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Antioxidant Activity of Fibres Originating from Traditional Varieties of Polish Flax Plants

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Abstract
This paper describes the examination of raw flax fibres in terms of their antioxidant activity. Fibres originating from five varieties of flax plants cultivated in Poland: Artemida, Modran, Sara, Nike and Luna were extracted with the application of different methods: dew retting and water retting. The extraction method has an influence on the fibre chemical composition, resulting in different levels of fibre antioxidant properties. The antioxidant activity of flax fibres was evaluated with the application of ferric ion reducing antioxidant power (FRAP). The results of the study indicated differences in the bioactivity of flax fibres linked with the method of their extraction applied as well as the lignin and phenolic acid content in the fibre chemical composition.

Key words: *flax fibres, antioxidant activity, dew retting, water retting, lignin, ferulic acid, coumaric acid.*

Introduction

Flax fibres are known as favorable in contact with human skin. A study on linen clothing's influence on the human body conducted in steady conditions proved their positive effect in ensuring well-being, without causing the desynchronisation of muscle motor units, and thus without increasing of the tendency to tiredness of users [1]. Another study showed that wearing of natural fibre pyjamas with hydrophilic properties influences the activity of sebaceous glands positively, which improves resistance to skin diseases [2]. Researchers proved that linen clothes characterised by hydrophilic properties do not cause an increase in reactive oxygen species and oxidative stress as opposed to garments made of synthetic fibres characterised by hydrophobic properties [3]. Linen garments are known as very breathable and provide optimal comfort for users, especially in hot climate conditions. It allows for the exchange of air captured in the skin-clothing area through the fabric with the outer environment, giving the rapid escape of moisture (sweat) from the skin and thereby hindering bacteria growth. This is related to the higher water retention of flax in comparison to other fibres and particularly flax ability for moisture management.

There is no information in research literature on the original antioxidant properties of flax fibres obtained from traditional non-modified varieties in relationship to the fibre extraction method.

The current study was conducted to determine the inherent bioactivity of raw flax fibres and to find an explanation of this phenomenon by the assessment of lignin as well as phenolic acid contents in dew retted and water retted flax fibres. Phenolic acids are known as strong antioxidants, [4 - 8], especially ferulic acid, which is used, apart from other applications, as a cosmetic ingredient to protect the skin against the unfavorable effect of the environment and to act as an antiaging agent [6]. ***Figure 1*** presents oxidising phenolic compounds. Based on an effectively scavenging chain reaction and deleterious radicals and suppressing radiation induced oxidative reactions, phenolic acids serve for preserving the physiological integrity of cells exposed to both air and for impinging UV radiation [9, 10].

The presence of phenolic substances in flax fibres has been studied by a few researchers [11, 12], but there is no analysis of fibres antioxidant activity.

The aim of the current study was to investigate the antioxidant activity of fibres extracted from different flax plant varieties in relationship to the fibre extraction method applied, which has an effect on the fibre chemical composition.

Materials and methods

Flax fibres extracted from straw with the application of two different methods e.g. dew retting and water retting were used as the material for the investigation. The fibres obtained from five varieties: Artemida, Modran, Sara, Nike and Luna by dew retting and water retting were tested in order to determine differences in their chemical composition and biological activity. All flax varieties mentioned were developed by the Institute of Natural Fibres and Medicinal Plants, differing in their resistance against fungi and some harmful climatic conditions.

Generally dew retting is applied after the mechanical pulling of stalks and deseeding. Flax stems are spread evenly in a field and left for 3 - 7 weeks, depending on climatic conditions. In this time, microorganisms, mainly fungi, secrete enzymes that degrade the following substances: pectin, proteins, sugars, starch, fats and waxes, tannins, and minerals [13, 14]. Water retting is a traditional retting method where the flax stalks are held below the water surface in dams, ditches or slow running streams and rivers. Now water retting is conducted in modern retting tanks. In water-retting, a variety of

