



Natural Antioxidant Enrichment of Linseed Oil: Ultrasound-Assisted Maceration with Mullein Flowers (*Verbascum thapsus* L.)

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Abstract

This study investigated the effect of natural antioxidants from mullein flowers (*Verbascum thapsus* L.) on the quality and oxidative stability of linseed oil (*Linum usitatissimum* L.). Ultrasonic-assisted maceration was optimized by evaluating the effects of extraction time, ultrasonic power, and flower concentration. The optimal conditions for maximising oil oxidative stability were identified as 3.31 min of ultrasound, 40 W power, and 8.56% (w/w) flower content. Under these conditions, the oxidative stability of the oil improved significantly, with an average protective factor of 1.74. Although slight increases in hydrolysis and oxidation were observed, the oils remained within acceptable quality and safety limits. Maceration also enhanced oils' bioactive compounds content, increasing phenolic content from 68.82 to 92.57 to 368.65–419.02 mg GAE/100 g and flavonoids from 6.24 to 8.87 to 34.54–38.20 mg QT/100 g. Additionally, the macerated oils had higher antioxidant activity, chlorophyll, and carotenoid levels. While the maceration process led to a reduction in individual fatty acid groups, the loss was less pronounced than that caused by ultrasound treatment alone. Overall, ultrasonic maceration with mullein flowers effectively improved both the bioactive properties and oxidative stability of linseed oil.

Keywords Linseed oil · Maceration · Mullein · Oxidation · Natural antioxidant · Ultrasound

Introduction

Cold-pressed oils, such as linseed oil, are a valuable component of a daily diet. Linseed oil (*Linum usitatissimum* L.) is particularly notable for its high content of polyunsaturated fatty acids, including α -linolenic acid. This compound has health-promoting properties, such as normalizing blood pressure and reducing the concentration of triacylglycerols

in plasma. However, the high content of unsaturated fatty acids negatively impacts the oxidative stability of the oil, leading to a shorter shelf life and a faster decline in its nutritional value. Therefore, it is essential to explore methods to enhance its oxidative stability. In this context, using natural antioxidants has gained particular interest, especially in light of recent concerns regarding the potential toxicity of synthetic antioxidants [1–3].

Mullein (*Verbascum thapsus* L.), is a biennial flowering plant recognized for its numerous medicinal properties. Its expectorant properties clear mucus, making it practical for bronchitis, colds, and flu, while its mucilage soothes lung and throat irritation. It also contains verbascoside, an anti-inflammatory compound that reduces inflammation and relieves joint and muscle pain [4, 5]. Additionally, mullein has antibacterial and antiviral properties. Rich in antioxidants, mullein protects cells and boosts immunity. It is also used for earaches, skin infections, and gastrointestinal issues, often prepared as teas, tinctures, or salves. This plant contains numerous bioactive compounds, such as phytosterols, flavonoids, and iridoid glycosides, usually exhibiting antioxidant properties. As verified in previous research,

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adding mullein to linseed oil may significantly improve its oxidative stability [6–8].

The maceration process is the simplest way to introduce natural antioxidants into the oil. This process takes time, which is why various supporting techniques are used, including ultrasound. In recent years, ultrasound technology has gained popularity in the food industry [9–13]. Ultrasound-assisted extraction (UAE) has emerged as a promising green technology for the recovery of bioactive compounds from plant materials, offering specific advantages over conventional techniques. The application of high-frequency ultrasound waves induces acoustic cavitation, which disrupts plant cell walls, thereby enhancing solvent penetration and mass transfer. This mechanism facilitates the efficient extraction of intracellular compounds such as phenolics and flavonoids, often using smaller amounts of solvent and significantly reducing extraction time. In contrast to traditional methods like maceration or Soxhlet extraction, which may require several hours or even days, UAE can achieve comparable or superior yields within minutes [14]. When applied to oil-based systems, UAE enables the direct enrichment of edible oils under mild, non-thermal conditions that help preserve thermolabile antioxidants and reduce the risk of oxidation. This makes it particularly suitable for developing functional oils with extended shelf life and added health benefits. However, ultrasound technology also poses challenges. Prolonged sonication or excessive ultrasound power may degrade sensitive bioactive compounds or negatively affect oil stability. Therefore, careful optimization of parameters such as ultrasound power, treatment time, and plant material concentration is crucial to maximize efficiency while maintaining product quality. Despite these limitations, UAE remains a scalable, efficient, and clean-label alternative that supports the sustainable extraction of natural antioxidants for food and nutraceutical applications [15–18].

The possibility of using mullein flowers as a natural antioxidant has been previously studied [6]. However, previous research did not consider optimizing the maceration process, which is crucial for efficiently extracting antioxidant compounds. This study, therefore, aimed to develop optimal maceration conditions for linseed oil enriched with freeze-dried mullein flowers and to evaluate the impact of this process on the oil's quality and stability. By optimizing ultrasound parameters, it is possible to achieve precise control over the enrichment process, tailoring the oil's stability and quality to meet specific requirements. This research represents a novel approach, combining the natural bioactive properties of mullein flowers with cutting-edge ultrasound technology. In this research, the optimization process of ultrasound-assisted maceration (UAM) parameters for enriching linseed oil with natural antioxidants from freeze-dried mullein flowers is presented for the first

time. Unlike previous work, it combines process optimization with detailed chemical and oxidative stability analysis, demonstrating a novel, non-thermal, and scalable method to improve oil functionality using an underutilized medicinal plant. The study contributes to the field by offering a clean-label, plant-based alternative to synthetic antioxidants, expanding current knowledge on ultrasound-assisted enrichment of edible oils, and demonstrating a practical application of UAE in functional food formulation. It provides a valuable foundation for further innovations in sustainable lipid processing, nutraceutical development, and natural antioxidant delivery systems.

Materials and Methods

Research Material

The research material consisted of three batches of brown linseeds (*Linum usitatissimum* L.), which were cold-pressed to produce fresh linseed oil. Cold pressing of the oils was performed using a screw press from the Klarstein brand (Berlin, Germany). The oil temperature did not exceed 30 °C (averaging 28.2 °C). After the pressing process, the oils were centrifuged using an MPW-352 laboratory centrifuge (Warsaw, Poland) for 10 min at a speed of 5000 rpm.

To prepare macerates, mullein flowers (*Verbascum thapsus* L.) collected in the vicinity of the Kozienice Forest in the Mazowieckie Voivodeship in September 2023 were used. Immediately after collection, the flowers were frozen in a ProfiMaster PMU0452 shock freezer (GmbH, Germany) at −40 °C for at least 5 h. Subsequently, the material was freeze-dried for 48 h at a shelf temperature of 30 °C and a pressure of 63 Pa, using an Alpha 1–4 LSC plus device (Christ, Germany). Before the maceration process, the flowers were ground using a mortar, and the particle size was smaller than 2 mm.

Chemicals, Reagents and Media Used

All chemicals used in this study were of analytical grade. Gallic acid (2,2-diphenyl-1-picrylhydrazyl (DPPH)), sodium nitrate (NaNO₃), isooctane, and Trolox were purchased from Sigma-Aldrich (St. Louis, MO, USA). Quercetin, Folin–Ciocalteu reagent, methanol, aluminum chloride (AlCl₃), sodium carbonate (Na₂CO₃), sodium hydroxide (NaOH), hexane, methyl heptadecanoate, potassium hydroxide in methanol (2 M KOH) were obtained from Merck (Darmstadt, Germany). The standard mixture of 37 fatty acid methyl esters (FAMES), used for gas chromatographic identification of fatty acids, was obtained from

Restek (Bellefonte, PA, USA). All analyses were conducted using purified water produced HLP-SMART (Hydrolab, Gdańsk, Poland).

Preparation of Oil Macerates - Optimization of the Maceration Process

The prepared oil samples were subjected to ultrasound treatment using the UP200St ultrasonic homogenizer equipped with a titanium sonotrode S26d7 (Hielscher Ultrasonics GmbH, Teltow, Germany). The macerates were prepared by mixing cold-pressed linseed oil with specific amounts of freeze-dried mullein flowers (2.5, 5, and 10%). Subsequently, they were subjected to ultrasound treatment using selected power parameters (10, 20, and 40 W) and durations (2, 5, and 7 min). To ensure the temperature did not exceed 30 °C during ultrasound treatment, the samples were kept in a beaker with a water jacket and continuously monitored. The resulting macerates were filtered through gauze.

