

Oxidative Stability of Lipid Fraction of Cookies Enriched with Chokeberry Polyphenols Extract

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The effect of incorporation of different contents of chokeberry polyphenols extract (CPE) into cookie recipe on the oxidative stability of the lipid fraction of cookies was determined in the study. Margarine and butter cookies with different contents of CPE were prepared. Contents of primary (Peroxide value, PV) and secondary (Anisidine value, AV; thiobarbituric acid reactive substances, TBARS) lipid oxidation products and fatty acids profile were measured during storage. Different course of lipid degradation depending on both polyphenols content and storage time was shown. Cookies were characterised by a low PV (1.45 and 4.90 meq of O/kg of fat in margarine and butter cookies, respectively). The AV increased during storage both in margarine and in butter cookies. Losses of PUFA in margarine cookies (0.34%) apply mainly to α -linolenic acid. It appears safe to incorporate 100 mg and 250 mg of CPE into margarine cookies stored for 9 weeks and 1000 mg of CPE into butter cookies stored for 9 weeks. The incorporation of 1000 mg CPE into cookies resulted in a significant increase in the intensity of astringent taste, acidic taste, off-taste and off-flavour.

INTRODUCTION

Oxidation of lipids is undoubtedly one of the most common processes in the food system. The structure of foods is changing during processing, as a result of which lipids may become more exposed to oxygen. The main effect of lipid oxidation is the development of off-flavours and odours which shorten the shelf-life of foodstuffs and make them unacceptable for consumption. At the same time, the nutritional value and safety of foods decrease owing to the formation of deleterious primary and secondary compounds [Frankel, 1996], which are associated with a number of degenerative processes and disorders, such as cancer, inflammation and cardiovascular diseases [Marquez-Ruiz *et al.*, 2008]. Effective counteracting food lipid oxidation includes the use of antioxidants.

Since the beginning of the twentieth century synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have been commonly used. Although they are relatively cheap and efficient in reducing the rate of oxidative processes, their use is questionable because of concerns over their toxicity [Mildner-Szkudlarz *et al.*, 2009]. Another drawback of these antioxidants is that they are volatile and readily decompose at high temperatures [Martinez-Tome *et al.*, 2001]. Therefore a worldwide trend is observed aimed at minimizing or even avoiding the use

of synthetic additives and at replacing them with naturally occurring ones. Natural antioxidants are presumed to be safe since they occur in different plant foods as secondary metabolites. The compounds isolated directly from different parts of fruits and vegetables have become significant in food production as alternatives to synthetic antioxidants [Valenzuela *et al.*, 2003].

Application of some natural antioxidants to bakery products has been reported in several studies. Various polyphenols originating from plants (from apple, green tea, sweet potato, drumstick (*Moringa oleifera*), *Garcinia*), spices (cinnamon, cardamom, cloves, ginger, anise, rosemary, black cumin, curry, turmeric, coriander, sage), and cereal products (amaranth, oatmeal, rice bran extracts, buckwheat) had been successfully used as inhibitors of the oxidation process during storage [Bhanger *et al.*, 2008; Vergara-Valencia *et al.*, 2007; Mildner-Szkudlarz *et al.*, 2009; Abdel-Samie *et al.*, 2010; Huang & Li, 2011; Badei *et al.*, 2002; Nanditha *et al.*, 2009; Basuny *et al.*, 2012; Sudha *et al.*, 2007; Ishida *et al.*, 2003; Reddy *et al.*, 2005; Isobe *et al.*, 2004; Kozłowska *et al.*, 2014].

As previously reported, berries are typical components of a diet of the North-European consumers. Among them, chokeberry (*Aronia melanocarpa*) is the richest source of polyphenols (the content ranges from 2 to 8 g per 100 g of dry matter), which possess a strong antioxidative activity [Oszmianski & Wojdyło, 2005]. Chokeberry contains 9 flavonoids (*e.g.* rutin, hyperoside, isoquercetin), 4 anthocyanins (cyanidin 3-O-glucoside, cyanidin 3-O-galactoside, cyanidin

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3-O-arabinoside, cyanidin 3-O-xyloside), 2 phenolic acids (chlorogenic acid and neochlorogenic acid) and 5 quercetins (quercetin 3-O-glucoside, quercetin 3-O-galactoside, quercetin 3-O-rutinoside, quercetin 3-O-robinoside, quercetin 3-O-vicianoside) [Lee *et al.*, 2014; Šavikin *et al.*, 2014; Taheri *et al.*, 2013]. Phenolic compounds present in chokeberry fruits are responsible for many health benefits. Recently Šavikin *et al.* [2014] revealed their cytotoxic activity against cervix carcinoma, melanoma, colon and chronic myelogenous leukemia cell lines. Other authors indicated their cardioprotective, hepatoprotective and antidiabetic properties [Broncel *et al.*, 2010; Denev *et al.*, 2012]. Chokeberry fruits are commonly used for the production of juices, syrups, teas, jams, jellies and wines. Also chokeberry by-products – pomaces remaining after juicing process, are used as an enriching ingredient in bread making and sausage production [Bialek *et al.*, 2012]. The addition of chokeberry fruits or extracts to high-fat bakery products to inhibit the oxidation process seems to be very promising.

