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Research Article

Effect of Natural Antioxidants on the Stability of Linseed Oil and Fish Stored under Anaerobic Conditions

Kinga Śpitalniak-Bajerska,[1] Antoni Szumny,[2] Alicja Zofia Kucharska,[3] and Robert Kupczyński[1]

[1]*Department of Environment Hygiene and Animal Welfare, Faculty of Biology and Animal Science, Wroclaw University of Environmental and Life Sciences, Chelmonskiego 38c, 51-630 Wroclaw, Poland*
[2]*Department of Chemistry, Faculty of Biotechnology and Food Sciences, Wroclaw University of Environmental and Life Sciences, Norwida 25, 50-375 Wroclaw, Poland*
[3]*Department of Fruit, Vegetable and Plant Nutraceutical Technology, Faculty of Biotechnology and Food Sciences, Wroclaw University of Environmental and Life Sciences, Chelmonskiego 37, 51-630 Wroclaw, Poland*

Correspondence should be addressed to Robert Kupczyński; robert.kupczynski@upwr.edu.pl

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Vegetable and animal oils are susceptible to the oxidation of their lipid components on storage. Polyphenols from apple peels are investigated as potential natural antioxidants used for stabilizing polyunsaturated fatty acid and preventing oxidation. The aim of this study was assessing the antioxidant efficacy of apple pomace as natural antioxidant in linseed and fish oils, stored in anaerobic conditions. Apple pomace was added to the linseed and fish oils stored for eight weeks to evaluate the antioxidant activity of their polyphenolic components. The total phenolic content, activity of DPPH, $ABTS^{\bullet+}$, FRAP, acid value (AV), peroxide value (PV), and fatty acid profile were analyzed in storage tests. We found that apple pomace, regardless of the oil content of the formulation, was capable of blanking 2,2-diphenyl-1-picrylhydrazyl radicals. The highest ability to reduce Fe^{3+} ions occurred in the samples containing 30% of the fish oil. The use of apple pomace comprising polyphenolic compounds improves the stability of linseed and fish oils in storage tests. Polyphenols in apple pomace show a high antioxidant potential, as indicated by their values of DPPH, $ABST^{\bullet+}$, and FRAP. The addition of apple pomace resulted in limiting the acid and peroxide values of the samples during storage.

1. Introduction

Lipid oxidation is an essential reason for deterioration in the quality of edible oils [1, 2]. The lipid oxidation products formed adversely affect the quality of food articles by altering their sensory properties and reducing their nutritive value [2]. The products become toxic and may have a negative health effect when eaten, potentially leading to numerous diseases, e.g., heart conditions or cancer [2, 3].

Cold-pressed vegetable oils, especially those containing *n*-3 unsaturated fatty acids, are an important component of a healthy diet. Their specific chemical structure and the presence of reactive chemical bonds make them susceptible to peroxidation. Although it is a common practice in the food-processing industry to use synthetic antioxidants, such as BHA (butylhydroxyanisol), BHT (butylhydroxytoluene), TBHQ (*tert*-butylhydroquinone), natural antioxidants, including extracts from the seeds of oil plants, vegetable oils, extracts of plants such as rosemary, oregano olive waste cake extract, clove (*Syzygium aromaticum*), and cinnamon (*Cinnamomum zeylanicum*) are becoming more and more popular [4–6]. The synthetic antioxidants may have an adverse health effect, being toxic or cancerogenic, disturbing enzyme synthesis and activity [7]. In addition, commercial antioxidants (such as DHA) may damage DNA by binding nucleic acids and show cytotoxic or mutagenic activity [8].