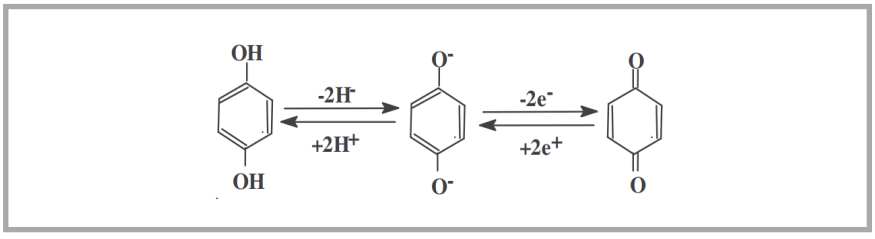

Figure 1. *Oxidizing phenolic compounds.*

Table 1. Conditions of the processes applied for fibre extraction

Fibre extraction method	Time	Temperature	Equipment
Dew retting	43 days	Ambient weather conditions	Field
Water retting	74 h	Water temp. 32 – 33 °C	Lab-scale tank

anaerobic bacteria is present and they are considered as the primary agents responsible for fibre release [15]. Several species of bacteria have been identified and investigated during tank retting, of which spore-forming *Clostridium* spp. have been shown to contribute considerably to pectin-degrading activity and, therefore, to retting.

All fibre types used for the study were extracted from flax plants grown in the same vegetation season. The parameters of processes applied for fibre extraction are presented in ***Table 1***.

Evaluation of flax chemical composition

Flax fibres contain in their chemical composition natural polymers like cellulose, lignin, hemicelluloses, pectin as well as waxes, fats, and others. The chemical composition of all the types of fibres extracted was evaluated with the use of relevant standards:

- Wax and fat content: Branch Standard BN – 86/7501-10
- Pectin content: a method developed at INF&MP
- Lignin content: Branch Standard BN – 86/7501-11
- Cellulose content: PN – 92/P-50092
- Hemicelluloses content: Branch Standard BN – 77/7529-02
- Phenolic acids, e.g. coumaric and ferulic acid, in flax fibres was assessed by the high-performance liquid chromatography (HPLC) method.

Additionally lignin extracted from dew retted fibres coming from different varieties of flax plants were tested to show the presence of phenols. Lignin was extracted from fibres according to the method described in Standard: BN – 86/7501-11. Fourier Transform Infrared Spectroscopy was used to determine the absorption spectra for lignin. The trials were performed with a spectrophotometer - FT-IR NICOLET iS10, (Thermo Scientific, USA) at infrared wavelengths of 350 – 4000 cm^{-1}.

Assessment of fibre antioxidant activity

Antioxidants are a large group of natural and synthetic compounds with the ability to reduce free radicals and prevent some amount of oxidative damage that destroys and depletes the skin function and structure while also preventing some of the degenerative effects in skin caused by sun exposure. In this study, two methods were used for the testing of flax fibre antioxidant capacity:

- Ferric Ion Reducing Antioxidant Parameter (FRAP)
- 2,2-Diphenyl-1-picrylhydrazyl Radical Scavenging Capacity Assay (DPPH) [16]

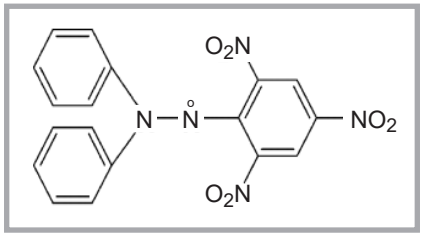

Figure 2. *DPPH radical.*

The DPPH* (***Figure 2***) radical is one of the few stable organic nitrogen radicals, which bears a deep purple colour. This assay is based on the measurement of the reducing ability of antioxidants toward DPPH*. The ability was evaluated by measuring the decrease in its absorbance. Antioxidant assays are based on measurement of the loss of DPPH colour at 515 nm after a reaction with test compounds, with the reaction being monitored by a spectrometer. The percentage of remaining DPPH* ($DPPH^*_{REM}$) is proportional to the antioxidant concentration, and the concentration that causes a decrease in the initial DPPH* concentration by 50% is defined as EC_{50}. The time needed to reach a steady state with EC_{50} is defined as TEC_{50}.

