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Investigation of the Effect of Mullein Flower Extract Obtained by Ultrasound-Assisted Extraction on the Oxidative Stability of Linseed Oil

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INTRODUCTION & AIM

Cold-pressed linseed oil is regarded as a nutritionally valuable product because it contains substantial amounts of polyunsaturated fatty acids, lignans, and a range of other biologically active components. At the same time, its richness in unsaturated fats makes it extremely vulnerable to oxidative degradation, which can diminish its flavor, reduce its quality, and shorten its storage stability. This challenge has intensified interest in plant-derived antioxidants that could serve as natural and safe stabilizing agents.

The present study set out to determine whether an extract from *Verbascum* (mullein) could help protect cold-pressed linseed oil against oxidative deterioration. The extract was prepared using an ultrasound-assisted technique, and the extraction parameters were carefully optimized to obtain a high concentration of antioxidant-active substances. Combining this modern extraction method with the search for effective natural additives provides a promising route for improving both the durability and overall quality of linseed oil.

METHOD

Material: Mullein flowers (*Verbascum nigrum* L.) collected near the Kozienicka Forest, Mazovian Voivodeship, Poland, August 2023.

Extract preparation: Optimized using DOE with central composite design and RSM; variables: ultrasound power (50–100%), time (10–30 min), solvent concentration (30–70%), plant-to-solvent ratio (20:1–40:1).

Linseed oils (3 batches) were analyzed for:

- **Oil hydrolysis:** AV (PN-EN ISO 660:2010)
- **Oxidation:** PV (PN-EN ISO 3960:2017-03), p-AnV (AOCS Cd 18-90), Totox indicator
- **Oxidative stability:** Rancimat (3 g, 20 L/h, 90 °C)
- **Phenolic and flavonoid content:** TPC, TFC
- **Antioxidant activity:** DPPH* (AA, AA HF, AA LF)
- **Fatty acid composition** (AOAC 996.01)

Table 2. Oils' qulaity features befor and after the addition of mullein extract in a concentration of 5%

Oil	AV [mg KOH/g]	PV [mEq O_2/kg]	p-AnV	TOTOX	Carotenoids [mg B-caroten/kg]	Chlorophylls [mg pheophitine a/kg]	Flavonoids [mg QE/100g]	Phenols [GAE/100g]	AA LF [mg Trolox/100g]	AA HF [mg Trolox/100g]
LO1	0.33^{a}	2.94^{bc}	0.46^{a}	5.91^{bc}	9.10^{a}	0.12^{a}	7.91^{a}	103.19^{a}	49.88^{a}	2.03^{a}
LO2	0.66^{b}	3.66^{c}	0.61^{a}	7.42^{c}	10.53^{a}	0.30^{a}	15.40^{bc}	120.34^{a}	58.05^{a}	2.75^{a}
LO3	1.70^{d}	1.94^{ab}	0.60^{a}	3.98^{ab}	12.45^{a}	0.92^{b}	12.31^{b}	122.05^{a}	53.00^{a}	3.71^{a}
LOE1	0.56^{b}	2.03^{ab}	1.51^{b}	4.86^{ab}	29.86^{b}	2.98^{d}	13.60^{b}	311.49^{b}	47.82^{a}	18.82^{b}
LOE2	1.00^{c}	2.16^{ab}	1.74^{b}	5.47^{abc}	27.77^{b}	1.22^{b}	21.07^{d}	430.41^{c}	46.04^{a}	18.73^{b}
LOE3	1.94^{e}	1.42^{a}	1.59^{b}	3.57^{a}	42.13^{c}	2.66^{c}	17.22^{c}	353.23^{b}	51.64^{a}	18.80^{b}

Table 3. Oils' fatty acid composition [%] before and after the addition of mullein extract in a concentration of 5%

Fatty acid	LO1	LO2	LO3	LOE1	LOE2	LOE3
C16:0	1.76	1.82	1.98	1.72	1.71	1.82
C18:0	4.63	3.72	4.89	4.71	4.25	4.67
C18:1	18.96	19.01	19.22	18.48	17.73	19.09
C18:2	15.44	16.19	14.75	15.23	15.46	14.69
C18:3	57.76	58.38	58.66	59.18	59.76	59.25
C22:1	0.98	0.51	0.31	0.40	0.58	0.29
C24:0	0.50	0.38	0.21	0.29	0.54	0.22
SFA	6.39 ^{a}	5.54 ^{a}	6.87 ^{a}	6.43 ^{a}	5.95 ^{a}	6.48 ^{a}
MUFA	18.96 ^{a}	19.01 ^{a}	19.22 ^{a}	18.48 ^{a}	17.73 ^{a}	19.09 ^{a}
PUFA	73.20 ^{a}	74.57 ^{a}	73.41 ^{a}	74.41 ^{a}	75.22 ^{a}	73.94 ^{a}
Other	1.47	0.89	0.52	0.69	1.11	0.51

