Tkachenko, Alina

Article

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Alina Tkachenko

COMPARATIVE STUDY OF THE ANTIOXIDANT PROPERTIES OF ORGANIC AND INORGANIC MELISSA

The object of research is organic and inorganic lemon balm. The subject of research is the antioxidant properties of these plants. One of the most problematic areas is that organic products have a slightly different chemical composition, which is due to the lack of fertilizers during their cultivation. The research hypothesis is that organic plants naturally release more phenols and polyphenols, which are needed to fight pests. At the same time, polyphenol compounds have antioxidant properties.

The work investigated the total content of polyphenols in organic and inorganic lemon balm. The content of polyphenols in terms of gallic acid was determined by the spectrophotometric method by reaction with the Folin-Chocalteu reagent. In order to determine the antioxidant properties, the content of the peroxide number and the acid number of the fatty base during storage were determined. As a fat base, a mixture of organic butter and organic rye oil was chosen in a ratio of 75:25. Dried lemon balm was added in the amount of 4 % to the mass of fat. Changes in the fat base were determined in three samples: without the addition of lemon balm, with the addition of dried inorganic and organic lemon balm. The samples were stored in a thermostat at a temperature of 50 °C for 10 days. Studies were conducted every 2 days. The amount of peroxides was determined by the iodometric method. The acid number content was determined every 5 days by the titration method. It has been established that organic lemon balm has better antioxidant properties. This is due to the content of polyphenolic substances. So, the content of polyphenols in ordinary lemon balm is 14.1 mg/g, and in organic – 26.5 mg/g. Research has established that the value of the peroxide number after 10 days of storage was 17 ½O for the sample with the addition of organic lemon balm and 22 ½O for the sample with the addition of ordinary lemon balm. The sample without the addition of lemon balm had a value of 25 ½O. The value of acid number of fat on the tenth day of storage was 1.4 for the sample with the addition of inorganic lemon balm and 1.1 for the sample with the addition of organic lemon balm. Further research is planned to be devoted to the effect of plant organic antioxidants on the lipid fraction of food products.

Keywords: *organic lemon balm, peroxide value, acid value, polyphenol content, antioxidant properties.*

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1. Introduction

Recently, there has been a significant increase in interest in identifying natural antioxidants that can extend the shelf life of products. Scientists have proven that the antioxidant activity of phenolic compounds is due to the fact that they bind heavy metal ions into low-active complexes, which leads to the quenching of free radical processes. Phenols are important for plants, as they participate in the process of growth and reproduction, as well as protect them from the action of pathogenic microorganisms [1].

It is worth noting that the content of phenolic compounds can be influenced by both the method of processing and the method of growing plants, growth environment, genotype, growth stage during harvesting, and the method of extraction [2].

Organic products are of particular interest to consumers recently. In the author's previous works, new formulations of organic confectionery products with improved consumption properties were investigated [3]. In addition, the primary production of organic products is developing rapidly, and Ukraine is among the TOP-10 countries exporting organic raw materials [4]. All this prompts a detailed study of the consumption properties of organic raw materials. However, the comparison of antioxidant properties of organic and inorganic plants is an understudied issue. These studies are relevant and can serve as a basis for the development of new types of natural antioxidants.

Oxidative transformations of fats are accompanied by the activation of free-radical reactions of lipid peroxidation and denaturation of carbohydrates and proteins, which are initiated and develop with the participation of free radicals – molecules or particles with high chemical activity. Catalysts can be tissue and microbial enzymes, metal ions of variable valence, light, heat. During oxidation, substances are formed that worsen the quality characteristics of the product. Schematically, the autooxidation process can be shown as follows:

1. *Formation of a free radical*, an active centre (chain formation):

$$RH+O_2 \xrightarrow{K_{01}} R^{\bullet}+HO_2^{\bullet}, \quad (1)$$

$$2RH+O_2 \xrightarrow{K_{02}} 2R^{\bullet}+H_2O_2, \quad (2)$$

where RH – a molecule of unsaturated or saturated fatty acid of triglyceride; R• – a free radical [5].

2. *Continuation of oxidation chains.* The free radical is unstable, has high chemical activity, and therefore interacts with oxygen quite intensively, forming a peroxide radical:

$$R^{\bullet}+O_2 \xrightarrow{K_{p1}} RO_2^{\bullet}. \quad (3)$$

It reacts with a new fatty acid molecule, again forming the original free radical and the main product of the oxidation reaction – fatty acid hydroxide [5]:

$$RO_2^{\bullet}+RH \xrightarrow{K_{p2}} ROOH+R^{\bullet}. \quad (4)$$

3. *Branching of chains.* Peroxide compounds are unstable and decompose with the formation of alkyl and hydroxyl radicals according to the scheme [5]:

