addition. The flavonol content of the apple pomace powder was 1313.84 mg/kg. This value was close to the result shown by Kruczek *et al.* [2023] (103.19 mg/100 g d.m.), although Ćetković *et al.* [2008] and Gumul *et al.* [2021] determined a lower content of quercetin glycosides (28.6–61.0 and 73.14 mg/100 g d.m., respectively). Nevertheless, as in our studies, literature data indicate quercetin 3-*O*-galactoside as the major flavonol of apple pomace [Gorjanović *et al.*, 2020; Gumul *et al.*, 2021; Kruczek *et al.*, 2023].

The most abundant flavan-3-ol, both in the pomace and in individual variants of cookies, was procyanidin B_2 (**Table 4**). Its content in cookies ranged from 52.45 (EA10) to 365.20 mg/kg (SA50) and in apple pomace it was 372.28 mg/kg. Procyanidin B_1 was present in smaller amounts – from 4.47 (EA10) to 38.04 mg/kg (SA50) in cookies and 231.09 mg/kg in apple pomace. According to earlier experimental studies, the content of procyanidin B_2 in apple pomace was lower and ranged from 2.61 to 16.00 mg/100 g d.m. [Gumul *et al.*, 2021; Schieber *et al.*, 2001].

The content of phloretin 2′-*O*-glucose ranged from 6.69 (SA10) to 43.48 mg/kg (SA50) and that of phloretin 2′-*O*-xyloglucose – from 3.46 (EA30) to 13.21 mg/kg (SA50) (**Table 4**). The presence of phloretin 2′-*O*-glucose in apple pomace has been previously reported by other authors within a broad content range from 0.7 to 18.0 mg/100 g d.m. [Ćetković *et al.*, 2008; Gumul *et al.*, 2021; Leyva-Corral *et al.*, 2016].

The phenolic compounds found in the lowest amounts were chlorogenic acid and anthocyanins (**Table 4**). The content of chlorogenic acid in cookies ranged from 3.60 (SA10) to 27.95 mg/100 g (SA50) and in apple pomace was 154.15 mg/100 g. Other authors determined chlorogenic acid in apple pomace as a major phenolic acid, with its content ranging from 8.2 to 41.6 mg/100 g d.m. [Ćetković *et al.*, 2008; Gorjanović *et al.*, 2020; Kruczek *et al.*, 2023; Leyva-Corral *et al.*, 2016].

The anthocyanin content of apple pomace was relatively low (**Table 4**). These polyphenolic compounds are found in red-colored apple varieties [Wojdyło *et al.*, 2008]. In our study, we used a commercially available pomace powder. According to the manufacturer's declaration, it was obtained from a by-product after squeezing juice from different varieties of apples with different fruit colors.

Table 4. Content of phenolic compounds (mg/kg) in apple pomace and shortbread cookies containing apple pomace.