Oils' Quality Assessment

To analyze the oil's initial quality and its macerates, acid value (AV), peroxide value (PV), and *p*-anisidine value (*p*-AnV) were determined using AOCS Official Method Cd 3d-63 [19], AOCS Official Method Cd 18-90 [20], and EN ISO 3960:2017-03 [21]. The total oxidation value (TOTOX indicator) was calculated as TOTOX indicator = $(2 \times \text{PV}) + p\text{-AnV}$.

Fatty Acids Composition Determination

Using gas chromatography, the fatty acid composition of oils before and after maceration was performed following the AOAC Official Method 996.06 [22]. Samples of oils' methyl esters were prepared by dissolving 0.1 g of the oil in 2 cm³ of hexane and 0.5 cm³ of 2 M methanolic KOH. After mixing and phase clarification, 1 cm³ of the hexane phase was placed in a vial. A ThermoScientific Trace 1300 gas chromatograph with an FID flame ionization detector was used to determine the fatty acid profile. An SGE BPX70 high-polar capillary column (60 m x 0.22 mm x 0.25 µm) separated the fatty acid esters. The temperature in the first four minutes of analysis was 100 °C and raised to 240 °C (rate of 3°C/min). The carrier gas was helium with a flow of 40 cm³/min. To identify the fatty acids, the standard mixture of 37 FAME methyl esters from Restek was used. Retention indices (RI) were calculated and compared with literature values to confirm compound identities. The results were reported quantitatively in g/100 g of oil using a standard internal C17:0.

Carotenoids and Chlorophylls Pigments Content

The chlorophyll pigment content was determined per the AOCS Official Method Cc 13i-96 [23]. For this purpose, the absorbance of the analyzed samples was measured at three wavelengths: $\lambda=630$ nm, $\lambda=670$ nm, and $\lambda=710$ nm. Results were expressed as mg of pheophytin/kg of oil. The carotenoid pigment content was determined according to the BS 684-2.20 method [24]. The analyzed samples were dissolved in a solvent, specifically iso-octane, and the absorbance was then measured at a wavelength of $\lambda=446$ nm. Results were expressed as mg β -carotene/kg of oil.

Total Phenolics Content Determination

The total content of phenols in the cold-pressed oils was determined using the Folin-Ciocalteu (F-C) reagent, according to the method described by Dewanto et al. [25]. The 0.125 mL of the oil extract was mixed with 0.5 mL of distilled water and 0.125 mL of Folin-Ciocalteu reagent. After 3 min, 1.25 mL 7% (m/v) sodium carbonate solution was added and made up to 3 mL with distilled water. After 1.5 h, the absorbance of the samples was measured at a wavelength of $\lambda=760$ nm. The extract for analysis was prepared by triple extraction of 3 g of the oil sample with 5 mL of methanol. The results of the quantitative determinations (total phenolic content – TPC) were presented as gallic acid equivalent (mg GAE/100 g of oil).

Total Flavonoids Content Determination

The total flavonoid content was determined according to the method described by Tohidi et al. [26] with modifications. To 5 µl of oil methanolic extract, prepared following the same extraction protocol as for phenolic analysis, 300 µl of 5% NaNO₃ was added. After 6 min, 300 µl of 10% AlCl₃ was added, and the samples were left for 5 min. After this time, 2 ml of 1 M NaOH and 2.5 µl of water were added. The samples were incubated for 15 min. The total flavonoid content (TFC) was expressed as mg of quercetin (QE) equivalent per 100 g.

Determination of the Total Antioxidant Activity by the Reduction in DPPH Free Radical

The analysis of total antioxidant activity was done using the methodology described by Pająk et al. [27]. The assay was performed for polar (aqueous) and nonpolar (lipid) fractions. The fractions were prepared by mixing 1 ml of oil and 1 ml of methanol. For the polar fraction, 3.9 ml of 0.1 mM DPPH solution was mixed with 0.1 ml of the sample's methanolic extract or oil, followed by incubation for

60 min. For the nonpolar fraction, a similar procedure was followed, except using ethyl acetate as the solvent instead of methanol. The DPPH radical scavenging activity in the analyzed samples was expressed in mM Trolox/l. The antioxidant activity was determined based on a Trolox calibration curve; the spectrophotometer was zeroed using pure solvent (methanol or ethyl acetate), and all measurements were referenced directly to the standard curve. The % of DPPH radical inhibition was calculated based on the absorbance of the blank sample (DPPH radical solution) and the absorbance of the oil sample.

Determination of Oxidative Stability and Protective Factor Using the Rancimat

The oxidation stability measurement in the Rancimat type 892 apparatus by Metrohm (Switzerland). The analysis was conducted at 90 °C for 3 g of sample at an air flow of 20 l/h [28]. The volatile oxidation products formed during the measurement were transferred along with the air stream to the measuring vessel containing 60 ml of deionized water. The determination of oxidative stability for each of the tested oils was performed in three parallel repetitions, of which their arithmetic mean was given as the final result.

The protective factor (PF) was calculated as the ratio of the induction time of the enriched oil sample to that of the control (non-enriched) sample.

Statistical Analysis of the Results

All experimental runs were performed in triplicate (three replicates) to ensure reliability and reproducibility of the results. Statistical analysis was performed using StatSoft, Inc. STATISTICA version 13.3 (StatSoft, Inc., Tulsa, OK, USA) and MiniTab version 21.1.0. It included group classification using one-way analysis of variance (ANOVA) and Tukey's test ($p \leq 0.05$). Additionally, Minitab software (version 20.2) was used to perform Response Surface Methodology (RSM). The protection factor of mullein flowers on linseed oil oxidation was related to the ultrasound power, time of maceration process, and amount of flowers by second-degree polynomial according to Eq. 1:

$$PF = b_0 + \sum_{i=1}^3 b_i X_i + \sum_{i=1}^3 b_{ii} X_i^2 + \sum_{i < j}^3 b_{ij} X_i X_j \quad (1)$$

where the coefficients b_i is related to the linear and b_{ii} to the quadratic effect of each input factor on the PF. The coefficient b_{ij} represents the interactions between two inputs.

Validation of the assumptions underlying the RSM model was carried out by analysing the residuals. Normality of residuals was assessed using normal probability plots and the Shapiro-Wilk test. Independence was tested by plotting

residuals against run order to identify any autocorrelation. Homoscedasticity (constant variance of residuals) was confirmed by examining residuals versus predicted values plots. All assumptions were satisfactorily fulfilled, confirming the adequacy of the RSM model. Significant terms were identified at $p \leq 0.05$.

Results and Discussion

Optimization of the Process of Obtaining Linseed Oil Macerates with Mullein Flower

The first stage of the work was optimizing the process for obtaining linseed oil (*Linum usitatissimum* L.) macerate with mullein (*Verbascum thapsus* L.) flowers using ultrasound-assisted maceration. The protective factor (PF) was chosen as the optimization parameter, calculated based on the induction times of the tested macerate samples (Table 1). A PF value greater than 1 indicates improved oxidative stability due to the addition of antioxidants from the mullein extract. A higher PF reflects greater protection, while values close to or below 1 suggest limited or no antioxidant effect. The influence of individual ultrasound-assisted maceration parameters on the protective factor (PF) of linseed oil enriched with mullein flowers was visualized using three-dimensional response surface plots.

