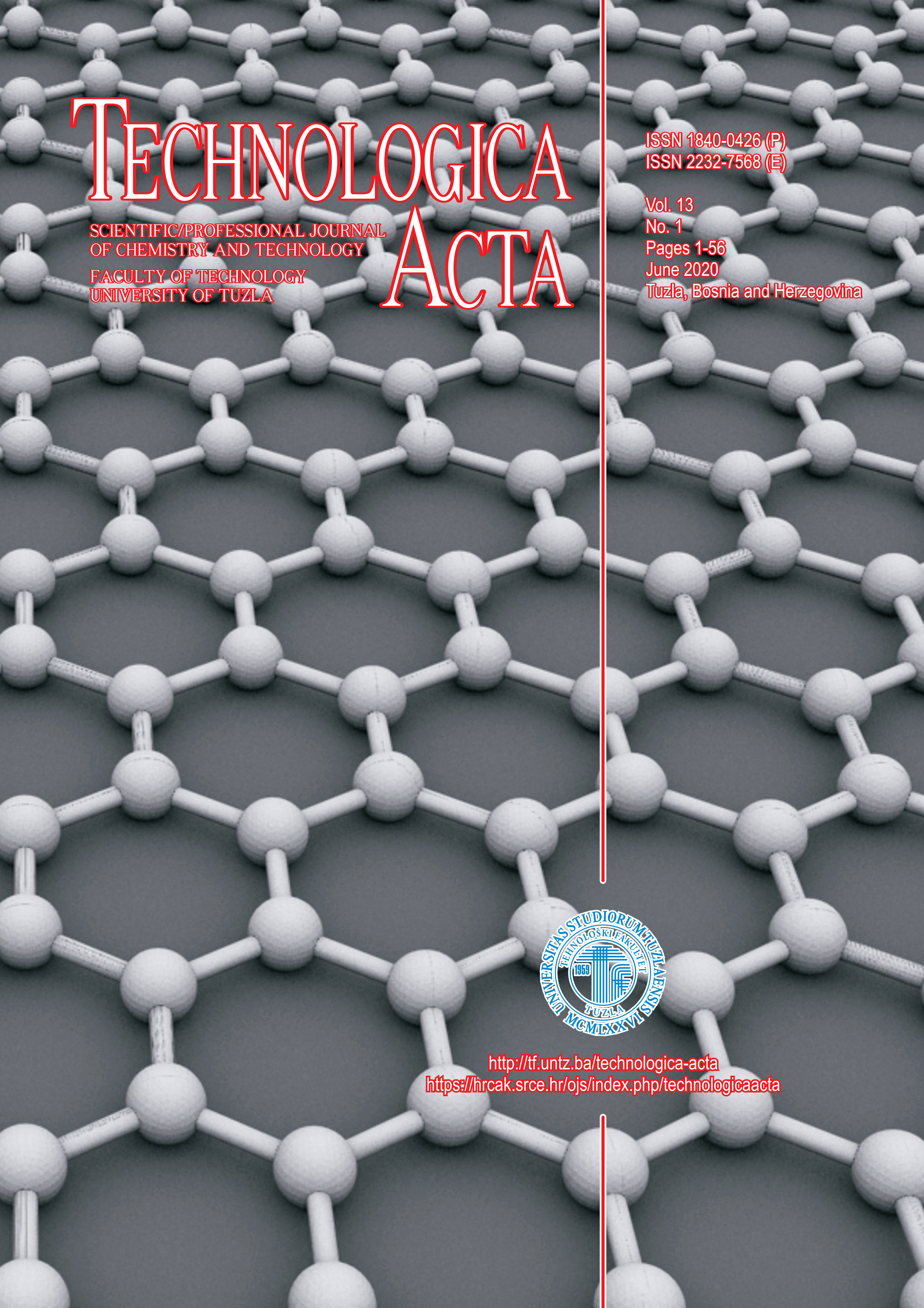

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CONTENT


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CHANGE IN THE QUANTITY OF ASCORBIC ACID AFTER THERMAL PROCESSING OF POTATO

ORIGINAL SCIENTIFIC PAPER

Amela Kusur✉, Aldin Karić, Martina Andrejaš, Hurija Alibašić

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ABSTRACT:
Potato isn’t eaten raw, it has to be thermally processed which influences the presence of the vitamin C considering its sensitivity, fast degradability, and a great loss of vitamin C is expected. Many different ways of thermal processing are used such as boiling, frying, baking and similar. The vitamin C loss depends on the different processing ways and potato preparation. This paper aims to show how much quantity of ascorbic acid that is kept in the potato after boiling it in its skin, without the skin and when cut into pieces, as well as after frying and baking in an oven and baking in a foil. The goal of thermal processing is to keep as many nutritional ingredients as possible, and this paper will show which way of processing and preparation is the most convenient for eating.

KEYWORDS: potato, vitamin C, thermal processing

INTRODUCTION

Potato, a vegetable that represents the main food after the grains, is the main representative of bulbous vegetables. This fact states that the potato is consumed largely and its advantages are affordable price, availability during the whole year, as well as the possibility to prepare various meals and to produce a wide range of species of this product. The chemical composition of the potato is rather variable depending on the type, climate, production methods, soil fertility and other factors. Carbohydrates that make around 75% of dry matter are the main source of energy. Potato also contains significant amounts of proteins, vitamins and minerals. It is also rich with enzymes and acids. The potato contains biologically high-value proteins that contain various essential amino acids among which lysin, valin and leucin are especially important [1].

The vitamin C that is essential for human diet, gives to the potato a special value. The vitamin C is unique because it does multiple physiological functions in the body and it is the only vitamin that has a role in almost all organism functions. The ascorbic acid is well-known by its antioxidative effect by which decreases the cancer risk, participates in the synthesis of collagen, carnitine and neurotransmitters, the synthesis and catabolism of tyrosine, as well as some other metabolism processes on the microsome level [2]. Since bone matrix contains collagen, the vitamin C is important for maintaining the connective tissue and wound healing as well as bone formation [2]. Collagen goes into the structure of the essential substance that surrounds the cell membrane and the lack of the vitamin C brings to the capillary fragility. The vitamin C can contribute to decreasing the risk of cardiovascular diseases, stroke prevention and it can cause mild lowering of systolic blood pressure [2].

In human diet, the potato can be used in a processed and unprocessed form. In its unprocessed form, it is still mostly used for human nutrition, especially in less developed countries. Fresh potatoes are baked, boiled or fried and used in many different ways in many different recipes. It is a very grateful food substance used for the preparation of various dishes in the household. It is considered that less than 50% of potatoes are used as fresh and the rest is used for industrial production in various product [3].

The transition to the use of processed food products also arises from the need to facilitate household and catering work. Potatoes are industrially processed into a range of food products for human consumption. According to the quantity of production and consumption in the world, the most important processed products are French fries, chips and mashed potatoes [4]. In the process of industrial potato processing, starch is obtained, which is an important raw material in the pharmaceutical, food, textile and other industries. Starch produces dextrin, starch syrup, glucose and alcohol, which are also widely used in various industries [1].

Ascorbic acid is very sensitive to the action of enzymes and to oxidation in all steps of food processing, storage and preparation. Significant losses of vitamins can occur at every stage of food preparation, including the processing of raw food itself, washing, removing inedible parts, chopping and mincing, cooking and other thermal processes, storage, etc. [2].

Various literature sources have cited different vitamin C content in potatoes. The vitamin C content certainly varies by many factors, and according to Vreugdenhil et al. [5] 8-54 mg/100 g is present. This paper shows how different preparation and thermal processing affect the loss of vitamin C in potatoes.

EXPERIMENTAL

The testing was performed on 7 potato samples: raw, boiled in the skin, the whole potatoes boiled without the skin, boiled without the skin and cut into cubes, fried in oil, baked in an oven and baked in an oven in a foil. Potato samples are shown in Figure 1. For all evaluation, the samples were ground in a blender or crushed in a saucepan. From the samples prepared in that way, 10 g were weighed and quantitatively transferred into a 100 mL graduated flask and supplemented with distilled water to the mark. Then the filtration was performed. Vitamin C is determined by the iodimetric method, and the procedure for the determination of vitamin C by iodimetric titration is based on its oxidation.

Exactly 25 mL of the solution containing vitamin C is pipetted into an Erlenmeyer flask, 5 mL of starch solution is added, and titrated with iodine solution (previously standardized) to the equivalence point when the blue color of the solution disappears. On the basis of the consumed volume of iodine solution, V (I_2), known concentration c (I_2), the content of vitamin C in the sample (g/250 mL) was calculated according to the expression:

$$m\,(\text{vit. C}) = c(I_2)\cdot V(I_2)\cdot M(\text{vit. C})\cdot R$$

$$m\,(\text{vitamin C}) \text{ - } [\text{g/100 g}]$$

where is:
R-dilution,
M (Vitamin C) = 176.12 g/mol [6].

The concentration of standard iodine solution (I_2) is calculated on the basis of the consumed volume of iodine solution (I_2) during standardization, and the known values for concentration and volume taken of the primary standard H_3AsO_4:

$$c(I_2) = \frac{c(H_3AsO_3)\cdot V(H_3AsO_3)}{V(I_2)}$$

$$c(I_2) \text{ - } [\text{g/mol}]$$

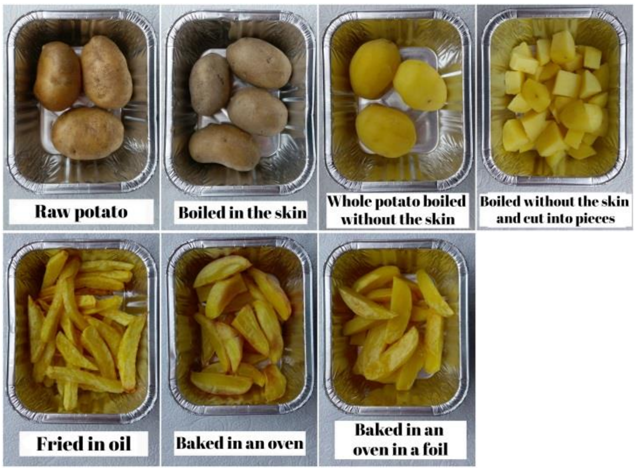

Figure 1. Potato samples

RESULTS AND DISCUSSION

The results of vitamin C content are shown in Table 1. Raw potatoes contain 52.84 mg/100 g of vitamin C. The highest loss was observed in potatoes boiled into cubes 38.75 mg/100 g (73.33%), then boiled potatoes without the skin with a loss of 29.94 mg/100 g (56.66%). Frying potatoes resulted in a loss of 24.66 mg/100 g (46.67%), followed by potatoes baked in a foil with a loss of 23.78 mg/100 g (45%). The most vitamin C is kept in the potatoes boiled in the skin and baked in the oven. When it comes to potatoes boiled in the skin, it shows a loss of 22.9 mg/100 g (43.34%) and baked in the oven 21.14 mg/100 g (40%). From the above results we can conclude that the best way of processing, that is, most of vitamin C is retained in baked potatoes, although potatoes boiled in the skin do not fall behind and certainly these thermal processing methods should be given the priority.

Table 1. The vitamin C content in the potatoes

Processing method	Vitamin C (mg/100 g)
Raw	52.84
Boiled in the skin	29.94
Whole potatoes boiled without the skin	22.9
Boiled without the skin and cut into pieces	14.09
Fried in oil	28.18
Baked in an oven	31.7
Baked in an oven in a foil	29.06

The loss of vitamins can be significantly reduced by careful food preparation. Frying, prolonged boiling, or a combination of multiple methods of food preparation, all increase the loss of vitamins. The longer the food processing time, the greater the losses, regardless of the procedure used [2].

Potatoes are vegetables that should never be eaten raw but must always be thermically processed. These methods have different losses of vitamin C. As it can be seen from the results, the largest loss of vitamin C is in boiled potatoes cut into cubes. Cooking increases digestibility but significantly affects the loss of vitamins since vitamin C belongs to the group of hydro-soluble vitamins (water-soluble vitamins) and is thermolabile. This sample was chopped, thereby significantly increasing the surface area and significantly destroying cells that come in contact with water, therefore a greater loss of vitamins is expected.

Whole potatoes boiled without the skin have a significantly lower loss, which can be explained by the previous statements, that is, the smaller surface is exposed to flushing and thus the smaller the loss of vitamins.

When it comes to boiling, it can be concluded that the best way to boil potatoes is to boil them whole with their skin. The skin contains a lot of nutrients, but also serves as a protective barrier that protects against nutrient loss. It used to be thought that all the nutrients of a potato were housed in a skin, however, this is not true. The percentage of ascorbic acid, niacin and pyridoxine has been shown to be approximately equal in the potato skin and meat, while the percentage of thiamine is higher in the meat [7].

A surprising fact during the analysis is that a significant amount of this vitamin was retained in potatoes fried in oil. Frying is an operation that involves immersing the product in hot oil (175 °C) until the desired product properties are obtained. During the process, moisture from the food surface migrates to the oil in the form of steam and the oil is absorbed in the food product. The type of oil, the temperature of the oil and the duration of frying greatly influence the final texture, taste and quality of the food. As the moisture evaporates, the product begins to dry [8]. Although the analysis shows considerable vitamin C content, fried potatoes should be avoided. First of all, a suitable frying oil should be chosen, and since deep frying is usually done at high temperatures (between 160 °C and 180 °C), in the presence of air and moisture, the frying oils and fats undergo physical and chemical degradation, which will affect the frying performance and stability of the fried products. In addition, highly oxidized oils can also produce polyaromatic hydrocarbons that are thought to have a carcinogenic effect [8].

The high content of vitamin C is observed in potatoes baked in the oven and baked in foil. This thermal processing method has proven to be the most advantageous and should be preferred when preparing potatoes.

Many researches have been done on this topic and the obtained results of the analysis are in accordance with the research of scientific papers. Babalola et al. [9] found that raw potatoes contained 79.3 mg/100 g of vitamin C, boiled potatoes lost 50.30 mg/100 g (63.43%), fried 43.30 mg/100 g (54.60%) and baked 41.30 mg/100 g (52.08%). So the biggest loss is boiling, and the least baking.

Also, according to Ikanone and Oyekan [10], raw potatoes contain 56.47 mg/100 mL of vitamin C. A maximum loss of 37.34 mg/100 mL (68.90%) was observed when the potatoes were boiled, while frying resulted in a loss of 30.44 mg/100 mL (53.90%). Therefore, different methods of thermal processing, as well as the preparation of potatoes themselves, namely grinding and peeling, result in different losses of vitamin C.

Some studies indicate that there may be three ways to increase the vitamin C content of potatoes: through cultivation, improved crop management and modification of the preparation process. Cultivation has a huge potential to increase the content of vitamin C, which involves the application of traditional or modern molecular genetics, as shown by many studies. Improved crop management involves the application of appropriate agro-technical measures as well as appropriate storage conditions. Modification of the preparation process can lead to a decrease in the oxidative and enzymatic degradation of vitamin C resulting from exposure to moisture, heat and air. These modifications include increasing the dimensions of the sliced products, reducing cooking time or temperature, preparing food with an intact skin, etc. [11].

CONCLUSION

Potatoes are a good source of nutrients, with a special value due to vitamin C (ascorbic acid present). Potatoes must be thermally processed before use, which improves digestibility, but on the other hand affects the loss of nutrients, especially the vitamin C. What the loss will be depends on the method of thermal processing and the preparation of potatoes. The highest loss of the vitamin C was observed in potatoes boiled into cubes and the lowest in potatoes baked in the oven. Therefore, the preference should be given to baking when preparing meals.

If boiling is preferred, then whole potatoes with their skin should be boiled. When preparing the potatoes, cutting should be avoided and, if necessary, the dimensions of the cut cubes should be larger. Preparing the potatoes with the skin is essential for preserving the nutrients. Although analysis has shown that frying potatoes resulted in a loss of vitamin C of 46.67%, potatoes fried in oil should be avoided because oils can produce polyaromatic hydrocarbons that are considered to have a carcinogenic effect.

Potatoes are great food for everyone and their importance in nutrition should not be underestimated.

