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Antioxidative, sensory and volatile profiles of cookies enriched with freeze-dried Japanese quince (*Chaenomeles japonica*) fruits

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Abstract

The study aimed at assessing effects of freeze-dried Japanese quince fruit (FJQF; 0-9%) added to cookies to improve their antioxidant attributes during storage, sensory and volatile characteristics and acceptability by consumers. Cookies containing FJQF had 2-3.5-fold higher radical scavenging activity and exhubited less secondary lipid oxidation products compared with the control cookies. Enriched cookies had higher contents of volatile hexanal, heptanal, octanal, 2-heptenal, (E) than control cookies. Acetic acid dominated in the volatile profile of enriched cookies (ranging 7.05 – 23.37%), hence intensities of acidic and citrus aroma were scored high. Cookies stored for 16-weeks showed increased amounts of hydrocarbons as compared with fresh cookies and new hydrocarbons were also generated, which were not detected in fresh cookies. The consumer panel indicated a higher preference for cookies containing 1.0 and 1.5% FJQF than those containing 6.0 and 9.0%.

Keywords: Freeze-dried Japanese quince, Cookies, Antioxidative properties, Volatile compounds, Lipid oxidation products

Introduction

Many fruits are gaining attention due to attractive flavour, as well as to diverse antioxidant, anticarcinogenic and antimutagenic substances they contain. Among them is the Japanese quince fruit (JQF; *Chaenomeles japonica*), whose fruits have a specific flavor, thus being well suited for industrial processing (Antoniewska, Rutkowska, & Adamska, 2017; Lesinska, Przybylski, & Eskin, 1988); JQF is acid in taste due to high content of malic, quinic and succinic acids (about 3.5% equivalent of malic acid), and has high antioxidant capacity due to a high content of vitamin C and about 20 polyphenolic compounds (Ros, Laencina, Hellin, Jordán, Vila, & Rumpunen, 2004), among them there were five representative compounds: chlorogenic acid,

catechin, procyanidin B1, epicatechin, and procyanidin B2 (Du et al., 2013). Also, JQF is rich in dietary fibre (32 g/100 g dry fruit) with substantial pectin content, as high as that of apple (Thomas, Guillemin, Guillon, & Thibault, 2003). Dry matter content in such fruits depends on weather conditions and ranges 13–18%.

Strugała et al. (2016) reported that JQF extract protected membrane lipids of erythrocytes and liposomes from oxidation induced by physicochemical factors. Moreover, JQF extract proved to be a more potent inhibitor of inflammatory enzymes than nonsteroidal anti-inflammatory drugs: indomethacin and ibuprofen.

The unique sensory properties of JQF make it widely applicable in the food industry, e.g. in producing juices, jams, liquors, puree, smoothies and candied fruits. Moreover, it is added to teas, yogurts, lemonades, ice cream, cottage cheese, and confectionery in order to improve sensory properties thereof (Nawirska-Olszańska, Kucharska, Sokół-Łętowska, & Biesiada, 2010; Nowicka, Wojdyło, Teleszko, & Samoticha, 2016). Due to its characteristics and composition, quince fruit juice should be useful for the food industry, especially as an acidulant with high antioxidant properties (Ros et al., 2004). The uses of JQF were markedly improved by freeze-drying, a popular technique of prolonging the shelf life of fruits (Agudelo, Barros, Santos-Buelga, Martinez-Navarrete, & Ferreira, 2017).

In bakery and in cookies industry, antimicrobials, antioxidants and antibrowning additives are commonly used to preserve products for longer time. However, possible toxic properties of synthetic additives urge to search natural and safe alternatives. Many papers were devoted on using in cookie manufacturing ingredients deriving from fruits like black currant powder, papaya pulp flour, chokeberry extract, grape marc extract and sour cherry pomace extract, not only as a source of polyphenols and other nutritive compounds, but also as an effective antioxidant (Bialek, Rutkowska, Bialek, & Adamska, 2016; Molnar, Brnčić, Vujić, Gyimes, & Krisch, 2015;

Pasqualone, Bianco, Paradiso, Summo, Gambacorta, & Caponio, 2014a; Šaponjac et al., 2016; Varastegani, Zzaman, & Yang, 2015).

In this study, various additions of freeze-dried Japanese quince (*Chaenomeles japonica* (Thunb.) Lindl. ex Spach) fruits were applied to obtain functional cookies with promising antioxidant attributes ensuring safety during storage, unique sensory and volatile characteristic and a high acceptability by consumers.

2. Materials and Methods

2.1. Preparation of freeze-dried Japanese quince fruits

Japanese quince (*Chaenomeles japonica* [Thunb.] Lindl. ex Spach) fruits were obtained from the Polish Academy of Sciences Botanical Garden – Center for Biological Diversity Conservation in Powsin (Warsaw, Poland). Harvesting was done manually when the fruits reached full maturity (September 2017). The collected fruits were surface-cleaned with a clean and dry cotton cloth and then washing in water; seed nests were manually removed, and fruits were freeze-dried using Christ Alpha 1-2 LDplus lyophilizer (Martin Christ Gefriertrocknungsanlagen GmbH, Germany). The freeze-dried Japanese quince fruits (FJQF) were ground to powder (mean particle size at around 280 μm) using a household grinder and used to prepare cookies in relative amounts appropriate to the recipe.

2.2. Preparation of cookies

Cookies were prepared in seven variants differing in FJQF content. Control cookie samples (C-cookies, C-samples) were prepared without FJQF (0%), whereas enriched cookies contained 0.5, 1.0, 1.5, 3.0, 6.0 or 9.0% of FJQF. Ingredients for cookie manufacturing were purchased from a local market (Warsaw, Poland).

The cookie making process consisted of mixing refined wheat flour (608 g) containing 0.4 g/100 g ash, margarine (133 g) and powdered sugar (117 g), using a mixer (Zelmer ZFP1100C, Poland) for 5 min, adding egg yolks (142 g) and mixing for 3 min again. For preparing other 6 variants of

dough, the amounts of wheat flour were reduced according to FJQF addition to obtain enriched cookies.

The dough was cooled down in a refrigerator (1 h at 4 °C), then cut into slices 5 mm thick, from which circular shapes, 50 mm wide, were cut. Baking was carried out at 170 °C for 17 min in an electric oven (Hendi 225516, The Netherlands). After cooling (24 h from baking), the cookies were packed in ecological cellulose film (20 μm thickness, permeability to: water vapour – 20 g/m^2. 24 h, oxygen – 5 cc/m^2. 24 h) packages, eight cookies each. Next, packages with cookies were put into cardboard box without access of light, and kept at 20 ± 1°C for 16 weeks.

2.3. Chromatographic analysis of vitamin C

Vitamin C was extracted with meta-phosphoric acid and perchloric acid solution. The mixture was centrifuged at 5000 rpm for 5 min and filtered through filter paper (POCH, S.A., Poland). The chromatographic separation was performed on HPLC apparatus (Waters Corporation, USA) equipped with a column (Symmetry RP C18; 150 mm, ID 4.6 mm, dp 5 μm). Vitamin C was detected using a DAD detector (Waters 2487, Waters Corporation, USA) at 245 nm wavelength. The mobile phase was a mixture of aqueous solutions of $(NH_4)H_2PO_4$ (80/20, v/v) and meta-phosphoric acid, the flow rate amounting to 0.8 mL/min. The injection volume of the sample uploaded to HPLC was 10 μL. The sum of ascorbic and dehydroascorbic acids (vitamin C content) was determined after reduction of dehydroascorbic acid to ascorbic acid using dithiothreitol.

2.4. Determination of carotenoids and chlorophylls

Carotenoids were extracted from FJQF with hexane/ethyl ether 1:1 (v/v). Unsaponificable matters (UM) were isolated by saponification of extracted lipids overnight at room temperature using 0.5 N NaOH in methanol. The UM were extracted from soap solutions three times with hexane/ethyl ether 1:1 (v/v). The solvents were removed on a rotary evaporator at 40 °C.

Chlorophylls were extracted as follows*:* 3g of FJQF was mixed in a mortar with anhydrous sodium sulfate. The powder was placed on the filter paper and flushed with 20 ml of ethyl ether twice. Than the filtrate was transferred to a round bottom flask and densified using a rotary evaporator.

Total carotenoids and chlorophylls were determined spectrophotometrically using a Specord 40 (Analytik Jena AG, Germany) device. Light absorbance at 668 nm (chlorophylls) and at 442 nm (carotenoids) was measured.

2.5. Chromatographic analysis of polyphenol compounds

Polyphenols were extracted from FJQF with 30% methanol acidified with 1% acetic acid and containing 1% ascorbic acid. The extraction was performed by incubation for 20 min under sonication (Sonic 6D, Polsonic, Warsaw, Poland). The details of sample preparation were presented elsewhere (Kolniak-Ostek & Oszmiański, 2015).