Consumers' awareness regarding prevention of cancer and other diet-related diseases has greatly increased in recent years. It is common knowledge that among the main causes of the above-mentioned disorders are formation of free radicals and oxidation processes [Marquez-Ruiz *et al.*, 2008]. Therefore, a growing interest is observed in antioxidant-supplemented foods. The aim of producers, processors and scientists has been to increase the nutritional value of such food products. Food-stuffs improved in this way can be produced on the commercial scale and offer not only convenience but also health-promoting properties to consumers. However, to produce the expected effects, enriched confectionery products should not only be rich in nutritive compounds, but also willingly consumed.

In the present study, a chokeberry polyphenol extract (CPE) was used as a source of antioxidants in cookies prepared with two different types of fat. The main aim of this study was to investigate the oxidative stability of fats in cookies prepared with different contents of this ingredient.

MATERIALS AND METHODS

Materials

Ingredients for cookies manufacturing (wheat flour, butter, margarine, eggs, sugar powder) were purchased at the local market. A chokeberry polyphenol extract containing 52.6% (w/w) of polyphenols was obtained free of charge from Agropharm company (Tuszyn, Poland). Detailed phenolic profile is presented in Table 1 [Scientific expert opinion by Prof. Oszmianski]. All other solvents and reagents were of analytical grade and were purchased from Sigma Aldrich (Poland).

Preparation of a solution of a chokeberry polyphenol extract

A solution of citric acid was prepared by dissolving 3.58 g of citric acid in 225 mL of water. Doses of 100 mg, 250 mg and 1000 mg of CPE were dissolved in 10 mL of the earlier prepared citric acid solution in order to stabilize and uniformly mix the formulation with dough.

Preparation of cookies

Cookies were prepared according to our own recipe in 4 variants differing in the content of CPE: with 100 mg,

TABLE 1. Content of phenolic compounds in chokeberry polyphenol extract (CPE) (acc. to Oszmianski).

Phenolic constituents	Mean ± SD (%)
Phenolic acids (total):	12.27
Neochlorogenic acid	6.03 ± 3.39
Chlorogenic acid	6.24 ± 1.91
Flavonols	1.95 ± 1.00
Anthocyanins (monomeric) (total):	23.52
Cya-3-galactoside	15.53 ± 5.08
Cyan-3-glucoside	0.79 ± 0.24
Cyan-3-arabinoside	6.17 ± 2.44
Cyan-3-xyloside	1.03 ± 0.46
Procyanidins	14.9 ± 2.66
Total phenolic compounds	52.64

250 mg and 1000 mg of CPE per kg of dough, and control (0 mg of CPE). The content of polyphenols in each sort of cookies was 52.6 mg, 131.5 mg, 526 mg and 0 mg per kg of dough, respectively. The following proportions of ingredients were used to prepare cookies: wheat flour (500 g), powdered sugar (250 g), fat – margarine or butter (312 g), egg yolks (4 pcs). Fat (butter or margarine) was added to sifted wheat flour and minced with a mixer (type HR 7677/A, Phillips, Austria), then egg yolks and powdered sugar were added. All ingredients were kneaded until completely merged. The dough was sheeted to a thickness of 5 mm and cut into circular shapes using a cookie cutter with 50 mm diameter. Then cookies were placed on a tray and baked at 180°C for 15 min in a convection oven with forced air circulation (Electrolux AR 85, Italy). Average weight of one cookie was 14.5 ± 0.05 g. Cooled cookies were packed in non-hermetized containers suitable for contact with food and stored at 19°C, without access to light for 18 weeks (to mimic in-shop storage conditions). Samples for analysis were collected after 0, 3, 6, 9, 12, 15 and 18 weeks of storage.

Methods

Oxidative stability

Lipids were extracted according to Polish Standard [PN-A 88021:1971] by the Soxhlet method using hexane as an extracting solvent. Stability of the lipid fraction of cookies was analysed periodically by determining the peroxide value (PV), anisidine value (AV), concentration of thiobarbituric acid reactive substances (TBARS) and by TOTOX value. PV was assayed according to ISO 2001:3960 by titration method. The results were expressed as meq of O/kg of fat. The AV was determined using a Milton Roy spectrophotometer at 350 nm according to ISO 2006:6885. TBARS content was determined spectrophotometrically at 532 nm according to the modified method of Ohkawa *et al.* [1979]. Results were expressed as µg MDA/g of fat. TOTOX was calculated from the following formula: TOTOX = 2 PV + AV. The reference value for TOTOX was calculated by substituting the permissible PV and proposed AV into the formula.

