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TABLE OF CONTENTS

DETERMINATION OF ASCORBIC ACID AND CHLOROPHYL PIGMENTS IN VARIOUS GREEN EDIBLE GARDEN PLANTS

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Abstract. *In a comparativ study, the concentration of Chl A, Chl B, Carotenoids, vitamin C in some green leaves from plants used as raw or cooked food were determined and analysed. The studied samples were Spinach (Spinacia oleracea), Dill (Anethum graveolens) Sorrel (Rumex acetosa), Parsley (Petroselinum crispum) and Patience dock (Rumex patientia) and were taken from two different localities in Bihor county, Romania, with different environmental conditions, cultivated in soils where no fertilizers were used. Results showed that the environment was not decisive in this case, an important role was due to the nature of the analysed species. However, it was observed that in the sorrel samples, which had the highest concentration of vitamin C, the oxidative process of Chl A was slowed down. The highest concentrations of Chl A and Chl B were obtained for spinach samples, followed by dill, and for sorrel, parsley and stevia, the concentrations were lower and very close. The highest concentrations of carotenoids were found in spinach leaves, and the lowest values were recorded for patience dock. The highest concentration of vitamin C was found in sorrel samples. This was almost 25 times that of spinach, 6 times that of parsley, 3 times that of stevia and double that of dill*

Keywords: chlorophyll, caroten, vitamin C, Spinach, dill, sorrel, parsley, patience dock

INTRODUCTION

Vitamin C, chlorophylls and carotenoids have proven over time that they play an important role both for plants and for our health, showing antioxidant properties [1, 2]. Carotenoids are a class of yellow-orange, fat-soluble compounds that are found in green leaves, but are not visible because their colour is covered by that of the green pigments, chlorophylls [3, 4]. Of these carotenoid pigments, the most active is β-carotene, which can be converted into vitamin A. Some carotenoids are absorbed intact and then deposited in various tissues of the body and are considered excellent sources of provitamin A. Vitamin A, for example, is essential for vision as it contributes to the formation of proper eye cells and good night vision [4]. It also strengthens the immune system, supports the health of the skin and mucous membranes, and is also involved in the development of bones and teeth. Vitamin A is important for vision, for the growth and formation of bones, the body's resistance to infections, it has a role in the growth mechanism [5]. The lack of vitamin A produces vision disorders, skin lesions, chronic diarrhea, susceptibility to infections. Due to the conjugated double bond system, carotenoids can interact with free radicals and manifest their antioxidant properties [6].

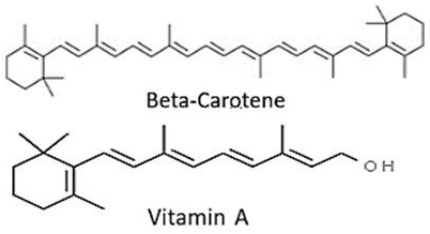


Fig. 1 Chemical structures of β-carotene and vitamine A

The leaf green pigment is chlorophyll, which absorbs light and allows the endothermic transformation of CO_2 and water into carbohydrates, necessary for the growth and development of the plant, accompanied by the release of oxygen, so necessary for life [7]. Chlorophyll is continuously synthesized by plants in the presence of both optimal light and optimal temperature, but it is not a very stable compound because it breaks down easily in strong light. The most important and abundant are chlorophylls A and B. Chlorophyll A (Chl A) is the major pigment and chlorophyll B (Chl B) in higher plants has a lower concentration, in many of them it is around a quarter of the concentration of chlorophyll A. Chl A has a deep blue-green colour and is directly involved in both photosystem I and photosystem II and is the most important pigment in the photosynthesis process. Chl B is light green and is more involved in photosystem II [8]. Carotene also absorbs light, but the light energy absorbed by carotene is transferred to chlorophyll, which uses it in photosynthesis. Carotene is a much more stable compound than chlorophyll, that's why after the disappearance of chlorophyll the leaf turns yellow [9, 10].

Ascorbic acid, named vitamin C is water-soluble, and can only be obtained from exogenous sources and cannot be stored in the body, so a regular intake is necessary to maintain proper health [10, 11]. It supports the functioning of the immune system, as it has strong antioxidant properties, which helps to neutralize free radicals, protecting cells from oxidative stress [12, 13].

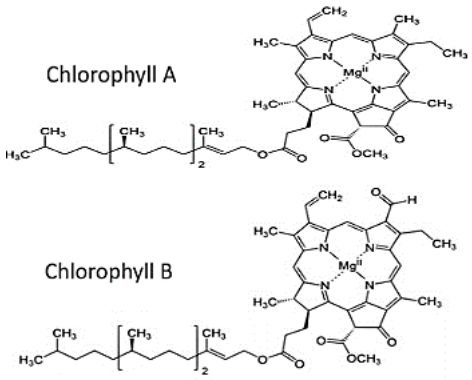


Fig. 2 Chemical structures of Chlorophyll A and Chlorophyll B

Easily oxidizable, Vitamin C is sensitive to heat and air. It is also essential for the production of collagen, which is essential for healthy skin, gums and connective tissues. Vitamin C helps absorb iron, which helps produce energy and prevent anemia. Lack of vitamin C can have serious consequences. The most well-known disease resulting from a lack of vitamin C is scurvy [14-16].

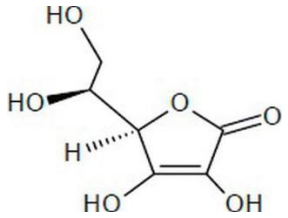


Fig. 3. Chemical structure of vitamin C

In this work, the concentration of chlorophyll pigments, carotenoids and vitamin C in edible green leaves used in kitchen preparations, both fresh and cooked, was determined.

MATERIALS AND METHODS

The determinations were made for green leaves samples from five different species, which were mature at the time of harvesting: Spinach (*Spinacia oleracea*), Dill (*Anethum graveolens*) Sorrel (*Rumex acetosa*), Parsley (*Petroselinum crispum)* and Patience (*Rumex patientia*). The samples were collected in April 2023 from two gardens that differ in soil structure, altitude, exposure to light and with small differences in humidity and temperature of the air. No fertilizers were used in both gardens.

The first sampling site was in Diosig village, Bihor county, Romania, a rural area. The second sampling site was in Oradea city, Bihor county, an urban area. The samples were taken from different points of the space where they were cultivated and for each species 25 samples were analyzed. Samples were transported to the laboratory in sealed bags in the dark, and measurements were taken 12 hours after collection.

Evidence coding of samples

1. DSA for spinach sample from Diosig
2. DAG for dill sample from Diosig
3. DRA for sorrel sample from Diosig
4. DPC for parsley sample from Diosig
5. DRP for patience dock sample from Diosig
6. OSA for spinach sample from Oradea
7. OAG for dill sample from Oradea
8. ORA for sorrel sample from Oradea
9. OPC for parsley sample from Oradea
10. ORP for patience dock sample from Oradea

Determination of Chl A, Chl B and carotenoids concentration was carried out by the spectrophotometric method from extracts in 90% acetone of green leaves pigments [17-19]. Approximately 0.02 g leaf sample was mortared in calcium carbonate mixture and the green pigments were extracted in 10 mL 90% acetone. The extract was filtered and the absorbances were recorded with a T60 UV-VIS spectrophotometer at 470, 645 and 662 nm. Measurements were performed three times for each analysed sample.

The method is based on the different solubility of pigments in certain organic solvents. Thus, the maximum absorption for Chl A in 90% acetone is at 662.6 nm, while for Chl B is at 645.6 nm. Because the two absorption maxima of chlorophyll A and B are very closed, we can say that at 662.6 nm Chl B will also have a significant absorbance, and at 645.6 nm Chl A will also absorb. The next Lichtenthaler equations were used to calculate the pigments concentration in samples [19]:

$$Chl\ A\ (mg/g) = (11.75 \cdot A_{662} - 2.35 \cdot A_{645}) \cdot 10/1000/m_{sample}\ (g) \quad (1)$$

$$Chl\ B\ (mg/g) = (18.61 \cdot A_{645} - 3.96 \cdot A_{662}) \cdot 10/1000/m_{sample}\ (g) \quad (2)$$

$$Carotenoids\ (mg/g) = (1000A_{470} - 2.270ChlA - 81.4\ ChlB)/227x10/1000/m_{sample}\ (g) \quad (3)$$

where

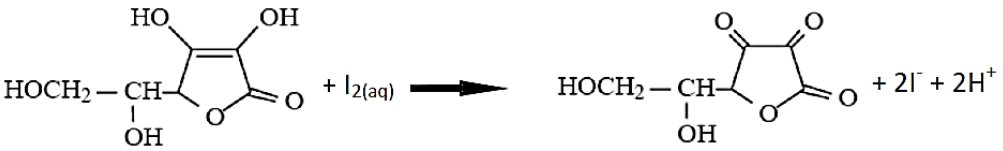
Chl A is chlorophyll A concentration (mg/100 g green leaf)

V is leaf extract volume in 90% acetone (mL)

m_{sample} is mass of leaf sample (g)

Chl B is chlorophyll B concentration (mg/100 g green leaf)

Vitamin C was determined by an indirect method, based on the oxidation of vitamin C with iodine resulting from a reaction between potassium iodide and potassium iodate in acidic medium [20]. When all of the vitamin C was oxidized, the iodine excess turned the starch indicator blue. The chemical oxidation reaction of vitamin C with iodine is:



The iodine necessary for the oxidation of vitamin C was obtained from an acidic reaction mixture, IO_3^-/I^-:

$KIO_3 + 5\ KI + 6\ HCl \rightarrow 3\ I_2 + 6KCl + 3\ H_2O$

In an Erlenmeyer flask, vitamin C was extracted in 20 mL of distilled water from 2 g of green leaves and 0.5 mL of 1% KI solution, 0.5 mL of 2% HCl and 2 mL of 1% starch were added. The sample was immediately titrated with 0.001N KIO_3 to a blue colour shift. The blank sample was distilled water. The concentration of vitamin C was determined with the equation:

$$c\%\ (mg\ \%) = \frac{8{,}8(V_S - V_B)}{m} \quad (4)$$

.

where

V_S is 0,001N KIO_3 volume from sample titration (mL);

V_B is 0,001N KIO_3 volume from blank titration (mL);

m is mass of leaf sample (g)

RESULTS INTERPRETATION

The data were processed with an Excel Microsoft Office 2021 program. In Table 1 and Fig. 4 the data obtained for the average concentrations of Chl A, Chl B and Carotenoids, respectively the Chl A/Chl B ratio for 25 samples from each analysed leaf species are recorded. Also, standard deviations were recorded in Table 1

Tabel 1. Chl A, Chl B and Carotinods concentrations in green leaves after extraction in 90% acetone

	Sample Code	Chl A (mg/g)	Chl B (mg/g)	Chl A/Chl B	Carotenoids (mg/g)
1	DSA	1.0721±0.082	1.3182±0.073	0.8133±0.078	1.0849±0.074
2	DAG	0.5774±0.074	0.6453±0.086	0.8948±0.076	0.7828±0.076
3	DRA	0.4812±0.061	0.4943±0.080	0.9730±0.075	0.819±0.078
4	DPC	0.4104±0.053	0.429±0.071	0.9566±0.064	0.6447±0.060
5	DRP	0.4033±0.064	0.5354±0.048	0.7533±0.056	0.3842±0.051
6	OSA	0.9588±0.112	1.1629±0.104	0.8245±0.107	1.0757±0.098
7	OAG	0.6025±0.084	0.6627±0.065	0.9092±0.072	0.8632±0.075
8	ORA	0.5038±0.065	0.4817±0.062	1.0458±0.063	1.0124±0.066
9	OPC	0.4877±0.057	0.5539±0.068	0.8804±0.062	0.7042±0.059
10	ORP	0.4332±0.075	0.5144±0.082	0.8421±0.076	1.0069±0.076

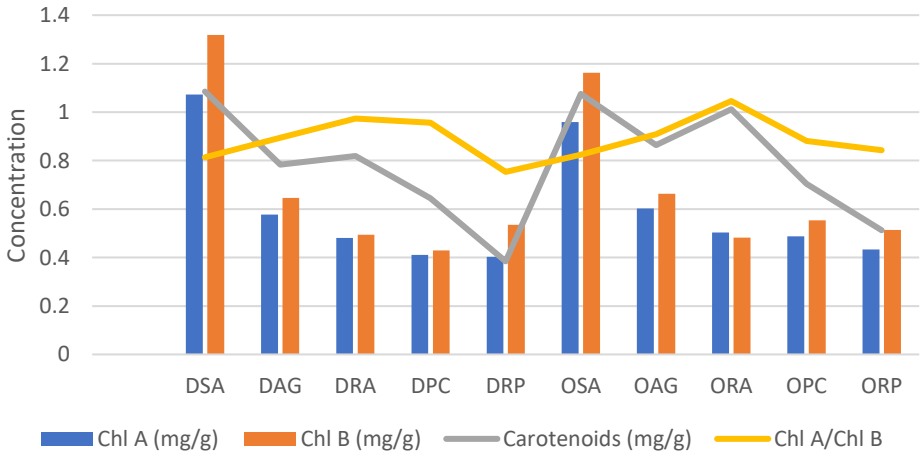


Fig. 4. Variation of average concentration of Chl A, Chl B, Carotinoids and Chl A/Chl B rate

In Figure 4, it can be seen first of all that the results obtained for the same species of green leaves from the two sampling points, Diosig and Oradea, are quite similar, and that the results were not significantly affected by the sampling point and variation. of environmental factors. In general, the concentrations of Chl A, Chl B and carotenoids were slightly higher in Oradea than in Diosig, this difference may be due to the positioning on a sunnier garden area in Diosig than in Oradea, as it is known that stronger light can lead to chlorophyll destruction. Chl A/Chl B ratios (Table 1) were slightly higher in Oradea than in Diosig, in agreement with what was previously discussed, that sun exposure was better in Diosig than in Oradea, where the garden is located in a garden area shaded.

The highest concentrations of Chl A and Chl B were obtained for spinach samples (DSA and OSA), and the lowest were found for Chl A in patience dock (DRP and ORP) and Chl B in parsley samples (DRP) in Diosig, respectively in patience dock (ORA) from Oradea, with the mention that both in Diosig and in Oradea both Chl A and Chl B had lower values for parsley, sorrel and patience dock.

The Chl A/Chl B ratio had the highest values for sorrel leaves (DRA and ORA) and were the only leaves with a ratio of ~1.00, indicating that there is more Chl A here a been kept unoxidized, which is important. from the point of view of photosynthesis. Low values of Chl A/Chl B ratios < 1, or were in this case due to the fact that leaf samples were not analysed immediately after harvest.

In the case of carotenoids, the highest concentration values were recorded in both situations for spinach leaf samples (DSA and OSA) and the lowest concentrations were found for patience dock leaves (DRP and ORP).

In Fig. 5, 6 and 7 the maximum and minimum values of Chl A, Chl B and carotenoids obtained for the 25 samples from each leaf species studied are recorded and their position compared to the average value obtained and recorded in Table 1 is noted.

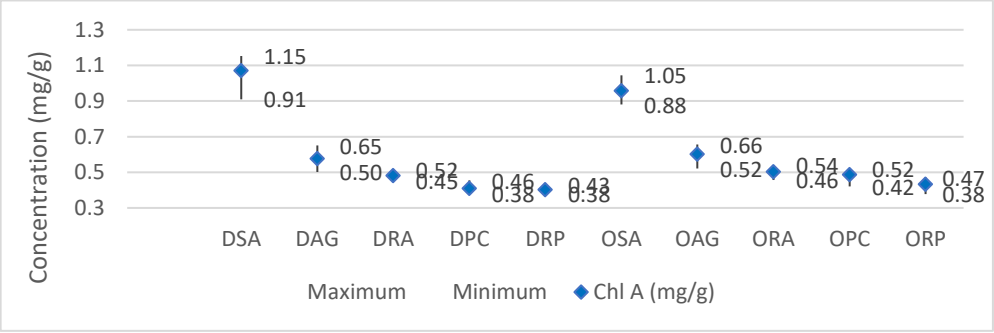


Fig. 5. Minimum and maximum of Chl A concentrations in green leaves samples

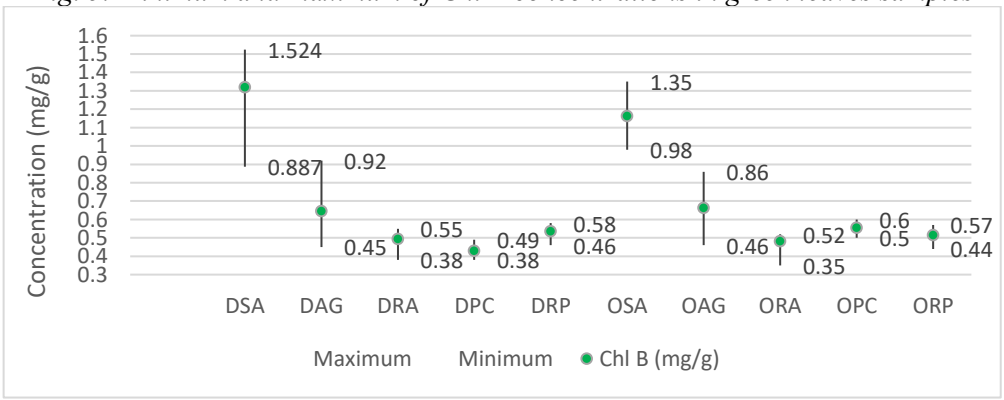


Fig. 6. Minimum and maximum of Chl B concentrations in green leaves samples

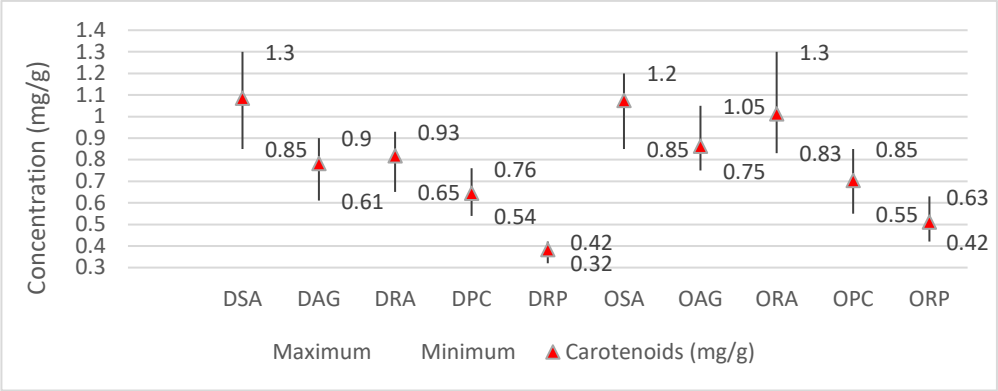


Fig. 7. Minimum and maximum of Carotinoide concentrations in green leaves samples

In Table 2, the values obtained for the average concentrations, the standard deviations, the maximum and minimum values of vitamin C in the analysed green leaf samples are noted. The differences in the concentration of vitamin C in the analysed green leaves can be seen in Figure 8. As in the case of leaf pigments, the variation in the concentration of vitamin C in similar leaf samples was not significantly different between the samples taken from both sampling points, Diosig and Oradea, which shows that environmental factors did not have an important influence on it. Here it can be seen that the highest amount of vitamin C was determined in the sorrel samples (DRA, ORA) by the chosen method, and the lowest concentration was recorded in the spinach samples (DSA, OSA). The concentration of vitamin C found in sorrel was almost 25 times that of spinach, 6 times that of parsley, 3 times that of patience dock and double that of dill:

The concentration of vitamin C determined in the leaves increases in the order:Spinach < Parsley < Patience dock < Dill < Sorrel

Table 2. Vitamin C concentrations in studied green leaves samples

	Sample Code	Concentration (mg%)	Minimum	Maximum
1	DSA	2.64±0.15	2.46	2.97
2	DAG	26.4±2.49	23.37	29.25
3	DRA	51.92±4.08	47.62	57.33
4	DPC	7.92±0.85	7.17	8.94
5	DRP	16.72±1.12	15.02	18.21
6	OSA	3.04±0.27	2.66	3.42
7	OAG	24.55±1.92	22.33	25.82
8	ORA	57.63±4.75	51.98	63.24
9	OPC	6.6±0.71	5.85	7.14
10	ORP	18.36±1.4	16.14	20.31

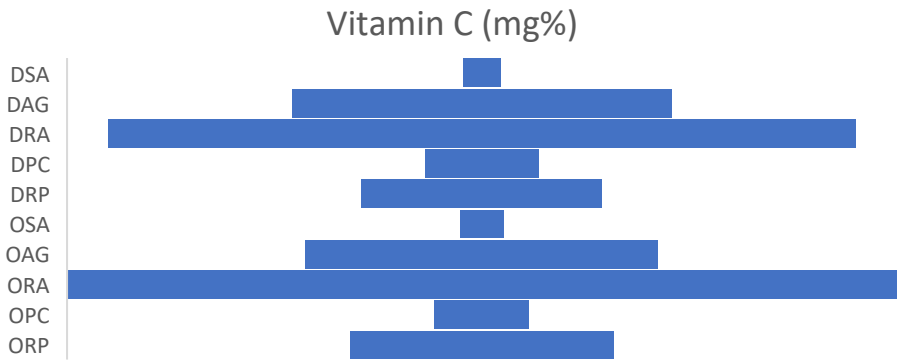


Fig. 8. Vitamin C in studied green leaves samples

Since both the highest vitamin C concentrations and the best Chl A/Chl B ratios were recorded for sorrel leaves (DRA and ORA), an attempt was made to see if there was a constant variation relationship between the two. As can be seen in Fig. 9 between the two variables there is no linear relationship, but nevertheless it seems that vitamin C contributes through its antioxidant activity to the quantitative reduction of Chl A oxidation.

