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INNOVATIVE TECHNOLOGIES FOR OBTAINING NEW EMULSIONS, BASED ON PINE OIL AND SURFACTANTS

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Abstract: *New emulsions were obtained by innovative technologies based on pine oil and 2 surfactants: sodium dodecyl sulfate and Tween® 80 mixture: Ps- pine oil/ sodium dodecyl sulfate/water; Pt- pine oil / Tween® 80/water; Pst- pine oil/ sodium dodecyl sulfate and Tween® 80 (ratio 1:1)/water, for different concentrations of pine oil, in order to improved surface properties with applications in leather industry. Pine oil has a strong antimicrobial and antifungal effect due to its content in: vitamins C and E, phytosterols, fatty acids, antioxidants and amino acids. The order of introduction of the components in innovative technologies, the working conditions and especially the choice of the concentration of surfactants >CMC, is essential in the solubilization of pine oil and obtaining the new emulsions. The emulsions were characterized by optical microscopy with pine oil at 23-60°C. The changes in the aggregation process were observed for each type of emulsion, the influence of temperature and the solubilization of pine oil. Dynamic light scattering (DLS) for the emulsions showed the stability, concentration, particle size, polydispersity, zeta potential. The antimicrobial properties were analyzed by microbiological tests. FTIR measurements highlighted the interaction mechanism of surfactants with pine oil from the new emulsions. The leather samples were microbiologically tested and antimicrobial, antifungal effects were observed. The new emulsions are original due to the successful inclusion of pine oil with high potential for improved surface properties with applications in leather industry.*

Key words: *new emulsions, innovative technologies based on pine oil and surfactants, leathers processed*

1. INTRODUCTION

This paper presents innovative technologies to created new emulsions, based on pine oil and 2 surfactants: sodium dodecyl sulfate and/or Tween® 80, in order to improved surface properties with applications in leather industry.

Pinus sylvestris, the Scots pine (UK), Scotch pine (US), Baltic pine, (M. Gardner; 2013) or European red pine is a species of tree in the pine family *Pinaceae* that is native to Eurasia.

It can readily be identified by its combination of fairly short, blue-green leaves and orange-red bark. *Pinus sylvestris* is an evergreen coniferous tree growing up to 35 metres in height (J. Bispham; 2015) and 1 m in trunk diameter when mature, exceptionally over 45 m tall and 1.7 m in trunk diameter on very productive sites.

The tallest on record is a tree over 210 years old growing in Estonia which stands at 46.6 m.

Fig. 1*: Image of Pinus sylvestris (J. Bispham; 2015)*

Pine oil is an essential oil obtained from a variety of species of pine, particularly Pinus sylvestris. Typically, parts of the trees that are not used for lumber- stumps, are ground and subjected to steam distillation. As of 1995, synthetic pine oil was the "biggest single turpentine derivative." Pine oil is a higher boiling fraction from turpentine. Both synthetic and natural pine oil consists mainly of α-terpineol, a C_{10} alcohol (214–217°C). Other components include dipentene and pinene. The detailed composition of natural pine oil depends on many factors, such as the species of the host plant. Synthetic pine oil is obtained by treating pinene with water in the presence of a catalytic amount of sulfuric acid. This treatment results in hydration of the alkene and rearrangement of the pinene skeleton, yielding terpineols. Pine oil is used as a cleaning product, disinfectant, sanitizer, antimicrobial and antifungal. It is effective against Brevibacterium ammoniagenes, the fungi Candida albicans, Enterobacter aerogenes, Escherichia coli, Gram-negative enteric bacteria. Due to its properties and beneficial effect on health, pine oil is used in: cosmetics, medicine, pharmacy and food (M.Gscheidmeier, F. Helmut; 2000).