There are earlier reports on the applicability of fruit-processing waste for the protection of lipids in foods against changes resulting from oxidation [9, 10]. The antioxidant properties of fruit extracts depend on both the level and the qualitative composition of phenolic compounds in the formulation. Apple pomace is a food-processing waste identified in numerous studies as a valuable source of natural antioxidants and bioactive compounds [2, 11, 12]. Usually, the waste from fruit pressing in juice production is used as a farm animal feed or as a fertilizer. It is also used for improving the stability of meat-based products or brown rice-based cracker [13, 14]. Fruits are a valuable source of phenolic compounds [15]. On the contrary, the use of certain vegetable oils or fish oil for feeding animals facilitates changes of the fatty acid profile in meat or milk [16, 17]. Recent years have witnessed particular interest in the use of fatty additives potentially increasing CLA or *n*-3 acids [18, 19]. Earlier reports demonstrated a considerable usefulness of apple pomace for improving the stability of fats in samples stored in aerobic conditions [20]. Therefore, the aim of the present research was to assess the antioxidant efficacy of apple pomace as natural antioxidant in the storage of linseed and fish oils in anaerobic conditions.

2. Materials and Methods

2.1. Experimental Design. The study involved vegetable (linseed) oil and animal (fish) oil with different fatty acid profiles, especially with respect to their content of long-chain fatty acids. The linseed oil (BiqOIL Laboratorium) and the fish oil (PPHW Tronina, Poland) were purchased on the wholesale market. The "Cortland" variety apple pomace was a waste material from "Maciejowy Sad" juice production. The apple pomace was dried in a laboratory oven at 60°C (Pol-Eko Aparatura, type SLW 115 TOP+, Poland) before being ground and mixed with the oils to obtain samples with 10% and 30% by weight of the oil. Samples of 50 g were packed and sealed using a vacuum packing machine (TEPRO SA Model PP3, Poland) and stored at a room temperature for 56 days. The samples were subjected to storage tests, including determination of total polyphenols by the Follin–Ciocalteu method [21], 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical binding power [22], antioxidant activity by the $ABTS^{\bullet+}$ method [23], the ferric reducing antioxidant power of the extracts (FRAP) [24], and determination of acid value (AV) and peroxide value (PV). The analyses were performed on Day 0 and after Day 7, 14, 28, and 56 of storage.

2.2. Content of Fatty Acids. The fatty acid profile of the test samples was analyzed by gas chromatography coupled with mass spectrometry, whereby the compositions of the obtained methyl esters were determined.

2.3. Preparation of Fatty Acid Methyl Esters (FAME). The tests were carried out using the methodology developed by Maślak et al. [25]. About 50 mg of the fat test sample was hydrolyzed with 7% methanolic solution of potassium hydroxide (2 mL) for 5 minutes at the boiling point of the solvent. The resulting fatty acid salts were esterified by addition of 3 mL 14% methanolic solution of boron trifluoride and kept at its boiling point for 5 minutes. On completion of the reaction, 15 mL cyclohexane was added, and the organic fraction was washed with sodium hydrocarbonate (2×10 mL) and brine, obtaining a neutral pH. The organic solution was dried over anhydrous sodium sulfate. After concentrating the solvent, the resulting esters were kept at −20 °C until GC.

2.4. Chromatographic Analyses. The fatty acid profiles of the test samples were analyzed using gas chromatography coupled with mass spectrometry (Saturn Chrompack 2000/2000). Separation was effected using a nonpolar column ZB WAX-MS ($30\,m \times 0.25\,mm \times 0.25\,\mu m$ film from Zebron, Phenomenex). The measurements were carried out by ionization using electrons (so-called "electron impact", EI) at the following conditions: ionization 70 eV, rate 1 scan per second, and split ratio 1 : 40. The temperature program was as follows: heating rate 5°C/min from 80°C to 200°C, then 25°C/min to 260°C, injector temperature 220°C, and helium gas carrier at 1 mL per minute. The analyses were carried out with ion collection in the range (m/z) from 39 to 300 with EI ionization of 70 eV, 1 scan per second.

The acids in the test samples were identified based on three different analytical methods: (1) comparison of Kovac retention indices, as found by the logarithmic method with respect to linear *n*-alkanes, with reference values from the NIST14 and Adams databases; (2) mass spectra comparison between the unknown compound and the spectrum in the NIST14 database; and (3) comparison of retention times and mass spectra of available chromatographic standards, Sigma-Aldrich, mix of 37 chromatographic standards.

