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Antioxidative activity of sage (*Salvia officinalis* L.), savory (*Satureja hortensis* L.) and borage (*Borago officinalis* L.) extracts in rapeseed oil

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The antioxidant activity (AA) of acetone oleoresins (AcO) and deodorised acetone extracts (DAE) of sage (*Salvia officinalis* L.), savory (*Satureja hortensis* L.) and borage (*Borago officinalis* L.) were tested in refined, bleached and deodorised rapeseed oil applying the Schaal Oven Test and weight gain methods at 80 °C and the Rancimat method at 120 °C. The additives (0.1 wt-%) of plant extracts stabilised rapeseed oil efficiently against its autoxidation; their effect was higher than that of the synthetic antioxidant butylated hydroxytoluene (0.02%). AcO and DAE obtained from the same herbal material extracted a different AA. The activity of sage and borage DAE was lower than that of AcO obtained from the same herb, whereas the AA of savory DAE was higher than that of savory AcO. The effect of the extracts on the oil oxidation rate measured by the Rancimat method was less significant. In that case higher concentrations (0.5 wt-%) of sage and savory AcO were needed to achieve a more distinct oil stabilisation.

Keywords: Antioxidant activity, sage, savory, borage, acetone oleoresin, deodorised acetone extract, rapeseed oil.

1 Introduction

Lipid oxidation is a major cause for the deterioration of fat-containing food. Furthermore, it initiates other undesirable changes in food affecting its nutritional quality, wholesomeness, safety, colour, flavour, and texture. Autoxidation of polyunsaturated lipids involves a free radical chain reaction that is generally initiated by exposure of the lipids to light, heat, ionising radiation, metal ions, or metalloprotein catalysts. Therefore the inhibition of free radical autoxidation by antioxidants is of great practical importance in preserving polyunsaturated lipids from deterioration. Synthetic compounds, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and *tert*-butylhydroquinone (TBHQ) are widely used antioxidants due to their low cost, high stability and effectiveness. However, possible toxicological side effects of synthetic antioxidants on human health were reported [1]. In addition, the general trend towards reducing the use of synthetic food additives resulted in an expansion of the search for natural substances possessing antioxidative properties [2–7].

Various spices, aromatic and medicinal herbs as well as other plants can accumulate significant amounts of strong antioxidative compounds; therefore their use in processed

foods is a promising alternative to synthetic antioxidants [8]. Natural products isolated from spices and herbs can act as antioxidants either solely or synergistically in mixtures with other natural and/or synthetic additives [9].

Most investigations of the antioxidant properties of plants were performed using different model systems. The spices were evaluated either as whole ground plant material or as their crude and purified extracts. An early study of *Chipault* et al. demonstrated the antioxidant activity of 32 spices and herbs and their solvent extracts both in edible oils and in oil-in-water emulsions [10]. The authors found that rosemary and sage possess a distinctive antioxidant activity. Prior to practical use in the food industry, any spice or its extract should be comprehensively tested in the real food under practical storage conditions; a three-step procedure was recently suggested for such testing [8].

Most studies are focused on testing the antioxidative effects of rosemary and its extracts. Dried rosemary is a widely used herb in processed foods both in flavouring and lipid stabilisation [11]. However, the strong and characteristic flavour of rosemary is a limiting factor for its use regardless of its well-established and very high antioxidative power. For instance, the flavour of rosemary is more intensive and therefore more easily detectable in lower concentrations in meatballs than that of summer savory, Chilean oregano and sage. This observation increases the interest in other herbs and spices, since some of them remain sensorically more acceptable when added in larger amounts [8].

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The antioxidative activity (AA) of some other Labiatae herbs, particularly sage (*Salvia officinalis* L.) is also well documented [4, 5, 7, 9, 12–17]. However, some aromatic, spicy and medicinal plants, including summer savory (*Satureja hortensis* L.) and borage (*Borago officinalis* L.) were less intensively studied. Madsen et al. [8] reported the AA of summer savory in a model system consisting of meat and an oil-in-water emulsion dressing. They found that the AA of rosemary in meat was only slightly higher than that of summer savory [18]. Borage seeds were studied mainly as a natural source of γ -linolenic acid. Recently Wettasinghe and Shahidi reported about the antioxidant and free radical-scavenging properties of extracts obtained from borage meal [19, 20].