Fig. 2*: Pinus sylvestris essential oil in a clear glass vial (M.Gscheidmeier, F. Helmut; 2000).*

Tween 80 is a polyethylene sorbitol ester, also known as Polysorbate 80, PEG (80) sorbitan monooleate, polyoxyethylenesorbitan monooleate (M.G. Hertog, E.J.Feskens, D.Kromhout, M.G Hertog, P.C.Hollman, M.G.Hertog, M.Katan; 1993). It has been used as emulsifying agent for the preparation of stable oil-in-water emulsions (D.S. Varasteanu; 2014). Tween is a group of non-volatile surfactant derivatives derived from glycerol esters (C.M Enescu; 2014). The most important usage of Tween is its application as an oil absorber and emulsifier (K.Walczak-Zeidler, A.Feliczak-Guzik, I. Nowak; 2012). Sodium dodecyl sulfate (SDS), also called sodium lauryl sulfate (SLS), is an anionic tenside used as a cleaning and hygiene product (H.Kallio, B. Yang, P. Peippo; 2002).

In this research the new emulsions created and leathers processed with them were analyzed by FTIR-ATR spectroscopy, DLS, optical microscopy and microbiological tests.

2. EXPERIMENTAL

Materials and Methods

In order to obtain new emulsions the following materials have been used: sodium dodecyl sulfate and Tween 80 from Sigma-Aldrich; pine oil from company “BIOCA”.

The experimental techniques used in this paper consist in: a zetasizer-nano "MALVERN" equipment, with measuring range between 0.3 nm- 60.0 microns and zeta potential determination with an accuracy of +/-2%; an FTIR-ATR spectrophotometer JASCO; optical microscopy with an ELTA 90 Medical Research S.R.L. equipment. A number of 3 samples of emulsions: **Ps, Pt, P**st were prepared in the working conditions: sodium dodecyl sulfate or/and Tween 80 at 1:1 ratio, temperature=50°C for 30 minutes with pine oil, fig.3. The Pst emulsion variant with the two surfactants sodium dodecyl sulfate and Tween 80 in a 1:1 ratio was selected because it is the most stable over time (1 month). The way of introducing surfactants and pine oil in obtaining emulsions is very important. The surfactant micellar solution is always made in water at a concentration above the micellar critical concentration- CMC and then the pine oil is added drop by drop and mixed. The chosen temperature is 50^{o}C for a good solubilization of the pine oil in the surfactant micelles. When there are two surfactants, micellar solutions in water are made separately for them, then the two solutions are mixed and mixed micelles in water are obtained. In the solution of mixed micelles, the pine oil is introduced drop by drop, stirring and at the appropriate temperature. In the end, the emulsion is obtained with pine oil solubilized in the mixed micelles. The yield of multiple drop formation decreases rapidly as the homogenization time increases. New emulsions are formed and the properties derive from the surfactants used, as well as the conditions and working parameters. This phenomenon is controlled by the concentration of: pine oil, surfactants, temperature, pH=4.

Fig. 3*: Image of new structured emulsions a)* ***Ps****- pine oil/ sodium dodecyl sulfate/water;* ***Pt****- pine oil/ Tween® 80/water;* ***Pst****- pine oil / sodium dodecyl sulfate and Tween® 80 (ratio 1:1)/water*

The leathers were processed by cross spraying with the three obtained emulsions: **Ps, Pt,Pst** with a quantity of 0.5 l for 8 times on a leather surface from 10 cm.

Method used for microbiologically tests: -replication of the bacteria used in the test: *Aspergillus niger*, *Staphylococcus aureus ATCC* 6538 (gram-positive). We work with a pure, freshly propagated culture;

- dry sterilization of laboratory glassware in an oven at 180^{o}C;
- preparation of the culture medium, characteristic of the test bacteria used, namely: Nutrient Agar for *Aspergillus niger* and Mannitol Salt Agar for the genus *Staphylococcus aureus*;
- wet sterilization in the autoclave and Erlenmayer vessels with culture media;
- the samples must be circular, with a diameter of 25±5 mm.

Prepare the agar volume for the bottom layer without bacteria. Place (10 ± 0.1) ml in each sterilized Petri dish and allow the agar to solidify. Prepare the amount of agar for the top layer and cool to 45^{0}C on a water bath. Inoculate 150 ml of agar with 1 ml of bacterial working solution (1-5 x 108 cfu/ml). Shake the container vigorously to distribute the bacteria evenly. Add (5±0.1) ml to each Petri dish and allow the agar to solidify. The samples are placed on the surface of the nutrient medium and then incubated at 37^{0} C

3. RESULTS AND DISCUSSIONS

Obtaining new emulsions based on pine oil and surfactants

New emulsions were obtained using 2 surfactants, sodium dodecyl sulfate and Tween 80, in which pine oil were introduced. According to novel innovative technologies in fig.4, three types of new emulsions were made: **Ps, Pt, Pst.** The antimicrobial and antifungal effect were improved with the increase in the amount of pine oil.

