CHEMISTRY & CHEMICAL TECHNOLOGY

Vol. 5, No. 2, 2011 Chemistry

Tamara Misharina, Margarita Terenina and Natalia Krikunova

INHIBITION OF 2-HEXENAL AUTOOXIDATION BY ESSENTIAL OILS FROM CLOVE BUD, LAUREL, CARDAMOM, NUTMEG AND MACE

Emanuel Institute of Biochemical Physics, Russian Academy of Sciences
4 Kosygina str., 119334 Moscow, Russia;
Tmish@rambler.ru

Received: November 27, 2009 / Revised: April 15, 2010 / Accepted: June 18, 2010

Abstract. The antioxidant properties and stability during the storage of hexane solutions of 5 individual essential oils from clove bud (*Caryophyllus aromaticus* L.), cardamom (*Elettaria cardamomum* L.), laurel (*Laurus nobilis* L.), nutmeg (*Myristica fragrans* Houtt.), and mace (*Myristica fragrans* Houtt) were studied by the method of capillary gas-liquid chromatography. We assessed the antioxidant properties by the efficiency of autooxidation inhibition of aliphatic aldehyde (*trans*-2-hexenal) into the corresponding carbon acid. The essential oil of clove bud had the maximal efficiency of inhibition of 2- hexenal oxidation (83 %), which did not depend on oil concentration in model solution. Antioxidant properties of essential oils of nutmeg, mace and laurel was connected with substituted phenols, and depended poorly on oils concentration in model systems. The stronger dependence of the antioxidant activity on the oil concentration in model solution was found for cardamom essential oil. We studied the changes in the essential oils composition during the storage of their hexane solutions for 100 days in the light and compared it with the stability of pure essential oils stored for a year.

Keywords: essential oils; inhibition, 2-hexenal, oxidation, aliphatic aldehyde.

1. Introduction

The aim of the work is to study and compare antioxidant properties of 5 essential oils containing substituted phenols in the model system of autooxidation of 2-hexenal, assess the influence of essential oils composition on their antioxidant activity, and to study the composition stability of essential oils during their autooxidation in solutions and in pure oils. This study is the continuation of the systematic investigation of antioxidant properties of natural essential oils biological activity.

Many spices and their essential oils have been recognized to have medicinal properties and possess many beneficial effects on health, such as antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial, hypolipidemic, antimutagenic, anticarcinogenic potential, *etc*. Essential oils of many plants possess an intensive and pleasant aroma [1-3]. Due to these properties essential oils have been actively used for a long time in different industries, mainly in perfumes (fragrances and aftershaves), in food (as flavorings and preservatives) and in pharmaceuticals [3, 4]. As a rule, essential oils are a complex mixture of organic substances with different functional groups, mainly terpenoids and substituted phenols. The composition of essential oils determines their organoleptic properties and biological activity including the antioxidant one [5-8]. The antioxidant activity of many essential oils is not surprising in view of the presence of phenol groups. It is well known that almost all phenols can function as antioxidants of lipid peroxidation because they trap the chain-carrying lipid peroxyl radicals [9-11]. Plant phenolics are multifunctional and can act as reducing agents, hydrogen-donating antioxidants and singlet-oxygen quenchers [12-14]. As a rule, antioxidant activity of essential oils is higher than of their individual components. This fact indicates the existence of synergetic effects due to the complex composition of oils [15, 19, 20].

For the estimation of antioxidant properties of substances or their mixtures, many different methods are used. It was showed that the value of antioxidant activity significantly depends on the method of its estimation, which is why published data obtained by different methods is practically not comparable. Besides, antioxidant properties of essential oils depend on the qualitative and quantitative composition of systems under test [6, 10, 13, 20, 21]. One of the simple and informative methods of the quantitative assessment of antioxidant activity is based on the inhibition of speed of lower aldehyde autooxidation, for example hexanal, in the presence of antioxidant substances [7, 8, 20, 22]. This method was used successfully for the estimation and comparison of antioxidant properties of a

number of essential oils [20, 22-25]. We used this method in the study of antioxidant activity of essential oils.

