Research Article

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Lyophilized apples on flax oil and ethyl esters of flax oil - stability and antioxidant evaluation

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Abstract: The research aimed to assess the effect of whole lyophilized apples added to flax oil or flax ethyl esters for oxidation processes and fatty acid profiles. The samples were washed with nitrogen and sealed in PE pouches. The analyses were performed on day 0 and afterwards on 7th, 14th, 28th, 56th and 84th day of storage. The antioxidant capacity was determined by stating in the samples DPPH, ABTS^{••}, and FRAP. Fatty acid profiles of the test samples were analyzed using GC-MS. The addition of lyophilized apples did not limit the decrease in C18: 3, *n*-3 after storage. The reduction of free radicals (ABTS, DPPH) was the lowest in samples with ethyl esters mixed with lyophilized apples rather than in the case of specimens that were a mixture of flax oil and lyophilized apples. The addition of lyophilized apples limited the decrease of IV and PV in ethyl esters, and in the case of flax oil AV. Obtained data show the possibility of wider usage of apple for the improvement of stability of the ethyl esters or flax oils and at the same time there are the real possibilities of the development of such kinds of preparations for the feeding industry.

Keywords: linseed oil, lyophilized apples, polyphenols, antioxidant capacity, flax ethyl esters.

1 Introduction

Increased awareness of the health-promoting properties of polyunsaturated fatty acids (PUFA) has generated great interest in the food industry and consumers of vegetable and animal oils, as well as products made from them [1,2,3]. However, foods and products containing a lot of polyunsaturated fatty acids (PUFAs) easily undergo oxidation processes. Oxidation of PUFA during its storage has an adverse effect on organoleptic properties (taste, color, texture, smell) and nutritional value. Consumption of food products containing oxidized lipids negatively affect the antioxidant mechanisms and immunological functions in the human and animal body [4,5].

The durability of vegetable oils depends on many factors, mainly on the quality of seeds, fatty acid composition, production conditions, distribution conditions, storage, oxygen availability, presence of free fatty acids, monoacids and diacylglycerols, transition metal ions, phospholipids, enzymes, dyes and antioxidants [6,7]. The fatty acid profile has a significant influence on the oxidative stability and physicochemical properties of vegetable oils [8].

Stability of *n*-3 fatty acids in flax oil during storage are moderate due to their natural autooxidation processes [9], as well as degradation of naturally occurring other compounds i.e. cyclolinopeptides. The most important issue for the practical usage of flax oil is bitterness that occurs over time [10]. Although there are cyclolinopeptide low flax varieties, their cultivation is problematic and on the food market it is unavailable. The one easy way of cyclolinopeptide reduction is hydrolysis and transesterification to flax ethyl esters (FEE).

During the FEE production, cyclolinopeptides are eliminated from oil. One of these cyclolinopeptide E types is strictly responsible for the bitter off-taste during

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the storage due to methionine oxidation. On the other hand, fatty acid ethyl esters have proven to have better bioavailability [12,13]. The technology of FEE production is becoming more available for consumers and they seem to be viable alternative for flax oil [14].

Among the various antioxidant additives used to extend the usefulness of fats, there are natural additives, e.g. herbal extracts from herbs, synthetic antioxidants [15, 16], as well as extracts from oilseeds rich in antioxidants, both lipophilic, e.g. tocopherols or hydrophilic phenolic compounds [17,18]. In the case of synthetic antioxidants, although commonly used in the food and feed industry, there were discovered to be possible toxic effects. [19,20] Williams et al. 1999, Huber and Rupasinghe 2009).

Sources of natural antioxidants that may prolong the usefulness of food for vegetable oils are also fruits; fruit extracts including their waste parts like pomace [21,22]. The pomegranate and apple peel extract inhibited the increase in the peroxide value in corn oil [21]. Kurhade and Waghmare [22] used ethanolic banana peel extract as a natural antioxidant for sunflower and soybean oil. Apple peel extract has also proved to have a beneficial effect on the increase of milk antioxidative capacity [23] and lipid peroxidation inhibiting activity [20]. The extracts rich in polyphenols from apples inhibit the oxidation of cholesterol in minced meat stored in a refrigerator [24] and inhibit the production of carcinogens during cooking [25].

