Antioxidative and Antimicrobial Effects of Some Natural Extracts in Lard

S. SEKRETÁR[1]*, Š. SCHMIDT[1], M. VAJDÁK[1], L. ZAHRADNÍKOVÁ[1] and J. ANNUS[2]

[1]Department of Food Science and Technology and [2]Department of Chemical Physics, Faculty of Chemical and Food Technology STU, Bratislava, Slovak Republic, *E-mail: stanislav.sekretar@stuba.sk

Abstract: The extracts from 17 ready available plants were prepared by 95% ethanol extraction in Soxhlet extractor. The crude extracts were examined for their antioxidation properties using the automatized swift test (Rancimat) and lard as a substrate. The extracts with the most promising results (*Rosmarinus officinalis* and *Salvia officinalis*) were chromatographed on silica gel column and the fractions so obtained were explored separately. Second fraction from the chromatography of rosemary extract exhibited the antioxidation activity (induction period 8 h at 0.002% wt.) comparable with the activity of butylated hydroxytoluene (BHT) at 12.5 times higher concentration (induction period 11.45 h at 0.025% wt.). The antimicrobial efficiency of these extracts was determined with the aid of three dimensional agar method on four selected microorganisms (*Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*). The most effective were extracts from agrimony, black tea, rosemary and sage. Extracts possessing good antioxidation activity also exhibited antimicrobial efficiency.

Keywords: antimicrobials; antioxidants; extracts; rosemary; sage

INTRODUCTION

Synthetic phenolic antioxidants (BHT, BHA, TBHQ) are commonly used in fats and oils to retard oxidation [1], however, their safety has been questioned [2, 3] and therefore some natural alternatives for this antioxidants have been studied [4]. The published data comparing the effectiveness of various natural antioxidants are often difficult to interpret because of different methodology, particularly the choice of methods utilizing various oxidation conditions. Also the use of crude extracts and the occurrence of complex interfacial phenomena in oils and food emulsions has further compounded the analytical problems [5]. With the use of Rancimat method [6] we evaluated the influence of 17 ethanolic plant extracts on the oxidative stability of lard. The efficacy of these extracts was compared with commonly used synthetic antioxidant BHT. The relatively high content of flavonoids in some extracts prompted us to test also their antimicrobial activity against four common microorganisms.

EXPERIMENTAL

Materials and methods. Ethanolic extracts from ready available plants were analyzed by TLC chromatography and some of them (containing flavonoid compounds) were selected for further experiments: stinging nettle *(Urtica dioica)*, thyme *(Thymus serpyllum)*, chamomile *(Matricaria chamomilla)*, calendula (*Calendula officinalis)*, agrimony *(Agrimonia eupatoria)*, horsetail *(Equisetum arvense)*, hawthorn *(Crataegus oxyacantha*), milfoil *(Achillea millefolium)*, lady's mantle *(Alchemilla vulgaris)*, roasted coffee *(Coffea)*, black tea *(Thea)*, melissa *(Melissa officinalis)*, sage *(Salvia officinalis)*, rosemary *(Rosmarinus officinalis)*, ginger (*Zingiber officinale)* were available commercially, red currant meal *(Ribes rubrum)* and black currant meal *(Ribes nigrum)* (purchased from Palma-Tumys enterprise).

Lard (commercial-grade): IV = 59.8 (g I_2/100 g), AV = 0.8 (mg KOH/g), PV = 2.9 (meq O_2/g), SV = 199 (mg KOH/g), M.p. = 30.4°C. FA (% wt.) composition (determined by GC of methylesters): 14:0 (1.43),

16:0 (24.76), 16:1 (2.96), 18:0 (13.65), 18:1 (48.15), 18.2 (7.15), 18:3 (0.52), 20:0 (0.25), 20:1 (1.12).

BHT (Aldrich). GTK agar (Imuna). Ethanol (95%), *iso*-hexane, diethyl ether, chloroform, methanol (Lachema). All the solvents were distilled before use. Microorganisms: *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* (CM Brno).

Preparation of the extracts. Starting material (20–50 g) was 4 h successively extracted in 500ml Soxhlet extractor with 95% ethanol. The crude extract (obtained after evaporation of the solvent on rotary vacuum evaporator) was directly used for further experiments.

