Effects of Human Skin Surface Lipids on Icons Painting Layer

ANA-MARIA BUDU[1], ION SANDU[2]*, VIORICA VASILACHE[2], ATENA-ELENA SIMIONESCU[3], IRINA CRINA ANCA SANDU[4]

[1]Alexandru Ioan Cuza University of Iasi, Faculty of Geography and Geology, 22 Carol I, Blvd., 700506 Iasi, Romania

[2] Alexandru Ioan Cuza University of Iasi, ARHEOINVEST Interdisciplinary Platform, 22 Carol I Blvd., 700506, Iasi, Romania

[3] George Enescu University of Arts, 29 Cuza Vodă Str., 700040, Iasi, Romania

[4] Universidade de Évora, Laboratório HERCULES, Palácio do Vimioso, Largo Marques de Marialva, 8, 7000-809, Évora, Portugal

*Icons, by their purpose, are paintings exposed to a frequent interaction with the human factor during the religious services, which leads to the formation of a lipid film that retains dirt and soot on their surface, changing the aspect. This study analyzes the effects of the skin surface lipids layer on materials that are used in panel paintings (ground layer, linseed oil mixed with ochre, egg emulsion, and dammar varnish). For this purpose there were used two methods: CIE L*a*b* colorimetry and FT-IR spectroscopy, which emphasized colour changes and also modifications at a molecular level under lipids influence.*

Keywords: skin surface lipids, linseed oil, varnish, FT-IR, colorimetry

Pertaining to the easel paintings group, the icons are generally realized on wood panels, and they have a ground layer made of animal glue and $CaCO_3$ or $CaSO_4$, on which pigments mixed with linseed oil or egg emulsion are applied; the painting is protected with a varnish obtained from resins dissolved in organic solvent (turpentine, alcohol). The use of icons in the religious cult implies a direct contact with people, especially when the paintings are not protected with glass. As a result, skin surface lipids are deposited on the painting surface, creating, in time, a greasy layer in which dirt (dust, smoke) is included, modifying aesthetically the icons' aspect [1-5].

The skin surface lipids layer (fingers, lips, forehead) has a thickness of 0.5-4 μm and consists of the sebum secreted by the sebaceous glands and of the lipids that are surrounding the cells from the stratum corneum (palm level) [6], released through the desquamation of the superficial layer of corneocytes [7]. Sebum has a complex composition, being made of families of triglycerides and diglycerides, free fatty acids (50-60%), wax esters (20-30%), squalene (10-16%), and cholesterol esters (2-4%) [8, 9]. Triglycerides have in composition saturated and unsaturated fatty acids, with a simple or branched chain, which can have up to 26 carbon atoms. Under the influence of microbial enzymes from the follicular duct the triglycerides are decomposed into mono- and diglycerides, fatty acids and glycerine [10]. The fatty acids characteristic to sebum are sapienic acid (16:1, Δ6) and sebaleic acid (18:2, Δ5, 8) [11]. Other fatty acids that appear in sebum composition are lauric (C12:0), palmitic (C16:0) and oleic (C18:1, cis-9) acid [12].

Wax esters are obtained through the esterification of saturated or monounsaturated fatty acids (C12-C29), normal or with a branched chain, with fatty alcohols (C24-C27), fatty hydroxyacids and diols. Esters that have a very long chain, made of fatty hydroxyacids with 30-34 carbon atoms or diols with 32 carbon atoms are found in small amounts [13].

Squalene ($C_{30}H_{50}$) is a triterpenoid, precursor of cholesterol, which has a branched molecule with 30 carbon atoms and double bonds on Δ2, Δ6, Δ10, Δ14, Δ18, and Δ22 position [9]. However, in sebocytes squalene is not further transformed in cholesterol. It is considered that the small percent of cholesterol from sebum is released through the destruction of the basal membrane of sebocytes [13, 14]. Once it enters the follicular duct the cholesterol is esterified [15].

The lipids from stratum corneum comprise o variety of compounds like ceramides (50%), cholesterol (25%) and cholesterol esters (cholesterol sulphate, 2-5%); free fatty acids (15%), triglycerides and squalene [10,16,17]. Ceramides are made of sphingosine (an unsaturated amino-alcohol with 18 carbon atoms) and a fatty acid with a long chain (it can have up to 34 carbon atoms). The free fatty acids have long chains with 14-24 carbon atoms, mostly saturated or monounsaturated (C18:1) [18]. There are small amounts of phospholipids (approximately 4%), but they are also rich in oleic acid (C18:1) [17].