FA content

Methyl esters of fatty acids (FAME) were prepared by transmethylation of fat samples using a mixture of con-

centrated H₂SO₄ (95%) and methanol according to AOCS 2000 Official Method Ce 2–66. The FAMES were analysed by gas chromatography (GC) using an Agilent 6890N (USA) chromatograph equipped with flame ionization detector (FID), a split/splitless injector, operated with a split ratio of 1:50, and a capillary column with a stationary phase of high polarity (100 m × 0.25 mm I.D., film thickness 0.1 μm; Rtx 2330 Restek). The analyses involved a programmed run with temperature ramps. The oven temperature was initially 120°C for 12 min then ramped to 155°C at 5°C/min and held for 50 min. The temperature was then ramped again at 2°C/min to 210°C and held for 35 min. Injector and detector temperatures were maintained at 250°C, the carrier gas (helium) flow rate was 0.9 mL/min. Fatty acid composition of cookies was determined at the beginning and at the end of storage. Peak areas were corrected by the response factors for FAME responses of FID, and area% of FAME was appropriately converted to weight% of FA. A butter reference standard (CRM 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to determine the recovery rates and correction factors for individual FAs in butter cookie samples while the Supelco 37 No. 47885-U standard (Sigma Aldrich) was applied for fatty acid identification and recoveries of FAME in margarine cookies. The contents of individual FAs were expressed as g/100 g FA.

Sensory evaluation

The sensory evaluation of fresh cookies was performed using the scaling method [Barylko-Pikielna & Matuszewska, 2014]. Ten experienced panellists (4 males and 6 females; 27–46 years of age) participated in the study. They scored each attribute on a 100 mm unstructured scale in marked borders prepared in accordance with Polish Standard [PN-ISO 4121: 1998]. The subjects were requested to evaluate the intensity of sensory attributes of samples three times in the following order: aroma (buttery, acidic and off-flavours), taste (buttery, sweet, acidic, astringent and off-taste), colour, crispness. Cookies were delivered in coded plastic containers in a random order. Every panellist was provided with spring water to rinse mouth between samples. The results from the unstructured scale were converted to numerical values (from 0 to 10 units).

Statistical analysis

All chemical analyses were conducted in triplicates. Data were reported as means ± standard deviation (SD). All SD were within 3% of the reported mean values. Results of chemical analyses were subjected to two-way factorial ANOVA. STATISTICA 9.1PL software (StatSoft, Inc. (2010) was used, the level of P<0.01 was considered significant. Results of sensory assessment were subjected to one-way ANOVA with the P<0.05 significance level.

RESULTS

Primary oxidation products in lipids extracted from cookies

The average fat content was 25.3±0.7% in margarine cookies and 25.4±0.5% in butter cookies. Changes in the content of primary oxidation products expressed as PV are shown

in Figure 1. The PV obtained from margarine cookies ranged from 0.57 to 1.45 meq of O/kg of fat. In cookies with CPE, the highest increase in PV was determined after 9 weeks of storage. In the case of butter cookies, the concentration of primary lipid oxidation products assayed at the 9th week of storage was in the range from 0.39 to 4.90 meq of O/kg of fat. PV value differed significantly regarding to content of CEP and storage time in margarine and butter cookies.

Secondary oxidation products in lipids extracted from cookies

Concentrations of secondary lipid oxidation products expressed as AV and TBARS are shown in Figure 2 and Figure 3, respectively. The content of *p*-anisidine reacting substances was increasing during storage in both types of cookies and reached the maximum level after 18 weeks of storage. The AV in cookies prepared with margarine ranged from 4.70 to 22.92, and in cookies with butter from 5.30 to 18.60.

In margarine cookies, the extended time of storage was associated with increased concentration of secondary lipid oxidation substances measured as TBARS. In butter cookies, the maximum TBARS value was observed after the 9th week of storage irrespectively of CPE content.

TOTOX value of lipids extracted from cookies

The calculated TOTOX values for lipids extracted from cookies are presented in Figure 4. In both types of cookies, the TOTOX values increased during storage, *i.e.* from 5.30 to 20.00 in butter cookies and from 4.70 to 25.81 in margarine cookies. The TOTOX values significantly differed according to both CPE content and time of storage in both types of cookies.