Analysis of polyphenols was carried out using an ACQUITY Ultra Performance LC system (UPLC) equipped with a photodiode array detector (PAD) and with a binary solvent manager (Waters Corporation, Milford, USA), coupled with a quadrupole time-of- flight (Q-TOF) micro Mass Spectrometer (MS; Waters, Manchester, UK) with an electrospray ionization (ESI) source operating in negative and positive modes. Separation of individual polyphenols was carried out using a UPLC BEH C18 column (1.7 μm, 2.1 mm x 50 mm, Waters Corporation, Milford, MA, USA). The mobile phase consisted of aqueous 0.1 % formic acid and 100 % acetonitrile. Samples (10 μl) were eluted according to the linear gradient described previously by Oszmiański, Kolniak-Ostek and Biernat (2015). Detection wavelengths were set to 280 nm (flavan-3-ols and hydroquinones), 320 nm (phenolic acids), 340 nm (flavones) and 360 nm (flavonols).

The conditions of MS were: a source block temperature of 130°C, desolvation temperature of 350 °C, capillary voltage of 2.5 kV, cone voltage of 30 V and a desolvation gas (nitrogen) flow

rate of 300 l/h. Calibration curves were prepared according to Kolniak-Ostek (2016). All experiments were done in triplicate. The results were expressed in milligrams per 100 g of dry matter.

2.6. Determination of antioxidative properties of FJQF and cookies

Ethanolic extracts of FJQF and cookies for determining radical scavenging activity and total phenolic content were prepared as previously reported (Antoniewska, Rutkowska, Martinez Pineda, & Adamska, 2018). Total phenolic content (TPC) was analyzed using Folin-Ciocalteu's reagent at 725 nm with gallic acid as the standard, according to Singleton and Rossi (1965) procedure with modifications (Antoniewska et al., 2018). Radical scavenging activity of examined extracts on 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH) was analyzed according to the modified method of Sanchez-Moreno, Larrauri, and Saura-Calixto (1998) presented in detail by Antoniewska et al. (2018) using the Specord 40 (Analytik Jena AG, Germany) device.

The antioxidant activity was expressed as percent capacity of scavenging the DPPH radical according to the following equation:

$$\% \text{ DPPH} = (A_{DPPH} - A_t)/A_{DPPH} \times 100$$

where A_{DPPH} – absorbance of the blank sample, A_t – absorbance of the analysed sample.

2.7. Determination of lipid oxidation products in cookies

Fat was extracted from cookies according to Folch, Lees, and Sloane-Stanley (1957), using a 2:1 chloroform/methanol (v/v) mixture. Stability of the lipid fraction of cookies was analyzed at two-week intervals. The hydroperoxide value (PV) as the content of primary products of lipid oxidation was determined by a standard titration method (ISO 3960, 2012) and the PV was expressed as mEq O/kg of fat. Anisidine value (AnV) as the content of carbonyl compounds was determined spectrophotometrically (Specord 40, Analytik Jena AG, Germany) at 350 nm according to ISO 6885 (2008).

2.8. Microbiological analysis of cookies

The numbers of mold and yeast colonies were determined according to PN-ISO 21527-2 (2009) and to previously reported procedure (Antoniewska et al., 2018).

2.9. Determination of volatile compounds

Volatile compounds of FJQF and of cookies were determined by headspace solid phase micro-extraction (SPME) coupled with gas chromatography/mass spectrometry (GC/MS) (6890N GC, 5975 MS Agilent, USA). Before analysis, the SPME fiber 50/30 μm coated with Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) (Supelco, Bellefonte, PA, USA) was conditioned by heating in a GC injection port at 270 °C.

Samples (5 g) were placed in 20 mL vials, closed with silicone-teflone sealing cap and heated at 40 °C for 30 min to stabilize concentrations of volatiles in the headspace. The extraction of volatile compounds was carried out by exposing SPME fiber in the headspace of the sample at 40 °C for 40 min. Then, the SPME fiber was quickly transferred to the GC injection port operating in the split-less mode.

Volatile compounds were separated on a HP-5MS column (30 m × 0.25 mm × 0.25 μm film thickness, 5%-diphenyl-95%-polydimethylsiloxan; Agilent, USA). Helium was used as the carrier gas with a linear velocity of 0.9 mL/min. Chromatographic separation was conducted as follows: oven temperature was held for 10 min at 38 °C, then increased up to 200°C (4 °C/min gradient) and held for 2 min, than raised to 250 °C at 20 °C/min, and that final temperature was held for 7 min. The MS was programmed as follows: interface 150 °C, source 230 °C, ionization energy 70 eV with a multiplier voltage of 1670 V, and scanning range 33-350 m/z (amu). Data were acquired using MSD ChemStation program (Agilent, USA). Individual peak identification was based on the comparison of their mass spectra with the reference mass spectra of National Institute of Standards and Technology (NIST 08) and Wiley 8th Ed. libraries. Mass spectra of respective volatiles were affirmed by comparing linear retention indices (LRI) calculated (using Automated Mass Spectral Deconvolution and Identification System software, USA) relatively to

a series of standard alkanes (alkane standard solution C8-C20, Sigma-Aldrich) with NIST 08 library LRI database. The quantities of volatile compounds were expressed as percentages of the total identified signal. The analyses were carried out in triplicates.

2.10. Sensory evaluation of cookies

The sensory evaluation of cookies was conducted using an unstructured 10 cm linear scale ranging from "no intensity" to "very high intensity" according to Baryłko-Pikielna and Matuszewska (2014). Ten experienced panelists (5 males and 5 females; 30-42 years of age) who participated in the study scored the intensity of every attribute three times in the following order: aroma (buttery, acid, citrus and off-aroma), taste (buttery, sweet, acid, citrus), color "light" to "dark" and fracturability. After cooling, the cookies were delivered in coded plastic containers in a random order. Every panelist was provided with spring water to cleanse the palate between tasted samples. The results from the analogous scale were converted to numerical values (0 to 10 units). The assessment was conducted twice: at the beginning (24 h following baking) and after 16 weeks of storage.

The overall consumer's degree of liking cookies was estimated using a hedonic category scale. The consumer panel consisted of 42 male and 83 female subjects (faculty students aged 20-26 years). Cookies were evaluated using nine-point structured hedonic scale with the extremes "dislike extremely – 1" and "like extremely – 9".

2.11. Data analysis

The results of sensory analysis, lipid oxidation products and antioxidative potential were subjected to two-way ANOVA followed by Tukey's post-hoc test, the level of $P < 0.05$ being considered significant. Statistical analyses were performed with GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA). All results for given volatile compounds were graphically

related to the amounts of FJQF (0-9%) added to cookies and R^2 values were computed for the regression (0, 1st or 2nd degree) fitting the data.

3. Results and discussion

3.1. Antioxidant properties of FJQF

FJQF proved to be remarkably rich in antioxidant compounds (Table 1), especially in vitamin C (287 mg/100 g), that latter being comparable with freeze-dried sea buckhorn fruits, 2-times higher than in freeze-dried cranberry (Sadowska, Dybkowska, Rakowska, Hallmann, & Świderski, 2017) or blackcurrant, and five times as high as in oranges and strawberries (Hägg, Ylikoski, & Kumpulainen, 1995). Carotenoids content in FJQF was higher than in apples or bananas, but 3–4-fold lower than in watermelon or apricot (Saini, Nile, & Park, 2015).

Application of UPLC/PDA/MS technique enabled determining the contents of 11 phenolic compounds in FJQF belonging to 3 groups, with phenolic acids dominating, compared with flavan-3-ols and flavonols (total content: 3630 mg/100 g FJQF). Phenolic profile of FJQF contained valuable protocatechic acid (about 1364 mg/100 g FJQF), considered to be an effective antioxidant in vitro in both lipid and aqueous media than BHT - the strongest synthetic antioxidant used in bakery products (Li, Wang, Chen, & Chen, 2011). The second most abundant compound was caffeic acid, for which high intestinal absorption in cell lines (intestinal ischemia–reperfusion model) was demonstrated. Since caffeic acid has a stronger antioxidant activity than that of chlorogenic acid, and that latter undergoes hydrolysis into caffeic acid in the intestine, it is possible that caffeic acid plays a major role in the protective effect of chlorogenic acid against ischemia–reperfusion injury (Sato et al., 2011).

Also, spectrophotometric, rapid assay using TPC (Granato et al., 2018) confirmed high content of phenolic compounds in FJQF - 4165 mg GAE/100 g of FJQF. Such impressive value of TPC measured using Folin-Ciocalteu assay was also reported in fresh fruits Japanese quince (about

2000 mg GAE/100 g) (Du et al., 2013). Thus antioxidant activity of FJQF measured by the DPPH method (Table 1) was remarkable and markedly exceeded that reported by other authors in Japanese quince syrup, candied fruit slices or jams enriched with JQF (Nawirska-Olszańska et al., 2010).

Insert Table 1

The antioxidant capacity of FJQF depended on the synergy of diverse compounds – ascorbic acid and polyphenols, possessing high number of hydroxyl groups: epicatechin, epigallocatechin gallate, epigallocatechin and procyanidines A2 and B2 (921 mg/100 g FJQF). Previous studies indicated the crucial role of flavan-3-ols and procyanidins as major determinants in creation of antioxidant potential of JQF fruits (Du et al., 2013; Strugała et al., 2016). Those authors also showed that JQF extract inhibited the pro-inflammatory enzymes of the cyclooxygenase group.

