10.1515/umcschem-2015-0020

ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. LXX, 2 SECTIO AA 2015

The influence of water on the estimation of antioxidant properties of essential oils

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This study discusses the influence of water content in measuring system on the estimation of antioxidant activity of essential oils. The presented data show that the antioxidant activity of thyme, clove, summer savory and basil essential oils strongly depends on water concentration what has important impact on the estimation of correct and reliable antioxidant activity of examined essential oils.

Keywords: antioxidant activity, essential oils, water impact.

1. INTRODUCTION

Currently known negative action of free radicals on the human and animals implicates more and more interest in research concerning antioxidant activity of substances which prevent living organism from the damaging influence of these reactive species. Hazardous action of free radicals results from their oxidation activity towards biomolecules, e.g. proteins, amino acids, lipids or DNA [1–4]. This process frequently leads to the injury of cell and its death. The auto-oxidation process of lipids initiated by free radicals has been also recognized as a major process of food deterioration [5]. During this process, the sensory and nutritional quality of foods is lost [6]. The negative activity of free radicals is eliminated

and reduced by the application of antioxidants, i.e. compounds inhibiting the oxidation process in living organisms and in fat-based foods [7].

A lot of antioxidants are used in medicine and industry. Generally, they are divided into two groups: natural and synthetic. The second group is widely used as food additives to provide protection against oxidative degradation of foods. Recently performed toxicological studies have shown, however, that some synthetic antioxidants, e.g butylhydroxy-toluene, butylhydroxyanisole, propyl gallate or tert – butylhydroquinone, cause side effects [8–10]. Such findings results in growing interest of researchers and consumers in the antioxidant properties of natural compounds. Some attention has been paid to essential oils, known since the middle ages not only due to their pleasant or unpleasant aroma but also due to their antibacterial, antifungal, anti-inflammatory and antioxidant activity [11–13].

They are a number of methods for measuring the efficiency of antioxidants [14]. Irrespective of the applied method, a lack of correlation between antioxidant activities determined on the same material using different assays is very often observed in literature [15]. Moreover, antioxidant activities of the same compound estimated by the same assay in various laboratories are also frequently different [16–17]. These statements are also true in the case of antioxidant properties of essential oils. The differences in antioxidant properties of given essential oil can be caused by essential oil contaminants which do not exhibit antioxidant activities but affect the result of the antioxidant activity estimation. As results from [18–19], the estimation of BHT antioxidant properties is strongly influenced by concentration of water in measuring system. It is obvious, that water is main contaminant of essential oils prepared by steam distillation process. Hence, the question concerning the influence of water content on the estimation of the antioxidant activity of essential oils arises in a natural way. The present study discusses the differences in the antioxidant activity of chosen essential oils estimated by the ABTS and DPPH methods in the systems differing in water content.

2. EXPERIMENTAL

2.1. Materials and reagents

Thyme, summer savory, basil and clove buds essential oils were obtained by means of steam distillation. Thyme and summer savory, cultivated in eastern Poland, were purchased from a local herb planter.

Basil, clove buds were purchased at a local market. The herbs were air-dried, cut and stored at +8°C. Immediately before essential oil isolation, an appropriate amount of plant material was ground and its exactly weighed portion was subjected to the distillation process.

2,2'- diphenyl-1- picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate (di-potassium peroxdisulfate) were purchased from Sigma Aldrich (Poznań, Poland). Methanol was purchased from the Polish Chemical Plant - POCh (Gliwice, Poland). Water was purified on a Milli-Q system from Millipore (Millipore, Bedford, MA, USA).

2.2. Steam distillation

Steam distillation process was performed for 3 h applying the Deryng apparatus, a Clevenger - type apparatus described in detail in the Polish Pharmacopea V, which contained a plant sample (50 g) and 600 cm^3 of water. The distillation time was measured after the fall of the first drop of the distillate. The separated essential oils were dried by freezing and, after filtration, stored at +4°C until further experiments.

2.3. Solutions of essential oils

The solutions of essential oil from: thyme, clove, summer savory and basil were prepared dissolving 60 mm^3 of a given essential oil in 5 cm^3 of methanol. Before antioxidant measurements, the obtained solutions were diluted 1:20 in the same solvent.