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ANTIMICROBIAL ACTIVITY OF LIPOSOMAL AND NON-LIPOSOMAL VAGINAL SUPPOSITORIES WITH *ORIGANUM COMPACTUM* ESSENTIAL OIL

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
***Origanum compactum* (Lamiaceae) is an endemic species of oregano from Morocco, and the main components are carvacol and thymol, which are considered to have antimicrobial activity. Essential oils can be unstable, poorly soluble in water and poorly delivered to target cells. The incorporation of essential oils into liposomes can reduce their irritant effect, while at the same time prolonging the action of the preparation itself as well as increasing its effectiveness. The aim of our study was to investigate antimicrobial activity of liposomal and non-liposomal vaginal suppositories, and see if there are any differences in antimicrobial activity. Examination of the antimicrobial activity of vaginal suppositories was examined in the same way as the antimicrobial activity of the essential oil, by the disk diffusion method. There were used standard bacterial strains from ATCC collection: *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Enterococcus faecalis* (*E. faecalis*) ATCC 51299, *Escherichia coli* (*E. coli*) ATCC 25922, *Candida albicans* (*C. albicans*) ATCC 10231. Liposomal vaginal suppositories had a smaller inhibition zones probably due to the slower release of active components, but still have an advantage over non-liposomal vaginal suppositories because they reduce the irritating potential of the essential oil.**

KEYWORDS: vaginal suppository, liposome, essential oil, *Origanum compactum*, antimicrobial activity

INTRODUCTION

Vaginitis is one of the most common infectious diseases of the female genital system. The most common causes of vaginitis are candidiasis and bacterial vaginosis [1]. The incorporation of antimicrobial drugs into liposomes (and other nanosystems) could significantly improve local vaginal therapy for fungal and bacterial infections. In this way, it would be possible to use lower doses of the drug than with conventional (classic) forms of drugs [2]. The vaginal cavity is an important area of the reproductive tract and acts as a favorable site for drug administration due to avoiding the first-pass effect, large permeable area, rich vascularization, and relatively low enzyme activity [3].

Vaginal suppositries [4] are dosed drug preparations intended for vaginal application. They are usually spherical or conical shape. They are solid at room temperature, while they dissolve in vaginal secretions at body temperature.

Cocoa butter and similar fatty substances, macrogols or mixtures of gelatin, glycerol and water are most often used as a bases. The carrier may, if necessary, contain emulsifiers and other excipients as well as preservatives. Suppositories and vaginal suppositories are made in the main practice by the method of melting and pouring into molds. This method implies that the drug substances are dissolved or uniformly suspended or emulsified in a dissolved base and the mixture is poured into appropriate molds. The most commonly used bases are different types of Witepsol, which belong to the semi-synthetic lipophilic base. They have advantages over cocoa butter because they have a defined melting point, and are chemically more stable.

Origanum compactum Benth. (Lamiaceae) is an endemic species of oregano from Morocco, and the main components are carvacol and thymol, which are considered to have antimicrobial activity. The antibacterial properties of *Origanum compactum* (*O. compactum)* essential oil have been investigated in many studies and the obtained results indicate strong antimicrobial activity [5,6]. It is one of the strongest antibacterial and antifungal oils. It is known for its beneficial effects on respiratory, fungal, urogenital and other infections. A possible explanation may be that carvacrol and thymol participate in the breakdown of the outer membrane of bacteria, releasing lipopolysaccharides (LPS) and inducing an increase in the permeability of the cytoplasmic membrane to ATP [6].

Essential oils can also be unstable, poorly soluble in water and poorly delivered to target cells. However, the incorporation of essential oils into liposomes can reduce their irritant effect, while at the same time prolonging the action of the preparation itself as well as increasing its effectiveness. Liposomes are considered highly desirable as drug carriers because they may contain hydrophilic, hydrophobic, and amphipathic substances (drugs), and are physiologically acceptable also because of their similarity to biological membranes and biodegradability [7,8]. These spherical phospholipid vesicles consist of a phospholipid bilayer that surrounds water.

The aim of our study was to investigate antimicrobial activity of liposomal and non-liposomal vaginal suppositories, and see if there are any differences in antimicrobial activity.

MATERIALS AND METHODS

MATERIAL

O. compactum essential oil from Pranarom International (Ghislenghien, Belgique) was used as the active component of the vaginal suppositories (Table 1, Table 2). It was obtained by hydrodistillation at low pressure and stored at 4 °C. The chemical composition of this essential oil was determined by gas chromatography.

Table 1. Basic information about *O. compactum* essential oil

Basic information about *O. compactum* essential oil (taken from the Quality Specification)	
Botanical name	*Origanum compactum*
French name	Origan compact
Lot number	OF31140
Origin	PRANAROM - MAROKO
Part of the plant	Flower tops
Distillation date	06/2017
Out of date	01/2023

[a] (taken from the Quality Specification)

Table 2. Physical characteristics of *O. compactum* essential oil

Physical characteristics of *O. compactum* essential oil	
Physical state	Clear liquid
Colour	Golden yellow
Odour	Characteristic, phenolic
Density (20 °C)	0.940
Density (15 °C)	0.943
Refractive index (20 °C)	1.505
Optical rotation (20 ºC)	+ 0.25 °
Flashpoint	64.4 °C

[a] (taken from the Quality Specification)

The chromatogram of *O. compactum* essential oil is shown in Figure 1, with carvacrol (46.01%, $t_{ret,}$= 82.4 min), thymol (16.81%, $t_{ret,}$= 80.7 min), γ-terpinene (15.61%, $t_{ret,}$= 24.3 min) and p-cymene (8.08%, $t_{ret,}$= 25.9 min) as the main components.

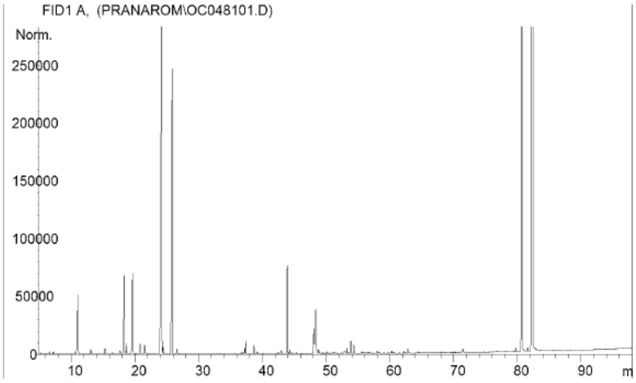

Figure 1. Chromatogram of *O. compactum* essential oil

PREPARATION OF VAGINAL SUPPOSITORIES

Vaginal suppositories were made in two series: non-liposomal (with *O. compactum* essential oil) and liposomal (with liposomal dispersion of *O. compactum*) (Table 3). The method of pouring into molds was used.

Vaginal suppositories were stored in the refrigerator at a temperature of 4 ºC. After production, a visual inspection was performed, and the following parameters were recorded: shape, color, odor, presence or absence of fissures.

Liposomal dispersion was prepared in the laboratory by using high pressure homogenizer (Emulsiflex-C3, Avestin, Canada) at 500 bar in five cycles, and pre-mixing of Phosal 40 IP and *O. compactum* essential oil with Aqua ad injectabilia [9,10]. This dispersion contained 9.3% of essential oil.

Table 3. Formulations of non-liposomal and liposomal vaginal suppositories

Components	Non-liposomal vaginal suppositories	Liposomal vaginal suppositories
***O. compactum* essential oil**	0.25 g	-
Liposomal dispersion with *O. compactum*	-	2.69 g (0.25 g of essential oil)
Cera alba	2.50 g	2.50 g
Span 60	0.30 g	0.30 g
Witepsol	6.95	4.51 g

[a] (quantities for 5 vaginal suppositories)

WEIGHT VARIATION OF VAGINAL SUPPOSITORIES

The recommended mass of the vaginal suppositories with respect to the available molds was 2 g. The prepared vaginal suppositories were evaluated for weight variation according to method of British Pharmacopoeia [11,12]. Twenty vaginal suppositories from each series were weighed and the average values with standard deviation values were calculated.

DISINTEGRATION TEST

The disintegration test determines whether the vaginal suppositories disintegrate at the prescribed time, under certain experimental conditions in a suitable medium. This test was performed by using a magnetic stirrer [13] set at 300 rpm. Phosphate buffer pH 4.5 was used as a medium and temperature was 37 ± 0.5 ºC, which according to the given conditions mimics the vaginal environment. The disintegration time was recorded when Witepsol completely melted. According to the recommendations of the British Pharmacopoeia, the disintegration time of vaginal suppositories and suppositories should not exceed 60 minutes [12].

EXAMINATION OF ANTIMICROBIAL ACTIVITY OF MANUFACTURED VAGINAL SUPPOSITORIES

The antimicrobial activity of vaginal suppositories was examined in the same way as the antimicrobial activity of the essential oil, by the disk diffusion method [14,15]. Both types of vaginal suppositories were tested: liposomal and non-liposomal. The prepared vaginal suppositories were dissolved in dimethyl sulfoxide (DMSO). Vaginal suppositories were completely dissolved in DMSO, but the temperature must be taken into account, because at lower temperatures than 37 ºC it hardens. Mueller Hinton (HiMedia, India) agar plates were inoculated with bacterial and fungal suspensions. There were used standard bacterial strains from ATCC collection: *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Enterococcus faecalis* (*E. faecalis*) ATCC 51299, *Escherichia coli* (*E. coli*) ATCC 25922, *Candida albicans* (*C. albicans*) ATCC 10231. Depressions with metal cylinders were made on each plate, in which 100 µL of solution was introduced. The plates were incubated at 37 °C for 24 hours. After incubation, the sizes of the inhibition zones, in millimeters, were measured, in triplicate. Already measured inhibition zones of essential oil were used to compare the antimicrobial effect of vaginal suppositories, and a comparison was also made with commercial vaginal suppositories (Neo – penotran forte, Embil Pharmaceuticals CO LTD) .

RESULTS AND DISCUSSION

Both types of vaginal suppositories contained 0.05 g of *O. compactum* essential oil, and the only difference in the recipe were phospholipids, ie liposomes in vaginal suppositories with liposomal dispersion. Both were white color, solid at room temperature, with a strong characteristic scent of oregano oil (Table 4, Figure 2).

Table 4. Physical properties and characterization of prepared vaginal suppositories

Properties	Non-liposomal vaginal suppositories	Liposomal vaginal suppositories
Shape	Conical	Conical
Colour	White	White
Odour	Strong, characteristic scent of oregano oil	Strong, characteristic scent of oregano oil
Fissuring	-	-
Weight variation (g)	2.089 ± 0.0447	2.0265 ± 0.0573
Disintegration time (min)	18.16 ± 0.150	31.35 ± 1.35

Figure 2. Vaginal suppositories (own photo)

Both types of vaginal suppositories had an average weight about 2g. The weight variation test complies with the regulations of the British Pharmacopoeia, which states that the standard deviation should be less than 5%. Non-liposomal vaginal suppositories had a faster disintegration time, due to the simpler formulation, ie the absence of phospholipids.

After incubation for 24 hours, inhibition zones (mm) were measured. The obtained results showed a really strong antimicrobial activity of vaginal suppositories (Table 5), because only a small amount of vagitoria solution was introduced on the petri dish (100 µL). Inhibition zones greater than 20 mm indicate the high sensitivity of microorganisms [16,17]. Liposomal vaginal suppositories have almost the same activity as non-liposomal vaginal suppositories (Figure 3, Figure 4), but liposomal preparations have some other advantages such as reduction of irritation potential of essential oil and prolonged action of the preparation itself.

Table 5. Antimicrobial activity of prepared vaginal suppositories (non-liposomal and liposomal)

Name of the organism	**Inhibition zones (mm) of undiluted *O. compactum* essential oil (50 µL)**	**Inhibition zones of Neo – penotran forte vaginal suppositories (100 µL)**	**Inhibition zones of non-liposomal vag. supp. (mm)**	**Inhibition zones of liposomal vag. supp. (mm)**
E. coli ATCC 25922	35 ± 0.5	24 ± 1,73	16 ± 1.0	10 ± 0.02
E. faecalis ATCC 51299	31 ± 0.57	34,06 ± 2,08	21 ± 0.02	19 ± 1.0
S. aureus ATCC 25923	35 ± 1.15	42,6 ± 2,51	11 ± 1.0	14 ± 1.0
C. albicans ATCC 10231	25 ± 1.0	48 ± 2,0	28 ± 1.0	18 ± 0

[a] mean ± SD (n=3)

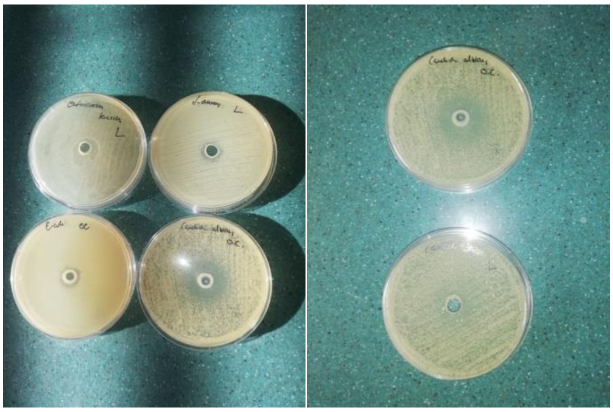

Figure 3. Inhibition zones caused by prepared vaginal suppositories

Both types of vaginal suppositories had the largest inhibition zones for *C. albicans*, and it can be concluded that they have a strong antifungal activity. The inhibition zones were smaller for liposomal vaginal suppositories due to the gradual release of components from liposomes. Many other studies have testified to the positive aspects of encapsulating essential oils [18,19,20]. Neo - penotran forte vaginal suppositories had a larger inhibition zones compared to manufactured vaginal suppositories, but they contain a larger amounts of active ingredients (750 mg of metronidazole and 200 mg of miconazole) than manufactured vaginal suppositories (50 mg of *O. compactum* essential oil).

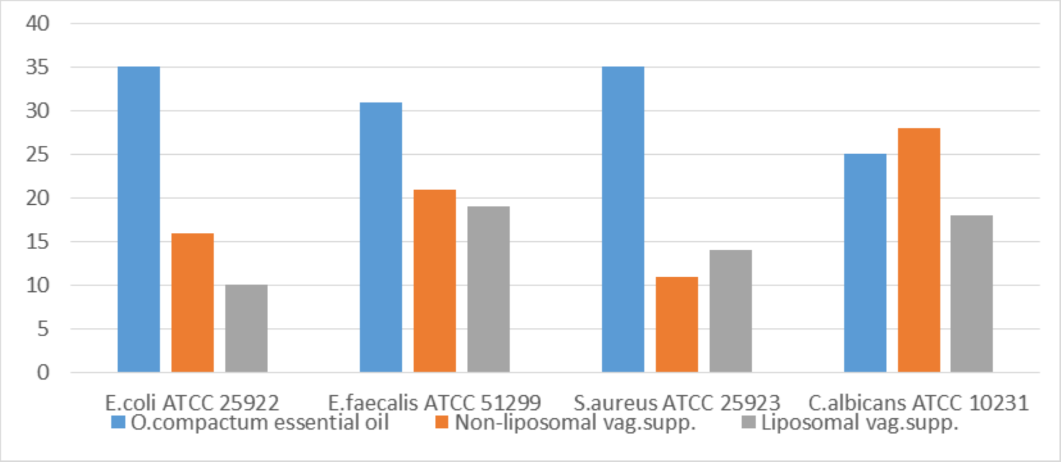

Figure 4. Antimicrobial activity of non-liposomal and liposomal vaginal suppositories

CONCLUSION

Based on the results of this research, it can be concluded:

- *Origanum compactum* essential oil has shown a strong antimicrobial activity on all tested microorganisms.
- To the best of our knowledge, vaginal suppositories with *O. compactum* essential oil (liposomal and non-liposomal) were prepared and tested for antimicrobial activity for the first time.
- A very small amounts of vagitoria solutions were tested, but vaginal suppositories also showed antimicrobial activity on all tested strains, especially for *C.albicans* strain.
- Liposomal vaginal suppositories had a smaller inhibition zones probably due to the slower release of active components, but still have an advantage over non-liposomal vaginal suppositories because they reduce the irritating potential of the essential oil.

ACKNOWLEDGEMENTS

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LEMON BALM (*MELISSA OFFICINALIS* L.) LEAVES PRE-DRYING BY USING LOW ENERGY LASER BEAM

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
A laboratory laser beam drying method for lemon balm (*Melissa officinalis L.*) is presented. The aim of this study was to determine the possibility of using lasers as a pre-treatment for drying the lemon balm leaves and also to determine the variation of water content during the treatment. Leaves were illuminated from one side during a period of 180 s with 100 mW and 200 mW lasers. Both sources of coherent light were in the red part of the visible spectrum with wavelengths of 650 nm. Freshly harvested lemon balm leaves with 75.64% of water content were used for the analysis. Low-energy laser beam treatment for a period of 180 s resulted in the reduction of water content of 0.307% for 100 mW laser treatment and 0.31% for the 200 mW laser treatment. Amounts of energy emitted by low energy lasers are relatively small. Therefore, lasers thermal influence is negligible so it can be concluded that laser activity on lemon balm leaves is based solely on biostimulation. The proposed method allows effective reduction of water content without causing damage to the plant material. For practical application, dryers with integrated laser diode network were proposed. Low energy coherent radiation treatment represents an inexpensive and environmentally safe method that does not pollute the environment. This method also provides close control of the process, and in spite of the greater cost of energy, the overall increase in drying efficiency and throughput can bring about significant economic savings.