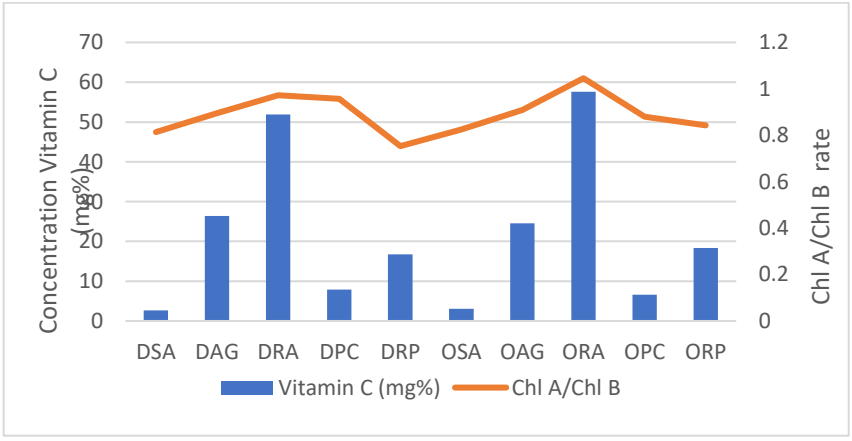


Fig. 9. Variation of Chl A/Chl B rate with vitamin C concentration for studied green leaves samples

CONCLUSION

In this work, the concentration of Chl A, Chl B, Carotenoids and vitamin C in green leaves samples from different species, grown in two sampling points from Bihor County, Romania, Diosig village and Oradea city, was determined. For all the parameters studied, there were no registered significant differences between the two sampling points, which shows that the influence of environmental factors did not have an important impact. However, slightly higher values of Chl A and Chl B concentrations in the samples collected from Oradea were attributed to the shady area where they were cultivated compared to those from Diosig. Based on the results from the two sampling points, we can say that the concentration of Chl A decreased in the sequence: spinach > dill > sorrel > parsley > patience dock. For Chl B, small differences were noticed between the two sampling points, with a change in the hierarchy of the last three species, due to the average concentrations found very close to each other, so that the series becomes spinach > dill > sorrel ~ parsley ~ patience dock. Carotenoids concentration varied similarly in both sampling points and decreased in the order: spinach > sorrel >

dill > parsley > patience dock. The highest concentration of Vitamin C was recorded in sorrel leaves, and it seems that it plays an important role as an antioxidant in keeping the concentration of Chl A constant and higher in the leaves, the main photosynthetic pigment.

REFERENCES

1. Zeece, M. (2020), Vitamins and minerals, *Introduction to the Chemistry of Food*, Vol 5, pp. 163-212
2. Baj, T., Sieniawska, E. (2017), Vitamins, *Pharmacognosy*, Vol. 13, pp. 281-292
3. Merhan, O. (2017), The Biochemistry and Antioxidant Properties of Carotenoids [Internet]. Carotenoids. InTech, Available from: http://dx.doi.org/10.5772/67592
4. Fiedor, J., Burda, K. (2014), Potential role of carotenoids as antioxidants in human health and disease, *Nutrients*, Vol. 6(2), pp. 466-88.
5. Rigby, S. H., Schwarz, K. B. (2001), Nutrition and Liver Disease, Vol. 39, pp. 601-613
6. Engelking, L. R. (2015), Textbook of Veterinary Physiological Chemistry, pp. 254-259
7. Lin, C., Popescu, S. C., Huang, S.C., Chen, Y.C. (2015), A novel reflectance-based model for evaluating chlorophyll concentration of fresh and water stressed leaves, *Biogeosciences*, Vol. 12, pp. 49–66
8. Amer, J. (1991), Regulated Chlorophyll Degradation in Spinach Leaves during storage, *Horticultural Crops Quality Laboratory*, pp. 58-62
9. Lisiewska, Z., Kmiecik, W., Korus, A. (2006), Content of vitamin C, carotenoids, chlorophylls and polyphenols in green parts of dill (Anethum graveolens L.) depending on plant height, *Journal of Food Composition and Analysis*, Vol. 19(2–3), pp. 134-140
10. Igual, M., Uribe-Wandurraga, Z.N., García-Segovia, P., Martínez-Monzó, J. (2022), Microalgae-enriched breadsticks: Analysis for vitamin C, carotenoids, and chlorophyll a, *Food Science and Technology International*, Vol. 28(1), pp. 26-31
11. Rucker, R. B., Steinberg, F. (2004), Vitamin C, *Encyclopedia of Biological Chemistry*, pp. 367- 371
12. Kildahl-Andersen, G., Naeess, S.N., Aslaksen, P.B. (2007), Studies on the mechanism of the CarrPrice blue colour reaction, *Organic &Biomolecular Chemistry*, Vol. 5(18), pp. 3027-3033
13. Chawla, J., Kvarnberg, D. (2014), Hydrosoluble vitamins, *Handbook of Clinical Neurology,* Vol. 59, pp. 891-914
14. Murakami, K., Iria, K., Shimizu, T. (2015), Potential Role of Vitamin C in the Prevention of Alzheimers Disease, *Diet and Nutrition in Dementia and Cognitive Decline*, Vol. 61, pp. 663-668
15. Vikram, V.B., Ramesh, M.N., Prapulla, S.G. (2005), Thermal degradation kinetics of nutrients in orange juice heated by electromagnetic and conventional methods, *Journal of Food Engineering*, Vol. 69, pp. 31-40
16. Choula-Diaz, J. L., Marroquin, D. L., Pramanick, B., Nieto-Arguello, A. (2018), Synthesis of colloidal silver nanoparticle cluster and their application in ascorbic acid detection by SERS, *Colloids and Surfaces B: Biointerfaces*, pp. 329-335
17. Egbuonu, R. (2017), Evaluation of colorimetric determinations of vitamin A in foods, *Thesis in food technology*
18. Blake, J. A., Morgan, J.J. (1975), An improved colorimetric procedure for the analysis of vitamin A, *Can. J. Chem,* Vol. 54, pp.1757-1764
19. Karakaş, V. E., Öztürk, Ş., Oran, S. (2017), Comparison of Photosynthetic Pigment Contents in Lichen Samples were Collected from Different Localities in Bursa, *J. Biol. Environ. Sci.,* Vol. 11(33), pp. 121-127
20. Gloria, M., Gonzalez, M. (2006), Determination of vitamin C in tropical fruits, A comparative evaluation of methods, *Food chemistry*, pp. 654-664

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A COMPARATIVE STUDY OF DIFFERENT BAKING FLOURS

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Abstract.

A comparative study between the properties of the main flours used in baking, wheat, rye, rice and corn, is discussed in this paper. The analyses were mainly carried out on an aqueous extract of these. It was found that the pH increases slightly over time with the increase in the degree of ionic dissociation in aqueous solution. The highest pH value (pH 7.6) was recorded for rice flour, which also obtained the highest concentration of cations compared to the rest of the samples. A higher acidity and a lower pH were found for wheat and rye flours, found to be richer in gluten and proteins. The best rheological properties were found for wheat flour. The highest enzymatic activity was recorded for corn flour and the lowest was recorded for wheat.

Key words wheat, rice, corn, rye, gluten, proteins, enzymatic activity

INTRODUCTION

Over time, man has sought to find different sources of food, and one of the most important was cereals due to its richness, both in nutritional and energetic value. A great advantage of using cereals was that the grains/seeds could be easily preserved throughout the year, even in harsher conditions, while preserving their properties. Another advantage was the possibility of easy processing of them with rudimentary methods, which did not require sophisticated technological procedures. This explains why cereals were one of the first food resources of humans [1-12].

Agricultural plants that are cultivated to obtain grains are known as cereals. The most common cereals cultivated in Romania are: wheat, corn, barley, oats and rye. Cereals belong to the

Gramineae family, also known as starchy agricultural products, due to their high starch content. These cereals are rich in sugars, proteins, vitamins, mineral salts and fats, all of which are necessary for both humans and animals. They have a high nutritional value, because the ratio between protein substances, those containing nitrogen, and non-protein substances, represented by fats and carbohydrates, is one corresponding to a normal diet [13-17].

The production of different types of wheat, rye and corn flour is ensured by the milling industry, which is essential for ensuring human nutrition, and the technologies for processing flours and cereal grains have undergone a process of evolution and adaptation to our needs over time. Flours constitute the basis for bakery products, which have recently become more and more diverse, trying to cover different requirements depending on the degree of health, age and taste preferences and habits of consumers [18-23].

In this paper, we proposed to do a comparative study of the physicochemical properties of different types of flour (wheat, rye, rice and corn), in which we monitored pH, conductivity, acidity, hydration capacity, humidity, wet gluten, deformation index, soluble proteins, and enzymatic activity, respectively, in order to determine which of the 4 types of flour is the best, from all points of view.

EXPERIMENTAL PART

In the practical part of our study, we aimed to determine several characterization parameters of four categories of flours, different by the nature of the seeds from which they were obtained. These parameters were pH, acidity, conductivity, hydration capacity, humidity, wet gluten, deformation index, soluble proteins, enzymatic activity, calcium ion concentration and total cations content at pH 10. The samples used were flours from rye, rice, wheat and corn (maize).

For each flour variety, ten samples were analyzed and the average values obtained were recorded. The standard deviation of the values was below 10% for all determinations performed.

pH determination

The pH value is the indicator of the proton concentration in a water solution of flour extracts. Although a good part of the flour components are not water-soluble substances, there are also components with a high solubility, capable of dissociating into ions in an aqueous medium, such as enzymes, proteins, monoglycerides, inorganic anions and cations, some vitamins, etc. For pH determination, an aqueous extract

was prepared from 10 g flour dissolved in 100 mL distilled water. The pH was measured with an InoLab Multi720 multimeter after 5 minutes and one hour after extracts preparation.

Conductivity determination

The conductivity determination was performed immediately after pH determination at room temperature in the aqueous extracts prepared in the same mode like above, by dissolving 10 g of flour in 100 mL of distilled water. Conductivity was measured with an InoLab Multi720 multimeter.

Acidity determination

The aqueous extracts of the flour samples obtained by dissolving 10 g of flour in 100 mL of distilled water were titrated with a 0.1 N NaOH solution, in the presence of 1% phenolphthalein. Acidity was expressed in the number of moles of NaOH required to neutralize a number of protons corresponding to a 100 g sample of flour and is calculated with the mathematical relationship:

$$A\% = \frac{c \cdot F \cdot V \cdot 100}{m} \quad (1)$$

Where: c is NaOH concentration, (0,1 N); F is 0,1 N NaOH correction f ctor (F=0,9524); V is titration volume of 0,1 N NaOH (mL), m is the mass of analyzed flour sample, corresponding to 10 g flour dissolved in 100 mL water, (g)

Determination of hydration capacity

The hydration capacity represents the amount of water absorbed by 100 grams of flour and is based on determining the amount of flour that must be added to a known amount of water to obtain a dough of normal consistency by kneading. The hydration capacity of flours is a very important property because it affects the quality of bakery products that can be obtained from it. It can affect the structure and properties of finished products. It depends on the composition of the flour, more precisely the seeds from which the flour was prepared and the technology used to grind the grains. The hydration capacity of wheat flour is due to gluten, while in rye it increases due to the presence of carbohydrates. In a bowl or mortar with pestle, 10 mL of water was placed, and flour was added until a ball of normal consistency was achieved [24-25]. The dough balls were weighed, and the hydration capacity was determined with the following formula:

$$HC\ (\%) = \frac{V \cdot 100}{(m_1 - m_2)} \quad (2)$$

V is the volume of distilled water used to knead the flour ball (mL); m_1 is the mass of the flour ball kneaded with water (g); m_2 is the mass of distilled water used to knead (g).

This was calculated knowing that the density of distilled water in the laboratory is 0,9892 g/cm^3

Moisture Determination

Moisture refers to the amount of water found in 100 grams of flour and is determined by drying flour samples in an oven at 110 ℃ for 2 hours. The sample is weighed on an analytical balance in a weighing bottle, dried in an oven, allowed to cool in a desiccator, and the cooled and dried sample is weighed again. From the difference between the two masses, the moisture is obtained, which is expressed in percentage by the formula [24-25].:

$$M\,\% = \frac{(m_i - m_f)\cdot 100}{m_i} \quad (3)$$

m_i is the initial mass of the sample weighed before drying (g);

m_f is the final mass of the sample weighed after drying (g)

The values obtained for moisture are listed in Table 1 and were used to determine the following parameters:

1. Mass heat capacity (J/kg K): C_p=1675+25,11U (4)
2. Thermal conductivity (W/m K): λ=0,029+0,0003T+0,00285U (5)
3. Thermal diffusivity (m^2/s): $a\cdot 10^8$=23,2+0,045U (6)

Determination of wet gluten and deformation index

The amount of wet gluten is determined by kneading a 100 g flour sample with 2% NaCl solution. Take 50 g of each flour sample, put it in a mortar, where it is mixed with 25 ml of 2% NaCl solution. A consistent crust is obtained, which is washed with 2% NaCl solution dropped from a container under continuous hand kneading until the sample remains free of starch. If more drops of NaCl solution are passed over the flour sample, the solution that drains must be clear. The remaining piece of dough is shaken and weighed. The resulting mass is used to determine wet gluten. Wet gluten is calculated with the formula:

$$G\% = \frac{m_f\cdot 100}{m_i} \quad (7)$$

Where m_f is the mass of dough remaining after kneading (g) and m_i is the mass of flour sample (g)

From the resulting product after washing with 2% NaCl, 5 g dough is modeled in a sphere and its diameter is measured. This is left to rest for 30 minutes at 30° C and the diameter is determined again. The deformation index is a parameter depending on the enzymatic activity, which is triggered by kneading and is higher in a more intense enzymatic activity. A high deformation index means a lighter, fluffier dough. A deformation index less than 6 mm indicates a weak gluten, while a deformation index greater than 20 mm indicates a very high enzymatic activity. As mentioned above, ideal temperature for this experiment should be 30 ℃, but it was done at lab temperature, 22 ℃. The deformation index of the flour was determined with formula:

$$DI\ (mm) = d_f - d_i \quad (8)$$

where d_i is the initial diameter of the dough ball (mm) and d_f is the final diameter of the dough ball (mm)

The values obtained for wet gluten (G%) and deformation index (ID) are given in the table 2. Images of the steps taken in this study are presented below [24-25]..

Determination of soluble protein concentration

The amount of soluble protein was determined from flour extracts. For the determination of total soluble protein, the method with the biuret reagent containing Cu(II) ions that form a chelate complex with proteins was used. To plot the calibration curve, standard albumin solutions were prepared with concentrations between 0.5 and 5 mg/mL. The absorbances of the prepared standard solutions were read at room temperature after 20 minutes, at 546 nm, with a UV-VIS T60 spectrophotometer. The blank sample was distilled water, and the control sample was the sample without albumin. The calibration curve is shown in Figure 1, and the equation used to determine the concentration is:

$$C_{SP}(\frac{mg}{mL}) = 117{,}234A \quad (9)$$

where A is absorbance and c is the protein concentration (mg/mL)

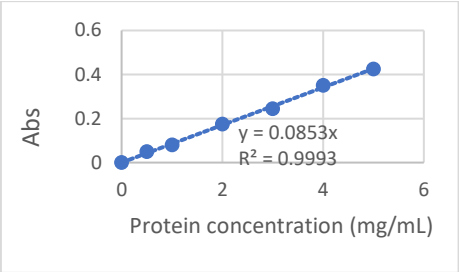

Fig. 1. Calibration plot of protein standards by the biuret method at 546 nm

The flour samples were prepared by dissolving 10 g of flour in 100 ml of 2% NaCl solution. The samples were gently shaken for a half an hour, then left to stand and filtered. The filtrate was the solution in which the quantitative determination of soluble proteins was performed.

The sample preparation is done according to the data in the following table, and the absorbances are measured at 546 nm, after 20 minutes at room temperature. Protein concentration values expressed in mg/mL [26].

Determination of enzymatic activity

The enzymatic activity of wheat depends largely on the biological maturity of the grain, the type of cereal, the climatic conditions of the harvest period and the degradations to which the grain was subjected before or after harvest, such as pest attacks or microbial infections. The amount of enzymes originating from the wheat grain that reach the flour is influenced by the degree

of extraction, and the technological regime of the mill influences their activity by conditioning the substrate. Amylases, proteases, lipases, phosphatases, oxidases and peroxidases are the main enzymes present in the wheat grain. Usually, they are located in the aleurone layer, on the periphery of the endosperm and in the embryo.

This experiment measured the enzymatic activity of the analyzed flour samples. The enzymes were extracted from the flour in aqueous solution for one hour and their activity was measured by their action on hydrogen peroxide. Under enzymatic action, hydrogen peroxide decomposes into water and oxygen.

This dual behavior practically allows hydrogen peroxide to transform under the action of both an oxidase and a reductase, practically this reaction is very versatile and has a positive response to a greater number of enzymes, such as alpha-amylases, proteases, oxidases, peroxidases, proteases, lipoxygenases, etc.