Fatty acid composition of lipids extracted from cookies

Results of FA profile analysis of experimental cookies are presented in Tables 2 and 3. The assayed FAs were classified into the following groups: SFA (saturated FA), MUFA (monounsaturated FA) and PUFA (polyunsaturated FA) in margarine cookies. In butter cookies SFA were divided into short chain saturated FA (SCSFA) and long chain saturated FA (LCFA) and also groups of odd- and branched chain FA (OBCFA) were isolated. The average content of PUFA in margarine cookies accounted for 15.69 g/100 g FA while in butter cookies for only 5.18 g/100 g FA.

Interactions

Significant (P<0.01) CPE content × storage time interactions were found for PV and TBARS and for AV and TOTOX values in both types of cookies (data not shown).

Sensory analysis by experts

The results of sensory assessment of fresh cookies by experts are presented in Figure 5. Sensory rating differed regarding CPE content in both types of cookies. Intensity ratings of buttery flavour and taste, sweet taste as well as crispness decreased with increasing level of CPE in cookies. Increasing the content of CPE resulted in higher ratings of the intensity of acidic aroma and taste, colour, off-flavour and off-taste.

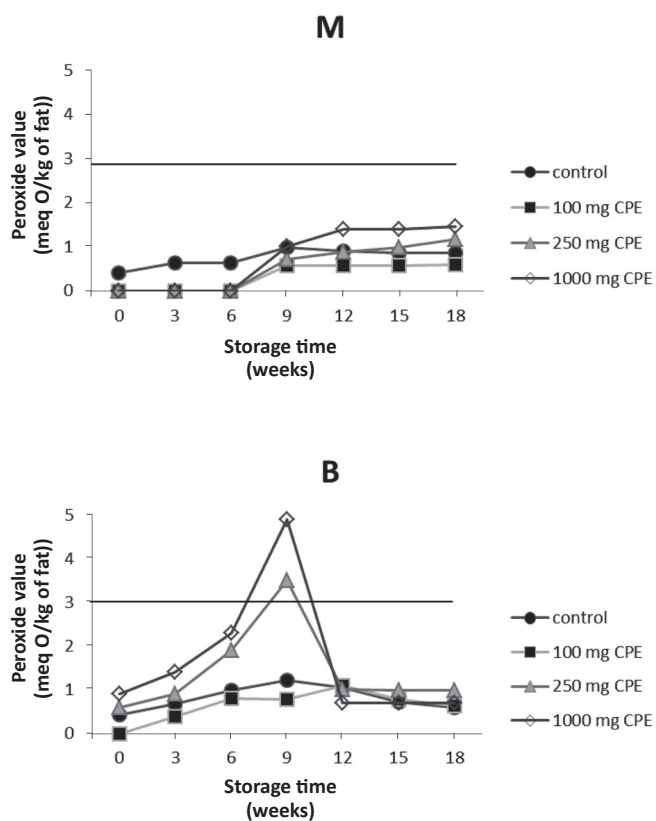


FIGURE 1. Changes in the content of primary oxidation products in cookies during storage (M – margarine cookies, B – butter cookies).

- Solid horizontal line – the acceptable PV value (3.0 meq O/kg of fat)

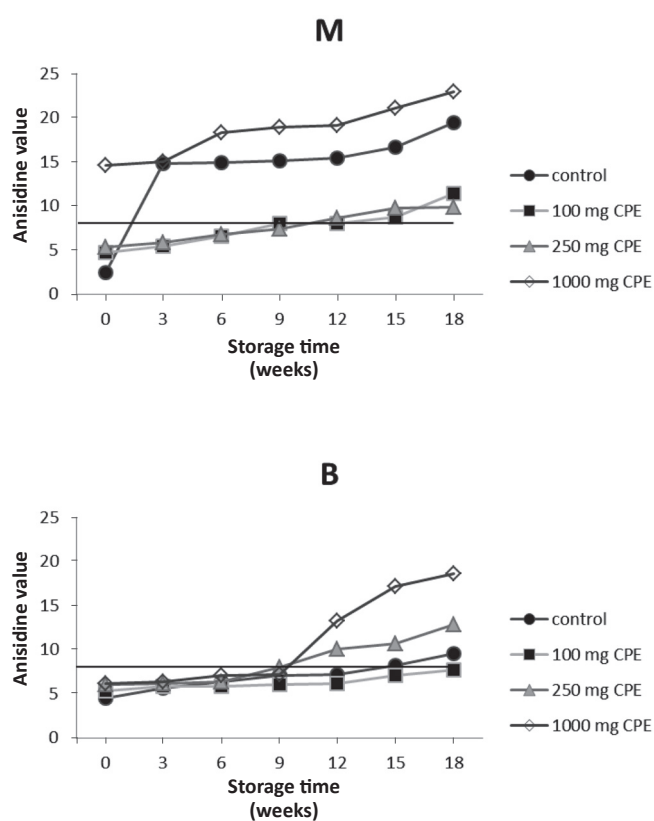


FIGURE 2. Changes in the anisidine value in cookies during storage (M – margarine cookies, B – butter cookies).

