The Effect of Lavender Essential Oils on Collagen Hydrolysate

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The aim of this study was to develop new natural compounds based on collagen hydrolysate modified with lavender essential oils. The essential oils were extracted by hydrodistillation from aerial parts of Lavender officinalis L. and Lavender stoechas L. subsp. stoechas with the yield of 3.0 and 3.6% respectively. The chemical composition determined by GC-MS analyses showed that fenchone (45.47%), 1.8 cineol (19.47%) and camphor (17.23%) were the main components for L. stoechas oil, while for L. officinalis, linalool (28.00%) linalyl acetate (43.96%) and camphor (5.94%) were found in high concentrations. The main groups of essential oils were identified also by FT-IR. Natural compounds with 75:25, 50:50 and 25:75 ratios (w/w) were prepared by collagen hydrolysate with 6000 Da and lavender essential oils. The modifications of collagen hydrolysate as a result of lavender essential oil interactions were determined by FT-IR. The results showed that L. stoechas essential oil was linked stronger on collagen due to higher content of ketones comparing with L. officinalis which is rich in alcohols and esters. The essential oils were bonded on side chain of amino acids from collagen hydrolysate. The new obtained compounds could be used as basic natural ingredients in medical, pharmaceutical and cosmetic fields, as they are products with added value, keeping the therapeutic properties of both collagen and essential oils.

Keywords: L. officinalis and L. stoechas essential oils, collagen hydrolysate, GS-MS, FT-IR spectroscopy

During the past decades the safety of medical, pharmaceutical and cosmetic products and their ingredients has attracted increasing attention. Due to their properties, plant and animal ingredients with functional and biological activity are continuously researched because of growing consumer demand. In the recent years the interest in using essential oils in food ingredients, perfumes, aromatherapy and pharmaceuticals has been raised. Essential oils have been shown to have antibacterial, antifungal, antiviral and antioxidant properties due to their biologically active compounds [1]. The genus *Lavandula* is an important member of *Lamiaceae* family which contains many different species [2] and Lavender's essential oils are popular as a complementary medicine and as an additive to many cosmetic and perfume products [3]. The most common of *Lavandula* species reported as having medicinal value such as *L. dentata, L. angustifolia (officinalis), L. latifolia, L. intermedia, L. stoechas and L. dhofarensis* have found application in perfumes and cosmetics a long time ago. In Turkey, mainly two species, *Lavandula stoechas* and *Lavandula officinalis* and their subspecies and hybrid forms grow wildly or are cultivated [4-6]. Although essential oils are very popular for medicinal use they can be toxic in direct contact with tissue, especially skin. On the other hand, collagen, the most used natural polymer in regenerative medicine is non-toxic and biocompatible, haemostatic, synergic with bioactive components [7]. The collagen-based products are applied in numerous fields, including pharmaceutical, cosmetic, food, packaging industry, medicine as biomaterials for tissue engineering, drug delivery, wound dressing and gene therapy [8,9]. However, collagen itself cannot treat the infected tissue because bacteria can use it as substrate [10].

So far, lavender oil (*L. angustifolia*) microencapsulated into solid waste of chromium-tanned leather was studied by Ocak [11], essential oils of bergamot and lemongrass incorporated into films of gelatin from marine tropical fish were developed by Ahmad et al. [12] and citrus essential oils were incorporated into gelatin from fish skin of tilapia by Tongnuanchan et al. [13] with the aim of using in packaging with antimicrobial activity. Gómez-Estaca et al. [14] studied biodegradable gelatin-chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. These recent studies showed the trends for combination of plant with animal materials in order to obtain natural products with improved properties and added value. However, there is no previously reported research about new natural ingredients based on collagen hydrolysate (medical grade) and lavender oil to be used in medical, pharmaceutical and cosmetic fields.

The aim of our study was to develop and characterize natural compounds based on collagen hydrolysate and lavender (*L. officinalis* and *L. stoechas)* essential oils (EO) and to investigate if the properties are modified during their processing.