The objective of this study was to assess and compare antioxidative properties of acetone oleoresins and deodorised acetone extracts of sage, savory and borage leaves in rapeseed oil by using different methods.

2 Materials and methods

2.1 Materials

Sage, savory and borage leaves were obtained from the collection of aromatic plants of the Lithuanian Institute of Horticulture in 1998. All herbs were harvested during the full flowering period. The leaves were dried at 30 ± 2 °C in a ventilated oven Vasara (*Utenos krosnys*, Utena, Lithuania). Joint Stock Company “Obeliu Aliejus” (Obeliai, Lithuania) donated fresh, fully refined, deodorised rapeseed oil, containing no synthetic antioxidants. The rapeseed oil had an initial peroxide value (PV) of 4.5 meq kg^{-1} , an erucic acid content of 0.5%, a linolenic acid content of 9.8%, and a total tocopherols content of 767 mg kg^{-1} (α -tocopherol = 176 mg kg^{-1} ; γ -tocopherol = 537 mg kg^{-1} as determined by HPLC). Rapeseed oil “Rapso” from VOG AG Company (Linz, Austria) containing 9.1% of linolenic acid and 300 mg kg^{-1} of tocopherols was used for Rancimat tests.

The following chemicals were used: 2,6-di-*tert*-butylhydroxytoluene (BHT) (Aldrich, Steinheim, Germany), acetone (pure, OBR PR, Plock, Poland), hexane (analytical grade, Merck, Darmstadt, Germany), sodium thiosulphate (Sigma, Deisenhofen, Germany), chloroform (pharm. grade), acetic acid (98%, pure) and potassium iodide (all from Lachema, Neratovice, Czech Republic).

2.2 Preparation of herb oleoresins and deodorised extracts

Acetone oleoresins (AcO) were obtained by extracting 15 g of ground material with 900 ml of acetone in a Soxhlet extractor during 6 h. Afterwards the solvent was evaporated in a R114 rotavapour apparatus using a

B480 water bath (60 °C) and a B169 vacuum pump (all from Büchi, Donau, Switzerland). Although various solvents were previously used to isolate active antioxidative substances, acetone frequently proved to be the most efficient solvent for the extraction of antioxidative substances from sage and rosemary [21–23]. The extracts were finally dried in a SPT 200 vacuum drier (Horyzont, Krakow, Poland) at 25 ± 2 °C and 0.08 MPa. The following yields of the extracts were obtained: borage AcO – 7.16 wt-%, DAE – 3.7 wt-%; savory AcO – 14.4 wt-%, DAE – 7.9 wt-%; sage AcO – 22 wt-%, DAE – 20 wt-%. Dry extracts were stored in a freezer below -18 °C until use.

For the preparation of deodorised acetone extracts (DAE), the essential oils were removed by hydrodistillation of the ground herb in a Clevenger type apparatus for 3 h, the liquid was decanted, and the residue was dried at 30 °C in a ventilated drying oven. The dry residue was extracted with acetone as described above.

2.3 Preparation of oil samples

Rapeseed oil stability and consequently the AA of the extracts were assessed applying the Schaal Oven Test [23] and weight-gain methods [21]. The samples were kept at 80 °C in the dark. Three replications were performed for every sample.

The extract was added directly to 80.0 g of rapeseed oil at a concentration of 1000 ppm (0.1 wt-%) and dissolved by using an ultrasonic bath (ASTRA-SON™, model 9HT, Heat Systems Ultrasonics, NY, USA). Afterwards, three aliquots, 25.0 g each, were weighed into 150 ml glass beakers for further analysis. BHT at a concentration of 200 ppm (0.02 wt-%) and sage oleoresin (AcO) were used for comparison purposes. The samples were stored in a forced air supply thermostat oven (HS 122A ZPA, Chirana, Praha, Czechoslovakia) at 80 °C for 85 h in case of the Schaal Oven Test and for 150.5 h to apply weight-gain methods. A blank sample was prepared under the same conditions without any additive.