Based on the obtained oxidative stability results of the oils, it cannot be unequivocally concluded that increasing ultrasound power or the amount of plant material extended the induction time. The highest values, and thus the best oxidative stability, were observed in the samples from Batch I. Oils from this batch also exhibited the longest initial induction time (9.28 h) compared to the other two batches—8.70 h for Batch II and 8.84 h for Batch III. Figure 1 shows the interaction between flower quantity (Q) and ultrasound power (P). The PF increased with higher power and a moderate flower-to-oil ratio (~6–8%). However, excessive amounts of flowers (>8%) did not significantly improve PF, possibly due to matrix saturation or limited diffusivity. Lower PF values were observed at low power (10–15 W), regardless of flower quantity. The relationship between ultrasound power (P) and time of extraction (T). Increasing power (up to ~30 W) and time (up to ~5–6 min) positively affected PF, likely due to enhanced cavitation and better release of phenolic antioxidants. However, at prolonged extraction times (>6 min), no further improvement was seen, indicating a potential degradation of sensitive compounds. The interaction between time (T) and flower quantity (Q) shows the highest PF was obtained at moderate extraction times (~4–5 min) and mid-range flower addition

Table 1 Optimization process based on macerates induction time (IT) and calculated protection factor of mullein flowers (PF)

Mass of flowers (%)	Power (W)	Time (min)	Batch I		Batch II		Batch III	
			IT	PF	IT	PF	IT	PF
2.5	10	2	10.71 ^a	1.14 ^a	11.04 ^b	1.27 ^{ab}	10.48 ^a	1.19 ^a
		5	13.66 ^h	1.46 ^{ef}	11.60 ^d	1.33 ^{cd}	11.83 ^f	1.34 ^{cde}
		7	11.37 ^c	1.19 ^a	12.44 ⁱ	1.43 ^{ghij}	11.93 ^g	1.35 ^{de}
	20	2	11.02 ^b	1.17 ^a	13.17 ^o	1.51 ^{lm}	12.12 ^h	1.37 ^{ef}
		5	14.70 ^k	1.57 ^h	11.41 ^c	1.31 ^{bc}	11.53 ^d	1.30 ^{bcd}
		7	10.91 ^{ab}	1.16 ^a	13.51 ^p	1.55 ^{mn}	12.48 ⁱ	1.41 ^{fg}
	40	2	15.48 ^m	1.65 ^{ij}	11.06 ^b	1.27 ^{ab}	11.37 ^c	1.29 ^{bc}
		5	13.45 ^{gh}	1.43 ^{de}	11.95 ^f	1.37 ^{def}	12.54 ^j	1.42 ^{fg}
		7	12.13 ^d	1.29 ^b	12.57 ^k	1.45 ^{ijk}	12.68 ^k	1.43 ^g
	5	2	16.00 ^{no}	1.71 ^j	12.51 ^j	1.44 ^{bij}	11.35 ^c	1.28 ^b
10	10	5	16.80 ^p	1.79 ^k	12.13 ^g	1.39 ^{efgh}	15.03 ^s	1.70 ^l
		7	12.62 ^e	1.34 ^{bc}	13.83 ^r	1.59 ^{no}	13.35 ^m	1.51 ^h
	20	2	14.44 ⁱ	1.54 ^{gh}	15.14 ^w	1.74 ^{pr}	12.43 ⁱ	1.41 ^{fg}
		5	15.98 ^o	1.70 ^j	11.96 ^f	1.38 ^{defg}	12.84 ^l	1.45 ^g
		7	13.93 ⁱ	1.49 ^{fg}	13.79 ^r	1.59 ^{no}	12.73 ^k	1.44 ^g
	40	2	14.95 ^l	1.59 ^{hi}	14.28 ^t	1.64 ^o	14.10 ^p	1.60 ^{lk}
		5	13.30 ^g	1.42 ^{de}	12.37 ^h	1.42 ^{fghi}	15.42 ^w	1.74 ^l
		7	13.61 ^h	1.45 ^{def}	15.55 ^x	1.79 ^r	15.28 ^t	1.73 ^k
	10	2	13.51 ^{gh}	1.44 ^{def}	12.63 ^l	1.45 ^{ijk}	10.72 ^b	1.21 ^a
		5	19.86 ^t	2.12 ⁿ	12.63 ^l	1.45 ^{ijk}	12.82 ^l	1.45 ^g
20	20	7	12.46 ^e	1.33 ^{bc}	13.05 ⁿ	1.50 ^{klm}	11.75 ^c	1.33 ^{bcd}
		2	15.62 ⁿⁿ	1.66 ^j	13.93 ^s	1.60 ^{no}	13.86 ^o	1.57 ^{ij}
		5	17.34 ^r	1.85 ^{kl}	10.62 ^a	1.22 ^a	13.45 ⁿ	1.52 ^{hi}
	40	7	11.06 ^b	1.18 ^a	12.91 ^m	1.48 ^{kl}	12.09 ^h	1.37 ^{ef}
		2	17.56 ^r	1.87 ^l	15.06 ^a	1.73 ^p	15.07 ^s	1.71 ^l
		5	18.97 ^s	2.02 ^m	11.68 ^e	1.34 ^{cde}	15.36 ^u	1.74 ^l
	7	7	13.04 ^f	1.39 ^{cd}	17.43 ^y	2.00 ^s	14.53 ^r	1.64 ^k

Values in the column marked with the same letter do not differ statistically significantly, assuming $\alpha=0.05$. The analysis was conducted in three replicates

(~6–8%). Very short extraction times or low quantities of flowers resulted in reduced antioxidant efficacy.

Based on the conducted oxidative stability results and the protective factor, it was concluded that no clear trend was observed in the stability increase depending on the power, treatment time of the ultrasound, or the amount of plant material used in the maceration process. However, in most variants, the longest induction time was achieved for samples subjected to ultrasound at a maximum of 40 W (Fig. 1). For the studied linseed oils, it was also shown that higher power favors higher protective factor values. However, excessive power increases may lead to the degradation of bioactive substances, as indicated by Linares and Rojas [29].

The duration of ultrasound treatment also played an important role in the obtained results. It was found that, in many cases, a longer treatment time (7 min) led to worse results than shorter times (5–2 min). These results are consistent with Soares et al.'s [30] observations. The authors noted that after reaching an optimal time point, further extending ultrasound exposure led to the degradation of active compounds. Similarly, the optimal treatment time for the studied linseed oils was 3.36 min (from the tested 2, 5, and 7 min), indicating the need for careful selection of ultrasound exposure time.

The amount of plant material used also significantly impact the oxidative stability of the studied linseed oils. It was shown that using freeze-dried mullein flowers in a proportion of 10% led to the highest protective factor values in most cases across all three batches. Too little plant material may lead to incomplete extraction, while too much may limit cavitation efficiency and mass transport. In practice, the right amount of plant material ensures maximum use of ultrasound energy and more effective release of nutrients and bioactive compounds [31, 32].

Parameter Desirability and Optimal Conditions

The selection of optimal ultrasound-assisted maceration (UAM) conditions was based on the desirability approach, where the goal was to maximize the protective factor (PF) as the sole response variable. The design of the experiment (DOE) was used to apply the RSM. Due to the different influence of operating parameters on oils' oxidative stability, the experimental data were fitted to a quadratic model. The obtained equation from the statistical software was used to calculate the optimization parameters (Eq. 2):

$$\begin{aligned} \text{PF} = & 6.93 + 27.2410^{-1}Q \\ & -1.710^{-2}P + 11.3110^{-1}T - 3.5510^{-1}Q^2 \\ & +7.510^{-4}P^2 - 9.6110^{-2}T^2 + \\ & 13.9610^{-3}QP7.2810^{-2}QT - 4.40 \cdot 10^{-3}PT \end{aligned} \quad (2)$$

where: Q – quantity of flowers, P – ultrasound power, T – maceration time.

The best parameters included the amount of mullein flowers – 8.56%, ultrasound power – 40 W, and treatment time – 3.31 min, with predicted PF – 1.79.

Response surface methodology also quantifies the relationship between the controllable input parameters and the obtained response surfaces. Statistically, testing of the model was performed by the Fisher's statistical test for analysis of variance (ANOVA). For each term of the regression model, the associated probability was evaluated. All results were summarised in Table 2.

The Influence of the Maceration Process on the Oxidative Stability of Linseed Oil

Based on the obtained optimization parameters, samples of oils were prepared for further tests to assess the composition and quality of oils after adding mullein flowers. Control measurements were samples of crude cold-pressed linseed oil and oil-treated only with ultrasound without adding flowers to check the effect of ultrasounds alone on the stability of the oil and possibly exclude the falsification of the final result.