Experimental part

Plant material: Aerial parts of *Lavandula officinalis* L. and *Lavandula stoechas* L. subsp. *stoechas* were harvested at full flowering stage in July 2011 from the botanical gardens, Field Crops Department of Mustafa Kemal University.

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Characteristics	Values
Dry substance, %	4.48
Amide nitrogen in dry substance, %	15.26
Ash in dry substance, %	4.52
Primary amino groups in dry substance, %	0.76
Fatty in dry substance, %	Free
pH for solution of 5%	3.73

Table 1
BASIC CHARACTERISTICS OF COLLAGEN HYDROLYSATES

Isolation of the essential oils: dried at 35 °C in oven, to constant weight, aerial parts of *Lavandula officinalis* L. and *Lavandula stoechas* L. subsp. *stoechas* (100 g) were extracted by hydrodistillation with 1 L distilled water for 3 h using Neo-Clevenger apparatus. Yield of obtained oils (*Lavandula officinalis* L. and *Lavandula stoechas* L. subsp. *stoechas*) were 3.0 and 3.6% respectively. The oils were dried over anhydrous sodium sulfate and then stored in dark color (amber) glass bottles, at -4°C ready for GC-MS analysis.

GC-MS analysis: Analysis of the essential oils carried out by using Thermo Scientific Focus Gas Chromatograph equipped with MS, auto sampler and TR-5MS (5% Phenyl Polysilphenylene-siloxane, 0.25 mm x 60 m i.d, film thickness 0.25). The carrier gas was helium (99.9%) at a flow rate of 1 mL/min; ionization energy was 70 eV. Mass range m/z 50-650 amu. Data acquisition was scan mode. MS transfer line temperature was 250°C, MS Ionization source temperature was 220°C, the injection port temperature was 220°C. The samples were injected with 250 split ratio. The injection volume was 1 μL. Oven temperature was programmed in the range of 50 to 220 °C at 3°C /min. The structure of each compound was identified by comparison with their mass spectrum (Wiley). The data were handled using Xcalibur software program. The retention indices (RIs) were calculated for all volatile constituents using a homologous series of n-alkane standard solutions C_8-C_{20} (Fluka, product no. 04070) and C_{21}-C_{40} (Fluka, product no. 04071).

Collagen hydrolysate was prepared by acidic hydrolysis of calf pelt at 125°C during 8 h according to the technology previously described [15]. The obtained liquid hydrolysate was then dried by freeze-drying using the program previously described [16]. To determine its molecular weight sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed. The results indicate that collagen hydrolysate contains a mixture of amino-acids and polypeptides with an average molecular weight of about 6000 Da. The basic characteristics are obvious in data presented in table 1.