3.2. Volatile compounds of FJQF

The analysis of volatile compounds in the headspace of FJQF using SPME yielded a total of 35 identified and quantified compounds (Table 1). The identified volatile compounds consisted of aldehydes (10 compounds), terpenes (10), ketones (5), alcohols (6), carboxylic acids (3), furan compounds (3) and other compounds (3). Carboxylic acids predominated in FJQF, the principal one being acetic acid (58.36%), the next two – octanoic and hexanoic acids. According to published reports, those compounds were not detected in volatile profile of the fresh juice obtained from *Chaenomeles* taxa, or in fruits of Chinese quince *Pseudocydonia sinensis* (Jordán, Vila, Hellin, Laencina, Rumpunen, & Ros, 2003; Choi, Lee, Lee, & Kim, 2018). However, acetic, malic, formic and succinic acids were assayed in quince fruits using GC in substantial amounts, and considered responsible for disapproval of the fresh fruits. The high content of

acetic acid in quince was due to over-ripening, while organic acids provide information about maturation stage of the fruit (Trigueros, Pérez-Alvarez, Viuda-Martos, & Sendra, 2011). Our results were, generally, in agreement regarding aldehydes, however some qualitative differences were found (Jordán et al., 2003). Aldehydes are probably derived from unsaturated fatty acids, e.g. linolenic and linoleic acids, via oxygenation and sequential transformation by lipoxygenase during maturation of fruits (Choi et al., 2018). Volatile compounds detected in FJQF, such as 2-hexenal, (E) and hexanal, proved strongly antimicrobial activity towards pathogen microorganisms at low concentrations (Ayseli & Ayseli, 2016).

3.3. Antioxidant properties of cookies

The enrichment of cookies with FJQF resulted in a significant increase in antioxidant properties as compared with C-samples (Fig. 1). It should be pointed out that the addition of 0.5% FJQF to cookies resulted in two-fold increase of the radical scavenging activity, whereas highest addition (9%) resulted in 3.5-fold increase, as compared with C-samples (DPPH values: 22.58% - C-cookies, 81.94% - cookies with 9% FJQF). Summing up, higher level of incorporation of FJQF into cookie formulation resulted in improving the antioxidant activity of the final product (Fig.1), like when using papaya or chokeberry (Bialek, Rutkowska, Bialek, & Adamska, 2016; Varastegani et al., 2015). Some polyphenols in FJQF, like epicatechin, significantly promoted the antioxidant capacity accompanied by a strong inhibition of glycation toxicants formation (Zhang, Chen, & Wang, 2014). Our results confirmed the reports that high contents of functional ingredients rich in polyphenols are associated with increased antioxidant activity of biscuits and muffins due to high amounts of polyphenols (Antoniewska et al., 2018; Krystjan, Gumul, Ziobro, & Korus, 2015).

It should be also noted that highly enriched cookies (9% FJQF) exceeded FJQF in DPPH activity (Fig 1). That indicated an important role of not only polyphenols, but also of compounds formatted during Maillard reaction (MR), in the antioxidant potential of cookies. Similar results

were obtained by Varastegani et al. (2015) in cookies highly substituted with papaya pulp flour. Other studies revealed that MR products, mainly melanoidins assayed in bakery products, were able to scavenge the peroxyl and ABTS radicals (Michalska, Amigo-Benavent, Zielinski, & Dolores del Castillo, 2008; Nooshkam, Varidi, & Bashash, 2019).

During the 16 weeks of storage, a significant decrease ($P < 0.05$) in radical scavenging activity of cookies was noted (Fig. 1). These observations are consisted with the findings of other authors who reported decreased antioxidant capacity in cookies enriched with cherry pomace encapsulated in whey and soy proteins, black currant powder and chocolate coat, and in muffins containing buckwheat flakes and amaranth flour blend (Antoniewska et al., 2018; Molnar et al., 2015; Šaponjac et al., 2016).

The inclusion FJQF, rich in polyphenols, into cookies, reduced the decrease of antioxidant capacity of enriched, stored cookies; those containing 9 or 1.5% FJQF lost only 15% of DPPH (Fig.1) in contrast to C-cookies (33% loss of DPPH). Moreover, FJQF contained procyanidins, regarded as stabilizing compounds. It was reported that the decomposition rate of phenolic compounds depends on their structure, the decrease of polymeric procyanidins being lower than that of other phenolic groups (e.g. anthocyanins) during a 6-month storage in smoothies obtained from different fruits (Nowicka et al., 2016). The high loss of anthocyanins (66.67%) was also observed in cookies enriched with pomace extract and stored for 4 months (Šaponjac et al., 2016).

3.4. Oxidative stability of cookies during storage

Oxidative stability of fat extracted from cookies, expressed as amounts of hydroperoxides measured by PV, was significantly ($P<0.05$) related to the FJQF content and storage time (Fig. 1). As compared with C-samples, lower amounts of hydroperoxides were found in enriched cookies containing 0.5 to 1.5% FJQF due to the presence of phenolic and other compounds having antioxidant potential. Similar results were reported for enriched cookies and bakery

products with plant components rich in antioxidants: green and yellow tea leaves, chokeberry extract, cherry pomace extract, black currant and jostaberry powder as natural ingredients to inhibit lipid oxidation (Bialek et al., 2016; Gramza-Michałowska et al., 2016; Molnar et al., 2015; Šaponjac et al., 2016). Also, substitution of wheat flour by other plant ingredients (e.g. oat, buckwheat, purple wheat, papaya pulp flour) in formulas was made in order to improve antioxidant properties of bakery products (Cognat, Shepherd, Verrall, & Stewert, 2014; Pasqualone et al., 2015; Varastegani et al., 2015).

Insert Fig. 1

We noted that higher amounts of FJQF in the formula were associated with higher values of PV, especially when cookies were enriched with 6 or 9% of FJQF (Fig. 1). This observation is in agreement with results of other authors who found that plant components rich in antioxidants used in formulas may act as a pro-oxidants in cookies (Bialek et al., 2016).

Oxidative stability of fat extracted from cookies was studied also by measuring secondary oxidation products using AnV method. Generally, FJQF-enriched cookies exhibited high AnVs due to the presence of volatile compounds with carbonyl groups, that reacted with p-anisidine. This is shown by AnVs in fresh cookies, which ranged from 2.74 to 12.98 (Fig. 1, Table 1).

Our results support the hypothesis that hydroperoxides - primary lipid oxidation products – may undergo degradation, leading to the generation of a range of secondary products, such as aliphatic aldehydes, alcohols, ketones and hydrocarbons. We found that after the 12-week storage, the breakdown of hydroperoxides in fat extracted from cookies was manifested by decreased PV and, simultaneously, by increased AnV (Fig. 1). It could have resulted from the oxidation of unsaturated fatty acids (mainly PUFA) in the lipid fraction of cookies as was previously reported in oat cookies and in muffins enriched with pumpkin seed flour during

storage (Bialek, Rutkowska, Adamska, & Bajdalow, 2016; Cognat et al., 2014). In our study, this phenomenon was manifested by increased AnVs in fat extracted from cookies stored for 12 weeks as compared with AnV of fresh cookies. In the case of C-cookies, a 4.5-fold increase of AnVs was noted, whereas in enriched cookies the increase of AnV was lower, 2- to 3-fold, depending on FJQF content.

3.5. Sensory and consumer evaluation and microbiological profile of cookies

Addition of FJQF into the cookie formulation significantly ($P < 0.05$) affected sensory rating of attributes of cookies (Table 2). Intensity ratings of buttery aroma decreased with increasing the content of FJQF, while acidic and citrus aroma increased, due to the presence of volatile compounds in FJQF (Table 1). Off-aroma was rated very low (0.80-1.61 scores) even in highly enriched cookies, probably because of a high intensity of acidic taste (Table 2). Increasing amount of FJQF in the formulation significantly ($P < 0.05$) decreased the intensity of buttery and sweet tastes of cookies with simultaneous increase in the intensity of acidic and citrus taste. Cookies containing highest amount of FJQF (9.0%) had low intensity of buttery and sweet tastes, associated with strong intensity of acid taste and aroma.

Insert Table 2

The ratings of cookie color increased with increasing level of cookie enrichment with FJQF. Similar effect was reported for cookies supplemented with sour cherry pomace extract (Šaponjac et al., 2016) and bee pollen (Krystjan et al., 2015). Like in case of the antioxidant potential, it could be affected not only by the content of FJQF, but also by the Maillard and caramelization reactions during the baking process (Molnar et al., 2015).

Incorporation of FJQF reduced the rating of cookie fracturability from 7.58 for C-samples to 3.3 for samples containing 9.0% FJQF (Table 2). It could have resulted from the dominating share of fibres (32g/100g dry fruit), especially of pectins and cellulose-like polysaccharides, in the carbohydrate composition of FJQF (Thomas et al.,2003).