The human perspiration contains Na^+, Cl^+, K^+, Ca^{2+}, Mg^{2+}, lactates, urea, ammonia, bicarbonate, amino acids, a part of these substances being metabolism products [19-21]. Proteins and peptides with an antimicrobial role can also be found [21].

The materials used to create panel paintings have also a complex composition. The animal glue used for the ground layer preparation is based on collagen that has in its structure amino acids like glycine (H_2N-CH_2-COOH), alanine (H_3C-$CH(NH_2)$-COOH), proline and hydroxyproline ($C_5H_9NO_3$) [22]. Linseed oil contains (in descending order of concentrations) linolenic (C18:3), oleic (C18:1), linoleic (C18:2), palmitic (C16:0), and stearic (C18:0) acids [23]. These fatty acids suffer an autoxidation process under the influence of light and temperature, resulting small mass volatile products, and also compounds with large molecules, which lead to an increased viscosity of the linseed oil and to the formation of the dry film [24]. The egg yolk dissolved in water is used as a medium for colours and contains lipids (triglycerides, phospholipids and cholesterol), proteins, polysaccharides and minerals (Zn, Na, Ca, Mg). The main fatty acids that are found in lipids are palmitic, oleic and linoleic acids, the saturated and monosaturated acids having a higher concentration [25, 26]. Having a composition similar to linseed oil, the oxidative polymerisation processes in egg yolk are likewise [27].

The dammar varnish is based on a triterpenic resin mainly composed of dammaradienol ($C_{30}H_{50}O$), dammaradienone ($C_{30}H_{48}O$) and dammarenolic acid ($C_{30}H_{50}O_3$), and

* *email: ion.sandu@uaic.ro*

also has a polymeric fraction (polycadinene $-(C_{15}H_{26})_n$) [28, 29]. Under light influence this resin suffers oxidation processes, isomerization, cleavage, polymerization, condensation and dehydration reactions, resulting polymeric structures and other compounds like ketones, quinones etc. [30].

The purpose of this study is to analyze the effect of skin surface lipids film, probably mixed with perspiration products, formed on the painting layer as a result of use and manipulation, on the degradation of varnish, painting layer (pigments and medium) and ground layer.

Experimental part

Materials and methods

For this experiment there were made 8 samples (2x2 cm) using Balsa wood as a support, on which a ground layer made of $CaCO_3$ and animal glue (8% concentration) was applied. Of the 8 samples, 2 remained covered only with the ground layer (fig. 1a and a'), the others being covered with linseed oil mixed with ochre pigment (fig. 1b and b'), egg emulsion obtained from egg yolk dissolved in water 1:3 (fig 1c and c') and dammar varnish (fig. 1d and d'). The samples with linseed oil and ochre and dammar varnish were previously coated with shellac, to prevent their absorption into the ground layer. The samples noted with a, b, c, and d were considered as reference, while the samples noted with a', b', c' and d' were covered with skin surface lipids, prepared according to the following procedure.

The skin surface lipids used in this experiment were taken from the forehead and hands of a few human subjects, with the help of 16 cotton wads soaked in acetone [31]. Afterwards each wad was dispersed in 3 mL acetone, to enable lipids to pass from the wad into solvent. The dispersions were mixed and submitted to evaporation. The lipid deposit formed at the bottom of the recipient was spread in a thin layer on the samples a', b', c, and d'. In order to avoid the samples contamination with lipids found on hands, they were manipulated using gloves.