Table 2
COMPOSITION OF THE TWO STUDIED ESSENTIAL OILS (% OF PEAK AREAS) DETERMINED BY GC-MS

RT	RI	*LS* oil, %	*LO* oil, %	Components
2.60	935	0.19	0.19	2,2-dimethoxybutane
3.30	1001	0.14	0.03	(-)-Bicyclo[3.2.1]oct-2-en-7-one
3.45	1013	0.10	0.05	Tricyclene
3.64	1028	0.75	0.19	α-pinene
3.98	1051	0.07	0.18	Toluene
4.35	1072	1.11	0.32	Camphene
5.14	1112	0.20	0.07	2-α-pinene
5.45	1127	0.08	0.04	Sabinene
5.54	1131	0.08	-	2,4(10)-thujadien
6.50	1170	-	0.15	Myrcene
6.89	1184	0.04	-	α-terpinene
7.41	1201	0.61	0.48	dl-Limonene
7.61	1208	19.47	3.97	1.8 Cineol
8.56	1242	-	0.30	Cis-Ocimene
8.84	1251	0.13	-	γ-terpinene
9.08	1258	-	0.29	α-ocimene
9.17	1261	-	0.48	3-octanone
9.66	1275	0.63	0.07	O-cymene
9.76	1278	-	0.39	Acetic acid, hexyl ester
10.03	1286	0.04	-	α-terpinolene
12.06	1345	0.08	-	Propanoic acid, hexyl ester
12.15	1347	-	0.12	Propanoic acid, 2-methyl hexyl ester
13.97	1394	45.47	0.07	Fenchone
14.87	1419	-	0.78	Butanoic acid, hexyl ester
15.26	1430	-	0.11	Hexyl-2-Methyl butyrate
15.68	1441	0.19	0.93	Linalool oxide
15.97	1449	-	0.10	Hexyl isovalerate
16.57	1464	0.12	0.04	Trans-sabinene hydrate
16.71	1468	0.59	-	α-fenchyl acetate
16.78	1470	-	0.57	Trans-Linalool oxide
16.99	1475	0.57	-	(+)-Cycloisosativene
17.47	1486	0.30	0.03	α-Cubebene
18.33	1508	17.23	5.94	Camphor
18.70	1519	0.08	-	Sativene

19.73	1547	0.12	-	Trans-2-pinanol
19.90	1551	0.29	28.00	Linalool
20.25	1560	-	43.96	Linalyl acetate
20.35	1563	0.14	-	Pinocarvone
20.88	1576	1.09	0.09	Borneol acetate
21.08	1581	0.63	-	D-Fenchyl alcohol
21.39	1589	-	0.93	Trans-Caryophyllene
21.77	1598	0.16	0.05	4-terpineol
22.17	1609	-	1.67	Lavandulyl acetate
22.61	1621	0.34	0.23	Myrtenal
23.66	1650	0.22	0.21	Trans-Pinocarveol
23.77	1653	-	0.43	β-Cadinene
24.13	1663	0.07	-	Cryptone
24.32	1668	-	0.43	α-Farnesene
24.38	1669	0.24	-	p-Menth-1en-8-ol
24.55	1674	0.28	-	Cis-Verbenol
24.69	1677	-	0.23	Lavandulol
24.93	1683	0.97	-	Myrtenyl acetate
25.26	1692	0.94	-	α-Terpinenyl acetate
25.36	1694	0.45	3.88	2-Methylisoborneol
25.53	1699	0.00	0.11	Germacrene D
25.84	1707	0.10	-	α-Selinene
26.52	1727	0.35	0.10	D-Carvone
26.82	1736	-	0.03	Epoxylinalool
27.37	1751	0.53	0.05	α-Amorphene
27.66	1759	-	0.22	Linalyl 3-methylbutanoate
28.69	1787	0.20	-	(-)-Myrtenol
29.56	1811	-	0.52	Epoxylinalool
30.02	1825	0.14	-	Calamenene
30.26	1832	0.17	-	Carveol 1
30.81	1848	0.18	0.06	p-Cymen-8-ol
31.90	1880	0.19	-	Germacrene D-4-ol
32.84	1906	0.04	-	Calacorene
33.66	1932	0.10	-	Torreyol
34.66	1962	-	0.43	Limonene oxide
34.83	1967	0.30	0.59	Caryophyllene oxide
36.42	2016	0.39	-	Viridifloral
37.62	2055	0.05	-	α-Copaene
38.23	2074	1.26	-	Viridiflorol
41.16	2201	0.49	-	Globulol
42.49	2236	-	0.34	α-Bisabolol

Samples preparation

Collagen hydrolysate: essential oil (CH:EO) compounds with 75:25, 50:50 and 25:75 ratio (w/w) were prepared and a volume of 80% distilled water was added. The obtained suspensions were freeze-dried using the method previously described [16]. The obtained powders were analyzed by FT-IR.

FT-IR analysis: The FT-IR spectra for collagen hydrolysate modified by essential oils and control one were recorded using a FT-IR 6000 spectrophotometer with ATR reflection system MK II Golden Gate Single (Jasco). The spectra were scanned in absorption mode at 4 cm^{-1} resolution.