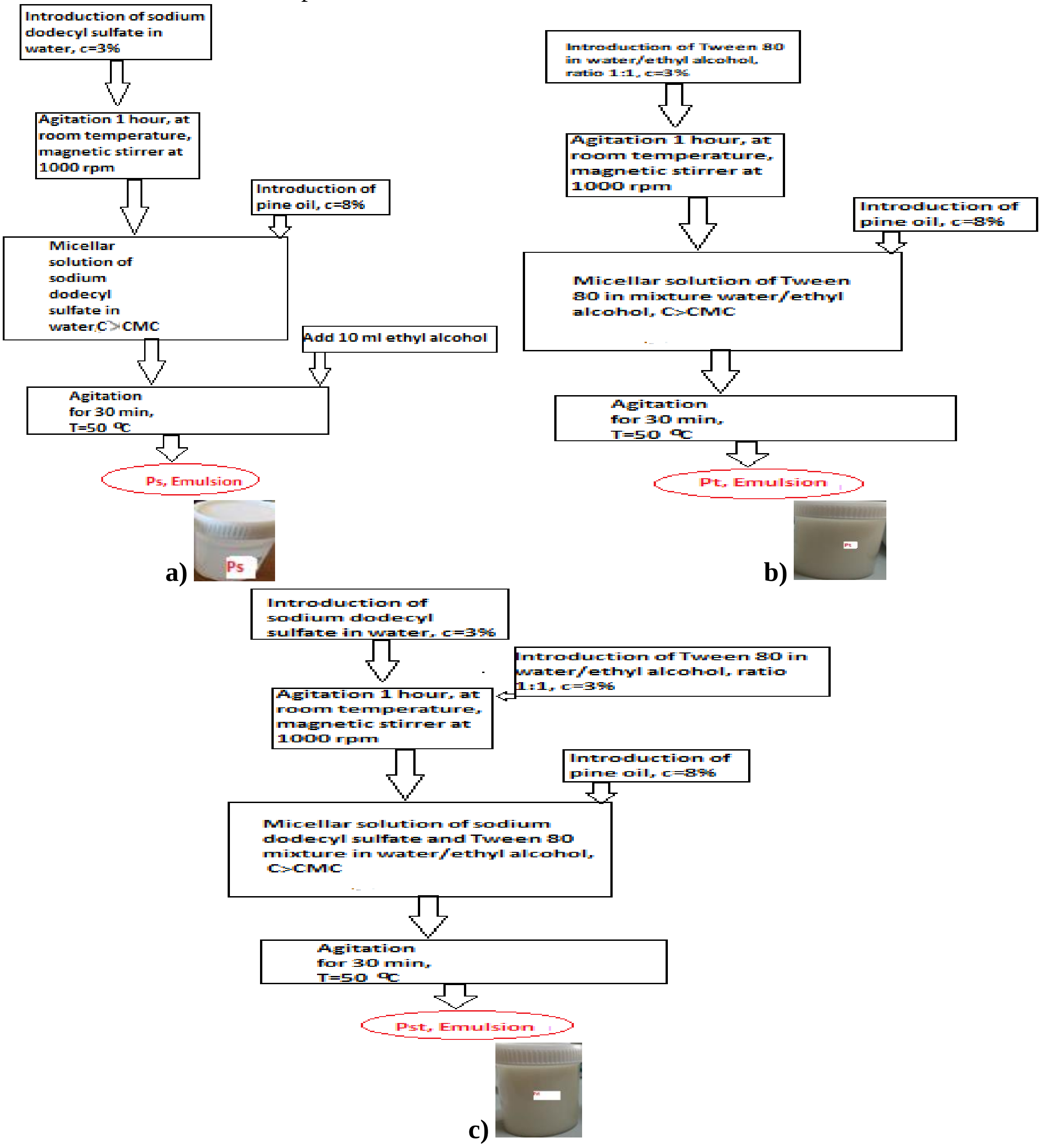


***Fig. 4**: Innovative technologies for obtaining 3 type of emulsions with pine oil: Ps-a); Pt-b); Pst-c)*

Mechanism of pine oil solubilization in surfactant micelles

In this research, the interaction of pine oil with 2 surfactants, sodium dodecyl sulfate and Tween 80 was investigated. A mechanism for the solubilization of pine oil in micelles was proposed, fig. 5.