2. Experimental

The fresh samples of essential oils from clove bud (*Caryophyllus aromaticus* L.), laurel (*Laurus nobilis* L.), cardamom (*Elettaria cardamomum* L.), nutmeg (*Myristica Fragrans* Houtt.) and mace (*Myristica fragrans* Houtt) (company "Plant Lipids Ltd.", India) have been studied.

For the estimation of antioxidant properties of essential oils and their mixture, 600 μl *trans*-2-hexenal (3 μl /ml) and 400 μl undecane (2 μl /ml) (an internal standard) were dissolved in 200 ml of *n*-hexane. The solution was separated into 3 ml aliquots, which were placed in 5 ml glass vials and then 10 μl (3.33 μl /ml), 50 μl (16.5 μl /ml), or 210 μl (70 μl /ml) of essential oils were added. Oil was not added in the control sample. Each sample was prepared twice; the control sample was prepared three times. The samples in vials with stoppers were stored in light under room temperature for 100 days. Every week the vials were opened and blown with 10 ml of air with the help of a pipette. The quantitative content of 2-hexenal and components of essential oils in the vials were determined by the method of capillary gas chromatography after every 10 days from the beginning of storage.

Gas chromatography analysis of essential oils samples and control sample was carried out on a Kristall 2000M chromatograph (Russia) with a flame ionization detector and an SPB-1 fused silica capillary column (50 m x 0.32 mm, phase layer 0.25 μm). The samples were analyzed at the column temperature programming from 333 to 523 K with the speed of 8^{o}/min under the temperature of detector and injector at 523 K. The rate of carrier gas helium through the column was 1.5 ml/min. We analyzed 2 μl of hexane solutions at once. The identification of components in oil samples was carried out on the basis of the retention indexes by their comparison with literary [26] or experimental data obtained by us. The quantitative content of 2-hexenal and essential oils components was calculated by the ratio of peak areas, which corresponded to the substances and internal standard. The oxidation extent of 2-hexenal and essential oils components (%) was determined in reference to their content in the initial samples.

3. Results and Discussion

The main component of clove bud oil was eugenol (80.3 %), which is responsible for its odor and antiseptic and antioxidant properties. Other major components were eugenyl acetate (10.1 %) and caryophyllene (6.3 %). Nutmeg oil was obtained by steam distillation of the kernels of nutmeg, which are the dried fruits of *Myristica Fragrans* Houtt. Mace oil was obtained from the coverings of nutmeg. The nutmeg oil contained ca. 90 % monoterpene hydrocarbons, mainly sabinene (30.7 %), α-pinene (14.0 %), β-pinene (10.8 %), and γ-terpinene (5.8 %). Major oxygen-containing constituents were terpinen-4-ol (6.7 %) and phenol ether derivatives: safrole (0.9 %), myristicin (2.4 %) and elemicin (1.2 %). Mace oil contained the same substances, but their content was differed: sabinene (20.5 %), α-pinene (21.2 %), β-pinene (16.0 %), γ-terpinene (5.8 %), terpinen-4-ol (4.6 %), safrole (1.6 %), myristicin (2.8 %), and elemicin (0.3 %). Cardamom oil was produced from the seeds of *Elettaria cardamomum* (L.) Maton (Zingiberaceae). This essential oil contained 41.2 % of 1.8-cineole and 39.2 % of α-terpinyl acetate. The content of monoterpene hydrocarbons, linalool, geraniol, linalyl, and geranyl acetates was *ca.* 1–2 %. Trace constituents like unsaturated aliphatic aldehydes may be important for the typical cardamom aroma. Laurel leaf essential oil was obtained from leaves of evergreen tree *Laurus nobilis* L. The main components of this oil were 1.8-cineole (49.8 %), α-terpinyl acetate (9.6 %), eugenol (7.1 %), linalool (4.4 %), sabinene (9.5 %), α-pinene (5.8 %), β-pinene (4.6 %), α-terpineol (2.3 %), and terpinen-4-ol (1.7 %). Using of the method of capillary gas chromatography allowed us to estimate the changes in the content of each oil component in the model systems with different oil concentrations and in pure oil during autooxidation, and also to distinguish the oxidation products of the main components. Furthermore, due to the comparison of oil composition and the oxidation rates of components, we managed to reveal some regularity, which enables us to predict and to regulate oil composition in order to obtain stable mixtures. This is very important because essential oils are currently widely used in industry and medicine. Usually the recommended storage time for oils is 1 year; however, nobody has studied yet what really happens with oils during this period. Earlier we studied the changes in the composition of coriander, laurel, marjoram, and fennel oils in the process of storage of pure oil samples in the dark and in the light but in bottles of dark glass [27-29]. It was established that the main process is the oil components oxidation. Thus, we found, and then it was proved in works [10, 11], that cyclic monoterpenes hydrocarbons α- and γ-terpinenes are completely oxidized into aromatic hydrocarbon *p*-cymene. We also found oxidation products of other components of essential oils – oxides, alcohols and aldehydes [26-29].