Results and discussions

Table 2 and figure 1 report the composition of *L. stoechas* (*LS*) and *L. officinalis* (*LO*) essential oil samples, determined by GC-MS.

As we can see in figure 1 and table 2, 54 and 48 peaks of components were identified for *L. stoechas* and *L. officinalis* essential oil respectively. Fenchone (45.47%), 1.8 cineol (19.47%) and camphor (17.23%) were the main components for *L. stoechas* oil, while for *L. officinalis*, linalool (28.00%) linalyl acetate (43.96%) and camphor (5.94%) were found in high concentrations. The components varied depending on plant genotype, cultivation, area etc [17]. Hassiotis [18] found 45.19% fenchone and 9.90% camphor in *L. stoechas* oil from North part of Greece and Giray et al. [5] presented 32.03% fenchone and 14.71% camphor in the same oil obtained from *L. stoechas* of experimental field of Çukurova University, Adana. L. officinalis essential oil has been described to have in its composition 26.5% linalyl acetate and 20.9% linalool [17] and 35.96-36.51% linalool and 21.74-14.42% linalyl acetate [4].

Groups of chemical compounds participating in chemical compositions of lavender essential oils are presented comparatively in figure 2.

The ketones are present in *L. stoechas* oil in high amount (63.40%) while the esters (47.44%) and alcohols (34.82%) are predominant in *L. officinalis* oil. The main ether found in both oils was 1.8 cineol. The results were confirmed also by FT-IR spectral analyses presented in figure 3.

The FT-IR spectrum of *L. officinalis* displayed strong peaks at 2967 and 2924 cm^{-1} characteristic for asymmetric stretching vibration of the aliphatic CH_3 and CH_2 groups respectively. The peak from 1736 cm^{-1} can be attributed to stretching vibration of the ester carbonyl functional group of linalyl acetate and lavandulyl acetate. Other notable peaks appeared at 1449 and 1369 cm^{-1} due to CH_2 asymmetric and symmetric deformation respectively. The bands from 1109 and 1017 cm^{-1} could be attributed to (C-O-C) of 1,8 cineol. The band from 3461 cm^{-1} could be attributed to broad OH stretch from linalool. The *L. stoechas* essential oil FT-IR spectrum is dominated by bands at 1738 cm^{-1}atributted to its main components, fenchone and