Compound	SA0	SA10	SA30	SA50	EA0	EA10	EA30	EA50	AP
Cyanidin 3-*O*-glucoside	nd	1.85±0.09^{b}	5.39±0.29^{ab}	9.28±0.57^{a}	nd	2.35±0.11^{b}	8.17±2.56^{a}	9.43±0.35^{a}	73.82±6.20
Cyanidin 3-*O*-galactoside	nd	nd	2.99±0.22^{a}	4.49±1.52^{a}	nd	nd	nd	4.03±0.95^{a}	35.60±2.02
Quercetin 3-*O*-rutinoside	nd	nd	7.10±0.14^{c}	14.19±1.65^{a}	nd	2.25±0.33^{d}	10.68±0.22^{b}	12.24±1.23^{ab}	47.74±4.18
Quercetin 3-*O*-galactoside	nd	17.41±0.75^{c}	55.27±5.17^{b}	108.66±2.24^{a}	nd	17.35±1.28^{c}	93.40±13.87^{a}	93.98±1.43^{a}	501.55±6.04
Quercetin 3-*O*-glucoside	nd	nd	9.88±0.38^{b}	19.97±0.37^{a}	nd	3.18±0.39^{c}	16.93±2.57^{a}	18.09±0.32^{a}	97.85±0.07
Quercetin 3-*O*-xyloside	nd	nd	11.45±0.76^{b}	24.75±0.68^{a}	nd	2.98±0.23^{c}	21.61±3.46^{a}	22.42±0.23^{a}	135.10±9.03
Quercetin hexoside	nd	7.19±0.04^{c}	23.95±0.71^{b}	47.35±0.94^{a}	nd	6.15±0.36^{c}	41.15±5.56^{a}	41.28±0.87^{a}	261.69±15.88
Quercetin 3-*O*-rhamnoside	nd	8.11±0.05^{c}	27.09±1.65^{b}	54.56±1.64^{a}	nd	6.44±0.45^{c}	46.36±6.05^{a}	45.93±1.73^{a}	269.91±19.02
Chlorogenic acid	nd	3.60±0.35^{d}	13.17±1.16^{c}	27.95±0.28^{a}	nd	3.88±0.15^{d}	19.68±1.12^{b}	23.52±1.99^{b}	154.15±0.80
Procyanidin B_2	nd	69.55±1.05^{d}	208.79±9.99^{c}	365.20±0.04^{a}	nd	52.45±4.00^{d}	231.84±10.88^{c}	295.38±15.63^{b}	372.28±8.26
Procyanidin B_1	nd	5.90±0.62^{d}	16.42±3.21^{c}	38.04±2.77^{a}	nd	4.47±0.91^{d}	23.85±2.21^{bc}	26.51±1.52^{b}	231.09±26.90
Phloretin 2′-*O*-xyloglucose	nd	nd	5.91±0.82^{b}	13.21±0.96^{a}	nd	nd	3.46±0.00^{c}	3.48±0.22^{c}	17.72±0.47
Phloretin 2′-*O*-glucose	nd	6.69±0.10^{c}	22.11±2.93^{b}	43.48±1.16^{a}	nd	6.97±1.17^{c}	41.88±3.25^{a}	42.37±3.46^{a}	290.67±8.59
Total phenolic compounds	**nd**	**120.30±2.30^{d}**	**409.51±13.50^{c}**	**771.15±2.13^{a}**	**nd**	**108.46±7.93^{d}**	**560.56±26.47^{b}**	**637.09±8.85^{b}**	**2,515.35±34.56**

Values (mean of three replications) ± standard deviation followed by the same letter (a, b, c,…), in the same row, are not significantly different (p>0.05; Tukey's test); apple pomace (AP) was not subjected to statistical analysis. SA, sucrose-sweetened shortbread cookies; EA, erythritol-sweetened shortbread cookies; 0, 10, 30 and 50 after EA and SA, percentage (by weight) of replacing wheat flour with apple pomace in the cookie recipe; nd, not detected.

The sweetener used was found not to affect the content of phenolic compounds in the individual variants of the products.

Antioxidant capacity

Two methods were used to determine the antioxidant capacity of shortbread cookies and apple pomace – ABTS and ORAC assays. The results of both determinations showed that the antioxidant capacity of cookies increased significantly with the increase in pomace share in their recipe (**Table 5**). This applied to the cookies with both sweeteners. $ABTS^{\bullet+}$ scavenging activity ranged from 0.022 (EA0) to 0.363 mmol TE/100 g (SA50) and ORAC from 1.116 (EA0) to 2.253 mmol TE/100 g (SA50). In most cases, cookies sweetened with sucrose were characterized by significantly higher antioxidant capacity than those sweetened with erythritol. This was most likely due to the fact that sucrose, unlike erythritol, participates in Maillard reactions. The antioxidant properties of processed foods exposed to high temperatures also result from the formation of compounds during the Maillard reaction or heating of reducing sugars for example, caramelization products, Amadori compounds, reactive reductones, premelanoidins, and melanoidins [Billaud *et al.*, 2003]. Michalska *et al.* [2008] studied the influence of baking rye bread on the formation of Maillard reaction products and their antioxidant activity. They showed that the Maillard reaction products formed during baking, mainly melanoidins, were able to scavenge the peroxyl radicals and ABTS cation radicals and reduce the Folin-Ciocalteu reagent. Also, the study conducted by Patrignani *et al.* [2016] on cookies showed that the Maillard reaction products, apart from their key role in imparting color and flavor to baking products, had antioxidant properties. Therefore, the higher antioxidant capacity of cookies determined in our study and associated primarily with a higher proportion of pomace (and thus a higher content of bioactive compounds) could also result from the formation of Maillard reaction products upon high-temperature baking. Moreover, Žilić *et al.* [2016] showed that the highest antioxidant activity was determined in cookies with the addition of ammonium bicarbonate, which proves that the Maillard reaction proceeds at an increased rate under alkaline conditions, resulting in an increase in antioxidant activity.