- Solid horizontal line – the acceptable AV value (8.0)

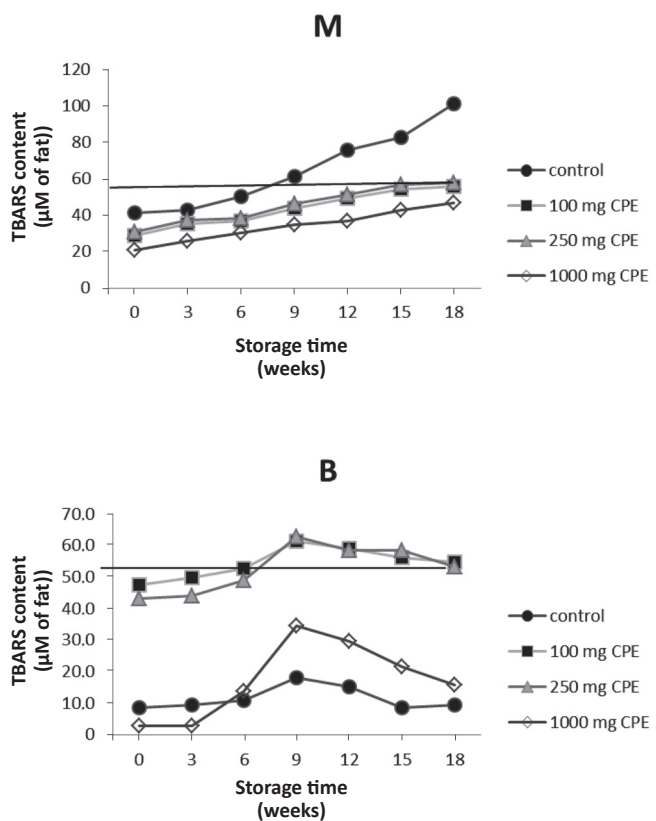


FIGURE 3. Changes in the TBARS content in cookies during storage (M – margarine cookies, B – butter cookies).

- Solid horizontal line – the acceptable TBARS value (57.6 µg MDA/g of fat)

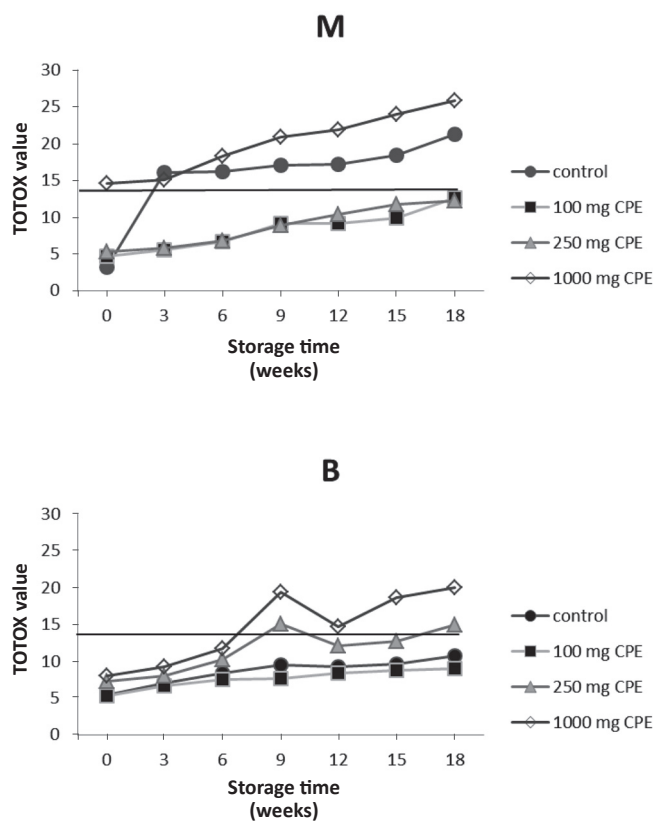


FIGURE 4. Changes in the TOTOX value in cookies during storage (M – margarine cookies, B – butter cookies).

- Solid horizontal line – the acceptable TOTOX value (14.0)

TABLE 2. Fatty acid composition^a of margarine cookies (g/100 g FA).