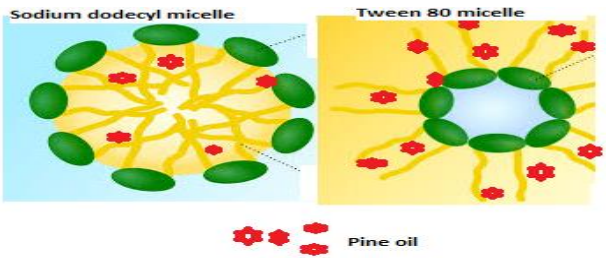


***Fig. 5**: Proposed mechanism for solubilization of pine oil in tensides: sodium dodecyl sulfate or Tween 80*

The effect of the length of the carbon chain on the interaction was analyzed by FTIR-ATR spectroscopy. The experimental results suggested that Tween 80 was most efficient out of the 2 surfactants taken for the study. The order of stability is given as sea pine oil-Tween 80 > pine oil - sodium dodecyl sulfate. Pine oil is hydrophobic and gets stuck in the core of the micelles but also on the alkyl ends of the hydrophobic chains. For Tween 80, the amount of solubilized pine oil is higher than in the case of sodium dodecyl sulfate, because interaction forces are responsible.

Characterization of the new emulsions obtained and the leathers processed

The optical microscopy images from figure 6 (a-f) show that all 3 emulsions obtained (at room temperature or 50 degree) are structured like irregular shapes, due to the influence of interaction between surfactants and pine oil.

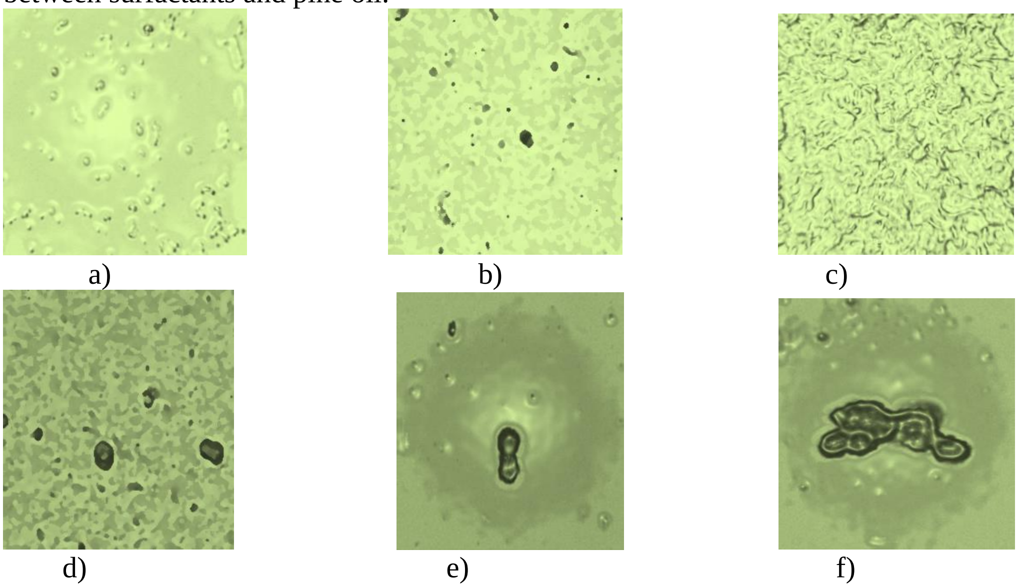


***Fig. 6**: Optical microscopy images of emulsions: Ps at room temperature-a); Pt at room temperature-b); Pst at room temperature-c); Ps at T=50ºC-d); Pt at T=50ºC-e); Pst at T=50ºC*

The average particle sizes of new structured emulsions showed dimensions between: (14-1020 nm), confirming the formation of the complex aggregates, table 1

The three types of emulsions were analysed by dynamic light scattering (DLS), table 1.