In order to understand the relationship between antioxidant capacity of cookies and phenolic compound contents, a correlation analysis was carried. For each group of phenolic compounds, the correlations were positive and significant with the value of correlation coefficient (r) much higher than 0.6 (**Table S1** in Supplementary Materials). For $ABTS^{\bullet+}$ scavenging activity, the strongest correlation was shown with chlorogenic acid (r=0.9952) and for ORAC with phloretin 2′-*O*-glucose (r=0.9929).

Gumul *et al.* [2021] conducted studies aimed at evaluating the health-promoting properties of gluten-free bread prepared with the addition of apple pomace. They showed that the 5% pomace addition contributed to a 66-fold increase in antioxidant activity. In our research, the $ABTS^{\bullet+}$ scavenging activity of cookies with 10% pomace increased almost 4 times compared to the cookies without apple pomace (**Table 5**). This concerned cookies with both sucrose and erythritol. For ORAC, the increase was 1.5 times for the sucrose-sweetened cookies and 1.2 times for the erythritol-sweetened ones. These results were in agreement with the study of Zlatanović *et al.* [2019] who reported that the addition of apple pomace to cookies contributed to a 3–5.5-fold increase in antioxidant activity compared with the control sample. The lower antioxidant activity of products sweetened with erythritol than with sucrose was previously found by Nowicka & Wojdyło [2016], who checked, among others, the stability of polyphenols and antioxidant properties of cherry puree prepared with the addition of various sweeteners.

Table 5. Antioxidant capacity measured as $ABTS^{\bullet+}$ scavenging activity (ABTS assay) and oxygen radical absorption capacity (ORAC), and α-amylase, α-glucosidase and pancreatin lipase inhibitory activity of apple pomace and shortbread cookies containing apple pomace.

Cookie variant/apple pomace	ABTS assay (mmol TE/100 g)	ORAC (mmol TE/100 g)	α-Amylase inhibitory activity	α-Glucosidase inhibitory activity	Pancreatin lipase inhibitory activity
			IC_{50} (mg/mL)		
SA0	0.032±0.004^{f}	1.153±0.065^{e}	221.8±4.00^{a}	1374.9±7.9^{a}	16.28±0.88^{a}
SA10	0.116±0.008^{d}	1.697±0.041^{c}	20.3±2.8^{d}	1149.3±8.6^{d}	16.84±0.86^{a}
SA30	0.236±0.005^{c}	1.939±0.018^{b}	13.1±1.4^{de}	1106.8±7.9^{d}	11.18±1.11^{bc}
SA50	0.363±0.005^{a}	2.253±0.052^{a}	<0.5±0.0^{e}	931.3±9.5^{f}	7.97±0.32^{c}
EA0	0.022±0.001^{f}	1.116±0.058^{e}	202.3±4.9^{ab}	1202.6±3.9^{c}	16.76±1.22^{a}
EA10	0.082±0.002^{e}	1.353±0.037^{d}	198.7±1.4^{b}	1287.3±4.7^{b}	13.04±0.76^{ab}
EA30	0.260±0.013^{c}	1.985±0.012^{b}	186.4±5.8^{b}	1131.2±9.4^{d}	9.92±1.05^{bc}
EA50	0.316±0.009^{b}	2.070±0.038^{b}	138.1±3.6^{c}	976.4±7.4^{e}	7.26±0.56^{c}
AP	1.715±0.065	8.760±0.171	26.4±1.1	395.5±4.5	2.28±0.32

Values (mean of three replications) ± standard deviation followed by the same letter (a, b, c,…), within the same column, are not significantly different (p>0.05; Tukey's test); apple pomace (AP) was not subjected to statistical analysis. TE, Trolox equivalent; IC_{50}, half maximal inhibitory concentration; SA, sucrose-sweetened shortbread cookies; EA, erythritol-sweetened shortbread cookies; 0, 10, 30 and 50 after EA and SA, percentage (by weight) of replacing wheat flour with apple pomace in the cookie recipe.