The Ferric Ion Reducing Antioxidant Power (FRAP) assay takes advantage of electron-transfer reactions, ***Figure 3***. The ferric reducing activity (FRAP) of the fibres extracts were estimated according to the method developed by Benzie and Strain [17]. The reaction mixture contained 300 mmol/l of acetate buffer, 10 mmol/l of 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mmol/l of HCL and 20 mmol/l of $FeCl_3 \cdot 6H_2O$. The working FRAP reagent was freshly prepared by mixing 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of $FeCl_3 \cdot 6H_2O$. The freshly prepared mixture was incubated at 37 °C in a water bath for five minutes and then a blank reading was taken spectrophotometrically at 593 nm. After that, 30 µl of the extract or standard and 90 µl of distilled water were added to 900 µl of the working FRAP reagent. Absorbance was measured at 0 min immediately upon addition of the working FRAP reagent after vortexing. Thereafter an absorbance reading was taken after four minutes [18, 19]. Three repetitions of the antioxidant test were conducted for each fibre sample.

Results

Chemical composition of flax fibres

The results of tests of the chemical composition of the materials used in the current study i.e. flax fibres extracted from different varieties of fibrous plants with the use of dew and water retting are shown in ***Table 2***.

The results of evaluation of the chemical composition of flax fibres indicates diversity in the share of particular components in different plant varieties, which is strongly related to the fibre extraction method.

All varieties of water retted flax fibres tested were characterised by a higher content of cellulose and lower content of lignin and hemicellulose in comparison with the relevant dew retted fibres. It means that the process of water retting caused better removal of non-cellulosic compounds (excluding waxes) from fibres, giving them properties that were more suitable for textile processing i.e. better quality and spinability.

On the other hand, all the varieties of dew retted flax fibres showed a higher share of lignin, the compound related to the bioactivity of the fibres. Analysis of flax varieties indicated that water retted Artemida showed the highest cellulose content, but dew retted Modran and Sara contained the largest amount of lignin.

The statistical analysis of results of flax chemical composition was conducted to verify if the differences are statistically significant. Analysis with the use of Shapiro–Wilk and U Mann–Whitney and T-Student tests for a confidence interval of

Table 2. Chemical composition of flax fibres extracted from fibrous plants.

Fibre	Fibrous plant variety	Lignin		Cellulose		Waxes and fats		Hemicelluloses		Pectin	
		content, %	SD, %	content, %	SD, %	content, %	SD, %	content, %	SD, %	content, %	SD, %
Dew retted	ARTEMIDA	6.33	0.41	64.86	0.64	1.30	0.02	19.31	0.14	1.32	0.17
	MODRAN	7.96	0.11	64.16	1.22	1.72	0.03	21.64	0.13	1.44	0.09
	SARA	7.42	0.22	62.21	0.22	1.63	0.02	22.30	0.14	1.20	0.13
	NIKE	6.40	0.41	64.31	0.87	1.19	0.03	20.91	0.02	1.72	0.16
	LUNA	5.18	0.17	69.61	0.14	1.06	0.13	16.86	0.04	2.12	0.19
Water retted	ARTEMIDA	3.51	0.04	74.01	0.49	4.40	0.00	14.57	0.05	0.58	0.15
	MODRAN	3.52	0.02	72.42	1.00	4.25	0.02	15.30	0.31	1.43	0.23
	SARA	3.18	0.13	70.76	0.14	4.43	0.11	17.31	0.17	2.15	0.08
	NIKE	3.05	0.13	71.31	0.91	4.41	0.12	15.55	0.19	1.35	0.07
	LUNA	2.56	0.21	72.77	0.35	4.49	0.02	13.91	0.08	0.65	0.02

95% confirmed significant differences in the share of particular components of fibres for $p < 0.05$.

The test for phenolic substance content in flax fibres proved that all varieties of dew retted flax fibres contained some amount of ferulic acid (***Figure 4***) and coumaric acid (***Figure 5***), which are known as strong antioxidants. The phenolic acid content in flax fibres is shown in ***Table 3***. In that test, phenolic acids could be detected only in dew retted fibres, because in the case of water retted fibres traces of the acids were extremely weak and their detection by the HPLC method applied was impossible.

Apart from the fact that all varieties of dew retted flax fibres contained a higher amount of lignin and hemicellulose than in comparison to water retted fibres, HPLC analysis of the fibre proved that the dew retted fibres contained some amount of phenolic acids, while higher amounts of phenolic acid occurred in Modran and Sara varieties. It is known that ferulic acid is a component of a primary cell wall and is bonded with lignin and hemicellulose in plants [7, 8]. ***Figure 6*** (see page 44) shows the FTIR spectra determined for lignin extracted from dew retted fibres coming from different flax varieties. Characteristic absorption bands in infrared shown in ***Table 4*** allowed to find the phenols at the spectra of extracted lignin.