Generally, the storage of cookies significantly ($P < 0.05$) affected rating intensities of many sensory indices (Table 2). Stored cookies were rated lower regarding buttery aroma and taste as compared with fresh cookies. Decreased intensity of acidic and citrus aromas were scored in cookies contained higher FJQF amounts (1.5-9.0%). Also the sensory panel found a significant decrease of intensity of acidic, citrus and sweet tastes after storage but not for all samples. Storage did not significantly influence rating intensity of off-aroma, except C-cookies and those containing 6 and 9% FJQF. Storage significantly affected fracturability rating: cookies became softer, slightly gummy and less fragile than fresh samples, especially those with higher amount of FJQF (6 and 9%). This resulted from increased fiber content in highly enriched cookies, as fibers markedly contribute to the hydration properties associated with hydroxyl groups in the fiber structure, thus enabling more water interactions via hydrogen bonding (Varastegani et al., 2015).

The ratings of acceptability of cookies by young consumers are shown in Table 3. Hedonic acceptability of cookies was significantly ($P < 0.05$) related to FJQF content in the formulation. In general, the consumer panel indicated a higher preference for cookies containing 1.0 and 1.5% FJQF than for containing higher amounts of FJQF (6 and 9%). About 20% consumers scored cookies containing highest amount of FJQF as “dislike a lot”, and about 6% as “dislike extremely”. No such ratings pertained to cookies containing 1, 1.5 or 3% of FJQF. The low hedonic preferences of acceptability of cookies containing 6 or 9% of FJQF was probably due to high acidic taste, low buttery taste and low fracturability. Similar effect was found in cookies in which wheat flour was substituted by orange pulp (5, 15 and 25g /100 g of flour). The

acceptability of highly substituted cookies (25 g/100 g flour) was scored lower (4.89 points) than those containing lower amount of orange pulp (7.28 points) (Larrea, Chang, & Martinez-Bustosc, 2005).

Insert Table 3

Throughout the 16 weeks of storage, no yeast or molds, with counts exceeding 10 (colony forming units) CFU/g, were found in cookies. An increased level of molds (3.5 x 10^4 CFU/g, data not shown) was found only in C-cookies stored for 18 weeks. Therefore, samples stored for over 16 weeks were not subjected to sensory evaluation or further analyzes. The use of plant materials rich in phenolic compounds may effectively extend the shelf life of products by reducing or eliminating spoilage by microorganism (yeast and molds) and also may preserve the foods by reducing lipid oxidation, as it was reported to have significant antioxidant activity (Negi, 2012). Having in mind this statement and our results, enrichment of cookies with FJQF may become a good alternative to synthetic additives.

3.6. Volatile compounds of cookies

The variety of volatile compounds in cookies, determined by SPME/GC-MS, showed a dependence on FJQF content, as well as to oxidative and thermal reactions. Those compounds included aldehydes (18), ketones (2), carboxylic acids (6), furanes (4), hydrocarbons (23), alcohols (5), terpenes (4), esters (4) and pyrazines (2). Three groups of volatile compounds in cookies were distinguished: volatiles detected only in fresh cookies, volatiles detected in fresh as well as in stored ones, and those detected only in stored cookies (Table 4). Many of them were previously identified in enriched bakery products (Cognat et al., 2014; Mohsen, Fadel, Bekhit, Edris, & Ahmed, 2009; Pasqualone et al., 2014a; 2015).

Insert Table 4

Among volatiles detected only in fresh cookies, acetic acid was the most abundant one (Fig. 2). As expected, it was present in FJQF-enriched cookies at levels up to over 6-fold higher than in C-cookies depending on the amount of FJQF, and was responsible for strong acid taste and aroma of cookies (Tables 2 and 4). The origin of low amounts of acetic acid detected in C-cookies could be the oxidation of ethanol (Pasqualone et al., 2014a).

The fresh enriched cookies were rich in volatile aldehydes: hexanal, octanal, 2-heptenal, (E) and 2-octenal, (E). The contents of those compounds increased with increasing FJQF content as confirmed by high R^2 values (Table 4, Fig. 2). Other identified aldehydes: nonanal, 2-nonenal, (E) and decanal were detected at significantly higher levels in C-cookies than in enriched cookies, as they were apparently formed as secondary lipid oxidation products in the baking process (Cognat et al., 2014). While C6 aldehydes were characterized by pleasant odor notes, other ones like heptanal, 2-heptenal, (E), octanal and nonanal were responsible for off-flavors (Pasqualone et al., 2015). Addition of FJQF to cookies contributed to their citrus aroma and taste generated by two terpenes – D-limonene and (+)-3-Carene (Fig. 2, Table 4).

Insert Fig. 2

Maillard reaction (MR) contributed to the formation of several volatile compounds: Strecker aldehydes, furan compounds, pyrazines and pyrydines. The most abundant among MR products was furfural, responsible for the sweet aroma of cookies. Furanones are mainly associated with caramel-like, sweet, fruity and burnt odor impression (Mohsen et al., 2009). Significantly higher levels of furfural were detected in cookies enriched with FJQF than in C-cookies. Also cookies with FJQF distinguished by higher amounts of other furan compounds

(e.g. 2-furanmethanol). This difference was probably due to different pH of the two types of cookies, because pH values lower than 7 (such as in the enriched cookies) favor the formation of furan-derivatives, while higher pH induces preferential formation of pyrazines (Jousse, Jongen, Agterof, Russel, & Braat, 2002; Pasqualone et al., 2014a). Thus, no pyrazines were detected in enriched cookies contained highest FJQF addition. An inhibitory effect of acetic acid on pyrazine formation was previously reported (Pasqualone et al., 2014a). Three Strecker aldehydes were detected in cookies: 2-methylbutanal, 3-methylbutanal, benzaldehyde; these could have been derived from some amino acids, e.g. leucine, isoleucine or phenylalanine (Fig. 2; Mohsen et al., 2009; Pasqualone et al., 2015). Intermediates from MR were shown to act as free radical scavengers, inhibiting the propagation of free radicals (Mohsen et al., 2009). In this study, the antioxidant properties of cookies were derived not only from FJQF, but also from the generation of MR products during baking.

Finally, 2,2,4,6,6-pentamethyl-heptane was detected in substantial amounts; its content decreased with increasing FJQF content in cookies, probably due to flour contamination. Also, Pasqualone, Paradiso, Summo, Caponio and Gomes (2014b) identified 2,2,4,6,6-pentamethyl-heptane in semolina samples and ascribed their exogenous origin to the production process and transportation to pasta factory.

The storage of cookies for 16 weeks contributed to changes in the contents of many volatile compounds and also generated formation of new compounds (Table 4). In general, the hydrocarbons showed a noticeable increase in comparison with their yields in fresh cookies (R^2 =0.677 and R^2=0.431). Hexane was the predominant compound in stored cookies, their content increasing 5 – 10-fold (depending on the content of FJQF) as compared with fresh cookies (Fig. 2). Moreover, 12 new hydrocarbons, not detected in fresh cookies, were generated during storage, e.g. isooctane and heptane, whose content was higher in C-cookies than in enriched cookies. Regarding aromatic hydrocarbons, in stored cookies the level of toluene increased and

styrene was generated, although their levels were generally low (Table 4). Their generation resulted from the decomposition of hydroperoxides, very labile species that may undergo degradation generating a complex array of secondary products, such as aliphatic aldehydes, hydrocarbons, alcohols, ketones and organic acids (Bialek et al., 2016). Hydrocarbons (heptane, octane) are perceived as products of oleic acid autooxidation (Cognat et al., 2014). The process of autoxidation of lipids also sparked the creation of four new carboxylic acids (pentanoic, hexanoic, octanoic, nonanoic), not detected in fresh samples (Table 4).

The dominating new compound in stored cookies was ethyl acetate, whose retention time (2.38 min, data not shown) indicated that it was generated from acetic acid via esterification with alcohol (Fig. 2). The storage-induced generation of ethyl acetate in wheat flour was reported (Dong, Hu, Sun, Zhang, Wu, & Wang, 2018). Also two other new esters in stored cookies were detected, probably due to esterification of butanoic acid.

In stored cookies, hexanal and butanal were detected in low quantities. These two aldehydes, as well as pentanal, 2-hexenal and 1-pentanol, are responsible for the perception of rancidity of cookies and bakery products, as linoleic acid - of oxidative degradation products (Cognat et al., 2014; Mohsen et al., 2009). Because of their low levels in enriched cookies, sensory panelists scored the off-aroma in cookies low. Also, the two mentioned aldehydes (2-heksenal-2E and 2 hexenal-E) were detected only in C-cookies.

During storage, the contents of three Strecker aldehydes and of other MR compounds – furfural and 2-furanmethanol in cookies, decreased (Fig. 2). It was also confirmed by sensory lower intensity of buttery and sweet aroma of stored cookies as compared with fresh cookies. The decrease of aldehydes in stored cookies most likely resulted in lowering scoring intensity of buttery aroma of cookies (Table 2).

Summing up, we noted increased levels of hydrocarbons in stored cookies, as well as new hydrocarbons arising during storage. We thus regard hydrocarbons as process-related contaminants of cookies.