For the estimation of antioxidant properties of essential oils we used a model system of 2-hexenal daylight induced autooxidation, which is used in the similar studies [7, 8, 20-25]. As a criteria for the estimation of antioxidant properties of essential oils we used the quantity of 2-hexenal, which remained unoxidized after 40 days in reference to the initial quantity (%). Fig. 1 presents the

obtained results of relative antioxidant activity values of the 5 studied essential oils. All model systems included essential oils in two concentrations: 3.3 μl/ml and 16.5 μl/ml. For the essential oils of cardamom and mace we studied an additional third system in which the content of oils was 70 μl/ml of hexane solution. As is clearly seen from Fig. 1, the concentration of oils influenced their antioxidant activity for all oils with the exception of clove bud oil. It is noteworthy that the increase in activity was usually not in proportion to the growth of essential oil concentration. The systems with minimal content of laurel, mace, and nutmeg oils (3.3 μl/ml) had antioxidant activity more than 50 %. A 5-time increase of the content of these oils was accompanied by the increase of antioxidant activity by 20–30 %, and further four-time increase in the content of mace oil (from 16.5 to 70 μl/ml) led to only 6 % growth of oil activity. The cardamom oil in diluted solution (3.3 μl/ml) had very low antioxidant activity – only 20 %. A 5-time increase in concentration of this oil (from 3.3 to 16.5 μl/ml) led to 3.5-time increase of activity. The further increase in concentration of cardamom essential oil up to 70 μl/ml led to only 3 % increase of antioxidant activity.

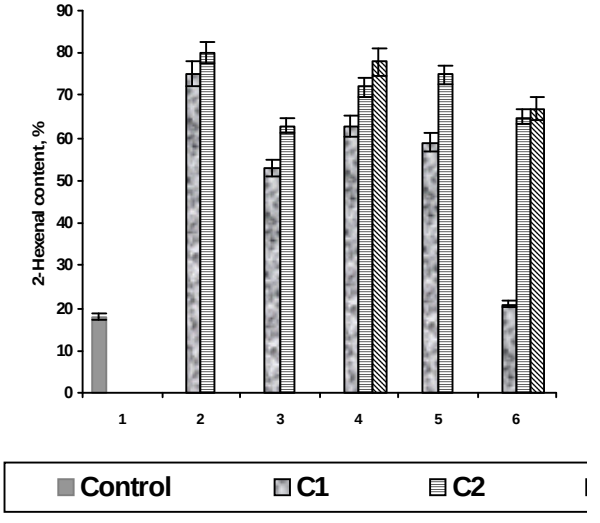

Fig. 1. Relative antioxidant activity of essential oils: control (2-hexenal) essential oils (1); clove bud (2); nutmeg (3); mace (4); laurel (5) and cardamom (6). Concentration of essential oils (μl/ml): C1 - 3.3, C2 - 16.5 and C3 - 70