The method is based on the addition of an excess amount of hydrogen peroxide to a sample of enzymatic extract. The excess hydrogen peroxide is titrated in an acidic medium with potassium permanganate. The determinations are carried out in parallel with a blank sample, in which the enzymatic activity is thermally deactivated. The blank sample will allow the exact assessment of the hydrogen peroxide strictly associated with the enzymatic activity. The enzymatic extract is prepared by dissolving 10 g of flour in 100 mL of distilled water for one hour. Over 20 mL of filtered enzymatic extract, 3 mL of 1% H_2O_2 is added and after 30 minutes, the remaining undecomposed hydrogen peroxide is titrated with 0.1N $KMnO_4$ (F 0,9259) in the presence of 5 mL of 10% H_2SO_4. The difference between the amount of 0.1N $KMnO_4$ used in both, sample and blank, corresponds to the hydrogen peroxide decomposed by enzymes. Enzymatic activity is expressed in enzymatic units equal to the number of micromoles of hydrogen peroxide decomposed by 1ml of enzymatic extract for 1 minute [27].

$$UI(\mu molH_2O_2/mL \cdot min) = \frac{1{,}7F_{KMNO_4}(V_B - V_S)1000}{M_{H_2O_2} \cdot V \cdot t} \quad (10)$$

Where: V is volume of both sample and blank from experiments (mL), V_B is volume of 0.1 N $KMnO_4$ from blank titration (mL), V_M is volume of 0.1 N $KMnO_4$ from sample titration (mL), $M_{H_2O_2}$ is molar mass of hydrogen peroxide (g/mol, t is the time reaction under enzyme (min).

Determination of cation concentration

The method consists in reaction between metal cations from flour samples and EDTA at pH 10, expressed as the number of mmoles of EDTA per 100 g of flour sample. The samples were prepared by dissolving 10 g of flour in 100 mL of water for one hour. 2 mL of pH 10 buffer solution, 0.1 g of eriochrome T black indicator were added to each sample and titrated with 0.01 M EDTA solution (F=1.0204).

The calculation formula used to determine the number of mmoles of cation in 100 g of flour sample is as follows [28]:

$$Cations\ (mmoli/100\ g) = \frac{c_{EDTA} \cdot V_{EDTA} \cdot F_{EDTA}}{m} \cdot 10 \quad (11)$$

Where: V_{EDTA} is volume of EDTA 0.01 M from sample titration (mL) , c_{EDTA} is concentration of EDTA , F_{EDTA} is factor of 0.01 M EDTA , m is sample mass (g)

Determination of calcium ion concentration

The same method mentioned above is used to determine the concentration of calcium ions, with the difference that the titration with 0.01 N EDTA is carried out at pH 12-13 in the presence of murexide indicator. The concentration of calcium ions is calculated with the formulas [28]:

$$Calcium\ (mmoli/100\ g) = \frac{c_{EDTA} \cdot V_{EDTA} \cdot F_{EDTA}}{m} \cdot 100 \quad (12)$$

RESULTS AND DISCUSSION

pH and acidity determination

In Figure 2 it can be seen that for all flour samples analyzed the pH values are around pH 7. The highest pH value was recorded for rice flour (pH 7.6) and the lowest for corn flour (pH 6.4). In contrast, after one hour, the pH values decreased slightly for the rye, rice and wheat samples, except for corn flour, for which the pH remained constant. This decrease in pH value corresponds to a slight increase in acidity for the first three flour samples. The increase in acidity can have two main causes: a triggering of enzymatic activity by dissolving the flours in water, but also a promotion of the release of free, protonated compounds from the macromolecular structure of polysaccharides, peptides and other organic compounds, which would favor the increase in the number of free protons. In general, the hydrophilic part of the organic compound in flour contains protons. The weak basic character of rice extract is due to the high content of metal cations whose hydrolysis reaction in water is weakly basic.

In Fig. 3 it can be seen that the highest acidity is shown by the rice sample, while the lowest acidity was recorded in the aqueous extract of corn flour. It would be expected that the pH variation would vary in the same way as the acidity, i.e. where the sample has a lower pH (acidic environment) the highest acidity value would be obtained. Practical results, as can be seen in the figure below, have shown that due to the complexity of the samples and the large number of compounds in the solution this rule is no longer valid.

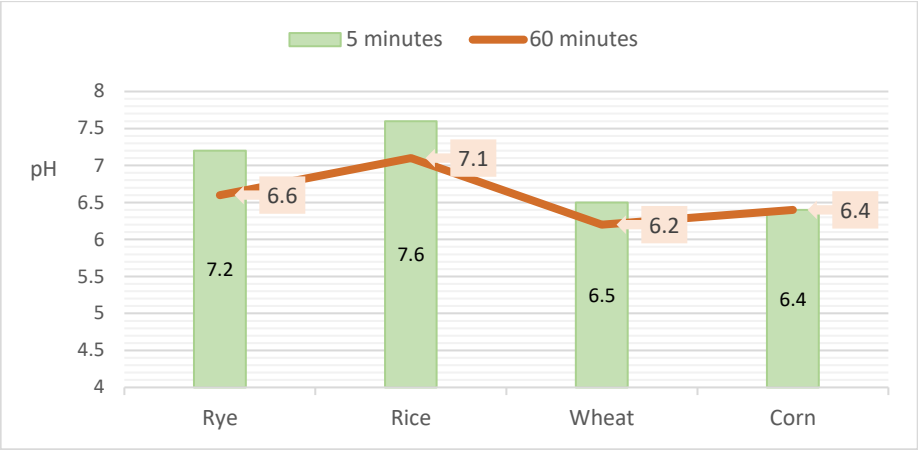

Fig. 2. pH of flour sample extracts after 5 and 60 minutes

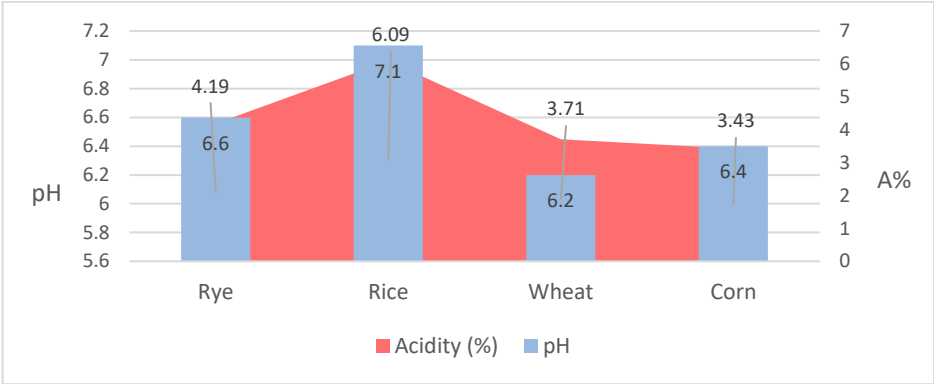

Fig. 3. Comparative variation of pH and acidity of flour sample extracts in water

2. Conductivity determination

In figure 4 it can be seen that the highest conductivity was recorded for the solution prepared from corn flour, which would indicate a higher charge of anions and cations with high mobility of this solution, more short molecules with high ionic mobility and also, a higher degree of ionic dissociation.

On the other hand, the lowest conductivity was obtained in the wheat flour solution, which shows that here there would be the highest concentration of large molecules with low ionic mobility.

Determination of hydration capacity

The results obtained for hydration capacity are recorded in figure 5. As can be seen, in cold distilled water, corn flour could not be kneaded. Literature data indicate a hydration capacity also for corn flour significantly lower than that of wheat flour, due to the lack of gluten. The hydration capacity of the other three flours analyzed increased in the order: rice < rye < wheat, which emphasizes the importance of gluten.

Moisture determination

The observations and discussions made regarding the data obtained in Table 1 are as follows:

- moisture value varies from 10% for rye flour to 13.22% for the wheat sample;
- mass heat capacity is around 2 kJ/kg K for all samples, with very close values:
- thermal conductivity of the samples was around 0.95 W/m K at 22°C, the temperature in the laboratory at the time of the determinations
- thermal diffusivity was around 23.7·108 m2/s for all four flour samples analyzed.

Determination of wet gluten and deformation index

In table 2 it can be seen that again for the corn flour sample the experiment could not be carried out. The highest amount of wet gluten is found in wheat flour, almost 40% followed by rye (34%), and the last in the series is rice with a content of less than 10%. In contrast, the deformation index was much higher in the case of dough resulting from wheat flour compared to that from rice or rye, which indicates a more intense enzymatic activity in wheat flour than in rice or rye.

Moreover, despite a fourfold higher concentration of gluten in rye compared to rice, the deformation indices were almost identical, which shows that the enzymatic activity in rye flour was very low. This happened despite the property of the gluten protein to provide elasticity to the dough. A parallel variation of the two parameters wet gluten and deformation index is shown in figure 6.

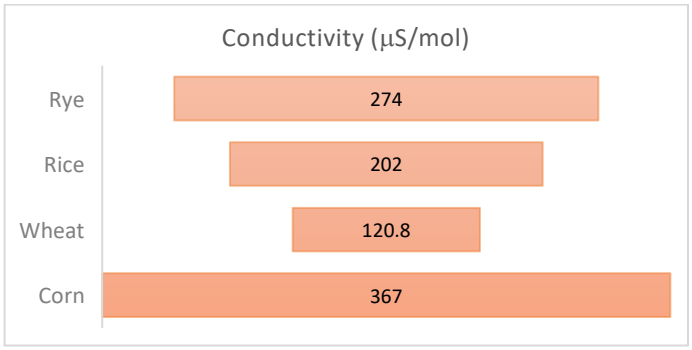

Fig. 4. Conductivity values of flour sample extracts in water after one hour

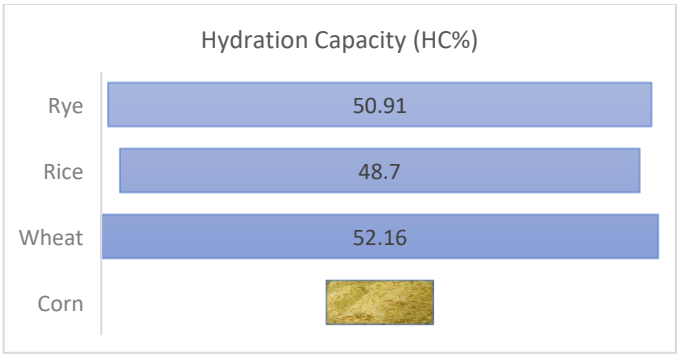

Fig.5. Hydration capacity of flour sample extracts in water

Tabel 1. Moisture determination and parameters depending by samples humidity

Crt. No.	Sample	M%	Cp (kJ/kg K)	Λ (W/m K) (22 °C)	$a \cdot 10^8$ (m^2/s)
1	Rye	10.01	1.926	0.943	23.65
2	Rice	11.00	1.951	0.945	23.69
3	Wheat	13.22	2.007	0.952	23.79
4	Corn	11.18	1.956	0.946	23.70

Tabel 2 Humid gluten and deformation index for flour samples

Crt. No	Sample	m_i (g)	m_f (g)	G%	d_i (mm)	d_f (mm)	DI (mm)
1	Rye	50	16.8277	33.66	18	22	4
2	Rice	50	4.1888	8.38	20	23	3
3	Wheat	50	19.6976	39.40	34	22	12
4	Corn	50	-	-		50	-

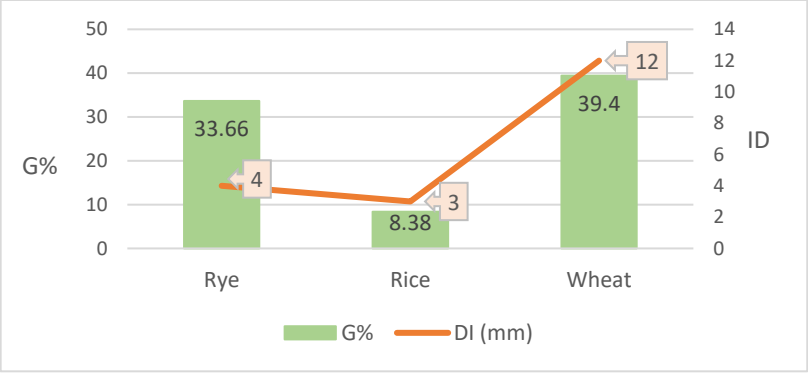

Fig. 6. Humid gluten and deformation index for flour samples

Determination of soluble protein concentration

To express the soluble protein concentration in grams in 100 g of solid sample, the following relationship is used:

$$SP(g/100\ g) = \frac{10C_{SP}}{m} \quad (12)$$

Where, C_{SP} is the soluble protein concentration (mg/mL) and m is the mass of the flour sample dissolved in distilled water for extract preparation (g). The total protein values were obtained from the label of the products used. The percentage of soluble protein in the total protein of the flours is determined with the relationship:

$$\%SP = \frac{SP \cdot 100}{TP} \quad (13)$$

Where TP is the total proteins expressed in grams in 100 grams of flour sample. All these data are recorded in the following table. In Table 3 it can be seen that the highest amount of soluble proteins is in the wheat flour extract, and the aqueous extract with the

lowest protein content is that of rice flour. In practice, the percentage of soluble protein in the total protein found in the analyzed flour samples decreased in the order:

wheat > rye > corn > rice.

Determination of enzymatic activity

Figure 7 below compares the enzymatic activities of the four flour extracts analyzed expressed in enzymatic units, i.e. in micromoles of hydrogen peroxide decomposed by 1ml of enzymatic extract for 1 minute. In this image it can be seen that the highest enzymatic activity was found in the corn flour extract (0.32), and the lowest in the wheat flour (0.17).

Determination of cation concentration at pH 10 and calcium ions in aqueous flour extracts

As can be seen in the data of figure 8, after analyzing the results obtained from determining the concentrations of calcium and all cations in flour samples, the following observations can be made: the sample with the highest concentration of cations, including calcium ions, is rice flour. It can be said that rice flour is the richest in minerals. In contrast, corn flour has the highest content of calcium ions among all the analyzed samples, but otherwise it is quite poor in minerals. The poorest flour in minerals and calcium is the wheat flour sample.

Tabel 3. Soluble proteins from flour samples extracts in water

Crt. No.	Samples	M (g)	C_{SP} (mg/ml)	SP (g/100 g)	TP (g/100 g)	%SP
1	Rye	10	0.188	0.188	7.2	2.61
2	Rice	10	0.117	0.117	9.4	1.24
3	Wheat	10	0.246	0.246	6.9	3.57
4	Corn	10	0.176	0.176	11.5	1.53

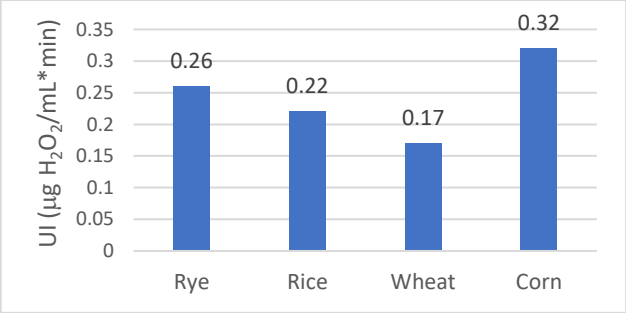

Fig. 7. Enzymatic activities of flour sample extracts in water after 30 minutes

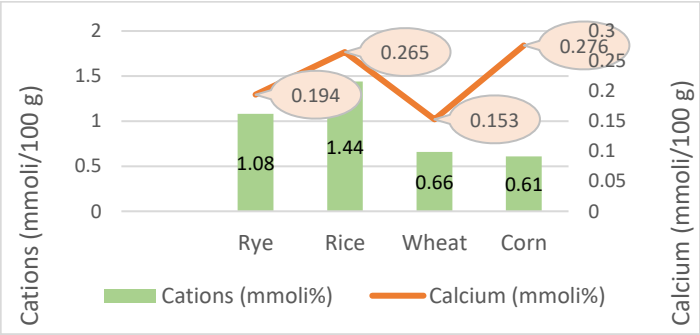

Fig. 8. Total cations and calcium concentrations in flour sample extracts in water

CONLUSIONS

After determining the pH, values between 6.4 and 7.6 were obtained. The highest value was recorded for rice, and the lowest value, 6.4, was recorded for corn. As was also observed in the pH variation diagram, the pH values in the case of rice, rye and wheat decreased slightly over time, with the exception of corn flour where the pH value remained constant. It was found that the solution with the highest anionic and cationic charge was determined in the corn flour solution, while the wheat flour solution presented the lowest ionic charge. Due to the fact that rice flour is the only one that tends towards a weakly acidic pH among the 4 types of flour, it can be used by patients with celiac disease, gastritis, diarrhea, etc. The highest hydration capacity was shown by wheat flour, which is due to the presence of gluten in its structure. This indicates that wheat flour is the best for baking, while corn flour is the poorest in quality, as it is not elastic and cannot be kneaded. The highest cation load, including calcium ions, was determined in the rice flour sample. Of the 4 types of flour, rice flour is the richest in minerals. The sample with the highest concentration of calcium ions was represented by corn flour. The poorest sample in minerals is represented by wheat flour. The richest sample in protein is wheat flour, followed by rye flour, while the poorest in protein is rice flour. The highest enzymatic activity associated with the decomposition of hydrogen peroxide was recorded for corn flour, followed by rye and rice, and the lowest enzymatic activity was recorded for wheat flour [1-24].

REFERENCES

[1] Stępniewska, S., Cacak-Pietrzak, G., Szafrańska, A., Ostrowska-Ligęza, E., Salamon, A., Kowalska, H. (2024). Assessment of the Baking Properties of Rye Flour Based on the Polysaccharide Content and Properties, Appl. Sci., Vol. 14, pp. 2772. https://doi.org/10.3390/app14072772

[2] Opopol, N. (2006). Nutriţia în sănătatea publică / Nicolae Opopol, Galina Obreja, Angela Ciobanu ; Univ. de Stat de Medicină şi Farmacie “Nicolae Testemiţanu”, Şc. Management în Sănătate Publică. - Ch. : Bons Offices, ISBN 978-9975-80-013-6 150 ex. 613.2

[3] Michalska A., Ceglinska A., Amarowicz R., Piskula M.K., Szawara-Nowak D., Zielinski H..(2007). Antioxidant contents and antioxidative properties of traditional rye breads, J Agric Food Chem., Vol.55(3), pp. 734-40. doi: 10.1021/jf062425w. PMID: 17263468.