Our research focused on the use of whole lyophilized apples as a source of natural antioxidants added to flax oil or ethyl esters made of flax oil. In addition to the basic chemical composition, preserved in the lyophilization process, apples preserved in this way are characterized by a favorable taste, and their form allows their long storage [26,27]. The aim of the study was to evaluate the effect of lyophilized apples (rich in antioxidant compounds) on the increase of the stability of flax oil or flax oil ethyl esters. We hypothesize that lyophilized whole apples and targeted storage conditions can affect oxidation processes and the fatty acid profile of flax oil and flax oil ethyl esters.

2 Methods

2.1 Materials and sample procedure

The research involved the use of linseed oil (BiqOIL Laboratorium s.c.) and a mixture of ethyl esters of fatty acids obtained from flax oil (FLC Pharma Sp. z o.o.) and lyophilized, afterwards powdered, Cortland' type apples (Industrial Automation Industry). The purity of the flax

ethyl esters was above 98% as assessed by ^1H NMR (in CDCl_3) spectra.

The samples were thoroughly mixed with lyophilized apples. The lyophilized apples were mixed with the oil or ethyl esters to obtain samples containing 10 g oil / esters in 25 g of lyophilized apples. The method of obtaining ethyl esters of polyunsaturated fatty acids is covered by patent protection [28,29]. The production technology is based on transesterification of linseed oil (virgin oil) with the ethyl alcohol in the presence of an inorganic catalyst. The process is carried out under anaerobic conditions at a temperature of 15-35°C. Next, unreacted bioethanol is removed, and the glycerol phase is separated from the crude ester phase in gravity separators. Purification of esters is carried out by centrifugation, and then the esters obtained are subjected to further purification by releasing residual alcohol with nitrogen gas (at a temperature of up to 50°C) and by sedimenting the remaining glycerine phase.

The samples were packed in PE string bags and tightly sealed by welding the edge with a plastic welder (B2BPartner Inc.).

The mixing, portioning and packaging procedure was carried out in a modified atmosphere. Before closing the bag, the preparation was thoroughly flushed with nitrogen. The product was packed, in the presence of nitrogen to protect polyunsaturated fatty acids from oxidation into polyethylene sacks. Samples prepared in this way were subjected to storage tests including the determination of total polyphenol content by the Folin-Ciocalteu method, strength of 2,2-diphenyl-1-picrylpyrazole radicals (DPPH), antioxidant activity by the ABTS $^{*+}$ method, reducing extracting force (FRAP) and marked acid value (AV) peroxide value (PV) and iodine value (IV). The analyses were performed in 5 independent replicates on day 0 and after 14, 28, 56 and 84 days of storage for total pthenolic (n=45), DPPH (n=90), FRAP (n=90), ABTS (n=90) and in 6 independent replicates on day 0 and after 7, 14, 28, 56 and 84 days of storage AV (n=72), PV (n=72), IV (n=72). The conditions during the repetition of the analysis, were the same.

2.2 Determination of fatty acids

The fatty acid profile of the test samples was analysed by gas chromatography coupled with mass spectrometry (Saturn Varian GC-MS Chrompack 2000/2000, USA), whereby the compositions of the obtained methyl esters were determined.

2.3 Preparation of fatty acids methyl esters (FAME)

The tests were carried out using the methodology developed by Maślak et al. [30]. 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 the 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 of cyclohexane was added, and the organic fraction was washed with sodium bicarbonate (2 x 10 mL) and brine, to obtain a neutral pH. The organic solution was dried over anhydrous sodium sulfate. After evaporation of the solvent, to volume ~0,8 mL, the resulting esters were kept at -20°C until GC was performed.