2.4 Methods of assessing antioxidant activity

The Schaal Oven Test, also referred to as an Oven Test, involves heating the sample at elevated temperatures until it becomes rancid by smell or taste or reaches a suitable end-point based on the peroxide value (PV), the conjugated diene or carbonyl value [3]. PV was determined by the Cd 8–53 method of the American Oil Chemist's Society [24]. The induction period (IP) was expressed as the time during which PV increased to 20 meq kg^{-1} [25]. Ultraviolet absorption (UV) was measured by the IUPAC method 2.505 (ISO 3656 method) [26].

The weight-gain method, designated as oxygen absorption method, is based on measuring the increase of an oil sample weight due to oxygen binding in the course of lipid oxidation [21]. In that case IP was determined when the weight of the oil sample increased by 0.2%. The relative activity of the antioxidants was expressed by the protection factor (PF) which is calculated by dividing the IP of rapeseed oil with added antioxidant by the IP of the blank oil sample.

The AAs of the extracts were also assessed by the Rancimat method at 120 °C in the Rancimat 679 apparatus (Metrohm AG, Herisau, Switzerland). The oxidation process was followed in 5 ± 0.0001 g oil samples at the air velocity of 20 l h^{-1} . The extracts were added at 120 °C by slow mixing. In the course of an oil oxidation the curves of oxidation are drawn automatically and the apparatus measures the IP. The PF was calculated as described above.

The following scale has been proposed for the assessment of the antioxidant power depending on the PF values: 1.0–1.5 (very low), 1.5–2.0 (low), 2.0–2.5 (medium), 2.5–3.0 (high), >3.0 (very high) [27]. Three replicates were analysed; the standard deviations were in the range of 0.01–10% for the PV method, 0.01–2% for UV and 0.01–0.1% for the weight increase method.

3 Results and discussion

3.1 Effect of herb extracts on the oil peroxidation

The curves in Fig. 1 demonstrate PV changes in rapeseed oil with different extracts. Derivative characteristics, IP and PF are presented in Tab. 1. It should be mentioned that the PV is a widely used measure of the primary lipid oxidation indicating the amount of peroxides formed in fats and oils during oxidation. Rapeseed oil oxidation was measured at timed periods during 35.3 h of storage. During this time the PV of the blank sample (1) increased to 173 meq kg^{-1} .

The results given in Fig. 1 and Tab. 1 show that all extracts reduced the oxidation rate of rapeseed oil at 80 °C in terms of formation of peroxides. As assessed by PF and IP (Tab. 1), the stability of the samples with 0.1% plant extract additives was considerably higher than that of the samples with 0.02% BHT. It is known, that the effectiveness of antioxidants varies depending on the food and on the processing and storage conditions. BHT is very effective in animal fats but less effective in vegetable oils; BHT may also be lost during heating because of its volatility. TBHQ is known to be a very effective antioxidant for vegetable oils, and it is more stable at high temperatures than BHT [28]. Most likely, plant antioxidant substances are more effective than the BHT at a high tem-

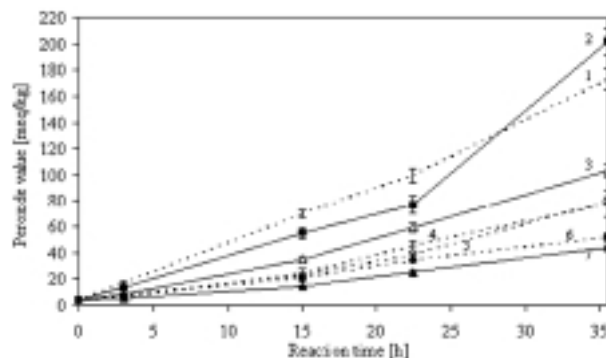


Fig. 1. Effect of plant extracts on the formation of peroxides in rapeseed oil at 80 °C (1 – blank, 2 – BHT 0.02%, 3 – savory AcO 0.1%, 4 – borage DAE 0.1%, 5 – sage DAE 0.1%, 6 – savory DAE 0.1%, 7 – sage AcO 0.1%).

Tab. 1. Antioxidant characteristics of plant extracts in rapeseed oil calculated on the basis of peroxide value measurements.