[4] Siyuan, S., Tong, L., Liu, R.H. (2018). Corn phytochemicals and their health benefits, Food Science and Human Wellness, Vol. 7(3), pp. 185-195, ISSN 2213-4530, https://doi.org/10.1016/j.fshw.2018.09.003.
[5] Modipuram, Meerut UP. (2013). Assessment of functional properties of different flours, African Journal of Agricultural Research, Vol. 8(38), pp. 4849-4852.
[6] Dziki, D., Krajewska, A., Findura, P. (2024). Particle Size as an Indicator of Wheat Flour Quality: A Review, Processes, Vol. 12, pp. 2480. https://doi.org/10.3390/pr12112480
[7] Ikram A., Saeed F., Arshad M.U., Afzaal M., Anjum F.M. (2021). Structural and nutritional portrayal of rye-supplemented bread using Fourier Transform Infrared Spectroscopy and Scanning Electron Microscopy, Food Sci Nutr., Vol. 9(11), pp. 6314-6321. doi: 10.1002/fsn3.2592. PMID: 34760261; PMCID: PMC8565228.
[8] Rathnayake H.A., Navaratne S., Navaratne C. (2022). Effect of Process Variables on Rice Flour Functional Properties, and Porous Structure of Rice and Wheat-Based Leavened Food Products, Food Technol Biotechnol., Vol. 60(1), pp. 99-108. doi: 10.17113/ftb.60.01.22.7238. PMID: 35440882; PMCID: PMC8990982.
[9] Ronie, M.E., Hasmadi, M. (2022). Factors affecting the properties of rice flour: a review, Food Research, Vol. 6 (6), pp. 1 – 12
[10] Mazzola, A.M., Zammarchi, I., Valerii, M.C., Spisni, E., Saracino, I.M., Lanzarotto, F., Ricci, C. (2024). Gluten-Free Diet and Other Celiac Disease Therapies: Current Understanding and Emerging Strategies, Nutrients, Vol. 16, pp. 1006.
[11] Matsuda T. (2019). Rice Flour: A Promising Food Material for Nutrition and Global Health, J. Nutr. Sci. Vitaminol. (Tokyo), Vol. 65(Supplement), S13-S17. doi: 10.3177/jnsv.65.S13. PMID: 31619613.
[12] Tian Y., Sun J., Li J., Wang A., Nie M., Gong X., Wang L., Liu L., Wang F., Tong L. (2024). Effects of Milling Methods on Rice Flour Properties and Rice Product Quality: A Review, Rice Science, Vol. 31(1), pp. 33-46, ISSN 1672-6308, https://doi.org/10.1016/j.rsci.2023.11.002.
[13] Ishikawa D., Sawa I., Sekiyama Y., Horigane A.K., Okunishi T., Fujii K., Fujii T. (2017). Study on the Change in Powder Properties of Rice Flour by Different Milling Processes. J. Appl. Glycosci., Vol. 64(4), pp.109-114. doi: 10.5458/jag.jag.JAG-2016_016. PMID: 34354503; PMCID: PMC8056928.
[14] Wang T., Cao Y., Zhang W., Luo X., Feng W., Wang R., Yi C., Ai Z., Zhang H. (2023). Physicochemical Properties of the Rice Flour and Structural Features of the Isolated Starches from Saline-Tolerant Rice Grown at Hazard, B., Trafford, K., Lovegrove, A. et al. (2020). Strategies to improve wheat for human health, Nat Food 1, pp. 475–480. https://doi.org/10.1038/s43016-020-0134-6
[15] Different Levels of Soil Salinity, J. Agr. & Food Chem., Vol. 71 (45), 17353-17361DOI: 10.1021/acs.jafc.3c05449
[16] Qin Y., Liu C., Jiang S., Cao J., Xiong L., et al. (2016) Functional Properties of Glutinous Rice Flour by Dry-Heat Treatment. PLOS ONE, Vol. 11(8): e0160371. https://doi.org/10.1371/journal.pone.0160371
[17] Mejía-Terán, A., Blanco-Lizarazo, C.M., Mateus, E.L., Sotelo-Díaz, I. (2024). Technofunctional and physicochemical properties of corn flours as potential food

ingredients, Applied Food Research, Vol. 4(1), pp. 100427, ISSN 2772-5022, https://doi.org/10.1016/j.afres.2024.100427

[18] Oyeyinka, S. A., Bassey, I. A. V. (2023). Composition, Functionality, and Baking Quality of Flour from Four Brands of Wheat Flour, J. of Culinary Science & Tech., pp. 1–21. https://doi.org/10.1080/15428052.2023.2191874

[19] Shewry P..R, Hey S.J. (2015). The contribution of wheat to human diet and health, Food Energy Secur. Vol. 4(3), pp. 178-202. doi: 10.1002/fes3.64. Epub 2015 Aug 14. PMID: 27610232; PMCID: PMC4998136.

[20] Németh, R., & Tömösközi, S. (2021). Rye: Current state and future trends in research and applications, Acta Alimentaria, Vol. 50(4), pp. 620-640. https://doi.org/10.1556/066.2021.00162

[21] Meenu, M., Kaur, S., Yadav, M. et al. (2024). Impact of inherent chemical composition of wheat and various processing technologies on whole wheat flour and its final products, Cereal Research Commun. https://doi.org/10.1007/s42976-024-00544-0

[22] Cappelli, A., Cini, E. (2021). Challenges and Opportunities in Wheat Flour, Pasta, Bread, and Bakery Product Xie, D., Li, X., Li X., Ren, S. (2023). Effect of gluten protein levels on physicochemical and fermentation properties of corn dough, International J. of Food Science & Technology, Vol 59(1), https://doi.org/10.1111/ijfs.16793.

[23] Production Chains: A Systematic Review of Innovations and Improvement Strategies to Increase Sustainability, Productivity, and Product Quality, Sustainability, Vol.13, pp. 2608. https://doi.org/10.3390/su13052608

[24] Brănzaru, I. (2004). Manual pentru cultura de specialitate Industria Alimentară, Editura Oscar Print Bucureşti.

[25] Mărgărit, E.R.(2021). Metode de analiză în industria panificației – Rețete și instrucțiuni tehnologice , Ed. Zven, Târgoviște, Romania, ISBN 978-606-9745-20-5664.6

[26] Hayes M. (2020). Measuring Protein Content in Food, An Overview of Methods. Foods, Vol. 9(10), pp. 1340. doi: 10.3390/foods9101340. PMID: 32977393; PMCID: PMC7597951.

[27] Cărăban, A., Bota, S. (2019). Lucrări practice Biochimie II, Editura Universităţii din Oradea, Romania

[28] Petrehele A. I. G. (2016). *Îndrumător de lucrări practice în chimia anorganică generală*, Ed. Universităţii din Oradea, Oradea, Romania

CALCIUM CONTENT IN THE SEEDS OF SOME PLANTS CONSUMED AS FOOD

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Abstract

This research focus on the content of Calcium in the seeds of some plants consumed as food, such as beans and lentils. Dry seeds of white, red and variegated beans (Phaseolus vulgaris) and green and red lentils (Lens culinaris) were subjected to the study. The method for determining Calcium was titration with potassium permanganate after the precipitation of Ca ions as oxalates, a method used in food chemical analysis laboratories, because it is an inexpensive e reliable method. In the case of beans, no very large differences were reported between the calcium content depending on the variety (155-198 mg/100g). It is noted, however, that the less pigmented grains have a higher Calcium content. The same conclusion can be drawn in the case of lentils. Taking into account the daily requirement of calcium (1200 mg for an adult), a daily portion of about 500-600 g of beans or lentils could ensure a good diet from this point of view.

Key words

Permanganate titration, Calcium content, *Phaseolus vulgaris, Lens culinaris*

INTRODUCTION

Legumes are the second most important group of plant crops after cereals in Human nutrition [1]. This fact is due to the low cost of cultivation, nutritional properties and beneficial physiological effects [2]. The most widespread edible legumes are: beans, peas, chickpeas, lentils, soybeans, peanuts and lupins. Legumes are widely cultivated for their nutritional properties, the high content of the grains in protein substances, the varied content in mineral substances. The mineral content of legumes varies depending on the type and variety of legumes [3]. Some studies have reported that regular consumption of legumes reduces people's susceptibility to chronic diseases such as cardiovascular disease, diabetes, cancer and excess body weight. This may be due to the high content of protein, dietary fibre, essential fatty acids and isoflavones. [4]

Calcium is the most abundant element found in our body in quantities greater than 5 g, exactly 1.5-2 kg of calcium, of which 99% is stored in bones and teeth, and 1% is used, mainly, for the acid-base balance in the body. When we don't have enough calcium in our diet to correct the acidic environment, it is pulled from the bones, which can increase the risk of osteoporosis. Serum calcium, from the blood, remains mostly constant in the body, using the deposits in the bones. Calcium is a mineral that depends on vitamin D for proper absorption and is especially important for maintaining healthy bones throughout life. Even if you eat a lot of green vegetables, calcium supplementation should be considered. [5] The calcium requirement is 1000-1300 mg per day, depending on gender and age, but it is not advisable to exceed the limit of 2000 mg per day as an adult. [6] Calcium is also contained in a wide range of seeds, which you can consume either as such, or you can add them to your morning cereals,

salads or other preparations. A 28 g serving of sunflower seeds contains approximately 50 mg of calcium. The same amount of chia seeds contains about 179 mg of calcium, and a single tablespoon of sesame contains 88 g of calcium. Half a cup of white beans contains about 100 mg of calcium, and one cup of soybeans, cooked without salt, contains 260 mg of calcium. [7]

This paper presents the study carried out on the content of Ca in the seeds of some plants consumed as food such as beans and lentils. Dry seeds of white, red and variegated beans (*Phaseolus vulgaris*) and green and red lentils (*Lens culinaris*) were subjected to the study. The method for determining Ca was the titration with potassium permanganate after the precipitation of Ca ions as oxalates, a method used in laboratories for the chemical analysis of food products.

EXPERIMENTAL

Analysis method

The method of analysis used was the titration with potassium permanganate after the precipitation of Ca ions as oxalates, a method used in laboratories for the chemical analysis of food products [8]. The method is a volumetric redox method based on the reaction:

$$5C_2O_4^{2-} + 2MnO_4^- + 16H^+ \rightarrow 2Mn^{2+} + 10CO_2 + 18H_2O$$

Reagents:

HCl solution: 25 mL HCl conc. diluted in 100 mL distilled water

Saturated ammonium oxalate solution

H_2SO_4 4N

Potassium permanganate standard solution 0.01 N. The analysed seeds were purchased from the supermarket in January 2024.

The analysed products

Figure 1. Investigated products
Beans (*Phaseolus vulgaris*) white, red and variegated (manufacturer Solaris) Lentils (*Lens culinaris*) green and red (manufacturer Simply).

Samples preparation

A quantity of about 50 g of each seed was crushed with the help of an electric grinding device (household grinder).

The powder was then placed in the oven at 550°C for calcination. The resulting ash was dissolved in HCl and transferred to a 50 mL volumetric flask. For each analysed product, 2 such solutions were prepared and analysed in duplicate. The solution obtained by dissolving the ash was treated with ammonium oxalate to precipitate Ca. The precipitate was separated by filtration and redissolved in H_2SO_4 4N.

Titration of solutions with potassium permanganate

The obtained solutions were titrated hot (75-85°C) with 0.01 N $KMnO_4$ solution until the pink colour persisted for 30 s.
Analyses were made in duplicate (on the 2 solutions prepared from ash) for each analysed product and the average value of the 2 results was calculated.

Calculations [10]
Ca/100 g analysed sample= 2000VN/m

Where:
V= volume in mL of permanganate solution used for titration
N= the normality of the potassium permanganate solution used for titration
m= mass of analysed material (g)

Results

The results are presented in table 1 and for comparisons in fig.2. The data obtained are in good agreement with those from the specialized literature (table 1).

Table 1. The results obtained from the titrimetric determination of calcium ions from the analysed samples:

	mg Ca^{+2}/100 g	mg Ca^{+2}/100 g, data from literature
White beans	198	150 *
Red beans	155	
Variegated beans	178	
Green lentils	150	210**
Red lentils	135	

*https://www.feedtables.com/content/common-bean
** https://www.feedtables.com/content/lentils

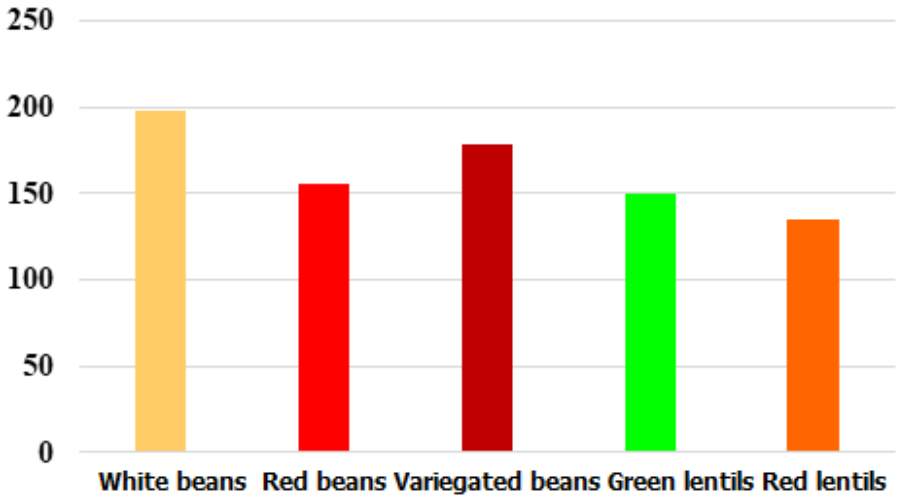

Figure 2. The calcium content of investigated varieties of beans and lentils

CONCLUSIONS

The obtained results demonstrated that dried beans and lentils are important foods for the ingestion of essential minerals necessary for human health.

In the case of beans, no very large differences were reported between the calcium content depending on the variety (155-198 mg/100g). It is noted, however, that the less pigmented grains have a higher Ca content. The same conclusion can be drawn in the case of lentils.

Beans and lentils are a good source of calcium in the daily diet.

Taking into account the daily requirement of calcium (1200 mg for an adult [9]), a daily portion of about 500-600 g of beans or lentils could ensure a good diet from this point of view).

Disadvantages of the titrimetric method of analysis:

The titrimetric method of calcium analysis can be subject to errors due to the sequence of steps that must be completed to prepare the solutions to be titrated (precipitation of calcium ions as oxalate, taking the precipitate and dissolving it). Difficulties also arise in terms of maintaining the solution to be analysed at 75-85ºC during the titration.

Advantages of the method:

The main advantage of the method is the fact that it does not require expensive instruments and if the person doing the analysis has experience in the field the results obtained can be reliable comparable to those obtained using Atomic Absorption, a method that is much more expensive and that not all laboratories have endowment

REFERENCES

[1]. Levetin-Mcmahon, (2008), Legumes. In: *Plants as a Source of Food*. 5th Edn., the McGrawHill, New York, USA, 207-209.

[2]. Moreno-Jiménez M. R. and all (2015), Phenolic composition changes of processed common beans: their antioxidant and anti-inflammatory effects in intestinal cancer cells, *FoodRes Int.*, 79-85.

[3]. Bolarinwa I. F., Al-Ezzi M., Carew I., Muhammad K., Nutritional Value of Legumes in Relation to Human Health, (2019) Advance Journal of Food Science and Technology, 17(5), pp. 72-85

[4]. Kouris-Blazos A., (2016), Health benefits of legumes and pulses with a focus on Australian sweet lupins. Asia Pac. *J. Clin. Nutr.,* 25(1), 1-7.

[5]. Kanis J.A., Passmore R., Calcium supplementation of the diet, BMJ (1989), doi: 10.1136/bmj.298.6667.137

[6]. https://ods.od.nih.gov/factsheets/Calcium-HealthProfessional/

[7]. https://www.healthline.com/nutrition/15-calcium-rich-foods

[8]. Pearson's Chemical analysis of Foods, Egan at al. 1981, Churchill Livingstone

[9]. https://www.health.harvard.edu/staying-healthy/how-much-calcium-do-you-really-need

[10]. IS: 7874 (part II) 1975/ https://law.resource.org/pub/in/bis/S06/is.7874.2.1975.pdf

SPECTROPHOTOMETRIC DETERMINATION OF ANTIOXIDANT ACTIVITY, CONTENT OF PHENOLS AND FLAVONOIDS FROM A VEGETABLE EXTRACT

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Abstract: *This paper presents the spectrophotometric analyzes of the plant extract of Polygonum aviculare L. herba (PAH). The Folin-Ciocâlteu method was used to determine the total polyphenol content, while for the evaluation of the total content of flavonoids was also used a colorimetric method. The antioxidant activity of the PAH extract was determined using the DPPH method.*
Keywords: UV-VIS spectrophotometry, antioxidant activity, Folin – Ciocâlteu method

INTRODUCTION

Plant extracts have numerous applications and are obtained from both wild and cultivated plants. Currently, plants are increasingly studied for their therapeutic actions. The separation and understanding of the chemical structure of plant extracts constitute the decisive step in explaining their characteristic therapeutic action.

Polygonum aviculare L. herba is an annual herbaceous plant, from the family Polygonaceae, with the stem usually lying on the ground or ascending, with small, elliptical leaves and white-pink or greenish hermaphrodite flowers[1] (Figure 1). The root is pivoting and plant is spread in all areas of the country, in cultivated or uncultivated places, on the sides of roads, in ditches and in empty places. It blooms from may until September[2]. For medicinal purposes, the aerial parts are harvested, by uprooting the plant and removing the roots and the lower, woody parts[3].

Figure 1. Plant *Polygonum aviculare L. herb*

The plant *Polygonum aviculare L.herba* is an annual weed propagated by seeds, with a fusiform root, very different in habitus and shape of the leaves from one year to another. It contains as chemical components: phenolic compounds, flavonoids which are quercetin, kaempferol, myricetin, avicularin, (Figures 2-5) etc. Recently, new flavonoids have been identified, such as: 5,7-dihydroxy-4'-methoxyflavane, 5,7-dihydroxy-6-methoxyflavane, and 5,7-dihydroxyflavane. The species contains tanning substances such as: silicon salts, vitamins A, K, and C, sterols, fats, sugars, mucilages, mineral substances rich in free and combined salicylic acid, volatile oil, triterpene saponins, and anthraquinone derivatives. Flavonoids are represented by flavonols, flavones, isoflavones, catechins, phenols existing in vegetable products and which through their structure possess antioxidant properties that give them the ability to actively intervene in the control of oxidative reactions in the body[4].

Natural antioxidants are a series of atoms joined by chemical molecules that help protect other molecules by inhibiting the oxidation process. For the use of antioxidants of vegetable origin, we chose the species *Polygonum aviculare L.*

Spectrometric methods are a particularly broad and diverse category of analytical methods, its applications appearing in different fields such as chemical, pharmaceutical, medicine, agriculture, food quality control, etc. The property of substances to selectively absorb electromagnetic radiation is the basis of absorption spectrophotometry and is used for identification, determination of purity and dosing[5].

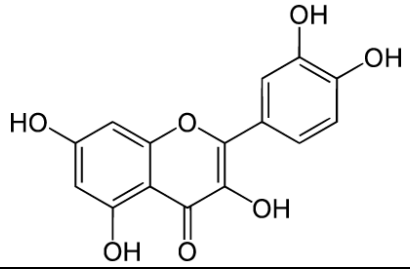


Figure 2. Chemical structure of quercetin.

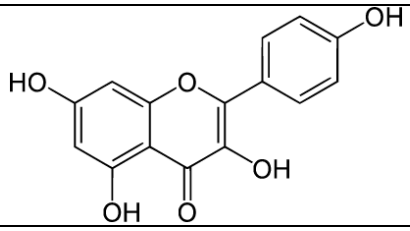


Figure 3. Chemical structure of kaempferol

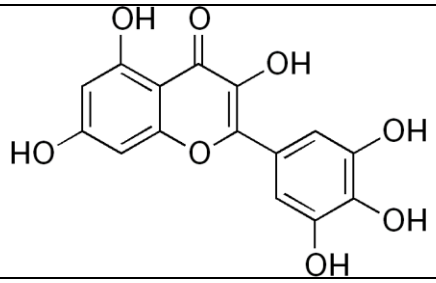


Figure 4. Chemical structure of myricetin

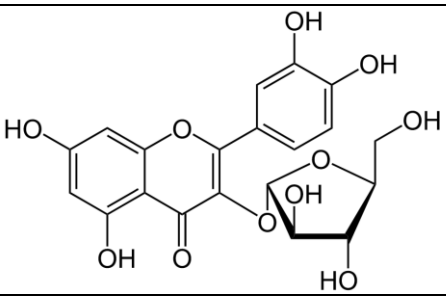


Figure 5. Chemical structure of avicularin

MATERIALS AND METHODS

The molecular absorption spectrometry in UV-VIS based on the absorption by molecules of light radiation from the range of 200-780 nm was applied in this research.