KEYWORDS: pre-treatment, drying, laser power, water content, lemon balm

INTRODUCTION

Lemon balm (*Melissa officinalis L.*) is a well-known medicinal plant. Leaves of this plant have been also widely used in cooking to add flavour to dishes and for the preparation of water infusions due to its pleasant aroma [1]. Lemon balm leaves are a rich source of various bioactive compounds that have strong antioxidant activity [2]. Lemon balm has been highly researched due to its many potentially therapeutically desirable effects such as sedative, antispasmodic, antibacterial, antiviral, anti-inflammatory, and sedative effect [3]. Therefore, the plant has been used for the treatment of mental diseases, various cancers, cardiovascular and respiratory problems, and as a memory enhancer, cardiac tonic, antidepressant, sleeping aid and antidote [4].

Leaves or top parts of the plant are picked before flowering when they contain the largest amount of essential oil [5], which due to its aromatic properties is used in aromatherapy, cosmetics, fragrance industry, and as a spice in the preparation of beverages and various sweet and salty foods [6].

Drying of plant material is a most important preprocessing step before storing for a longer time period but this process requires large financial costs. Non-invasive technique such as laser can be applied as pre-treatment of drying. Previous research on low energy lasers has shown that their application causes more positive reactions to plant material, which provides opportunities for their widespread use in agriculture and processing of agricultural products [7]. The use of pre-treatment in the drying process effectively shortens the drying time, and also contributes to the preservation of bioactive compounds. According to Danilov and Leontchik [8] and Mujumdar [9] only 20 to 60% of the heat supplied to the dryer is used for water evaporation, 5 to 25% for material heating, the rest are heat losses. One of the ways to achieve energy savings is application of different physical principle for drying process, such as use of radio frequency, laser radiation, NIR, microwave and vacuum drying. Starzycki et al. [10] indicates that alternative energy sources such as low energy lasers could be a feasible tool to perform the pre-drying process. Mujumdar [9] indicates that energy during the electromagnetic radiation drying is transferred directly to the material that is heated. Therefore, the energy is not expended by heating the air, or parts of equipment as in conventional drying methods, so this kind of drying leaves enough space for energy savings. According to Jović et al. [11], Nenadić et al. [12] and Nenadić et al. [13] beside water content removing, laser beam can also be successfully applied in fungi removing process. This is also an important fact because by reducing the number of undesirable microorganisms, the possibility of infection with the toxic products of their metabolism is also reduced. However, exposing the material to higher power lasers can remove too much water and cause serious damage to the plant even if exposure period is very short [11].

Therefore, the aim of this study was to determine the possibility of using lasers as a pre-treatment for drying the lemon balm leaves and also to determine the variation of water content during the laser treatment.

MATERIALS AND METHODS

PLANT MATERIAL

Lemon balm plants were grown according to the standard procedure in the experimental field of the Department of Vegetable Crops, Faculty of Agriculture University of Zagreb. Lemon balm leaves was harvested in the early morning hours and immediately after harvesting fresh material was delivered to the laboratory of the Department of Agricultural Technology, Storage and Transport at Faculty of Agriculture, University of Zagreb where all the analyses were performed. Leaves were manually cleaned up from impurities. Only whole, healthy and undamaged leaves were considered for treatment. Water content of fresh leaves was determined by a standard AOAC [14] procedure in laboratory dryer INKO ST40T (Croatia) in the temperature range of 105 °C and at atmospheric pressure until the mass of the substance remains constant.

LASER TREATMENT

Two main parts laser treatment equipment are laser beam source and micro-objective. Two laser beam sources with 100 mW (model HLM1845) and 200 mW (model HLP18130) (China) output power were used to expose lemon balm leaves to beam energy. Both laser beam sources have the same output wavelength of 650 nm. Different power outputs were used to determine whether there is any effect of power to the water content removal process.

In order to perform pre-drying the dryer with integrated laser diode net (3 × 5) lasers were proposed. Dryer consists of drying chamber with inspection window, air output and input. Measuring instruments were wet thermometer, dry thermometer and electronic balance. The drying chamber is made of polyurethane insulation panels, coated on both sides with stainless steel (X6CrNiTi18-10) sheets, which ensures quality thermal insulation. Drying chamber internal dimensions are 300 × 300 × 400 mm. For testing purposes in presented study laboratory tests were performed on one segment of the laser diode net. The experiment was set up, as shown in Figure 1.

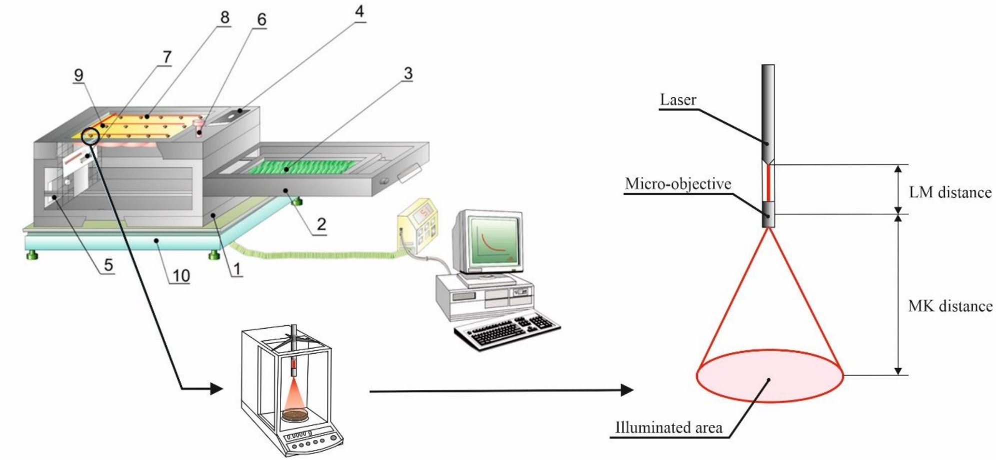

Figure 1. Experimental set-up of the drying process with laser beam
(where: 1 – dryer, 2 - frame for biological material, 3 – biological material (lemon balm leaves), 4 – air output, 5- air input, 6 - thermometer (dry), 7 - thermometer (wet), 8 – inspection window, 9 - laser diode net (3 x 5 lasers), 10 - electronic balance) [7].

Approximately 1.5 g of leaves per repetition were placed in elementary (thin) layer on illuminated area, 100 mm in diameter under the laser source. Illuminated area surface is calculated by using following formula:

$$A = r^2\pi \text{..} (1)$$

Distance (LM) between laser source and micro-objective was 70 mm and distance (MK) between micro-objective and leaves was 320 mm.
Intensity of laser radiation within the illuminated area for a 100 mW laser is calculated using the following equation:

$$Intensity = \frac{E_{laser}(W)}{Illuminated\ area\ (m^2)} \times treatment\ period\ (s) \text{.......} (2)$$

The energy of individual photon is calculated by following equation:

$$E_{individual\ photon} = h\upsilon = \frac{hc}{\lambda} \text{..................................} (3)$$

where:

- h – Planck's constant (6.626 x 10^{-34} Js),
- υ – photon frequency for semiconductor laser (4.61538 x 10^{15} s^{-1}),
- c – speed of light (2.99 x 10^8 ms^{-1}),
- λ – wavelength (650 nm).

Numbers of photons from the individual laser sources are calculated by using equation:

$$n_{photons} = \frac{E_{laser}}{E_{photon}} \text{...} (4)$$

In addition to the above knowing the laser output power and period of treatment, total energy emitted by the source of laser light during 180 seconds is calculated by using equation:

$$E_{laser} = E_{ivdividual\ photon} \times n_{photons\ 100mW} \times treatment\ period \text{...} (5)$$

Plate with prepared leaves is placed on the laboratory analytical balance (Sartorius BP 221S, Göttingen, Germany) with measurement accuracy class I scale interval 0.1 mg. Leaves are illuminated only from one side during 180 seconds. During the pre-drying process, reduction in the weight of the samples was measured and recorded every 30 seconds. During the drying process, laboratory environment humidity was between 80-84% and atmosphere pressure was in the mean 1014 hPa.

RESULTS AND DISCUSSION

According to Dinoev et al. [15] physiological changes occurring in the treated plant material mostly depend on the laser wavelength and intensity of radiation. Illuminated area surface of 100 mm in diameter according to equation (1) was 7.85 $\times 10^{-3}$ m^2.

According to equation (2) intensity of laser radiation for 100 mW laser were 2292.99 Wsm^{-2} and 4585.98 Wsm^{-2} for a 200 mW laser.

The energy of individual photon calculated by equation (3) were 3.06 × 10^{-18} J.

Numbers of photons from the individual laser sources calculated from the equation (4) were 3.27 × 10^{16} photon s^{-1} for 100 mW laser and 6.54 × 10^{16} photon s^{-1} for 200 mW laser.

Total energy emitted by the lasers during 180 seconds calculated using equation (5) were 18.01 J for 100 mW laser and 36.02 J for 200 mW laser.

According to the results, it is obvious that the amounts of energy emitted by lasers are relatively small. Therefore, lasers thermal influence is negligible so it can be concluded that laser activity on lemon balm leaves is based solely on biostimulation.
Beside wavelength of electromagnetic radiation and intensity of radiation, interaction between laser light and plant material also depends on the water content and the structure of plant material. The initial water content of lemon balm leaves was 75.64%. The loss of water content during laser pre-treatment is shown in Figure 2. The results are presented as mean values of 30 repetitions (n=30).

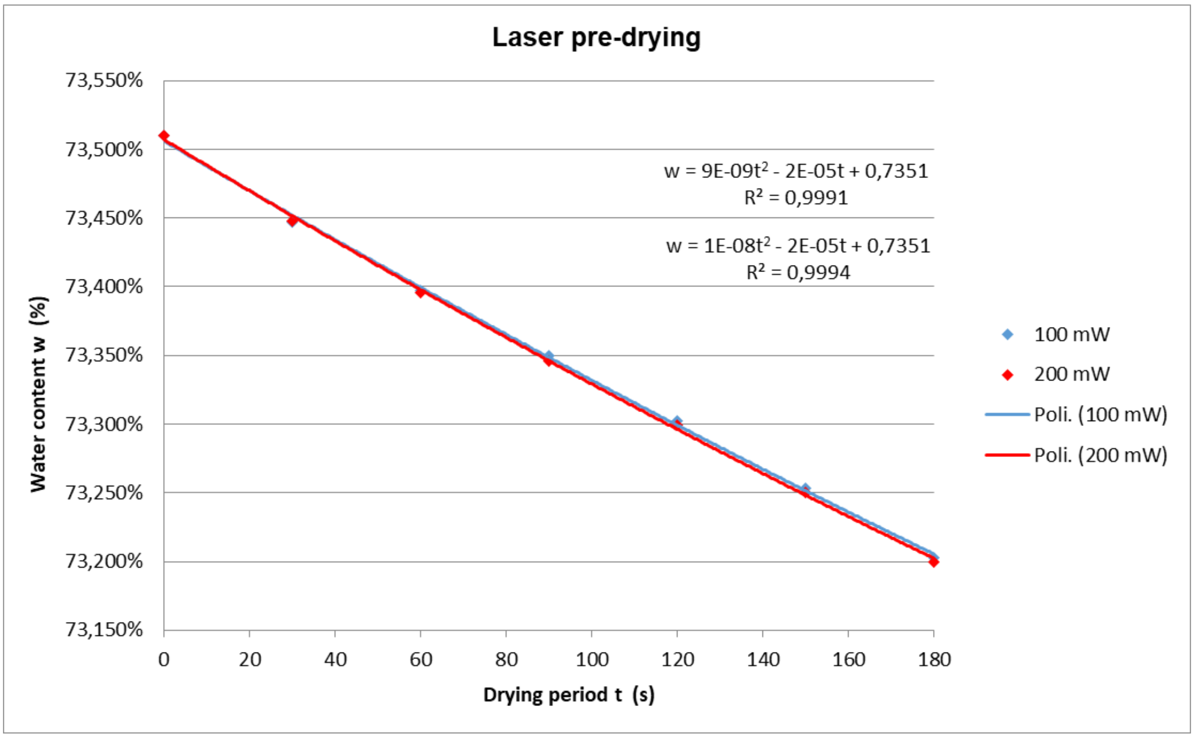

Figure 2. Effect of 100 mW and 200 mW laser pre-treatment on water content of lemon balm leaves

Based on the obtained data, second order polynomial equations were calculated. Pre-drying by 100 mW laser takes place according to the equation $w = 0.7351 - 2\times10^{-5}t + 9\times10^{-9}t^2$ with a very high coefficient of determination $R^2 = 0.9991$. Pre-drying by 200 mW laser takes place according to the equation $w = 0.7351 - 2\times10^{-5}t + 1\times10^{-8}t^2$ with also a very high coefficient of determination $R^2 = 0.9994$.

By deriving second order polynomial equations the slopes of the lemon balm leaf drying curves were determined and the following expressions were shown in table 1.

Table 1. Derivations of second order polynomial equations

Pre - treatment	**Derivations of second order polynomial equations (*dw/dt*)**
100 mW	$dw/dt = - 2\times10^{-5} + 18\times10^{-9}t$
200 mW	$dw/dt = - 2\times10^{-5} + 2\times10^{-8}t$

The derivatives of the equations have a negative sign, which indicates a decreasing function, and confirms that this is a process of drying. It is also evident from the derivations that the slopes of the drying curves differ from each other depending on the method of treatment. Therefore, the results of both 100 mW and 200 mW lasers show positive aspects in water removing process. By comparing the above equations, *i.e.* their coefficients, it can be reliably concluded that the application of a 200 mW laser results in a somewhat faster release of water from the lemon balm leaves. According to Moore et al. [16] reason for this water removing process is a better energy transfer during laser treatment than in convective drying, probably due to Förster - Dexter's energy transmission in dipole molecules. In addition, these processes need further careful investigated in order to find balance between laser power and its biological effect on treated materials.

Laboratory tests carried out on a single section of laser diode network shows that proposed dryer with integrated laser diode net (3 × 5 lasers) can be successfully used for pre-drying purposes.

CONCLUSION

Experiments have shown that a low energy laser can be used for the pre-drying of lemon balm leaves. Treatment with a low energy laser beam for 180 seconds caused a decrease in the water content by 0.307% with 100 mW laser and 0.31% with 200 mW laser. Obtained results indicate a realistic possibility of applying laser radiation in a technological process that precedes conventional drying. Application of laser drying needs to be considered as cheap and environmentally friendly method that does not pollute the environment and at the same time allows significant energy savings. Also this method of pre-treatment creates favourable conditions for the automation of process.

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RAGWEED (*AMBROSIA ARTEMISIIFOLIA* L.) BIOMASS AS A SOURCE FOR ENERGY PRODUCTION

PROFESSIONAL PAPER

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ABSTRACT:
Due to their rapid spread and reproduction, invasive plant species adversely affect agricultural production, the biodiversity of other species, as well as human health. They can be used as a potential raw material for energy production after mechanical removal to reduce their population without using herbicides. Ragweed belongs to the group of foreign invasive species, mainly as a weed, it can be found in crops, and its pollen causes allergies in humans. This paper aimed to investigate the energy properties of ragweed and the possibility of its use in thermal energy production. The results show that ragweed is a solid energy source for direct combustion, with a slightly higher proportion of ash, which negatively affects the energy properties.

KEYWORDS: invasive species, ragweed, biomass, energy potential

INTRODUCTION

Ragweed is a native species of North and South America, and it can be found in all states of the United States except Alaska. The first records of ragweed in the United States date from 1838 in Michigan. It was shipped from America to Europe in 1863. It was found in red clover seeds, alfalfa, and cereal seeds, and its spread began from European ports: from Rijeka to the rest of Croatia and the Danube Valley, from Trieste and Genoa to northern Italy and from Marseille to the Rhone Valley [1]. Croatian herbologist Kovačević was the first to mention a ragweed presence in Croatia in 1940 in Podravina, Posavina, and Slavonia. Today it is widespread in most of Croatia, and it dominates in the continental part. Along with Croatia, Hungary, Italy, and France are European countries that have the biggest problem of ragweed pollen pollution. Ragweed is found in abandoned habitats, along railroads, roads, roads, riverbanks, and streams, in neglected gardens and poorly cultivated fields [2].