The inhibition of 2-hexenal oxidation by clove bud oil was practically independent on the concentration and amounts to 75–78 %. Antioxidant activity and composition stability of clove bud oil were high. In both hexane solutions oil has not changed the composition for 100 days. Pure individual oil has also been stable while being stored in the dark for 2 years and in the light for 8 months. We did not notice oxidation even of traces amounts of monoterpene hydrocarbons in stored clove bud oil.

Antioxidant activity of nutmeg and mace essential oils increased from 53 to 69 % and from 63 to 78 %, accordingly, with the increase of their concentration in model solutions (Fig. 1). Thus, activity of mace oil was 10 % higher than that for nutmeg oil. These oils were close in the quantitative and qualitative content of their components. The main compounds in oils were monoterpene hydrocarbons and alcohol terpinen-4-ol. The aroma of this species is due to phenol derivatives – safrole and myristicin, the content of which in both oils was from 0.9 to 2.6 %. These compounds together with terpene hydrocarbons were responsible for the antioxidant activity of oils. During the autooxidation of oils in hexane solutions for 40 days, only 3-4 time decrease of the content of γ-terpinene was observed while the content of its oxidation product, *p*-cymene, increased. During storage of these pure oils for 4 months, the content of γ-terpinene did not change but the storage for 1 year led to practically complete oxidation of γ-terpinene and oxidation of 50 % of caryophyllene with the formation of caryophyllene oxide.

Laurel and cardamom essential oils had close composition of the main components – 1,8-cineole and terpinyl acetate; the difference in aroma was due to the presence in cardamom oil of about 1 % of neryl and linalyl acetates and nerolidol. Laurel essential oil in hexane solution at the concentration of 3.3 μl/ml inhibited the oxidation of 2-hexenal 1.5 times more effectively than cardamom essential oil (Fig. 1). Laurel oil, after 40 days of autooxidation in the light in hexane solution, remained stable. We previously established that individual pure laurel oil had not changed its content in storage in the dark for 2 years [29]. The stability of cardamom essential oil as well as its antioxidant activity depended on its concentration in hexane solution. So, under the concentration of 3.3 μl/ml the quantity of sabinene decreased five times and terpinyl acetate decreased two times, α- and γ-terpinenes were oxidized completely. In more concentrated solutions, only α- and γ-terpinenes were oxidized completely; the content of other components in solutions with oil concentration of 16.5 and 70 μl/ml changed in the same way. It is noteworthy that antioxidant activity of cardamom oil was increasing with the increase of its concentration in the model system (Fig. 1). Probably, they were mainly α- and γ-terpinenes, which were responsible for the antioxidant activity properties of this oil. During the storage of pure cardamom essential oil, after 100 days we noticed the significant oxidation of α- and γ-terpinenes; the quantity of sabinene decreased by 65 % and that of terpinyl acetate decreased by 35 % (Fig. 2). The reason of the changes in laurel and cardamom essential oil behavior was probably that laurel oil contained approximately 7 % of eugenol and 2 % of methyl eugenol, which possesses strong antioxidation properties. Due to their presence, the laurel oil has higher antioxidant activity and was relevant to oxidation. Adding to the cardamom essential oil at least 0.5 % of clove bud oil, which contained approximately

80 % of eugenol, significantly increased the relevance of all components to oxidation in comparison with individual cardamom oil (Fig. 2). In many cases, the antioxidant activity of the essential oils could not be attributed to the major compounds, and minor compounds might play a significant role in the antioxidant activity due to synergistic effects [15, 19, 20]. For instance, in *Melaleuca* species (cajuput oil or tea-tree oil), essential oil containing 1,8-cineole (34 %) and terpinen-4-ol (19 %) exhibited stronger antioxidant activity than those with high methyleugenol (97 %) or 1,8-cineole (64 %) contents [3].