1.Spectrophotometry analysis of phenols[6]

The determination of the content of total polyphenols from vegetable sources was done by measuring the optical density of a primary extract, which, by complexing with the Folin-Ciocâlteu reagent, absorbs at the wavelength λ = 750 nm. The total amount of polyphenols is expressed relative to a gallic acid calibration curve.

Reagents and materials: ethanol 40% of analytical purity, Folin-Ciocâlteu reagent 0.1 N, sodium carbonate (5%), distilled water, gallic acid - standard (purity degree 99%); analytical balance, ultrasonic bath, UV-VIS Biotek multidetection spectrophotometer. For the preparation of the standard, precisely weigh 25 mg of gallic acid using an analytical balance and place it into a 25 ml volumetric flask. Add 15 ml of 40% ethanol and sonicate until completely dissolved, resulting in a solution with a concentration of 1 mg/ml. Fill the solution with the solvent up to 25 ml. From this solution were done the dilutions in order to prepare the standard solutions of: 0.5mg/100 ml, 0.75mg/100 ml, 1mg/100ml, 1.25mg/100ml. It was taken 1 ml of standard solution and inserted into a 100 ml volumetric flask, add 60-70 ml of distilled water, stir, then add 5 ml of Folin-Ciocâlteu reagent and homogenize. After 1 minute and before 8 minutes, there were added 14 ml of 7.5% sodium carbonate solution. This moment was noted as moment "0" and homogenized again to a volume of 100 ml with distilled water. Thus, the dilution of the standard solution of 1 mg/100 ml was obtained. After 2 hours, the absorbance was read at λ = 750 nm compared to the reference prepared in the same way.

2.Spectrophotometric determination of flavonoids[7,8]

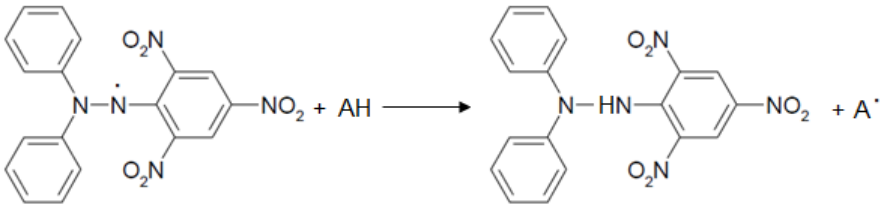
In order to evaluate the total content of flavonoids it was used the colorimetric method with aluminum chloride; for the standard calibration curve was used quercetin.

The quercetin stock solution was prepared by dissolving 5.0 mg quercetin in 1.0 ml methanol, followed by the preparation of a standard quercetin solution with serial dilutions using methanol (5 - 200 μg/ml).

Absorbance was read at a wavelength of 520 nm with a UV-VIS spectrophotometer - 1601 Shimadzu. The total flavonoid concentration was calculated based on the calibration line and expressed as quercetin equivalent (QE) mg/100 g in the extract.

3. Spectrophotometric determination of antioxidant activity[9,10]

The antioxidant activity of the PAH extract was determined using the DPPH method. Known as a powerful radical "trap" for neutralizing or suppressing free radicals, DPPH is a solid material of black crystalline powder consisting of stable free radical molecules. By this method is measured the antioxidant levels within complex biological systems. The basic reaction for determining the antioxidant activity by the DPPH method:



$I\%(t) = A_{517nm}(t)/ A_{517nm}(t = 0)\cdot 100$

I%(t) depending on the concentration of the solutions

Solution of 1 mg/ml was diluted to final concentrations of 200, 100, 50 µg/ml in ethyl alcohol 96%. The antioxidant activity was determined as follows: 1.5 ml of the solution to be analyzed is mixed with 2.5 ml of DPPH 0.004% solution, then left in the dark for 30 minutes, after which the absorbance was read at 517 nm; the solution decolorizes over time as DPPH reacts with free radicals in the system, and the progress of the reaction is monitored spectrophotometrically. As reference it was used absolute ethanol.

The antioxidant activities of the samples are evaluated from the dependence curves of the relative absorbance (A%), as a ratio between the absorbance at time t and the initial absorbance (at t= 0) corresponding to the control solution:

$$A\%(t) = \frac{A_{517nm}(t)}{A_{517nm}(t=0)} \cdot 100$$

The lower the A%, the higher the antioxidant activity of the studied sample.

RESULTS AND DISCUSSION

1. *Determination of total polyphenols from the PAH extract*
2.

The values of absorbances for gallic acid were read at a wavelength of 750 nm. The calibration curve is shown in Figure 6.

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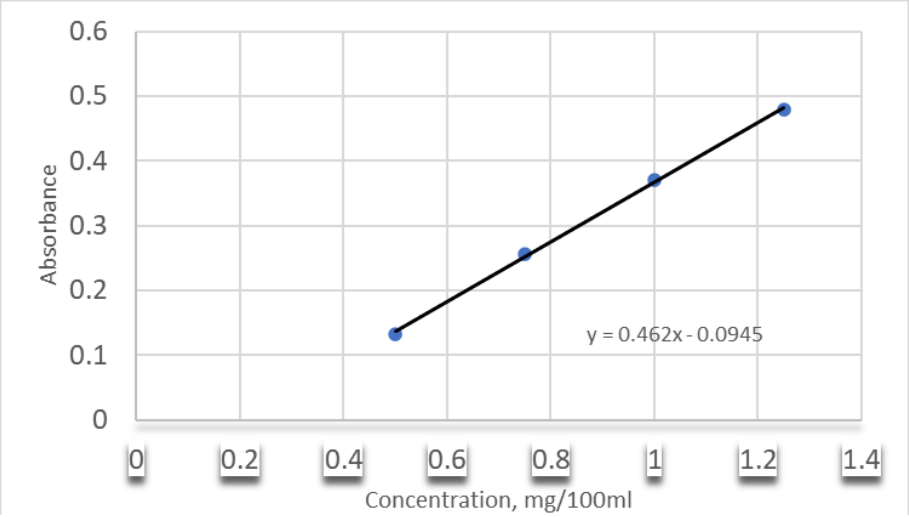

Figure 6. Calibration line of gallic acid

After 2 hours, were read the absorbances of the samples at λ = 750 nm compared to the control. From the equation of the calibration curve: x =(y + 0.0945)/0.462, was calculated the amount of total polyphenols (expressed in mg/mL and µg/1 mL plant extract). The results obtained for the four solutions from the vegetable extracts are presented in table 1.

Table 1. The content of total polyphenols in the plant extracts of PAH

Samples of PAH extract	The solution 1 (1mg/1 ml)	The solution 2 (200 µg/1 ml)	The solution 3 (100 µg/1 ml)	The solution 4 (10 µg/1 ml)
Absorbance value $A_{750}(t)$	0.594	0.321	0.234	0.095
The amount of polyphenols mg/ml	1.490	0.899	0.711	0.410

The results of the Folin - Ciocâlteu method are very well correlated with the results obtained by antioxidant analyses.

The total polyphenol content was calculated as equivalent of gallic acid (GAE/100 g) extract, based on the gallic acid calibration line (y=0.462x-0.0945 R^2 = 0.9993).

2. Spectrophotometric determination of flavonoids

Values for absorbances of quercetin were read at wavelength of 520 nm. It was plot the calibration line (Figure 7) and, based on it, the total content of flavonoids in each solution of *Polygonum aviculare L. herba* (PAH) can be expressed as an equivalent quercetin concentration (Table 2).

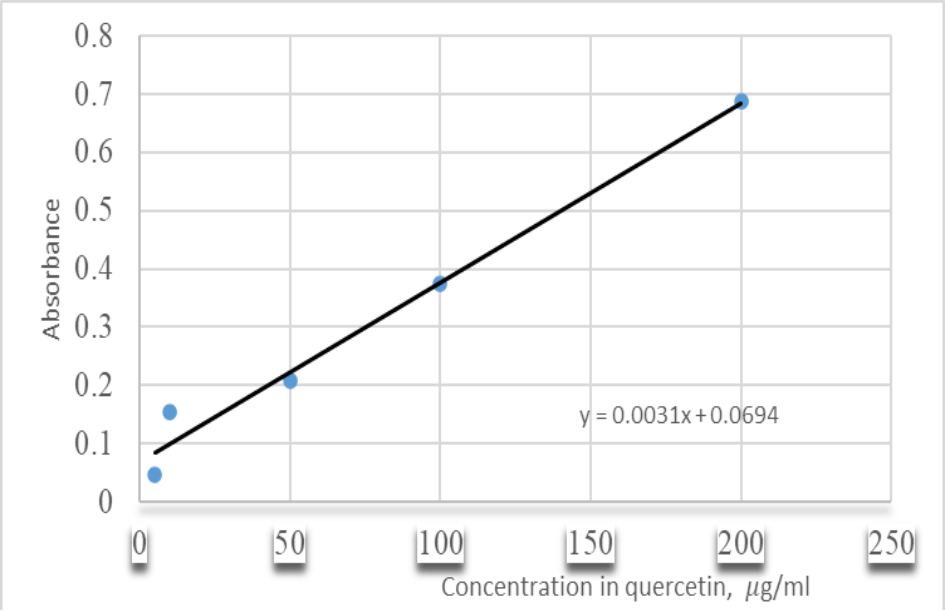

Figure 7. Calibration line of quercetin

Table 2. Total content of flavonoide expressed as equivalent concentration of quercetin from solutions of *Polygonum aviculare L. herba* (PAH)

Samples of PAH extract	The solution 1 (200 µg/1 ml)	The solution 2 (100 µg/1 ml)	The solution 3 (50 µg/1 ml)
Absorbance value $A_{520}(t)$	0.522	0.434	0.196
The amount of quercetin (μg /ml)	146	117.6	40.8

Flavonoids or flavonoid pigments are phenolic compounds in the molecule of which there are condensed heterocycles, benzopyran or benzofuran, in which the heteroatom is oxygen. Another benzene nucleus condenses to this dicycle[11].

Among the more well-known flavonoid pigments are derivatives that originate from: flavan, flavena, flavanone, flavone, chalcone, aurone.

From the heterocyclic rings of flavonoids through hydroxylation, methoxylation, hydrogenation, elimination reactions, the generate the great diversity of flavonoid pigments[11].

3. Antioxidant activity by the DPPH method

The absorbances values at 517 nm of the analyzed samples, based on which the antioxidant activity was calculated for $A_{517\ (t=0)} = 0.108$, are presented in Table 3. The absorbance variation of the extract of *Polygonum aviculare L.* is presented in Figure 8.

Table 3. Values for absorbances, A%(t) for the 4 solutions of PAH extract at 517 nm.

Samples of PAH extract	The solution 1 (1mg/1 ml)	The solution 2 (200 µg/1 ml)	The solution 3 (100 µg/1 ml)	The solution 4 (50 µg/1 ml)
Absorbance value $A_{517}(t)$	0.0577	0.0691	0.0732	0.0834
Inhibition %	53.4259	63.9814	67.7777	77.2222

The antioxidant capacity measured by the DPPH method had high values. Thus, the PAH extract showed the table values of the antioxidant activity measured by the DPPH method.

Thanks to these tests, the antioxidant capacities of the plant product *Polygonum aviculare L. herba* were confirmed.

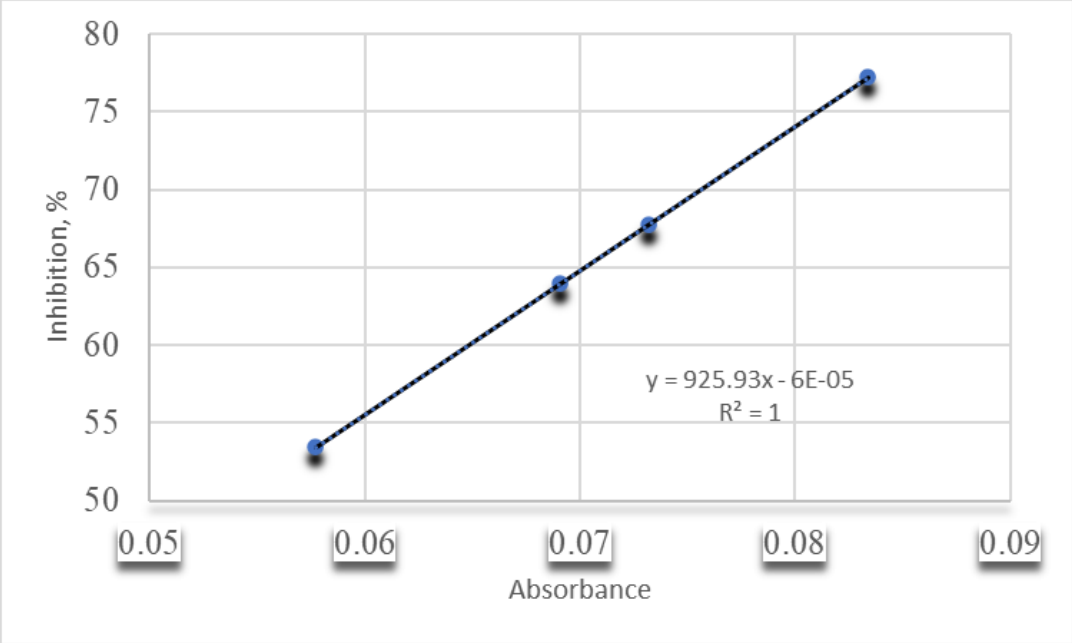

Figure 8. Inhibition, % or antioxidant activity for the extract of *Polygonum aviculare L.*

CONCLUSIONS

The aim of the work was to highlight the antioxidant properties through spectrophotometric analyzes of the extract of the *Polygonum aviculare L.*

The antioxidant activity of a system can be estimated by spectrophotometric monitoring of the color reactions that take place at the time of DPPH reduction, on account of the tested substrate – in our case, solutions from the vegetal extract.

The DPPH solution in contact with the analyzed extract discolors, and the progress of the reaction is monitored spectrophotometrically at 517nm.

The DPPH method is used to determine the antioxidant activity of pure phenolic compounds as well as natural extracts[12]. Some researchers have reported that most phenolic compounds react slowly with DPPH, requiring 1-6 hours. This suggests that, using DPPH, the antioxidant activity must be evaluated over time and the interference of the DPPH color over time with that of samples containing anthocyanins will lead to an underestimation of the antioxidant activity.

The antioxidant activity was evaluated by the DPPH method (was used a 0.004% concentration solution in 96% ethyl alcohol, a solution used as a standard antioxidant).

The Folin - Ciocâlteu method was used to evaluate the total content of polyphenols.

To evaluate the total content of flavonoids, was used the colorimetric method with aluminum chloride, for the standard calibration curve, was used quercetin.

The obtained results indicated that the ethanolic solutions of *Polygonum aviculare L. herba* have a significant antioxidant activity.

REFERENCES

[1] SAFTA M., *Super-Antioxidanţi naturali în alimentaţie şi medicină,* Secretul sănătăţii şi longevităţii, Ed. Sudura, Timişoara, 2002.

[2] NEAMŢU G., *Substanţe naturale biologic active, vol*.I, Vitamine, Ed. R.A. Bucureşti, 2001.

[3] Stryer, L., *Biochemistry*, 2nd Ed., W.H. Freeman Co., San Francisco, 1981.

[4] HOLLMAN, P.C.H., KATAN, M.B., *Absorption, metabolism and health effects of dietary flavonoids in man*, Biomed. Pharmacather, 51, 305-310, 1997.

[5] GHEŢIU, M.M., *Chimia Organică,* Ed. Tehnica-Info, Chişinău, 1999.

[6] CIOBANU D., *Chimia produselor alimentare-investigaţii analitice*. Editura Tehnica-Info, Chişinău, 2002.

[7] ANDERSEN, M.O., MARCHAM, K.R., *Flavonoids: chemistry, biochemistry and application*, Taylor & Francis. Group. NW, Boca Raton, U.S.A., 2006.

[8] BRAND-WILLIAMS, W., CUVELIER, M.E., BERSET, C., *Use of a free radical method to evaluate antioxidant activity*, Lebensm.-Wiss. Technol. 28, p. 25-30, 1995.

[9] HERDAN, J.M., GIURGINCA, M., MEGHE, A., *Antioxidanţi*, Ed. Tehnică, Bucureşti, 1995.

[10] CONDRAT, D., LUPEA, A.X., Elimination activity of free radicals evaluation of ethanol extracts from phanerogram plants by DPPH method, *Scientific and Technical Bulletin,* Arad, 12, p. 45-50, 2007.

[11] BODEA, C., FARCĂŞAN, V., NICOARĂ, E., SLUSANSCHI N., *Tratat de Biochimie vegetală, Partea I, Fitochimie,* vol. I-III, Acad. R.S. România, Bucureşti, 1964-1966.

[12] WANG, J., *Analytical Electrochemistry,* Vch Verlagsgesellschaft, 2000.

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GRAVIMETRIC STUDIES ON THE CORROSION OF STEEL IN AN ACID ENVIRONMENT WITH A GREEN INHIBITOR

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Abstract: *Green inhibitors are environmentally friendly substances obtained by their extraction by various methods in alcoholic or hydroalcoholic solvents, such as maceration or Soxhlet extraction. Added in small quantities, eco-inhibitors reduce or cancel metal corrosion in aqueous solutions, by forming a film that adsorbs on the metal surface. The extract's inhibitory effectiveness depends critically on the concentration of its active ingredients. In this work, the electrochemical behavior of steel in the presence of an inhibitor derived from a plant extract is evaluated using gravimetric measurements.*
Key words: plant extract, green inhibitor, gravimetry, corrosion, mild steel, acid environment*.*

INTRODUCTION

Corrosion of metallic materials is a rather problematic phenomenon in modern technology, impacting both safety and efficiency, as well as maintenance costs. By definition, corrosion is the process of degradation of metals as a result of their interaction with the surrounding environment.

The process involves redox reactions at the metal/non-metal interface, with electron transfer through the metal and ion transport through the solution [1]

There are various factors that influence the rate of corrosion, including: the nature of the metal, the composition of the electrolyte, the condition of the surface, the pressure, the temperature and the flow rate of the solution. To ensure the efficiency and safety of installations, corrosion control is essential and involves the choice of suitable materials, thermal, mechanical or chemical treatments, as well as the use of protective coatings or electrochemical methods of protection.

One of the known and used protection methods is the treatment of the corrosive environment with corrosion inhibitors [1-22].

green corrosion inhibitors or eco-inhibitors are characterized by the absence of toxic compounds or heavy metals and biodegradability.

So, green-inhibitors can be both organic and inorganic, deriving from natural or biological sources, such as plants or drugs [2-6]. They act by forming a protective film or by adsorbing to the metal surface, and are often classified as micro-inhibitors, having significant effectiveness in preventing corrosion.

Recent studies have investigated the effectiveness of various natural extracts as corrosion inhibitors. For example, extracts of peach peel, orange peel, argan, mahogany leaves or essential plant oils have demonstrated the ability to protect metals against corrosion in acidic environments[2-6]. The efficiency of these extracts was evaluated by various methods such as gravimetric, electrochemical and mass loss analyses.

METHODS AND MATERIALS

Green inhibitor preparation

The preparation process involves the preparation of a hydroalcoholic solution, with a ratio of 1:1 between distilled water and ethyl alcohol p.a.

For each type of extract, a specific amount of dried plant was weighed and added to the water-alcohol solution. The tinctures were left to macerate for 24 hours at room temperature, protected from light to prevent oxidation. After maceration, the extracts were filtered to remove solids and stored in cold dark glass containers to prevent degradation under the influence of solar radiation (Figure 1 a).