Table 1: *Results of DLS for 3 emulsions: Ps, Pt, Pst*

Sample	*Average diameter (nm)*	*% Intensity*	*Zeta Potential (mV)*
Ps	*14* *50* *107*	*20* *60* *20*	*-50*
Pt	*29* *300*	*30* *70*	*-35*
Pst	*600* *1020*	*21* *79*	*-69*

The leathers were processed by spraying with the three obtained emulsions: **Ps, Pt,Pst** and were marked **Psl, Ptl, Pstl** (figure 7) and then analyzed spectrophotometrically FTIR-ATR.

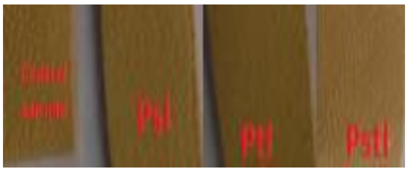


Fig. 7*: Image of the leathers processed with 3 emulsions: Ps, Pt, Pst and a control sample*

From figure 8 it can be seen that the largest amount of pine oil is found in the leather treated with the P**st** emulsion (the spectrum intensity is the highest in the entire spectral range).

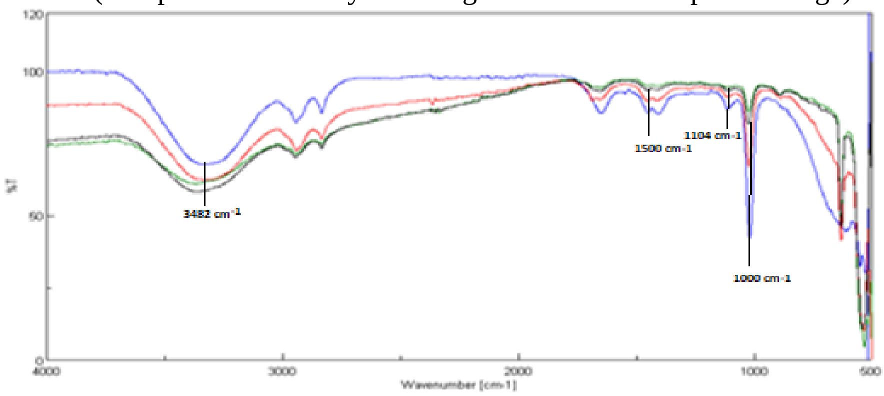


Fig. 8*: Overlay of FTIR-ATR spectra for leathers processed with emulsions: Psl---, Ptl--, Pstl--, control sample---*

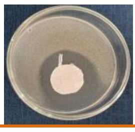
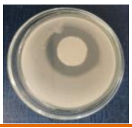
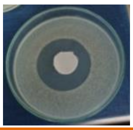
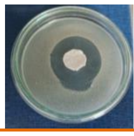
The P**st** emulsion is the most stable >2 month. The absorption maximum at the wavenumber =3482 cm^{-1} is the result of the overlap of the CH_2 deformation with the asymmetric CH_3 deformation (the intensity of the absorption maximum being proportional to the number of CH_2 and CH_3 groups present). The range of wavenumbers:1500–1000 cm^{-1} (1500 cm^{-1}, 1104 cm^{-1}, 1000 cm-^{1}) is specific to pine oil that have a high content of phenolic compounds and flavonoids.

The microbiological tests of leathers processed with 3 emulsions to the attack of *Staphylococcus aureus ATCC 6538* and *Aspergillus niger*, carrying out analysis three days from inoculations, are presented in table 2. Specimens of the material to be tested are placed on two-layer agar plates. The lower layer consists of a culture medium without bacteria while the upper layer is seeded with the selected bacteria. The level of antibacterial activity is assessed by examining the area of bacterial growth in the area of contact between the agar and the test tube, and if applicable the area of the zone of inhibition around the test tube.