Column chromatography. Sage and rosemary extracts (2 g) were further fractionated on silica gel 100/40 (60 g) packed in a column (i.d. 3 cm, length 100 cm) using hexane: diethylether and finally 95% ethanol as eluants. Three fractions were obtained by stepwise gradient elution (200 ml of each) with 5, 15 and 50% of diethyl ether in isohexane, fourth fraction was eluted with 95% ethanol. The progress of the chromatography was monitored by TLC chromatography on silica gel plates (chloroform: methanol = 97:3 as eluant, UV detection).

Antioxidation activity. Rancimat method [6, 7] was used at the following conditions: 2.5 g of lard + extract (0.025–0.002% wt.), 100°C, air flow = 200 ml/min.

Antimicrobial activity. Three dimensional agar method [8] was used at 0; 0.1; 0.5; 1; 1.5; 2; 3 and 5% wt. of the extract in 95% ethanol.

RESULTS AND DISCUSSION

Antioxidant activity of extracts

Table 1 shows the antioxidant activity of the ethanol extracts and BHT in lard at the concentration 0.025% wt. The most effective extracts (rosemary and sage) were tested at the concentration 0.01% wt. because at the concentration 0.025% the induction period is too high (> 20 h) and side reactions in lard may occur at elevated temperature. Probably due its volatility BHT exhibits only moderate antioxidant activity at these conditions (100°C, air

Table 1. Inhibition of lard oxidation with 0.025% wt. of plant extracts

Plant extract	Induction period[1] (IP) (h)	Protection factor (IP/IP0)
Control – lard	2.90 = IP0	1.00
Thyme	3.20	1.10
Milfoil	3.88	1.34
Black currant meal	3.06	1.06
Red currant meal	4.95	1.71
Lady's mantle	4.12	1.42
Ginger	14.40	4.97
Hawthorn	3.69	1.27
Melissa	4.24	1.46
Agrimony	3.47	1.20
Horsetail	3.15	1.09
Chamomile	2.75	0.94
Stinging nettle	2.95	1.02
Black tea	8.00	2.76
Roasted coffee	6.00	2.07
Calendula	3.16	1.09
Rosemary[2]	9.87	3.40
Sage[2]	7.70	2.66
BHT	11.45	3.95

[1]mean of the two determinations, [2]determined at 0.01% concentration of the extract

flow = 200 ml/min.). Some extracts contain less volatile flavonoid antioxidants and their observed antioxidant activity is comparable to BHT one (ginger, rosemary, sage). The problem is their thermal decomposition. The crude extracts are not suitable as food antioxidants directly, because they contain dyes or heavy metals which accelerate oxidation of fats (generation of active oxygen species). This effect was observed for stinging nettle rosemary, sage and chamomile extracts. Crude rosemary and sage extracts are very effective antioxidants. Their activity was further enhanced after separation of undesirable prooxidants by column chromatography. Figure 1 shows the antioxidant activity of rosemary chromatography fractions. The most effective was second fraction followed by third fraction. The fourth fraction exhibits prooxidative effect and first fraction is inactive. The efficiency of this second fraction (8.3 h at 0.002% wt.) is comparable with BHT at 12.5 times higher concentration (IP = 11.45 h at 0.025% wt.). The similar results we obtained for sage extracts' fractions (Figure 2). Here the prooxidants were first and fourth chromatography fractions.

Antimicrobial activity of extracts

It is known that phenolic antioxidants in food inhibit the microbial growth [9]. With the use of three dimensional agar method all the extracts were tested against four microorganisms that can be found in common food and cosmetic products. The selected results of assays by this method are summarized in Table 2. Extracts containing polyphenolic compounds (rosemary, sage and their chromatography fractions) were effective

Table 2. Antimicrobial properties of 5% ethanolic solution of selected plant extracts expressed as inhibition zone (mm)

Plant extract	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Black tea	1.55	1.55	1.18	1.15
Agrimony	5.83	0	0	3.75
Rosemary	3.20	0	0	5.60
Rosemary 2. fr.	3.90	0	0	6.30
Sage	4.25	0	0	6.28
Sage 2. fr.	3.13	0	0	5.18
Sage 3. fr.	5.50	0	0	4.78