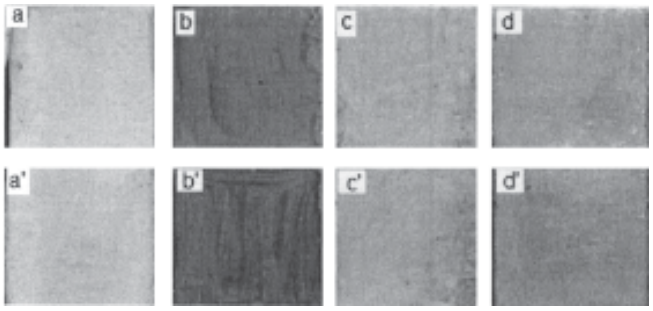

Fig.1 Painting layer samples that were exposed to artificial ageing: a – ground layer, b – linseed oil with ochre pigment, c – egg emulsion; d – dammar varnish (a, b, c, and d – reference samples; a', b', c', and d'- samples coated with skin surface lipids)

All the samples were exposed to artificial ageing for 20 hours, at 105°C (the equivalent of approximately 10 months of exposure at 20°C) [32] using a thermoregulation oven type AIR Concept (FIRLABO). The samples were analyzed before and after artificial ageing with the help of a FT-IR spectrometer, model TENSOR 27, coupled with a microscope HYPERION 1000, both systems being produced by Brüker Optic Equipment, Germany.

Every 4 h, during the artificial ageing treatment, the colour changes of the samples were measured with a spectrophotometer Lovibond RT 300. The colorimetric analysis of the samples was made in CIEL*a*b* system and it showed the changes occurred on each of the 3 axis, L* (luminosity), a* axis from green (-a*) to red (+a*) and b* axis from blue (-b*) to yellow (+b*), and colour variations ΔE were calculated using the formula [33-37]

$$\Delta E^* = [\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}]^{1/2}$$

Results and discussions

In figure 2 there are presented the spectra of the ground layer samples (fig. 2a – reference and figure 2a' sample coated with skin surface lipids) before (I) and after artificial ageing (II). These spectra present peaks characteristic to $CaCO_3$ (1330.83, 889.10 cm^{-1}) and animal glue (3312.72, 2979.47, 2873.72, 2515.45 cm^{-1}) [38-40]. The peaks specific to the animal glue protides became more pronounced, as a result of adding with the lipids peaks. The peak at 1659.82 cm^{-1}, specific to protides, is smaller after lipid coating. Comparing the spectra of the a' sample before (I) and after (II) artificial ageing it was noticed that the peaks of the initial sample, at 1546.37 si 1511.86 cm^{-1}, specific to organic salt (stearates, acetates), disappear, and the one characteristic to carbonyls (1584.90 cm^{-1}) increases [41]. Also, the peaks at 1279.11 and 1170.32 cm^{-1}, specific to esters, which initially were more distinctive, are less pronounced. There were not observed big differences between FTIR spectra of the samples with simple ground layer (a) and lipid coated ground layer, both artificially aged (fig. 2, II). The spectrum for the lipid coated ground layer has peaks at 1752.06, 1261.90 cm^{-1} characteristic to esters, while at 1584.90 cm^{-1} appears a peak corresponding to carbonyls from acids.

According to colorimetric analysis of sample a, realized in CIEL*a*b*, the values varied on L* axis from $L^*_0 = 94.88$ to $L^*_{20} = 94.83$, and on a* axis from +0.01 to -0.13 (they changed towards green). On b* axis the values increased from +4.84 to +6.00. For the sample a', coated with skin surface lipids, the variations on L* axis were small, from 91.19 to 91.43, but they were more evident on a* axis,