Lignin extracted from all varieties of flax showed a broad band at 3410 – 3460 cm^{-1}, attributed to the hydroxyl groups in phenolic structures. The highest absorbance in that band was observed in the case of lignin extracted from Sara flax. Differences between the absorbance of lignin from other flax varieties in this band were inconsiderable. C-O stretching vibration in band 1140 – 1230 cm^{-1} confirmed the presence of phenols. In this case, the absorbance was the highest for lignin obtained from the Modran variety of flax, while the lowest was observed for lignin extracted from the Luna variety. Other flax varieties showed similar levels of absorbance in that band. The level of phenolic acid absorbance shown by the FTIR test confirmed the investigation of Holser, who evaluated ferulic and p-coumaric acid spectra in the 650 - 1500 cm^{-1} region [23]. The FTIR spectra of lignin extracted from fibres coming from different varieties of flax plant confirmed the results of HPLC fibre analysis that proved that Modran and Sara flax fibres contained the highest level of phenolic acids.

*Table 3. Phenolic acid content in flax fibres (*the non-determined trace amounts of the acids equal to detection limits are shown in unit mg/ml of the calibration curve according to the test method).*

Fibre	Fibrous plant variety	Content of coumaric acid, mg/100 g	SD coumaric acid	Content of ferulic acid, mg/100 g	SD ferulic acid
Dew retted	ARTEMIDA	5.580	0.118	4.645	0.092
	MODRAN	6.136	0.448	5.128	0.385
	SARA	6.021	0.394	5.014	0.329
	NIKE	5.688	0.293	4.739	0.241
	LUNA	5.708	0.984	4.859	0.803
Water retted	ARTEMIDA	< 0.0022 mg/ml*	-	< 0.0019 mg/ml*	-
	MODRAN				
	SARA				
	NIKE				
	LUNA				

