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Food Chemistry 83 (2003) 585–593

Food Chemistry

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Resveratrol and its antioxidant and antimicrobial effectiveness

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Received 23 October 2002; received in revised form 18 March 2003; accepted 18 March 2003

Abstract

Resveratrol (3,5,4′-trihydroxystilbene) was prepared by total chemical synthesis. Water solubility, solubility of resveratrol in water/ethanol mixture (0–40% v/v) at temperatures of 0–60 °C, antioxidation effect of resveratrol, tested in a mixture of sunflower oil–rapeseed oil, or in margarine emulsion, and antimicrobial activity of resveratrol against yeasts and moulds were tested. Water solubility of resveratrol was low and it rose as ethanol concentration and temperature increased. The solubility of resveratrol was 40 mg l^{-1} (20 °C) when the ethanol concentration achieved 10% v/v. Resveratrol behaved as a slight antioxidant against oxidation of triacylglycerols of sunflower and rapeseed oils. Resveratrol did not achieve the effectiveness of BHT or ascorbylpalmitate. When tested in margarine emulsion w/o, resveratrol did not achieve the effectiveness of ascorbic acid. In vitro, resveratrol behaved as an antimicrobial against filamentous fungi *Penicillium expansum* and *Aspergillus niger*.

Keywords: Resveratrol; Antioxidant; Lipid oxidation; Oxidation in emulsions; Antimicrobial effect

1. Introduction

Resveratrol, as a biologically active compound, belongs to the phytoalexins, secondary plant metabolites, which are produced de novo or are increasingly formed as a response to stress or to attack by nonpathogenic or avirulent bacteria, viruses or fungi (Langcake, Cornford, & Pryce, 1979; Langcake and McCarthy, 1979; Langcake & Pryce, 1976). Resveratrol was the main component of plant extracts that were used for centuries in traditional medicine to treat human diseases (Adrian, Jeandet, Breuil, Levite, Deborg, & Bessis, 2000; Palomino, Góméz-Serranillos, Slowing, Carretero, & Villar, 2000).

Trivially named, resveratrol is 3,4′,5-trihydroxystilbene (Fig. 1). It is obvious that two geometrical isomers of resveratrol exist, *E*- (*trans*-) and *Z*- (*cis*-). In plant materials, a mixture of these two isomers occurs, although the *trans*-isomer usually predominates.

Contemporary interest in monitoring the presence and effectiveness of resveratrol was caused by the existence of the so-called ''French paradox'' (Soleas, Diamandis, & Goldberg, 1997a, 1997b). It has been found out and statistically confirmed that, in certain parts of France, the death rate caused by coronary artery diseases is lower despite relatively high fat consumption in the human diet. The consumption of wine is one of the dietary factors that might partially explain the low mortality caused by atherosclerosis (Renauld & De Lorgeril, 1992). This fact led to an assumption that the enjoyment of wine could act against the effect of high-fat-diet and therefore limit incidence and extent of coronary artery diseases.

More than 200 articles have been published on resveratrol alone in the last few years (Jeandet, Douillet-Breuil, Bessis, Debord, Sbaghi, & Adrian, 2002), mostly concerning biological properties of resveratrol, e.g. antioxidizing effect (Kimura, Ohminami, Okuda, Baba, Kozawa, & Arichi, 1983), anti-atherosclerotic effect, effect on the cardiovascular system (Arichi, Kimura, Okuda, Baba, Kozawa, & Arichi, 1982; Diamandis, Soleas, & Goldberg, 1995; Frankel, Waterhouse, & Teissedre, 1995; Pace-Asciak, Rounova, Hahn, Diamandis, & Goldberg, 1996), anti-mutagenic effect (Uenobe, Nakamura, & Miyazawa, 1997) and chemo-protective advantage against cancer proliferation (Jang et al.,

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doi:10.1016/S0308-8146(03)00157-2

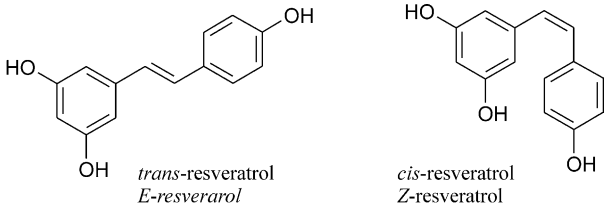

Fig. 1. *E*-Resveratrol and *Z*-resveratrol.

1997). Plant polyphenolic compounds, being substances with anti-oxidizing properties, can inhibit destructive tumor growth (Savouret & Quesne, 2002).