2.4. Methods

The antioxidant activity of the examined solution of essential oils was determined by DPPH and ABTS method. The scavenging of radicals by potential antioxidant was measured for these methods.

2.4.1. DPPH method

An aliquot (2940 mm^3) of methanolic $DPPH^{\bullet}$ solution (24 $\mu g/cm^3$) was mixed in a 4 cm^3 test tube with essential oil solution (60 mm^3). Before measurement, each mixture was vigorously shaken during 30 s and immediately transferred into a quartz cuvette (1 cm × 1cm × 3.5 cm). The decrease in absorbance at 516 nm was registered in continuous manner during 60 minutes employing a UV Probe-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Subsequent readings were taken at regular intervals (60 s).

The inhibition percent (I) was calculated according to the following equation [20]:

$$I(\%) = \left(1 - \frac{A_t}{A_{t=0}}\right) \cdot 100\%$$

where $A_{t=0}$ and A_t are the values of absorbance of $DPPH^{\bullet}$ at 0 min and at time equal to (t) min, respectively. The inhibition percent was estimated after 60 min essential oils/ $DPPH^{\bullet}$ reaction.

2.4.2. ABTS assay

Generation of ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium] radical cation was performed according to Ref. [21]. The $ABTS^{\bullet+}$ solution was prepared by the reaction of 5 ml of a 7 mM aqueous ABTS solution and 88 mm^3 of 140 mM (2.45 mM final concentration) potassium persulfate ($K_2S_2O_8$) solution. The mixture was incubated in the dark for 16 h. The formed radical cation was then diluted in methanol until the initial absorbance value of 0.7 at 744 nm was reached.

2000 mm^3 of the prepared ABTS radical cation solution was mixed in a 4 cm^3 test tube with essential oil solution or main essential oil component solution (20 mm^3). The reaction mixture was stirred vigorously for 30 seconds and poured into cuvettes (1cm × 1 cm × 3.5 cm). The decrease in absorbance was recorded in a continuous manner during 60 minutes at 744 nm employing UV Probe-1800 spectrophotometer (Shimadzu, Japan).

Inhibition percent (I) was calculated from the following equation:

$$I(\%) = \left(1 - \frac{A_t}{A_{t=0}}\right) \cdot 100\%$$

where $A_{t=0}$ and A_t are the values of absorbance of $ABTS^{\bullet+}$ at 0 min and at time equal to (t) min, respectively. The inhibition percent was estimated after 60 min essential oils/ $ABTS^{\bullet+}$ reaction.

2.5. The influence of water concentration on the antioxidant activity of examined essential oils

The systems described in Table 1 and Table 2 were used for the estimation of the influence of water on the antioxidant activity of examined essential oils by DPPH and ABTS methods, respectively. The

inhibition percents (I) were calculated following the procedure described above.

Table 1. Volume (in mm^3) of the individual components used for formation of the examined systems

System component	System number						
	1	2	3	4	5	6	7
$DPPH^{\bullet}$ in MeOH[1]	2000	2000	2000	2000	2000	2000	2000
Methanolic solution of essential oil [2]	60	60	60	60	60	60	60
MeOH	940	930	920	910	890	860	840
Water	–	10	20	30	50	80	100
Total volume	3000						

1 – the concentration of methanolic solution of $DPPH^{\bullet}$ equals 0.024 mg/cm^3,
2 – the concentration of methanolic solution of examined essential oils equals 0.5 mg/cm^3.

Table 2. Volume (in mm^3) of the individual components used for formation of the examined systems.

System component	System number							
	1	2	3	4	5	6	7	8
$ABTS^{*+}$ in MeOH	2000	2000	2000	2000	2000	2000	2000	2000
Methanolic solution of essential oil [1]	20	20	20	20	20	20	20	20
MeOH	80	70	60	50	40	30	20	0
Water	0	10	20	30	40	50	60	80
Total volume	2100							

1 – the concentration of methanolic solution of examined essential oils equals 0.5 mg/cm^3.