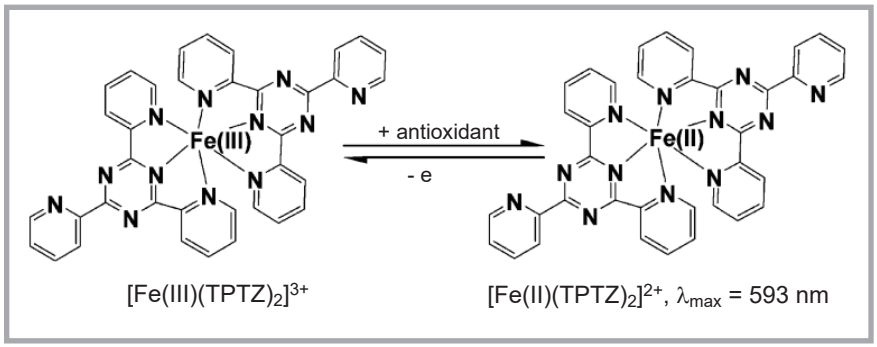

Figure 3. *FRAP - electron-transfer reaction.*

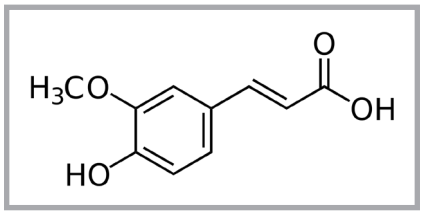

Figure 4. *Ferulic acid.*

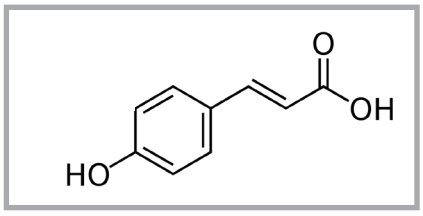

Figure 5. *p-coumaric acid.*

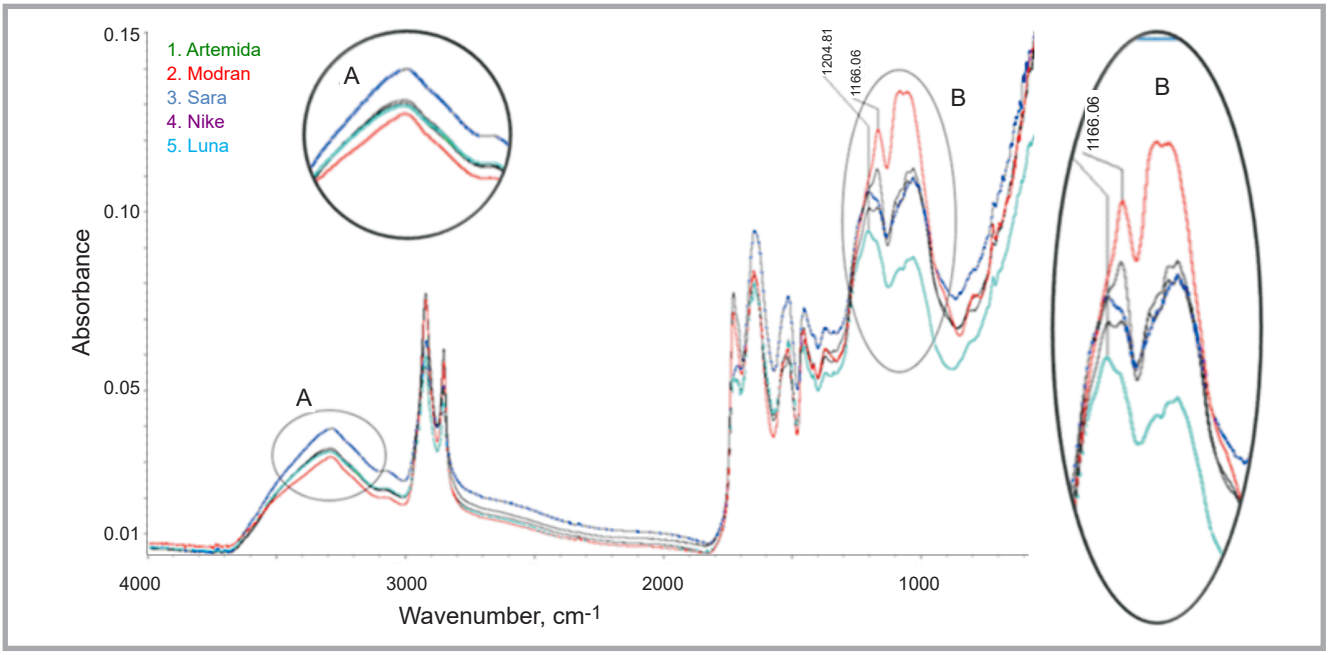

Figure 6. *FTIR spectra of lignin extracted from fibres obtained from different varieties of flax plant: 1. – lignin extracted from Artemida fibre, 2. - lignin from Modran fibre, 3. – lignin from Sara fibre, 4. – lignin from Nike fibre, 5. – lignin from Luna fibre.*

Table 4. *Characteristic absorption bands in infrared [20 - 22].*

Bonds	Compound type	Band range, cm^{-1}
C-H stretching vibrations	aromatic rings	2800 - 3000
C-H bending vibrations (in-plane)	aromatic rings	1000 - 1100
C-H bending vibrations (off-plane)	aromatic rings	675 - 870
C-H bending vibrations	methyl group -CH_3 methylene group -CH_2-	1430 - 1470 and 1375 1430 - 1470
C-C stretching vibrations	aromatic rings	1500 - 1600
C-C stretching vibrations	quaiacyl ring	1270
C-C stretching vibrations	syringyl ring	1330
C-O stretching vibrations	phenols carboxylic acids	1140 - 1230 1250
C=O stretching vibrations	aldehydes and ketones carboxylic acids	1675 - 1725 1680 - 1725
O-H stretching vibrations	phenols carboxylic acids	3200 - 3600 2500 - 3000
O-H bending vibrations	carboxylic acids	1400 and 920