2.4 Chromatographic analyses

The fatty acid profiles of the test samples were analysed using gas chromatography coupled with mass spectrometry (Saturn Varian GC-MS Chrompack 2000/2000, USA). Separation was performed using a non-polar column ZB WAX-MS (30 m x 0.25 mm x 0.25 µm film Zebron, Phenomenex). The measurements were carried out by ionization using electrons (the so-called “electron impact”, EI) at the following conditions: ionization 70 eV, rate 1 scan per second, 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, helium gas carrier at 1 mL per minute. The analyses were carried out with ion collection in the range (m/z) from 39 to 380 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 retention times and mass spectra of available chromatographic standards, Merck, mix of 37 FAME chromatographic standards 2) mass spectra comparison between the unknown compound and the spectrum in the NIST14 database; 3) comparison of Kovac retention indices, as found by the logarithmic method with calculated according to linear *n*-alkanes (C-7 to C-40, Neochem), with reference values from the NIST14 databases.

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 + sodium bisulphate, 1mL/L) to obtain a volume of 5 mL, keeping in a water bath for 15 min and cooling for 2 hours. The resulting solution was filtered. The following parameters were determined for the extracts:

- 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 a gallic acid (GA) equivalent.- 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical binding power, which works by reacting with the antioxidants in the test sample; they 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.
- Antioxidant activity by the method using ABTS^{•+}; it enables the quantitative assessment of free-radical scavenging ability of a given component to quench the stable ABTS^{•+} radical (2,2'-azine-bis acid (3-ethylenebenzothiazoline ABTS^{•+}). Absorbance was measured at wavelength $\lambda = 734$ nm.
- The ferric reduces antioxidant power of the extracts (FRAP), by measuring the ability of 2,4,6-tripyridyltriazine (TPTZ) to reduce Fe ion; when reacted with an antioxidant, the Fe (III) compound gives a colored product which is determined at 595 nm.

Table 1: Fatty acid profile of formulations during storage.

Fatty acids	Flax oil	Flax oil	Flax oil+ lyophilized apples	SEM	P-value	Ethyl esters	Ethyl esters	Ethyl esters + lyophilized apples	SEM	P-value
	Storage time (day)									
	0	84	84			0	84	84		
C 16:0	5.30	5.41	5.48	0.07	0.62	3.84 ^A	6.77 ^B	5.73 ^C	0.37	0.01
C 16:1	0.03 ^A	0.02 ^A	0.34 ^B	0.04	0.01	0.10 ^A	0.06	0.01 ^B	0.02	0.04
C 17:0	0.02 ^A	0.03 ^A	0.20 ^B	0.03	0.01	0.50 ^{Aa}	0.80 ^b	0.98 ^B	0.08	0.01
C 18:0	3.97 ^A	4.37 ^B	4.58 ^B	0.09	0.01	5.97 ^A	5.27 ^B	5.10 ^B	0.13	0.01
<i>c9</i> C 18:1	20.75 ^A	21.07 ^B	20.12 ^C	0.12	0.01	19.92 ^A	22.50 ^B	22.11 ^C	0.34	0.01
<i>t9</i> C 18:1	0.79 ^A	0.86 ^A	1.12 ^B	0.05	0.01	0.28 ^A	0.02 ^B	0.75 ^C	0.09	0.01
C 18:2 <i>n</i> -6	15.81 ^A	16.49 ^B	16.07 ^C	0.09	0.01	17.02 ^a	17.02 ^A	16.86 ^{Bb}	0.04	0.03
C 18:3 <i>n</i> -6	0.03 ^A	0.01 ^A	0.63 ^B	0.09	0.01	Nd	nd	nd	nd	nd
C 18:3 <i>n</i> -3	52.73 ^A	51.01 ^B	50.35 ^C	0.31	0.01	51.32 ^A	47.57 ^B	48.46 ^C	0.48	0.01
C 20:0	0.12 ^A	0.19 ^B	0.16 ^C	0.01	0.01	nd	nd	nd	nd	nd
C 20:1 <i>n</i> -9	0.15 ^A	0.18 ^B	0.26 ^C	0.02	0.01	nd	nd	nd	nd	nd
C 20:2 <i>n</i> -6	0.13 ^A	0.18 ^B	0.26 ^C	0.04	0.01	nd	nd	nd	nd	nd
C 20:4 <i>n</i> -6	0.06 ^A	0.09 ^A	0.34 ^B	0.07	0.01	nd	nd	nd	nd	nd
Σ PUFA _n -3	52.73 ^A	51.01 ^B	50.35 ^C	0.05	0.01	51.32 ^A	47.57 ^B	48.46 ^C	0.04	0.01
Σ PUFA _n -6	16.03 ^A	16.77 ^B	17.30 ^C	0.02	0.01	17.02 ^a	17.03 ^a	16.86 ^b	0.03	0.04