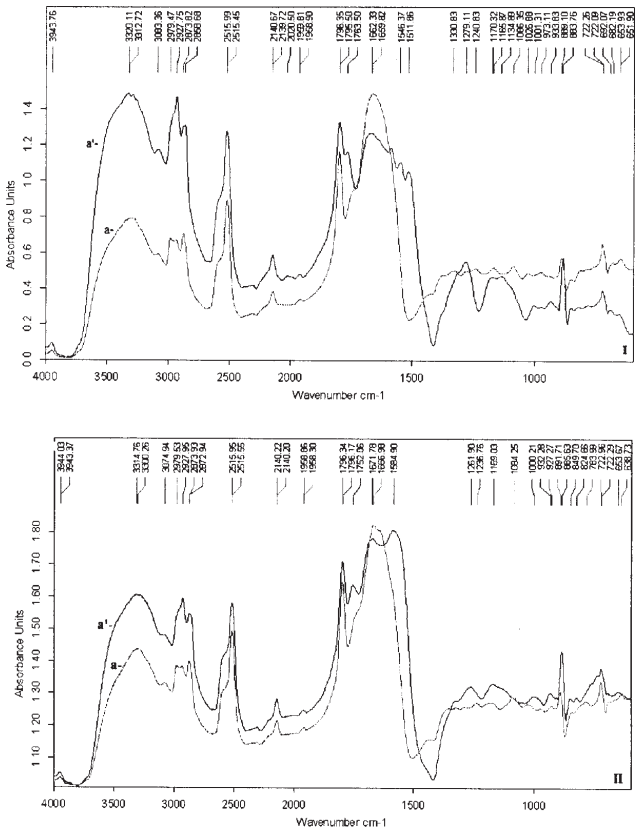

Fig. 2. FTIR spectra of the ground layer samples (reference and lipid coated) before (I) and after (II) exposure to artificial ageing

from +0.27 to -0.10, and on b* axis, from +7.98 to +11.67. The ΔE* values were calculated using the formula presented above, and they are presented in figure 3, which highlights the differences between the two samples.

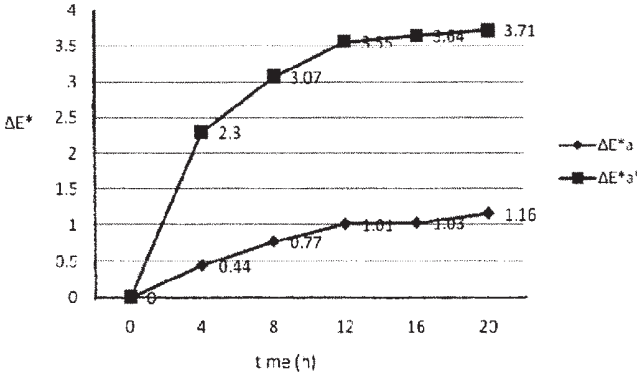

Fig. 3. The graphic for ΔE* values of samples a and a'

The FTIR spectra for the artificially aged samples with linseed oil mixed with ochre pigment (b and b') are similar in the first half (3282.31-1757.12 cm^{-1}), presenting peaks for carbonyl group (2968.64, 2866.40 and 2515.11 cm^{-1}) and esters (1757.12 cm^{-1}) (fig. 4). Further the spectrum for sample b presents a peak at 1635.68 cm^{-1}, corresponding to –OH, which in sample b' spectrum is smaller, appears at 1681.07 cm^{-1}, followed by a zone with a peak specific for stearates at 1517.26 cm^{-1}, shifted to the left compared to the sample b peak at 1466.69 cm^{-1}. In the spectrum for sample b we can observe a peak at 1385.31cm^{-1}, characteristic to acetates (organic salt), which in sample b' decreases in intensity. The peaks for esters at 1277.84 and 1188.49 cm^{-1} are more evident in sample b' spectrum compared to sample b, where the peak is smaller (1265.12 cm^{-1}) and is followed by a peak for acids (1308.88 cm^{-1}). Also, the peak for acetates at 977.29 cm^{-1} is smaller in sample b' compared to the sample b.

Comparing the spectra of sample b' before (I) and after artificial ageing (II) it can be noticed that the peak specific to esters at 1280.40 cm^{-1} (I) is shifted at 1277.84 cm^{-1} (II) and has a lower intensity. The same thing was observed in the case of peaks that are specific to acetates and esters at 1139.55 and 1103.76 cm^{-1} (I), which cumulated at 1113.76 cm^{-1} (II). The peaks specific to carboxyl group at 2964.13 and 2866.63 cm^{-1}, remain almost unchanged at 2968.64 and 2866.40 cm^{-1} after artificial ageing.

The colorimetric analysis of sample b presents a decline of values on L* axis, from 71.10 to 66.15, an increase on a* axis from +15.71 to +18.13 and a diminution on b* axis from +64.55 to +58.49. Sample b' had smaller values than sample b on L* (from 69.09 to 58.89) and b* axis (+ 61.79 to +50.67), but the differences between the final and initial measurements are greater for sample b'. On a* axis values are similar to those for sample b (from +15.38 to +18.59). In figure 5 are presented the colour variations ΔE* for both samples (noted ΔE^*_b for the reference sample and ΔE^*_b for the lipid coated sample).