Ragweed being a weed in crops can cause significant damage. It mechanically suffocates the crop, depriving the plant of the above-ground and underground space. It reduces the yield and quality of cultivated plants, reduces the amount of water and nutrients in the soil, lowers the soil temperature, and makes tillage difficult. It can be a transient host to pests and plant disease agents. Ragweed thrives well in dry habitats and has moderate nutrient requirements. But it can also grow on bright, hot, or different habitats on neutrophilic soils, regardless of soil quality. The requirements for temperature and light are high, so ragweed is a heliophilic and thermophilic plant. The optimum temperature for germination is 20 to 22°C. The vegetation period of the plant lasts from 150 to 170 days depending on ecological conditions. Ragweed pollen is one of the most potent pollen allergens and causes serious health problems for a specific part of the population. In people who are allergic to ragweed pollen, allergic rhinitis occurs during flowering in August and September. The ragweed plant has a firm spindle root that is overgrown with lateral roots. The lateral roots of the plant provide strength and excellent absorption of water and nutrients. The root is short, compact, and branched. It usually does not penetrate deep into the soil, so it can take root on shallow and compacted soils and can be easily pulled out of the soil mechanically. Ragweed has an upright, herbaceous, and branched stem that forms a bush in the upper part. The colour of the stem can be from green-gray to reddish. The height is usually between 20 and 120 cm, and in optimal conditions, it can reach a height of 2 m. The leaves are alternately placed on the stem and pinnately divided into narrow, elongated-lanceolate lobes and overgrown with small hairs. The reverse of the leaf is gray-green, and the face is dark green. The back of the leaf is lighter in colour and hairier than the epidermis of the face of the leaf. The size of the leaves is between 5 and 10 cm. The flowers of ragweed are small, unisexual, tubular, and yellow. Ragweed is a monoecious plant and develops male and female flowers on the same plant. The male flowers are small and tubular, turned downwards, and are grouped in a head-shaped inflorescence. The heads contain 10–15 tiny yellowish flowers composed of five petals with dark lines between them). The fruit of ragweed is round ovoid hornbeam or achenia, in which seed is wrapped in a tight shell. The pods contain 5 to 7 prickly growths on one side, with the central growth being the longest. The fruit's diameter is about 5 mm, the length from 1.5 to 5 mm, and the thickness from 1.0 to 1.7 mm. The plant can produce up to 3,000 pods per year that retain germination for up to 40 years. Ragweed reproduces solely by seed. Apart from "primary dormancy" ragweed seeds also have "secondary dormancy" which occurs if the seeds fail to germinate due to unfavourable conditions [3,4,5,6].

Today, ragweed is found in almost all parts of Croatia. It is most widespread between the rivers Sava and Drava. Galzina et al. [1] conducted a three-year study (2004-2006) on the prevalence of ragweed in Croatia. Of the 521 municipalities surveyed, ragweed was found in 490 municipalities. According to the results of their research, ragweed is present in the entire interior of Croatia. It is found as a weed in the fields of sunflower, corn, sugar beet, and soybeans. It is present along roads, railways, and highways, and on abandoned land. In the coastal and mountainous parts of Croatia, it is present in a much smaller presence.

Since the optimal way to destroy ragweed is its mechanical removal before flowering to prevent further spread, the question arises whether the biomass remaining after the mechanical removal of ragweed is a useful resource in energy production. Namely, one of the underused energy sources today is biomass precisely because of the limited resources in its production. Ways to get energy from biomass are different. Plants can be directly grown as biomass for energy production, or plant residue from agricultural production, organic waste, and even invasive plant species such as ragweed. The oldest way of directly converting biomass into energy is combustion. Today, various processes of converting biomass into energy or fuel have been developed. Besides being renewable, biomass and its products, are also sufficiently similar to fossil fuels, so their direct replacement is possible. Biomass can be directly converted into energy by combustion, thus producing steam for heating in industry and households, and obtaining electricity in small thermal power plants. Some plants provide oil that can be used in diesel engines. Biochemical processes (anaerobic and alcoholic fermentation, esterification) produce biogas, bioethanol, and biodiesel. Thermochemical conversions (combustion, gasification, and pyrolysis) result in direct energy production. Dry distillation (heating without the presence of air) can produce methanol, acetone, charcoal, and other products from biomass [7,8].

Biomass of invasive plant species, including ragweed, has the potential for energy production due to its rapid spread and reproduction. Due to its spread and a large amount of generated biomass, this paper examined the possibility of using its biomass through energy recovery while reducing the need for herbicide use. Therefore, this study aimed to determine the possibility of using ragweed biomass as a potential raw material for energy production, or as a raw material for direct combustion. Analysis of ragweed biomass will be carried out after its mechanical removal from nature, guided by the fact that its invasiveness and a large amount of biomass poses a threat to biodiversity, is highly allergenic, and causes considerable damage to agriculture. Its energy potential will be determined with the aim of direct combustion and heat production.

MATERIALS AND METHODS

In this paper, research was conducted on ragweed biomass. The collection of materials was carried out at five locations in the city of Zagreb, the Republic of Croatia. Materials were collected on abandoned land and along roads. The first location is the sub-Sljeme zone of the Markuševec district the second location is the Dubrava district, the third location is Maksimir Park near the Faculty of Agriculture, the fourth location is the Sava embankment, and the fifth location is near Jarun Lake.

After collecting samples, the research was conducted in the laboratory of the Department of Agricultural Technology, Storage and Transport, University of Zagreb, Faculty of Agriculture.

Samples were analysed by the following standard methods: dry matter content (HRN EN ISO 18134-2: 2017); ash content (HRN EN ISO 18122: 2015), coke (EN 15148: 2009), fixed carbon (calculated), volatile matter content (EN 15148: 2009), carbon, hydrogen, nitrogen content (HRN EN 15104: 2011), sulphur (HRN EN 15289: 2011), the content of cellulose, hemicellulose (NREL / TP-510-42623) and content of lignin (NREL / TP-510-42618) and higher and lower calorific value (EN 14918: 2010).

The analyses were preceded by the grinding of the samples in a laboratory mill (IKA Analysentechnik GmbH).

RESULTS AND DISCUSSION

The water content in biomass is a significant factor as it affects the fuel's energy value. Dry biomass has a higher calorific value because no energy is wasted on evaporation. The moisture content in biomass varies from 10 to 50% [9]. According to Ross et al. [10] (2008), the optimal water content ranges between 10 and 15%. The method of obtaining energy from biomass depends on the water content. Raw materials with low moisture content (<50%) are required for heat treatment, while raw materials with high moisture content can be used for biochemical processes [7].

Table 1. Share of water in the wet and dry samples in investigated ragweed biomass

Samples location	Wet sample (%)	Dry sample (%)
I	59.09	10.02
II	58.50	9.21
III	61.08	11.46
IV	57.33	10.81
V	67.87	12.97
Average	**60.84**	**10.93**

Table 1 shows the mean values of the water content of analysed biomass samples from different locations in Zagreb. The mean value of the water content in the dry sample is 10.93%, which is acceptable because, according to Ross et al. [10], the optimal water content is between 10% and 15%.

Table 2. Share of ash, coke, fixed carbon and volatile substances in the studied ragweed biomass

Samples location	Ash (%)	Coke (%)	Fixed carbon (%)	Volatile substances (%)
I	10.61	15.68	5.07	74.30
II	11.52	18.82	7.30	71.97
III	10.79	16.93	6.14	71.61
IV	11.34	19.09	7.75	70.10
V	12.14	17.58	5.44	69.45
Average	**11.28**	**17.62**	**6.34**	**71.49**

Ash is an inorganic part of the fuel that remains after complete combustion. The ash content can vary from 1% to 40% [11]. According to Parmer [9], wood has less than 1% ash, while crops and residues have higher ash content. According to Bilandžija et al [12], the ash content in agricultural biomass ranges from 2% to 25%. The substances that produce the ash have no calorific value, and its desirable value should be as low as possible. Table 2 shows the mean value of the ash content in the dry matter of the analysed samples of ragweed biomass, depending on the location. The average value of the ash content of the samples is 11.28%, which is a high content compared to some energy crops that are targeted for this purpose.

Coke is secondary coal formed at higher temperatures. It is the rest of the dry distillation, and by its increasing, it increases fuel quality [13]. Table 2 shows the mean values of the coke content of the analysed ragweed biomass samples. The average value of the coke content of the analysed samples is 17.62%. However, there was a significant difference in the coke content between the samples, which ranged from 15.68 to 19.09%. Yet, the obtained values of coke in ragweed biomass have favourable amounts of coke for energy production.

The fixed carbon content is, in addition to the ash, a solid residue after combustion or release of volatile substances. Increasing the fixed carbon increases the calorific value, thus improving the quality of the biomass. Table 2 shows the fixed carbon content in ragweed biomass samples at different locations which averaged 6.34%, which is significantly less compared to wheat biomass containing 21% fixed carbon, barley containing 18%, and wood biomass containing 17% fixed carbon [7]. Due to the low content of fixed carbon, the value of biomass raw material for energy production decreases.

Volatiles are components that are released at high temperatures [7]. The volatile matter content of ragweed biomass samples from different locations is also shown in Table 2. The range of volatile matter content in the samples is from 69.45 to 74.30%, and the mean value of volatile matter content in the samples is 71.49%, which is higher volatile matter content compared to wheat straw [14], but lower compared to miscanthus [15].

Biomass contains different amounts of cellulose, hemicellulose and lignin, and small amounts of other components (lipids, proteins, simple sugars, and starches). The ratio of cellulose to lignin is one of the essential factors in determining the suitability of a particular plant species for energy production [7]. It is desirable to have the lowest possible cellulose content and hemicellulose in the biomass in the combustion process. Biomass with higher lignin content is more suitable for direct combustion processes [14].

Table 3. The share of cellulose, lignin and hemicellulose in the studied ragweed biomass

Samples location	Cellulose (%)	Lignin (%)	Hemicellulose (%)
I	30.87	24.16	17.70
II	27.54	27.16	13.84
III	28.44	24.81	15.79
IV	25.28	27.46	13.15
V	25.66	28.29	16.90
Average	**27.55**	**26.38**	**15.48**

Table 3 shows the proportion of cellulose, hemicellulose, and lignin in ragweed biomass samples from different locations. The cellulose content averaged 27.55%. The average cellulose content for wheat straw is 45 to 50%, and for wood biomass 40 to 50%. The share of lignin in analysed samples averaged 26.38%, which was in wheat 15 to 20%, and 20 to 25% for woody biomass [7]. The share of hemicellulose ranged, on average, 15.48%, which is lower than the biomass of wheat, 20 to 25%, or wood biomass, which is 25 to 30%.

Table 4 shows the total carbon, nitrogen, hydrogen, oxygen, and sulphur in ragweed biomass samples.

Table 4. The share of carbon, hydrogen, nitrogen, sulphur, and oxygen in the studied ragweed biomass

Samples location	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Sulphur (%)	Oxygen (%)
I	61.68	5.36	7.55	0.31	25.10
II	64.99	6.54	8.85	0.34	19.38
III	57.51	6.18	8.36	0.37	27.58
IV	61.41	6.49	7.91	0.28	23.91
V	59.00	5.42	9.47	0.30	25.81
Average	**60.92**	**6.00**	**8.43**	**0.32**	**24.36**

The fundamental element of biomass is carbon, which makes up 30 to 60% of the dry matter, depending on the ash content [16]. Higher carbon content increases the energy value of biomass. Carbon is not free in biomass, but in organic compounds with oxygen, hydrogen, nitrogen, and sulphur. During combustion, carbon binds to oxygen and gives off significant amounts of heat energy. In total combustion, if combustion takes place with enough oxygen, CO_2 is released [17]. The mean value of the carbon content in the samples is 60.92%. In the research of Bilandžija (2014) on miscanthus culture, the percentage of carbon ranged from 48.55 to 48.72%. The carbon content of wood biomass is 51.6%, and of wheat 48.5%. According to these crops, ragweed has a significantly higher carbon content and, therefore, is suitable for energy production.

Hydrogen is, in addition to carbon, the essential component of the fuel, and biomass contains, on average, 5 to 6% hydrogen. Higher hydrogen content increases the energy value. The hydrogen content of the analysed samples was around 6.00%. According to Parmer [9], miscanthus contains 5.8% hydrogen, wood biomass 6.3%, and wheat 5.5% hydrogen. The analysed ragweed samples have a slightly higher hydrogen content than miscanthus and wheat crops, but a lower content than woody biomass.

Nitrogen is a macronutrient that is important for plant growth (Jenkins et al., 1998). The nitrogen content in biomass varies from 0.2% to more than 1% [9]. During combustion, nitrogen is released in the elemental state and acts as an inert ingredient, which means that it neither burns nor gives off heat. It negatively affects the activity of the elements with which is combined and reduces the calorific value. It can create undesirable nitrogen oxides (NO_x) that pollute the environment [17]. Nitrogen in the examined ragweed samples averaged 8.43% in all samples. The nitrogen content of miscanthus is 0.5%, and 0.3% of wheat [7]. Compared to other crops, ragweed tested contains large amounts of nitrogen, which reduces its calorific value.

Most of the biomass fuels contain less than 0.2% sulphur, with a few exceptions with a higher amount of 0.5 to 0.7% [9]. Sulphur in fuel can be combustible and non-combustible. Combustible sulphur is usually bound to organic matter or is in combination with metals. Non-combustible sulphur is stably bound in the form of calcium sulphate, which remains mainly in the ash during and after combustion [17].. Sulphur oxides (SO_x) are formed during combustion and affect environmental pollution. Biomass usually has a low sulphur content, and its combustion does not significantly contribute to sulphur emissions. In the analysed samples, the average sulphur value in ragweed biomass was 0.32%, which is higher compared to miscanthus, wheat, and wood biomass, which have 0.1% sulphur or less [7].

According to Jenkins et al. [16], the oxygen content in biomass ranges from 30% to 40%. The presence of oxygen in the fuel is undesirable because the oxygen does not burn, but it participates in combustion. It is most often found in compounds with other elements. It makes them non-combustible, so it reduces the effect of combustible elements with which they are combined, resulting in a decrease in the calorific value of fuel [17]. The oxygen content in the samples averaged 24.36%. The oxygen content in miscanthus is 43%, in wheat 44.5% [9], and in wood biomass 41.5% [7]. According to these cultures, the tested samples of ragweed have significantly lower oxygen content; therefore, they are better raw material for combustion processes.

The calorific value (higher and lower heating value) of fuel represents the amount of heat generated during the complete combustion of a certain amount of fuel [17]. The higher heating value (Hg) is the amount of heat generated by the complete combustion of a certain amount of fuel. Produced gasses are cooled to a temperature of 25 ° C and moisture is eliminated from them as condensate. The lower heating value (Hd) is the amount of heat generated by the complete combustion of a certain amount of fuel. Produced gasses are cooled to a temperature of 25°C, and the moisture in them remains in a vapor state, and the condensate heat remains unused. Table 5 shows the higher and lower heating values of ragweed samples from different locations.

Table 5. Higher and lower heating value in the investigated ragweed biomass

Samples location	Higher heating value (MJ/kg)	Lower heating value (MJ/kg)
I	16.60	15.43
II	15.93	14.50
III	16.49	15.14
IV	14.83	13.41
V	16.42	15.24
Average	**16.05**	**14.74**

The average higher heating value of ragweed biomass is 16.05 MJ / kg, and the average lower value of samples is 14.74 MJ / kg. In a study conducted by Bilandžija et al. [12], miscanthus has a higher heating value of 18 MJ / kg, which is slightly higher than the tested samples of ragweed biomass. Wheat straw (24.72 MJ / kg), barley (25.35 MJ / kg), oats (25.70 MJ / kg) and triticale (24.87 MJ / kg), from the research of Grubor et al. [14] their samples also have more eminent heating values than ragweed values investigated.

CONCLUSION

Based on the analysis conducted on ragweed biomass from five different locations in the city of Zagreb, the following can be concluded:

1. Analysis of ragweed biomass from different areas does not cause excessive deviations between samples,
2. Biomass has a favourable content of water, coke, volatile substances and heating values, but too high ash content and low content of fixed carbon. The lignocellulosic composition of ragweed is beneficial because it has desirable amounts of lignin and does not have too high a cellulose content. As for the elemental composition of biomass, ragweed samples have a favourable carbon and hydrogen content, but excessively high nitrogen.
3. According to the above data, ragweed biomass is a suitable raw material for the direct combustion process with unfavourable ash content. It could be used in the energy production process.