The letters A, B, in the same rows indicate statistically high significant differences ($p < 0.01$)

The letters a, b, in the same rows indicate statistically significant differences ($p < 0.05$)

SEM - standard error of the means,

nd = < 0.01 or not detected.

The results of antioxidant capability of DPPH, ABTS^{•+} and FRAP were expressed in $\mu\text{mol Trolox/g}$ of the sample.

2.6 Analysis of peroxide value, acid and iodine value

Determination of the peroxide value (PV), acid value (AV) and iodine value (IV) was performed by the method described in the previous work [31]. All designations were made in triplicate for each sample.

2.7 Statistical analysis

The numerical values obtained during the tests were subjected to statistical analysis using the Statistica v. 10.0. Arithmetic means ($\bar{X}$), standard deviations (SD) are

included. Significance of differences between means were estimated using the Duncan test. The study of correlations between variables was carried out with the Spearman correlation (r).

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

The analysis of the fatty acids profile was aimed at demonstrating the differences between the composition of the unprocessed fat additive and the chemical esters obtained from ethyl esters of flax oil. The fatty acid profile of flax oil was typical for the raw material from which the oil was obtained and did not differ from the data contained in the literature [7,32,33,34]. In both preparations (Table 1) a high content of polyunsaturated fatty acids was found,

Table 2: Content of α -linolenic acid and average number of double bonds in the triacylglycerol molecule (TAG) determined on the basis on the analysis of NMR or GC-MS.

Item	Methods	Flax oil	Flax oil	Flax oil + lyophilized apples	SEM	P-value	Ethyl esters	Ethyl esters	Ethyl esters + lyophilized apples	SEM	P-value
		Storage time (day)									
		0	84	84			0	84	84		
C18:3 <i>n</i> -3 ¹ (g/100g)	GC-MS	52.73 ^A	51.01 ^B	50.35 ^C	0.05	0.01	51.32 ^A	47.57 ^B	48.46 ^C	0.06	0.01
	NMR	55.16 ^A	53.92 ^a	53.27 ^{bb}	0.01	0.01	55.75 ^A	51.22 ^B	51.92 ^B	0.07	0.01
C=C ²	GC-MS	2.06	2.07 ^b	2.03 ^a	0.05	0.04	2.07 ^a	1.99 ^b	2.00 ^b	0.02	0.04
	NMR	2.11 ^a	1.95 ^b	1.93 ^b	0.02	0.03	2.12 ^A	2.08 ^A	1.87 ^B	0.03	0.01

The letters A, B, in the same rows indicate statistically high significant differences ($p < 0.01$)

The letters a, b, in the same rows indicate statistically significant differences ($p < 0.05$)

SEM - standard error of the means,

¹ C18:3 *n*-3: content of α -linolenic acid in g/100 g of fat; ² C=C- average number of double bonds

and in both cases it was over 90%. This result is consistent with other Research [34]. The composition of ethyl esters also confirms previous publications [3]. The flaxseed oil and flax oil ethyl esters used in the research were characterized by a high content of C 18: 3 *n*-3 (respectively 52.73 and 51.32 g / 100g fat). The addition of lyophilized apples to esters or flax oil slowed down the content of C 18: 3, *n*-3 slightly after 3 months of storage.