Considering the obtained results, it can be inferred that adding freeze-dried mullein flowers and using ultrasound-assisted maceration contributed to an increase in the oxidative stability of linseed oil, with the protection factor (PF) ranging from 1.73 to 1.75 (average 1.74, predicted 1.79), which higher than PF obtained in previous work [6]. The highest oxidative stability at a temperature of 90 °C, and thus the longest oxidation time, was observed in linseed oil enriched with mullein flowers and subjected to ultrasound maceration (LOM1). The oxidation induction time for this sample was 16.02 h, nearly 7 h longer than crude oil. The results obtained for other oils with the addition of plant material (LOM2 and LOM3) were similarly high—15.24 h and 15.31 h, respectively—compared to samples without adding mullein flowers. However, the best protective effect of mullein, and thus the highest PF value, was achieved for oil sample LOM2.

The protection factor (PF) obtained for linseed oil enriched with freeze-dried mullein flowers via ultrasound-assisted maceration ($\text{PF} \approx 1.74$) notably exceeds the PF values reported for other herbs such as oregano, thyme, and saffron in argan oil, which typically show moderate improvements without precise PF quantification. Literature data indicate PF increases in the range of 1.36–1.39 for various herbal infusions obtained by ultrasound, suggesting that the mullein extract provides a superior antioxidative effect under the applied conditions [33, 34].

Fig. 1 3D surface chart of PF (protective factor) dependency on Q (flowers quantity), T (time), and P (power), parameters of the maceration process using ultrasound

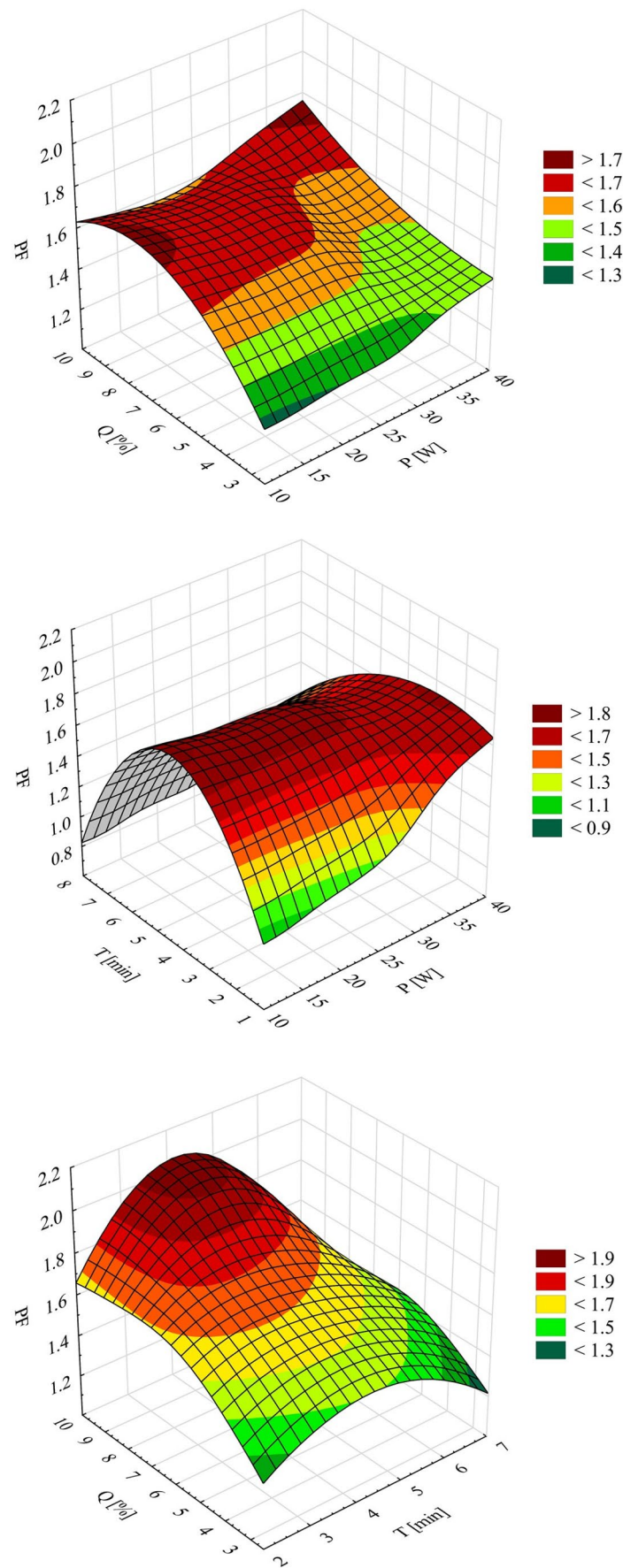


Table 2 Analysis of variance of the experimental results

Source	Degrees of freedom	Adjusted Sum of Squares	Adjusted Sum of Squares	F-value	p-value
Model	9	1.19	0.13	4.27	0.000
Linear	3	0.92	0.31	9.92	0.000
Q, a quantity of flowers	1	0.63	0.63	20.38	0.000
P, ultrasound power	1	0.29	0.29	9.39	0.003
T, maceration time	1	0.02	0.02	0.54	0.467
Square	3	0.34	0.11	3.60	0.018
Q ²	1	0.27	0.27	8.75	0.004
P ²	1	0.00	0.00	0.14	0.714
T ²	1	0.06	0.06	1.92	0.170
2-Way interaction	3	0.14	0.05	1.48	0.227
Q×P	1	0.08	0.08	2.42	0.124
Q×T	1	0.05	0.05	1.66	0.202
P×T	1	0.01	0.01	0.36	0.551
Error	71	2.21	0.03		
Lack-of-Fit	17	0.35	0.02	0.60	0.877
Pure Error	54	1.86	0.03		
Total	80	3.40			

Based on ANOVA results, it can be concluded that the protection factor (PF) of examined linseed oils is highly dependent linearly on Q and P, with associated probabilities $p < 0.05$ and $p = 0.000$ and 0.003 . Additionally, PF depends on the square of the flower quantity ($p = 0.004$, $p < 0.05$)

Table 3 Linseed oil hydrolysis and oxidation degree, total oxidation value and induction time before and after maceration and ultrasound treatment

Oil	AV (mg KOH/g)	PV (mEq O ₂ /kg)	p-AnV	TOTOX (2PV + p-AnV)	IT (h)
LO1	0.78 ^a	1.25 ^{bc}	0.01 ^a	2.50 ^{bc}	9.28 ^b
LO2	0.67 ^a	1.39 ^{cd}	0.07 ^c	2.79 ^{cd}	8.70 ^b
LO3	0.67 ^a	1.03 ^{abc}	0.01 ^a	2.06 ^{abc}	8.84 ^b
LOU1	0.67 ^a	0.52 ^a	0.04 ^b	1.08 ^a	7.65 ^a
LOU2	0.61 ^a	0.75 ^{ab}	0.07 ^c	1.56 ^{ab}	7.24 ^a
LOU3	0.62 ^a	0.69 ^{ab}	0.08 ^c	1.46 ^{ab}	6.98 ^a
LOM1	1.63 ^c	1.95 ^{de}	0.07 ^c	3.96 ^d	16.02 ^c
LOM2	1.44 ^b	2.20 ^e	0.12 ^d	4.52 ^e	15.24 ^c
LOM3	1.46 ^{bc}	1.80 ^{de}	0.12 ^d	3.72 ^{de}	15.31 ^c

Values in the column marked with the same letter do not differ statistically significantly, assuming $\alpha = 0.05$. The analysis was conducted in three replicates

Fresh oil samples (control samples) exhibited good stability and did not differ significantly at the statistical significance level of $p\text{-value} \leq 0.05$. The induction time of crude linseed oils was comparable to values reported in the literature [35–37]. The application of ultrasound significantly reduced the oxidation induction time of linseed oils, although the values obtained did not differ significantly

from one another in statistical terms. This effect is likely due to the generation of free radicals induced by ultrasound treatment, which can accelerate oxidation processes and consequently shorten the induction period [38].

The Effect of Linseed Oil Maceration with Mullein Flowers on the Degree of Hydrolysis and Oxidation of Oils

Change in the Degree of Linseed Oil Hydrolysis

The tested linseed oils were characterized by low hydrolysis degree immediately after pressing. The maceration process led to a more than twofold increase in the acid value (AV). The results presented in Table 3 indicate no statistically significant differences between crude oil samples and those subjected to ultrasound treatment at a significance level of $p\text{-value} \leq 0.05$. An evaluation of hydrolytic changes in the tested samples suggests they maintained good quality even after the maceration process. The observed acid values, ranging from 0.61 to 1.63 mg KOH/g, remain well below the threshold specified in the Codex Alimentarius [39] for cold-pressed oils (≤ 4 mg KOH/g).