$$ROOH \xrightarrow{K_{d1}} RO^{\bullet}+HO^{\bullet}. \quad (5)$$

New molecules of fatty acids and oxygen are involved in this process [5]:

$$2ROOH \xrightarrow{K_{d2}} RO_2^{\bullet}+RO^{\bullet}+H_2O, \quad (6)$$

$$ROOH+RX \xrightarrow{K_{d3}} RO^{\bullet}+Y^{\bullet}. \quad (7)$$

4. *Chain breakage* – the destruction of free radicals during lipid autoxidation occurs as a result of the interaction of radicals according to biomolar reactions [6]:

$$R^{\bullet}+R^{\bullet} \xrightarrow{K_{t1}} M_1, \quad (8)$$

$$R^{\bullet}+RO_2^{\bullet} \xrightarrow{K_{t2}} M_2, \quad (9)$$

$$RO_2^{\bullet}+RO_2^{\bullet} \xrightarrow{K_{t3}} M_3. \quad (10)$$

Phenolic compounds have a significant antioxidant effect [6]. Previous studies have found that the essential oils of *Melissa officinalis L.* have a good potential for antioxidant activity and can be used in products containing lipids. Lemon balm is a rich source of antioxidants, particularly from the group of phenolic compounds. Its antioxidant activity is associated with phenolic compounds such as citronellal and neral [7]. Considering the development of organic agriculture, a comparative study of the antioxidant capacity of organic and inorganic types of lemon balm is quite important.

There are also data where aqueous extracts of 6 different herbs of the *Lamiaceae* family (ditania, lemon balm, peppermint, sage, siderite, and sweet marjoram) have been tested for antioxidant properties. The extracts were tested for their activity against lipid oxidation in comparison with an aqueous tea extract. Lemon balm extract was shown to be rich in bound forms of phenolic compounds such as hydroxycinnamic acids and flavonoids, rosmarinic and caffeic acids. In another study, the essential oil, ethanol extract, and decoction of 10 plant species were analyzed for acetylcholinesterase enzyme activity and antioxidant activity. *Melissa officinalis* and *Mentha suaveolens* demonstrated the ability to inhibit acetylcholinesterase above 50 % in the essential oil fraction [8]. *Melissa officinalis* demonstrated both high inhibitory capacity for acetylcholinesterase and antioxidant activity. In addition, *Melissa officinalis* showed significant antioxidant activity only in polar fractions. The antioxidant activity of different fractions of *Melissa officinalis* extract was evaluated. The ethyl acetate fraction showed the highest content of flavonoids, as well as antioxidant activity compared to other studied fractions [9].

Lemon balm extract has the ability to absorb both synthetic and natural free radicals. This is of great significance as it indicates that the extract may have the potential to prevent oxidative damage in vivo by preventing free radical-mediated oxidative stress. *Melissa officinalis* has a strong ability to absorb the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) [10].

Previous studies have confirmed the high phenol content and radical scavenging activity of extracts of *Melissa officinalis L.* In the study, *Melissa officinalis* had a very high level of phenolic compounds (13.2 mg GAE/100 g dry weight). Such compounds as quadranose III, salvianic acid A, rosmarinic acid, and luteolin were identified from the dried stems of lemon balm leaves [11].

The source [12] evaluated the phenolic profiles of different samples of lemon balm. Profiles were compared to understand differences between cultured, in vitro cultured and commercial (bag and pellet) samples. Rosmarinic acid was the most common compound. In addition, dimers, trimers, and tetramers of caffeic acid were first identified and quantified in lemon balm. Only one flavonoid — luteolin-30-O-glucuronide — was detected in all samples. In general, cultured and in vitro cultured samples contained the lowest amount of phenolic compounds; otherwise, commercial samples showed the highest content.

Considering the above, the antioxidant properties of lemon balm are scientifically proven, but the properties of organic lemon balm are poorly understood. Depending on the method of cultivation, the absence of agrochemicals in organic samples, the antioxidant effect may differ [13]. That is why the study of antioxidative properties of organic lemon balm and their comparison with non-organic is quite relevant.

The aim of research is a comparative study of the antioxidant properties of organic and inorganic plants (organic and inorganic lemon balm). Taking into account the specified goal, the tasks of the research are:

1. To analyze the content of polyphenolic compounds in organic and inorganic plants.
2. To analyze changes in the peroxide value of the fat base during storage.
3. To analyze changes in the acid number of the fatty base during storage.

2. Materials and Methods

The object of research is organic and inorganic lemon balm (Fig. 1). *The subject of research* is the antioxidant properties of these plants.

Fig. 1. Lemon balm is organic and non-organic

For a comparative study of antioxidant properties, a mixture of butter was used as a fat base in the proportions shown in Table 1. Lemon balm was used in dried form.