Generally, stilbenes are recognized as biologically active compounds that have antifungal activities against various pathogens (Jeandet et al., 2002). The most described antimicrobial effect of resveratrol and other stilbenes is that against the common grapevine pathogen, *Botrytis cinerea*, which causes significant losses for vineyard owners worldwide. In host–plant tissues, the elevated levels of resveratrol may slow down or inhibit the spread of *B. cinerea* infection. Some strains of the highly pathogenic *B. cinerea* circumvent this defence by detoxifying resveratrol though an oxidative process. Cichewicz, Kouzi, and Hamann (2000) confirmed production of three new (restrytisols A–C) and three known (resveratrol *E*-dihydromer, leachinol F, and pallidol) oxidized resveratrol dimers that were evaluated for their anti-HIV-1, cytotoxic, and cyclooxygenase (COX) I and COX II activities (Cichewicz et al., 2000). Langcake and Pryce (1976) were the first ones who tested the biological activity of resveratrol. They established that the ED_{50} (i.e. effective dose or concentration required for 50% mortality) of resveratrol upon dormant conidia of *B. cinerea* or spores of *Cladosporium cuccumerinum* was more than 200 mg ml^{-1} (Jeandet et al., 2002). Adrian, Jeandet, Veneau, Weston, and Bessis (1997) established that resveratrol had real inhibitory effects on conidial germination of *B. cinerea* liquid cultures when used at concentration ranging from 60 to 160 mg ml^{-1} (resveratrol solubility was ensured by concentration of a minute quantity of ethanol, less than 4%). Antimicrobial activity of resveratrol has also been published against bacteria, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and dermatophytes (Man-Ying Chan, 2002). Man-Ying Chan (2002) found out that 171–342 mg of resveratrol per litre, when dissolved in dimethyl sulfoxide, inhibited the growth of *S. aureus*, *E. faecalis*, *P. aeruginosa*. The growth of dermatophytes was inhibited at 25–50 mg l^{-1} of resveratrol. Pterostilbene (*E*-3,5-dimethoxy-4′-hydroxystilbene), the stilbene monomer synthesised by vine leaves stressed by infection, was also identified and proved to have more potent anti-fungal activity (Soleas et al., 1997).

Complex knowledge of resveratrol was obtained during the 1980s, due mainly to the development of instrumental analysis, especially HPLC, the most common method allowing the monitoring of incidence and concentration of resveratrol in grapevines—*Vitis vinifera* L. (*Vitaceae*) (Langcake et al., 1976, 1979; Palomino et al., 2000).

As it is a natural antioxidant, food application of resveratrol has several benefits. Antioxidants prevent food products from oxidation and consequently protect the product from nutritional and sensory damage. The first sign of damage of food could be changes of sensory properties, even though the appearance and nutritional quality might be typical or normal. The natural antioxidant advantage is that it has been consumed conventionally for many years in common beverages and foods. So, if it used as an antioxidant or an antimicrobial in foods, the examination of health effects in humans would not be necessary.

Murcia and Martínez-Tomé (2001) studied the antioxidant and prooxidant activities of resveratrol and compared their results to those of other antioxidants (butylated hydroxytoluene, butylated hydroxyanisole, phenol, propyl gallate, sodium tripolyphosphate, alfa-tocopherol, and vanillin) in lipids. They found that only butylated hydroxyacetone was better than resveratrol and that butylated hydroxyanisole, resveratrol, and propyl gallate inhibited the peroxidation in a concentration-dependent manner.

Resveratrol, in its chemically pure state, has not been described and parameters such as solubility, and antioxidant effect in food have not been sufficiently explored. To test the antifungal activity of resveratrol we used *B. cinerea* that has a characteristic metabolism (the ability to degrade resveratrol by an oxidative process) and the commonly occurring fungal strains *Aspergillus niger*, *Penicillium expansum*, and *Saccharomyces cerevisiae*.

2. Materials and methods

2.1. Synthesis

E-Resveratrol was obtained by total synthesis via diethyl-3,5-dimethoxybenzylphosphonate that was prepared by reaction of 3-5-dimethoxybenzylchloride and triethyl phospite. The *E*-3-4-5′-trimethoxystillbene was formed by a Wittig–Horner reaction between the above mentioned phosphonate and 4-methoxybenzaldehyde. This stillbene was demethylated by borontribromide to *E*-3-4-5′-trihydroxystilbene (*trans*-resveratrol) (Šmidrkal et al., 2001).