The increase in the acid value (AV) of oils after the maceration process may result from water residues in the flowers or the activity of hydrolytic enzymes, both of which can contribute to fat hydrolysis. Furthermore, mullein flowers are rich in organic acids, and ultrasound causes microscopic damage to plant cell walls, promoting the release of these compounds. Consequently, the AV may rise due to reactions between the organic acids from the mullein and KOH during titration [40].

Other researchers have also observed an increase in the degree of oil hydrolysis after the maceration process with other plant material. Ammar et al. [41] reported an increase in the acid value of olive oil macerated with prickly pear flowers. Although the increase was lower than in the present study, Soares et al. [42] also noted a slight rise in the content of free fatty acids in olive oil after maceration with rosemary and basil, using ultrasound. Similar results were obtained by Hrebień-Filisińska & Tokarczyk [43], who observed an almost 2-times increase in hydrolysis in fish oil after maceration with sage.

Change in the Primary Oxidation State of the Linseed Oil

The initial oxidation level in crude oils was low (1.03–1.39 mEq O₂/kg) but varied by production batch (Table 3), and the differences between batches were statistically significant at the $p \leq 0.05$ level. Oils treated with ultrasound had lower oxidation values, about half of fresh oils, likely due to peroxide decomposition [44]. Linseed oils with mullein flowers

subjected to maceration (LOM) had the highest oxidation levels. However, all oils had low peroxide value (PV), not exceeding the Codex Alimentarius [39] limit of 15 mEq O₂/kg for cold-pressed oils.

The introduction of natural antioxidants through ultrasound-assisted maceration leads to an increase in the degree of oil oxidation. This phenomenon was demonstrated in a study by Bhimjiyani et al. [35], where the addition of carotenoids extracted from sea buckthorn pomace using ultrasound-assisted extraction (UAE) increased the PV of the oil. Similarly, Odeh et al. [38] showed that adding herbs such as basil, fennel, oregano, and rosemary to cold-pressed linseed oil increased PV.

Adding freeze-dried mullein flowers contributed to the formation of peroxides in the tested oils. The primary cause of this phenomenon may be the increased degree of oil hydrolysis, leading to higher levels of free fatty acids (FFA). The rise in FFA content affects the surface tension of the oil, facilitating oxygen diffusion, and the FFAs themselves oxidize more rapidly than glycerol esters. Another contributing factor could be the introduction of mullein flowers into the oil, as they may contain pro-oxidant compounds [45]. Conversely, as Soares et al. [30] noted, using ultrasound, depending on process parameters (e.g., power, duration), can produce both beneficial and detrimental effects on oil quality parameters. They also assessed the impact of the process on the migration of volatile compounds, oxidative stability, and antioxidant properties of the oil. The results showed that flavoring olive oil with rosemary led to a deterioration in quality parameters, including increased PV. Conversely, using basil produced the opposite effect, reducing peroxide values.

Change in the Secondary Oxidation Degree of the Linseed Oil

The anisidine value analysis indicated low values for fresh oils, signifying proper pressing and high-quality raw materials. These low values reflect the oil's freshness and safety. Among the tested samples (Table 3), linseed oil showed significant differences in *p*-AnV values at a significance level of $p \leq 0.05$, with the values remaining low. Higher values (0.04 to 0.08) were found in samples subjected to maceration without plant material. The highest values were in samples macerated with freeze-dried mullein flowers. The *p*-AnV did not change significantly, and maceration did not result in a significant formation of secondary oxidation products. However, *p*-AnV did not change much after maceration, and these values were still low.

The increase in secondary oxidation products due to ultrasound action results from both the ultrasound treatment and the addition of mullein flowers. Studies have shown

that ultrasound can enhance the content of secondary oxidation products, such as malonic aldehyde (MDA), which significantly impacts the *p*-AnV, mainly in oils rich in polyunsaturated fatty acids. This is supported by the research of Halim & Thoo [46], who observed that ultrasonic treatment increased this parameter, reducing the oxidative stability of sunflower and palm oils. The application of ultrasound also caused an increase in the anisidine value of the tested samples. However, the raw material added to the oil is also significant. In the study by Yang et al. [47], the addition of pepper to sunflower oil caused a slight decrease in the anisidine value after the maceration process. On the other hand, in the research by Bittencourt Fagundes et al. [48], the addition of pink pepper to olive oil resulted in an increase in the anisidine value after both traditional maceration and ultrasound-assisted maceration. Relatively low *p*-AnV values in cold-pressed oils may be associated with the absence of high temperatures, as during the ultrasound maceration process, the sample temperature did not exceed 30 °C.

Change in Total Oxidation Degree of Linseed Oil

The total oxidation value (TOTOX) of the tested oil samples was presented in Table 3. The TOTOX values for the tested oils ranged from 1.08 to 4.52. Samples subjected to maceration with mullein flowers and ultrasound had the highest TOTOX values (3.72–4.52), because of high PV and *p*-AnV values. The lowest TOTOX value was observed in the sample treated only with ultrasound. TOTOX values for samples after maceration with flowers and crude oils showed no statistically significant differences at $p\text{-value} \leq 0.05$. These findings suggest that adding freeze-dried mullein flowers increased the overall oxidation level of the oils, while ultrasound-assisted maceration alone reduced the TOTOX parameter.

Adding plant material could contribute to higher quality parameter values in oils. This was also confirmed by the findings of Hrebień-Filińska & Bartkowiak [49], who reported that macerates obtained from sage and cod liver oil increased the total oxidation value. On the other hand, Ahmed et al. [50] showed a decrease and an increase in the TOTOX value of sunflower oil after maceration with saffron as a natural antioxidant, depending on plant concentration. The results and the literature data indicate that adding plant material to the oil may affect its total oxidation degree in two ways.

The Effect of Linseed Oil Maceration with Mullein Flowers on their Antioxidant Activity

Fresh linseed oils' antioxidant activity (AA) in the lipophilic fraction (LF) was greater than that of the hydrophilic

Table 4 Linseed oils' HF and LF fraction antioxidant activity and total antioxidant activity before and after maceration treatment

Oil	AA HF (mM Trolox/ml)	Inhibition HF (%)	AA LF (mM Trolox/ml)	Inhibition LF (%)	Total AA (mM Trolox/ml)
LO1	0.06 ^a	9.58 ^a	2.50 ^d	59.51 ^c	2.56 ^d
LO2	0.06 ^a	9.67 ^a	2.79 ^c	62.36 ^f	2.85 ^c
LO3	0.05 ^a	9.49 ^a	2.06 ^c	53.87 ^d	2.11 ^c
LOU1	0.03 ^a	9.21 ^a	1.08 ^a	42.82 ^a	1.11 ^a
LOU2	0.04 ^a	8.52 ^a	1.56 ^b	47.98 ^c	1.60 ^b
LOU3	0.03 ^a	9.07 ^a	1.46 ^b	45.58 ^b	1.49 ^b
LOM1	0.57 ^c	17.87 ^b	3.96 ^g	82.76 ^h	4.53 ^g
LOM2	0.46 ^b	16.54 ^b	4.52 ^h	88.95 ⁱ	4.98 ^h
LOM3	0.47 ^b	16.72 ^b	3.72 ^f	79.85 ^g	4.19 ^f

Values in the column marked with the same letter do not differ statistically significantly, assuming $\alpha=0.05$. The analysis was conducted in three replicates

fraction (HF). As noted by Prescha et al. [51] this reflects the higher content of lipophilic antioxidants (e.g., tocopherols) compared to hydrophilic antioxidants (such as phenolic compounds) in oils. The highest activity in the HF and LF was observed in samples subjected to ultrasound-assisted maceration with freeze-dried mullein flowers (LOD). In contrast, the lowest activity in both layers was found in linseed oil samples subjected solely to maceration (LOU). The results were presented in Table 4, and the samples did not differ significantly statistically at $p\text{-value}\leq 0.05$ for the hydrophilic layer. However, the remaining samples showed statistically significant differences both within groups and between each other.

