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Food Chemistry 92 (2005) 721–727
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant activity of natural resins and bioactive triterpenes in oil substrates

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Received 6 October 2003; received in revised form 31 August 2004; accepted 31 August 2004

Abstract

Natural resins that possess biological properties (*Pistacia lentiscus* var. Chia, *Commiphora myrrh*, *Boswellia serrata* and *Gum storax*) and the bioactive triterpenes (oleanolic acid and ursolic acid) were studied for their antioxidant activities. Lard, corn oil, olive oil and sunflower oil were used as oil substrates for the antioxidant assay.

Pistacia lentiscus resin showed significant antioxidant activity in each of the oil substrates examined; the best concentration of the resin presenting the highest activity depended on the substrate. The combination of *P. lentiscus* resin with citric acid presented a synergistic effect in both sunflower oil and corn oil. Essential oils of *C. myrrh* and *B. serrata* resins and the triterpenes, ursolic and oleanolic acid, presented satisfactory antioxidant activity in sunflower oil. In lard, *P. lentiscus* and *B. serrata* showed good antioxidant activity while, in virgin olive oil, *P. lentiscus* resin and its essential oil presented high antioxidant activity.

It can be concluded that *Pistacia lentiscus* resin and the essential oils of *P. lentiscus*, *C. myrrh* and *B. serrata* can be used in pharmaceutical and cosmetic preparations, and in functional foods, due to their antioxidant effects in oil substrates.

Keywords: *Pistacia lentiscus*; *Commiphora myrrh*; *Boswellia serrata*; Mastic gum; Antioxidant activity; Oven test

1. Introduction

Oils and fats are susceptible to oxidation. Traditionally, chemically synthesized compounds, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are used as antioxidants in oil products. However, some of these compounds have been questioned for their safety (Bran, 1975; Whysner, Wang, Zang, Iatropoulos, & Williams, 1994). Therefore, the use of natural antioxidants is now becoming important.

In this research, natural resins and bioactive triterpenes are studied for their antioxidant effect on several oils and animal fats. Plants produce a variety of antioxidants against molecular damage from reactive species and thus certain natural products could play a preventive role due to their antioxidant properties. This research is a continuation of our investigations on exploiting bioactive natural products with prospects for use in pharmaceutical and cosmetic preparations as antioxidants.

All natural resins that were selected to be examined for their antioxidant activity have been reported to possess medicinal properties. In the present study, the natural resins *Pistacia lentiscus* var. Chia, *Commiphora myrrh*, *Boswellia serrata* and *Gum storax* were studied for their possible antioxidant activities. *Pistacia lentiscus* resin (known as mastic gum) has been reported to possess anticancer activity (Duke, 1983), antiulcer activity (gastric and duodenal) (Al-Said, Ageel, Parmar, & Tariq, 1986) and also haemostatic, immunostimulant and antimicrobial properties on *Salmonella* and *Staphylococcus* (Block, 1999; Duke, 1983). Also, *P. lentiscus*

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doi:10.1016/j.foodchem.2004.08.033

resin has been reported to have a fairly good antibacterial activity against *Helicobacter pylori* (Bona, Bono, Daghetta, & Marone, 2001; Huwez, 1999; Huwez, Thirwell, Cockayne, & Ala'Aldeen, 1998) and inhibits in vitro LUL oxidation (Andrikopoulos, Kaliora, Assinopoulos & Papageorgiou, 2004). The essential oil of *Pistacia lentiscus* resin has been reported to cure dyspepsia and peptic ulcer (Duke, 1983), and presents antimicrobial activity (Tassou & Nychas, 1995) and its use is wide-spread in cosmetics.