2.2. Assay of resveratrol solubility in water or in water/ethanol mixture

The test of resveratrol solubility was carried out in water or in water/ethanol mixture (0–40% v/v) at temperatures from 0 to 60 °C. When the equilibrium was achieved (approximately after 2 h), the measurement of

the concentration of resveratrol in the solution was done by a UV-spectrophotometer, model Cary 50 Conc ($\lambda = 300$ nm, cuvette 1 cm; spectrophotometer, Varian Australia PTY Ltd. Germany)

2.3. Assay of antioxidant activity of resveratrol

An active oxygen method (Oxidograph, Mikrolab Aarhus, Brabrand, Denmark) was used to measure the oxidation stability of sunflower oil or rapeseed oil in relation to antioxidant effect of resveratrol (tested conditions: temperature 110 °C, sample 5 g of oil, rate 1 s^{-1}). To help dispersion of resveratrol in bulk oil, two emulsifiers of a distilled monoacylglycerol type (Danisco Ingredients, Wien, Austria) were used in proportion 1:9 w/w: emulsifier MAG1 (Dimodan CPT, iodine value 80) or MAG2 (Dimodan OT, iodine value 60). We used butylated hydroxytoluene (2,6-di-*tert*-butyl-4-methylphenol; 99%, Fluka Chemie GmbH, BRD) and L-ascorbyl palmitate (6-O-palmitoyl-L-ascorbic acid; Fluka Chemie GmbH, BRD) as comparative antioxidants.

Induction period (IP, measured in hours) was evaluated to compare the oxidation stability of sunflower oil or rapeseed oil (Table 1) and was determined by the method of the tangents to the two parts of the oxidation curve. Measurements on Oxidograph apparatus were duplicated and mean values with standard deviations were reported for each case (Table 2).

To test the antioxidant activity of resveratrol in a model soft margarine emulsion, we prepared an emulsion w/o: 70% of fat phase (acid value 0.15 mg KOH. g^{-1}; C14:0 0.4%, C16:0 19.4%, C18:0 5.5%, Σ C18:1 36.9% from that *trans*-C18:1 21.4%, C18:2 33.7%, C18:3 3.7%, C20:1 0.4%; SFC_{10} 25.8%, SFC_{20} 11.6%, SFC_{30} 3.1%, SFC_{40} 0.6% by p-NMR, ISO 8292-1991), 0.3% w/w of emulsifier D (Setuza a.s., CZ, content of monoacylgylcerol 70% w/w) and 30% of distilled water adjusted to pH 4 by lactic acid as a water phase. Tested emulsion was prepared with the aid of a semi-scale laboratory apparatus Stephan (U M 5 universal, A. Stephan u Söhne GmbH&Co, Hamelen, Germany). Fat phase (1750 g) with added emulsifier D (7.5 g) was placed into a mixing vessel and water phase (242.5 g) was added after heating to 45 °C. Before addition to fat phase, the water phase, after elimination of oxygen by argon, was heated up to boiling point under Ar-atmosphere. After the emulsification (temperature 45 °C, 3 min, agitation 1500 min^{-1}) the emulsion was cooled by cold water in the mixing vessel (step by step from 45 to 30 °C during 5 min, agitation 1500 min^{-1}, following 8 min from 30 to 25 °C, agitation 3000 min^{-1} and finally during 4 min from 25 to 21 °C, agitation, 1500 min^{-1}. Both the emulsification and the cooling were carried out under Ar-atmosphere. Reservatrol was dissolved in 50 μl of ethanol (95% v/v) and then added to the water phase. The polypropylene pots were filled with the prepared margarine emulsion (250 g) and the cap tightly covered the margarine emulsion surface, so that between the cup and the margarine surface there was no air. The margarine was stored at 15 °C for 13 weeks. The samples were taken from the surface layer (2–3 mm) and from the core of the margarine emulsion (the inner part) in regular time periods and the peroxide value (EN ISO 3960: 2000) as mmol 1/2 O_2 kg^{-1} was evaluated. Ascorbic acid was used as a comparative antioxidant to resveratrol.

Table 1
Characteristics of used refined vegetable oils[a]

Characteristic	Sunflower oil	Rapeseed oil
Acid value (mg KOH/g)	0.14	0.13
Iodine value (g I_2/100 g)	126.8	114.2
Oleic acid content (%)	21.2	60.1
Linoleic acid content (%)	65.3	19.8
Linolenic acid content (%)	0.1	9.4
Peroxide value (meq/kg)	0.43	0.29

[a] Source: Palma Tumys a.s. Slovak Republic.