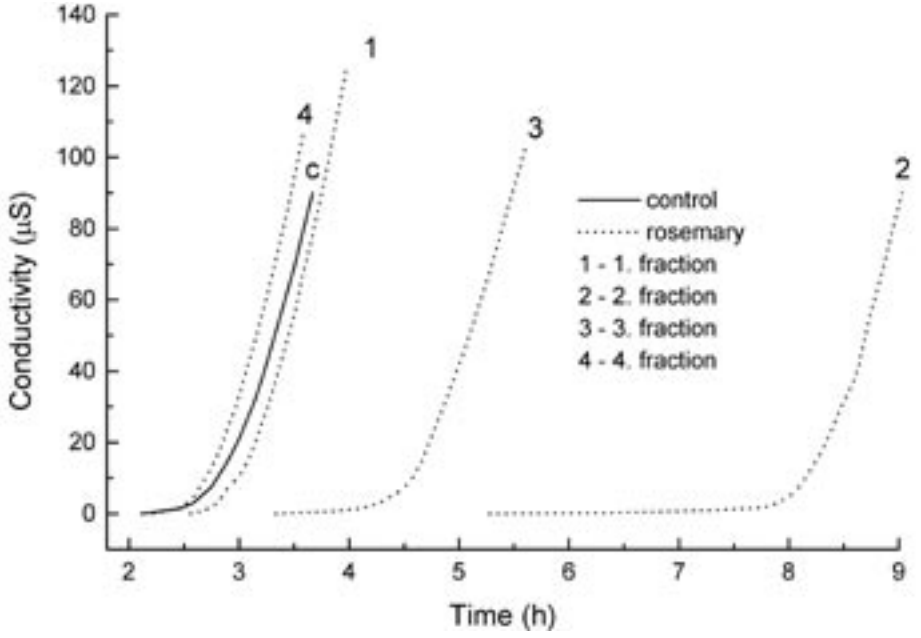

Figure 1. Oxidative stability of lard with fractions (0.002% wt.) from the chromatography of rosemary extract

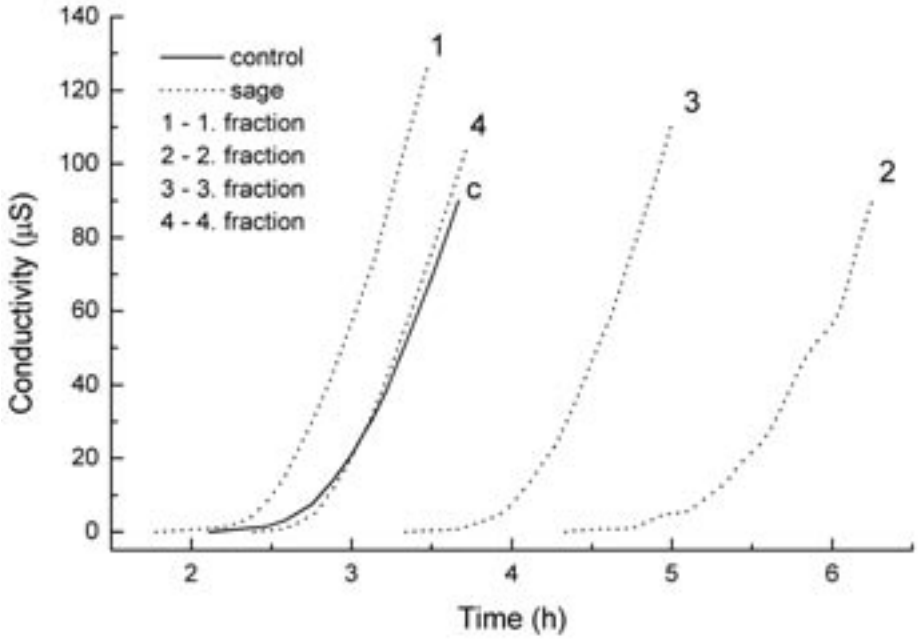

Figure 2. Oxidative stability of lard with fractions (0.002% wt.) from the chromatography of sage extract

against *Bacillus subtilis* and *Staphylococcus aureus*, black tea extract inhibited the growth of all four microorganisms. The good relationship between their antioxidant and antimicrobial activities is probably due to polyphenols' content in these extracts. Good antimicrobial and weak antioxidant activities of agrimony extract show that there are some other effective constituents present in it.

CONCLUSIONS

This work confirmed the close relation between antioxidative and antimicrobial efficiency of extracts.

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