Commiphora myrrh (commonly known as myrrh) is extensively used as a sedative and in treating disorders of the oral cavity. It is used in cosmetics, in mouthrinses and for treatment of gingivitis (Tipton, Lyle, Babich, & Dabbous, 2003). Several studies report that myrrh acts against amenorrhoea, leucorrhoea and as an antiulcer agent (Duke, 1983). Gum storax (styrax), from the Liquidambar family, is used in the pharmaceutical industry for its antiseptic properties (Pastorova, Weeding, & Boon, 1998) and contains oleanolic acid derivatives. Finally, *Boswellia serrata* resin (commonly known as olibanum resin or frankincense) has been established to possess anticancer properties and help as a syphilis cure (Duke, 1983). It was used for treatment of inflammatory diseases in the traditional Ayurvedic medicine in India (Krohn, Rao, Raman, & Khalilullah, 2001), since its main component is boswellic acid, with established anti-inflammatory activity (Rios, Recio, Manez, & Giner, 2000). It is also used in pharmaceutical mouthwashes, since it helps in preventing dental caries (Duke, 1983). All these resins have been reported to have anti-inflammatory properties that seem to be related to antioxidant activity and they contain pentacyclic triterpenes, a class of bioactive natural products, probably responsible for the anti-inflammatory activity. Dammar resin, isolated from plants belonging to the family Dipterocarpaceae, contains dammarane type triterpenes.

Several bioactive triterpenes have also been examined for their possible antioxidant activities; all of them exert significant biological properties. Ursolic acid (3β-hydroxy-urs-12-en-28-oic acid; Fig. 1) and its isomer, oleanolic acid (3β-hydroxy-olea-12-en-28-oic acid; Fig. 1), which is a constituent of *Pistacia lentiscus* resin, are among the best known bioactive triterpenes. Both ursolic and oleanolic acid are effective in protecting against chemically induced liver injury in laboratory animals; they present anti-inflammatory and antihyperlipidemic properties, antitumor-promotion effects, are non toxic, have been used in cosmetic and health products and have also been proposed for skin cancer prevention (Liu, 1995; Rios et al., 2000). This study is a continuation of our efforts to evaluate natural products, for possible antioxidant activity, and that can be used in pharmaceutical and cosmetic formulations and food supplements.

2. Materials and methods

2.1. Chemicals

Lard was rendered from fresh pig fat, purchased from a local butcher's shop. Sunflower oil (Osolio) and corn oil (Corona) were purchased from a retail market. Virgin olive oil samples were kindly provided by an olive processing plant, located in the area of Chalkida (owner: A. Antoniou, Greece). Caffeic acid (Sigma Chemicals, Steinheim, Germany) was used as a reference antioxidant substance. Citric acid (Sigma) was used as metal chelator for sunflower and corn oils and for its possible synergistic action with natural resins.

Pistacia lentiscus var. Chia resin and essential oil of *P. lentiscus* resin (normal & liquid collection), were kindly donated by Mr. J. Perikos (Mastic Gum Growers' Association, Chios, Greece). Essential oil was distilled from resins collected in two ways: the traditional way, with incisions in the trees of *P. lentiscus* (normal collection) and the new way, with hormones, where resin is in the form of a liquid, and not tears (liquid collection).

Boswellia serrata and *Commiphora myrrh* resins were kindly donated by Dr. N. Argyriades (Vioryl S.A., Athens, Greece), while *Gum storax* and *Dammar* resin were purchased from Sigma and Fluka Chemika (Buchs, Switzerland), respectively. Essential oils of *C. myrrh* and *B. serrata* were purchased from Ath. Germanos Co. (Athens, Greece). The resin of *P. lentiscus* was sub-

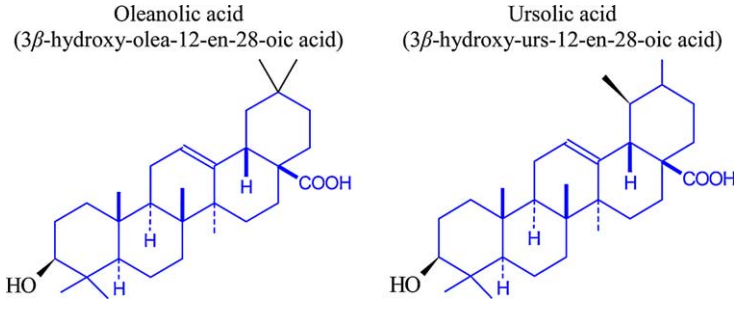

Fig. 1. Chemical structure of the triterpenes tested for their antioxidant activity.