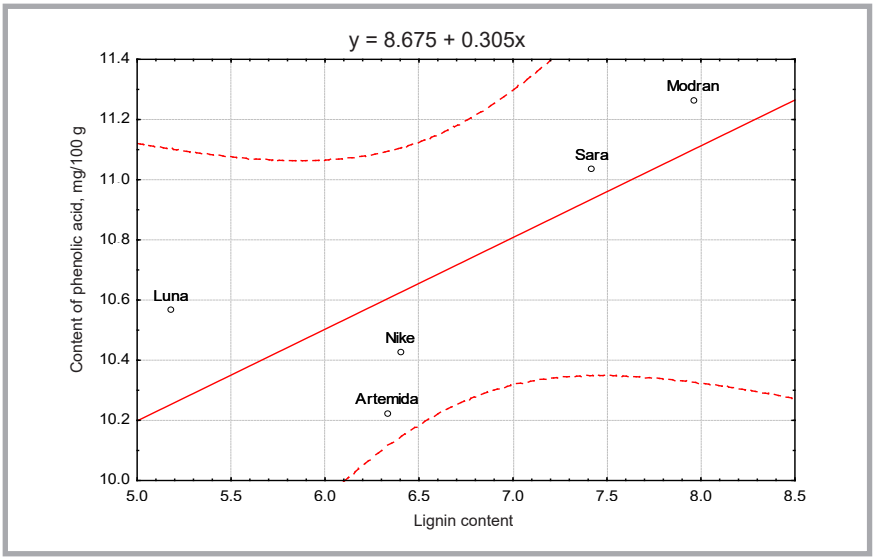

Figure 7. *Relationship between lignin and phenolic acid content in dew retted flax fibres.*

The relationship between phenolic acid and lignin content in the dew retted flax fibres tested was analysed and presented in ***Figure 7***. The relationship between lignin and phenolic acid content was high (***Figure 7***), as confirmed by correlation analysis - the Pearson correlation coefficient was 0.76. The analysis of correlation was conducted regardless of the low number of observations in order to have preliminary information about the relationship between the main fibre components and bioactivity. All varieties of water retted flax fibres were characterised by lower amounts of lignin, as well as they had only trace amounts of phenolic acid, unable to detect precisely by the method used. The reason for this is clear because ferulic acid is easily soluble in water and can be readily removed from fibres by water during the retting process. Coumaric acid is poorly soluble in water and its removal could be partial.

Antioxidant activity of flax fibres

FRAP tests were conducted for different sample concentrations. It is known that the value of FRAP increases with increasing sample concentration.

The results of the test of the antioxidant activity of flax fibres assessed with the use of the FRAP method shown in ***Figure 8*** proved that dew retted fibres extracted from the following varieties: Modran, Sara and Luna have higher ability for Fe ion reduction from Fe^{+3} to Fe^{+2}