Table 1

Characteristics of research samples

Sample No. 1	Fat base for FCP (organic butter + organic castor oil 75:25 %)
Sample No. 2	Fat base for FCP (organic butter + organic castor oil 75:25 %) + 4 % dried lemon balm powder
Sample No. 3	Fat base for FCP (organic butter + organic castor oil 75:25 %) + 4 % dried organic lemon balm powder

Note: FCP – flour confectionery products

Stabilizing properties of additives were determined by indicators of oxidation – peroxide and acid number.

The method of determining the peroxide number is based on the reaction of the oxidation products of oils and fats (peroxides and hydroperoxides) with potassium iodide in a solution of acetic acid and chloroform and the subsequent quantitative determination of the released iodine with a solution of sodium thiosulfate by the titrimetric method.

To determine the acid value, weigh 3–5 g of the studied oil with an accuracy of 0.01 g into a conical flask with a capacity of 150–200 cm^3, add 50 cm^3 of a neutralized mixture of ethanol and ethyl ether (1:2) and shake the contents. If the oil does not dissolve, then the flask must be heated in a water bath and cooled to a temperature of 15–20 °C. Add 3–5 drops of a 1 % alcoholic solution of phenolphthalein and, with constant stirring, titrate the sample with a 0.1 N alcoholic solution of potassium or sodium hydroxide until a faint crimson color appears, which does not disappear within 30 seconds [9].

To determine the content of polyphenolic substances, the weight was mixed with a fat base and heated in a water bath at t=600 °C for 2 hours. The content of polyphenols in terms of gallic acid was determined by the spectrophotometric method by reaction with the Folin-Ciocalteu reagent [14].

3. Results and Discussion

Phenolic compounds are an important class of organic substances with pronounced antioxidant properties. Plant polyphenols are characterized by high biological activity, and they are increasingly and successfully used in medicine and pharmacology as substances with neuroregulatory, biostatic, immunomodulating, and antitumor activity [12]. The content of polyphenolic substances is shown in Fig. 2.

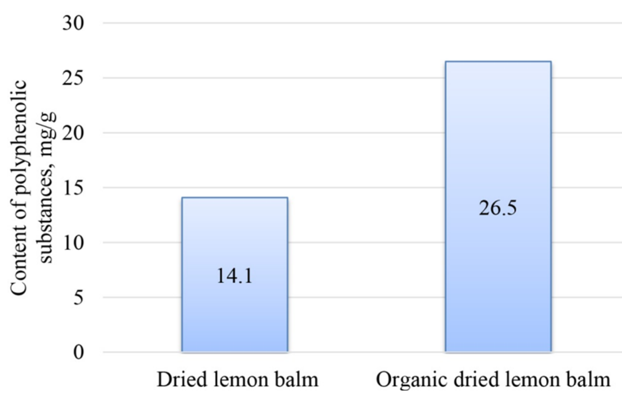

Fig. 2. The content of polyphenolic substances in the studied samples

As can be seen from Fig. 2, the amount of polyphenolic substances in organic lemon balm almost doubles. To confirm the stabilizing effect of organic lemon balm, the peroxide value of the fat base was determined by the accelerated kinetic method. The results of the research are shown in Fig. 3.

As can be seen from Fig. 3, the fatty base without the addition of lemon balm was the most prone to the accumulation of peroxides during storage. Already on the second day of storage, the peroxide number was 2.5 ½O mmol/kg, while the sample with the addition of inorganic lemon balm had an indicator of 1.8 ½O mmol/kg. In the sample with the addition of organic lemon balm, the peroxide value was 1.2 ½O mmol/kg. At the end of storage, the trend did not change, and the sample without antioxidant additives had the highest peroxide value. The value of the peroxide number in the sample with the addition of lemon balm was 15.4 % lower than the control. The value of the peroxide value in the sample with the addition of organic lemon balm was 34.6 % lower. That is, the stabilizing effect of organic lemon balm is much better in terms of peroxide value. In Table 2 shows the values of the acid number of the fatty base at the beginning of storage, on the 5th and on the 10th day of storage. When fat is stored, there is an accumulation of free fatty acids, that is, acidity increases. An increase in acidity indicates a decrease in the quality of fat.