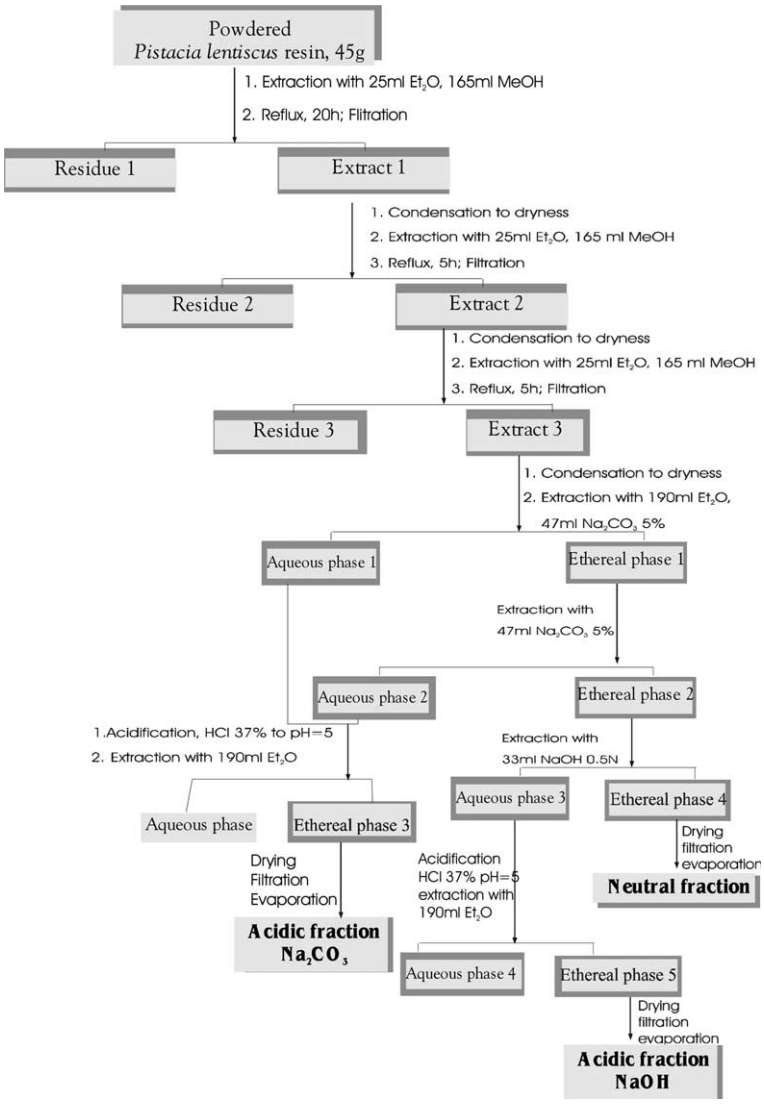

Fig. 2. Subfractionation of the resin *Pistacia lentiscus* var. Chia to a neutral fraction and two acidic ones (NaOH and Na_2CO_3).

fractionated according to Fig. 2, in order to examine the possible antioxidant activity of several fractions. Thus, the acidic fraction (NaOH), the acidic fraction (Na_2CO_3) and the neutral fraction of *P. lentiscus* resin were obtained. The following triterpenes were tested for their possible antioxidant activities in oils: ursolic acid (Sigma), and oleanolic acid (Sigma) (Fig. 1).

For the determination of peroxide values acetic acid, chloroform, potassium iodide (KI), soluble starch and sodium thiosulfate ($Na_2S_2O_3$), all purchased from Merck (Damstadt, Germany), were used.

2.2. Preparation of samples

All samples tested were added to oils in a solution form so that homogenization could be achieved. A *P. lentiscus* var. Chia solution 0.04% w/v in dichloromethane was prepared as follows: 2 mg *P. lentiscus* resin were added to 5 ml dichloromethane and the solution was stirred in a Vortex apparatus. 1 ml of each solution was added to 5 g of each oil substrate, solvent was evaporated and all samples were put into the oven. Each oil substrate used was placed, without additives, in the oven as of standard. All solutions of each sample were prepared by the same above-described procedure. Caffeic acid was diluted in absolute ethanol.

2.3. Antioxidant assay

Oil oxidation was assessed by the oven test. Each oil sample (5 g) was transferred to a series of open transparent glass bottles. A specific concentration (%w/w oil) of each additive tested for antioxidative activity, was added in a solution form, and the solvent was afterwards removed. The solvent selected each time was either dichloromethane (DCM), ethanol or methanol (all from Merck, pro analysis grade), depending on the solubility of each compound, in order to facilitate the

incorporation into the oil as a solution. A control sample was prepared under the same conditions without adding any additives.