A

b

Figure 1. Preparation of green inhibitor solutions by hydroalcoholic maceration and alcoholic extraction.

The Soxhlet method is a continuous extraction technique used to

obtain soluble substances from solid or semi-solid materials using a suitable solvent. The process involves putting the extraction material into an extractor, placed above a solvent in a lower vessel. By heating the solvent, it evaporates, passes through the extraction material and condenses, collecting the soluble substances.

The cycle is repeated continuously until the desired substances are completely extracted. For the preparation of eco-inhibitors, the dried plants were placed in a Soxhlet extractor and ethyl alcohol was used as a solvent. The extraction process continued for several hours until the soluble substances were completely extracted (Figure 1 b).

The gravimetric method

The gravimetric method consists in calculating the corrosion rate of a metal or alloy. Gravimetric determinations are made over time, the experiments can last from a few hours to a few months depending on the metal/environment system studied [1].

The corrosion rate or the gravimetric index is calculated from the ratio between the variation in the mass of the metal sample (Δm) and the product between time (t) and the surface of the sample (S) with the relationship:

$$V_{cor} = \Delta m / (t\ S)\ ,\ g/m2h \qquad (1)$$

RESULTS AND DISSCUSIONS

An inhibitor is a chemical substance that is used to reduce the corrosion rate of the corrosive medium by adding it in small amounts. Inhibitors can cause an anodic reaction, a cathodic reaction, or occasionally both.

Although they are used to prevent general corrosion, many of these inhibitors do not work well when it comes to localized attacks such as crevice corrosion.

Figure 2 shows the dependence of the corrosion speed (gravimetric index) on the volume of eco-inhibitor used, in the studied time interval of 1, respectively 7 days, in 1N HCl aqueous solution.

Table 1 presents comparative data from other plant extracts, the data measured after 7 days of immersion in the corrosive aqueous media environment It also shows the optimal concentration of extracted green-inhibitors correspondingly, the efficiency of the examined green-inhibitors, in percentage on carbon steel in various aqueous environments [3,4,7,8].

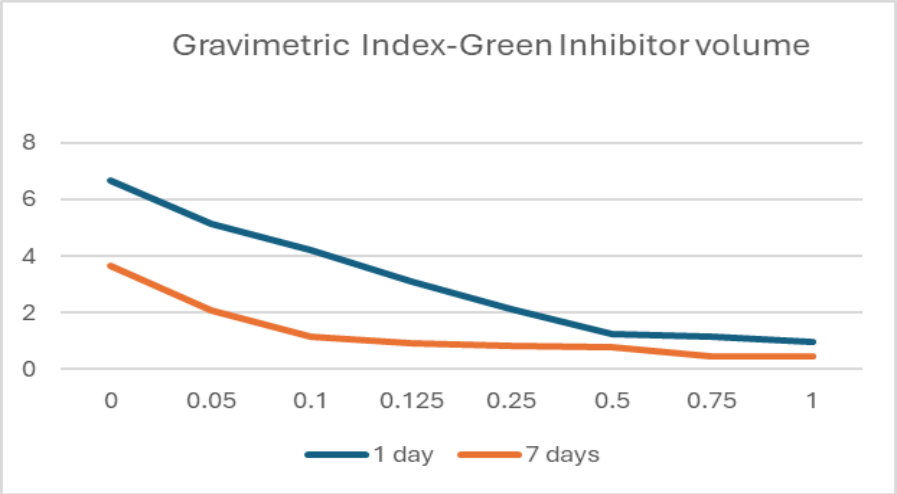

Figure 2. Variation of the gravimetric index depending on the volume of green-inhibitor and time.

Table 1. .Comparative green-inhibitor extracts

No.	Plant	Concentration (10^3 g/l)	Inhibition Efficiency, %
1	*Galium verum*	0.75	88.97
2	*Lavandula Stoechas*	2	61.97
3	*Calendula officinalis*	1.25	92.31
4	*Hypericum perforatum*	2.66	39.50
5	*Urtica dioica*	-	92.24

CONCLUSIONS

The plant extract showed promise as a corrosion inhibitor for the selected metal system carbon steel/1 N HCl solution. The higher the eco-inhibitor concentration, the lower the corrosion rate (gravimetric index), the rates are in the range 2.1 - 10.5 g/m2h, meaning that the metal is somewhat resistant to corrosion. Even after 7 days of exposure, carbon steel is in the same category, with the mention that the values are still lower. Either the use of a higher concentration of eco-inhibitor or longer exposure to determine if corrosion resistance increases over time is required as solutions. These results provide a promising perspective for the further development of these green eco-inhibitors and their application in industry to protect metallic materials against corrosion in a sustainable and environmentally friendly way. The research has been funded by the University of Oradea, within the Grants Competition "Scientific Research of Excellence Related to Priority Areas with Capitalization through Technology Transfer: INO - TRANSFER - UO", Project No. 327/2021.

REFERENCES

1. Rajni Ratti, Industrial applications of green chemistry: Status, Challenges and Prospects, SN Applied Sciences (2020) 2:263 | https://doi.org/10.1007/s42452-020-2019-6

2. Bensouda, Z., El Assiri, E.H., Sfaira, M. et al. Extraction, Characterization and Anticorrosion Potential of an Essential Oil from Orange Zest as Eco-friendly Inhibitor for Mild Steel in Acidic Solution. J Bio Tribo Corros 5, 84 (2019). https://doi.org/10.1007/s40735-019-0276-y

3. Mohammed A. El-Hashemy, Amal Sallam, The inhibitive action of Calendula officinalis flower heads extract for mild steel corrosion in 1 M HCl solution, Journal of Materials Research and Technology, Volume 9, Issue 6, 2020, Pages 13509-13523.

4. Mohammad Ramezanzadeh, Ghasem Bahlakeh, Zahra Sanaei, Bahram Ramezanzadeh, Studying the Urtica dioica leaves extract inhibition effect on the mild steel corrosion in 1 M HCl solution: Complementary experimental, ab initio quantum mechanics, Monte Carlo and molecular dynamics studies, Journal of Molecular Liquids, Volume 272, 2018, Pages 120-136.

5. Badea, G. E.; Dzitac, S.; Marin, L.; Petrehele, A. I. G.; Porumb, C.; Badea, P.G.; An Investigation On The Electrochemical Behavior Of Steel In The Presence Of An Eco-inhibitor. 17th International Conference on Engineering of Modern Electric Systems (EMES), Oradea, Romania, 2023, 1, doi: 10.1109/EMES58375.2023.10171763.

6. G.E. Badea, O. D. Stănăşel, S. Bota, S. Dziţac, C. Ionaș, P.G.Badea, C.M.Stănăşel, Database With Eco-Inhibitors Of Corrosion For Carbon Steel In Acid Environment, Analele Universităţii Din Oradea - Fascicula Chimie, AUOFC Vol.XXVIII(29) , 2022, Pg.23-26,

7. N. Belarbi, F. Dergal, I. Chikhi, S.Merah, D. Lerari, K.Bachari, „Study of anti-corrosion activity of Algerian L. stoechas oil on C38 carbon steel in 1 M HCl medium" Int J Ind Chem 9, 115–125, 2018.

8. Buğra Karahan, Alper Turhan, Iskender Ince, Hakan Ekinci, Aylin Albayrak, Ahmet Cakir, Inhibition of EN 10204 Steel in by a Mixture of Hypericum Perforatum Plant Extract and Nitrite Based Inorganic Inhibitors, Conference: The European Corrosion Congress 2011, Stockholm.

9. Rani BEA, Basu BBJ (2012) Green inhibitors for corrosion protection of metals and alloys: an overview. Int J Corros 2012:1–15

10. Popoola LT (2019) Organic green corrosion inhibitors (OGCIs): a critical review. Corros Rev 37:71–102

11. El Ibrahimi B, Jmiai A, Bazzi L, El Issami S (2020) Amino acids and their derivatives as corrosion inhibitors for metals and alloys. Arab J Chem 13:740–771

12. Marzorati S, Verotta L, Trasatti SP (2019) Green corrosion inhibitors from natural sources and biomass wastes. Molecules 24:48

13. Montemor MF (2016) Fostering green inhibitors for corrosion prevention. In: Hughes A, Mol J, Zheludkevich M, Buchheit R (eds) Active protective coatings. Springer Ser Mater Sci, vol 233. Springer, Dordrecht.

14. A.Cojocaru, I.Maior, D.I.Vaireanu, Lingvay C, Lingvay I, S.Caprarescu, G.E. Badea, Ethanol extract of fagus sylvatica leavesas an eco-friendly inhibitor for carbon steel corrosion in acidic solutions, Journal of Sustainable energy nr.1.vol 3, pg.66-71, 2010

15. Afia, L., Salghi, R., Bazzi, L., Errami, M., Jbara, O., Al-Deyab, S. S., and Hammouti, B. Testing Natural, compounds: Argania spinosa Kernels,Extract and Cosmetic Oil as Ecofriendly Inhibitors for Steel Corrosion in 1 M HCl. Int. J. Electrochem. Sci., 2011, 6:5918-5939.

16. A.li, I. Inhibitory Effect of Leaf Extract of Khaya senegalensis (Mahogany) on C-steel Corrosion in 1.0 M Hydrochloric Acid Solution. Int. J. Electrochem. Sci., 2016, 11: 2130-2141.

17. Pinky Sisodia, S. Khalid Hasan, Corrosion Inhibition of Mild Steel by Phytochemicals in Acidic Media, Journal of Corrosion Science and Engineering, 16, 2013.

18. Prabakaran, M., Hemapriya, V., Kim, SH. et al. Evaluation of Antioxidant and Anticorrosion Properties of Epipremnum aureum. Arab J Sci Eng 44, 169–178 (2019).

19. Zhao, B., Han, W., Zhang, W. et al. Corrosion inhibition performance of tannins for mild steel in hydrochloric acid solution. Res Chem Intermed 44, 407–423 (2018).

20. Belarbi, N., Dergal, F., Chikhi, I. et al. Study of anti-corrosion activity of Algerian L. stoechas oil on C38 carbon steel in 1 M HCl medium. Int J Ind Chem 9, 115–125 (2018).

21. Oguzie EE (2008) Evaluation of the inhibitive effect of some plant extracts on the acid corrosion of mild steel. Corrosion Science 50(11):2993–2998.

22. Desai PS (2015) Hibiscus rosa-sinensis (Jasud) leaves extracts used as corrosion inhibitors for mild steel in hydrochloric acid. E-jpmr 2(1):470–485

IDENTIFICATION AND SEPARATION OF SOME AMINO ACIDS FROM PLANTS THROUGH TLC

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Abstract:

Amino acids are compounds with mixed function (compounds that have different functional groups in their molecule) that contain -NH_2 and -COOH groups in their molecules.

Amino acids are named using the word "acid" followed by "amino" and the name of the corresponding acid. The prefixes "di", "tri", etc. indicate the number of amino and carboxyl groups. Essential amino acids reach the body through food and contribute to the good functioning of the entire body. Amino acids are the building blocks of proteins, indispensable elements in the diet of any human being. The lack of a single amino acid is felt in general health. Currently, a number of approximately twenty-two amino acids are known. Of these, eight are essential, they cannot be synthesized by the body and, therefore, must be taken from food. Amino acids help us to reduce the level of stress, to sleep well, to be energetic and to fight the manifestations of diseases. They are, in fact, the most important nutrients in the body.

Key words: Amino acids ,-NH_2 and -COOH groups , good functioning, twenty-two amino acids, important nutrients, level of stress.

1. INTRODUCTION

Amino acids are compounds with mixed function (compounds that have different functional groups in their molecule) that contain -NH_2 and -COOH groups in their molecule. [1-]

The general formula of amino acids is: [6]

$$R-\underset{\underset{NH_2}{|}}{CH}-\ COOH$$

Amino acids are named using the word "acid" followed by "amino" and the name of the corresponding acid.

Essential amino acids reach the body through food and contribute to the good functioning of the whole body. Amino acids are the building blocks of proteins, indispensable elements in the diet of any human being. The lack of a single amino acid is felt in general health. Currently, a number of approximately twenty-two amino acids are known. Of these, eight are essential, they cannot be synthesized by the body and, therefore, must be taken from food.[2-3]

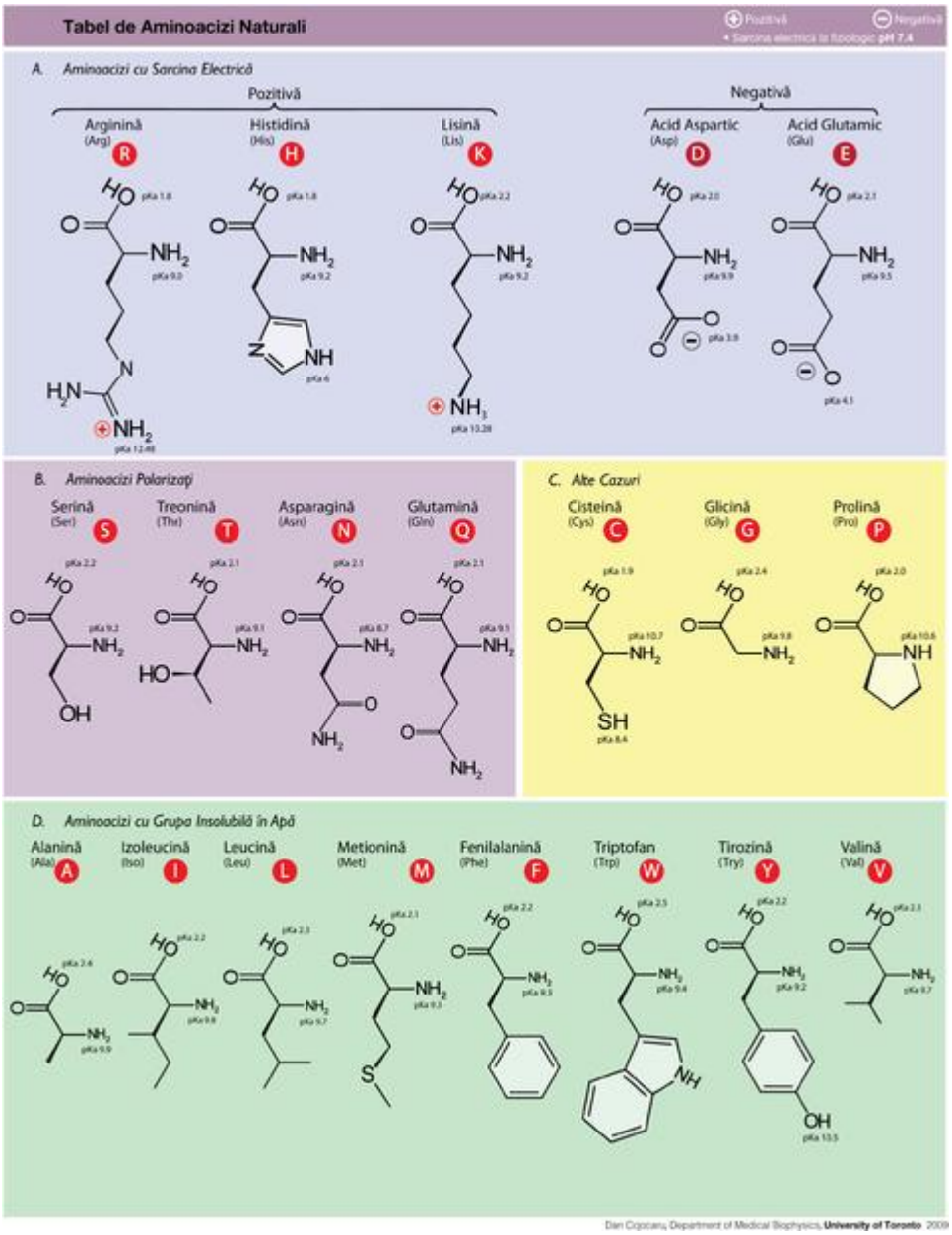

Fig. 1. Some natural amino acids [1]

The 8 amino acids that cannot be synthesized are called essential or "indispensable". The term "essential" is applied in nutrition to any nutritional component that cannot be produced in the body, and, consequently, must not be missing from the diet. it refers not only to amino acids, but also to other substances: fats (omega 3, omega 6), vitamins.[4]

Essential amino acids are particularly important during growth (especially in infants). The 8 essential amino acids are: Isoleucine (Ile), Leucine (Le), Lysine (Lys), Methionine (Met), Phenylalanine (Phe), Threonine (Thr), Tryptophan (Trp) and Valine (Val). Two semi-essential amino acids are added to them, but which often appear alongside essential amino acids in the official terminology: cysteine (Cis) and tyrosine (Tyr). Some specialists include histidine (His) on the list of essential amino acids, but this is indispensable only in infants, adults being able to synthesize it. Essential amino acids are necessary for the synthesis of important biological substances in the body: thyroid hormones, catecholamines (phenylalanine /tyrosine), serotonin (tryptophan), histamine (histidine), etc. Amino acids help us to reduce the level of stress, to sleep well, to be energetic and to fight the manifestations of diseases. They are, in fact, the most important nutrients in the body. Here are these pillars of life and from which foods we can procure them[4-5].

Valine - its deficiency in the diet causes a decrease in food consumption and movement coordination disorders;

Lysine - is part of tryptophan and methionine. Insufficiency of lysine in the diet has the consequence of blood circulation disorder, reduction of the number of erythrocytes in the blood and hemoglobin content, causes muscle fatigue, disorders in bone calcification, headache, vertigo, nausea, vomiting, anemia;

Methionine - normalizes the metabolism of lipids and phospholipids in the liver and is recommended for the prevention and treatment of atherosclerosis. Methionine is necessary for the functioning of the adrenal glands and for the synthesis of adrenaline;

Threonine - in its absence, body mass decreases and growth slows down;

Tryptophan - participates in the synthesis of albumin and globulin and the maintenance of nitrogen balance, in the synthesis of serum proteins and hemoglobin, nicotinic acid and plays an important role in the prevention of pellagra;

Phenylalanine - participates in normalizing the function of the thyroid gland and adrenal glands;

Histidine - participates in the synthesis of hemoglobin. The decarboxylation of histidine contributes to the formation of

histamine, which dilates the vessels, increases the permeability of their walls;
Leucine - contributes to the normalization of nitrogen balance, protein and carbohydrate metabolism;
Isoleucine - is part of the body's proteins. The lack of isoleucine in the food ration causes a negative nitrogen balance;[3,5]

The sources of essential amino acids are dietary proteins. High biological value or complete proteins contain all the essential amino acids (for example, milk proteins, egg), while incomplete proteins (vegetable proteins, except soy protein, which is a complete one) are missing one or more essential amino acids.[3]

2. EXPERIMENTAL

The identification and separation of some amino acids from linden flowers (*Tilia Europea*) by thin layer chromatography (TLC), using two elution systems, was studied.

The main work stages were:

a) extraction

b) separation and identification of amino acids

After picking the plant, it was left to dry for several days in the laboratory, at room temperature, (the temperature must be constant , between 20-22 ^{o} C), until it reaches about 60 – 65 % of the initial mass. In order to be able to extract the amino acids from the analysed plant, the dry leaves were placed in a mortar and with the help of a pestle we mixed until a fine powder was formed. In order to extract the free amino acids from the dry plant, 5 g of it were used and macerated with 50 ml of 80% methyl alcohol.The proteins contained in the extract were removed by precipitation with a 25 % aqueous solution of potassium phosphotungstate, followed by centrifugation. The obtained extracts are used for carrying out identification reactions and for the separation of amino acids compounds on a thin layer of cellulose.