2.5. Analysis of Phenolic Content and Antioxidant Activity. The phenolic content and antioxidant activity of the formulations were determined using the UV-2401PC spectrophotometer from Shimadzu.

The extract for analyses was prepared by weighing 0.5 g of the formulation and adding enough 50% aqueous solution of methanol (50% aqueous solution of methanol + SO_2 (1 ml/l)) to obtain a volume of 5 ml, keeping on water bath for 15 min, and cooling for 12 hours. The resulting solution was filtered. The following parameters were determined for the extracts.

2.5.1. Total Phenolic Compound Content Analysis. Total phenolic content by the Follin–Ciocalteu method in which phenol compounds, among other ones, form a colored complex with the Follin–Ciocalteu reagent (a mixture of sodium tungstate, sodium molybdate, and lithium sulfate in a medium composed of phosphoric and hydrochloric acids); the complex is green-blue. After oxidation, the complex was analyzed spectrophotometrically at wavelengths starting at 765 nm. The phenolic content was calculated as gallic acid (GA).

2.5.2. Free-Radical Scavenging Ability by the Use of a DPPH Radical. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical binding power, which consists in reacting the antioxidants present in the test sample, reduce the stable nitrogen radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), thereby decreasing absorbance measured at 517 nm. The active radical solution is purple, and its discoloration indicates that the previously unpaired electron has been paired. The intensity of discoloration of the DPPH solution after addition of the solution containing the antioxidants is a measure of their free-radical scavenging ability.

2.5.3. Thiobarbituric Acid Reactive Substances (TBARS) Assay. Antioxidant activity by the method with $ABTS^{\bullet+}$ enables the quantitative assessment of free-radical scavenging ability of a given component to quench the stable $ABTS^{\bullet+}$ radical (2,2′-azine-bis acid (3-ethylenebenzothiazoline $ABTS^{\bullet+}$). Absorbance was measured at wavelength $\lambda = 734$ nm.

2.5.4. Ferric Reducing Antioxidant Power (FRAP) Assay. The FRAP is based on the reduction of ferric 2,4,6-tris(2-pyridyl)-1,3,5-triazine [Fe(III)-TPTZ] to the ferrous complex at low pH, followed by spectrophotometric analysis. Quantitative analyses were performed by the external standard method using ferrous sulfate as the reference standard and correlating the absorbance (λ 593 nm) with the concentration.

The results of antioxidant capability of DPPH, $ABTS^{\bullet+}$, and FRAP were expressed in μmol Trolox/g of sample. Determination of the peroxide value (PV) as an indicator of the original products of oxidation was performed according to the procedure described in PN-ISO 3960-2016 [26], whereas the acid value (AV) was found by the method described in PN-ISO 660-2010 [27]. Determinations of PV and AV were carried out for pure linseed and fish oils and for the formulations prepared by mixing the oil with the apple pomace, in triplicate for every sample.

The data of apple pomace and linseed oil included in Tables 1–4 and Figures 1 and 2 are from the PhD dissertation of Kinga Śpitalniak-Bajerska.

2.6. Statistical Analysis. A statistical analysis of the numerical values, obtained in the tests, was performed using the Statistica 10.0 software (StatSoft, Tulsa, OK, USA), a two-way analysis of variance (ANOVA). The significance of differences between the means was assessed using the Duncan test. Determined arithmetic means ($\overline{x}$), standard deviations (SD), and standard error of mean (SEM) were also determined.

3. Results and Discussion

Total phenolic content and the antioxidant activity (DPPH, FRAP, and $ABTS^{\bullet+}$) in the "Cortland" apple pomace before addition of the fats are shown in Table 5. Total polyphenols changed immediately after seven days. Their level was mainly shaped by storage times ($p < 0.01$), whereas the effect of the linseed or fish oil content was insignificant. The lowest reduction of total polyphenols was found in pure pomace. The addition of 10% of the fish or linseed oil exerted a lesser effect on changes in the phenolic content during storage than a higher oil dosage (30%) (Table 1). However, the decrease in polyphenols was smaller for the test samples containing the apple pomace with the linseed oil. The most observable decrease in that value was detected, on Day 7 of the tests, for samples with 30% of linseed or fish oil. In apple peels alone, total phenolic content may range from 150 to 700 mg GA/100 g [2] or be as high as 830.9 mg GA/100 g [28]. Earlier studies by the present authors indicate that the phenolic content of apple pomace was higher—1385.44 mg GA/100 g [20]. Differences in the concentrations of active compounds in the apple pomace tested are attributable to many factors, including the fruit variety, cultivation, and weather conditions, as well as the juice production process conditions.