The rate of oil oxidation was monitored by the increase of peroxide values (PV). 1 ± 0.1 g of each oil sample was weighed and subjected to iodometric determination (AOCS, 1990). The experiments were performed twice. Values in tables represent means of two determinations. In all cases, relative error was below 10%. Oven temperature used was 65 °C, in order to achieve accelerative oxidation.

3. Results and discussion

The antioxidant activities of natural resins and bioactive triterpenes were studied in sunflower oil, corn oil, lard and olive oil, three oil substrates with different concentrations of polyunsaturated fatty acids. The possible antioxidant activity of natural resins and triterpenes, compared to caffeic acid, was initially studied in sunflower oil at 65 °C (Table 1). In this group of experiments, natural resins were studied in typical concentrations of 0.05, 0.1 and 0.15% w/w in sunflower oil. *Pistacia lentiscus* (0.1 and 0.15% w/w oil) reduced the oxidation rate of sunflower oil while at low concentrations (0.05%), it increased oil oxidation. *Gum storax*, added at 0.05% w/w to sunflower oil, decreased oil oxidation while, upon increasing its concentration (0.1, 0.15%), the oxidation rate of sunflower oil was increased. Dammar resin increased oxidation rate of sunflower oil at each concentration tested. Essential oils of *Boswellia serrata* and *Commiphora myrrh* significantly retarded oil oxidation, while *P. lentiscus* essential oil increased oil oxidation rate (prooxidant activity).

The three subfractions of *Pistacia lentiscus* (neutral, acidic NaOH and acidic Na_2CO_3) retarded oil oxidation, with the acidic NaOH fraction presenting the highest antioxidant activity. Finally, ursolic and oleanolic acid, examined at a concentration 0.05% w/w oil (same as caffeic acid), exhibited significant antioxidant activity, similarly to caffeic acid, with ursolic acid presenting the highest activity.

The antioxidant activities of the several samples in sunflower oil decreased in the following order:

Resins: Gum storax (0.05% w/w) > *P. lentiscus* (0.1 & 0.15% w/w) > *Dammar* resin (0.05% w/w) > *P. lentiscus* (0.05% w/w) > *Dammar* resin (0.1% w/w) > Gum storax (0.1% w/w) > *Dammar* resin (0.15% w/w) > Gum storax (0.15% w/w).

Essential oils: *C. myrrh* (0.05% w/w) > *C. myrrh* (0.1% w/w) > *B. serrata* (0.1% w/w) > *B. serrata* (0.05% w/w) > *P. lentiscus* (0.05% w/w) > *P. lentiscus* (0.1% w/w) > *P. lentiscus* liquid collection (0.05%% w/w) > *P. lentiscus* liquid collection (0.1%% w/w).

Subfractions of P. lentiscus resin: acidic fraction NaOH > neutral fraction > acidic fraction Na_2CO_3.

Triterpenes: ursolic acid > oleanolic acid.

Table 1
Antioxidant activities (PV) of natural resins and bioactive triterpenes in sunflower oil (oven test: 65 °C)