Conclusions

- Enrichment of cookies with freeze-dried Japanese quince fruits significantly improved the antioxidant potential of cookies as shown by a 2–3.5 fold increase in DPPH values in cookies (depending on the content of fruits). Also, the addition of freeze-dried Japanese quince fruits substantially decreased the loss of antioxidant potential during the storage for 16 weeks as compared with the control cookies.

- The content of the freeze-dried Japanese quince fruits had a marked effect on the volatile profile of cookies, namely higher contents of hexanal, heptanal, octanal, 2-heptenal, (E) than in the control cookies. These compounds were not derived from the oxidation of cookies but from the added fruits. Acetic acid was dominating in the volatile profile of enriched cookies; that was associated with acidic taste and higher amounts of furan compounds as compared with control cookies. Storage of cookies resulted in a noticeable increase in hydrocarbons as compared with their yields in fresh cookies; also, new hydrocarbons not detected in fresh cookies, were generated resulting in low scores of off-aroma in stored cookies.

- The unique aroma and taste of freeze-dried Japanese quince fruits are responsible for giving strong intensity of acid and citrus aroma and taste, masking others tastes and aromas in cookies, especially in those highly enriched. The consumer panel indicated a higher preference for cookies containing 1 and 1.5% of freeze-dried Japanese quince fruits than those containing higher amounts: 6 and 9%.

- The obtained results evidenced a positive effects of using freeze-dried Japanese quince fruits in manufacturing cookies containing no artificial additives: higher radical scavenging activity, prevented generation of lipid-derived compounds, unique sensory attributes and high

acceptance by consumers. Freeze-dried Japanese quince fruits could be regarded as a possible solution to extend the shelf-life of cookies, enhancing their microbiological safety during storage.

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Declaration of interests

The authors of the present work declare no conflict of interests.

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Tables and figures captions:

Table 1: Components of freeze-dried Japanese quince fruits (FJQF)

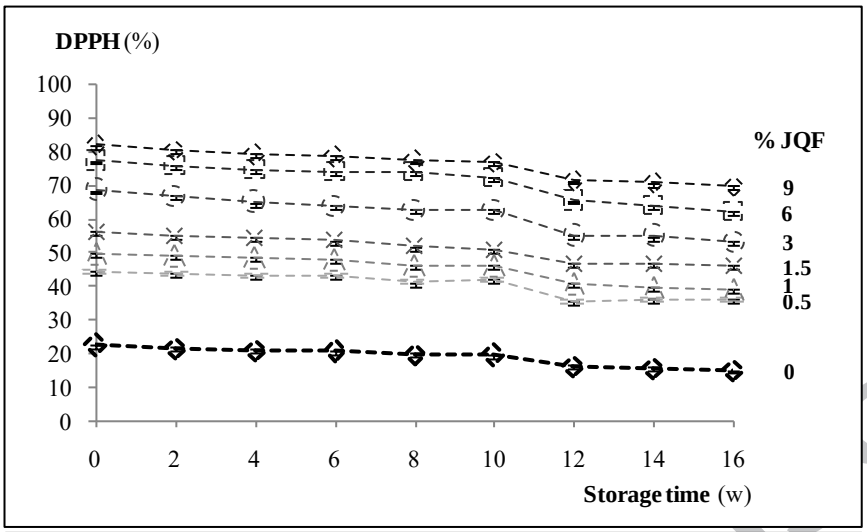
Table 2: Sensory intensity of attributes of the enriched (0.5-9%) and control cookies (0%) – fresh and stored for 16 weeks

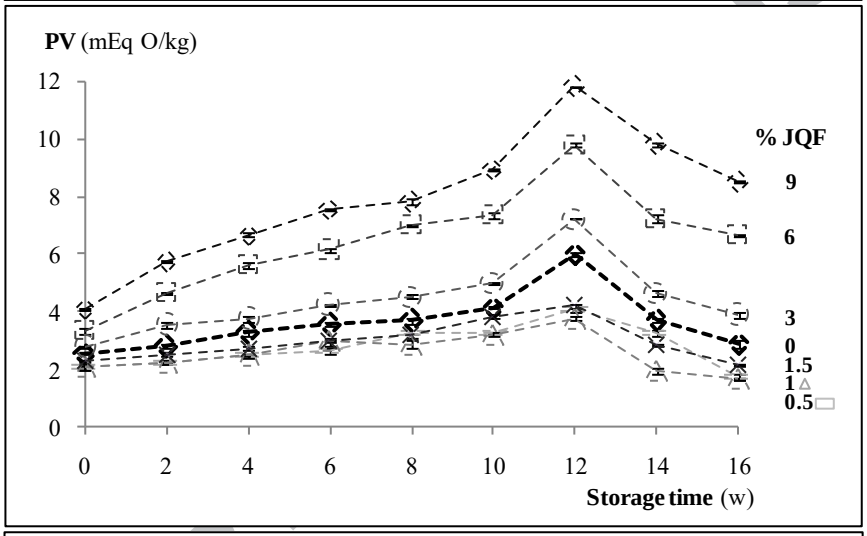
Table 3: Assessment of hedonic acceptability of the enriched (0.5-9%) and control cookies (0%) (n=125)

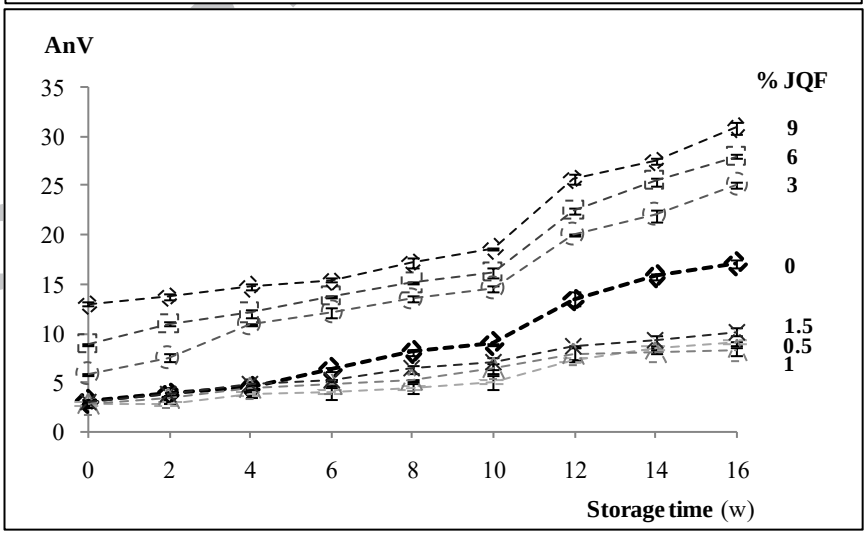
Table 4: Volatile compounds of the enriched (1-9%) and control cookies (0%) - fresh and stored for 16 weeks (means ± SD, n=3)

Figure 1. Radical scavenging activity on DPPH (DPPH, %), hydroperoxide value (PV, mEq O/kg fat) and anisidine value (AnV) of the enriched (0.5-9%) and control cookies (0%)

Figure 2. Volatile compounds of the enriched (0.5-9%) and control cookies (0%) - fresh (●) and stored (▲) for 16 weeks