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INFLUENCE OF PROCESS CONDITIONS ON REDUCTION OF SILICON AND CALCIUM IMPURITIES IN ALUMINUM SOLUTION

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
As it is known, during regular conditions Bayer's process is used for production of alumina of a 99.0 % grade of purity. In order to obtain high-purity alumina which can then be used for special purposes, additional purification is performed in relation to the application of impurities, and, in the first hand removal of silica (Si) and calcium (Ca). One of the most effective ways of removing these compounds is the process of desilication. The method consists of treating an aluminate solution with lime that binds silica and calcium to tricalcium-aluminate (TCA) which is insoluble and therefore easily separated of the solution. The experimental research examined the impact of process parameters (temperature, time, concentration of added lime) on the efficacy of purifying aluminate solution from Si and Ca, which has a practical and theoretical contribution to aluminate solution research. Synthetic aluminate from non-metallurgic alumina and pure sodium-alkaline (NaOH) is used, in the caustic ratio in the range of 1.45-1.55. Classic volume analysis and analysis using optical emigration spectroscopy (ICP-OES) were used to determine the contents of aluminum and impurities. The experimental research found that short intervals are adverse because soluted compounds $Ca(OH)_2$ and tetra-calcium aluminum are formed. Also, during longer reaction time and higher temperatures there was an increase in the contents of Si and Ca in the aluminum, because some components from the limestone were dissolved. Small amounts of lime are adverse because there is an increase in calcium content in the solution since part of the lime dissolved, without interacting with the present impurities, while large quantities led to large aluminum loss due to the formation of TCA.

KEYWORDS: aluminate solution, high purity alumina, lime

INTRODUCTION

The mentioned process of Al_2O_3 production is based on the precipitation of aluminum hydroxide crystals, as polymorphs of gibsite from caustic solutions of sodium aluminate [1]. The chemical basis of the Bayer process can be summarized via the following reversible chemical equation:

$$Al(OH)_{3(s)} + NaOH_{(aq)} \leftrightarrow NaAl(OH)_{4(aq)} \tag{1}$$

A solution of sodium aluminate was obtained by leaching the hydrated alumina from bauxite with a hot NaOH solution [2,3]. In the second part of the Bayer process, aluminum hydroxide is added to the sodium aluminate solution to act as a crystallization germ. As a result, $Al(OH)_3$ crystals are segregated, which are converted into anhydrous Al_2O_3 by calcination, while the regenerated alkali is returned to the process [4,5].

Minerals in bauxite such as rutile (TiO_2), hematite (Fe_2O_3), magnetite (Fe_3O_4), organic bitumens are insoluble in NaOH and as such can be removed as waste sediment, known as "red mud" [6]. In addition to aluminum oxide, other minerals present in bauxite react with NaOH solution to form a soluble compound, e.g. compounds of silicon, phosphorus (P_2O_5), vanadium (V_2O_5), gallium (Ga_2O_3), sulfides (FeS_2), minerals magnesite ($MgCO_3$), siderite ($FeCO_3$)… [7]. Due to their nature, they represent a technological problem because they can be found in the final product. The most common compounds in bauxite are silicon compounds, which can be free as quartz

(SiO_2) or bound in kaolinite (Al_2O_3•$2SiO_2$•$2H_2O$) or other aluminosilicates. Their removal is the most common problem in Bayer's production process [8]. They react with the alkali to form a solution of sodium silicate:

$$SiO_2 + 2NaOH \rightarrow Na_2SiO_3 + H_2O \qquad (2)$$

$$Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O + 6NaOH \rightarrow 2NaAlO_2 + 2Na_2SiO_3 + 5H_2O \qquad (3)$$

The formed sodium silicate reacts with sodium aluminate to form permutite ($Na_2O \cdot Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$), which is insoluble and precipitates [2]. Although the solution is purified from silicon in this way, the basic problem lies in the fact that binding Na_2O and Al_2O_3 also occurs, which represents a pure loss of alkali and aluminum.

By adding lime, one part of the dissolved silicon will bind into a low soluble hydrogranate:

$$\begin{aligned}&3Ca(OH)_2 + 2NaAlO_2 + mNa_2SiO_3 + (4-m)H_2O = \\ &= 3CaO \cdot Al_2O_3 \cdot mSiO_2(6-2m)H_2O + 2(1+m)NaOH\end{aligned} \qquad (4)$$

This compound does not contain NaOH, which allows it to be reused in the process [6]. In addition to the basic role of purification of silicon solution, the addition of lime increases the leaching rate and the degree of alumina utilization [9]. The mechanism of action is such that it destroys the insoluble membranes of sodium titanate that envelop $Al(OH)_3$ particles, thus enabling their contact with the alkali [10,11,12].

Despite the process of purification of bauxite by the Bayer process, the corresponding impurities remain in the solution and later crystallize together with aluminum hydroxide. **Table 1** shows the content of non-metallurgical and metallurgical alumina.

Table 1. Composition of metallurgical and non-metallurgical alumina [13]

Component	Metallurgical alumina		Non-metallurgical alumina	
	Typical values [%]	The limit values [%]	Typical values [%]	The limit values [%]
Al_2O_3	98.8-99.0	98.7	98.8-99.0	98.7
SiO_2	0.005-0.010	0.015 max	0.005-0.010	0.015 max
Fe_2O_3	0.008-0.012	0.018 max	0.008-0.012	0.018 max
Na_2O_{total}	0.30-0.35	0.42 max	0.15-0.20	0.25 max
CaO	0.018-0.025	0.030 max	0.018-0.025	0.030 max
P_2O_5	0.0002-0.0005	0.0009 max	0.0002-0.0005	0.0009 max
TiO_2	0.0020-0.0027	0.003 max	0.0020-0.0027	0.003 max
ZnO	0.017-0.020	0.022 max	0.017-0.020	0.022 max

As can be seen from **Table 1**, 1-1.3 % of impurities can be found in the final product, where SiO_2, CaO, Fe_2O_3, ZnO and Na_2O make up the largest part. As mentioned, silicon and calcium compounds are removed by desilicatization by adding calcium compounds to the solution (mainly lime is used) which has the role of binding the present silicon to the insoluble compound [14]. Fe_2O_3 and ZnO are removed by the addition of finely precipitated hydroxide which acts as a crystallization germ, while Na_2O is removed by the hydrothermal washing method [15,16,17].

Silicon dioxide (SiO_2) aggravates most of the industrially important properties of alumina, and is undesirable (but usually unavoidable) in the product. First, silicon is a harmful pollutant in aluminum metal, and especially in industrial alumina powder [4]. A bigger problem occurs with the application of alumina in special processes. Thus, a very important question is what is the composition of the alumina used as a raw material for the production of alumina-based ceramics, given that silicon contamination is a problem in all advanced applications of ceramics. Silicon is harmful in the high-quality ceramic industry because it concentrates on grain boundaries and makes alumina sensitive to static fatigue caused by moisture; it is a serious problem if it occurs in dental or orthopedic implants, reduces refractoriness when alumina is used as a refractory material, reduces hardness [18,19,20].

The subject of this research is the removal of silicon and calcium from the aluminate solution by the addition of calcium oxide in order to obtain special (advanced) types of high purity alumina. For this purpose, the aluminate solution was treated with lime during the experiment because it is the most industrially and commercially acceptable, most efficient, does not change the caustic ratio and does not contaminate the solution. This paper also investigates the influence of process parameters (desilicatization time, temperature and amount of added lime) on the process of silicon and calcium removal.

EXPERIMENTAL

MATERIALS AND METHODS

Non-metallurgical hydrate obtained in the factory "Alumina" d.o.o. Zvornik and alkali NaOH were used as raw materials for obtaining ultra-pure aluminum hydroxide.

The leaching rate of aluminum hydroxide is optimized by the addition of lime ($CaO_{active} = 90.93\%$).

SYNTHESIS OF SYNTHETIC ALUMINATE

To obtain a volume of 1 l of synthetic aluminate with caustic modulus α_k=1.50 (tolerance 1.45 - 1.55), 204 g of sodium hydroxide and 260 g of dry non-metallurgical hydrate were weighed on an analytical scale. The measured NaOH was transferred to a 2 l beaker and then was added water up to 350 ml. This solution was first heated and then dry non-metallurgical hydrate was added moderately. The resulting solution was further heated and allowed to boil for 15 min.

After that, the obtained aluminate solution was cooled to room temperature and transferred to a 1 l Erlenmeyer flask, with water addition to the line marked on the neck of the flask. Volume (100 ml) of the solution was taken to determine Al_2O_3, Na_2O_k, Ca and Si in the initial sample, while the rest was transferred to a beaker and heated to the synthesis temperature. Finally, a given amount of lime is added to that synthetic solution.

Upon completion of the synthesis, the solution was filtered using a Büchner flask and funnel (a white filter strip was used). The filtrate as well as the initial sample was subjected to classic volumetric analysis and analysis using optical emigration spectroscopy to determine the content of Al_2O_3, Na_2O_k, Ca and Si. The precipitate left on the strip was washed with 1 l of hot distilled water and then dried for 2 h at 105°C to remove a coarse moisture.

RESULTS AND DISCUSSION

INFLUENCE OF TIME ON THE PROCESS OF PURIFICATION OF ALUMINATE SOLUTION

Determination of the influence of time on the purification of silicon and calcium was performed at a temperature of t = 65 °C, the amount of additional lime of 20 g/l for different times of 10 - 50 min. **Table 2** shows the results.

Table 2. Influence of time as a process parameter on the purification of aluminate solution

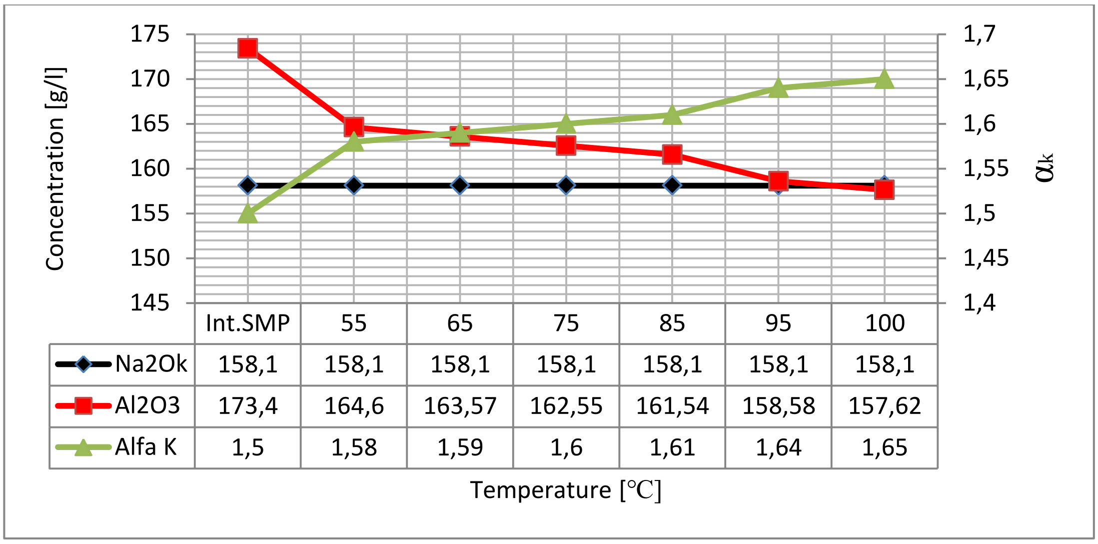
Method of analysis		**Time [τ]**					
		Initial sample	**10 min.**	**20 min.**	**30 min.**	**40 min.**	**50 min.**
Classic volumetric analysis							
	Na_2O_k [g/dm^3]	156.55	156.55	156.55	156.55	156.55	156.55
	Al_2O_3 [g/dm^3]	169.83	168.30	164.73	163.88	163.26	161.97
	α_k	1.52	1.53	1.56	1.57	1.58	1.59
ICP-OES							
	Ca [ppm]	9.08	1.36	2.28	1.72	0.96	1.68
	Si [ppm]	78.16	30.8	23.68	23.44	20.08	18.88
Granulation TCA							
	ds50% [μm]		6.18	7.47	6.19	8.06	9.08

The concentration of Na_2O_k remained the same even after 50 min., which confirms the fact that the hydrogranate was formed by addition of lime, which does not bind alkali. The amount of Al_2O_3 in the solution decreases from 169.83 g/dm^3 in the initial sample to 161.97 g/dm^3 after 50 min. of reactions, with a corresponding increase in the caustic module. The Si content decreases over time, while the Ca content is the lowest after 40 min. The reason for that is that insoluble tricalcium aluminate was formed. A further increase in time leads to an increase in the Ca content in the solution because certain components from the industrial lime have dissolved. From the previous table, it is clear that at a temperature of

65°C and at amount of 20 g/l of lime, the time after process should be stopped is 50 min. Although there was a slight increase in the calcium content of the solution, at that time the silicon content was the lowest.

INFLUENCE OF TEMPERATURE ON THE PROCESS OF PURIFICATION OF ALUMINATE SOLUTION

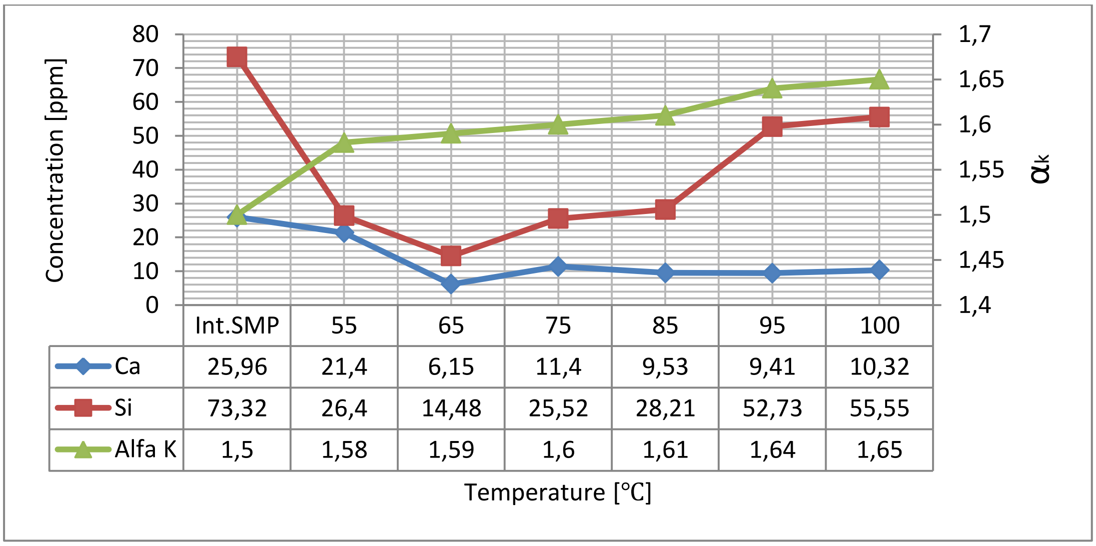
Figures 1 and **2** show the influence of temperature on the content of aluminate solution by the addition of 20 g/l of lime and after 120 min. of reaction.



	Int.SMP	55	65	75	85	95	100
Na2Ok	158,1	158,1	158,1	158,1	158,1	158,1	158,1
Al2O3	173,4	164,6	163,57	162,55	161,54	158,58	157,62
Alfa K	1,5	1,58	1,59	1,6	1,61	1,64	1,65

Figure 1. Dependence of caustic ratio and concentration of Na_2O_k and Al_2O_3 on temperature

As with the influence of time as a process parameter, even with a change in temperature there is no significant change in the content of caustic soda. The aluminum content decreases with increasing temperature, which is followed by an increase in the caustic module.