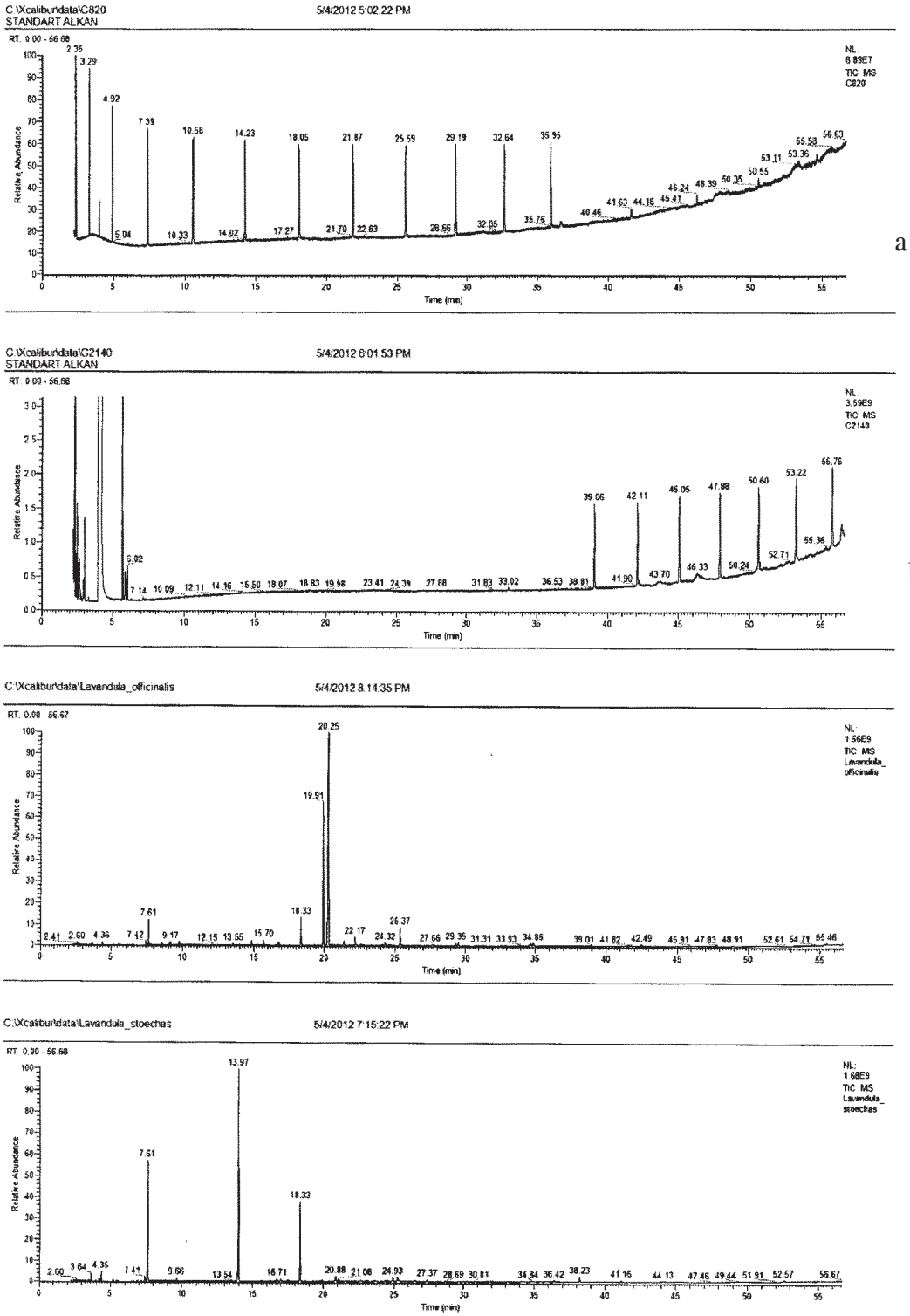

Fig. 1. Chromatographs of: a) standard alkane series C_{8-20}; b) standard alkane series C_{21-40};c) *Lavandula officinalis* L. essential oil; d) *Lavandula stoechas* subsp. *stoechas* L. essential oil

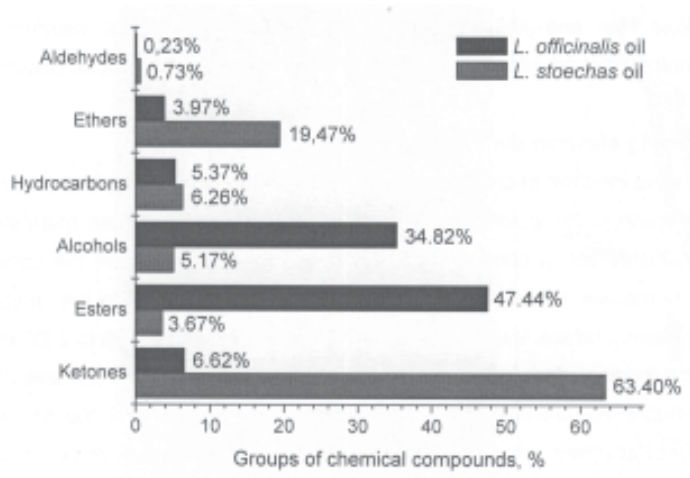

Fig. 2. Groups of chemical compounds participating in chemical composition of lavender essential oils