Antioxidant activity of the alcoholic extracts of apple pomace is shown in Tables 2–4. The findings are presented in the same units, as TEAC (Trolox equivalent antioxidant capacity). Differences in the antioxidant activity among the three methods are attributed to differences in the responses of iron and the radicals to the amount and quality of the compounds present in the apple pomace. On Day 7, the highest DPPH radical quenching ability was detected for the apple pomace enriched with 10% of linseed oil (Table 2). No changes in their activity with respect to DPPH radical were observed for extracts with 10% of fish oil (15.2 vs. 14.55 μmol Trolox/g sample). Lower activity with respect to DPPH radical was observed at the end of the experiment in the samples containing 30% of linseed oil, which also showed a stronger drop in antioxidant activity. The study revealed a greater decrease in activity towards DPPH after 56 days for pure pomace and apples mixed with the linseed oil than with the fish oil, regardless of the fat content. The linseed oil contains natural antioxidants such as tocopherols, tocotrienols, carotenoids, phenolic compounds, and sterols [29, 30]. The fact that the test samples with the linseed oil showed lower antioxidant activity could be explained by the antagonistic effect of the antioxidants in the oil and in the apple pomace, which could also intensify the oil oxidation process.

After seven days, the highest Fe^{3+} reducing power was observed for the samples of apple pomace mixed with 10% linseed oil (Table 3). The storage tests confirmed a significant ($p < 0.01$) effect of oil dose on the value of FRAP. Higher contents of the fish oil and linseed oil resulted in lower values of FRAP. On Day 56, the highest Fe^{3+} reducing power was observed for the apple pomace with 10% of the fish oil. There was a decrease in the value of FRAP over time; however, the changes were not significant. On Day 28, the value of FRAP dropped in all the groups by a comparable fraction. On Day 7, the ABTS tests showed similar values for the apple pomace samples with 10% and 30% of the linseed oil (29.06 and 29.65 μmol Trolox/g, respectively). On the final day, the antioxidant activity towards $ABTS^{\bullet+}$ radicals was higher for the apple pomace enriched with the fish oil (especially for the 10% samples), as compared with those mixed with the linseed oil (Table 4).

In this paper, dried apple pomace was used as the natural source of antioxidants. Many authors who have tested apples

Table 1: The content of total polyphenols in the test formulations during storage (mg GA/100 g).

Total phenolic mg GA/100 g (ml)	Storage time (day)					SEM	p value of time
	0	7	14	28	56		
Pure pomace	613.74^{Aa}	585.57	555.60	538.29	530.20	16.89	0.01
Pomace + fish oil 10%	563.96^{Ab}	423.81^{A}	411.36^{A}	369.4^{A}	279.99^{Ba}	17.59	<0.01
Pomace + linseed oil 10%	571.31^{Ab}	451^{A}	375.59^{B}	298.01^{Ba}	308.79^{Bb}	17.46	<0.01
Pomace + fish oil 30%	441.36^{B}	354.93^{Ba}	279.82^{B}	290.43^{B}	197.59^{A}	14.79	0.01
Pomace + linseed oil 30%	454.36^{B}	389.75^{Bb}	319.9^{C}	279.3^{Bb}	218.97^{A}	17.20	<0.01

The letters A, B, and C in the same columns indicate statistically high significant differences ($p < 0.01$). The letters a and b in the same columns indicate statistically significant differences ($p < 0.05$). SEM, standard error of the mean.

Table 2: The antioxidant activity (DPPH) apple pomace together with the tested oils (μmol Trolox/g).