A declining tendency of unsaturated fatty acids in the tested formulations was demonstrated, while simultaneously there was an increase in the concentration of the saturated and monounsaturated fatty acids. A similar result was obtained in the case of storing rapeseed expeller with the addition of synthetic antioxidants [35]. In turn, micro-encapsulated linseed oil did not show a decrease in PUFA and deterioration of quality indicators during storage [36].

On the last day of the tests, a decrease in the average number of double bonds in the samples was observed ($p < 0.01$) (Table 2). NMR analysis confirmed the results obtained for the iodine value, for which the decrease during storage tests was greater in samples with the addition of lyophilized apples of 1.40 mg I₂ / 100 g in flax oil and 1.52 mg I₂ / 100 g in esters compared to samples without the addition of lyophilized apples. In the case of the GC-MS method, slight changes were found in the average number of double bonds in samples with flax oil and with ethyl esters.

The highest total polyphenol content (day "0") was found in samples containing only lyophilized apples (1495 mg GA / 100g) (Table 3). Naturally occurring in flax

oil and in esters, substances with antioxidant properties could increase the amount of polyphenols [3]. In our own studies in samples containing flaxseed oil or esters mixed with lyophilized apples, the content of polyphenols on day "0" was significantly lower than in the lyophilized apples themselves, so the issue requires further research. A significant ($P < 0.01$) decrease of this value was found on day 56 of the tests by 18.51% for freeze-dried apples, for samples containing esters 12% and by 11.67% for samples with flax oil and lyophilized apples. In other studies, the total polyphenol content in apple peels was 150-700 mg GA / 100 g [20] or 830.9 mg GA / 100 g [2].

Samples containing esters were characterized by lower activity against the DPPH radical during the tests compared to samples with flax oil or containing only lyophilized apples (Table 4). A significant decrease in the scavenging capacity of the DPPH radical was observed on day 14 for samples with oil or esters and with lyophilized apples without added fat ($P \leq 0.01$). In flax seed oil alone, the activity against DPPH was higher than in ethyl esters, but in which the ability to DPPH radical quenching was more stable during tests. After two weeks of storage in the samples involving esters it was observed that a significantly ($P \leq 0.01$) higher decrease in assay values to the radical ABTS occurs, rather than in the samples with flax oil. The decrease in activity for samples containing only lyophilized apples was negligible.

In our study, the correlation coefficient between the total polyphenol content and the antioxidant activity of lyophilized apples indicated a fairly strong relationship in the case of DPPH ($r = 0.72$), and lower in the case

Table 3: Content of total polyphenols in the test formulations during storage (GA mg/100g).

Item		Storage time (day)					SEM	P-value
		0	14	28	56	84		
Total phenolic mg GA/100g	Ethyl esters + lyophilized apples	912.84 ^A	893.04 ^A	856.03 ^B	806.32 ^C	805.05 ^C	7.40	0.01
	Flax oil + lyophilized apples	925.43 ^A	915.98 ^B	894.59 ^C	839.53 ^D	836.30 ^E	5.15	0.01
	Lyophilized apples	1495.18 ^A	1385.87 ^B	1360.73 ^B	1218.48 ^C	1215.51 ^C	15.86	0.01

The letters A, B, in the same rows indicate statistically high significant differences ($p < 0.01$)

The letters a, b, in the same rows indicate statistically significant differences ($p < 0.05$)

SEM - standard error of the means,

Table 4: Antioxidant activity (DPPH, FRAP, ABTS **) during the storage period of fat preparations.