	Int.SMP	55	65	75	85	95	100
Ca	25,96	21,4	6,15	11,4	9,53	9,41	10,32
Si	73,32	26,4	14,48	25,52	28,21	52,73	55,55
Alfa K	1,5	1,58	1,59	1,6	1,61	1,64	1,65

Figure 2. Dependence of Si and Ca contents on temperature

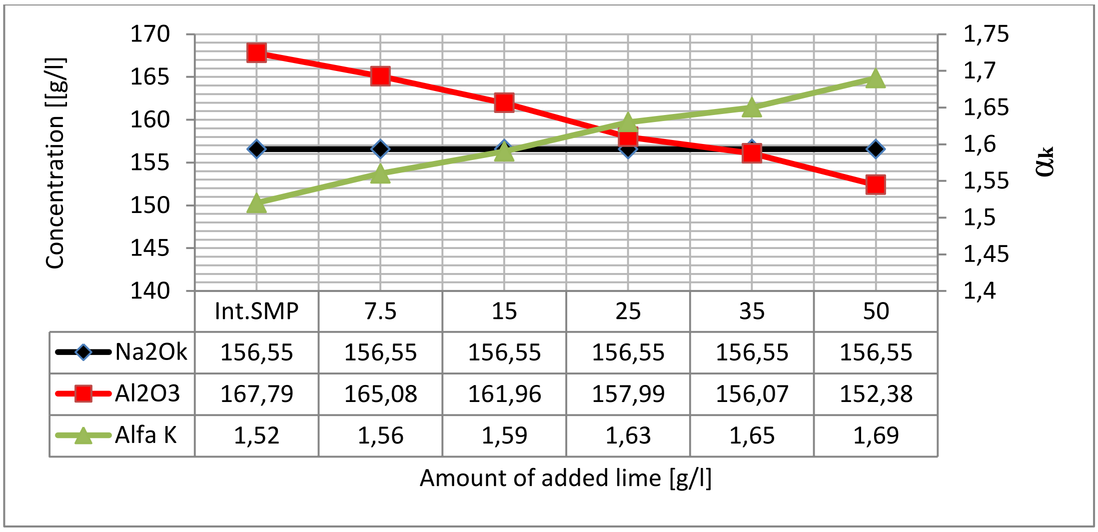
At temperatures below 55°C, calcium removal is very slow, and only 17 % of the starting amount is removed. From the previous diagram it can be seen that the concentration of calcium is the lowest at 65°C, at which its concentration is 6.15 ppm. Unlike calcium, the removal of silicon is more intensive at lower temperatures (removed even 65 % Si at t = 55°C), while at a temperature of 65°C, similar to calcium, the maximum amount of silicon is removed. At this temperature, the minimum concentration of Si and Ca is observed on the graph of the dependence of concentration on temperature (**Figure 2**). At higher temperatures, there was an increase in the solubility of the components present in the lime, which in total increases the concentration of impurities in the final aluminate. Therefore, this temperature is adopted as optimal and future industrial processes of obtaining high purity alumina can take place on it.

INFLUENCE OF THE AMOUNT OF ADDED LIME ON THE PROCESS OF PURIFICATION OF ALUMINATE SOLUTION

Determination of the influence of the concentration of added lime was performed at the following process parameters:

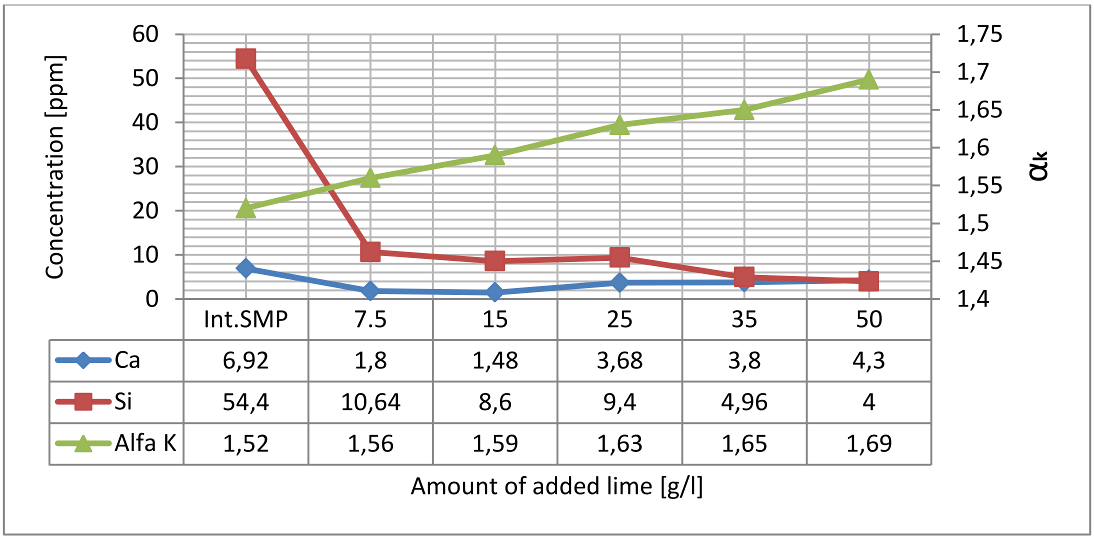
t = 65 °C
τ = 120 min.
c_{lime} = 7.5 - 50 g/l

Even at different concentrations of added lime, the amount of sodium present in the solution will not change and will remain the same as in the starting sample. As the amount of added lime increases, the Al_2O_3 content in the solution decreases. Increasing the concentration of calcium present leads to a larger number of collisions with aluminum molecules from the aluminate, which results in the formation of insoluble tricalcium aluminate (**Figure 3**).



	Int.SMP	7.5	15	25	35	50
Na2Ok	156,55	156,55	156,55	156,55	156,55	156,55
Al2O3	167,79	165,08	161,96	157,99	156,07	152,38
Alfa K	1,52	1,56	1,59	1,63	1,65	1,69

Figure 3. Dependence of the caustic ratio and concentration of Na_2O_k and Al_2O_3 on the amount of added lime.



	Int.SMP	7.5	15	25	35	50
Ca	6,92	1,8	1,48	3,68	3,8	4,3
Si	54,4	10,64	8,6	9,4	4,96	4
Alfa K	1,52	1,56	1,59	1,63	1,65	1,69

Figure 4. Dependence of Si and Ca content on the amount of added lime

Figure 4 shows the dependence of the content of Si and Ca in the solution on the amount of added lime. The addition of relatively small amounts of lime is advantageous because there is a pronounced removal of calcium and silicon in the solution. The addition of 15 g/l of lime removes most of the calcium and it lagged only 1.48 ppm. With further addition of lime, the value of silicon constantly decreases, while the content of calcium in the solution increases. After 120 min. of reactions and at a temperature of 65°C, depending on whether it is desired to remove more calcium or silicon, two different optimal amounts of added lime are adopted: 15 g/l and 50 g/l of added lime. The addition of 50 g/l of lime removes most of the silicon due to the formation of insoluble hydrogranate, but in some cases this is not desirable because larger amounts of impurities pass from industrial lime into solution and because the Al_2O_3 content decreases due to insoluble tricalcium aluminate. Therefore, the process can take place with the addition of 15 g/l of lime, whereby larger amounts of calcium are removed with significantly lower losses of aluminum.

CONCLUSION

The aim of this study was to determine the influence of process parameters on the removal of silicon and calcium from non-metallurgical hydrate, which can then be used as a precursor for obtaining special types of alumina. Unlike the Bayer process where bauxite is the starting material, in this case the non-metallurgical hydrate was converted to a soluble form by the addition of alkali and then treated with lime to remove Si and Ca. In order to determine the parameters at which the largest amount of impurities will be removed, the optimal temperature, reaction time and the amount of added lime during the desilication process were determined.

In the process carried out at a temperature of 65°C and the amount of additional lime of 20 g/l, the optimal duration of the desilication process is 50 min. During this time, most of the silicon is removed because an insoluble compound of hydrogranate, which contain Ca and Si, is formed, which purifies the solution. The desilication process, which was carried out at τ = 120 min., c_{CaO} = 20 g/l and temperatures in the range of 55 - 100°C, determined the mechanism of removing impurities from the solution. Calcium and silicon had the lowest value at a temperature of 65°C, why is this temperature considered as optimal on which future processes could be based. Higher temperatures led to the dissolution of individual components from the lime, which resulted in an increase in the impurity content in the final solution. Finally, the influence of the amount of added lime on the desilication process was determined. The addition of 50 g/l of lime removes most of the silicon, but due to the formation of insoluble hydrogranate, large losses of aluminum are expressed. Therefore, in special cases, 15 g/l of lime is added, removing most of the calcium with negligible alumina losses.

During future research, additional optimization is needed to determine the concentration of lime and its full effect on calcium removal. For that purpose, research should be performed by adding lime in the range c_{CaO} = 7.5 - 15 g/l. The next round of optimization should be performed at the recommended value of the amount of lime addition and temperature, while the time range will be extended in relation to this research and will be in the range of 10 - 130 min. Also, in order to obtain high purity alumina, in addition to desilication as a method of removing Si and Ca, it is necessary to include a method based on the addition of crystallization initiators which remove zinc (Zn) and iron (Fe), and a method of hydrothermal washing which removes sodium (Na). By optimizing the parameters from the purification procedures, high-purity aluminum hydroxide was synthesized, which can be used for special purposes due to its physical and chemical properties.

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COMPARISON OF BROWN SOIL OF DIFFERENT EXPOSURES IN THE AREA OF THE NATIONAL PARK “UNA” WITH SPECIAL EMPHASIS ON THE DISTRIBUTION OF CADMIUM, NICKEL AND ARSENIC

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
The paper presented results of a comparison of brown soils of different exposures at two different sites within the National Park „Una“. The sites were related to the southwestern exposure (SW) and the northeastern exposure (NE) of the same land use (orchard). The main objective of the research, in addition to the physical and chemical parameters of soil quality, was to determine the distribution of the total content of cadmium (Cd), nickel (Ni) and arsenic (As). The total content of these elements werw observed in composite samples at two depths of 0 – 10 and 0 – 20 cm and horizons profile. Their total content was measured by atomic adsorption spectrometry – AAS. The results were statistically analyzed using Kruskal - Walisovog test at the significance level of $p \leq 0.05$ using correlation coefficient χ^2. The results showed a single legality of the distribution of Cd, Ni and As in samples of soil profile, while the average soil samples showed unique legality of the distribution of observed elements.

KEYWORDS: brown soil, distribution, cadmium, nickel, aresenic

INTRODUCTION

The area of the National Park „Una“ has always aroused the interest of many scientists and researchers [1]-[3]. In particular, the issues of distribution of toxic elements in the soil have been dealt with, both in our country and the world, by many researchers coming from different functional areas: industrial [4]-[6], urban [7], landfills [8] and protected areas [9]-[11]. Especially the problem of deposition of various emissions under the influence of wind as a transmission factor was dealt with [12]-[16] and found that depending on the direction, intensity and frequency of wind can transmit various emissions (dry and wet) over long distances from the source. There is also monitoring of deposition monitoring, for example within the UN organization[1], within the EEA Agency[2], etc. In Bosnia and Herzegovina, air quality monitoring is performed at the entity level (F BH, RS and BD).

This paper presents the results of a comparison of brown soil of different exposures of the same land use (orchard) at an inclination within the NP „Una“. The research was performed with the aim of comparing soil samples from two localities of the same soil type (brown soil, opposite exposures (southwest – SW and northeast – NE), with the same sampling depths of average samples and approximately the same altitudes. The study has placed a special emphasis on the distribution of the total content of cadmium (Cd), nickel (Ni) and arsenic (As) in the average samples (0 – 10 and 0 – 20 cm) and by depth of the soil profile. Selected research sites were located on the slopes of the southwestern (SW) and northeastern (NE) exposures in the Cukovi settlement. The first site SW – southwest exposure (altitude: 400 m, wind speed: 6 m/s, pressure: 1354.56 hPa) and the second locality NE – northeast exposure (altitude: 448 m, wind speed: 11 m/s, pressure: 1388.42 hPa). Both sites have the same land use (orchard). The northeastern locality is otherwise exposed to the prevailing wind gusts (Figure 1).

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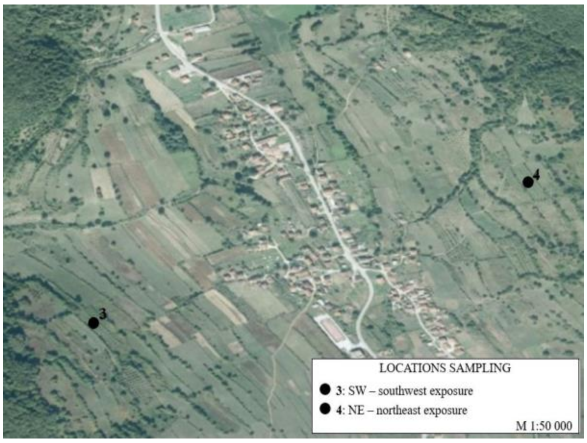

Figure 1. Slopes of opposite exposures in the settlement of Ćukovi

According to pedological maps of BH [17] and pedological interpreters (Bihac 3), brown soils alternate from deep to very shallow at selected localities. Lighter mechanical composition – sandy loam to sandy clay in the upper layers, and the depth of the soil profile heavier – clay and heavy clay. The soils are porous (P) and have medium and moderate capacities for water (K_w) and air (K_a). The soils are weakly humus, poor in potassium (K_2O) and phosphorus (P_2O_5), slightly richer in calcium carbonate ($CaCO_3$). Their color is usually brown and yellow-brown.

MATERIALS AND METHODS

This paper presents results of the field research, sampling and laboratory testing. All analyzes of soil samples were determined according to standardized methods: Mechanical composition (Modified method B pipettes, ISO 11277); Structure (Method by Sekera); The rights specific gravity, Porosity (Conventional method, ISO 11508); The volume spacific gravity (Gravimetric method by Kopecky, ISO 11272); Actual humidity, Capacity of soil for water and Air capacity (Gravimetric method, ISO 11465); Humus (Spectrophotometric method, ISO 10694); $CaCO_3$ (Volumetric method ISO 10693); pH (Electrometric method, ISO 10390); El.conductivity (Electrometric method, ISO 11265); P_2O_5 and K_2O (Al method, ISO 19730) and The total content of metals and metalloids in the soil (Extraction HCl/HNO_3 = 3:1, ISO 11466). A total of two pedological profiles were opened at the sites of research and average samples were taken from two soil depths: 0 – 10 cm and 0 – 20 cm. Preparation of soil samples was carried out at the Biotechnical Faculty of the University of Bihac. All analyses of soil samples were carried out in the laboratory of the Institute of Soil Science, Agrochemistry and Reclamation of the Faculty of Agricultural and Food Sciences, University of Sarajevo, except for the content of arsenic that was carried out at the Faculty of Agriculture, University of Zagreb, and the Institute for Medical Research and Occupational Health in Zagreb. Statistical analysis was performed by the Kruskal – Wallis test at the level of significance of $p \leq 0.05$. To determine the relation and distribution of Cd, Ni and As in the soil we used the correlation coefficient (χ^2) [18], [19]. Data were statistically analyzed using SPSS 17. The maximum allowable amounts of total contents were determined in accordance with the current regulations [20] in relation to the soil texture (MRL) and the use of soil in organic farming (MRL-OF). Used are orthophoto imagery in the scale of 1:5000 and 1:75000, topographic maps and GIS.

RESULTS AND DISCUSSION

Results of the study on the comparision of brown soils with the same soil use but opposite exposures provide:

Description of the brown soil profile at the site of the southwestern (SW) exposure (Figure 2)

Site: the slope of the southwestern exposure – SW (N 44° 39.810′, E 16° 04.059′)
Vegetation: orchard (plum)
Use: orchard
Parent substrate: limestone

Ah	0-19 cm: surface layer interwined with roots, jellow-brown in colour, with fine granular structure and sandy loamy clay texture (sand = 47.9 %, powder = 25.2 %, clay = 26.9 %)
A/(B)	19-42 cm: transitional horizon, dark yellow-brown colour with evident sprouted roots and clay loam texture (sand = 38.0 %, powder = 32.1 %, clay = 29.9 %)
(B)	42-68 cm: cambic horizon, no visible plant roots, dark yellow-brown colour with skeleton (20%) and clay loam texture (sand = 31.0 %, powder = 36.0 %, clay = 33.0 %)
(B)	68-100 cm: sceleton (> 30%) with fine granular structure and clay texture (sand = 28.0 %, powder = 33.0 %, clay = 39.0 %).
C	>100 cm: coarse limestone

Description of the brown soil profile at the site of the norteast (NE) exposure (Figure 3)

Site: the slope of the norteast exposure – NE (N 44° 39.571′, E 16° 03.537′)

Vegetation: orchard (plum)
Use: orchard
Parent substrate: limestone

Ah	0-19 cm: sufrace layer interwined with roots, dark jellow-brown in colour, with granular structure and sandy clay texture (sand = 62.1 %, powder = 1.1 %, clay = 36.8 %)
(B)	19-45 cm: adhesive layer with skeleton (> 50%) and clay texture (sand = 27.6 %, powder = 29.1 %, clay = 43.3 %)
C	>45 cm: crumbly coarse limestone

Figure 2 – 3. Profiles of soil at the sites SW (left) and NE (right)

Physical properties of soil at the sites of SW and NE

The stability of the structural aggregates in the depth of the profile was very good to good. The mechanical composition in the surface layer was lighter – sandy loamy clay, in deeper layers – clay. The soils were porous, with medium soil water and moderate soil air capacities.