2.4. Antifungal activity of resveratrol

P. expansum DBM 4061, *A. niger* DMF 0801, *B. cinerea* DBM 4111 and *S. cerevisiae* Sherry DBM 181 were used to test the growth inhibition activity of resveratrol (11 mg l^{-1} and 22 mg l^{-1}). Two microgrammes of resveratrol were dissolved in 2 ml of ethanol (95% v/v) and an aliquot was added to the growth media to give the tested concentrations of resveratrol (11 mg l^{-1} and 22 mg l^{-1}). The method evaluated by Kim, Lee, and Roh (2000) was used to test the growth inhibition of filamentous fungi and yeast in liquid media. A suspension of fungal spores or yeast cells (0.1 ml, concentration 1.10^6 per 1 ml) in saline diluent was inoculated into the cultivation medium containing 20% (w/w) sucrose, 1.5% (w/w) malt extract and 2% (w/w) yeast extract. Cultivation was done in a shaker (45 min^{-1}, amplitude 2) for 3 days at 22 °C. Then the cultivation medium was filtered through cellulose nitrate filter (pore diameter 0.45 μm, Sartorius, Germany) which was dried at 60 °C for 24 h and weighed. Percentage of inhibition was calculated as follows:

$$\%\ inhibition = [(P - M) - (R - M_R)]100/(P - M)$$

where P is the cellulose nitrate filter dried mass after fungal cultivation without resveratrol, R is the cellulose nitrate filter dried mass after fungal cultivation with resveratrol, M_R is the cellulose nitrate filter dried mass when only cultivation medium with dissolved resveratrol was filtered through and M is the cellulose nitrate

Table 2
Statistic of measurement of antioxidant activity of resveratrol in sunflower oil or in rapeseed oil tested by Oxidograph apparatus

Number of measurement (x)		Induction period at 110 °C (h)[a]	Standard deviation	Significance level[b] ($IP_{CO}-IP_x$)[c]	Significance level[b] ($IP_{x1}-IP_{xi+1}$)[c]
Sunflower oil		**3.65**	**0.03**	+	
1	0.01% resveratrol	3.70	0.02	+	
2	0.02% resveratrol	3.71	0.01	+	n.s.
3	0.05% resveratrol	3.77	0.02	+	+
4	0.1% resveratrol	3.91	0.01	+	+
1	0.01% resveratrol (in mixture with MAG 1)[a]	3.68	0	+	
2	0.02% resveratrol (in mixture with MAG 1)[a]	3.73	0	+	+
3	0.05% resveratrol (in mixture with MAG 1)[a]	3.75	0	+	+
4	0.1% resveratrol (in mixture with MAG 1)[a]	3.73	0.1	+	n.s.
1	0.01% resveratrol (in mixture with MAG 2)[a]	3.81	0.02	+	
2	0.02% resveratrol (in mixture with MAG 2)[a]	3.77	0.01	+	+
3	0.05% resveratrol (in mixture with MAG 2)[a]	3.77	0.02	+	n.s.
4	0.1% resveratrol (in mixture with MAG 2)[a]	3.93	0.01	+	+
1	0.01% BHT	4.05	0.02	+	
2	0.02% ascorbylpalmitate	3.82	0	+	+
Rapeseed oil (RO)		**5.83**	**0.07**	+	
1	0.01% resveratrol	6.15	0.01	+	
2	0.02% resveratrol	6.15	0	+	n.s.
3	0.05% resveratrol	6.13	0.01	+	+
4	0.1% resveratrol	6.29	0.05	+	+
1	0.01% resveratrol (in mixture with MAG 1)[a]	5.85	0.02	+	
2	0.02% resveratrol (in mixture with MAG 1)[a]	5.85	0.05	+	n.s.
3	0.05% resveratrol (in mixture with MAG 1)[a]	5.84	0.06	+	n.s.
4	0.1% resveratrol (in mixture with MAG 1)[a]	6.14	0.06	+	+
1	0.01% resveratrol (in mixture with MAG 2)[a]	6.06	0.07	+	
2	0.02% resveratrol (in mixture with MAG 2)[a]	6.12	0.05	+	n.s.
3	0.05% resveratrol (in mixture with MAG 2)[a]	6.20	0.04	+	+
4	0.1% resveratrol (in mixture with MAG 2)[a]	6.31	0	+	+
1	0.01% BHT	5.97	0.06	+	
2	0.02% ascorbylpalmitate	6.58	0	+	+

[a] The mixture contained 10% w/w of resveratrol and 90% w/w of monoacylglycerol.
[b] Significance is reported on t-test ($n=10$), + $P<0.05$.
[c] IP is induction period, CO is refined oil (sunflower oil or rapeseed).

filter dried mass when only cultivation media without dissolved resveratrol was filtered through. Method precision was determined for the inhibition effect of the filamentous fungi, *A. niger* and *P. expansum*; there were 24 trials carried out and the average was calculated with standard deviations 4.8% and 10.1%, respectively.