Item		Storage time (day)					SEM	P-value
		0	14	28	56	84		
DPPH $\mu\text{mol Trolox/g}$	Ethyl esters + lyophilized apples	25.21 ^A	22.63 ^B	21.29 ^B	18.76 ^C	15.46 ^D	0.55	0.01
	Flax oil + lyophilized apples	28.05 ^A	23.80 ^B	22.65 ^B	21.23 ^C	21.29 ^C	0.41	0.01
	Lyophilized apples	44.44 ^A	37.75 ^B	35.54 ^C	33.19 ^C	27.33 ^D	0.94	0.01
	Ethyl esters	0.36 ^A	0.30 ^B	0.28 ^C	0.28 ^C	0.25 ^D	0.04	0.01
	Flax oil	1.91 ^A	1.15 ^B	1.10 ^{BC}	1.03 ^C	0.79 ^D	0.05	0.01
FRAP $\mu\text{mol Trolox/g}$	Ethyl esters + lyophilized apples	31.37 ^A	28.19 ^B	26.95 ^C	25.98 ^D	26.36 ^D	0.26	0.01
	Flax oil + lyophilized apples	35.37 ^A	32.71 ^B	33.49 ^C	34.53 ^D	33.65 ^C	0.14	0.01
	Lyophilized apples	54.6 ^A	52.93 ^B	51.12 ^C	50.53 ^D	48.65 ^E	0.29	0.01
	Ethyl esters	1.19 ^A	1.10 ^B	1.03 ^C	0.99 ^D	0.98 ^D	0.01	0.01
	Flax oil	3.05 ^A	2.79 ^B	2.51 ^C	2.23 ^D	2.09 ^E	0.05	0.01
ABTS $\mu\text{mol Trolox/g}$	Ethyl esters + lyophilized apples	43.08 ^A	45.06 ^B	33.34 ^C	34.48 ^C	32.43 ^D	0.76	0.01
	Flax oil + lyophilized apples	47.07 ^A	46.61 ^A	32.52 ^B	33.70 ^B	43.6 ^C	1.15	0.01
	Lyophilized apples	78.32 ^A	65.81 ^B	58.24 ^C	53.78 ^D	54.33 ^D	1.32	0.01
	Ethyl esters	1.21	1.13	1.10	1.07	1.05	0.01	0.12
	Flax oil	3.95 ^A	3.49 ^B	3.31 ^B	3.13 ^C	3.11 ^C	0.05	0.01

The letters A, B, in the same rows indicate statistically high significant differences ($p < 0.01$)

The letters a, b, in the same rows indicate statistically significant differences ($p < 0.05$)

SEM - standard error of the means

Table 5: Mean acid value (AV) of formulations during storage (mg KOH/g).

Item	Storage time (Day)						SEM	P-value
	0	7	14	28	56	84		
Flax oil	1.05 ^{Aa}	1.22 ^A	1.83 ^{Ab}	4.79 ^B	5.08 ^c	8.62 ^D	1.67	0.01
Flax oil + lyophilized apples	1.55 ^A	2.80 ^B	3.34 ^C	3.91 ^D	3.88 ^D	4.47 ^E	1.96	0.01
Ethyl esters	0.28 ^A	0.27 ^A	0.29 ^A	0.36 ^B	0.40 ^B	0.46 ^B	0.03	0.01
Ethyl esters + lyophilized apples	0.38 ^A	1.24 ^B	1.44 ^B	2.9 ^C	4.18 ^D	5.04 ^D	0.87	0.01

The letters A, B,C,D... in the same rows indicate statistically high significant differences ($p < 0.01$)

The letters a, b, in the same rows indicate statistically significant differences ($p < 0.05$)

SEM - standard error of the means

of ABTS ($r = 0.65$). A big impact on the relationship between the total polyphenols and antioxidant activity has qualitative composition comprising of compounds of fruit [37]. Many studies confirm the high antioxidant activity of apples or apple pomace [20,38,39] and the high correlation coefficient of antioxidant activity with the total polyphenol content [40,41,42]. In similar studies, ethanolic extracts from frozen apple peels, inhibited the oxidation of fish oil more strongly than synthetic antioxidants such as BHT (butylated hydroxytoluene) [2]. Also, green tea extract to a greater extent than BHA (butyl hydroxyanisole) inhibited the peroxidation processes in fish oil [43]. In our own research on the last day of storage tests, the free radical quenching activity (ABTS, DPPH) was the lowest in samples with ethyl esters mixed with lyophilized apples. Higher antioxidant activity of flax oil samples may have resulted from the difference in the content of natural substances with antioxidant properties [32]. During the tests, the reducing power of FRAP was subject to fewer changes in the flax oil samples than in the case of samples with the addition of esters. A large proportion of antioxidants have reducing properties. In the FRAP method, the indicator is Fe^{3+} , which is reduced to Fe^{2+} forming a colored complex with 2,4,6-tripyridyl-S-triazine (TPTZ). The increase in the absorbance of this complex shows a high content of antioxidants in the examined material. Choe and Min [44] also indicate how important it is to choose the right antioxidant for a particular type of fat in order to avoid antagonistic effects.