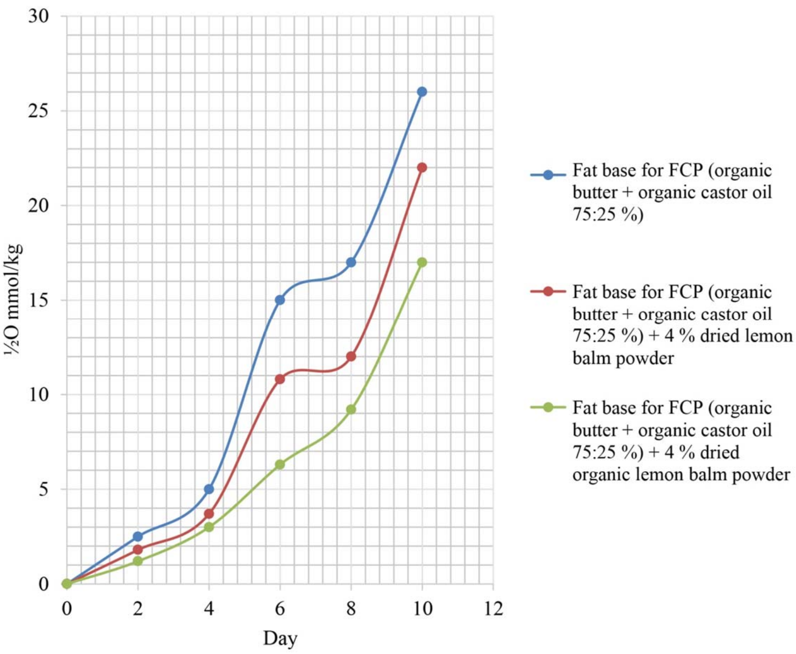

Fig. 3. Changes in the peroxide value of the fat base during 10 days of storage in a thermostat

Table 2

The effect of antioxidant additives on the change in the acid number of the fatty base, mg of KOH

Additives, % by weight of fat	Storage duration, days		
	0	5	10
Fat base for FCP (organic butter + organic castor oil 75:25 %)	0.25–0.01	0.85–0.02	2.00–0.01
Fat base for FCP (organic butter + organic castor oil 75:25 %) + 4 % dried lemon balm powder	0.25–0.01	0.41–0.01	1.40–0.02
Fat base for FCP (organic butter + organic castor oil 75:25 %) + 4 % dried organic lemon balm powder	0.24–0.02	0.31–0.03	1.10–0.02

As can be seen from the Table 2, during 10 days of storage, the value of the acid value increases significantly. Specifically, the sample had an acid value of 2.00 mg KOH after 10 days of storage, while a similar sample with lemon balm had an acid value of 1.40 mg KOH. The sample with the addition of organic lemon balm had the lowest acid value at the end of storage.

The obtained data give reason to believe that organic lemon balm has better antioxidant properties. This is confirmed by a study of the peroxide and acid number during fat storage. Better antioxidant properties in organic lemon balm are provided due to the higher content of phenolic substances. Their content is higher in organic products, since plants are not exposed to the action of agrochemicals and naturally release a higher content of phenols.

The strong side of the research is that it is the first time that the higher antioxidant activity of organic raw materials compared to inorganic ones has been proven. This supports the research hypothesis, which is that organic plants naturally contain higher levels of polyphenolic compounds.

A weakness of the study is that only one type of lipid fraction and one percentage ratio of fat and antioxidants were taken for the study. For a more complete coverage of scientific data, it is worth examining several samples of the lipid base with different proportions of the addition of lemon balm.

Prospects for further research comparing other organic and inorganic plants that have antioxidant properties.

The influence of martial law conditions. Limited access to laboratories, which today are located in the occupied territories, became a significant obstacle to conducting research during martial law. That is why, in the article, only the total content of phenols is given, but their qualitative composition is not investigated.

The market for organic products is still limited due to the high cost of raw materials. The introduction of organic antioxidants can significantly affect the cost of raw materials. However, taking into account its positive effect on the preservation of the lipid base, economic calculations of the effect of adding organic raw materials should be made.

4. Conclusions

1. The amount of polyphenolic substances in organic lemon balm almost doubles and amounts to 26.5 mg/g. At the same time, the amount of polyphenolic substances in inorganic lemon balm was 14.1 mg/g. This supports the research hypothesis, which is that organic plants naturally contain a higher content of polyphenolic compounds.

2. At the end of storage, the sample without antioxidant additives had the highest peroxide value. The value of the peroxide number in the sample with the addition of lemon balm was 15.4 % lower than the control. The value of the peroxide value in the sample with the addition of organic lemon balm was 34.6 % lower. That is, the stabilizing effect of organic lemon balm is much better in terms of peroxide value.

3. The unspiked sample had an acid value of 2.00 mg KOH after 10 days of storage, while a similar sample with lemon balm had an acid value of 1.40 mg KOH. The lowest acid value at the end of storage was the sample with the addition of organic lemon balm and was 1.20 mg of KOH.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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Data availability

The manuscript has no associated data.

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Alina Tkachenko, *PhD, Associate Professor, Department of Commodity Research, Biotechnology, Examination and Customs, Poltava University of Economics and Trade, Poltava, Ukraine, e-mail: alina_biaf@ukr.net, ORCID: https://orcid.org/0000-0001-5521-3327*