The FTIR spectrum after the artificial ageing of the sample with egg emulsion coated with lipids c' (fig. 6) presents a higher peak at 1759.25 cm^{-1} specific for esters and a peak with a lower intensity characteristic to -OH at 1685.46 cm^{-1}. The peak at 1471.76 cm^{-1} (specific for acetates) in the sample c' spectrum is shifted much to the left at 1550 cm^{-1}, after artificial ageing, and its intensity decreases. The peak at 1557.10 cm^{-1} (for carboxyl group) [42] is shifted to 1552.70 cm^{-1} and decreases very much in intensity, suggesting a lower concentration of esters and organic salts. Also, the strong peak at 879.37 cm^{-1}, specific

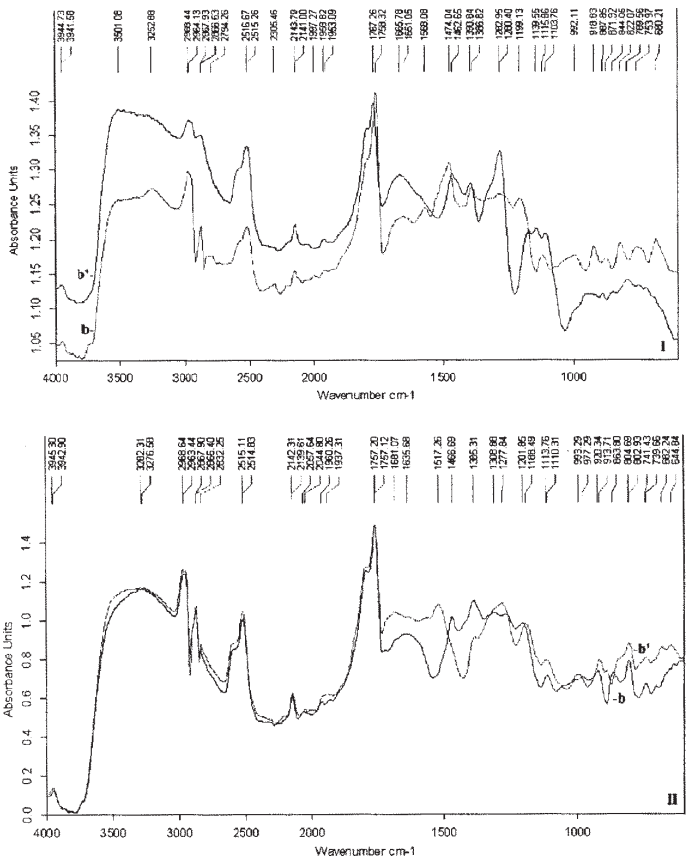

Fig. 4. FTIR spectra of linseed oil mixed with ochre samples (reference and lipid coated) before (I) and after (II) exposure to artificial ageing

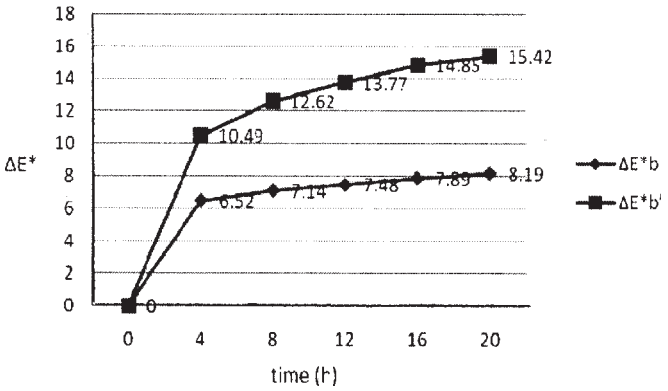

Fig. 5. The graphic for ΔE* values of samples b and b'

to aromatic C-H bond is shifted to 885.74 cm^{-1} after artificial ageing, and the peak at 719.11 cm^{-1}, characteristic for primary and secundary amides, moves to 724.77 cm^{-1}, and has a lower intensity due to ageing.

The CIE L*a*b* colorimetric analysis for samples c-c' shows smaller variations on L* and a* axis, and higher modifications on b* axis. Thus, for sample c, there are changes on L* axis from 92.7 to 90.35, on a* axis from la -1.82 to -0.06, while on b* coordinate they go from +23.60 to +27.23. Sample c' has lower values than sample c on luminosity axis and varies from 90.58 to 88.52. The changes on a* axes are, also, small: from -0.39 to -0.11. In turn, on b* coordinate the values increase from +13.08 to +27.59. In figure 7 are emphasized the colour variations, ΔE^*_c and ΔE*c', and the differences appeared between the two samples.