3. Results and discussion

3.1. Determination of resveratrol solubility in water or in ethanol

Water solubility of resveratrol was low at 20 °C and it did not significantly depend on temperature or on the ethanol concentration up to 10% v/v. If the ethanol concentration in water rises (more than 20% v/v), the resveratrol solubility increases. In 10% v/v ethanol, 40 mg l^{-1} (20 °C) resveratrol was dissolved. Obviously in wine, containing usually more than 10% v/v of ethanol, the resveratrol content is not limited by its solubility (content of resveratrol in wine ordinarily is 2–4 mg l^{-1}, max 10 mg l^{-1}). The resveratrol solubility significantly rises at ethanol concentrations higher than 30% v/v and at higher temperatures (Fig. 2).

Under similar conditions we found that resveratrol is not soluble in vegetable oils (detection limit was 0.1 mg l^{-1}) so that it was necessary to use an emulsifier to homogenise (to disperse) resveratrol in bulk oil.

3.2. Antioxidant effect of the resveratrol in sunflower oil and in rapeseed oil

To test the antioxidant effect of resveratrol comprehensively, two vegetable oils that differ in content of monoenic and polyenic fatty acids were used. The main

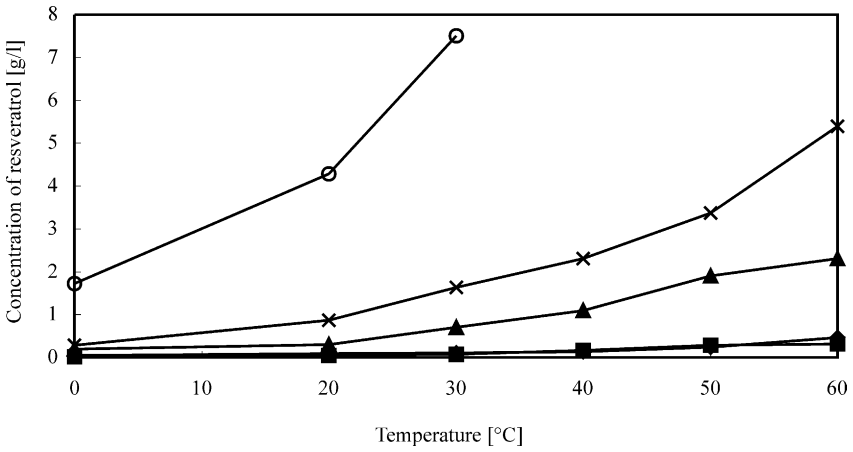

Fig. 2. Solubility of resveratrol in water or in water/ethanol mixture: ◆ solubility in water, ■ solubility in 10% ethanol, ▲ solubility in 20% ethanol, × solubility in 30% ethanol, **O** solubility in 40% ethanol.

fatty acid of sunflower oil is linoleic acid, whereas rapeseed oil contains mainly oleic acid, accompanied by linoleic acid and linolenic acid. As found, resveratrol itself is not soluble in bulk oil so, in order to disperse it in both oils, we had to use emulsifiers of the monoacylglycerol type. To eliminate of the effect of the monoacylglycerol emulsifier, two batches of samples with two defined food emulsifiers of different acyl compositions (MAG1, MAG2) were prepared: emulsifier MAG1 (Dimodan CPT, iodine value 80) and MAG2 (Dimodan OT iodine value 60).

Oxidation stability of sunflower oil itself was lower (IP was 3.65 h) than that of rapeseed oil (IP was 5.82 h). Resveratrol showed a low antioxidant effect against the oxidation of fatty acids of sunflower oil at concentrations 0.01–0.10% w/w (Fig. 3). The most effective antioxidant system of sunflower oil was butylated hydroxytoluene (BHT, 0.01% w/w) and ascorbylpalmitate (AP, 0.02% w/w); resveratrol (RESV 0.01–0.1% w/w) showed a lower antioxidant effect (Fig. 3). The antioxidant effects of 0.01% w/w of ascorbylpalmitate and resveratrol themselves were similar.

In rapeseed oil (Fig. 4), ascorbylpalmitate (AP, 0.02% w/w) was the most effective antioxidant. The effect of 0.01% w/w of resveratrol and BHT (0.01% w/w) itself on oxidation stability of rapeseed oil was similar to that of ascorbylpalmitate (Fig. 4).

We found that in sunflower oil, as well as in rapeseed oil, an emulsifier of the monoacylglycerol type influences the autooxidation of fatty acids and the oxidation stability of both oils. This influence was found to be stronger than that of all tested polar substances.