Additive	Added amount [%]	IP [h]	PF	PF scale*
Without additive (blank)	–	3.80	–	–
BHT	0.02	5.90	1.55	–
Sage AO	0.1	19.10	5.03	very high
Sage DAE	0.1	13.20	3.47	very high
Savory AO	0.1	8.60	2.26	medium
Savory DAE	0.1	14.40	3.79	very high
Borage DAE	0.1	12.75	3.36	very high

* PF assessment according to [27].

perature. In terms of retarding the formation of primary oxidation products the effectiveness of the extracts added at concentrations of 0.1% can be put into the following order: sage AcO > savory DAE > sage DAE > borage DAE > savory AcO. The AA of the all plant extract additives can be assessed as “very high” (PF >3), save savory AcO (3), which is ranked as a “medium” strength (PF = 2.26) antioxidant. It is worth noting that the AA of savory DAE (6) (PF = 3.9) was especially high, it possessed only a slightly lower AA than sage AcO (7) (PF = 5.03). Antioxidant properties of sage are well documented. It is also interesting to note that with regard to the rate of peroxide formation the activity of sage DAE (5) (PF = 3.47) was approximately 1.5 times lower than that of sage AcO.

Several factors can induce changes in the AA during the distillation of volatile constituents from the herbs. First, DAE does not contain essential oil components that are present in AcO. It was reported that such sage volatile

constituents as thujone, camphor, α -humulene, borneol, bornyl acetate, camphene, α - and β -pinene, β -caryophyllene, and aromadendrene possess some AA [29]. Secondly, volatile and non-volatile compounds, both possessing antioxidant and prooxidant properties can be present in the plants in the form of adjuncts with other molecules, *e. g.* glycosides. Such compounds can be released by hydrolysis or other cleavage processes taking place during hydrodistillation and consequently those compounds participate in the lipid oxidation processes [30]. And finally, heat- and water-induced chemical reactions can also change the activity of a complex extract system consisting of numerous compounds with different chemical and physical properties. The differences in the AA of savory AcO (3) and DAE (6) were quite significant as well. However, with regard to the rate of peroxide formation the activity of savory DAE (PF = 3.79) was approximately 1.7 times higher that of savory AcO (PF = 2.26). The essential oil of savory contains antioxidative compounds, namely carvacrol, thymol, β -caryophyllene, γ -terpinene, *p*-cymene, together with linalool, which was reported to possess a strong prooxidant effect [30]. However, it is difficult to conclude which of the three above-mentioned reasons is the most important one in increasing the AA of savory extract or in decreasing the AA of sage extract after removal of the essential oil from the whole herb material.

3.2 Effect of herb extracts on the UV absorbance in the oxidised oil

The effect of the extracts on the formation of primary and secondary oxidation products was assessed by measuring the changes in the UV absorption values at 232 and 268 nm during 76.5 h of rapeseed oil storage at 80 °C [26]. The absorbance at 232 nm as well as the PV, indicate the degree of primary oxidation and hence the for-

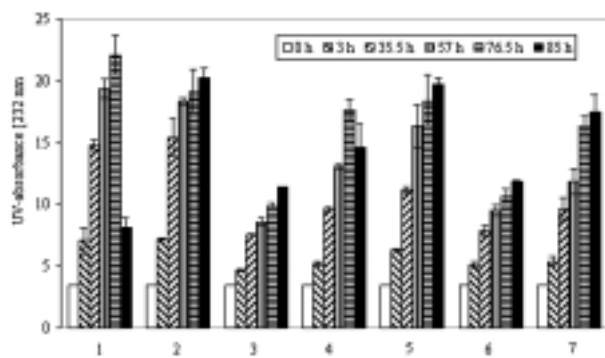


Fig. 2. Effect of plant extracts on the UV absorbance at 232 nm (conjugated dienes) in rapeseed oil at 80 °C (1 – blank, 2 – BHT 0.02%, 3 – sage AcO 0.1%, 4 – sage DAE 0.1%, 5 – savory AcO 0.1%, 6 – savory DAE 0.1%, 7 – borage DAE 0.1%).