3. RESULTS AND DISCUSSIONS

The clear solution, the result of centrifugation ,contains the following amino acids: leucine, histidine and valine. After centrifugation, the solution containing the free amino acids passes over a column of ion exchange resin (Amberlite R 120). The column is eluted with 40 ml of 10% ammonia solution. The obtained solution is evaporated to dryness on a water bath. For subsequent chromatography operations, the residue is taken up again with 1 ml of 30% isopropanol.

In order to separate and identify the free amino acids present in the studied plant, 2,5 % standard solutions in water were prepared from the amino acid standards listed above.

The separation and identification of amino acids was done by two-dimensional thin layer chromatography and microcrystalline cellulose plates were used.

The preparation of the cellulose plates is done as follows: 10 g of microcrystalline cellulose powder (Merck) is weighed on a pharmaceutical scale, to which 30 ml of water and 5 ml of methyl alcohol are added. If the cellulose suspension is prepared only in water, after drying its adherent layer on the inert glass support cracks, which prevents the migration of the mobile phase system by interrupting the capillaries formed along the stationary phase. The formed suspension is subjected to homogenization using a magnetic stirrer for 10 minutes in a warm place. The resulting homogenous paste is spread with the help of a thin layer spreader, on 20 x 20 cm plates, which were previously degreased to allow the stationary phase to adhere to the inert support. The plates thus obtained are left to dry at room temperature, on a perfectly flat surface, for 24 hours.

The thickness of the stationary phase layer (microcrystalline cellulose) was adjusted to 0.3 mm. At a distance of 3 cm from one of the ends of the plate, samples from the plant extracts to be analyzed can be applied with a micro syringe. For linden flowers , the mixture of solvents was used as the mobile phase: butanol: acetone: acetic acid: water = 35:35:7:23 (volumes). This mixture is homogenized well, by stirring, and left at room temperature for 24 hours, to separate the phases. The mixture is made directly in the separation funnel, and after 24 hours the two distinct phases are separated. For elution, the organic phase obviously saturated with water is used. An optimization of amino acid separation by two-dimensional TLC was attempted. For elution in the first direction, the system butanol : acetone : acetic acid : water = 35 : 35 : 7 : 23 was used. After drying the plates, the elution was done in the second direction using the mixture of solvents: methanol : water : pyridine = 80 : 20 : 5. The elution was done in the direction perpendicular to the first. Both elutions were done twice.

For visualization, a ninhydrin solution was used, obtained by dissolving 3.6 g of ninhydrin in 50 ml of butanol:acetone mixture (50:50 v/v). The ninhydrin solution was sprayed with the help of a special sprayer, homogeneously over the entire surface of the plate. In both situations, after elution, the plates are left at room temperature for a few hours, after which they are introduced for 20 minutes at 100^0 C. On the white background of the support, the distinct pink-reddish areas of the amino acids present in the analyzed sample appear. For identification, parallel to the plate on which the plant extract (linden flowers) was applied, two plates were eluted on which standard solutions of amino acids (1%) were pipetted, one in the first eluent and the other in the second eluent for determination of the R_f. By comparing

the R_f values in the two elution systems and the colors of the spots, the amino acids present were identified.

Tab. 1. The R_f values of the main amino acids in linden flowers using the first elution system

Nr.	Amino acid	R_f
1	leucină	0.484
2	valină	0.678
3	histidină	0.345

Tab. 2. The R_f values of the main amino acids using the second elution system

Nr.	Amino acid	R_f
1	leucina	0,362
2	valina	0,581
3	histidina	0,476

4. CONCLUSIONS

All the free amino acids present in the plant were identified and separated, with the help of thin layer chromatography,using two elution systems, a plant with multiple uses in pharmacology.

From the chromatogram it can be seen that for each amino acid present in the extract, the peak corresponding to it increased proportionally with the contribution of the concentration of each one in the extract.

Some components appear more intensely colored, others less intensely, related to the sensitivity of the reaction of the given component with the reagent used.

It has been proven, once again, that this method is very advantageous both from a financial point of view (low costs) and from a working time point of view.

5.REFERENCES

[1]. Hou Y, Wu G. Nutritionally Essential Amino Acids. Adv Nutr. 2018 Nov 01;

[2]. Reeds PJ. Dispensable and indispensable amino acids for humans. J Nutr. 2000 Jul;

[3]. https://www.medicalnewstoday.com/articles/324229#types

[4]. https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/amino-acids

[5]. Kubala Jillian. “Essential Amino Acids: Definition, Benefits, and Food Sources.” *Healthline*, 2022,

[6]. Cassata, Cathy. “What Are Amino Acids?.”, *EveryDay Health*, 2016, https://www.everydayhealth.com/amino-acids/guide/

STUDIES ON THE PHYSICO-CHEMICAL PROPERTIES OF SOILS FOR CONSTRUCTION

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Abstract: *Soil analysis is very important in the field of construction. As a result, laboratory determinations play an important role in engineering calculations. In this work, the analysis of different soil samples, from different depths, was studied. Granulometric analysis, determination of humidity, free swelling and content of organic matter (humus) were carried out.*
Keywords: soil, particle size fractions, variation of grain sizes with depth

INTRODUCTION

Earth, as far as its solid phase is concerned, is made up of particles of different sizes. One of the criteria for evaluating the type of soil is the size of the particles of which it is composed.

However, just knowing that there are particles of a certain size in a certain type of soil is not enough, being also necessary to determine the proportion of particles of different sizes that occur. The ratio of soil particles of different sizes varies from soil to soil and from layer to layer, determining the grain size composition and texture of the soil. The main particle size fractions are sand, dust and clay. A common method of size characterization is known as texture fractionation or grain size fractions. According to this argument, you can distinguish: sand (2 - 0.02 mm), dust (0.02 - 0.002 mm) and clay (less than 0.002 mm).

Clay is the granulometric fraction that plays a major role in determining a significant number of physical and chemical characteristics of the soil, especially due to its small particle size, the very high number of particles per unit volume and the very large specific surface area, which gives it the character of an active part of the soil matrix[1,8]. Clay presents the organic part of the soil and properties such as: absorption of water and exchangeable cations, adhesion, plasticity, contraction and swelling, heat of wetting, cohesion and the ability to form structural elements, through the aggregation of elementary soil particles.

Sand has completely opposite properties to clay. The basic particle sizes are comparatively larger, the number of particles per unit volume of soil is small, and the specific surface area is very small. This particle size fraction gives to the soil good permeability and aeration properties,

reduces water and nutrient retention, heat of wetting, cohesion, adhesion, swelling and shrinkage. The sand fraction can be divided into three sub-fractions: large, medium or fine sand[2,3,7]. The I.C.P.A system recognizes six textural classes of fine soil material, divided into 23 subclasses[13].

The granulometric composition represents the ratio in which the different particle size fractions are found expressed in mass units, relative to the total mass of a volume of earth. The laboratory method used to determine the granularity of a soil differs depending on the size of the particles. It can be applied: sieve sieving method for granules with a diameter greater than 2 mm, the sieving method for granules with diameters between 2 and 0.05 mm or the sedimentation method for granules with diameters smaller than 0.05 mm.

Sieving is considered complete if, when shaking each sieve with different mesh sizes over a piece of paper, the amount of the analyzed sample that passes through the sieve or screen in one minute does not represent more than 1% of the sieved material. The granular fractions remaining on each sieve, screen, or in the box are weighed. If the total sum of the masses of the granular fractions, including the remainder in the box, is more than 1% different from the total mass of the initial analyzed sample, the determination must be repeated. If the remainder left in the box is greater than 10% of the total mass of the initial analyzed sample, the determination must be supplemented with granulometric analysis using the sedimentation method. Moisture content is of great importance, as it influences the consistency state, and consequently, the resistance of the soil when considering construction loads. Soils with high swelling and shrinkage are those containing a large amount of clay. They have the property of significantly changing their volume when moisture changes occur. Soils with high swelling and shrinkage can be more or less active, depending on:

the percentage of clay with a diameter smaller than 0.002 mm, plasticity index, activity index, plasticity criterion, free swelling, shrinkage limit, volumetric shrinkage, maximum wetting heat, moisture content corresponding to the 15-bar section and swelling pressure[4,5,6].

In the case of soils with organic matter content, the consolidation process is very slow and non-uniform. Depending on the organic matter content, the following types of soils are distinguished: silts (with organic matter content < 5%), sludges (5 - 10%), peaty soils (10 - 60%)

and peat (> 60%)[6]. When characterizing the organic matter content present in soil, the percentage content relative to the mineral skeleton and the degree of decomposition are taken into account[13].

MATERIALS AND METHODS

Laboratory soil analysis has direct applications in the field of construction. The values obtained from these analyses have a significant influence on the foundation ground.

In this study, the analysis of various soil samples from different depths was undertaken. Granulometric analysis, moisture determination, free swelling, and organic matter (humus) content determination were performed. In Table 1 are presented the soil samples from different depths.

1.Granulometric analysis[11]

Due to the sedimentation over time of an initially homogeneous aqueous suspension, a variation in its density occurs, which is proportional to the sedimentation rate of the granules, depending on the size and density of the granules. The density of the suspension over time is measured with an aerometer, Casagrande type, calibrated at 20°C, with a reading range of 0.995-1.030, with a precision of 0.0005, where the position of the center of buoyancy depends on the density of the granules in suspension that have not yet settled and whose percentage mass depends on the density of the suspension at that time and the density of the granules.

Table 1. Soil samples

Sample no.	Depths, [m]
1	-0,50
2	-1,40
3	-2,40
4	-0,70
5	-1,60
6	-2,80

The aerometer readings are taken at the following cumulative time intervals from the start of the determination: 30 sec, 1 min, 2 min, 4 min, 8 min, 15 min, 30 min, 1 h, 2 h, 4 h, 16 h.

2.Moisture content determination

The determination of moisture content was carried out according to the methodology[10]. Moisture content was obtained as the mass of water lost by a soil sample when dried at 105 ± 2°C, relative to its dry mass. The result is expressed as a percentage.

3. Free swelling

The determination of free swelling was performed according to the methodology[12]. The method consists of determining the volume of sediment resulting from depositing, a previously

dried and ground soil sample with an initial volume of 10 ml, in distilled water.

4. Identification of the content of humus soluble in alkalis

The method consists of dissolving the humus from the soil sample in a sodium hydroxide solution, identifying the presence of organic matter. From the dry sample, approximately 10 grams of soil are weighed and introduced into a 100 ml graduated cylinder. In the cylinder, 50 ml of a 5% NaOH solution is added and allowed to stand for 1-2 hours. Then the cylinder is shaken well and filled with 5% NaOH solution up to 100 ml. The cylinder is shaken again and allowed to stand for 24 hours. After that, the color of the liquid layer above the sediment is checked[9].

RESULTS AND DISCUSSIONS

1.Results of granulometric analysis

Based on the obtained data, the diameters of the granules and the percentage content of granules are determined. The effective densities read on the aerometer are recorded as reduced densities R (Table 2). The amount of material retained on the sieve after washing the samples, in grams, for each soil sample was: 20, 23, 19, 13, 10, respectively 11. Based on the read values presented in Table 2 and calculations according to methods[11], the percentage content of granulometric fractions (Table 3) and granulometric curves were performed (Figure 1). In the granulometric curves you have the diameter of the sieve opening against the percent of passages through the sieve.

Table 2. Values read on the aerometer as reduced densities R

Sample no.	Reduced densities R at different time intervals										
	30 s	1 min	2 min	4 min	8 min	15 min	30 min	1 h	2 h	4 h	16 h
1	14.0	13.5	13.0	12.0	11.0	9.0	7.5	6.0	5.0	4.0	3.0
2	13.0	12.5	12.0	11.5	10.5	9.5	8.5	75	6.5	5.5	4.0
3	13.0	12.5	12.0	11.0	9.5	8.0	6.5	5.0	4.0	3.0	2.0
4	18.5	18.0	16.5	14.5	12.0	10.0	8.0	6.5	5.0	3.5	2.0
5	20.5	20.0	17.5	14.0	11.0	9.0	7.0	5.5	4.0	3.0	2.0
6	21.0	20.5	20.0	17.0	13.5	10.5	8.5	7.0	5.5	4.0	3.0

Table 3. Granulometry of the analyzed samples

Sample no.	Clay (Cl), %	Dust (Si), %	Sand (Sa), %	Soil name
1	7.1	33.7	59.2	Silty sand [siSa]
2	10.3	27.3	62.4	Silty sand [siSa]
3	3.9	33.7	62.4	Silty sand [siSa]
4	3.9	51.4	44.7	Sandy silt [saSi]
5	3.9	57.8	38.3	Sandy silt [saSi]
6	7.1	56.2	36.7	Sandy silt [saSi]

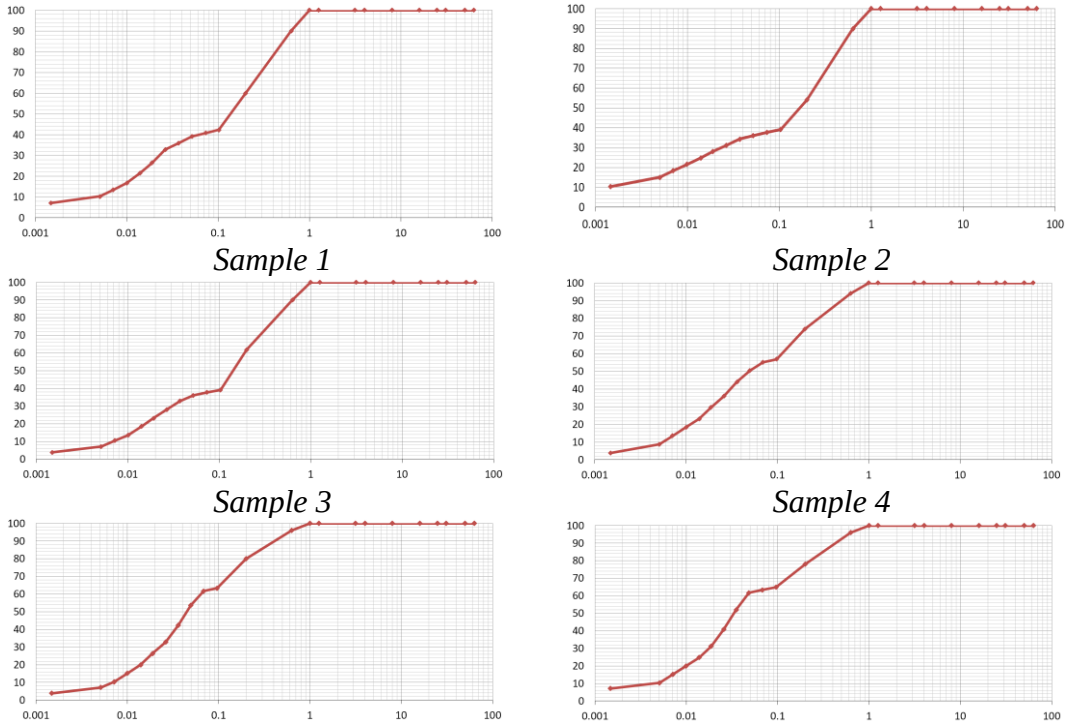

Figure 1. Granulometric curves

Based on the data in Tables 1 and 3, the variation of grain sizes is observed depending on the depth from which the samples were taken (Figure 3). The clay granulometric fraction is present in a small amount in each analyzed sample. At depths of -0.50 m, -1.40 m, and -2.40 m, the dominant granulometric fraction is sand; however, at depths of -0.70 m, -1.60 m, and -2.40 m, an increase in the percentage of silt can be observed.

2.Moisture content results

Based on the weighed values illustrated in Table 4, the moisture contents (w %) of the samples was calculated according to methodology[10], (Table 5).

$$w = \frac{m_w - m_d}{m_d - m_c} \cdot 100, \quad [\%]$$

where:

- m_w –mass of wet material + container tare, in grams;
- m_d –mass of dry material + container tare, in grams;
- m_c – container tare, in grams[10].

Table 4. Values of m_w, m_d, m_c of the analysed samples

Sample no.	m_w [g]	m_d [g]	m_c [g]
1	361.5	347.5	261.5
2	345.0	330.3	245.0
3	328.1	311.5	228.1
4	336.3	318.0	236.3
5	369.1	355.0	269.1
6	374.9	359.0	274.9

Based on the data in Table 5, the variation of the moisture content of the analyzed samples is observed. At a depth of -0.50 m, the soil has a relatively low moisture content; however, at a depth of -0.70 m, a significant increase can be observed (Figure 2).

Table 5. The moisture content of the samples, in %

Sample no.	*w* %
1	16.3
2	17.2
3	19.9
4	22.4
5	16.4
6	18.9

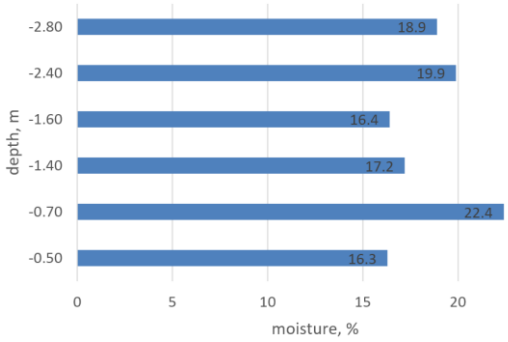

Figure 2. Variation of moisture content at different depths

At depths of -1.40 m and -1.60 m, the moisture content decreases compared to the moisture determined at a depth of -0.70 m. At depths of -2.40 m and -2.80 m, the moisture contents of the samples are relatively similar.

3. Free swelling results

Free swelling U_L is calculated using the equation:

$$U_L = \frac{V_f - V_i}{V_i} \cdot 100, \quad [\%]$$

the results being summarized in Table 7, where:

V_f – final volume of the sediment, in ml (Table 6);

V_i – initial volume of the soil, equal to 10 ml[12].

By data from Tables 1 and 7 you may notice the variation of the free swell of the analyzed samples. An increase in swelling values is observed from a depth of -0.50 m to a depth of -1.40 m. From a depth of -1.60 m to a depth of -2.80 m, the free swell decreases significantly.

Table 6. Initial and final volumes of the analysed samples

Sample no.	Initial volume, [ml]	Final volume, [ml]
1	10	13.5
2	10	14.0
3	10	14.5
4	10	13.5
5	10	13.0
6	10	12.5

Table 7. The free swelling of the analyzed samples.

Sample no.	U_L %
1	35
2	40
3	45
4	35
5	30
6	25

4. Results of the content of humus soluble in alkalis

The colors of the decanted layers correlated to the humus content in the case of the analysed samples are illustrated in Table 8.

Based on the data in Tables 1 and 8, the variation of organic matter content is observed. At a depth of -0.50 m, the amount of organic matter is between 2% and 5%, while at a depth of -0.70 m it is over 5%. With increasing depths, it can be observed that the organic matter content decreases. At a depth of -2.40 m, the humus content is below 1%, which represents a very small amount, and at a depth of -2.80 m it is between 1% and 2%, which also does not influence engineering calculations in the field of construction.

Table 8. The color of the decanted layer and the humus content

Sample no.	Color of the liquid	Humus content, %
1	yellow to	2…5
2	faintly	1…2
3	colorless	0…1
4	dark brown	> 5
5	yellow to	2…5
6	colorless	1…2

CONCLUSIONS

Various soil analyses, such as granulometric analysis, moisture determination, free swelling assessment, and identification of organic matter presence, play a significant role in the preliminary phases of construction projects. This study aimed to investigate the variation of certain physico-chemical properties of the soil with depth.