Inhibition of α-amylase, α-glucosidase and pancreatic lipase activity

The next stage of the study aimed to determine the ability of apple pomace and individual variants of cookies to inhibit the activity of α-amylase, α-glucosidase and pancreatic lipase, and respective data are shown in **Table 5**. Extracts obtained from cookies with apple pomace addition at various levels were tested at different concentrations. This allowed calculating the IC_{50} value (mg/mL). Enzymes such as α-amylase and α-glucosidase play a key role in the digestion of carbohydrates; hence, the inhibition of their activity plays a significant role in diabetes control [Tundis *et al.*, 2010]. Pancreatic lipase catalyzes the conversion of fat molecules into vitamins A, D, E, K, fatty acids and salts of bile acids [Lunagariya *et al.*, 2014]. For each enzyme, it was shown that the increasing addition of pomace in the cookie recipe was associated with an increased inhibition of its activity (lower IC_{50} values) (**Table 5**). The exception was α-glucosidase inhibitory activity of EA10 cookies, for which significantly ($p \leq 0.05$) higher IC_{50} values were obtained compared to the EA0 variant. The difference was only 84.7 mg/mL. Perhaps the addition of 10% pomace was insufficient to induce an inhibitory effect on α-glucosidase. For α-amylase inhibitory activity, the IC_{50} ranged from <0.5 (SA50) to 221.8 mg/mL (SA0). The ability of SA50 cookies to inhibit α-amylase activity was more than 400 times higher than that of cookies without added pomace (SA0). For α-glucosidase inhibitory activity, IC_{50} values ranged from 931.3 (SA50) to 1,374.9 mg/mL (SA0), and for lipase from 7.26 (EA50) to 16.76 mg/mL (SA10). It was also observed that the higher the addition of apple pomace was, the higher was the α-amylase, α-glucosidase, and pancreatin lipase inhibitory activity of cookies. Additionally, it was observed that shortbread cookies prepared with erythritol elicited significantly greater effect on the activity of α-amylase and α-glucosidase than the sucrose-sweetened ones. From the data presented in **Table 5**, it appears that the main factor influencing the increase in enzyme inhibition was the increasing proportion of apple pomace in cookie formula

Results of correlation analysis (**Table S1** in Supplementary Materials) showed that certain phenolic compounds, such as phloretin 2′-*O*-xyloglucose (r=–0.6220) and procyanidin B_2 (r=–0.5934), significantly affected the α-amylase inhibitory activity of the cookies. Significant correlations were found between α-glucosidase inhibitory activity of the cookies and contents of selected phenolic compounds, including quercetin 3-*O*--rutinoside (r=–0.4385), phloretin 2′-*O*-xyloglucose (r=–0.4289), quercetin 3-*O*-glucoside (r=–0.4198), and quercetin 3-*O*-xyloside (r=–0.4119). Phenolic compounds that had the greatest effect on lipase inhibition were procyanidin B_2 (r=–0.8841), quercetin 3-*O*--rutinoside (r=–0.8608), phloretin 2′-*O*-xyloglucose (r=–0.8375), quercetin 3-*O*-galactoside (r=–0.8262), and quercetin 3-*O*-glucoside (r=–0.8250).

The phenolic compounds contained in apples play a key role in the treatment of diabetes. One of the mechanisms is the inhibition of the activity of α-amylase and α-glucosidase, which reduces the rate of carbohydrate digestion. In addition, flavonoids have been shown to exert the anti-diabetic effect, affecting obesity, diabetes, insulin resistance, hyperglycemia, and hyperlipidemia [Alkhalidy *et al.*, 2018]. However, there are no results in the available literature showing the extent of inhibition of the aforementioned enzymes by sweet snack foods with apple pomace added. Researchers have focused more often on the technological properties of these types of products and sensory sensations after their consumption [Lyu *et al.*, 2020].