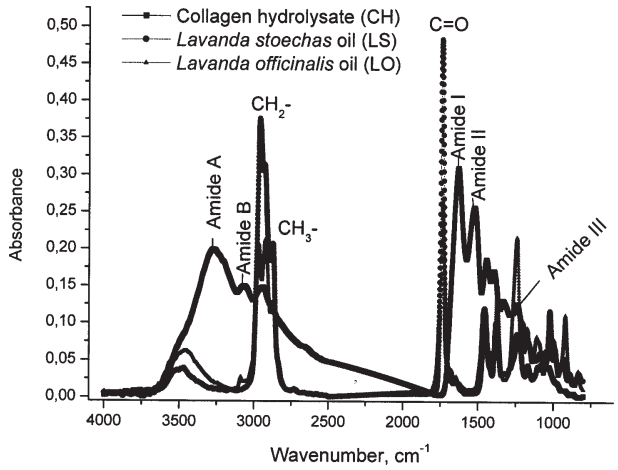

Fig. 3. FT-IR spectra of collagen hydrolysate, *Lavandula officinalis* L. and *Lavandula stoechas* subsp. *stoechas* L. essential oils.

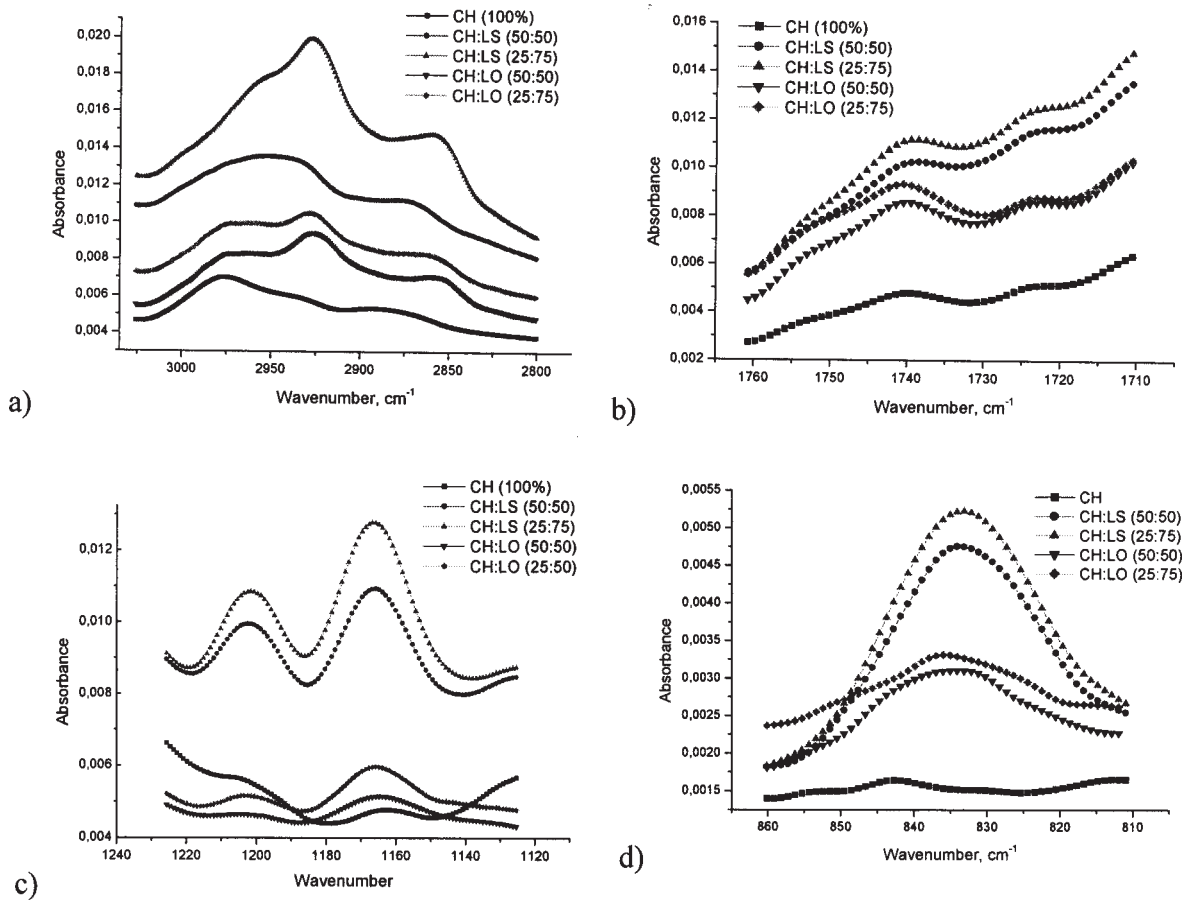

Fig. 4. Modification of FT-IR spectra of collagen hydrolystae by lavender esential oils at: a) 3025-2800 cm^{-1}; b) 1760-1710 cm^{-1}; c) 1225-1135 cm^{-1}; d) 860-810 cm^{-1}

camphor, both revealed intensive bands due to C=O stretching vibrations. The groups CH_3 and CH_2 from 2959 and 2928 cm^{-1} are characteristic for most components present in essential oils [19] as the FT-IR spectrum of *L. officinalis* essential oil showed. Also, characteristic bands can be observed for alcohol at 3511 cm^{-1} and for 1.8 cineol at 1020 cm^{-1} and 1374 cm^{-1} (CH_3-CO).

As we can see in figure 3, the collagen hydrolysate (CH) is characterized by amide A (3274 cm^{-1}), amide B (3068 cm^{-1}) bands, associated with NH stretching modes. Amide I (1632 cm^{-1}), amide II (1525 cm^{-1}) and amide III (1238 cm^{-1}) are characteristic for collagen in random coil protein which proved that the collagen was in hydrolysate form. Essential oils are an important source of natural products that can be modified chemically to generate a wide variety of polyfunctional compounds [20].

The FT-IR spectra for CH:EO compounds showed that amide A, B, I and II related with peptide bonds of collagen had no changes comparing with CH spectrum. This demonstrated that the studied essential oils did not destroy the secondary collagen structure. The main modifications due to interactions between collagen and essential oils are presented in figure 4 a-d for CH:EO compounds with 25:75 and 50:50 compositions. The compounds with 75:25 CH:EO did not show significant differences and they are not presented in figure 4.