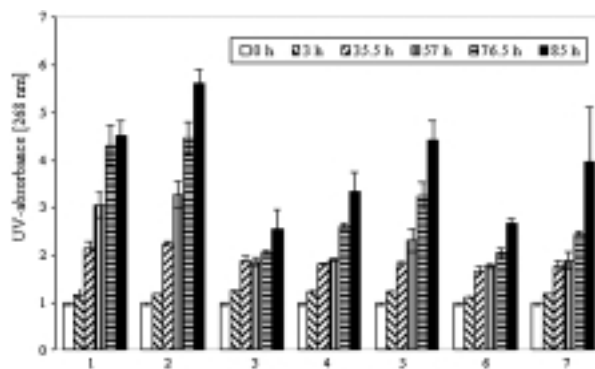


Fig. 3. Effect of plant extracts on the UV absorbance at 268 nm (conjugated trienes) in rapeseed oil at 80 °C (1 – blank, 2 – BHT 0.02%, 3 – sage AcO 0.1%, 4 – sage DAE 0.1%, 5 – savory AcO 0.1%, 6 – savory DAE 0.1%, 7 – borage DAE 0.1%).

mation of primary oxidation products such as conjugated dienes. The determination of conjugated dienes by UV spectrophotometry is related to the content of polyunsaturated hydroperoxides which are flavour precursors of oxidised lipids [3]. In general, the results obtained by UV absorption (Fig. 2) were closely correlated with the PVs.

The formation of secondary oxidation products, such as aldehydes and ketones, correlates with UV absorption at 268 nm. Fig. 3 shows that all measured samples with plant extract additives start to absorb more intensively after approximately 70 h of storage at 80 °C. The respective time for the blank sample and the sample with BHT was 30 h indicating the formation of secondary degradation products in the rapeseed oil. The changes in UV absorption at 268 nm which the samples showed were similar to those displayed by peroxides and conjugated dienes.

3.3 Effect of herb extracts on the oxygen binding rate during oil oxidation

Oxygen binding was determined by weighing rapeseed oil samples stored at 80 °C at timed periods during 150.5 h [21]. The curves of oxidation kinetics obtained by this method are provided in Fig. 4. The oxygen binding method also proved that all the investigated plant extracts possess an AA (Tab. 2).

The effect of the extracts added at a concentration of 0.1% on retarding oxygen binding by the rapeseed oil can be put into the following order: borage AcO > savory DAE > borage DAE > sage AcO > sage DAE > savory AcO. The plant extract additives exhibited a “very high” AA (PF>3) except for savory AcO (3). The effect of borage AcO (8) (PF = 6.75) on the retarding oxygen binding by the rapeseed oil was higher than that of borage

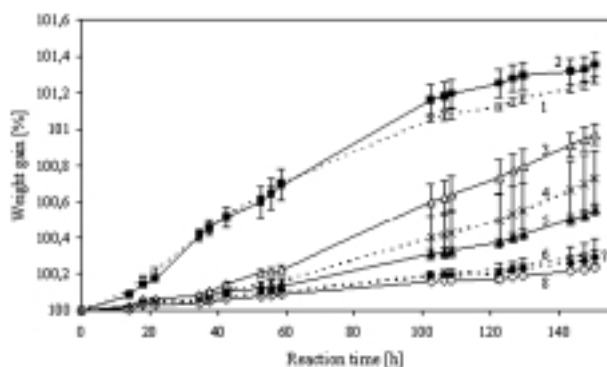


Fig. 4. Effect of plant extracts on the oxygen binding in rapeseed oil at 80 °C (1 – blank, 2 – BHT 0.02%, 3 – savory AcO 0.1%, 4 – sage DAE 0.1%, 5 – sage AcO 0.1%, 6 – borage DAE 0.1%, 7 – savory DAE 0.1%, 8 – borage AcO 0.1%).

Tab. 2. Antioxidant characteristics of plant extracts in rapeseed oil calculated on the basis of oxygen binding.

Additive	Added amount [%]	IP [h]	PF	PF scale*
Without additive (blank)	–	20.0	–	–
BHT	0.02	23.0	1.15	–
Sage AO	0.1	75.0	3.75	very high
Sage DAE	0.1	66.0	3.30	very high
Savory AO	0.1	51.5	2.57	high
Savory DAE	0.1	122.0	6.10	very high
Borage AO	0.1	135.0	6.75	very high
Borage DAE	0.1	107.5	5.37	very high

* PF assessment according to [27].