Consumer evaluation

The results of the consumer evaluation of the individual variants of cookies are shown in **Figure 1**. Products were rated for color, taste, odor, crispness and overall acceptability. According to the participants, the highest scores were given to SA0 and EA0 (overall acceptability 7.85 and 7.98, respectively). This can be attributed to the known characteristics of commonly consumed shortbread cookies. It was shown that the higher the addition of pomace was, the lower was the acceptance by potential consumers, but even with the 50% pomace addition the overall acceptability was at 5.76 (SA50) and 6.24 (EA50). The high scores may be related to the fact that apple pomace is a potential flavoring ingredient for short-crust pastry, which certainly affects its acceptance [Sudha *et al.*, 2007]. Studies aimed to evaluate apple pomace cookies were also conducted by Sudha *et al.* [2007], but in this case the maximum pomace addition was 30%. Other authors also evaluated the effect of apple pomace addition to bakery and confectionery products in terms of consumer acceptance [Sudha *et al.*, 2007, Usman *et al.*, 2020]. Products with 20% pomace addition usually had a pleasant apple taste and were well accepted by the panelists [Sudha *et al.*, 2007]. However, with the increase in pomace share in the cookie formula, their sensory characteristics were more often decreased; as low to moderate acceptability of cookies with 30%–50% pomace was reported [Usman *et al.*, 2020].

Interestingly, for each variant of cookies and each distinguishing feature, the use of erythritol instead of sucrose had a positive impact on the panelists' assessment (the difference in assessment between variants of cookies sweetened with sucrose and erythritol, depending on the distinguishing feature, ranged from 0.13 for the cookies without added pomace for overall acceptability to 1.08 for the products with 30% added pomace for taste) (**Figure 1**). A study by Laguna *et al.* [2013] led to different results, showing that replacing 50% sucrose with erythritol in shortbread cookies negatively affected consumer acceptance. These differences may be due to the fact that product analyzed in the present study were made with fruit pomace. The use of erythritol could affect the achievement of a cooling effect, which eliminated the aftertaste of the pomace.

Principal component analysis

Principal component analysis (PCA) was used to summarize the relationship between the variables (nutritional, bioactivity and sensory properties) and show the effect of apple pomace and sweetener addition in the design of a new snack product (**Figure 2**). The determined probability of the occurring dependencies