Sample	Peroxide value (PV)[a]			
	8 h	24 h	48 h	57 h
Sunflower oil	5	12.9	41.6	45
Sunflower oil + *P. lentiscus* var. Chia 0.15% w/w	4.9	11.1	25.7	30.6
Sunflower oil + *P. lentiscus* var. Chia 0.1% w/w	5.6	11.2	28.9	29.8
Sunflower oil + *P. lentiscus* var. Chia 0.05% w/w	6.5	18.7	50.9	59.5
Sunflower oil + Gum Storax 0.05% w/w	4.5	11.9	26.4	30.3
Sunflower oil + Gum Storax 0.1% w/w	5.1	12.2	25	59.9
Sunflower oil + Gum Storax 0.15% w/w	5.2	20.4	49.5	69.3
Sunflower oil + dammar resin 0.05% w/w	6.9	19.8	49.7	50.2
Sunflower oil + dammar resin 0.1% w/w	7.0	19.5	47.9	57.2
Sunflower oil + dammar resin 0.15% w/w	7.1	20.2	54.5	65
Sunflower oil + essential oil of *Boswellia serrata* 0,05% w/w	8.7	18.2	34.5	43.7
Sunflower oil + essential oil of *Boswellia serrata* 0.1% w/w	4.5	11.9	26.5	32.5
Sunflower oil + essential oil of *Commiphora myrrh* 0.05% w/w	5.2	13.5	24.5	30.3
Sunflower oil + essential oil of *Commiphora myrrh* 0.1% w/w	4.8	13.7	27.4	31.3
Sunflower oil + essential oil of *P. lentiscus* var. Chia 0.05% w/w	7.4	19	50.9	56.8
Sunflower oil + essential oil of *P. lentiscus* var. Chia 0.1% w/w	6	30.2	52.3	59.2
Sunflower oil + essential oil of *P. lentiscus* var. Chia liquid collection 0.05% w/w	5.9	19.9	60	60
Sunflower oil + essential oil of *P. lentiscus* var. Chia liquid collection 0.1% w/w	15.5	18.7	53.5	77.8
Sunflower oil + acidic fraction Na_2CO_3 *P. lentiscus* var Chia 0.1% w/w	5.8	13.5	30.3	36.7
Sunflower oil + neutral fraction *P. lentiscus* var Chia 0.1% w/w	4.7	12.9	27.7	35.9
Sunflower oil + acidic fraction NaOH *P. lentiscus* var Chia 0.1% w/w	4.2	10.8	26	30
Sunflower oil + ursolic acid 0.05% w/w	4.9	10.9	24	27
Sunflower oil + caffeic acid 0.05% w/w	7.9	13.3	23.8	30.1
Sunflower oil + oleanolic acid 0.05% w/w	5.2	12.6	26.2	35.3

[a] Values represent means (PV) ($n = 2$). In all cases relative error was below 10%.

In some cases, a prooxidant effect of the added resins was observed, which is probably due to the presence of metals in sunflower oil (iron ions). Prooxidant activity has been reported in several additives when sunflower oil is used as oil substrate. Hence, citric acid, which is a metal chelator, was added, together with the resins, and the results are presented in Table 2.

As shown, *Pistacia lentiscus* resin, at 0.05 and 0.1% w/w of oil, together with citric acid (0.02%), significantly decreased sunflower oil oxidation rate while, above 0.1% concentration of *P. lentiscus* resin, the antioxidant activity decreased. Citric acid showed synergistic antioxidant activity with *P. lentiscus* resin in sunflower oil. It is thus confirmed that sunflower oil is susceptible to the presence of metals and hence some compounds/fractions act in a prooxidant way. Sunflower oil is an unstable oil, since it is rich in linoleic acid and other polyunsaturated fatty acids. The best conditions, among those tested, that resulted in the highest antioxidant activity in sunflower oil, were *P. lentiscus* resin (0.05% w/w) + citric acid (0.03% w/w), followed by *P. lentiscus* resin (0.1% w/w) + citric acid (0.02% w/w). Therefore, in the presence of citric acid, *Pistacia lentiscus* resin presented antioxidant activity in sunflower oil.

In the second group of experiments, where corn oil was used as oil substrate for examining the possible antioxidant activities of *P. lentiscus* resin (oven test: 65 °C), citric acid was also added for its metal chelation activity (Hras, Hadolin, Knez, & Bauman, 2000). As shown (Table 3), *Pistacia lentiscus* resin, even at low concentrations (0.02%) together with citric acid, exhibited significant antioxidant activity in corn oil.

In the third group of experiments, lard was used as substrate in order to determine the possible antioxidant activity of natural resins and bioactive triterpenes (Table 4). Lard is a highly unstable fat, due to its high concentration of polyunsaturated fatty acids. As shown, *Pistacia lentiscus* retards lard oxidation rate and thus exerts antioxidant activity in lard, increasing with the increasing concentration of resin. A concentration of 0.05% w/w presented the highest antioxidant activity in lard.

Commiphora myrrh resin, at a concentration of 0.02% w/w in lard, increased oxidation while, upon increasing the concentration to 0.05% w/w, a slight antioxidant activity was observed. Finally, *Boswellia serrata* resin, added to lard at 0.02 and 0.05% w/w presented respectively slight and good antioxidant activities. Thus the antioxidant activities in lard of natural resins, for each of the concentrations tested, decreased in the following order:

P. lentiscus > *B. serrata* > *C. myrrh.*

Oleanolic acid, tested in lard, showed a slight antioxidant activity at 0.02% w/w concentration.