DPPH (μmol Trolox/g sample)	Storage time (day)					SEM	p value of time
	0	7	14	28	56		
Pure pomace	14.13	13.20	11.10	10.50	9.30	0.39	<0.01
Pomace + fish oil 10%	15.51	15.20^{A}	15.10^{A}	14.90^{A}	14.55^{A}	0.42	0.93
Pomace + linseed oil 10%	15.60	15.47^{A}	15.95^{A}	14.11^{A}	11.28^{B}	0.44	<0.01
Pomace + fish oil 30%	16.71	15.9^{A}	10.42^{B}	11.25^{B}	12.38	0.53	0.01
Pomace + linseed oil 30%	14.64	13.7^{B}	13.70^{A}	11.94^{B}	9.28^{B}	0.44	<0.01

The letters A and B in the same columns indicate statistically high significant differences ($p < 0.01$). SEM, standard error of the mean.

Table 3: The antioxidant activity (FRAP) of apple pomace together with the tested oils (μmol Trolox/g).

FRAP (μmol Trolox/g sample)	Storage time (day)					SEM	p value of time
	0	7	14	28	56		
Pure pomace	12.57^{A}	11.90^{Aa}	11.20^{Aa}	10.50^{A}	10.40^{A}	0.68	0.07
Pomace + fish oil 10%	21.20	20.82^{B}	18.6^{B}	16.84^{B}	13^{B}	0.79	0.03
Pomace + linseed oil 10%	23.41^{B}	22.2^{B}	19.36^{B}	17.06^{B}	12.57^{B}	0.91	<0.01
Pomace + fish oil 30%	22.31	22.0^{B}	17.56^{b}	17.78^{B}	10.95^{A}	0.48	0.14
Pomace + linseed oil 30%	23.48^{B}	17.1^{Ab}	15.67	16.72^{B}	11.36	0.82	<0.01

The letters A and B in the same columns indicate statistically high significant differences ($p < 0.01$). The letters a and b in the same columns indicate statistically significant differences ($p < 0.05$). SEM, standard error of the mean.

Table 4: The antioxidant activity ($ABTS^{\bullet+}$) of apple pomace together with the tested oils (μmol Trolox/g).

ABTS (μmol Trolox/g sample)	Storage time (day)					SEM	p value of time
	0	7	14	28	56		
Pure pomace	27.93	26.89	25.62^{a}	24.10^{A}	18.54^{A}	1.45	0.01
Pomace + fish oil 10%	36.30	35.56^{A}	24.58^{a}	17.35^{Ba}	14.18^{b}	1,69	<0.01
Pomace + linseed oil 10%	32.41	29.06	24.35^{a}	10.08^{C}	13.69	1.41	<0.01
Pomace + fish oil 30%	28.75	24.8^{B}	24.1^{a}	12.38^{b}	13.87	1.56	<0.01
Pomace + linseed oil 30%	34.62	29.65	19.93^{b}	14.74	11.54^{Ba}	1.50	<0.01

The letters A and B in the same columns indicate statistically high significant differences ($p < 0.01$). The letters a and b in the same columns indicate statistically significant differences ($p < 0.05$).

or apple pomace confirm their high antioxidant activity, although its specific value largely depends on the fruit variety [2, 31, 32]. Reduced content of polyphenols that we observed on Day 14 ($p < 0.01$) manifested itself also in lower antioxidant activity, both for the apple pomace and for the samples with the linseed oil. Any later changes in the polyphenolic content were less severe. High phenolic content in apples has a significant effect on their antioxidant activity [32]. Apple peel extract was proved to have a favorable effect on the inhibition of lipid peroxidation [2]. Synthetic antioxidants, with a potentially undesirable effect on human and animal health, are commonly used in the food-processing industry and animal feed production [2].