DAE (6) (PF = 5.37). The results obtained by this method (Tab. 2, Fig. 4) corresponded to the PVs and the UV absorbance data (Tab. 1, Figs. 1–3). The rate of weight increase was very low and consequently the inhibition of oxygen binding was very high in the samples with savory DAE (PF = 6.1), borage AcO (PF = 6.75) and DAE (PF = 5.37). It is worth noting, that some differences between weight-gain, PVs and UV data were observed. For instance, employing the weight-gain method the three above-mentioned extracts proved to be more effective than sage AcO (PF = 3.75), which was the strongest antioxidant according to PV and UV tests. It was reported that the weight-gain test is not sufficiently sensitive and the end-point is questionable because it requires too high a level of oxidation. Nevertheless, that is beside the point when flavour deterioration occurs and especially when vegetable oils containing linolenic acid and fish oils containing *n*-3 polyunsaturated fatty acids are concerned [3].

3.4 Effect of herb extracts on the oil oxidation assessed by Rancimat method

The AAs of the extracts obtained by the Rancimat method are summarised in Tab. 3. That method is often used to assess the stability of edible oils at high temperatures, e.g. 100–120 °C, that are more preferable for secondary oxidation reactions. The rate of peroxide formation at such temperatures becomes more dependent on the oxygen concentration due to the decrease of the solubility of the peroxides in the oil [31, 32]. The effect natural additives exert on the IP as determined by the Rancimat method was significantly lower at 120 °C than that determined at 80 °C employing the other used methods. It was reported that tocopherols at naturally occurring concentrations had no influence on the induction time in the Rancimat measurements [33]. A comparatively high concentration of sage AcO (0.5 wt-%) was required to achieve a “medium” rapeseed oil stabilisation effect. This result is in agreement with the results obtained by other authors. For instance, *Aruoma et al.* reported, that plants such as sage, thyme, oregano, rosemary, ginger extracts showed higher antioxidant indices in animal fats than in vegetable oils when tested with the Rancimat method at 110 °C [4]. Some increase in the stability was achieved by increasing the concentration of savory AcO to 0.5 wt-%, while borage additives were not effective at all the concentrations applied. It can be supposed that the extracts are less effective at higher temperature, however, most likely, the main reason for the differences in the AAs of the herb extracts are related to the differences in the methodical principles. Therefore, regarding the complexity of the lipid oxidation process the use of different methods for its assessment can give more comprehensive information,

Tab. 3. Antioxidant characteristics of plant extracts in rapeseed oil calculated on the basis of the Rancimat method.

Additive	Added amount [%]	IP [h]	PF	PF scale*
Without additive (blank)	–	5.95	–	–
Sage AO	0.05	6.65	1.12	very low
Sage AO	0.1	9.97	1.68	low
Sage AO	0.5	14.20	2.39	medium
Savory AO	0.05	5.97	1.00	very low
Savory AO	0.1	6.33	1.06	very low
Savory AO	0.5	7.65	1.29	very low
Borage AO	0.05	6.66	1.12	very low
Borage AO	0.1	6.15	1.03	very low
Borage AO	0.5	6.80	1.14	very low

* PF assessment according to [27].

especially when the effectiveness of multicomponent natural extracts is investigated.

4 Conclusion

It can be concluded that herbal oleoresins and deodorised extracts of sage (*Salvia officinalis*), savory (*Satureja hortensis*) and borage (*Borago officinalis*) at concentrations of 0.1% were effective in stabilising rapeseed oil during storage at 80 °C. The AA effect of herbs was less significant when the oil was analysed by the Rancimat method at 120 °C. The differences in the AA between AcO and DAE obtained from the same herbal material were determined and, in case of savory, DAE was the more effective antioxidant than AcO. It suggests that for practical applications the possible deodorisation of herbs by removing their volatile oil can be considered. For the first time a strong AA of crude acetone extracts from borage leaves has been reported. This makes a strong impact on expanding the investigations of antioxidant constituents present in borage and being able to protect the oil against oxidation.

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