In the fourth group of experiments, virgin olive oil was used as substrate for examining the possible antioxidant activity of *P. lentiscus* resin and its essential oil, since virgin olive oil is used in pharmaceutical and cosmetic preparations as a dispersion medium for additives

Table 2
Antioxidant activities (PV) of *P. lentiscus* var. Chia resin combined with citric acid in sunflower oil (oven test: 65 °C)

Sample	Peroxide value (PV)[a]					
	20 h	42 h	94 h	118 h	142 h	166 h
Sunflower oil	24.5	38.6	100	119	244	1157
Sunflower oil + citric acid 0.02%	15.3	37.1	70	96.9	120	843
Sunflower oil + citric acid 0.03%	15.2	36.4	77.9	107	165.7	970
Sunflower oil + *P. lentiscus* 0.05% + citric acid 0.02%	15.9	28.4	80.8	96.2	212	631
Sunflower oil + *P. lentiscus* 0.1% + citric acid 0.02%	12.5	28.8	65.5	99.2	101	579
Sunflower oil + *P. lentiscus* 0.15% + citric acid 0.02%	19.3	35	83.5	112	276	896
Sunflower oil + *P. lentiscus* 0.05% + citric acid 0.03%	17.2	24.7	62.8	81.8	101	234
Sunflower oil + *P. lentiscus* 0.1% + citric acid 0.03%	11.8	36.5	76.1	110	113	853
Sunflower oil + *P. lentiscus* 0.15% + citric acid 0.03%	14.1	34.6	84.7	108	166	983

[a] Values represent means (PV) ($n = 2$). In all cases relative error was below 10%.

Table 3
Antioxidant activities (PV) of *P. lentiscus* var. Chia resin combined with citric acid in corn oil (oven test: 65 °C)

Sample	Peroxide value (PV)[a]					
	20 h	42 h	94 h	118 h	142 h	166 h
Corn oil	14.0	21.7	58.3	83.2	90.3	210
Corn oil + citric acid 0.02% w/w	12.4	22.2	45.9	68.2	70.1	98.3
Corn oil + *P. lentiscus* 0.02% w/w + citric acid 0.02% w/w	11.3	19.1	44.8	67.8	74.3	78.4
Corn oil + *P. lentiscus* 0.05% w/w+ citric acid 0.02% w/w	11.0	16.0	48.7	60.8	78.1	82.5
Corn oil + *P. lentiscus* 0.1% w/w + citric acid 0.02% w/w	11.4	18.2	47.8	58.9	76.6	80.6

[a] Values represent means (PV) ($n = 2$). In all cases relative error was below 10%.

Table 4
Antioxidant activities (PV) of natural resins and bioactive triterpenes in lard (65 °C)

Sample	Peroxide value (PV)[a]			
	22 h	44 h	68 h	88 h
Lard	92.8	200	446	520
Lard + *P. lentiscus* var. Chia resin 0.05% w/w	81.3	149	375	361
Lard + *P. lentiscus* var. Chia resin 0.02% w/w	79.1	179	377	413
Lard + Oleanolic acid 0.02% w/w	55.0	194	352	452
Lard +caffeic acid 0.02% w/w	13.0	14.4	25.3	39.5
Lard + *Commiphora myrrh* resin 0.02% w/w	42.1	150	324	648
Lard + *Commiphora myrrh* resin 0.05% w/w	29.8	129	371	450
Lard +*Boswellia serrata* resin 0.02% w/w	51.8	149	319	470
Lard + *Boswellia serrata* resin 0.05% w/w	19.4	116	275	394

[a] Values represent means (PV) ($n = 2$). In all cases relative error was below 10%.