The band at 2976 cm^{-1} from collagen shifted to lower frequencies such as 2928 and 2859 cm^{-1} in the CH:LS and CH:LO respectively as it can be seen in figure 4a. These shifts to lower frequencies are due to CH_3 and CH_2 groups which are specific for essential oils due to high hydrocarbons content.

The CH-EO compounds showed C=O stretching vibrations at 1740 cm^{-1} (fig. 4b) as the effect of camphor and fenchone content in CH-LS and linalyl acetate in CH-LO.

Significant differences are seen for CH-LS and CH-LO in figure 4c at 1202 and 1164 cm^{-1} assigned to C-O-C from 1,8 cineol. CH-LS showed intensive bands comparing with CH-LO which is the cause of higher percentage of 1,8 cineol in LS (19.47%) comparing with LO (3.97%) .

In Figure 4d, CH-LS showed stronger IR bands due to CH_2 wagging vibrations between 810 and 860 cm^{-1} as a result of higher content of terpenes in LS than in LO.

Conclusions

Thus, we can conclude that the studied compounds based on lavender essential oils and collagen hydrolysates contain both collagen with un-modified secondary structure and essential oils with their main components. The *L. stoechas* essential oil was linked stronger on collagen due to the higher content of ketones comparing with *L. officinalis* which is rich in alcohols and esters. Although the essential oils are volatile they were bonded during freeze-drying on side chain of the amino acids from collagen composition as the FT-IR showed. The new obtained compounds could be used as basic natural ingredients in pharmaceutical and cosmetic fields being products with added value and having therapeutic properties of both collagen and essential oils.

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