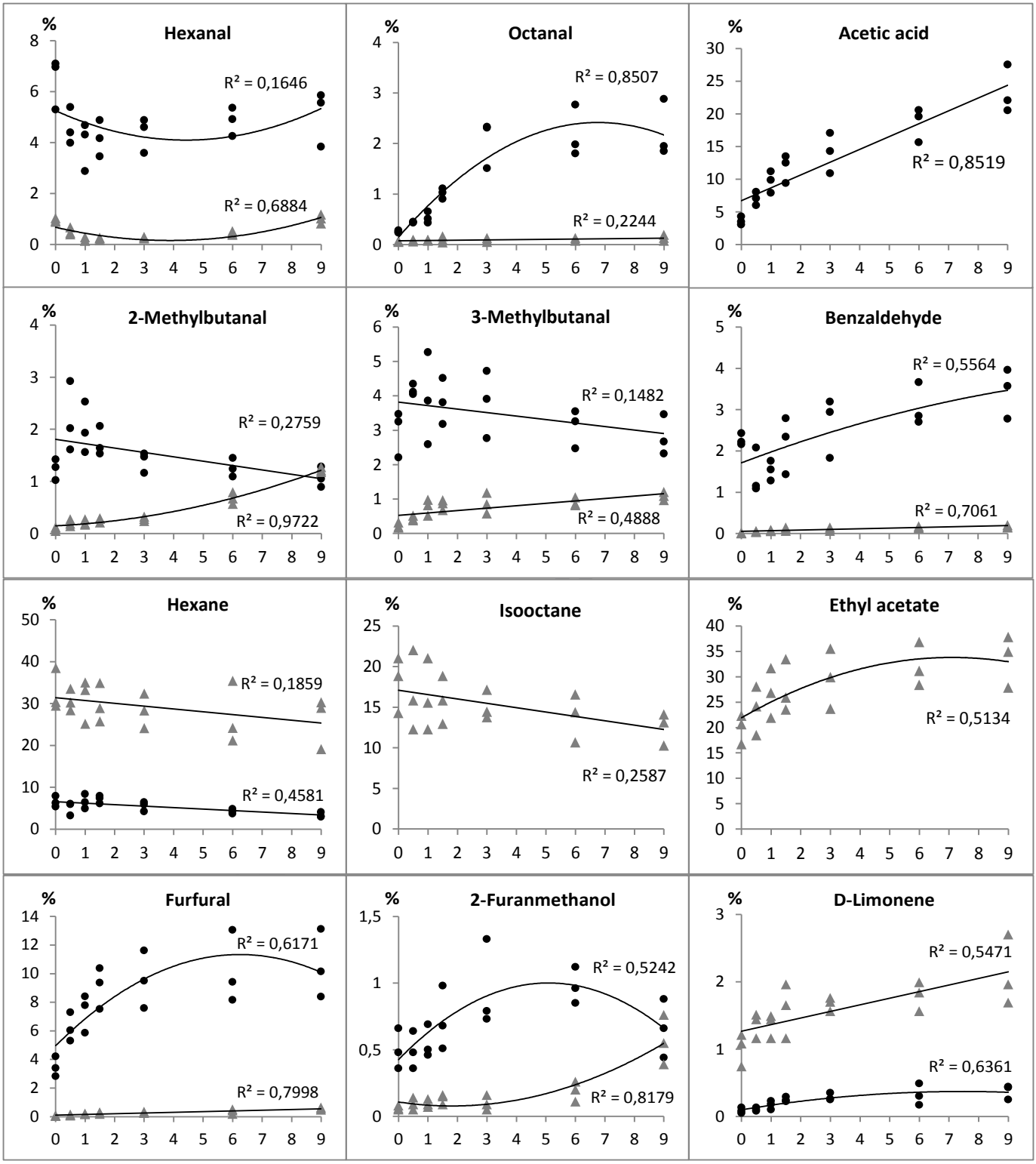


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Figure 2. Volatile compounds of the enriched (0.5-9%) and control cookies (0%) - fresh (●) and stored (▲) for 16 weeks

Table 1. Components of freeze-dried Japanese quince fruits (FJQF)

Component	Content, mg/100 g*
Vitamin C	287.3 ± 15.3
Total carotenoids	7.95 ± 0.65
Total chlorophyll	5.77 ± 0.07
Phenolic acids	
Protocatechuic acid	1364 ± 55
Neochlorogenic acid	51.21 ± 1.23
Chlorogenic acid	195.4 ± 4.1
Caffeic acid	1026 ± 28
Flavan-3-ols	
Epicatechin	364.1 ± 6.8
Epigallocatechin gallate	246.6 ± 4.4
Epigallocatechin	189.4 ± 2.8
Procyanidin A2	42.56 ± 1.95
Procyanidin B2	78.45 ± 2.09
Flavonols	
Isoquercetin	5.27 ± 0.18
Kaempferol	66.52 ± 2.46
DPPH, %	69.00 ± 4.58
TPC, mg GAE/100 g	4165 ± 24
Volatile compound	Relative content (%)
Alcohols	
1-Pentanol	0.15 ± 0.03
1-Hexanol	0.23 ± 0.02
1-Decanol	0.16 ± 0.02
1-Octanol	0.10 ± 0.00
1-Octen-3-ol	0.46 ± 0.05
Benzenemethanol	0.19 ± 0.01
Aldehydes	
2-Butenal	0.31 ± 0.02
Pentanal	2.89 ± 0.15
2-Pentenal, (E)	0.44 ± 0.03
Hexanal	2.51 ± 0.26
2-Hexenal, (E)	0.46 ± 0.09
Heptanal	1.08 ± 0.12
2-Heptenal, (E)	0.85 ± 0.02
Octanal	1.94 ± 0.10
Nonanal	1.03 ± 0.08
p-Menth-1-en-9-al	0.79 ± 0.06
Carboxylic acids	
Acetic acid	58.36 ± 3.96
Hexanoic acid	0.52 ± 0.02
Octanoic acid	1.36 ± 0.11
Furan compounds	
Furfural	1.45 ± 0.09

2-Methylfuran	3.27 ± 0.38
2-Acetylfuran	0.09 ± 0.01
Ketones	
2-Butanone, 3-hydroxy	0.48 ± 0.03
1-Octen-3-one	0.75 ± 0.10
α-Ionone	0.43 ± 0.07
Acetophenone	0.36 ± 0.12
2,6,6-Trimethyl-2-cyclohexene-1,4-dione	0.20 ± 0.01
Terpenes	
D-Limonene	1.83 ± 0.16
m-Cymene	0.29 ± 0.02
α-Pinene	0.23 ± 0.00
Camphene	0.15 ± 0.01
Carvone, (+)	0.14 ± 0.03
Ocimene, (E)	0.17 ± 0.02
Estragol	0.23 ± 0.09
Terpenol	0.64 ± 0.14
Terpinolen	0.85 ± 0.13
Ocimene, (Z)	0.09 ± 0.01
Others	
Butyrolactone	0.20 ± 0.01
Toluene	0.58 ± 0.12
Pentane	0.77 ± 0.07

* - mg/100 g of dry matter
DPPH - 2,2-diphenyl-1-picrylhydrazyl radicals
TPC - Total phenolic content
GAE – Gallic acid equivalents

Table 2. Sensory intensity of attributes of the enriched (0.5-9%) and control cookies (0%) – fresh and stored for 16 weeks

FJQF content (%)	Indices							
	Buttery aroma		Acid aroma		Citrus aroma		Off-arom	
	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	
0	$6.00^{a} \pm 0.35$	$4.98^{a,*} \pm 0.27$	$0.31^{a} \pm 0.15$	$0.35^{a} \pm 0.14$	$0.21^{a} \pm 0.11$	$0.27^{a} \pm 0.13$	$0.25^{a} \pm 0.17$	1.1
0.5	$5.17^{b} \pm 0.62$	$4.47^{b,*} \pm 0.36$	$1.74^{b} \pm 0.44$	$1.45^{b} \pm 0.22$	$1.52^{b} \pm 0.33$	$1.39^{b} \pm 0.25$	$0.80^{b} \pm 0.17$	0.
1.0	$5.05^{b} \pm 0.41$	$3.92^{c,*} \pm 0.29$	$1.96^{b} \pm 0.28$	$1.79^{b} \pm 0.32$	$1.86^{b} \pm 0.29$	$1.78^{c} \pm 0.21$	$0.90^{b} \pm 0.24$	0.
1.5	$4.43^{b} \pm 0.41$	$3.82^{c,*} \pm 0.38$	$3.69^{c} \pm 0.46$	$2.96^{c,*} \pm 0.29$	$2.70^{c} \pm 0.41$	$2.38^{d,*} \pm 0.26$	$0.95^{b,c} \pm 0.21$	1.
3.0	$3.06^{c} \pm 0.57$	$1.24^{d,e*} \pm 0.19$	$4.95^{d} \pm 0.32$	$3.41^{d,*} \pm 0.36$	$4.08^{d} \pm 0.26$	$2.91^{e,*} \pm 0.31$	$1.01^{b,c} \pm 0.21$	1.
6.0	$2.61^{c} \pm 0.69$	$1.55^{d,*} \pm 0.26$	$5.40^{d} \pm 0.41$	$4.12^{e,*} \pm 0.35$	$5.21^{e} \pm 0.32$	$3.35^{f,*} \pm 0.24$	$1.21^{c} \pm 0.18$	1.3
9.0	$2.50^{c} \pm 0.72$	$1.08^{e,*} \pm 0.17$	$6.47^{e} \pm 0.35$	$5.09^{f,*} \pm 0.28$	$6.31^{f} \pm 0.23$	$4.23^{g,*} \pm 0.31$	$1.61^{d} \pm 0.24$	2.4
FJQF content (%)	Buttery taste		Sweet taste		Acid taste		Citrus tas	
	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	
0	$7.14^{a} \pm 0.28$	$5.79^{a,*} \pm 0.26$	$7.19^{a} \pm 0.25$	$6.65^{a,*} \pm 0.40$	$0.72^{a} \pm 0.24$	$0.68^{a} \pm 0.18$	$0.24^{a} \pm 0.11$	0.
0.5	$5.69^{b} \pm 0.43$	$5.22^{b,*} \pm 0.60$	$5.89^{b} \pm 0.28$	$5.50^{b,*} \pm 0.39$	$2.60^{b} \pm 0.42$	$2.59^{b} \pm 0.29$	$2.09^{b} \pm 0.21$	2.
1.0	$5.61^{b} \pm 0.50$	$4.12^{c,*} \pm 0.44$	$5.81^{b} \pm 0.29$	$5.40^{b,*} \pm 0.32$	$3.95^{c} \pm 0.31$	$3.42^{c,*} \pm 0.26$	$3.47^{c} \pm 0.38$	3.
1.5	$4.29^{c} \pm 0.34$	$3.22^{d,*} \pm 0.40$	$4.31^{c} \pm 0.36$	$4.09^{c} \pm 0.32$	$4.69^{d} \pm 0.45$	$4.37^{d} \pm 0.28$	$4.59^{d} \pm 0.29$	3.
3.0	$3.95^{c} \pm 0.24$	$1.76^{e,*} \pm 0.34$	$4.03^{c} \pm 0.27$	$3.95^{c} \pm 0.29$	$6.68^{e} \pm 0.38$	$5.80^{e,*} \pm 0.32$	$6.15^{e} \pm 0.32$	4.0
6.0	$3.09^{d} \pm 0.23$	$1.21^{f,*} \pm 0.26$	$2.91^{d} \pm 0.33$	$2.78^{d} \pm 0.44$	$7.18^{e} \pm 0.35$	$6.84^{f,*} \pm 0.25$	$7.31^{f} \pm 0.33$	4.
9.0	$1.15^{e} \pm 0.23$	$1.13^{f} \pm 0.20$	$2.51^{d} \pm 0.41$	$2.13^{e,*} \pm 0.28$	$8.67^{f} \pm 0.29$	$8.64^{g} \pm 0.36$	$7.97^{g} \pm 0.26$	5.