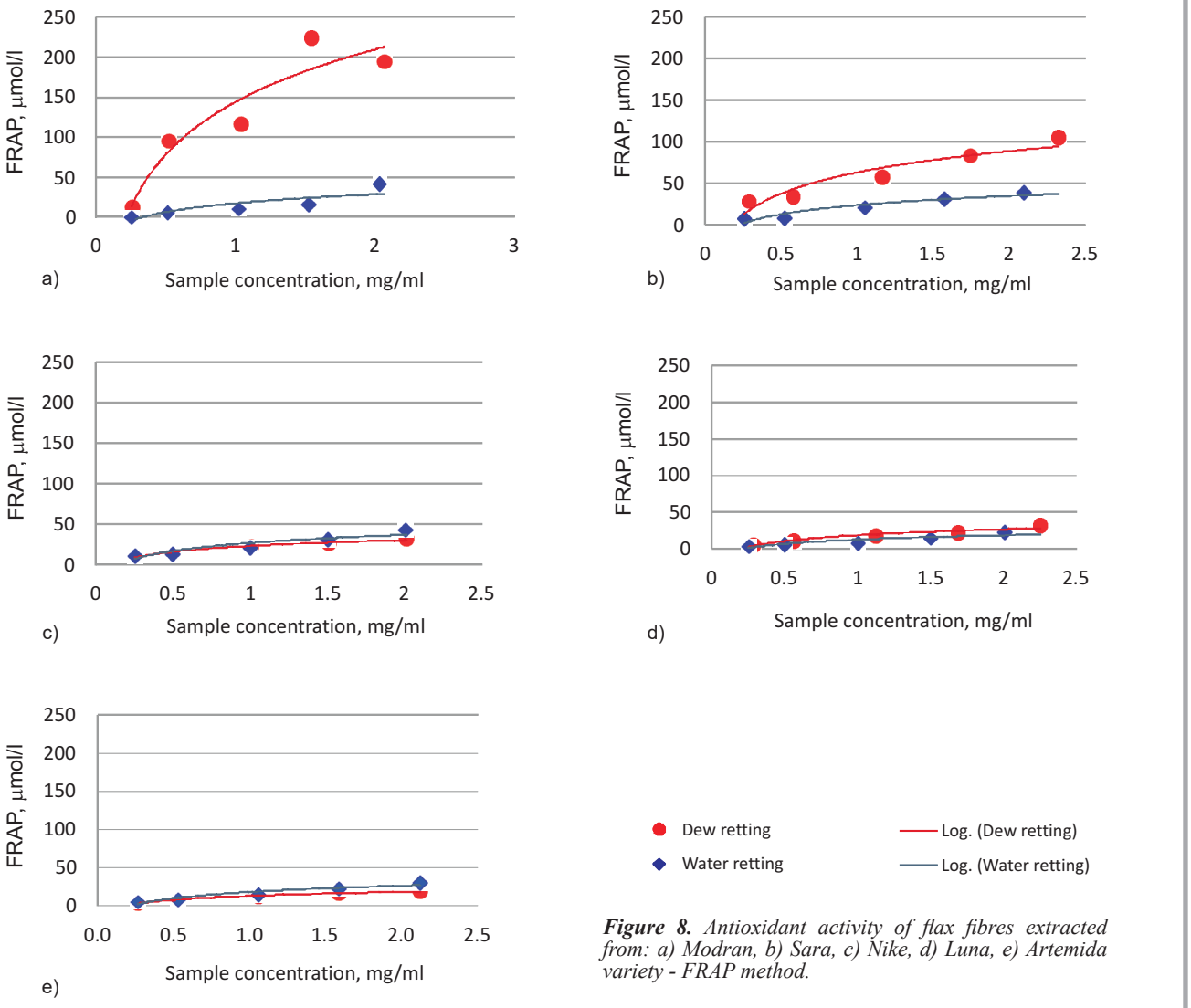

Figure 8. *Antioxidant activity of flax fibres extracted from: a) Modran, b) Sara, c) Nike, d) Luna, e) Artemida variety - FRAP method.*

in comparison to relevant water retted fibres. However, it must be noted that the value of FRAP for dew retted flax from the Modran variety was very high in comparison to other fibres. Dew retted fibres extracted from the Sara variety also showed a high FRAP value. The ability of fibres extracted from Luna, Nike and Artemida to reduce the Fe ions was very low and differences between dew and water retted fibres were similar (***Figures 8.c - 8.e***). The variations between the antioxidant activity of dew and water retted fibres are the most visible for flax varieties with high FRAP values, e.g. for Modran and Sara varieties (***Figures 8.a, 8.b***).

The antioxidant activity of flax fibres was strongly related to the phenolic acid content in the fibre extracts. From all samples tested, dew retted fibres extracted from the Modran variety showed the highest content of lignin as well as coumaric and ferulic acid, which resulted in the highest ferric reducing antioxidant power. The second best was Sara variety.

The relationship between the phenolic acid content and antioxidant activity of the dew retted flax fibres tested is presented in ***Figure 9*** (see page 46). The line of trend drawn in the diagram indicated a strong relationship between the fibre ability to reduce free radicals and the phenolic acid content in the fibres.

Statistical analysis conducted for preliminary evaluation of the relationship between the antioxidant activity of flax fibres tested by the FRAP method and the phenolic acid content indicated a strong correlation.

The coefficient of the Pearson linear correlation reached the following values:

- FRAP and both phenolic acid contents - 0.89
- FRAP and ferulic acid – 0.88
- FRAP and cumaric acid is 0.9

The correlation analysis confirmed very strong relationship between phenolic acid content and antioxidant activity of dew retted flax fibres.