Table 2: *Results of microbiological tests of leathers processed with 3 emulsions: Ps, Pt, Pst*

Sample	***Result, UFC/ml***	***R%***	***Log_{10} red***	***Sample***	***Result, UFC/ml***	***R%***	***Log_{10} red***
Aspergillus niger Inoculum concentration	$T_o=9,7x10^3$	-	-	*Staphyococcus aureus* Inoculum concentration	$T_o=9,2x10^3$	-	-
Psl	$T_{24}=3,6x10^3$	*90,6*	*2,5*	*Psl*	$T_{24}=4,8x10^3$	*99,5*	*2,1*
Ptl	$T_{24}=1$	*99,8*	*3,45*	*Ptl*	$T_{24}=3$	*99,6*	*3,9*
Pstl	$T_{24}=2,6x10^3$	*97*	*1,22*	*Pstl*	$T_{24}=1,5x10^2$	*97,8*	*1,8*

Table 3: *Images of Petri dishes obtained for leather supports treated with the obtained bioemulsions and microbiological testing*

Sample code	Zone of inhibition (mm)		Evaluation	
	A. niger	S. aureus	A. niger	S. aureus
Control sample	0	0	Insufficient effect	Insufficient effect
Psl	10 	7 	Satisfactory effect	Satisfactory effect
Ptl	11 	10 	Satisfactory effect	Satisfactory effect
Pstl	5 	10 	Satisfactory effect	Satisfactory effect

4. CONCLUSIONS

1.The aim of this research was fulfilled to develop new emulsions and to study the influence of surfactants and pine oil in obtaining structures with irregular shapes.The structures of new emulsions was demonstrated by optical microscopy.

2.The emulsions with particle sizes of 14- 1020 nm were obtained by DLS tests.

3. The new emulsions are original due to the successful inclusion of pine oil, with applications in leather industry.

4. A mechanism of solubilization of pine oil in micelles was proposed. Pine oil is hydrophobic and get stuck in the core of the micelles but also on the alkyl ends of the hydrophobic chains. For Tween 80, the amount of solubilized pine oil is higher than in the case of sodium dodecyl sulfate, because it has a larger hydrophobic chain. Van der Waals interaction forces are responsible.

5. The changes in the aggregation process were observed for each type of emulsion (Ps, Pt, Pst), the solubilization of pine oil by dynamic light scattering and optical microscopy.

6. In the process of finishing the leathers by spraying with 3 types of emulsions obtained compared to an untreated leather, was improved the antifungal, antimicrobial properties as well as appearance of the leathers.

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REFERENCES

[1] M.Gardner, M, "*Pinus sylvestris*". IUCN Red List of Threatened, doi:10.2305/IUCN.UK, 2013

[2] J.Bispham, "*Architectural Timber – History and Conservation*", In Taylor, J. (ed.). The Building Conservation Directory (22nd ed.). Tisbury: Cathedral Communications. p. 123–126, ISBN 978-1-900915-71-7. OCLC 931417684. 2015

[3] M.Gscheidmeier, F.Helmut, "*Turpentines, Pine Oil*", Ullmann's Encyclopedia of Industrial Chemistry. Weinheim: Wiley-VCH. doi:10.1002/14356007.a27_267. ISBN 978-3527306732, 2021

[4] M.G. Hertog, E.J.Feskens, D.Kromhout, M.G Hertog, P.C.Hollman, M.G.Hertog, M.Katan, *„Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study",* Lancet, 342, p.1007–1011, 1993.

[5] D.S. Varasteanu, "*Protein-based surfactants - Obtaining and uses", PhD Thesis, "Politehnica*" University of Bucharest, 2014**.**

[6].Enescu, C.M. „*Sea-buckthorn: A species with a variety of uses, especially in land reclamation. Dendrobiology*", 72, p.41–46. 2014

[7] K.Walczak-Zeidler, A.Feliczak-Guzik, I. Nowak, *„Oleje Ro´slinne Stosowane Jako Surowce Kosmetyczne-Leksykon"*, Wydawnictwo Cursiva: Kostrzyn, Poland,ISBN 9788362108206, 2012

[8] H.Kallio, B. Yang, P. Peippo, *„Effects of Different Origins and Harvesting Time on Vitamin C, Tocopherols, and Tocotrienols in Sea Buckthorn (Hippophaë rhamnoides) Berries"*, J. Agric. Food Chem., 50, p.6136–6142. 2002