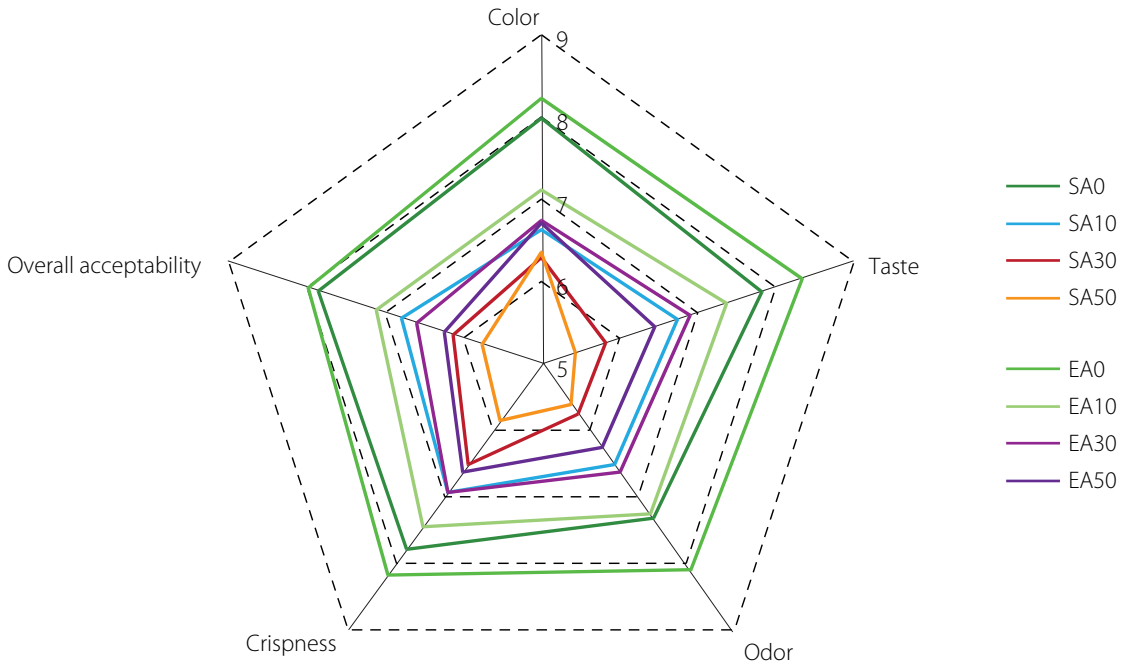

Figure 1. Consumer evaluation of shortbread cookies containing apple pomace. SA, sucrose-sweetened shortbread cookies with specific addition of apple pomace; EA, erythritol-sweetened shortbread cookies with specific addition of apple pomace; 0, 10, 30 and 50 after EA and SA, percentage (by weight) of replacing wheat flour with apple pomace in the cookie recipe.

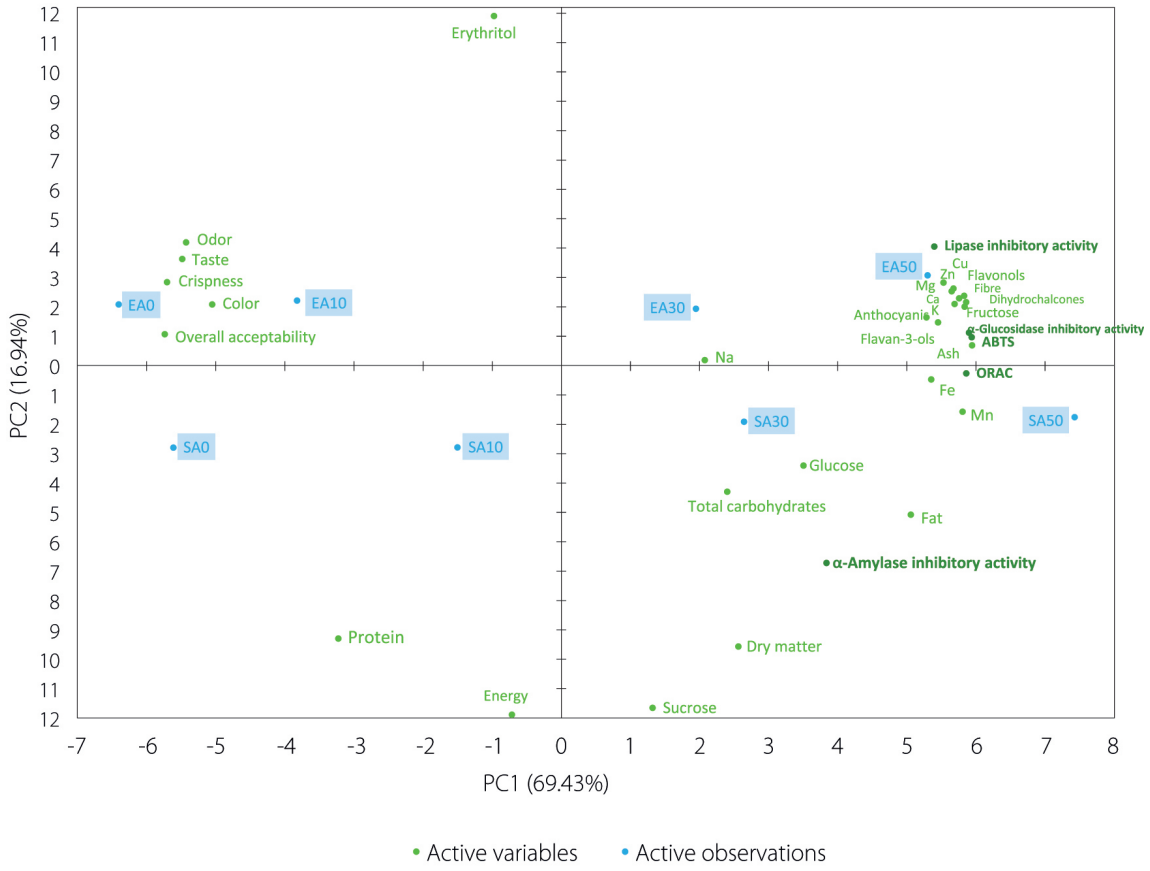

Figure 2. Principal component analysis (PCA) of the impact of erythritol/sucrose and apple pomace in cookies recipe on nutritional, bioactive and sensory parameters of cookies. SA, sucrose-sweetened shortbread cookies with specific addition of apple pomace; EA, erythritol-sweetened shortbread cookies with specific addition of apple pomace; 0, 10, 30 and 50 after EA and SA, percentage (by weight) of replacing wheat flour with apple pomace in the cookie recipe; d.m., dry matter.