Fatty acids	Control		100 mg CPE		250 mg CPE		1000 mg CPE	
	0 weeks	18 weeks	0 weeks	18 weeks	0 weeks	18 weeks	0 weeks	18 weeks
SFA	46.45±0.45	46.44±0.47	46.19±0.40	46.12±0.37	45.29±0.56	45.31±0.62	45.04±0.33	44.96±0.24
MUFA	37.84±0.36	37.61±0.38	37.79±0.21	37.32±0.34	38.15±0.45	37.71±0.37	38.06±0.19	37.10±0.32
PUFA:	15.71±0.33	14.83±0.27	15.69±0.19	15.51±0.23	15.75±0.19	15.66±0.13	15.83±0.14	15.64±0.21
C18:2 9c 12c	12.24±0.09	11.50±0.10	12.35±0.09	12.33±0.06	12.42±0.03	12.41±0.01	12.50±0.09	12.10±0.13
C18:3 9c 12c 15c	3.25±0.03	3.05±0.07	3.24±0.09	3.10±0.05	3.23±0.07	3.07±0.09	3.26±0.04	3.19±0.05

^aValues are expressed as means ± SD of 3 independent assays. SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids.

TABLE 3. Fatty acid composition^a of butter cookies (g/100 g FA).

Fatty acids	Control		100 mg CPE		250 mg CPE		1000 mg CPE	
	0 weeks	18 weeks	0 weeks	18 weeks	0 weeks	18 weeks	0 weeks	18 weeks
SCSFA	13.83±0.09	13.75±0.10	13.65±0.21	13.59±0.27	13.80±0.29	13.84±0.34	13.54±0.11	13.56±0.15
LCSFA	50.10±0.13	49.73±0.11	49.93±0.37	49.90±0.31	49.84±0.45	49.83±0.39	50.04±0.32	50.00±0.43
OBCFA	2.07±0.01	2.05±0.05	2.06±0.02	2.06±0.02	2.08±0.03	2.00±0.01	2.05±0.02	1.98±0.01
MUFA:	27.13±0.23	26.90±0.34	26.92±0.16	26.84±0.19	27.39±0.15	26.97±0.17	27.50±0.07	26.15±0.10
C18:1 11t	1.93±0.05	1.94±0.07	1.95±0.04	1.88±0.08	1.97±0.01	1.94±0.03	1.97±0.09	1.87±0.05
C18:1 9c	20.99±0.19	20.90±0.13	21.02±0.17	20.83±0.20	21.16±0.25	20.87±0.21	21.38±0.14	20.08±0.19
PUFA:	5.28±0.07	5.14±0.09	5.02±0.12	4.88±0.09	5.39±0.05	5.11±0.07	5.46±0.05	5.16±0.07
C18:2 9c 12c	3.35±0.08	3.20±0.02	3.09±0.03	2.99±0.02	3.41±0.03	3.36±0.01	3.51±0.01	3.20±0.02
C18:3 9c 12c 15c	0.80±0.01	0.70±0.03	0.79±0.07	0.77±0.05	0.80±0.02	0.70±0.09	0.81±0.08	0.77±0.03
CLA	0.87±0.02	0.86±0.05	0.88±0.02	0.86±0.03	0.89±0.07	0.86±0.04	0.91±0.09	0.83±0.04

^aValues are expressed as means ± SD of 3 independent assays. SCSFA – short chain saturated fatty acids, LCSFA – long chain saturated fatty acids, OBCFA – odd-and branched chain fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, CLA – conjugated linolenic acid.

DISCUSSION

Cookies with a high level of fats are willingly consumed owing to their high sensory values, however they are sources of undesirable compounds such as lipid oxidation products [Mildner-Szkudlarz *et al.*, 2009; Caponio *et al.*, 2013]. Recently, special interest has been given to the substances with antioxidant properties that naturally occur in plants. In the present study, a chokeberry polyphenol extract was used as an antioxidative agent due to the high content of polyphenolic compounds.

The primary lipid oxidation products are hydroperoxides, odorless and colorless but very labile species than can undergo degradation to generate a complex array of secondary products, such as aliphatic aldehydes, alcohols, ketones and hydrocarbons [Kolakowska, 2003]. Since the safety of lipid-containing food products depends on the degree to which oxidation had progressed, the simultaneous detection of primary and secondary lipid oxidation products helps to better characterize lipid quality. It is noteworthy that there is no ideal method to measure oxidative changes in food lipids. For this reason, the formation and degradation of both primary and secondary products of lipid oxidation should be monitored over time. The use of two or more methods assessing both primary and secondary oxidation products is, therefore, highly recommended [Pegg, 2001].