$^{a, b, c}$ - significantly different, depending on FJQF (freeze-dried Japanese quince fruits) content
* - significantly different, depending on storage time

Table 3. Assessment of hedonic acceptability of the enriched (0.5-9%) and control cookies (0%) (n=125)

Hedonic value	Number of assessors making hedonic choices						
	Content of FJQF (%) in cookies						
	0	0.5	1.0	1.5	3.0	6.0	9.0
Like extremely - 9	0	3	6	12	0	0	0
Like very much - 8	40	24	46	46	26	15	5
Like moderately - 7	33	30	30	32	51	25	23
Like a little - 6	16	23	20	16	18	23	14
Neither like or dislike - 5	14	12	10	9	13	22	12
Dislike a little - 4	11	18	6	7	13	18	22
Dislike moderately - 3	7	9	7	3	4	12	15
Dislike a lot - 2	4	6	0	0	0	10	26
Dislike extremely - 1	0	0	0	0	0	0	8
acceptability	6.32 [b]	5.90 [c]	6.78 [a]	7.02 [a]	6.42 [b]	5.30 [d]	4.30 [e]
Range	8-2	9-2	9-3	9-3	8-3	8-2	8-1

[a,b,c,d] - Means bearing the same superscript do not differ significantly from each other

FJQF – freeze-dried Japanese quince fruits

Table 4. Volatile compounds of the enriched (1-9%) and control cookies (0%) - fresh and stored for 16 weeks (means of 3 replicates ± SD)

Volatile compound (%)	Cc	Content of FJQF (%) in cookies										
		0	1.0	3.0	9.0	R^2		0	1.0	3.0	9.0	R^2
		Fresh cookies						Stored cookies				
Acetic acid	Ac	3.63 ± 0.52	9.67 ± 1.35	14.09 ± 2.52	23.37 ± 3.00	0.923	1	n.d.	n.d.	n.d.	n.d.	
Butanoic acid	Ac	0.28 ± 0.10	0.33 ± 0.11	0.41 ± 0.08	0.77 ± 0.18	0.858	2	n.d.	n.d.	n.d.	n.d.	
Pentanal	Ald	3.87 ± 0.25	3.41 ± 0.67	3.59 ± 0.68	3.67 ± 0.54	0.089	2	n.d.	n.d.	n.d.	n.d.	
Heptanal	Ald	0.85 ± 0.06	0.35 ± 0.06	0.61 ± 0.08	0.91 ± 0.21	0.527	1	n.d.	n.d.	n.d.	n.d.	
Methional	Ald	0.27 ± 0.05	0.30 ± 0.06	0.36 ± 0.03	0.29 ± 0.06	0.394	2	n.d.	n.d.	n.d.	n.d.	
2-Heptenal, (E)	Ald	1.07 ± 0.08	0.80 ± 0.11	1.15 ± 0.15	1.31 ± 0.11	0.654	1	n.d.	n.d.	n.d.	n.d.	
Benzeneacetaldehyde	Ald	1.23 ± 0.10	2.41 ± 0.70	2.89 ± 0.26	2.54 ± 0.55	0.773	2	n.d.	n.d.	n.d.	n.d.	
2-Nonenal, (E)	Ald	2.42 ± 0.10	1.15 ± 0.20	1.58 ± 0.35	2.02 ± 0.30	0.071	1	n.d.	n.d.	n.d.	n.d.	
2-Octenal, (E)	Ald	0.00 ± 0.00	0.06 ± 0.00	0.21 ± 0.03	0.32 ± 0.07	0.896	1	n.d.	n.d.	n.d.	n.d.	
2-Ethylhexene	Hc	0.34 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	N		n.d.	n.d.	n.d.	n.d.	
3-Ethylheptane	Hc	0.23 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	N		n.d.	n.d.	n.d.	n.d.	
2,2,4,4-Tetramethyloctane	Hc	4.03 ± 0.21	3.41 ± 0.47	2.47 ± 0.43	2.64 ± 0.29	0.788	2	n.d.	n.d.	n.d.	n.d.	
2,6-Dimethyl-octane	Hc	0.55 ± 0.05	0.60 ± 0.05	0.52 ± 0.09	0.48 ± 0.07	0.470	1	n.d.	n.d.	n.d.	n.d.	
Butylcyclopentane	Hc	0.15 ± 0.03	0.13 ± 0.03	0.09 ± 0.01	0.14 ± 0.01	0.500	2	n.d.	n.d.	n.d.	n.d.	
Undecane	Hc	0.28 ± 0.04	0.20 ± 0.00	0.28 ± 0.05	0.19 ± 0.05	0.316	2	n.d.	n.d.	n.d.	n.d.	
1-Hepten-3-one	K	0.33 ± 0.04	0.06 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	M		n.d.	n.d.	n.d.	n.d.	
1-Octen-3-one	K	0.00 ± 0.00	0.03 ± 0.02	0.14 ± 0.03	0.33 ± 0.06	0.956	1	n.d.	n.d.	n.d.	n.d.	
1-Pentanol	A	0.21 ± 0.04	0.36 ± 0.10	0.48 ± 0.06	0.57 ± 0.10	0.730	1	n.d.	n.d.	n.d.	n.d.	
1-Octanol	A	0.76 ± 0.14	1.07 ± 0.15	1.21 ± 0.20	1.50 ± 0.21	0.665	1	n.d.	n.d.	n.d.	n.d.	
Acetylfuran	Fc	0.17 ± 0.02	0.25 ± 0.08	0.87 ± 0.10	1.31 ± 0.27	0.932	2	n.d.	n.d.	n.d.	n.d.	
5-Methyl-2-furancarboxaldehyde	Fc	0.05 ± 0.01	0.07 ± 0.02	0.33 ± 0.06	0.98 ± 0.08	0.978	2	n.d.	n.d.	n.d.	n.d.	
2,6-Dimethylpyrazine	P	0.21 ± 0.02	0.15 ± 0.02	0.01 ± 0.00	0.00 ± 0.00	0.815	R	n.d.	n.d.	n.d.	n.d.	
2-Ethylpyrazine	P	0.34 ± 0.02	0.09 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.729	P	n.d.	n.d.	n.d.	n.d.	
β-Pinene	T	0.36 ± 0.02	0.17 ± 0.05	0.19 ± 0.01	0.20 ± 0.01	0.170	1	n.d.	n.d.	n.d.	n.d.	
(+)-3-Carene	T	0.10 ± 0.06	0.60 ± 0.14	0.59 ± 0.08	0.48 ± 0.03	0.525	1	n.d.	n.d.	n.d.	n.d.	
Propanoic acid, heptyl ester	E	0.73 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	N		n.d.	n.d.	n.d.	n.d.	
Butanal	Ald	1.35 ± 0.15	0.94 ± 0.12	0.92 ± 0.22	1.19 ± 0.05	0.144	1	0.39 ± 0.08	0.30 ± 0.05	0.29 ± 0.06	0.33 ± 0.02	0.072	1
3-Methylbutanal	Ald	2.98 ± 0.55	3.91 ± 1.09	3.80 ± 0.80	2.82 ± 0.48	0.385	2	0.23 ± 0.06	0.77 ± 0.19	0.87 ± 0.25	1.09 ± 0.09	0.699	1