(PC1 *vs.* PC2) was very high – 86.37%. Eigenvalues for PCA are shown in **Table S2** and **Figure S1** in Supplementary Materials. Both the addition of a sweetener (erythritol/sucrose) and the pomace had a significant effect on the active observation ranking of the cookies studied (**Figure 2**). The analyzed active observations were arranged opposite along the horizontal axis in the same order and distance. The acceptability of the analyzed sensory traits of products with erythritol and a low content of apple pomace (EA10) and without pomace (EA0) was higher compared to the other analyzed products. The highest addition of pomace was the least acceptable in this evaluation. In addition, a very strong relationship was observed between active observations (EA50 and SA50) and active variables, such as phenolic compounds (flavonols, dihydrochalcones, anthocyanins, flavan-3-ols), minerals (Zn, Cu, Mg, Ca, Na, Fe, Mn, K), antioxidant capacity (ABTS$^{\bullet+}$ scavenging activity and ORAC) and α-glucosidase inhibitory activity. Weaker impacts were measured between phenolic compounds or minerals and lipase inhibitory activity or α-amylase inhibitory activity. This was also confirmed by the correlation analysis, whose results are presented in **Table S1** in Supplementary Materials. In turn, PCA analysis showed that flavan-3-ols, anthocyanidins and dihydrochalcones exerted a stronger effect on α-glucosidase inhibitory activity and ABTS$^{\bullet+}$ scavenging activity than flavonols. By contrast, the activity of lipase inhibitory activity was influenced not only by flavonols but also by the presence of dietary fibers, and was unaffected by total carbohydrate or fat, and in the case of α-amylase inhibitory activity, the relationship was proportionally inverse. Protein content had no significant impact on the health-promoting potential of the analyzed products. It should be noted that the energy value was negatively correlated with the presence of erythritol and positively correlated with the content of sucrose.

CONCLUSIONS

The study compared 8 variants of shortbread cookies prepared with different addition levels of apple pomace and sucrose/erythritol. The most beneficial properties were obtained for the products with 50% added pomace. The content of dietary fiber increased about 8 times compared to the products without pomace addition, while the energy value decreased. Thirteen polyphenolic compounds, including quercetin glycosides, phloretin glycosides, cyanidin glycosides, procyanidins and chlorogenic acid, were identified in the cookies with added pomace. The antioxidant capacity and antidiabetic properties of cookies increased significantly as the share of pomace increased in cookie formula. The highest consumer acceptability was shown for the cookies without added pomace, but the cookies with 50% added pomace received relatively high scores. The highest scores given by panelists to SA0 and EA0 cookies result from the fact that the sensory values of traditional shortbread cookies (without the addition of other raw materials apart from the basic ones: wheat flour, butter, sweetener, egg yolks) are widely known and accepted by consumers. This is most likely due to a lack of a habit or an experience in consuming products with the addition of raw materials featuring functional properties. For each cookie variant, replacing sucrose with erythritol favorably influenced the consumer assessment. The study demonstrated that it was feasible to develop recipes of cookies that will offer high health benefits and at the same time will be accepted by consumers, which inscribes into a recent global trend that focuses on developing sustainable food formulas offering the mentioned values. Therefore, in the future, the possibilities of using fruit pomace should be expanded to minimize the problem of disposing its excess in an environmentally friendly way, while at the same time producing food of a high nutritional value and health quality, by incorporating this valuable functional ingredient into recipes.

ADDITIONAL INFORMATION

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CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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SUPPLEMENTARY MATERIALS

The following are available online at **http://journal.pan.olsztyn.pl/Effect-of-the-Addition-of-Apple-Pomace-and-Erythritol-on-the-Antioxidant-Capacity,187941,0,2.html**; **Table S1**. Spearman correlation analysis (*r*).; **Table S2**. Eigenvalues for principal component analysis.; **Figure S1**. Plot of eigenvalues and cumulative variability of principal component analysis.

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