3. RESULTS AND DISCUSSION

The antioxidant activity of essential oils estimated by different methods are discussed in many paper. In most cases the antioxidant properties of examined essential oils are largely related to differences in their quantitative and qualitative compositions. The influence of another factors, presence in essential oils (water content, metal ions or hydrogen ions) is generally omitted. Because in literature lack information about the influence of water on the antioxidant activity of essential oils, in this paper this impact was determined. The difference in reaction rates for the essential oils/radical system with and without water was labeled as (ΔI). Figure 1 presents the method of ΔI calculation in these experiments.

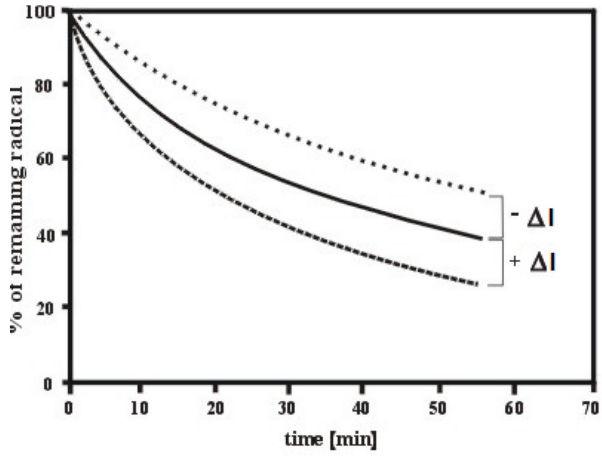

Fig. 1. The way of (ΔI) calculation. Solid line – kinetic curve in system containing only radical and pure antioxidant - reference system; dashed line – kinetic curve in system containing radical and antioxidant contaminated by component accelerating antioxidant/radical reaction rate; dotted line – kinetic curve in system containing radical and antioxidant contaminated by component decelerating antioxidant/radical reaction rate.

$DPPH^{\bullet}$ and $ABTS^{\bullet+}$, which were applied in these experiments, are two stable and colored free radicals that have been widely employed to determined antioxidant activity. $DPPH^{\bullet}$ is commercially available, whereas $ABTS^{\bullet+}$ must be generated during the oxidation of ABTS by oxidants such as $K_2S_2O_8$ or MnO_2. $DPPH^{\bullet}$ is a stable radical,

commercially available, with a deep purple colour whose reaction with other compounds leads to loss of colour at 516 nm. This radical is soluble only in organic media, while $ABTS^{\bullet+}$ is soluble in aqueous as well as in alcoholic media and its absorption at 744 nm is used. ABTS and DPPH radicals are neutralized by electron and/or hydrogen atom transfer from antioxidant to radical [22].

Figure 2 presents the influence of the water amount on the difference (ΔI) in reaction rates for the essential oils/$ABTS^{\bullet+}$ system with and without the water. ΔI values were estimated after 60 min of the essential oils/$ABTS^{\bullet+}$ reaction.

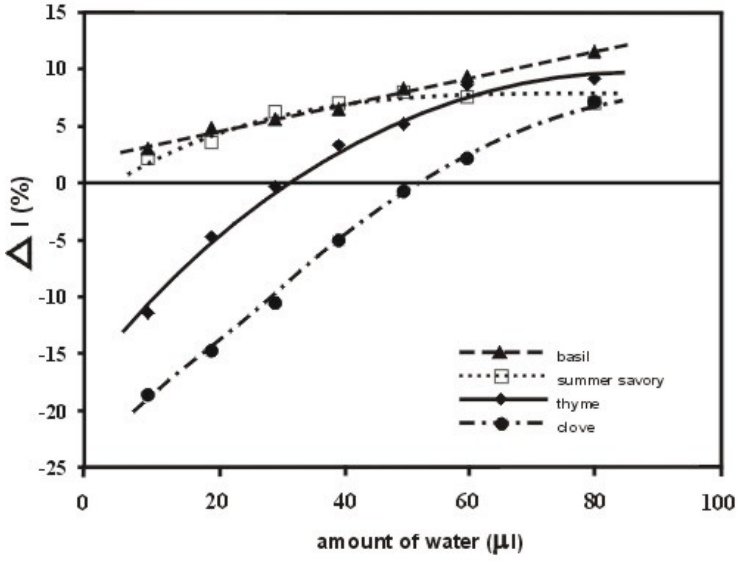

Fig. 2. The influence of water amount in the measuring systems on difference (ΔI) in reaction rates between examined essential oil and ABTS cation radical.