Mean values of real and bulk specific density also indicated that the soils were porous. Overview of the physical properties of the soil is provided in Table 1.

Table 1. The physical properties of the soil profile (Σn = 18)

Parameters	$X_{mv} \pm X_{se}$		min	max	V	σ
S	SW	3.16 ± 0.27	2.00	4.00	0.87	0.93
	NE	1.66 ± 0.33	1.00	3.00	0.66	0.81
P_{sg} (g/cm^3)	SW	2.61 ± 0.03	2.50	2.76	0.01	0.09
	NE	2.58 ± 0.01	2.56	2.60	0.00	0.01
V_{sg} (g/cm^3)	SW	1.42 ± 0.03	1.23	1.56	0.01	0.09
	NE	1.27 ± 0.02	1.21	1.33	0.00	0.04
P (%)	SW	46.35 ± 1.00	42.30	50.70	9.15	3.02
	NE	48.96 ± 0.54	48.00	49.90	0.90	0.95
K_w (%)	SW	38.46 ± 1.06	30.80	41.90	10.19	3.19
	NE	41.86 ± 0.24	41.40	42.20	0.17	0.41
K_a (%)	SW	6.81 ± 0.85	3.80	10.70	5.82	2.41
	NE	8.23 ± 0.46	7.40	9.00	0.64	0.80

SW – southwest exposure; NE – northeast exposure; X_{mv} – mean value, X_{se} – statistical error, min – minimum value, max – maximum value, V – variance, σ – deviation, n –numer of samples, S – soil structure, P_{sg} – the righs specific gravity, V_{sg} – the volume spacific gravity, P – porosity, K_w – capacity of soil for water, K_a – air capacity

Chemical properties of the soil profile and average samples 0 – 20 cm at sites SW and NE

In open profiles the reactions of pH values of SW and NE ranged from slightly acidic to alkaline. In the surface layer, the contents of P_2O_5, K_2O and humus were low and deteriorated rapidly with the depth. Total content of $CaCO_3$ was high with a tendency of increase with the profile depth. Mean values of measured electro-conductivity of the soil were not indicative of soil salinity at the profile.

Results of the observed chemical parameters in samples (0 – 20 cm) had similar characteristics as the parameters of the soil profile of the surface layer (Table 2 – 3).

Table 2. The chemical properties of the soil profile ($\Sigma n_{SW\text{-}NE}$ = 18)

Parameters		$X_{mv} \pm X_{se}$	min	max	V	σ
pH_{H2O}	SW	8.47 ± 0.04	8.19	8.67	0.02	0.14
	NE	7.62 ± 0.26	7.01	8.25	0.43	0.65
pH_{KCl}	SW	7.26 ± 0.02	7.13	7.41	0.005	0.07
	NE	6.27 ± 0.32	5.30	6.98	0.62	0.78
EC (µS/cm)	SW	209.03 ± 9.97	160	271	1193.70	34.55
	NE	149.60 ± 26.47	86	222	4204.20	64.84
K_2O (mg/100g soil)	SW	11.50 ± 1.09	7.20	19.10	14.32	3.78
	NE	9.56 ± 1.38	4.50	13.50	11.47	3.38
P_2O_5 (mg/100g soil)	SW	2.04 ± 0.22	1.08	3.45	0.58	0.76
	NE	1.63 ± 0.28	0.77	2.63	0.48	0.69
Humus (%)	SW	0.73 ± 0.16	0.34	1.94	0.34	0.58
	NE	1.63 ± 0.29	0.90	2.52	0.52	0.72
$CaCO_3$ (%)	SW	6.81 ± 0.30	5.13	8.80	1.14	1.07
	NE	3.61 ± 0.03	3.50	3.70	0.01	0.08

SW – brown deep soil on the southwestern exposure; NE – brown shalow soil on the northeast exposure, n – numer of samples, X_{mv} – mean value, X_{se} – statistical error, min – minimal value, max – maximum value, V – variance, σ – deviation

Table 3. The chemical properties of the average samples 0 – 20 cm ($\Sigma n_{SW\text{-}NE}$ = 18)

Parameters		$X_{mv} \pm X_{se}$	min	max	V	σ
pH_{H2O}	SW	7.74 ± 0.16	7.06	8.23	0.16	0.40
	NE	7.05 ± 0.05	6.91	7.29	0.02	0.14
pH_{KCl}	SW	6.79 ± 0.16	6.28	7.19	0.16	0.4
	NE	5.43 ± 0.04	5.30	5.55	0.01	0.11
K_2O (mg/100g soil)	SW	27.90 ± 3.52	16.50	36.80	74.61	8.63
	NE	12.23± 0.89	9.80	14.80	4.75	2.18
P_2O_5 (mg/100g soil)	SW	3.25 ± 0.64	1.08	4.89	2.51	1.58
	NE	2.50 ± 0.67	1.19	5.72	2.69	1.64
Humus (%)	SW	2,31 ± 0,20	1,73	2,77	0,25	0,50
	NE	2.65 ± 0.25	1.43	3.10	0.39	0.62
$CaCO_3$ (%)	SW	3.84 ± 0.20	2.93	4.42	0.26	0.51
	NE	2.32 ± 0.24	1.63	2.93	0.37	0.61

SW – southwest exposure; NE – northeast exposure; n – numer of samples; X_{mv} – mean value; X_{se} – statistical error; min – minimal value; max – maximum value; V – variance; σ – deviation

Distribution of the total contents of Cd, Ni and As in average samples

In average samples at the sites of SW and NE (0 – 10 and 0 – 20 cm), a significant difference ($p \leq 0.05$) in the total content of Cd, Ni and As was established (Table 4).

Table 4. Distribution of the total contents of Cd, Ni and As in average samples

Observed		$X_{mv} \pm X_{se}$	min	max	V	σ	χ^2	p
Deph 0 – 10 cm (Σn = 9)								
Cd (mg/kg)	SW	0.08 ± 0.03	0.08	0.09	0.00	0.05	4.50	$p \leq 0.05$
	NE	0.52 ± 0.01	0.52	0.54	0.00	0.01		
Ni (mg/kg)	SW	77.79 ± 0.01	77.80	78.70	0.00	0.01	4.35	$p \leq 0.05$
	NE	112.44 ± 0.03	112.40	112.50	0.03	0.05		
As (mg/kg)	SW	9.74 ± 0.03	9.70	9.80	0.03	0.05	3.97	$p \leq 0.05$
	NE	17.20 ± 0.00	17.20	17.21	0.00	0.01		
Deph 0 – 20 cm (Σn = 9)								
Cd (mg/kg)	SW	0.09 ± 0.01	0.08	0.11	0.00	0.01	9.54	$p \leq 0.05$
	NE	0.35 ± 0.07	0.17	0.54	0.03	0.19		
Ni (mg/kg)	SW	82.84 ± 2.05	77.80	88.00	30.59	5.53	8.45	$p \leq 0.05$
	NE	119.56 ± 2.90	112.40	126.70	60.83	7.79		
As (mg/kg)	SW	10.87 ± 0.50	9.70	12.10	1.53	1.23	8.45	$p \leq 0.05$
	NE	18.35 ± 0.48	17.20	19.50	1.57	1.25		

SW – southwest exposure; NE – northeast exposure; n – numer of samples, X_{mv} – mean value, X_{se} – statistical error, min – minimal value, max – maximum value, V – variance, σ – deviation, χ^2 – Kruskal – Wallis coefficient; p – level of significance

In the average samples at the sites of SW and NE (0 – 10 and 0 – 20 cm)), total mean contents of Cd and As were lower than the permitted levels of MRL and MRL-OF, while the content of Ni was exceeded MRL and MRL-OF. At the sites SW and NE in depth 0 – 10 cm (Cd_{SW} = 0.08 mg/kg; Cd_{NE} = 0.52 mg/kg; Ni_{SW} = 77.79 mg/kg; Ni_{NE} = 112.44 mg/kg; As_{SW} = 9.74 mg/kg; As_{NE} = 17.20 mg/kg), and the sites SW and NE in depht 0 – 20 cm (Cd_{SW} = 0.09; Cd_{NE} = 0.35; Ni_{SW} = 82.84 mg/kg; Ni_{NE} = 119.56 mg/kg; As_{SW} = 10.87 mg/kg; As_{NE} = 18.35 mg/kg).

Generaly the total contents of the analyzed elements at NE exposure (under the influence of stronger wind gusts) were higher compared to the opposite SW exposure (weaker wind gusts), which confirms the fact of possible atmospheric deposition of dry or wet emissions on wind-affected slopes (Figure 1 – 3).

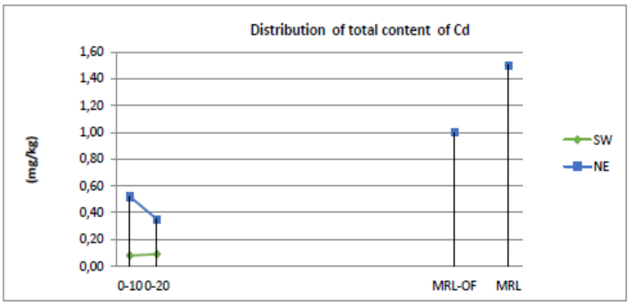

Figure 1. Distribution of the total content of Cd (0 – 10 and 0 – 20 cm)

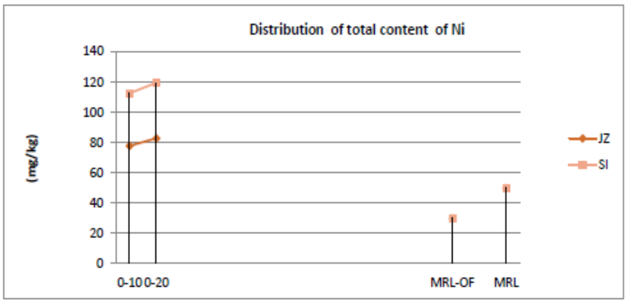

Figure 2. Distribution of the total content of Ni (0 – 10 and 0 – 20 cm)

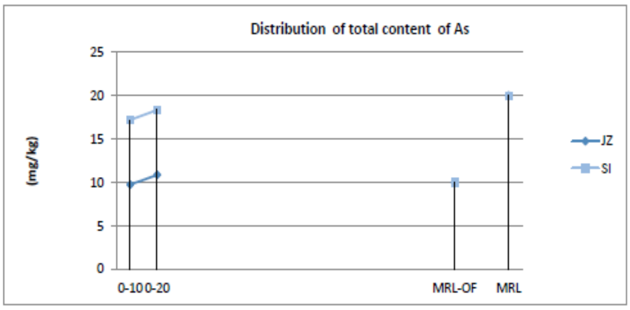

Figure 3. Distribution of the total content of As (0 – 10 and 0 – 20 cm)

Distribution of the total content of Cd, Ni and As in the soil profiles

Results of the analysed profiles SW and NE sites of the same use (orchard – plum) have shown significant differences ($p \leq 0.05$) in concentrations of Cd, Ni and As (Table 5).

Table 5. Distribution of the total content of Cd, Ni and As in the soil profiles ($\Sigma n_{JZ\text{-}SI} = 18$)

Observed		$X_{mv} \pm X_{se}$	min	max	V	σ	χ^2	p
Cd (mg/kg)	SW	0.11 ± 0.00	0.10	0.13	0.00	0.01	11.37	$p \leq 0.05$
	NE	0.48 ± 0.13	0.15	0.80	0.10	0.33		
Ni (mg/kg)	SW	95.00 ± 1.87	83.66	99.20	42.34	6.50	11.38	$p \leq 0.05$
	NE	160.01 ± 15.84	124.40	195.54	1506.81	38.81		
As (mg/kg)	SW	11.10 ± 0.32	10.00	12.70	1.28	1.13	11.47	$p \leq 0.05$
	NE	18.60 ± 0.42	17.50	19.80	1.08	1.04		

SW – southwest exposure; NE – northeast exposure; n – numer of samples; X_{mv} – mean value, X_{se} – statistical error, min – minimal value, max – maximum value, V – variance, σ – deviation, χ^2 – Kruskal – Wallis coefficient; p – level of significance

Observed in general in the examined profiles at the SW and NE sites, the total mean contents of Cd, Ni and AS in the SW exposure profile were higher in relation to the opposite NE (Figure 4 – 6). In the examined profiles, the total mean Cd content on the SW exposure was higher by 78%, for Ni and As by 41% in relation to the opposite NE exposure.

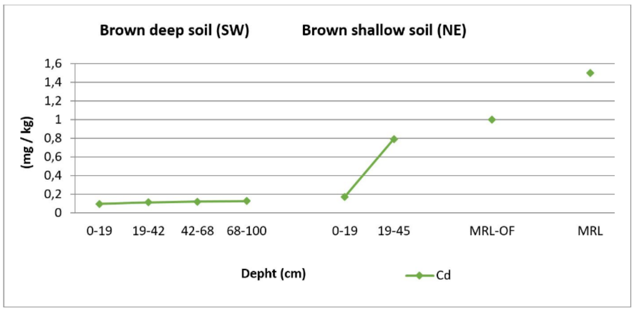

Figure 4. Distribution of the total content of Cd in soil profiles

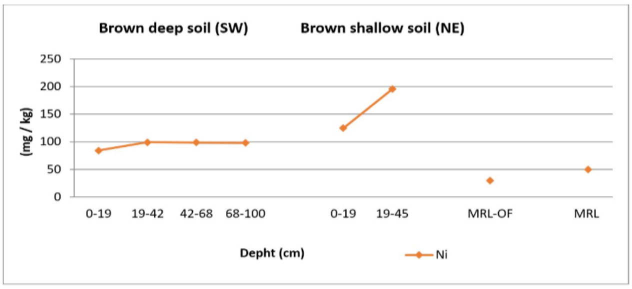

Figure 5. Distribution of the total content of Ni in soil profiles

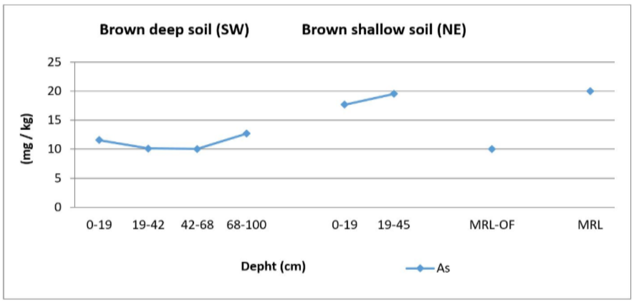

Figure 6. Distribution of the total content of As in soil profiles

CONCLUSION

At the sites of SW and NE, according to the physical and chemical parameters of soil, stability of structural aggregates declines from good to very good; mechanical composition of the soil in the surface layer – sandy loamy clay, and in the deeper ones – clay. The soils were porous, with medium soil water and moderate soil air capacities.

In general, the pH values at the sites of SW and NE ranged from weakly acidic to alkaline with an upward trend with the profile depth ($pH_{SW\ in\ H20}$ 8.19 – 8.67; $pH_{SW\ in\ KCl}$ 7.13 – 7.41; $pH_{NE\ in\ H20}$ 7.01 – 8.25; $pH_{NE\ in\ KCl}$ 5.30 – 6.98) and average samples ($pH_{SW\ in\ H20}$ 7.06 – 8.23; $pH_{SW\ in\ KCl}$ 6.28 – 7.19; $pH_{NE\ in\ H20}$ 6.91 – 7.28; $pH_{NE\ in\ KCl}$ 5.30 – 6.55).

In the average samples (0 – 10 and 0 – 20 cm) at the sites, total mean content of Cd were lower than the permitted levels of MRL-OF and MRL (Cd_{SW} = 0.08 – 0.11 mg/kg; Cd_{NE} = 0.17 – 0.54 mg/kg), while the contents of Ni nad As was exceeded MRL-OF and MRL (Ni_{SW} = 77.80 – 88.00 mg/kg; Ni_{NE} = 112.40 – 126.70 mg/kg; As_{SW} = 9.70 – 12.10 mg/kg; As_{NE} = 17.20 – 19.50 mg/kg). In the surface layer, contents of P_20_5, K_20 and humus were low and decreased with the depth of soil. Calcium carbonate content tended to increase slightly in depth ($CaCO_{3SW}$ 5.13 – 8.80 %; $CaCO_{3NE}$ 3.50 – 3.70 %). The measured levels of soil electrical conductivity were not indicative of the soil salinity (EC_{SW} 160 – 271 µS/cm; EC_{NE} 86 – 222 µS/cm).