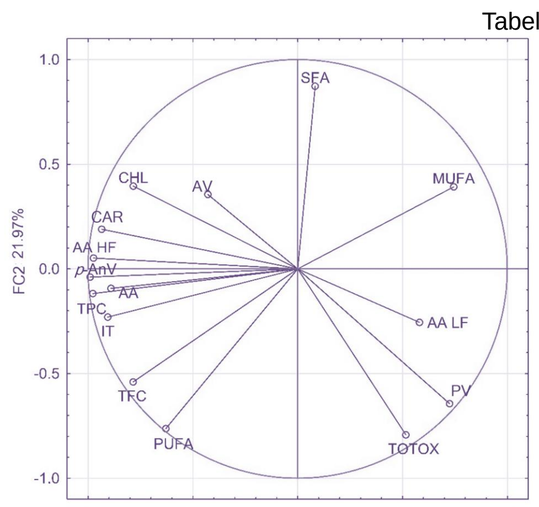

Fig 3. PCA analysis of the relationships between fatty acid composition, antioxidant content, and quality parameters of the oil.

Tabel 4. Correlation between oils' quality features and induction time

Quality factor	IT
AV	0.02
PV	-0.44
***p*-AnV**	**0.95***
TOTOX	-0.18
TPC	**0.97***
TFC	0.74
AA HF	**0.93***
AA LF	-0.70
AA	0.76
SFA	-0.12
PUFA	0.75
MUFA	-0.73
CHL	0.63
CAR	0.79