As results from Figure 2, in the case of thyme and clove essential oils, lower water concentration decelerates the essential oils/$ABTS^{\bullet+}$reaction (negative value of ΔI), whereas higher concentration accelerates this reaction (positive value of ΔI). In the case of summer savory and basil essential oils, the increase of water content results in an almost linear acceleration of the reaction kinetics over the whole range of water concentrations used. As was reported in [19], the increase of water content in system with BHT causes the increase of reaction rate of this standard antioxidant with ABTS cation radicals. The observed effect was explained by the structural changes in applied solvent (methanol) and/or

by the increase of the dissociation degree of the antioxidant resulting from the presence of water in measuring system. According to [19] the water molecules facilitate the transfer of electron and/or hydrogen from antioxidant to radical.

As it commonly known, essential oil is a complex mixture which, beside highly polar compounds, contains also less polar and nonpolar ones. In consequence, the explanation of the water presence influence on the essential oils/$ABTS^{\bullet+}$reaction rate in the same way as in the case of BHT/$ABTS^{\bullet+}$reaction rate seems to be insufficient and should be supplemented additionally by "polar paradox". The "polar paradox theory" states that polar antioxidants are more effective in less polar media, such as bulk oils, while nonpolar antioxidants are more effective in relatively more polar media, such as oil/water emulsions.

The influence of water volume on the differences (ΔI) in reaction rates for the essential oils/$DPPH^{\bullet}$ system with and without the water is presented in Figure 3.

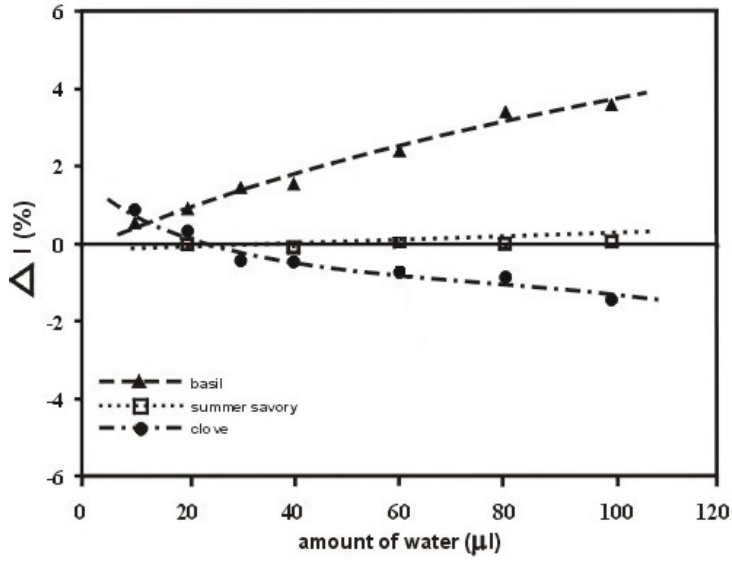

Fig. 3. The influence of water volume in the measuring systems on antioxidant activity of chosen essential oils estimated by DPPH method.

As results from the figure, the influence of water concentration increase on the reaction rate between essential oil and DPPH is different. In the case of basil essential oil the reaction rate acceleration is observed. For clove essential oil, the water concentration increase decelerates the rate of the reaction (ΔI changes from positive to negative values). The

influence of water concentration on reaction rate in summer savory/DPPH$^{\bullet}$ system is negligible. If the acceleration of the essential oils/DPPH$^{\bullet}$ reaction rate resulting from the water concentration increase can be explain in the same way as for essential oils/ABTS$^{\bullet+}$, i.e.:

- by the structural changes in applied solvent (methanol) or
- by the increase of the dissociation degree of the antioxidant resulting from the presence of water or
- by "polar paradox"

so far the decelerating influence of water on essential oils/DPPH$^{\bullet}$ reaction rate is difficult.