In the distribution of Cd, Ni and As in average samples from the sites of exposure SW and NE (0 – 10 and 0 – 20 cm), it was found that there is a significant difference ($p \leq 0.05$) in the total content of Cd, Ni and As.

In general, in the average samples (0 – 10 and 0 – 20 cm) there was a unique tendency of distribution (increase) of the total content of Cd, Ni and As on the NE exposure in relation to the opposite SW exposure. This confirms the fact that atmospheric deposits are deposited on the slopes of exposures that are under the blows of the dominant wind. In the profiles at the sites (SW and NE), significant differences ($p \leq 0.05$) were determined in the total content of Cd, Ni and As. At the sites of opposite exposures SW and NE, it was determined that the total mean content of Cd, Ni and As in the profiles at NE exposure was higher in relation to the opposite SW, with Cd by 78%, and with Ni and As by 41%.

In this study in the area of the National Park „Una“, in relation to the direction, intensity, dominance (frequency) of the wind, it was found that the total content of Cd, Ni and As at the impact NE exposure was higher. This confirms the fact of deposition in the soil that takes place with the help of wind as a transfer factor.

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SYNTHESIS, CHARACTERIZATION AND BIOACTIVITY OF SELECTED METAL COMPLEXES WITH IMINE LIGANDS

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
The chemistry of complex compounds containing imine ligands is attracting significant attention from researchers today. In this work, complexes of selected transition metals (Cu, Co, Ni and Fe) with imines based on ninhydrin and amino acids methionine and cysteine were synthesized. FTIR and UV/VIS spectroscopy were used for structural characterization. Antioxidant activity of the complex was analyzed by the FRAP method. The synthesized compounds showed a significant reducing ability, ranging from 221.94 to 756.30 µmol/L. *In vitro* antimicrobial activity was tested on strains from the ATCC collection. Inhibitory activity against the tested microorganisms was recorded, and the zones of inhibition ranged from 10-24 mm. Preliminary research shows that these compounds have biological potency, but more detailed *in vitro* and *in vivo* studies are required for their use.

KEYWORDS: metal complexes, imine, bioactivity, antibacterial agents, FRAP

INTRODUCTION

Schiff bases are synthetically accessible and structurally diverse compounds, typically obtained by facile condensation between an aldehyde, or a ketone with primary amines[1]. They are known in the name of Hugo Schiff who first reported the reversible acid-catalyzed condensation reaction between primary amine with carbonyl compounds[2]. These compounds are also known as imines with general structure R-CH=N-R′, where R and R′ is linear or cyclic alkyl and/or aryl group which may be differently substituted [3,4]. They aremost widely used organic compounds and can be coordinated with metals especially the transition metals to form Schiff base complexes [5,6]. As previously stated, Schiff bases are versatile metal complexing agents and have been known to coordinate all metals to form stable metal complexes with vast therapeutic applications [7]. Their wide spectrum of biological activities is the reason why they are very important in pharmaceutical industry. Most of them show biological activities including antibacterial, antifungal, antidiabetic, antitumor, antiproliferative, anticancer, herbicidal, and anti-inflammatory activities [8,9]. Also they show excellent catalytic activity in various reactions and in the presence of moisture [6]. Many of them havehigh thermal stability and they are reported as effective candidates in homogenous and heterogeneous catalysis [10,11]. Due to excellent ligating properties and relatively facile synthesis methods the imine based ligands are widely used in transition metal coordination chemistry. These complexes are used as model molecules for biological oxygen carrier systems [12,13]. The compounds of this type can be greatly modified by introducing different substituents providing very useful model compounds for investigation of different chemical processes and its effects [14,15]. It is important to emphasize the structural similarity between Schiff bases possessing different donor atoms (N, O, S, etc.) and biologically active compounds found in natural biological systems [16,17].

MATERIALS AND METHODS

¸ All chemicals used were of analytical grade and were used as received without any further purification.

PREPARATION OF THE METAL COMPLEXES

The synthesis of the complexes was performed according to described procedure [18]. Ninhydrin (0.01 mol) was transferred to a round bottom flask and dissolved in 25 mL of 96% ethanol, with stirring and heating. After dissolution, 0.005 mol of metal salts was added to the solution, and the mixture was stirred for 30 minutes. 0.01 mol of amino acid(methionine, cysteine)was then added to the flask and mixture was refluxed for 3 hours at 70-80 °C. The product was then filtered, washed with absolute ethanol and stored in a desiccator until analysis.

SPECTRAL CHARACTERIZATION

The products were characterized by FTIR and UV/VIS spectroscopy. Attenuated Total Reflection was used to record FTIR spectra. The samples were recorded in the wavelength range of 4000-525 cm^{-1}, on a Nicolet iS10 FT-IR spectrophotometer. The recordings were made in triplicate. Methanolic solutions at concentrations of 0.01 mg/mL were used to record the UV spectra in Perkin Elmer Lambda 25 UV/VIS spectrophotometer.

ANTIMICROBIAL ACTIVITY *IN VITRO*

Antimicrobial activities were investigated by diffusion method for reference bacterial strains *E.coli* (EC), *E. faecalis* (EF), *S. aureus* (SA), *B. subtilis* (BS), *L. monocytogenes* (LM) and *P. Aeruginosa* (PA). Solutions of the complex were prepared by dissolving the synthesized compounds in dimethyl sulfoxide. In the agar sterile drill-shaped holes were made ("wells") into which 100 μL of complex solutions of concentration 1 mg/mL were added. After the plates were left at room temperature for 15 min, the substance was diffused into agar, incubated at 37°C for 24 h.

FERRIC REDUCING ANTIOXIDANT POWER ASSAY (FRAP)

To prepare the calibration curve, solutions of $FeSO_4$ x $7H_2O$ was prepared in the concentration range of 200-1000 mmol/L. The volume of 3.0 mL FRAP reagent was measured in five tubes and 0.1 mL standard solutions were added there too. The absorbance was measured with regard to a blank sample (3 mL FRAP reagent and 0.1 mL of water). In each tube, 0.2 mL of metal complexes (concentration 0.10 mg/mL) and 6.0 mL of FRAP reagent were added. The samples were incubated in an aqueous bath for 30 minutes at 37°C, and the absorbance was measured at 593 nm with regard to a blank sample (6.0 mL FRAP reagent and 0.2 mL DMSO). Measurements were performed in triplicate.

RESULTS AND DISCUSSION

STRUCTURE OF THE COMPLEXES AND SPECTRAL ANALYSIS

The reaction scheme and the proposed structure of the complex are shown in Figure 1. The imine ligand synthesized from ninhydrin and amino acids coordinates metal centers as a tridentate ONO donor ligand. The oxygen atom of the carbonyl group of ninhydrin, the nitrogen from the imine bond and the oxygen atom from the carboxyl group of the amino acid part of the molecule participate in the formation of the bond. Exceptions are Co(II) and Ni(II) complexes with methionine in which the imine coordinates metal ions as a bidentate NO-donor ligand. In these two complexes, the oxygen atom of the carboxyl group of the amino acid does not participate in the formation of the bond.

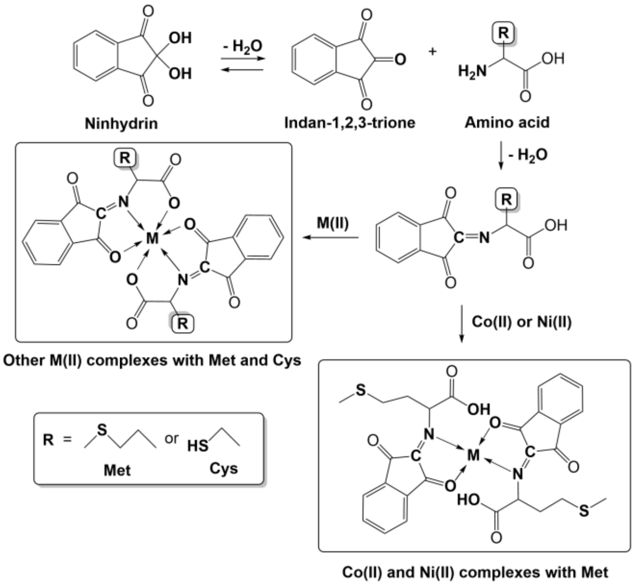

Figure 1. Reaction scheme and proposed structure of the metal complexes

Spectral data for the synthesized complexes, obtained by FTIR spectroscopy are shown in Table 1. At about 540 cm^{-1} on the FTIR spectrum was recorded a newly formed M-N bond. The absence of a band in the interval from 3000 cm^{-1} to 3500 cm^{-1} (except in the case of the Co(II) and Ni(II) complex with methionine) which is characteristic of the O-H bond from the carboxyl group, indicates the participation of oxygen atoms in the formation of the bond with the metal center. The FTIR spectra of ninhydrin and amino acids shows two strong absorption bands in the region around 1700 cm^{-1}

corresponding to the carbonyl group. Changes in the appearance of the bands in the area of about 1700 cm^{-1} also indicate the involvement of the carbonyl group in the formation of the complex. In the metal chelates, the band characteristic of the azomethine group is shifted to 1574-1520 cm^{-1} suggesting the coordination of the azomethine nitrogen atoms to the metal.

Table1. Spectral data of the synthesized complexes obtained by FTIR and UV/VIS spectroscopy

Compound	Infrared spectral data [cm^{-1}]						UV/VIS λ_{max} [nm]
	v(COO$^-$)	v(O-H)	v(C=N)	v(C=O)	v(C-N)	v(M-N)	
Fe(NinMet)$_2$	1612	-	1540	-	1202	567	226
Fe(NinCys)$_2$	1596	-	1540	1705	1200	551	206
Cu(NinMet)$_2$	1536	-	1559	1684	1162	534	208
Cu(NinCys)$_2$	-	-	1540	1700	1189	556	235
Co(NinMet)$_2$	-	3150	1521	-	1167	560	218
Co(NinCys)$_2$	-	-	1558	1705	1197	568	211
Ni(NinMet)$_2$	-	3200	1520	1716	1201	560	212
Ni(NinCys)$_2$	-	-	1574	1708	1236	546	222

ANTIMICROBIAL ACTIVITY

The results of antimicrobial activity of the synthesized complexes are shown in Table 2. In general, the greatest inhibition on the tested bacterial strains had the nickel complex containing cysteine. Then, significant antimicrobial activity show complexes Co(II) with cysteine and Cu(II) with methionine. All synthesized complexes in the study of antimicrobial activity showed its complete absence against *E.coli* and *P. aeruginosa*. In all three cases, they are gram-negative bacteria that have an outer membrane made of lipopolysaccharide (LPS) that is a barrier to metal complexes, which explains the absence of their antimicrobial activity.

Table2. Results of antimicrobial activity of the synthesized complexes

Compound	Inhibition zone [mm]					
	LM	EC	EF	BS	SA	PA
Co(NinMet)$_2$	19	-	-	-	-	-
Co(NinCys)$_2$	21	-	15	13	20	-
Cu(NinMet)$_2$	18	-	12	24	13	-
Cu(NinCys)$_2$	-	-	13	10	11	-
Ni(NinMet)$_2$	-	-	-	11	-	-
Ni(NinCys)$_2$	23	-	22	16	23	-
Fe(NinMet)$_2$	20	-	-	-	-	-
Fe(NinCys)$_2$	11	-	12	-	18	-

Antioxidant activity

The results of the antioxidant activity of the synthesized complexes obtained by FRAP method are shown in Table 3. Based on the obtained FRAP values (for concentration 0.1 mg/mL), it can be concluded that compounds containing methionine have a higher reduction potential. Observing the FRAP values of compounds containing different metals conclusion is that complexes with Co(II) generally have higher FRAP values in both cases, when they contain methionine (which is slightly higher) and when they containcysteine. The two complexes differ in structure since spectral analysis showed that the complex with methionine has an uninvolved oxygen atom of the O-H group (from the carboxyl part) and thus a different structure compared to the complex with cysteine. FRAP values of complexes containing Fe(II) range from 221.94 μmol/L with cysteine, which is the lowest FRAP value of the tested compounds, to 736.20 μmol/L with methionine as one of the highest FRAP values.

Table 3. Results of antioxidant activity of the synthesized complexes

Sample	FRAP value [μmol/L]
Co(NinMet)$_2$	660.16
Co(NinCys)$_2$	482.50
Cu(NinMet)$_2$	399.09
Cu(NinCys)$_2$	756.30
Ni(NinMet)$_2$	590.47
Ni(NinCys)$_2$	283.92
Fe(NinMet)$_2$	736.20
Fe(NinCys)$_2$	221.94

CONCLUSION

The synthesized metal complexes with methionine and cysteine showed significant antimicrobial and antioxidant activity *in vitro*.These compounds are interesting for further and more detailed research. Their proven bioactivity offers the possibility of application in biological systems and drugs.

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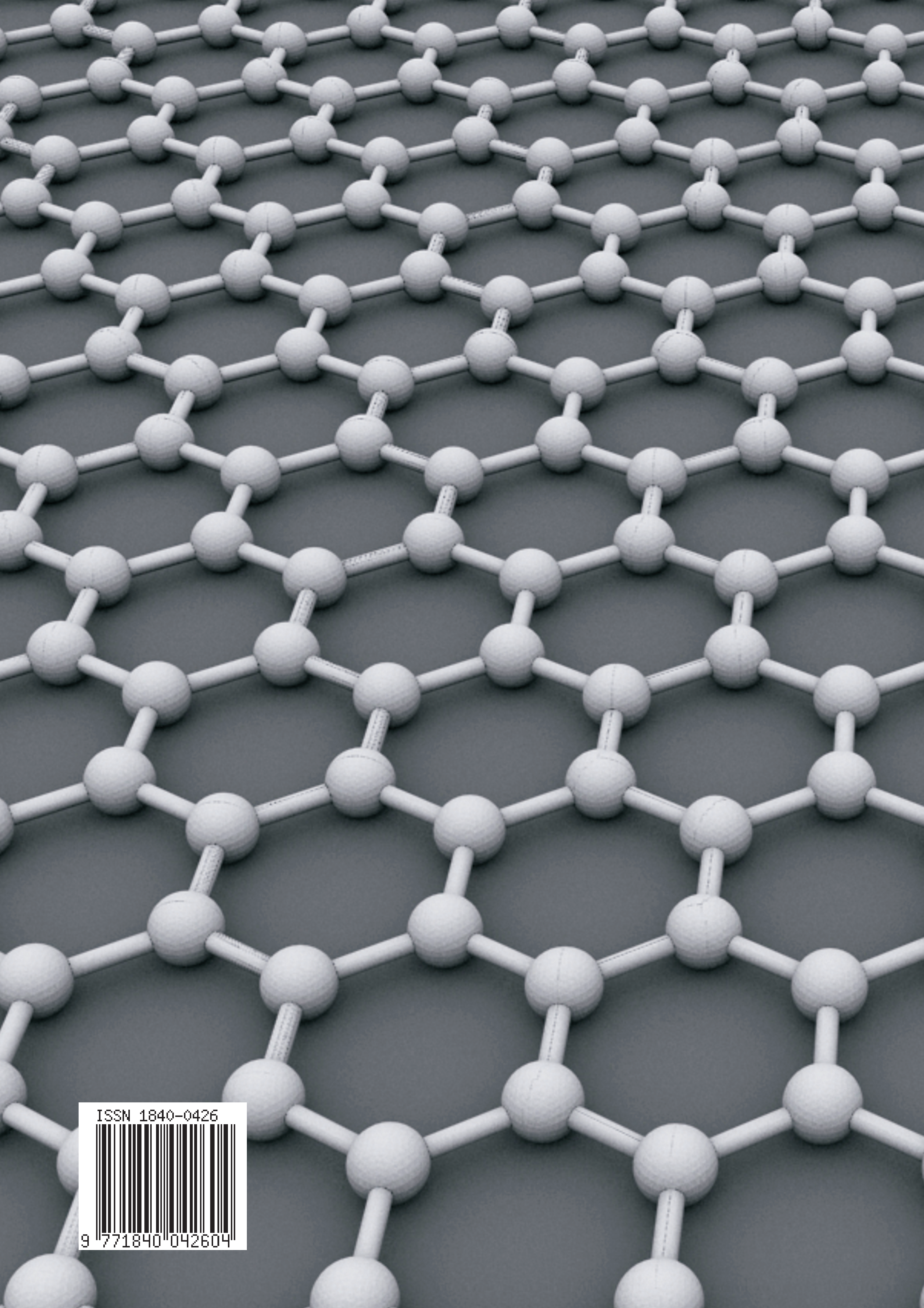

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