The FTIR spectra realized before and after artificial ageing of the dammar sample coated with lipids show a strong modification of peaks. Thus, before ageing (I) the

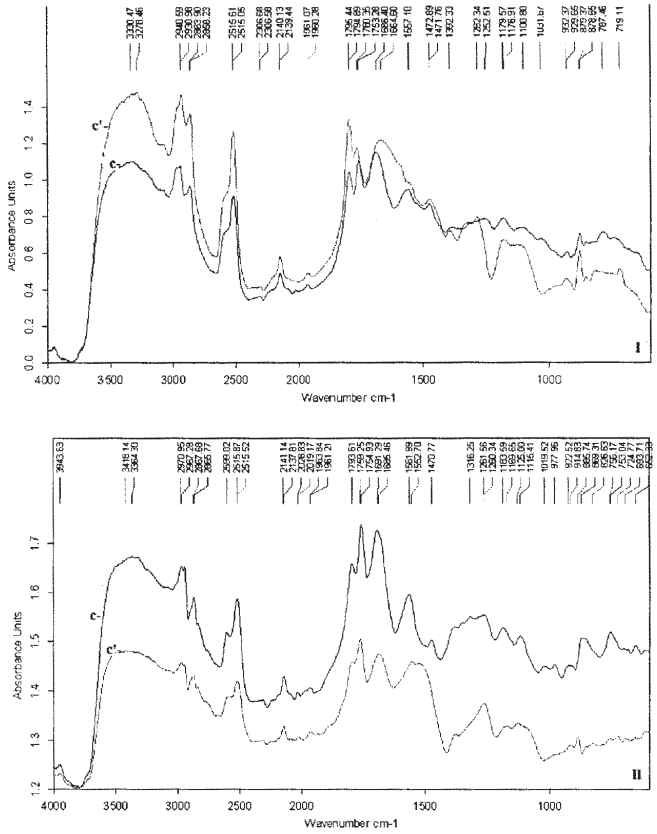

Fig. 6. FTIR spectra of egg emulsion (reference and lipid coated) before (I) and after (II) exposure to artificial ageing

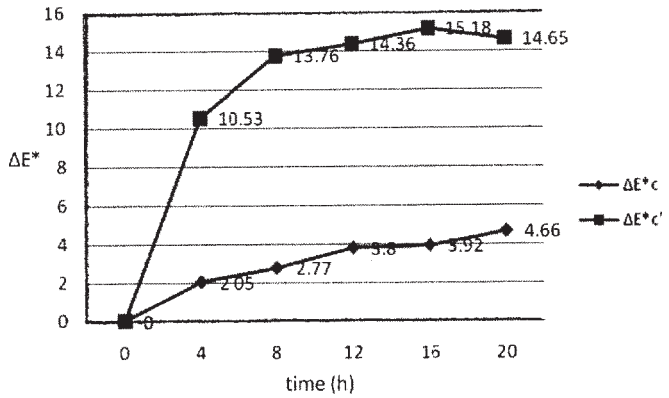

Fig. 7. The graphic for ΔE* values of samples c and c'