The metal ion reducing power is one of the mechanisms of action of antioxidants and is typical of secondary antioxidants [33]. A large number of antioxidants have reducing properties. In the FRAP method, Fe^{3+} ions are the indicator: they are reduced to Fe^{2+} forming a colored complex with 2,4,6-tripyridyl-*S*-triazine (TPTZ). An increase in the absorbance of that complex indicates a high level of

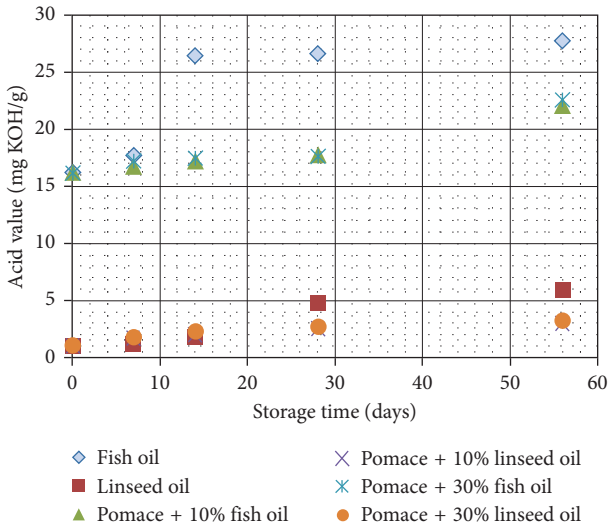

Figure 1: Changes in acid number (mg KOH/g) of the test oils stored with the addition of apple pomace.

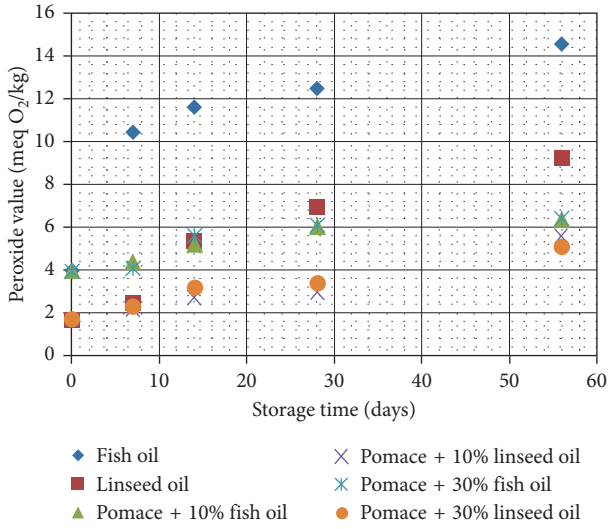

Figure 2: Changes in peroxide value (meq O_2/kg) of the test oils stored with the addition of apple pomace.

Table 5: The content of total polyphenols and antioxidant activity (DPPH, FRAP, and ABTS$^+$) of apple pomace (μmol Trolox/g).

Item	Initial sample ($\overline{x} \pm$ SD)
Total polyphenols	613.74 ± 19.19
DPPH	14.13 ± 0.89
FRAP	12.57 ± 2.56
ABTS	27.93 ± 6.83

antioxidants in the investigated material. On the final day of the experiment, the samples containing apple pomace with 30% of the fish oil showed the lowest Fe^{3+} ion ferric reducing power (10.95 μmol Trolox/g sample), whereas the highest value was observed in the samples with 10% of the fish oil (10.95 μmol Trolox/g sample) (Table 3). In similar studies, ethanolic extracts of frozen apple peels more strongly inhibited the oxidation of fish oil than synthetic antioxidants such as BHT (butylhydroxytoluene) [28]. Also a green-tea extract appeared to be more powerful than BHA (butylhydroxyanisol) in inhibiting the oxidation of fish oil [34].

Sea fish and sea animals in general, as well as the oils derived from them, are a rich source of EPA and DHA acids [35, 36]. Among oil plants, α-linolenic acid is abundant in linseed oil [37] as well as in *Camelina sativa* [38]. For analyses of the fatty acid profiles for fats using more complex matrices (dried apple pomace), the most recommendable solution is to use a gas chromatography apparatus coupled with a mass spectrometer (GC-MS). For the reasons stated above, the solution was applied in the present study.