Measurement of PV is the most common method used to determine the content of hydroperoxides formed during initial stages of the oxidation cascade. Some differences in the course of oxidative changes between two types of the analyzed cookies were observed depending on both CPE content and time of storage (Figure 1). In general, butter cookies contained higher amounts of hydroperoxides than cookies with margarine. The safe value for PV for bakery fats is 3 meq of O/kg of fat [Polish Standard, PN-A-86908:1966]. The content of primary lipid oxidation products in margarine cookies did not exceed this value even after 18 weeks of storage regardless of CPE content. In butter cookies with 100 and 250 mg content of CPE after 9 weeks of storage the PV was higher than the recommended value. Compared to the control cookies, the antioxidative effect of polyphenols incorporation was observed in margarine cookies with all CPE contents, especially in these containing 100 mg of CPE, because it was observed during the whole experiment. In butter cookies, only the content of 100 mg of CPE caused the inhibition of oxidative changes till the 12th week of storage.

The anisidine value (AV) is an indicator of the concentration of secondary lipid oxidation products generated during the decomposition of hydroperoxides [Shahidi & Zhong, 2005]. These unstable compounds were consequently broken down by a free radical mechanism to give hydroxyl free radical and lots of other compounds – alcohols, aldehydes, ketones

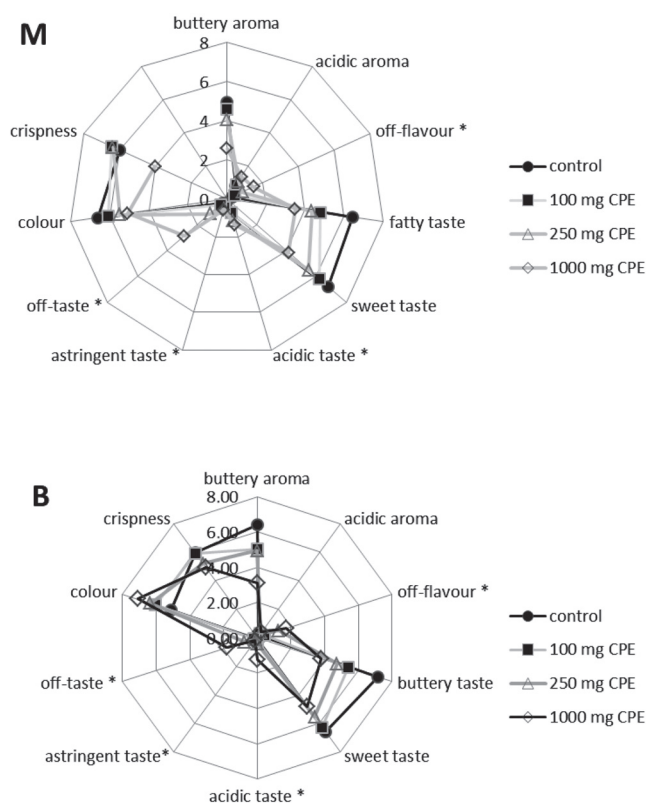


FIGURE 5. Sensory ratings of fresh margarine (M) and butter (B) cookies. Attributes differed significantly ($p < 0.05$) regarding CPE content is marked by *.

and malonaldehyde, called secondary oxidation products [Darughe *et al.*, 2012]. In the present study, in butter cookies initially an increase was observed in PV with time, which after reaching the maximum level began to decline. The PV decrease was accompanied by an increasing AV. This was due to the rapid decomposition of hydroperoxides to secondary oxidation products. This phenomenon was also confirmed by Aidos *et al.* [2002]. The high AV in cookies made with butter may be due to the content of volatile compounds responsible for the buttery flavor (Figure 2). Most of them are aldehydes and ketones, which can react with *p*-anisidine and overstate the results of AV determination [Stolyhwo & Rutkowska, 2013]. The proposed permissible concentration of secondary lipid oxidation products measured as AV is 8 [Podmore, 1992] and in case of margarine cookies it was only slightly exceeded at the end of storage in the cookies with 100 and 250 mg of CPE. The highest level of CPE potentiated the generation of secondary lipid oxidation products. This observation is in agreement with results of Darughe *et al.* [2012] who found that coriander essential oil rich in antioxidants in high concentration may act as a pro-oxidant. The antioxidative effect of chokeberry polyphenolic extract was observed in cookies containing 100 and 250 mg of CPE when compared to the control samples. In butter cookies, the reference value was exceeded only in cookies containing 250 and 1000 mg of CPE after 9 weeks of storage. The inhibitory effect of CPE was shown in the cookies with 100 mg of the chokeberry extract.

PV and AV reflect the oxidation level at early and later stages of oxidation reaction, respectively. TOTOX values reflect con-

tents of both hydroperoxides and their breakdown products, and provide a better estimation of the progressive oxidative deterioration of fats *e.g.* during storage [Shahidi & Zhong, 2005; Mildner-Szkudlarz *et al.*, 2009]. Exactly the same tendency as for AV was observed for TOTOX in the case of both margarine and butter cookies. Also by comparing to the control samples, the antioxidative effects were shown for butter cookies containing 100 mg of CPE and in margarine cookies with 100 and 250 mg of CPE.