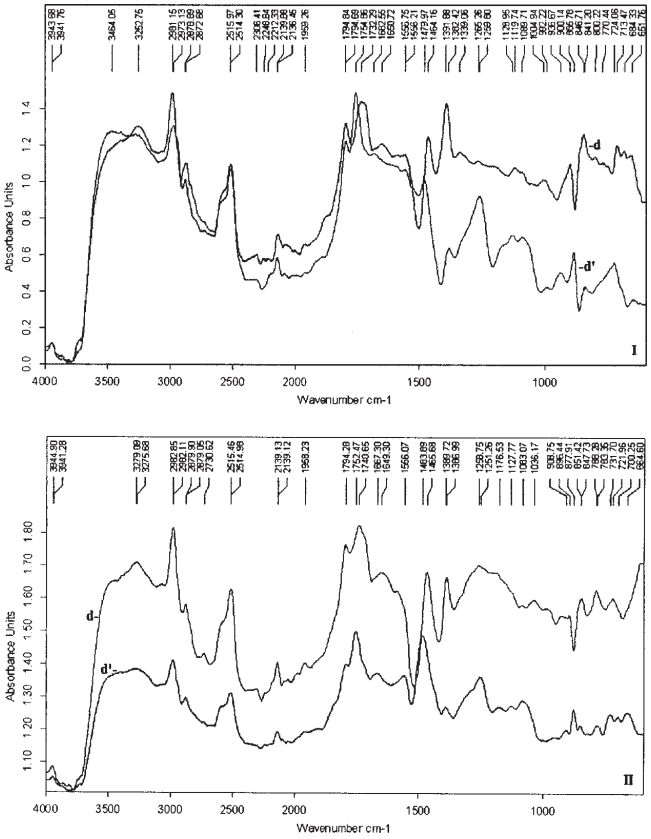

Fig. 8. FTIR spectra of dammar varnish (reference and lipid coated) before (I) and after (II) exposure to artificial ageing

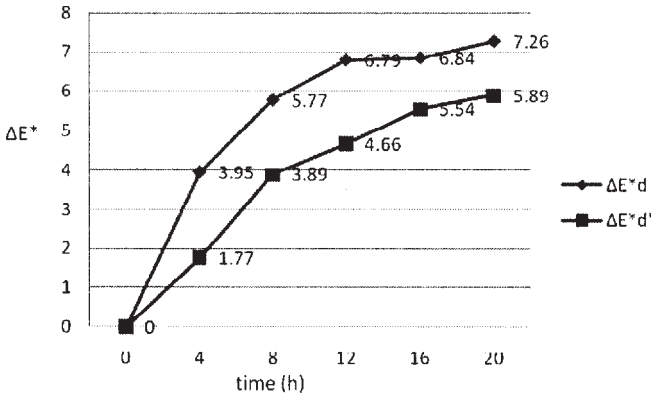

Fig. 9. The graphic for ΔE* values of samples d and d'

majority of peaks between 1500 and 4000 cm^{-1} has an approximately similar evolution, and after 1500 cm^{-1} the peaks for the sample d' gradually decrease in intensity comparing to the sample d spectrum. After artificial ageing (II) it is obvious a difference between the two spectra (d and d') on their entire length. Between the affected peaks, the one for the ester group, at 1176.53 cm^{-1} and for the organic salts of acetic acid, at 1083.07 cm^{-1}, are modified comparing to the spectrum before ageing (1128.95, 1089.71 cm^{-1}). Also, sample d presents a decrease of the peak at the 1556.07 cm^{-1} (carboxyl) and an enhancement of the peak at the 1258.75 cm^{-1}, characteristic to double bonds C=C [43]. The peak at the 1386.99 cm^{-1}, corresponding to $-CH_3$, is much lower in the spectrum for the lipid coated sample.

The CIE L*a*b* colorimetric analysis for sample d does not show great changes neither on L* axis (from 84.44 to 84.98), nor on a* axis (from +5.31 to +4.47), but they are more visible on b* coordinate (from +21.07 to +28.27). The same situation is in the case of the sample d', where variations are small on the L* axis (from 80.21 to 80.60) and a* axis (from +5.78 to +5.12). The differences are higher on the b* axis (from +21.71 to +27.55). ΔE* values are presented in figure 9 (ΔE^*_d, respectively $\Delta E^*_{d'}$). Comparing the data from figure 9 for the d-d' samples it can be observed a small difference between ΔE^*_d and $\Delta E^*_{d'}$, while for the previous samples the differences are higher. The values on b* axis are higher in the case of the sample d, compared to d', indicating a colour modification towards yellow.

Conclusions

The artificial ageing of the lipid coated samples lead to a decomposition of the organic salts (acetates). It also determined the decomposition of lipid esters for the ground layer sample and for the dammar samples, while for the linseed oil sample the concentration of esters became higher. The presence of lipids on the surface of varnish, linseed oil mixed with ochre and egg emulsion lead to colour changes, with a decrease of values on luminosity coordinate L* and with high variations on b* axis (from

blue to yellow), which shows a tendency to yellowing. The solution is the protection of the icons with glass or by encasing.

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