The fish oil tested herein was characterized by a higher content of C16:0, C16:1, and C18:1 *n*-9, lower content of C18:0, C18:2 *n*-6, C18:3 *n*-3, and total number of polyunstaturated fatty acids (PUFA) *n*-3, as compared with the linseed oil. The content of α-linolenic acid (ALA) was high in the linseed oil, compared with the fish oil. The storage of the fish oil mixed with the apple pomace for 56 days had no effect on its content of C18:3 *n*-3, whereas a decrease in the amount of that fatty acid was detected in the linseed oil samples mixed with the apple pomace (Table 6). The specific apple pomace used and the fact that the fish oil was stored in a sealed container were not found to have an effect on the level of EPA although that of DHA was reduced (4.98 vs. 5.12% at the beginning of the experiment). The addition of apple pomace more effectively limited the growth of the saturated acid content in the fish oil, in comparison with the linseed oil, on the final day of testing. Generally, after 56 days of storage, PUFAs increased in fish oil, whereas in linseed oil, it decreased.

The formulation tested in our study is applicable in animal feed production for modification of the fatty acid profile in animal tissues and milk. Supplementation of cow feeds with fish oil or a lipid complex based on fish oil and vegetable oils caused a considerable increase in the content of CLA in milk fat [36, 39]. Increasing the content of dietary FA (CLA, and *n*-3 FA) and maintaining a favorable ratio between *n*-3 and *n*-6 FA in milk will enhance the nutritive and therapeutic value of dairy products [40].

On the starting day of the experiment, AV was 16.17 mg KOH/g and 1.05 mg KOH/g in the fish oil and linseed oil, respectively (Figure 1). After 56 days of storage, AV increased to 27.69 mg KOH/g and 4.08 mg KOH/g for the fish and linseed oil, respectively ($p < 0.01$). The addition of apple pomace limited the increase as early as after 7 days of storage for the fish oil and after 14 days for the linseed oil, and it slowed down any further increase in AV. The slowest increase in AV during the experiment was observed for the samples with 10% of the linseed oil. For the linseed oil, the acid value did not exceed the cold-pressed oil values, as recommended in the Codex Alimentarius [41] (AV ≤ 4 mg KOH/g). By contrast with refined oils, the acid value for cold-pressed oils may increase due to the progressing hydrolysis (enzymes and trace amount of water in the oil and air moisture) [1]. For the fish oil, the value should be a maximum of 3 mg KOH/g, whereas for oils rich in phospholipids, the permissible

Table 6: Fatty acid content (%) in fish and linseed oil or in formulations (oil + apple pomace) before and after 56 days of storage under anaerobic conditions.

Fatty acid	Oils and preparations during the storage period					
	Fish oil	Fish oil after 56 d	Fish oil + apple pomace after 56 d	Linseed oil	Linseed oil after 56 d	Linseed oil + apple pomace after 56 d
C14:0	2.78	2.72	2.36	0.02	0.02	0.33
C15:0	0.27	0.23	0.34	nd	nd	nd
C16:0	12.85	11	11.94	5.29	5.39	6.15
C16:1 cis	4.40	4.39	4.65	0.03	0.02	0.34
C17:0	1.96	0.96	1.15	0.02	0.03	0.20
C17:1 cis	0.21	0.23	0.24	nd	nd	nd
C18:0	2.97	2.92	3.05	3.97	4.37	4.58
C18:1 *n*-9 cis	33.12	33.41	33.01	20.75	21.07	21.12
C18:1 *n*-9 trans	3.37	3.49	3.41	0.80	0.86	1.12
C18:2 *n*-6	10.90	11.29	11.6	15.81	16.49	17.07
C18:3 *n*-6	0.32	0.42	0.38	0.03	0.01	0.63
C18:3 *n*-3	4.12	382	3.97	52.73	51.01	47.35
C18:4 *n*-3	1.04	1.14	1.06	nd	nd	nd
C20:0	0.24	0.30	0.27	0.12	0.19	0.16
C20:1 *n*-9	5.03	5.39	5.26	0.15	0.18	0.26
C20:2 *n*-6	0.75	0.84	0.74	0.13	0.18	0.26
C20:3 *n*-6	0.37	0.46	0.37	nd	nd	nd
C20:3 *n*-3	0.65	1.45	1.7	nd	nd	nd
C20:4 *n*-6	0.53	0.58	0.31	0.06	0.09	0.34
C20:5 *n*-3	3.03	3.11	3.15	nd	nd	nd
C21:0	0.14	0.18	0.14	nd	nd	nd
C22:0	4.50	4.97	4.65	nd	nd	nd
C22:1 *n*-9	0.40	0.11	0.12	nd	nd	nd
C22:5 *n*-6	0.93	1.06	1.15	nd	nd	nd
C22:6 *n*-3	5.12	5.53	4.98	nd	nd	nd
Saturated FA	25.71	23.28	23.90	9.42	10.00	11.42
Unsaturated FA	74.29	76.72	76.10	90.49	89.91	88.49
Monounsaturated FA	46.53	47.02	46.69	21.73	22.13	22.84
Polyunsaturated FA	27.76	29.70	29.41	68.76	67.78	65.65
Total *n*-6 FA	13.80	14.65	14.55	16.03	16.77	18.30
Total *n*-3 FA	13.96	15.05	14.86	52.73	51.01	47.35