The maceration process of linseed oil with mullein flowers significantly increased the antioxidant content in the oil's lipophilic and hydrophilic fractions. Adding mullein flowers combined with ultrasound-assisted maceration in the lipophilic layer increased the inhibition coefficient from 53.87 to 62.35% to 79.85–88.95%. In the hydrophilic layer, this increase was from 9.49 to 9.67% to 16.54–17.87%. In contrast, using maceration alone, without the plant material, led to lower antioxidant activity values than fresh oils. This could be attributed to the partial oxidation of the oil during the maceration process with ultrasound, suggesting that the antioxidants in the oil were utilized to mitigate this oxidation process. The results indicate a positive effect of adding freeze-dried mullein flowers on enhancing the AA of oils. Mullein flowers are a source of various antioxidant groups, including flavonoids, iridoids, and phenolic acids (hydrophilic antioxidants), as well as carotenoids and phytosterols, which are classified as lipophilic antioxidants [52]. Their presence likely contributed to the increased antioxidant activity in both fractions of oils after their use in the maceration process. The effect of ultrasound-assisted maceration on the improvement of the antioxidant properties of oil is confirmed by the study of Achat et al. [53]. The best antioxidant properties of olive oil macerated with olive leaves were obtained using ultrasound, with an inhibition percentage of 86.2%. For comparison, the inhibition percentage for the conventional enrichment method was 65.0%, and for

Table 5 Content of chlorophylls, carotenoids, phenolics and flavonoids in analyzed linseed oil before and after maceration

Oil	Chlorophylls (mg pheophytin a/kg)	Carotenoids (mg β -carotene/kg)	Phenolics (mg GAE/100 g)	Flavonoids (mg QT/100 g)
LO1	0.16 ^c	1.04 ^d	92.57 ^c	6.24 ^c
LO2	0.13 ^b	0.82 ^b	68.82 ^a	9.69 ^e
LO3	0.09 ^a	0.90 ^a	84.40 ^{bc}	8.87 ^d
LOU1	0.21 ^d	1.10 ^c	136.60 ^d	18.26 ^f
LOU2	0.13 ^b	0.91 ^a	127.01 ^d	22.97 ^a
LOU3	0.08 ^a	0.88 ^c	79.37 ^b	22.73 ^a
LOM1	3.84 ^g	7.90 ^h	387.65 ^f	34.75 ^b
LOM2	3.51 ^f	6.75 ^g	368.23 ^e	38.20 ^g
LOM3	3.27 ^c	6.55 ^f	419.02 ^g	34.54 ^b

Values in the column marked with the same letter do not differ statistically significantly, assuming $\alpha=0.05$. The analysis was conducted in three replicates

the unfortified olive oil, it was 52.9%. The inhibition factor obtained for the ultrasound-assisted process they used is similar to that obtained in the case of mullein flowers.

Moreover, the use of ultrasound plays a significant role. Sonification, as a technique for enhancing the extraction of bioactive compounds from plant materials, has found widespread application, particularly in the food and pharmaceutical industries. This method relies on acoustic cavitation, during which mechanical forces cause, among other effects, the rupture of cell walls in plant material, thereby increasing extraction efficiency. It also expands the surface area in contact with the solvent, facilitating mass transfer [54].

The Effect of Linseed Oil Maceration with Mullein Flowers on their Chlorophylls and Carotenoid Pigments

Fresh linseed oils exhibited varied but low levels of chlorophyll and carotenoid pigments, ranging from 0.09 to 0.16 mg pheophytin a/kg and 0.82–1.04 mg β -carotene/kg, respectively (Table 5). Statistically significant differences were observed between the obtained values at a significance level of $p\text{-value}\leq 0.05$. The obtained results suggest that adding mullein flowers increased the levels of chlorophyll

and carotenoid pigments in the tested oils. The results show that using the maceration process alone did not directly affect the changes in pigment content in the tested samples; it did not lead to their breakdown or involvement in the oil oxidation process, but slightly impacted their extraction from the oil.

This increase of pigments in oil contributed to pigments present in mullein flowers. In a study by Amini et al. [55], the total carotenoid content in dried mullein flowers ranged from 1.56 to 5.43 g/kg of dw. Previous studies on mullein have indicated a high chlorophyll content in its leaves, but data on chlorophyll levels in flowers are lacking. It is presumed that the chlorophyll content in the flowers should be minimal due to their limited role in photosynthesis. However, the analyses confirmed the presence of chlorophyll pigments in the added mullein flowers. According to available information, small amounts of chlorophyll may be found in the green part of the floral calyx [56].

The Effect of the Maceration Process of Linseed Oil with Mullein Flowers on the Total Phenolic Content in the Oil

Fresh cold-pressed linseed oils showed the lowest phenolic compound content among the samples studied, ranging from 68.82 to 92.57 mg GAE/100 g, with significant differences at a p -value ≤ 0.05 (Table 5). Oils subjected to ultrasound and maceration with the addition of flowers, also enhanced by ultrasound, exhibited a statistically significantly higher content of phenolic compounds. The increase in phenolic compound content in oils following ultrasound treatment is attributed to the better extraction of this group of compounds from the oils [53]. The content of phenolic compounds was more than twice as high in the samples where ultrasound was applied. Nevertheless, the highest total phenolic content was found in the samples with the addition of mullein and subjected to ultrasound treatment (LOU).

The obtained results were primarily influenced by the presence of these compounds in the lyophilized mullein flowers. According to research, the content of phenolic compounds in mullein flowers ranges from 29.03 to 54.77 mg GAE/g dw, mainly including quercetin, chlorogenic acid, caffeic acid, rutin, and apigenin [55]. Previous research confirmed that after maceration, oils were enriched in, among others, gallic, p -hydroxybenzoic, and protocatechuic acids [6]. The addition of mullein flower extract significantly impact the phenolic compound content, thus contributing to the increase in the antioxidant activity of the oil. A positive effect of adding plant material to improve oxidative stability by increasing phenolic compound content was also noted by Odeh et al. [38].

The Effect of the Maceration Process of Linseed Oil with mullein Flowers on the Total Flavonoid Content in the Oil

Flavonoids in the polyphenol group exhibit significant diversity in structure and properties. They are among the most important bioactive compounds, and a considerable amount of them is present, among other places, in mullein. The highest flavonoid content was found in samples after ultrasound-assisted maceration with freeze-dried mullein flowers. These samples showed significant differences at p -value ≤ 0.05 , indicating that flavonoids from the mullein flowers were extracted during maceration (Table 5). Literature reports flavonoid content in mullein flowers ranging from 13.72 to 19.91 mg rutin equivalents (RE) per gram of dry weight, mainly diosmin and rutin [55]. Ultrasound alone increased flavonoid extraction, with maceration doubling their presence in the oils. The highest flavonoid content was in the LOD maceration oil (38.20 mg QT/100 g), compared to 18.26 mg QT/100 g after ultrasound treatment. A study by Ghasemzadeh et al. [57] confirmed that ultrasound improved bioactive compound extraction. The lowest flavonoid content was found in fresh, cold-pressed linseed oil, ranging from 6.24 to 9.69 mg QT/100 g (Table 5). Similar results were obtained in previous work [6].

The Effect of the Maceration Process of Linseed Oil with Mullein Flowers on Oils' Fatty Acid Composition

Since ultrasounds influenced the degree of oxidation of analyzed oils, it was decided to quantify the content of individual fatty acids to control the influence of this parameter on the content of essential but susceptible to oxidation polyunsaturated fatty acids.