The TBARS value measures the formation of secondary oxidation products reacting with thiobarbituric acid (mainly malondialdehyde - MDA) [Darughe *et al.*, 2012]. During lipid oxidation, MDA, a minor component of fatty acids with 3 or more double bonds, is formed as a result of the degradation of PUFA. MDA is probably formed from trienes by α - or β -unsaturated peroxides [Wheatley, 2000].

In margarine cookies (Figure 3), the control samples exhibited the highest TBARS content throughout the experimental period, showing great oxidative changes. Cookies containing CPE had a lower concentration of TBARS, which is in agreement with results of Darughe *et al.* [2012] and Izzreen & Noriham [2011] who evaluated antioxidative properties of coriander essential oil and some Malaysian herbal extract, respectively. In the case of both margarine and butter cookies, the samples with the highest amount of CPE were safe for consumption over the whole storage period, because the level of TBARS was lower than $57.6 \mu\text{g MDA/g}$ of fat, which is a reference value for rancidity [Izzreen & Noriham, 2011].

Interestingly, in both margarine and butter cookies the addition of 1000 mg of CPE inhibited the formation of TBARS to a greater extent than the lower amounts. The opposite situation was observed in case of secondary oxidation products measured as AV, where the highest level of polyphenol extract incorporated into cookies potentiated the oxidative changes. This observation leads to the conclusion that one method is insufficient to determine oxidative changes in food lipids. Many other substances may react with the TBA reagent and contribute to the increased absorption, causing an overestimation of the intensity of color complex. Interferences may come from additional absorption of other alkanals, 2-alkenals, 2,4-alkadienals, ketones, ketosteroids, acids, esters, proteins, sucrose, urea, pyridines and pyrimidines, also referred to as TBARS [Shahidi & Zhong, 2005]. Due to the non-specificity of the TBARS test, its results reflect the rancidity of food better than other conventional methods, especially in the case of off-flavors which are caused by volatiles from lipids and are affected by products of lipids interaction with nitrogenous compounds [Kolarkowska, 2003]. It is highly advisable to conduct further studies in this area to explain this phenomenon.

Measurement of changes in the fatty acid composition is useful for the identification of FA that are involved in the oxidation process. It is an indirect indicator of the extent of oxidation. During the storage of experimental cookies, losses were observed in the MUFA and PUFA content (Tables 2 and 3). A decreasing content of unsaturated FA was noticeable especially after the 18th week of storage. The effect of storage is more evident in the case of α -linolenic acid (ALA, *c*-9 *c*-12 *c*-15 C18:3) than in LA (linoleic acid, *c*-9 *c*-12 C18:2) because of the higher number of double bonds in the chain

[Frankel, 2005]. FA containing unsaturated bonds in their chain are susceptible to oxidation and their content decreases throughout storage, while the concentration of oxidation products increases [Izzreen & Noriham, 2011]. Changes in the ALA content are confirmed by the TBARS level in lipids extracted from cookies, while MDA is a product of oxidation of FA with 3 or more double bonds.

As evidenced by the two-way ANOVA, both CPE content and storage period had a significant impact on the quality of fat in the experimental cookies. Also Maisuthisakul *et al.* [2007] found that storage time was a significant factor for oxidative changes in rice crackers enriched with *Cratoxylum formosum* extract.

Chokeberry contains high amounts of proanthocyanidins which impart a specific astringent and bitter taste. This fruit is also exceptionally rich in anthocyanins, which is responsible for its deep, purple color [Wilkes *et al.*, 2014]. Therefore, depending on pH conditions, it may adversely affect the taste and color of confectionery products. That is why the sensory assessment of cookies enriched in CPE was conducted by the panel of experts. The increase in the level of CPE incorporated into cookies resulted in significant growth of intensity of astringent taste, acidic taste, off-taste and off-flavour (Figure 5). The sensory panel scored the highest intensity of above mentioned attributed in cookies containing 1000 mg of CPE. Our results are in agreement with these obtained by Górecka *et al.* [2010], who used raspberry pomace for cookies enrichment. Taking into account that desired by the consumers sweet and buttery taste were less intense, the evaluation of consumer acceptance of cookies with CPE seems to be of great importance.

CONCLUSION

Assessment of oxidative quality indicators of lipids extracted from cookies enriched with different levels of chokeberry polyphenols suggest that they may be used to extend the shelf-life of cookies. Regarding the oxidation degree, margarine cookies containing 100 and 250 mg of CPE seem to be safe for consumption within 9 weeks. It is also safe to incorporate 1000 mg of CPE to butter cookies when the time of storage is no longer than 9 weeks. However, the highest CPE content may decrease consumer acceptability of cookies, due to higher intensity of astringent and acidic taste as well as off-flavours.

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