nd = <0.01 or not detected.

value of AV is 30 mg KOH/g. The high acid value of the fish oil may have been caused by long storage period or a high content of free fatty acids. According to Rotkiewicz and Konopka [42], oils tend to be hydrolyzed at a rate which increases with the content of free fatty acids they have just after being pressed.

The fat oxidation process intensity, as measured with their PV, indicates the current amount of peroxides. Before using the apple pomace, the PV was 1.7 meq O_2 per 1 kg of the linseed oil and 3.99 meq O_2 per 1 kg of the fish oil (Figure 2). The permissible value for vegetable oils is <15 meq O_2/kg [41]. Long-term storage of linseed oil and apple pomace caused a lower increase in PV, in comparison with pure oil samples. The increase in the peroxide value for the linseed oil could be due to the fact that we used cold-pressed linseed oil, that is, a nonrefined oil containing accompanying compounds [43], such as primary and secondary products of oxidation, free fatty acids, colorants, metals, or incomplete triacylglycerols that could have intensified the oxidation process. The steepest increase in the peroxide curve was detected for the pure fish oil ($p < 0.01$). On the final day of the experiment, PV of the fish oil (10 and 30%) mixed with apple pomace was 6.35 and 6.43 meq O_2 per 1 kg, respectively, and 14.56 meq O_2 per 1 kg for the pure fish oil. The fish oil is highly susceptible to oxidation, which is attributed to its high number of 1,4-pentadiene systems in its structure, and the absence of endogenous antioxidants [44]. The linseed oil (which contains α-linolenic acid; three double bonds) is less susceptible to oxidation processes, in comparison with, for instance, EPA acid (five bonds) and DHA (six bonds), which are abundant in the fish oil [45]. The oxidation of α-linolenic acid may lead to the formation of seven trans isomers, whereas the oxidation of EPA with five bonds produces 32 isomers.

Anaerobic storage of the fish or linseed oils mixed with the apple pomace inhibited the increase of AV and PV in the stored samples. Aerobic conditions tended to result in higher increases in AV and PV, according to earlier reports [20].

4. Conclusions

The apple pomace was the source of phenolic compounds enhancement of oils stability, evidenced by peroxide and hydroxide value at the end of the study. After 56 days of storage, the total polyphenol contents in all tested combinations of fish or linseed oils with pomace were reduced (46 to 55%). However, apple pomace used in connection with oils, storage under anaerobic conditions did not significantly increased oxidation or degradation of fatty acids. The highest antioxidant capacity against $DPPH^{\bullet}$ radicals and ABTS radicals cation was found in combination of apple pomace with 10% of linseed oil content, and the lowest capacity was found in samples with 30% of linseed oil content. In animals, feeding could be solved in the proposed approach, which makes the product acceptable for livestock breeding. Pomace, as a relative rich source of polyphenols, could improve metabolic and oxidative processes in animals. Therefore, the further research in this direction is necessary.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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