3.3. Antioxidant effect of the resveratrol in model emulsion system of margarine emulsion

Water solubility of resveratrol at 15 °C (the recommended temperature of storage of the same type of margarine) was at the limit that could be tested for its antioxidant effect. Therefore, the tested concentration

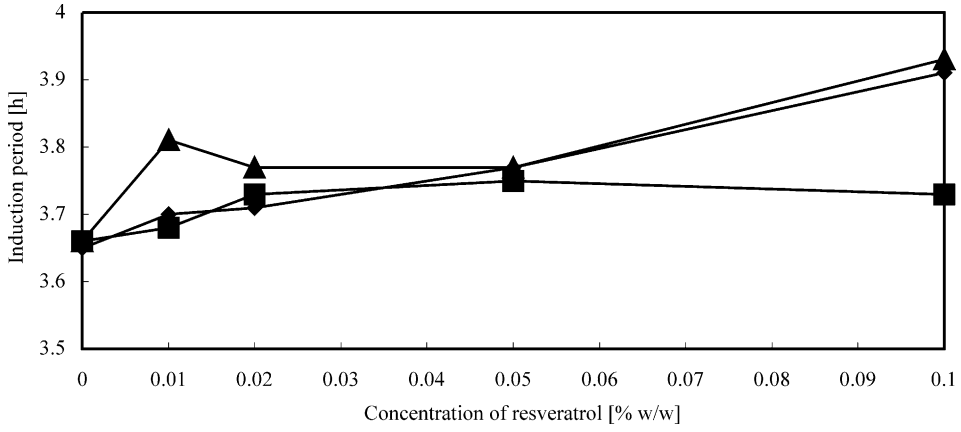

Fig. 3. Oxidation stability of sunflower oil with various concentrations of resveratrol: ◆ resveratrol; ■ resveratrol in emulsion containing MAG 1, ▲ resveratrol in emulsion containing MAG 2.

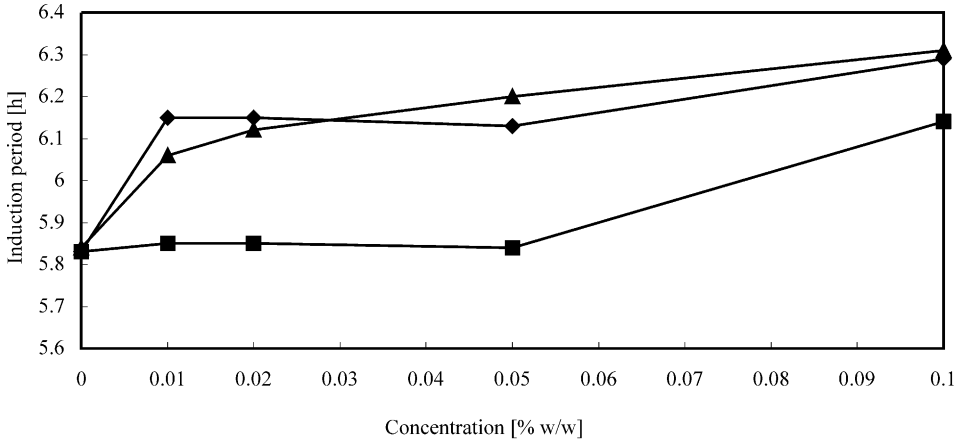

Fig. 4. Oxidation stability of rapeseed oil with various concentrations of resveratrol: ◆ resveratrol, ■ resveratrol in emulsion containing MAG 1, ▲ resveratrol in emulsion containing MAG 2.

was in the range 0.001–0.005% w/w (1–5 mg/100 g emulsion). Due to the high access of oxygen to or into the surface layer of the emulsion, the development of oxidation was expected to be different in different parts of the emulsion. Therefore we controlled the oxidation processes, both in the surface part (Figs. 5 and 6) and the inner part of the emulsion (Figs. 7 and 8). Ascorbic acid, that reduces hydroperoxide concentration for all long storage periods, was used as a comparative antioxidant (Figs. 5–8). At 0.01% w/w of ascorbic acid, the peroxide value did not get over 6 meq/kg in the inner part of the margarine emulsion. The effect of ascorbic acid (mainly 0.01% and 0.02% w/w) is obvious in the surface layer of margarine emulsion (Fig. 5) as well as in the inner part of margarine emulsion (Fig. 7). Compared to ascorbic acid, resveratrol showed low antioxidant effect, even when tested at maximal concentration that was possible in this system (0.005% w/w). We found a similar effect of resveratrol in the inner part (Fig. 8) as well as in the surface part (Fig. 6) of the margarine emulsion and the differences in reduction of peroxide value (about 2–3 meq/kg) became evident at later storage stages, mainly in the inner part of the margarine (Fig. 8). The addition of ascorbic acid (0.0025%) to resveratrol (0.0025%) did not affect the antioxidant effect of resveratrol alone in the inner part of the emulsion (Fig. 8). However, in the surface part of the emulsion, in which the oxidation process was extensive, resveratrol alone, or the mixture of resveratrol and ascorbic acid, presented similar effects to ascorbic acid (Fig. 5) up to 12 weeks of storage.