The maceration process did not alter linseed oil's overall fatty acid profile or the proportion of individual fatty acids. Therefore, it was decided to conduct a quantitative analysis. The results of the content of individual fatty acids obtained for the tested oils were presented in Table 6. The composition and content of fatty acids in the oils differ significantly before and after the maceration process at a significance level of p -value ≤ 0.05 . Before maceration, linseed oils exhibited a typical fatty acid composition [1, 2]. After ultrasound treatment, a decrease in the content of all fatty acids was observed. Specifically, the ultrasonic process significantly reduced PUFA levels (average of 11%) from 62 to 70 g/100 g. The greatest decrease was observed in the case of α -linolenic acid (C18:3 n3), whose content in the oil samples before maceration ranged from 53.56 to 55.02 g/100 g. After the maceration process, a statistically significant decrease in its content was noted, particularly in samples after maceration treatment. A decrease of approximately

Table 6 Fatty acid composition of analyzed linseed oils before and after maceration with mullein flowers [g/100 g]

Fatty acid	Oil sample								
	LO1	LO2	LO3	LOM1	LOM2	LOM3	LOU1	LOU2	LOU3
C16:0	4.17 ^a	3.92 ^b	4.23 ^a	3.67 ^c	3.34 ^d	4.02 ^a	3.28 ^d	3.18 ^c	3.91 ^b
C18:0	4.95 ^a	4.29 ^b	4.34 ^b	4.80 ^a	4.13 ^b	4.17 ^b	4.54 ^b	3.79 ^c	3.98 ^c
C18:1	18.89 ^a	18.95 ^b	18.75 ^a	17.46 ^{ab}	16.53 ^b	16.98 ^b	16.58 ^b	14.91 ^c	15.72 ^b
C18:2n6	15.04 ^a	14.98 ^a	14.93 ^a	13.64 ^b	13.08 ^b	13.30 ^b	12.96 ^{bc}	12.50 ^c	12.67 ^c
C18:3n3	54.98 ^a	53.56 ^a	55.02 ^a	50.95 ^b	50.33 ^b	51.70 ^b	49.77 ^c	47.48 ^c	49.62 ^c
C20:0	0.21 ^a	0.25 ^a	0.21 ^a	0.24 ^a	0.25 ^a	0.21 ^a	0.24 ^a	0.21 ^a	0.19 ^a
C21:0	0.23 ^a	0.19 ^a	0.22 ^a	0.19 ^a	0.20 ^a	0.21 ^a	0.18 ^{ab}	0.14 ^b	0.19 ^a
C22:0	0.32 ^a	0.32 ^a	0.30 ^a	0.29 ^a	0.28 ^a	0.27 ^a	0.30 ^a	0.27 ^a	0.27 ^a
C22:5n6	0.28 ^{ab}	0.33 ^a	0.25 ^b	0.26 ^b	0.29 ^{ab}	0.23 ^b	0.26 ^b	0.18 ^c	0.22 ^c
Other	0.35 ^a	0.30 ^a	0.31 ^a	0.23 ^b	0.25 ^b	0.26 ^b	0.15 ^d	0.18 ^c	0.20 ^{bc}
ΣSFA	9.88 ^a	8.97 ^b	9.30 ^{ab}	9.09 ^b	8.20 ^c	8.88 ^b	8.54 ^{bc}	7.59 ^c	8.54 ^{bc}
ΣMUFA	18.89 ^a	18.95 ^a	18.75 ^a	17.46 ^{ab}	16.53 ^b	16.98 ^b	16.58 ^b	14.91 ^c	15.72 ^b
ΣPUFA	70.30 ^a	68.87 ^a	70.20 ^a	64.85 ^b	63.70 ^b	65.23 ^b	62.99 ^{bc}	60.16 ^c	62.51 ^{bc}

Values in the column marked with the same letter do not differ statistically significantly, assuming $\alpha=0.05$. The analysis was conducted in three replicates

10% in MUFA content was also noted. The main representative of MUFA was oleic acid (C18:1). Before the maceration process, its content in the LO1–LO3 samples ranged from 18.75 to 18.95 g/100 g. After ultrasound treatment, a statistically significant decrease in this value was observed, particularly in the LOU1–LOU3 samples (14.91–16.58 g/100 g).

According to the literature, ultrasound treatment also affects saturated fatty acid decomposition. Kwiatkowski et al. [58] confirmed that hydroxyl radicals formed during oxidation can break down single carbon-carbon bonds, producing carbon dioxide and water, due to the combined effects of pyrolytic decomposition and oxidative degradation. Saturated fatty acids (SFA) constituted the smallest fraction of fatty acids in the analyzed linseed oils. Their total content before the maceration process ranged from 8.97 to 9.88 g/100 g, while after ultrasounds, a decrease was observed, particularly in the LOU1–LOU3 samples, where it dropped to 7.59–8.54 g/100 g.

Maceration of linseed oil with mullein flowers protected individual fatty acids from decomposition. The lower decrease in fatty acids after maceration with mullein flowers is probably due to their bioactive compounds. The protective effect of mullein bioactive compounds also results from the results obtained for the induction times of oils presented in Table 3. Even though the content of fatty acids decreased after maceration, these oils were characterized by very good stability. Nevertheless, it should be noted that ultrasound maceration led to lower fatty acid losses but increased peroxide and *p*-anisidine values (Table 3), therefore, indicators of oil oxidation. This may be explained by the fact that during maceration with flowers, free radicals occur [59]. It was confirmed that phenols, present in mullein flowers, can be oxidized to phenolic radicals, which then react with oxygen to generate hydrogen peroxide [60]. This

may increase the oil's peroxide value, even with reduced fatty acid degradation.

Influence of Individual Determinants on the Stability of the Examined Oils – Principal Component Analysis (PCA)

Principal Component Analysis (PCA) identifies key factors affecting the stability of the examined oils. The plot presents the projection of variables onto the first two principal components, which explain 94.85% of the total variance in the data – Factor 1 (73.05%) and Factor 2 (21.80%) (Fig. 2). This indicates that these two components effectively describe the relationships within the dataset.

Compounds with antioxidant properties (flavonoids, phenolics, chlorophylls, carotenoids, AA HF, AA LF, AV, HF, and LF inhibition, Total AA) exhibit a strong correlation. They are positioned in the upper right area of the plot. Their presence significantly impacts oil stability. The strongest correlation was observed for HF antioxidant activity ($r=0.99$), while the weakest was with flavonoid content ($r=0.75$). Oxidation indicators (PV, TOTOX, IT) also demonstrate a strong correlation with antioxidants, suggesting that natural bioactive compounds may assist in limiting degradation processes in oils; however, the maceration process also contributes to the increased oxidation level of oils. Fatty acid composition (PUFA, MUFA, SFA) is positioned at a 90° angle to IT, indicating a lack of correlation between these variables and no effect on the stability of oils. Moreover, fatty acid content was not highly correlated with oil oxidation degree, expressed as PV ($r<0.25$) or *p*-AnV ($r<0.55$). This suggests that the breakdown of fatty acids affects the levels of primary and secondary oxidation products and the increase in these parameters, which is