On the day of testing, the acid number in the case of ethyl esters was very low (Table 5), which is consistent with the AV value given by Sokola-Wysoczańska et al. [3]. The value of AV in flax oil determined at the same time was higher (1.05 mg KOH / g), however, it did not exceed the values recommended in Codex Alimentarius [45] for cold pressed oils ($\text{AV} \leq 4$ mg KOH / g). The higher AV value in flax oil was due to the fact that the cold-pressed flax

oil used in the experiment could contain accompanying compounds, which include oxidation products or free fatty acids [46]. The presence of free fatty acids is the essence of fat rancidity in animal feed and food products [47]. In the case of the production of ethyl esters, there is purification with potentially harmful or undesirable compounds naturally present in flax oil, while substances such as lignans, phytosterols, phospholipids, carotenoids are preserved [3]. This may cause a different effect of lyophilized apples on changes in quality parameters in esters during storage compared to flax oil. The slowest increase in AV during the experiment was noted for pure ethyl esters, while the addition of lyophilized apples significantly ($P \leq 0.01$) accelerated the increase in AV (Table 5). The addition of lyophilized apples limited this increase in the case of flax oil only after the 28th day of storage and slowed the further increase of AV. On day 84 of AV storage in flax oil without the participation of lyophilized apples, it significantly exceeded (8.62 mg KOH / g). The reported AV values in the tested samples regardless of the addition of lyophilized apples did not exceed the requirements of PN-EN-ISO-660:2010 [48], which allows AV for fats of up to 50 mg KOH / g, also the humidity of air during storage, then the susceptibility to release of free fatty acids in esters due to hydrolytic degradation increases [49]. However, Jakóbiec and Ambrozik [50] found no statistically significant increase in AV in methyl esters of rapeseed oil (AV 0.36 mg KOH / g) stored for 12 months in room conditions.

In our own research, the addition of lyophilized apples slowed the increase in the peroxide value in ethyl esters (Table 6). Regardless of the proportion of lyophilized apples, the peroxide value in the esters was maintained at a very high level during the course of the experiment. Long-term storage of flax oil with lyophilized apples resulted in a larger increase in PV compared to pure oil samples at the end of the experiment. The limit value for

Table 6: Mean peroxide value (PV) of formulations during storage (meq O₂/kg).

Item	Storage time [Day]						SEM	P-value
	0	7	14	28	56	84		
Flax oil	1.70 ^A	2.50 ^B	4.41 ^C	5.97 ^D	9.25 ^E	10.75 ^F	2.34	0.01
Flax oil + lyophilized apples	1.76 ^A	2.36 ^{Ba}	3.15 ^{Bb}	5.40 ^C	10.09 ^C	14.07 ^D	1.02	0.01
Ethyl esters	19.01 ^A	27.2 ^{Ba}	29.76 ^{Bb}	34.77 ^C	43.75 ^D	63.77 ^E	4.47	0.01
Ethyl esters + lyophilized apples	19.41 ^A	20.7 ^A	24.86 ^b	29.41 ^c	39.09 ^D	46.03 ^E	3.93	0.01

The letters A, B, C, D in the same rows indicate statistically high significant differences ($p < 0.01$)

The letters a, b, in the same rows indicate statistically significant differences ($p < 0.05$)

SEM - standard error of the means

Table 7: Mean iodine value (LI) of formulations during storage (mg I₂/100g).