As results from literature [23, 24], DPPH radicals, in contrast to ABTS cation radicals, are soluble only in organic solvent. The presence of small water amounts in the measuring system promotes of DPPH$^{\bullet}$ recombination. Higher concentration of water results in a partial coagulation of DPPH radicals. Hence, the deceleration of clove essential oil/DPPH$^{\bullet}$ reaction rate with the increase of water content in measuring system can be connected with the formation of non-reactive DPPH conglomerates. It is probable that clove essential oil decreases DPPH solubility in system containing more and more water amount.

4. CONCLUSIONS

According to the presented results, the antioxidant activity of the examined essential oils estimated by ABTS and DPPH methods strongly depends on water content in measuring system. The influence of water on the reaction rate between essential oil and radical can be explained by:

- the structural changes in applied solvent (methanol),
- the increase of the dissociation degree of the antioxidant resulting from the presence of water,
- "polar paradox",
- the formation of non-reactive radical conglomerates,

The demonstrated relationships reveal the difficulty in estimating of antioxidant activity of essential oils which are complex mixtures of the mutually interacting components.

REFERENCES

[1] I. Fridovich, *Annals of the New York Academy of Sciences*, **893**, 13, (1999)
[2] L.J. Ignarro, G. Cirino, A. Castini, C. Napoli, *Journal of Cardiovascular Pharmacology*, **34**, 879, (1999)
[3] J.M. McCord, *The American Journal of Medical Sciences*, **108**, 652, (2000)
[4] M. Zheng, G. Storz, *Biochemical Pharmacology,* **59**, 1, (2000)
[5] N.V. Yanishlieva , E.M. Marinova, M.H. Gordon, V.G. Raneva, *Food Chemistry*, **64**, 59, (1999)
[6] B. Tepe, D. Daferera, A. Sokmen, M. Sokmen, M. Polissiou, *Food Chemistry*, **90**, 333, (2005)
[7] O. Politeo, M. Jukic, M. Milos, *Food Chemistry*, 101, 379, (2007)
[8] H. Zhang, F. Chen, X. Wang, H.Y. Yao, *Food Research International*, **39**, 833, (2006)
[9] B.M. Ames, *Science*, **221**, 1256, (1983)
[10] M. Namiki, *Critical Review in Food Science and Nutrition*, **29**, 237, (1990)
[11] Í. Gülçin, Í.G. Şat, Ş. Beydemir, Ö.İ. Elmastaş Küfrevioğlu, *Food Chemistry*, **87**, 393, (2004)
[12] F. Bakkali, S. Averbeck, D. Averbeck, M. Idaomar, *Food Chemical Toxicology,***46**, 446, (2008)
[13] T.A. Misharina, A.L. Samusenko, *Applied Biochemistry and Microbiology*,**45**, 482, (2008)
[14] M. Antolovich, P.D. Prenzler, E. Patsalides, S. McDonald, K. Robards, *The Analyst,* **127**,183, (2002)
[15] T. Juntachote, E. Berghofer*, Food Chemistry*, **92**, 193, (2005)
[16] A.T. Mata, C. Proença, A.R. Ferriera, M.L.M. Serralheiro, J.M.F. Nogueira, M.E.M. Araŭjo, *Food Chemistry*, **103**, 778, (2007)
[17] M.J.T.J. Arts, J.S. Dallinga, H.P. Voss, G.R.M.M. Haenen, A. Bast, *Food Chemistry*, 88, 567, (2004)
[18] A.L. Dawidowicz, D. Wianowska, M. Olszowy, *Food Chemistry*, **131**, 1037, (2012)
[19] A.L. Dawidowicz, M. Olszowy, *European Food Research and Technology*, **232**, 837, (2011)
[20] W. Brand-Williams, M.E. Cuvelier, C. Berset, *LWT-Food Science and Technology,***28**, 25, (1995)
[21] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang,

[22] C. Rice-Evans, *Free Radical Biology and Medicine,* **26**, 1231, (1999)
[23] C. López-Alarcon, A.Denicola, *Analytica Chimica Acta*, **763**, 1, (2013)
[24] I. Gülçin, *Archives of Toxicology*, **86**, 345,(2012).
[25] I.I. Koleva, H.A.G. Niederlănder & T.A. Van Beek, *Analytical Chemistry*, **72**, 2323, (2000).