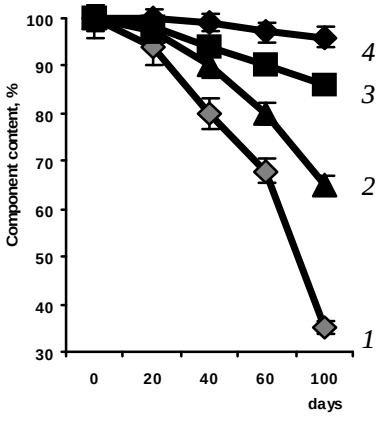

Fig. 2. The content of sabinene and α-terpinyl acetate (% from initial) in cardamom oil and its mixture with 0.5 % clove bud oil during the storage: sabinene and *α*-terpinyl acetate in cardamom oil (1, 2); sabinene and *α*-terpinyl acetate in the mixture of cardamom and 0.5 % of clove bud oils (3, 4)

Thus, the conducted research and literary data show that essential oils are effective natural antioxidants, which are able to compete with the synthetic ones. Antioxidant properties of essential oils are determined by their composition. Oils with high content of substituted phenols are able to significantly inhibit oxidation processes of labile unsaturated aldehydes even in low concentrations. Antioxidant properties of essential oils which did not consist of phenols were determined by monoterpene and sesquiterpene hydrocarbons and alcohols. The concentration of such oils (cardamom, for example) significantly influences their antioxidant properties. The stability of essential oil composition increased with the increase of their concentration in model solutions. The oxidation of substances in pure essential oils happened slower than in solutions.

4. Conclusions

The essential oil of clove bud had the maximal efficiency of inhibition of 2-hexenal oxidation (83 %), which was independent from oil concentration in model solution. Antioxidant properties of essential oils of nutmeg, mace and laurel were caused by substituted phenols and depended poorly on oil concentration in model systems. A stronger dependence of antioxidant activity on the oil concentration in model solution was found for cardamom essential oil. The stability of essential oil composition increased with the increase of their concentration in model solutions. The oxidation of substances in pure essential oils happened slower than in solutions.

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ІНГІБІТУВАННЯ АВТООКИСНЕННЯ 2-ГЕКСАНАЛЯ ЕТЕРНИМИ ОЛІЯМИ ГВОЗДИКИ, ЛАВРУ, КАРДАМОНУ, МУСКАТНОГО ГОРІХУ ТА МАЦИСУ

Анотація. *Методом капілярної газо-рідинної хроматографії вивчено антиоксидантні властивості і стабільність при зберіганні гексанових розчинів 5 індивідуальних етерних олій бутонів гвоздики (Caryophyllus aromaticus L.), кардамону (Elettaria cardamomum L.), лавру (Laurus nobilis L.), мускатного горіху (Myristica fragrans Houtt.) та мацису (Myristica fragrans Houtt). Проведено оцінку антиоксидантних властивостей за ефективністю інгібітування автоокиснення аліфатичного альдегіду (2-гексаналя) у відповідну карбонову кислоту. Встановлено, що максимальну ефективність інгібітування окиснення 2-гексаналя, яка не залежить від концентрації олії в модельному розчині, має етерна олія гвоздики (83 %). Антиоксидантні властивості етерних олій мускатного горіху, лавру і мацису пов'язані з присутністю заміщених фенолів і слабо залежать від концентрації олій в модельних системах. Встановлена значна залежність антиоксидантної активності від концентрації олії для етерної олії кардамону. Вивчено зміни в складі етерних олій в процесі зберігання гексанових розчинів протягом 100 днів на світлі. Проведено порівняння їх стабільності зі стабільністю чистих етерних олій, що зберігались протягом року.*

Ключові слова: *етерні олії, інгібітування, окиснення, 2-гексаналь, аліфатичний ангідрид.*