Murcia and Martínez-Tomé (2001) studied the antioxidant and prooxidant activities of resveratrol and other antioxidants (butylated hydroxytoluene, butylated hydroxyanisole, phenol, propyl gallate, sodium tripolyphosphate, α-tocopherol, and vanillin). They studied lipid peroxidation, but in a different system, not lipid emulsion. They reported that only butylated hydroxyanisole was better than resveratrol.

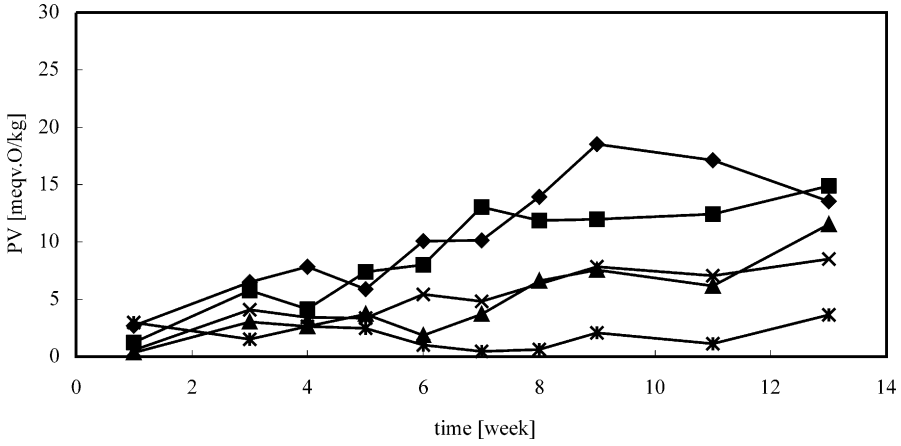

Fig. 5. Oxidation of the surface part of emulsion with different contents of ascorbic acid: ◆ control emulsion without ascorbic acid, ■ 0.0025% ascorbic acid, ▲ 0.01% ascorbic acid, × 0.02% ascorbic acid, ∗ fat base without ascorbic acid.

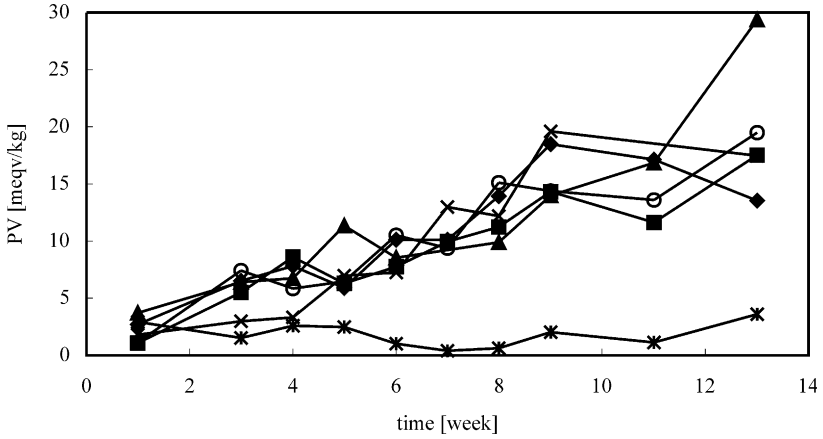

Fig. 6. Oxidation of the surface part of emulsion with different contents of resveratrol: ◆ control emulsion without resveratrol, ■ 0.00125% resveratrol, ▲ 0.0025% resveratrol, × 0.005% resveratrol, **O**% resveratrol and 0.0025% ascorbic acid, ∗ fat base without ascorbic acid.