2-Methylbutanal	Ald	1.24 ± 0.16	2.01 ± 0.40	1.39 ± 0.16	1.07 ± 0.16	0.525	1	0.09 ± 0.03	0.21 ± 0.04	0.28 ± 0.03	1.22 ± 0.04	0.986	2
Hexanal	Ald	6.45 ± 0.82	3.96 ± 0.78	4.36 ± 0.55	5.08 ± 0.89	0.406	2	0.95 ± 0.06	0.20 ± 0.06	0.26 ± 0.02	1.00 ± 0.14	0.830	2
Octanal	Ald	0.25 ± 0.02	0.53 ± 0.09	2.05 ± 0.38	2.23 ± 0.46	0.922	2	0.06 ± 0.01	0.08 ± 0.01	0.09 ± 0.03	0.13 ± 0.05	0.474	1
Nonanal	Ald	6.45 ± 0.77	2.47 ± 0.43	3.54 ± 0.49	4.13 ± 0.42	0.598	3	0.79 ± 0.14	0.64 ± 0.19	0.68 ± 0.07	0.58 ± 0.07	0.242	1
Decanal	Ald	0.70 ± 0.06	0.10 ± 0.02	0.20 ± 0.08	0.24 ± 0.09	0.817	3	0.16 ± 0.06	0.10 ± 0.00	0.09 ± 0.03	0.13 ± 0.02	0.515	2
Benzaldehyde	Ald	2.27 ± 0.12	1.53 ± 0.20	2.65 ± 0.59	3.44 ± 0.49	0.746	2	0.01 ± 0.00	0.08 ± 0.01	0.11 ± 0.03	0.18 ± 0.02	0.840	1
Hexane	Hc	6.53 ± 1.06	6.56 ± 1.40	5.50 ± 0.92	3.35 ± 0.50	0.677	1	32.77 ± 4.05	31.12 ± 4.29	28.27 ± 3.36	26.10 ± 4.97	0.431	1
Toluene	A Hc	0.12 ± 0.04	0.09 ± 0.01	0.10 ± 0.02	0.17 ± 0.02	0.756	2	1.43 ± 0.09	1.03 ± 0.02	0.21 ± 0.07	1.31 ± 0.52	0.848	3
2,2,4,6,6-Pentamethyl-heptane	Hc	23.89 ± 2.21	19.95 ± 2.40	12.76 ± 2.23	7.21 ± 1.58	0.924	2	1.07 ± 0.16	0.81 ± 0.07	0.72 ± 0.19	0.19 ± 0.04	0.872	1
Decane	Hc	0.59 ± 0.05	0.49 ± 0.08	0.37 ± 0.09	0.25 ± 0.06	0.838	3	0.22 ± 0.04	0.18 ± 0.03	0.16 ± 0.05	0.05 ± 0.03	0.683	2
Nonane	Hc	0.12 ± 0.02	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	M		0.19 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	N	
2-Furanmethanol	Fc	0.50 ± 0.12	0.55 ± 0.10	0.95 ± 0.27	0.66 ± 0.18	0.724	2	0.06 ± 0.01	0.10 ± 0.02	0.10 ± 0.05	0.57 ± 0.15	0.904	2
Furfural or 2-furancarboxaldehyde or 2-furaldehyde	Fc	3.48 ± 0.57	7.35 ± 1.09	9.56 ± 1.65	10.55 ± 1.96	0.786	2	0.06 ± 0.02	0.18 ± 0.02	0.29 ± 0.04	0.56 ± 0.07	0.894	1
α-Pinene	T	0.28 ± 0.00	0.49 ± 0.07	0.93 ± 0.11	1.63 ± 0.19	0.952	2	0.02 ± 0.00	0.09 ± 0.01	0.11 ± 0.03	0.17 ± 0.04	0.700	1
D-Limonene	T	0.09 ± 0.03	0.17 ± 0.05	0.29 ± 0.04	0.37 ± 0.09	0.798	2	1.01 ± 0.20	1.36 ± 0.15	1.67 ± 0.08	2.12 ± 0.43	0.740	1
1-Octen-3-ol	A	0.24 ± 0.02	0.22 ± 0.06	0.32 ± 0.05	0.60 ± 0.12	0.889	2	0.00 ± 0.00	0.11 ± 0.03	0.14 ± 0.04	0.45 ± 0.11	0.859	2
Ethyl acetate	E	n.d.	n.d.	n.d.	n.d.			19.89 ± 2.35	26.81 ± 3.99	29.71 ± 4.84	33.56 ± 4.18	0.716	2
Acetic acid, butyl ester	E	n.d.	n.d.	n.d.	n.d.			0.10 ± 0.00	0.03 ± 0.00	0.05 ± 0.03	0.09 ± 0.03	0.239	1
Butanoic acid, methyl ester	E	n.d.	n.d.	n.d.	n.d.			0.84 ± 0.08	0.37 ± 0.09	0.42 ± 0.09	0.79 ± 0.09	0.753	2
2,4-Dimethylpentane	Hc	n.d.	n.d.	n.d.	n.d.			1.54 ± 0.25	1.51 ± 0.16	1.59 ± 0.09	1.45 ± 0.16	0.202	2
Isooctane	Hc	n.d.	n.d.	n.d.	n.d.			18.06 ± 2.81	16.29 ± 3.61	15.12 ± 1.45	12.50 ± 1.63	0.509	1
Heptane	Hc	n.d.	n.d.	n.d.	n.d.			2.10 ± 0.21	1.28 ± 0.19	0.77 ± 0.22	2.57 ± 0.13	0.468	1
Hexadecane	Hc	n.d.	n.d.	n.d.	n.d.			0.08 ± 0.02	0.11 ± 0.02	0.18 ± 0.03	0.62 ± 0.09	0.952	2
1-Heptene, 5-methyl-	Hc	n.d.	n.d.	n.d.	n.d.			0.05 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	L	
2-Nonene, (E)	Hc	n.d.	n.d.	n.d.	n.d.			0.08 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	L	
Dodecane	Hc	n.d.	n.d.	n.d.	n.d.			0.18 ± 0.08	0.09 ± 0.02	0.06 ± 0.04	0.14 ± 0.05	0.585	2
Decane, 5-ethyl-5-methyl-	Hc	n.d.	n.d.	n.d.	n.d.			0.21 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	L	
Tetradecane	Hc	n.d.	n.d.	n.d.	n.d.			0.05 ± 0.02	0.02 ± 0.02	0.06 ± 0.02	0.08 ± 0.01	0.620	2
Nonadecane	Hc	n.d.	n.d.	n.d.	n.d.			0.05 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	N	
Cyclododecane	Hc	n.d.	n.d.	n.d.	n.d.			0.13 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	L	
Styrene	A Hc	n.d.	n.d.	n.d.	n.d.			0.09 ± 0.02	0.10 ± 0.02	0.12 ± 0.04	0.21 ± 0.04	0.744	2

Pentanoic acid	Ac	n.d.	n.d.	n.d.	n.d.	0.00 ± 0.00	0.08 ± 0.01	0.10 ± 0.03	0.18 ± 0.02	0.811	1
Hexanoic acid	Ac	n.d.	n.d.	n.d.	n.d.	0.00 ± 0.00	0.17 ± 0.06	0.21 ± 0.07	0.57 ± 0.05	0.924	3
Octanoic acid	Ac	n.d.	n.d.	n.d.	n.d.	0.00 ± 0.00	0.07 ± 0.03	0.18 ± 0.04	0.48 ± 0.04	0.914	1
Nonanoic acid	Ac	n.d.	n.d.	n.d.	n.d.	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.02	0.18 ± 0.03	0.960	2
(2E)-2-Octen-1-ol	A	n.d.	n.d.	n.d.	n.d.	0.00 ± 0.00	0.10 ± 0.05	0.12 ± 0.02	0.19 ± 0.04	0.696	1
Cyclopentanol	A	n.d.	n.d.	n.d.	n.d.	0.21 ± 0.06	0.17 ± 0.03	0.14 ± 0.03	0.33 ± 0.10	0.715	2
2-Hexenal, (2E)	Ald	n.d.	n.d.	n.d.	n.d.	0.05 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	L	
2-Hexenal, (E)	Ald	n.d.	n.d.	n.d.	n.d.	0.09 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	N	
Heptanal	Ald	n.d.	n.d.	n.d.	n.d.	0.07 ± 0.01	0.09 ± 0.02	0.09 ± 0.04	0.12 ± 0.02	0.327	2

FJQF – freeze-dried Japanese quince fruits; Cc –chemical classes of volatile compounds; A – Alcohols; Ac – Carboxylic acids; Ald – Aldehydes; Alk – Alkanes; AHc – Aromatic hydrocarbons; Hc – Hydrocarbons; E – Esters; K – Ketones; T – Terpenes, P – Pyrazines, Fc - Furan compounds; n.d. – not detected.

The number accompanying R^2 value corresponds to the degree of polynomial regression: 1 – linear; 2 – 2nd degree; 3 – 3rd degree

L - Correlation not applicable due to zero values at FJQF content exceeding 0

N – Correlation not applicable due to zero values at FJQF content exceeding 0.5

M - Correlation not applicable due to zero values at FJQF content exceeding 1.0

P - Correlation applicable to range 0 – 1.5

R – Correlation applicable to range 0 – 3

Highlights:

Cookies with Japanese quince fruits (JQF) had high antioxidative potential

Presence of acetic acid in enriched cookies intensified acidic and citrus aroma

Stored cookies were rich in hydrocarbons, including newly generated ones

Addition of JQF enhanced microbiological safety of stored cookies

Consumers preferred cookies containing 1 and 1.5% JQF to those containing 6 or 9%