Item	Storage time [Day]						SEM	P-value
	0	7	14	28	56	84		
Flax oil	175.07 ^A	174.95 ^A	173.84	172.67	171.40 ^B	167.64 ^C	0.95	0.01
Flax oil + lyophilized apples	174.13 ^A	170.31 ^B	169.53 ^B	169.28 ^B	169.90 ^B	166.24 ^C	0.78	0.01
Ethyl esters	176.57	175.97	176.61	175.54	176.09	175.83	1.01	0.05
Ethyl esters + lyophilized apples	176.44 ^a	174.37 ^b	174.71 ^b	174.27 ^b	174.99 ^b	174.31 ^b	0.25	0.03

The letters A, B, C, D in the same rows indicate statistically high significant differences ($p < 0.01$)

The letters a, b, in the same rows indicate statistically significant differences ($p < 0.05$)

SEM - standard error of the means

cold pressed vegetable oils has not been exceeded ($< 15 \text{ meq O}_2 / \text{kg}$) [45]. The increase in the peroxide value in the flax oil may have resulted from the fact that the experiment used cold pressed flaxseed oil, i.e. unrefined, containing accompanying compounds, which include primary and secondary oxidation products, free fatty acids, dyes, metals and incomplete triacylglycerols. These compounds have a big influence on the oxidative stability of oils and can interfere with the antioxidant effects of the added specimens. Also, too big an addition of antioxidants may aggravate the oxidation process. The reason may be a synergistic or antagonistic effect due to the overlap of the activity of native non-glyceride oils and antioxidants used [44].

In the samples with the addition of lyophilized apples, a greater decrease in the iodine value was found than in the samples with pure flax oil (Table 7). The same was true for esters, however, the addition of lyophilized apples slowed the decrease in IV compared to flax oil. The iodine value is an indicator of the degree of fat insensitivity, showing the fat's susceptibility to autooxidation. The average value of the iodine value in own studies was similar to the results

obtained by other researchers [34] (about $177 \text{ mg I}_2 / 100\text{g}$), while slightly lower than the value given by Przybylski [51] ($182\text{--}203 \text{ mg I}_2 / 100\text{g}$). The high volume of the iodine value in flax oil is determined by the high content of unsaturated fatty acids [51]. The decrease in the iodine value during the experiment may have resulted in a reduction in the number of double bonds due to the oxidation of PUFAs in the specimens.

Consuming fat rich in polyunsaturated fatty acids is important in every age. Unfortunately, the taste or form of flax oil or esters may not suit all consumers. Mixing fat additives with lyophilized apples can improve their taste and causing them to be more eagerly consumed, while the rich composition of compounds with antioxidant properties can extend the shelf-life of saturated fatty acids, which can make them an alternative to synthetic antioxidants. Our own research has shown great applicability of the use of whole lyophilized apples in order to increase fat stability.

4 Conclusion

During the experiment period, the sum of *n-3* PUFAs was reduced in flax oil combined with lyophilized apples and ethyl esters of flax oil applied to the lyophilized apples. The addition of lyophilized apples did not limit the decrease in C18: 3, *n-3* after 84 days of storage. The most intense changes in the total polyphenol content were found after 56 days of storage in freeze dried apples without flax oil or esters. Free radical quenching activity (ABTS, DPPH) and Fe³⁺ ion reduction polyphenols were the lowest in samples with ethyl esters mixed with lyophilized apples. Higher antioxidant activity of flax oil samples may have resulted from the difference in the content of natural substances with antioxidant properties. The addition of lyophilized apples reduced the decrease peroxide PV in ethyl esters, in the case of flax oil, the AV. The use of polyphenols contained in lyophilized apples has made it possible to improve the stability of flax oil and ethyl esters during the storage tests.

Conflict of interest: Authors (Śpitalniak, K., Szumny, A., Kucharska, A. Fogt A. and Kupczyński R.) declare that there are no conflicts of interest regarding the publication of this paper.

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