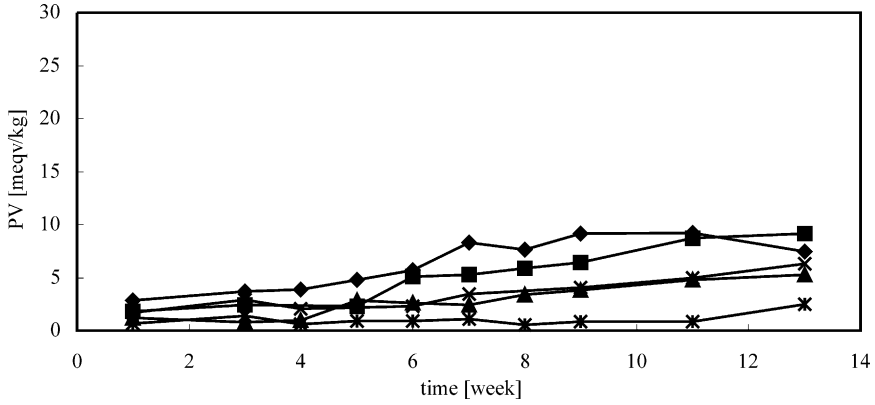

Fig. 7. Oxidation of the inner part of emulsion with different contents of ascorbic acid: ◆ control emulsion without ascorbic acid, ■ 0.0025% ascorbic acid, ▲ 0.01% ascorbic acid, × 0.02% ascorbic acid, ∗ fat base without ascorbic acid.

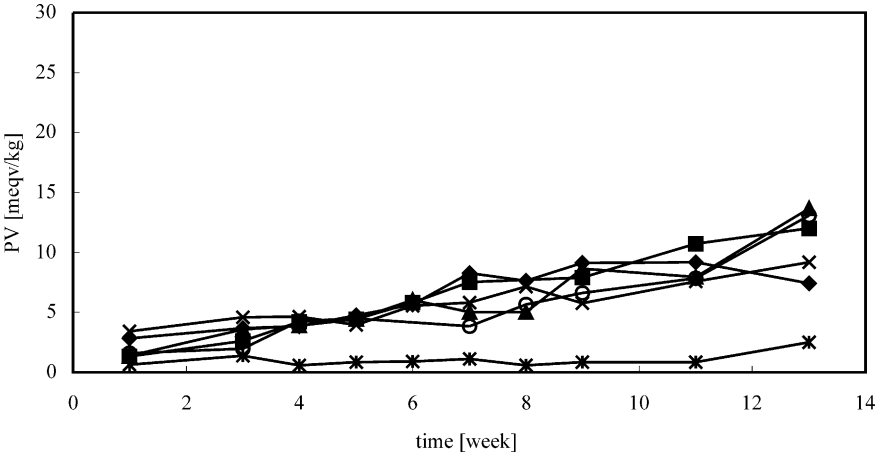

Fig. 8. Oxidation of the inner part of emulsion with different contents of resveratrol: ◆ control emulsion without resveratrol, ■ 0.00125% resveratrol, ▲ 0.0025% resveratrol, × 0.005% resveratrol, **O**% resveratrol and 0.0025% ascorbic acid, ∗ fat base without ascorbic acid.

Table 3
Growth inhibition of filamentous fungi and yeast in liquid media

Tested strains	Relative inhibition (%) at different resveratrol concentration	
	11 mg l^{-1}	22 mg l^{-1}
Saccharomyces cerevisiae	57.2	18.3
Penicillium expansum	16.1	21.6
Aspergillus niger	36.4	55.8

3.4. Inhibition of filamentous fungi and yeast by resveratrol in liquid media

The inhibition effect of two concentrations of resveratrol was tested dissolved in water–ethanol solution: 11 mg l^{-1} and 22 mg l^{-1} (Table 3). The yeast *S. cerevisiae* Sherry DBM 181 was inhibited more by the concentration 11 mg l^{-1} than by the higher concentration, 22 mg l^{-1}. *A. niger* was the most sensitive mould out of three tested genera. *P. expansum* DBM 4061 showed only a little sensitivity to resveratrol, but its inhibition by both tested concentrations of resveratrol was also evident. The results for the genus *B. cinerea* DBM 4111 were not credible, because this mould did not show sufficient growth in the liquid medium used. *B. cinerea* DBM 4111 strain, that usually stimulates stilbene production by infection of grapes, was reported to need special media to be cultivated. Cichewicz et al. (2000), after examining several formulations found that potato-dextrose broth (24 g l^{-1}), supplemented with 2 g l^{-1} of pectin, provided the best growth conditions for preparative-scale biotransformations with strain *B. cinerea*. Considering the error of the used method, we conclude that the inhibition effect of resveratrol on yeast strain *S. cerevisiae* Sherry DBM 181 and fungal strains *P. expansum* DBM 4061 and *A. niger* DMF 0801 in liquid media was proved.

To our knowledge, this is the first study to successfully apply synthetically prepared resveratrol to prevent the oxidation of margarine emulsion and to suppress food deterioration by mould strains.

Acknowledgements

This work was supported by the grant No. 525/99/1338 from the Grant Agency of Czech Republic and VEGA 1/9132/02 from Grant Agency of Ministry of Education of Slovac Republic.

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