RESULTS & DISCUSSION

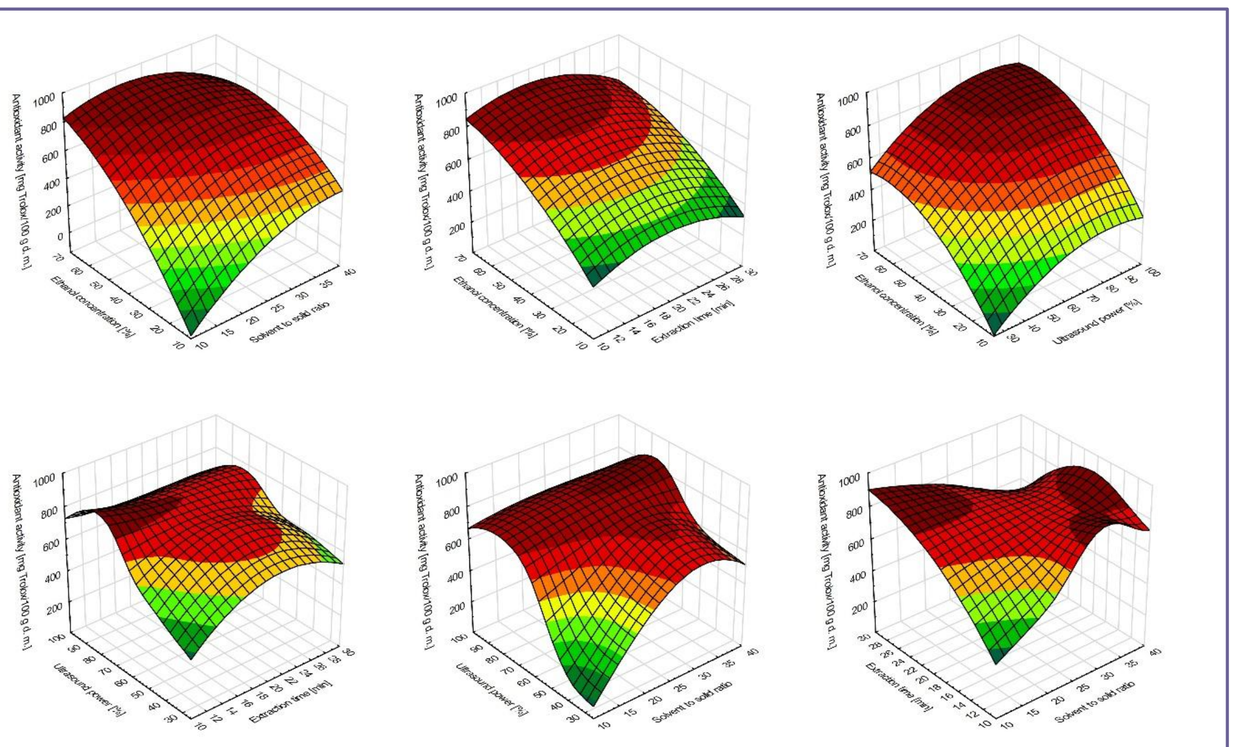

Fig 1. Effect of extraction parameters of mullein flower extract on its antioxidant activity (AA)

Table 1. Optimised parameters of mullein flowers extract obtaining and extract quality features

Power [%]	Time [min]	Ethanol concentration [%]	Solvent to solid [x:1]	AA [mg Troloxu/100 g s. m. ekstraktu]	TPC [mg GAE/100 g s. m. ekstraktu]	TFC [mg QE/100 g s. m. ekstraktu]
100	24.75	70	13.64	1014.24±25.89	7036.65±89.75	351.32±24.57

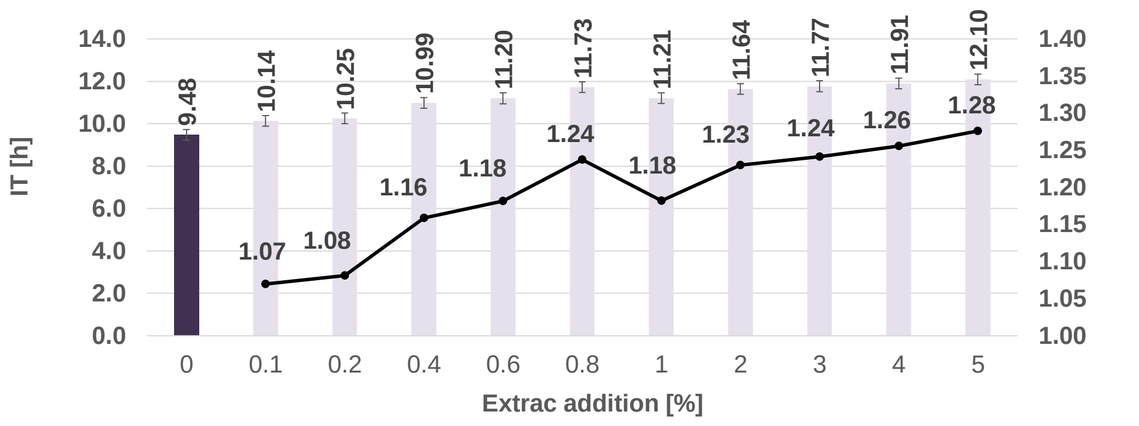

Fig 2. LO1 induction time before and after addition of mullein extract, calculated protection factor (PF)

CONCLUSION

The study showed that the efficiency and antioxidant activity of mullein flower extracts depend strongly on extraction parameters, with ultrasound power and solvent concentration having the greatest impact. Excess plant material or overly long extraction times reduced antioxidant activity due to compound degradation. The mullein extract enhanced the oxidative stability of flaxseed oil, with a 5% addition providing the longest induction time. It also significantly increased the oil's phenolic content, which strongly correlated with improved stability. The extract boosted antioxidant activity in the oil's hydrophilic phase due to its high phenolic levels. Overall, the results indicate that further optimization of the extraction process and exploration of other plant parts could yield extracts with even stronger bioactive properties.

FUTURE WORK

Future research could focus on improving the extraction process by testing devices capable of generating higher ultrasonic power, as well as exploring alternative extraction techniques such as microwave-assisted, enzyme-assisted, or supercritical CO_2 extraction. It would also be valuable to investigate other parts of the mullein plant—such as leaves or roots—to compare their phenolic content and antioxidant potential with those of the flowers. Detailed profiling of bioactive compounds using advanced analytical methods (e.g., LC-MS/MS or HPLC) could help identify which phenolics contribute most to oil stability. Further studies might examine the effectiveness of mullein extracts in different edible oils and emulsions, along with their stability during storage under various environmental conditions. Additionally, assessing the safety, sensory properties, and potential applications of mullein extracts in food or cosmetic products would support their practical use. Exploring synergistic effects with other natural antioxidants could also reveal combinations that provide even greater oxidative protection.