The results of FRAP tests of flax fibres indicated an inherent antioxidant activity of raw flax fibres; however, it was not the same for all flax plant varieties and was strongly related to the flax variety and method of fibre extraction. The value of the ferric reducing antioxidant power of the dew retted Modran fibres tested was the highest in comparison to the activity of the other varieties of flax plants. The dew retted Modran flax fibres tested showed strong antioxidant properties and can be used for textile manufacture and

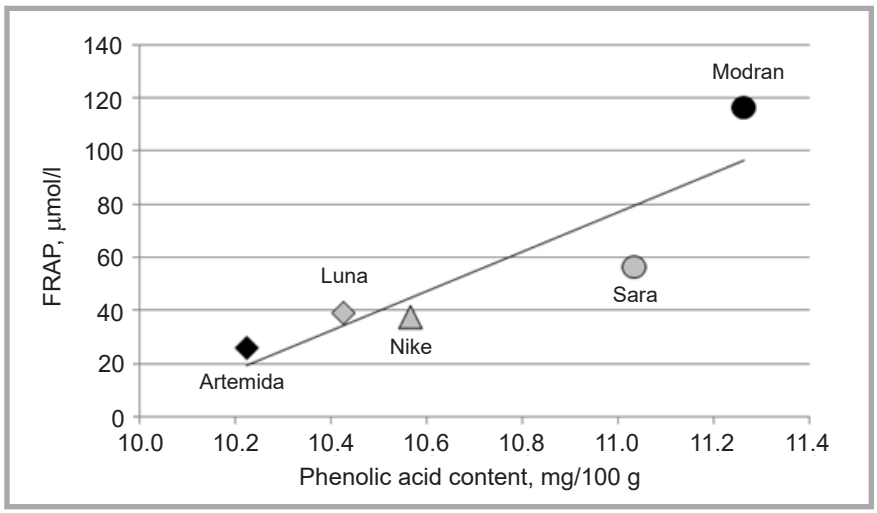

Figure 9. *Relationship between the phenolic acid content and antioxidant activity of dew retted flax fibres.*

serve as a barrier against free radicals coming from air and UV radiation and reaching human skin. Dew retted Sara fibres could also act for the neutralisation of free radicals; but their activity was not so strong as the Modran variety.

Results of the DPPH test were not so significant, reaching low values in the range from 1.21 (for Nike) to 3.28 (for Modran). Generally, in the FRAP method, the reaction runs very quickly due to employing metal ions and covering the largest number of components, whereas in the case of the DPPH radical method, the reaction is much slower and covers only the most reactive substances. For this reason, the values of the DPPH results are significantly lower in comparison to the FRAP method [24].

Conclusions

The current study conducted on flax fibres extracted from five varieties of fibrous plants: Artemida, Modran, Sara. Nike and Luna with the use of dew and water retting methods proved that unmodified flax fibres can show inherent antioxidant properties. The biological activity of the fibres tested was strongly related to the lignin content and related with the phenolic acid content in their chemical composition. The fibre antioxidant power was related to the method of their extraction applied, especially in case of the Modran and Sara varieties, where the dew retted fibres showed the highest activity, whereas water retted fibres show very weak biological activity in the tests conducted. The results of the study obtained allowed to draw the following conclusions:

1. The study proved that the antioxidant activity of the flax fibres tested were strongly related to the method of retting and to the flax plant variety, which had an effect on the chemical composition of the fibres.
2. Dew retted flax fibres obtained from three varieties tested were characterised by better antioxidant properties in comparison with respective water retted fibres. Two other flax varieties showed low antioxidant power and differences between dew and water retted fibres were slight.
3. Dew retted fibres contained a higher amount of lignin and, hence, phenolic acids in comparison to water retted fibres. The study proved there is a strong relationship between the content of lignin and phenolic acids bonded with it as well as the bioactivity of dew retted flax fibres.
4. Water retted flax fibres showed lower biological activity because the most active phenolic substances are water soluble and were removed during retting. The water retting process allows for removal of more non-cellulosic compounds, excluding waxes to obtain purer fibres. Water retted flax fibres extracted from all the flax plants tested were characterised by the highest cellulose content, which allowed to conclude that the quality of textile made of the fibres would be better than of respective dew retted fibres.
5. Flax fibres extracted from the Modran variety with the use of dew retting showed the highest antioxidant activity. The second best was the Sara variety, which is strongly related to the highest content of lignin and phenolic acids in their structure in comparison to other flax varieties.
6. Fibres were obtained from Artemida and Luna varieties with the highest cellulose content, which predisposes them as the best for textile processing, were characterised by weak biological activity due to low lignin and phenolic acid content.
7. The biological activity of flax fibres is inversely proportional to their textile quality, because the higher lignin content in flax fibres negatively influences textile processing.

Acknowledgment

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