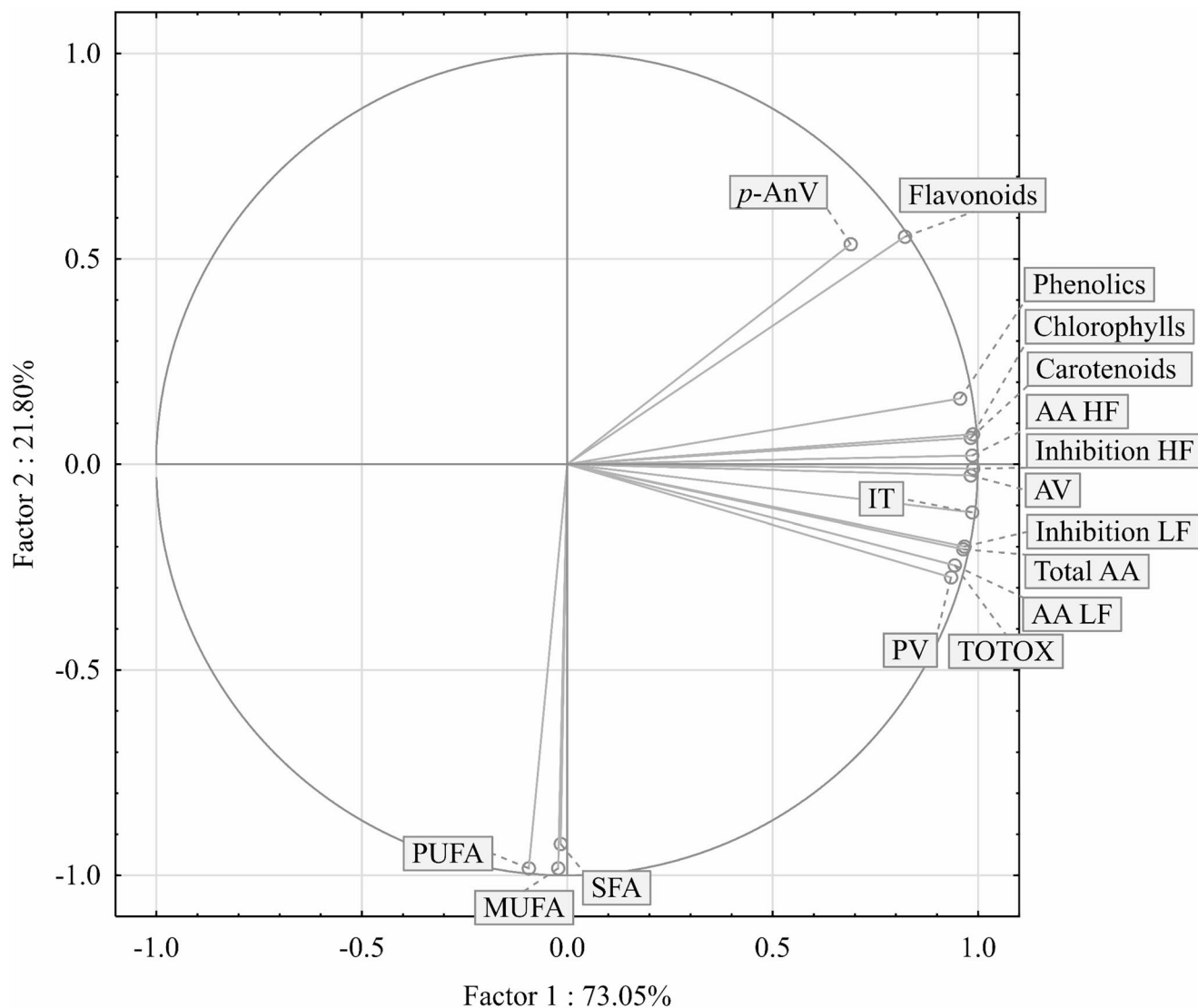


Fig. 2 Principal Component Analysis for linseed oils and their oxidative stability (IT) and chemical composition: hydrolysis degree (AV), primary (PV) and secondary oxidation products (p -AnV), total oxida-

tion value (TOTOX), fatty acid composition (SFA, MUFA, PUFA), antioxidant activity (AA, AA HF, AA LF), pigments (chlorophylls, carotenoids), phenols, and flavonoids content

associated with introducing compounds present in the flowers (Fig. 3). Variables are displayed on both axes, and color intensity indicates the strength and direction of correlations: red colors denote positive correlations, blue colors indicate negative correlations and white represents no correlation ($r \sim 0$). Clustering was performed using Euclidean distance and complete linkage, grouping parameters with similar correlation patterns.

Conclusions

The results of this study confirm that mullein (*Verbascum thapsus* L.) can serve as an effective source of bioactive compounds for stabilizing linseed (*Linum usitatissimum* L.

) oil against oxidation when applied through ultrasound-assisted maceration. The ultrasound-assisted process also led to notable increases in phenolic and flavonoid content, pigment levels, and mild increases in hydrolysis and oxidation indicators — all within acceptable ranges for cold-pressed oils. These changes demonstrate that enrichment can be achieved without compromising oil quality or safety.

From an industrial perspective, the findings of this study have direct applications in the development of functional edible oils with extended shelf life and enhanced health benefits. The ultrasound-assisted maceration process offers a scalable, efficient, and clean-label approach to fortifying linseed oil with natural antioxidants, aligning with consumer demand for minimally processed, plant-enriched foods. These enriched oils may be particularly valuable in

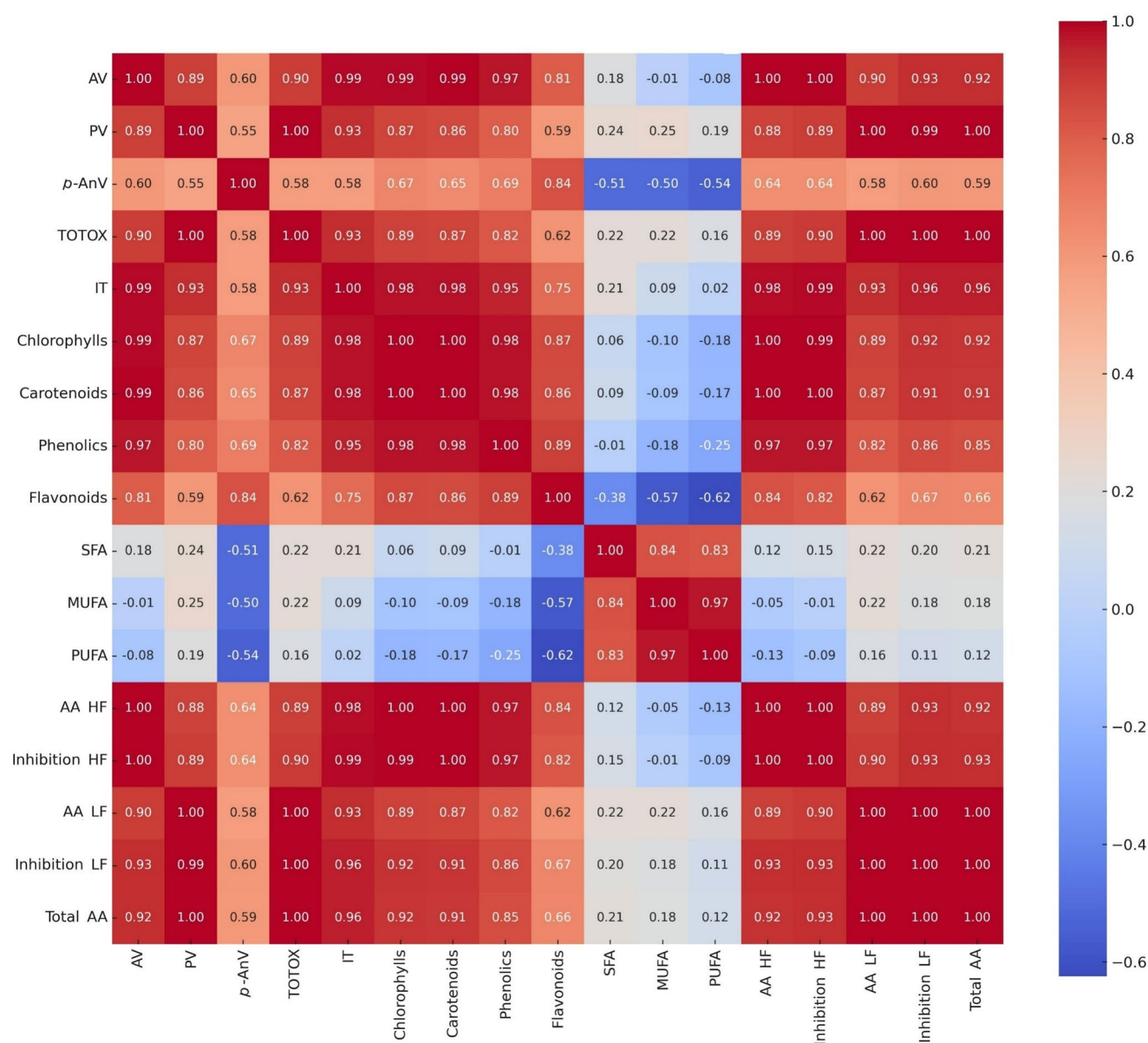


Fig. 3 Heatmap representing the Pearson correlation matrix of oxidative stability (IT) and chemical composition: hydrolysis degree (AV), primary (PV) and secondary oxidation products (*p*-AnV), total oxidation value (TOTOX), fatty acid composition (SFA, MUFA, PUFA),

antioxidant activity (AA, AA HF, Inhibition HF, AA LF, Inhibition LF), pigments (chlorophylls, carotenoids), phenols, and flavonoids content

food preservation, nutraceutical formulations, and culinary uses where oxidative stability is essential. Moreover, the ability to enhance oil functionality without compromising safety or quality parameters supports broader implementation in commercial oil production lines.

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Declarations

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