Table 5
Antioxidant activities (PV) of *P. lentiscus* resin and its essential oil in virgin olive oil (oven test: 65 °C)

Sample	Peroxide value[a]				
	139 h	237 h	308 h	359 h	431 h
Olive oil	20.6	21.1	39.0	50.2	88.2
Olive oil + *P. l entiscus* var. Chia 0.02% w/w	17.8	24.5	27.9	41.1	82.0
Olive oil +*P. lentiscus* var. Chia 0.05% w/w	14.4	25.0	30.3	41.9	72.1
Olive oil + *P. lentiscus* var. Chia 0.1% w/w	18.2	26.9	35.2	40.1	38.8
Olive oil + *P. lentiscus* var. Chia 0.15% w/w	17.8	25.7	28.0	41.50	39.2
Olive oil +essential oil of *P. lentiscus* var. Chia 0.02% w/w	18.4	27.7	30.3	41.3	48.5
Olive oil + caffeic acid 0.02% w/w	13.7	18.4	22.5	27.4	30.6

[a] Values represent means (PV) ($n = 2$). In all cases relative error was below 10%.

and is also used in foods. As shown (Table 5), *P. lentiscus* resin exhibits antioxidant activity, increasing with concentration increase. Thus, *P. lentiscus* resin added to olive oil at concentrations 0.1 and 0.15% w/w of oil, presented high antioxidant activity. The essential oil of *P. lentiscus* resin exhibited strong antioxidant activity in olive oil, even at low concentration (0.02% w/w). Olive oil proved to be the most stable oil among those tested, probably due to the presence of tocopherols and very low concentration of polyunsaturated fatty acids included, even without the addition of natural resins.

4. Conclusions

The possible antioxidant effects of the natural resins, *Pistacia lentiscus*, *Boswellia serrata* and *Commiphora myrrh*, and of the bioactive triterpenes, oleanolic and ursolic acid, were studied in lard and in vegetable oils, such as olive, corn and sunflower oils that can be used as oil bases and dispersion media for resins and triterpenes in cosmetic and pharmaceutical preparations.

In sunflower oil, the following samples presented antioxidant activity: *Pistacia lentiscus* resin (0.1, 0.15% w/w), Gum storax (0.05% w/w), essential oils of *B. serrata* and *C. myrrh*, the acidic (both NaOH and Na_2CO_3) and the neutral fraction of the *P. lentiscus* resin, and finally ursolic and oleanolic acid (strong antioxidant activity). When citric acid was used to examine its synergistic effect with *P. lentiscus* resin, due to its metal chelation properties, combination of *P. lentiscus* with citric acid significantly reduced sunflower oil oxidation rate.

Pistacia lentiscus resin (0.02% w/w), combined with citric acid, showed significant antioxidant activity in corn oil. In lard, 0.05% w/w of *P. lentiscus*, *B. serrata* and *C. myrrh* exhibited high, good and slight antioxidant activities, respectively. In olive oil, *P. lentiscus* resin, at concentrations of 0.1 and 0.15% w/w, showed high antioxidant activity and its essential oil, even at low concentrations (0.02%), strongly retards its oxidation. Olive oil can be used as the dispersion medium or lipophilic base for several pharmaceutical and cosmetic preparations.

Pistacia lentiscus proved to have strong antioxidant activity in almost all oil substrates examined. The best concentration of *P. lentiscus* resin varied among oil substrates. However, the results can hardly be compared because of the huge difference in the shelf-life of these oils, due to different concentrations of polyunsaturated fatty acids.

Resins, such as *Pistacia lentiscus*, *Boswellia serrata* and *Commiphora myrrh*, are natural products, used as active ingredients or additives in pharmaceuticals and cosmetics. The antioxidant activity (mainly of *P. lentis-*

cus) was established in oils, which is a strong reason for expanding the investigation of natural constituents responsible for the protection of oil against oxidation and stability of lipophilic pharmaceutical and cosmetic preparations. It is very interesting that *Pistacia lentiscus* resin can additionally be used as natural antioxidant in cosmetics and pharmaceutical preparations. Further studies may show its use in functional foods.

Acknowledgements

Prof. D. Boskou (Department of Chemistry, Aristotle University of Thessaloniki) is gratefully acknowledged for his useful recommendations. We gratefully acknowledge Mr. J. Perikos (Mastic Gum Growers Association) for kindly providing the samples of *Pistacia lentiscus* resin and its essential oil. Dr. N. Argyriades (Vioryl S.A, Athens) is also thanked for kindly donating us the samples of the resins, *Boswellia serrata* and *Commiphora myrrh*. Mr. A. Antoniou and N. Sotiras, Chemical Engineers, are thanked for their technical assistance.

Dr. A.N. Assimopoulou acknowledges IKY for a postdoctoral scholarship.

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