Granulometric analysis was conducted using the sedimentation method, which identified the percentage composition of granulometric fractions at different depths and also provided precise nomenclature for the respective soil types. Subsequently, secondary determinations were performed on the

identified soils: moisture determination revealed an irregular variation with depth. causes of these moisture levels. It is a variable characterized by constant fluctuation. Moisture can vary for several reasons, including the presence of the groundwater table, uncontrolled infiltration of meteoric water, and broken pipes near the sampling area, as observed in sample 4 at a depth of -0.70 m.

The next secondary determination was the analysis of free swelling. This parameter varies according to soil type. In this study, it was observed that soil swelling exhibits a linear increase up to a depth of -1.40 m, followed by a linear decrease from -1.60 m to -2.80 m.

This parameter does not allow for a definitive conclusion regarding the The final secondary determination in this work was the identification of organic matter present in the soil at various depths. Organic matter is generally found in the upper layers, which is corroborated by the results obtained. Due to plant residues and human contributions, a pronounced percentage of organic matter is observed up to a depth of -0.70 m. Up to this depth, organic materials can penetrate the soil due to human activities and natural phenomena. Below an approximate depth of -0.70 m, the content of organic matter decreases significantly.

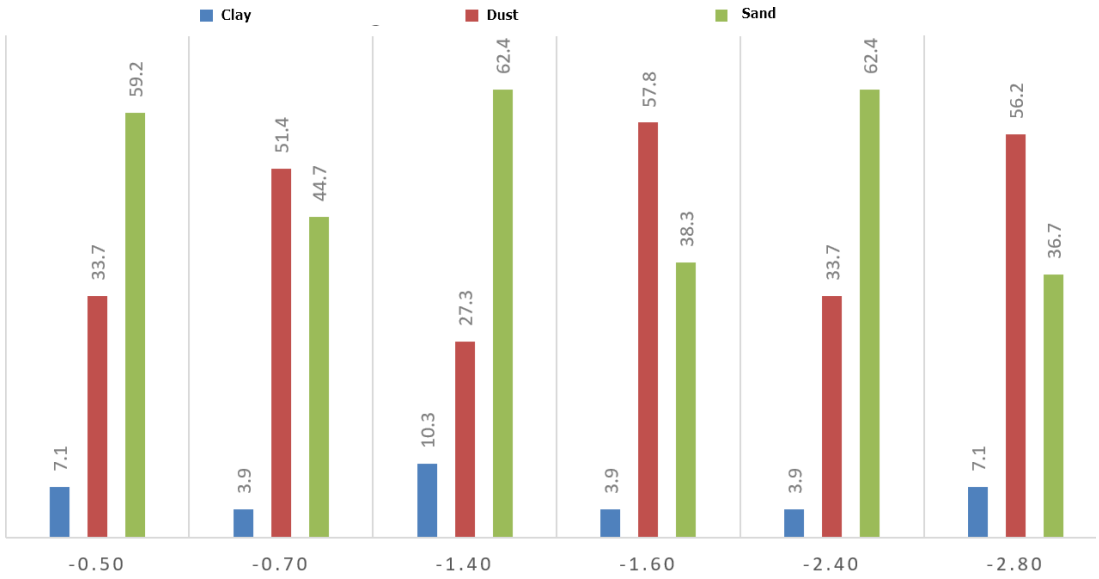

Figure 3. The variation of grain sizes determined according to depth

REFERENCES

[1] BĂNCILĂ I., *Geologie inginerească*, București, Editura Tehnică București, 1980.

[2] BEJAN F., *Fizica mediilor poroase*, https://e.uoradea.ro/, 2018.

[3] BERCHEZ O., BERCHEZ D., *Metode fizice și chimice pentru cercetarea solului*, Editura Universității din Oradea, 2020.

[4] BERCHEZ O., HODIŞAN M. F. *Geneza solurilor*, Editura Universităţii din Oradea, 2021.

[5] BUCUR N., LIXANDRU Gh., *Principii fundamentale de Știința solului*, Iași, Editura Dosoftei, 1997.

[6] MUNTEANU I., FLOREA I., *Ghid pentru descrierea în teren a profilului de sol și a condițiilor de mediu specifice*. ICPA București, MAPDR, Craiova, Editura Sitech, 2009.

[7] OBREJANU Gr., *Metode de cercetare a solului*, București, Editura Academiei Române, 1964.

[8] VASILE D., POPESCU C., *Pedologie*, Editura Universitaria, Craiova, 2003.

[9] STAS 7107/1-76 - Teren de fundare. Determinarea materiilor organice. Identificarea humusului solubil în alcalii. Determinarea conținutului de materie organică vegetala recentă.

[10] STAS 1913/1-82 - Teren de fundare. Determinarea umidității. Metoda de laborator. Metoda de șantier.

[11] STAS 1913/5-85 - Teren de fundare. Determinarea granulozității. Metoda sedimentării și metoda cernerii.

[12] STAS 1913/12-88 - Teren de fundare. Determinarea caracteristicilor fizice și mecanice ale pământurilor cu umflări și contracții mari. Determinarea umflării libere.

[13] *Sistemul Român de Clasificare a solurilor*, I.C.P.A București, 1980.

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STUDY OF SOME CHEMICAL AND BIOCHEMICAL PROPERTIES OF SEA BUCKTHORN FRUITS AND OIL

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Abstract: *In this paper we set out to determine some chemical and biochemical properties of sea buckthorn, using two sea buckthorn products, dried fruits and sea buckthorn essential oil. Both are products sold in our country and are prepared fresh, within the shelf life. Thus, we determined the acidity, vitamin C, iodine index, cation concentration and calcium ion concentration, comparatively, from dried fruits and sea buckthorn essential oil.*

Keywords: *Hippophaë rhamnoides L.*, aqueous extract, alcoholic extract, essential oil.

INTRODUCTION

Sea buckthorn is a medicinal and ornamental plant that belongs to the genus Hippophae of the Elaeagnaceae family. Sea buckthorn is also known as sea buckthorn, sea buckthorn. This plant is widespread in Europe, Asia and North America, preferring coastal areas and mountainous areas [1].

Sea buckthorn is a tall shrub with dense and thorny branches, narrow and silvery-green leaves. The fruits of sea buckthorn are small, spherical, reddish-orange in color and have a sour taste. These fruits are recognized for their rich content in vitamins (especially vitamin C), minerals, antioxidants, and essential fatty acids, such as omega-3, omega-6, and omega-7 [2, 3].

Due to its nutritional and therapeutic properties, sea buckthorn and products derived from it (sea buckthorn oil, sea buckthorn syrup, sea buckthorn tea) are used for medicinal, cosmetic and culinary purposes. Sea buckthorn can help strengthen the immune system, improve skin health, fight inflammation, and prevent some cardiovascular conditions [4, 5]. Sea buckthorn fruits (*Hippophae rhamnoides*) have a particularly rich nutritional content and offer many health benefits. They are an excellent source of essential vitamins and minerals, as well as powerful antioxidants, which help maintain

health. Sea buckthorn is one of the oldest remedies used in traditional and modern medicine. Sea buckthorn extracts are used in the treatment of certain diseases, such as diabetes, as well as to relieve symptoms of digestive disorders, such as irritable bowel syndrome or chronic constipation. Sea buckthorn may also help prevent and treat cardiovascular disease and maintain low cholesterol levels [6-8].

Sea buckthorn fruits are recognized as a significant source of bioactive compounds such as vitamins (C and E), carotenoids (α-carotene, lycopene, lutein and zeaxanthin), flavonoids (isoramnetin, quercetin, kampferol), organic acids, amino acids, as well as micro and macronutrients [9]. The bioactive composition of the fruits varies depending on the degree of maturity, size, species and geographical region [10, 11].

Due to its high content of vitamins, carotenoids and flavonoids, this miracle plant has been shown to act as a powerful antioxidant, protecting cells against oxidation, which aroused my interest in studying it in chemical laboratories. I chose to investigate the oily extract in sea buckthorn fruits (*Hippophae rhamnoides*) and its fruits, determining the concentration of calcium ions, vitamin C, which is a powerful antioxidant, and iodine index. This plant has gained global recognition due to its many biological benefits, especially for skin care [12, 13].

In conclusion, sea buckthorn is a valuable shrub due to its nutritional properties, and regular consumption of its fruits can provide numerous health benefits. In the form of an extract, sea buckthorn can also be found in various dietary supplements.

I. Description of sea buckthorn fruits

I.1 Sea buckthorn (*Hippophae rhamnoides*)

Sea buckthorn is a remarkably hardy species to drought and cold, with few claims on soil. It develops optimally on gravel, sand, unfertilized soils, as well as on steep slopes, which it stabilizes. It requires direct light to develop properly. Depending on the altitude, sea buckthorn is found from coastal to mountainous areas, with a preference for riverbeds in hilly areas, where the vitamin C content is higher.

I.1.1 Provenance and species

Baby buckthorn, scientifically known as *Hippophae rhamnoides* (syn. *Elaeagnus rhamnoides* L.), is a member of the family Elaeagnaceae. This flowering plant is native to cold temperate regions, between 27° and 69° latitude N and 7° W and 122° longitude E, having its origin in India.

Figure 1. *Sea buckthorn [14]*

I.1.2 Recognition characteristics

Sea buckthorn is a thorny shrub with simple leaves, covered with stellate hairs that give it a silvery hue. The flowers are hermaphroditic, greenish-yellow in color, and the fruits are bright orange, globular or ellipsoid, arranged in clusters. These fruits, which are false drupes, are distinguished by a very high content of vitamin C. General characteristics of the plant: It is a bushy shrub, with heights ranging from 1-3 (to 6) meters, highly branched, thorny, and with a general whitish tint due to the stellate hairs. The stem is branched, with dark brown bark, which turns into a furrowed rhytidom. The side spikes have strong thorns. The leaves are arranged alternately, they are narrow-lanceolate, with lengths of 1-6 cm, having only the obvious median vein. On the underside, the leaves are silvery-gray, with rusty scales. The dioecious unisexual flowers appear before the leaves, they are small and yellowish, grouped in globular inflorescences in male plants and more elongated in female ones. The fruits are false, ovoid, 7-8 mm, fleshy, orange, with a hard core, which remain on the branches over the winter. Flowering period: March-April.Raw material: *Fructus Hippophaë* - the fruits are harvested fresh, intact, globular in shape, either isolated or in groups of 2-3, having a yellow-orange color. The fruits have a characteristic faint odor and a sour, slightly bitter taste [1].

I.2. Sea buckthorn and the antioxidant effect

The term *Hippophae* comes from Latin, where "hippo" means horse, and "phaos" means flower or shine, which suggests in the literature the idea of "shining horse". The active ingredients in *Hippophae rhamnoides* have been used effectively in both the cosmetic industry and medicine. The valuable substances in sea buckthorn oil are responsible for maintaining the health and beauty of the skin, as well as for the proper functioning of the human body. With about 200 beneficial properties, *H. rhamnoides* oil is

a valuable additive in health and beauty products. Due to these qualities, *H. rhamnoides* has attracted attention for its use in cosmetics and medicine [15, 16].

I.3. Content of sea buckthorn fruits and its extracts

Although Sea buckthorn has been known for thousands of years for its healing properties, recent research has confirmed that sea buckthorn fruits are a rich source of vitamin C, vitamin A, vitamin B complex (B1, B2, B6 and B9), as well as vitamins E, K, P and F. In addition, Sea buckthorn contains microelements such as calcium, magnesium, phosphorus, potassium, iron, polyunsaturated fatty acids and carotenoids, including beta-carotene [15-17].

I.4. Activity of sea buckthorn fruits

Thanks to its composition rich in vitamin C, vitamin A, vitamin B complex (B1, B2, B6 and B9), as well as vitamins E, K, P and F, sea buckthorn offers numerous benefits for human health. In addition, it contains essential microelements such as which help maintain the health of the body.

Figure2. Sea buckthorn samples analyzed: dried sea buckthorn fruits and essential oil

calcium, magnesium, phosphorus, potassium, iron, polyunsaturated fatty acids and carotenoids, including beta-carotene. Scientific studies have shown that these components contribute to the treatment and prevention of several conditions, including cardiovascular diseases, cancer, gastrointestinal diseases. Sea buckthorn also has radioprotective, hepatoprotective, immunostimulating, anti-inflammatory effects and a strong antioxidant effect on the whole body [11-15].

MATERIALS AND METHODS

In this paper we set out to characterize two products used from sea buckthorn, dried fruits and sea buckthorn essential oil. Both samples are products sold in our country and are prepared fresh within the shelf life.

We chose these products because sea buckthorn is known for its rich properties in essential vitamins and minerals, as well as powerful antioxidants,

II.1. Determination of acidity

For the determination of acidity, the method of suspension in water was used. The studied sea buckthorn samples were titrated with a 0.1 N NaOH solution in the presence of the 1% phenolphthalein indicator, until the color of the extract from colorless to pink was observed.

For this determination, the samples were prepared differently.

In the case of dried sea buckthorn fruits, 10 g were weighed and extracted in 100 mL of distilled water. 2 drops of 1% phenolphthalein acid-base indicator were added and the sample was titrated with 0.1 N NaOH solution (F=0.9524), until the color of the solution changes to faint pink. The determination was repeated two more times and the average value of the results obtained was determined.

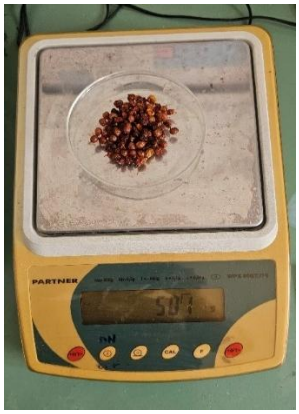


Figure 3. *Weighing of Dried Sea Buckthorn Fruit Solid Sample for Extract Preparation*

In the case of the dried sea buckthorn fruit sample, the acidity value was expressed in the number of NaOH mmoles required to neutralize a corresponding number of protons in a sample of 100 g sea buckthorn fruit and is calculated with the mathematical formula:

$$A\% = \frac{c_{NaOH} \cdot F_{NaOH} \cdot V_{NaOH} \cdot 100}{m_{probă}} \quad (1)$$

Where

- cNaOH is the normal concentration of the NaOH solution (0.1 N);
- FNaOH is the correction factor of the 0.1 N NaOH solution obtained by titration of a 0.1 N HCl sample (F=1.000)
- VNaOH is the volume of 0.1 N NaOH solution used in titration (mL);
- mprobe is the flour sample mass from which the extracts were prepared (g)

II.2. Determination of cation concentration

The method consists of complexing the metal cations present in the water, apart from the concentration of alkali metal ions, which we will express in the number of moles of EDTA consumed per 100 g of flour sample. The principle is based on the fact that EDTA (complexon III) reacts with metal cations in a molar ratio of 1:1.

The reagents used in this experiment are:

- complexon III, 0.01 M solution
- NH_4Cl/NH_3 pH 10 buffer solution
- black Eriochrome T solid NaCl marker
- 2N NaOH buffer solution
- Murexide Solid Indicator Moistened with NaCl

The determination of the concentration of calcium cations was done only in dried sea buckthorn fruits. The sample was prepared by dissolving 10 g of dried sea buckthorn fruits in 100 mL of

distilled water and they were left to be extracted for one hour. In each sample, 2 ml of pH 10 buffer solution, 0.1 g of black eriochrome T indicator were added and titrated with 0.01 M complexon III solution (F=1.0204) until the burgundy red color of the initial solution turned blue. For accuracy, three determinations were made. The calculation formula used to determine the number of cations mmoles in 100 g of sample is as follows:

Figure 4. *Samples of aqueous extract of dried sea buckthorn fruits before and after titration with EDTA 0.01M at pH 10*

$$Cations\ (mmols/100\ g\ sample) = \frac{c_{EDTA} \cdot V_{EDTA} \cdot F_{EDTA}}{m_{extract}} \cdot 100 = \frac{0{,}01 \cdot 1{,}0204 \cdot 100 \cdot V_{EDTA}}{10}$$

$$Cations\ (mmols/100\ g\ sample) = 0{,}10204 \cdot V_{EDTA}$$

In which

V_{EDTA} is the volume of 0.01 M EDTA used in titration (mL)

c_{EDTA} is the concentration of EDTA solution (0.01 M)

F_{EDTA} is the solution factor of EDTA 0.01 M

$m_{extract}$ is the flour mass from which the aqueous extract was prepared (10 g)

II.3. Determination of calcium ion concentration

The strict determination of the concentration of calcium ions by titration with EDTA (complexon III) is done at pH-12-13 in the presence of a murexide indicator, which when all calcium cations are complexed by EDTA interacts with the EDTA added in excess, with the change of color from pink to purple.

The reagents used in this experiment are:

• Complexion III 0.01 M (F=1.0204)

• NaOH 2 N

• Murexid indicator

Prepare the aqueous extract by adding 10 g of dried fruits to 100 mL of distilled water, left at room temperature for

one hour. In the Erlenmeyer glasses with the samples, add 2 ml NaOH 2 N solution and check the pH of the sample to be between 12-13. If the pH is lower, add 1 mL of NaOH 2N or more until the pH is adjusted. An additional 0.1 -0.2 g of murexide indicator shall be placed in the samples, so that the solution has a distinctive intense pink color, and titrated with 0.01 M complexon III solution until the color shift to purple. The experiment is repeated two more times.

Figure 5. *Sea buckthorn extract solutions before and after titration with 0.01 M EDTA in the presence of murexid for calcium determination*

The concentration of calcium ions expressed in mg Calcium per 100 g sample and in mmoli calcium per 100 g sample and is calculated with the formulas:

1. expressed in mg Ca/100 g sample

$$Calcium\ (mg/100\ g\ sample) = \frac{A_{Ca} \cdot c_{EDTA} \cdot V_{EDTA} \cdot F_{EDTA}}{m_{extract}} \cdot 100 = \frac{40{,}08 \cdot 0{,}01 \cdot 1{,}0204 \cdot 100 \cdot V_{EDTA}}{10}$$

$$Calcium\ (mg/100\ g\ sample) = 4{,}089 \cdot V_{EDTA}$$

2. expressed in mmoles Ca/100 g sample

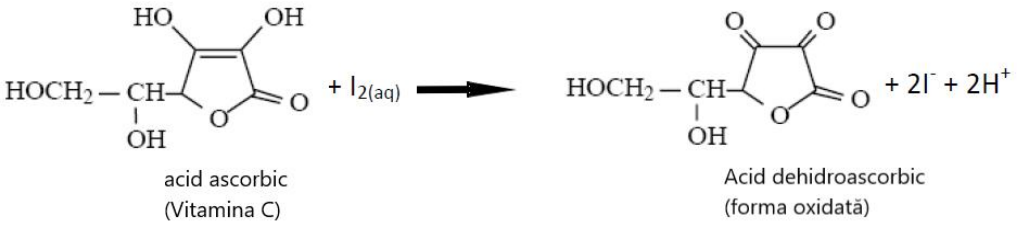
$$Calcium\ (mmols/100\ g\ sample) = \frac{c_{EDTA} \cdot V_{EDTA} \cdot F_{EDTA}}{m_{extract}} \cdot 100 = 0{,}10204 \cdot V_{EDTA}$$

where A_{Ca} is the atomic mass of calcium (40.08):

II.4. Quantitative determination of vitamin C

Vitamin C is determined by an indirect method, based on the oxidation of vitamin C with iodine resulting from a reaction between potassium iodide and potassium iodate in an acidic environment. When the entire amount of vitamin C has been oxidized, excess iodine will be formed, which will color the starch indicator blue.

The chemical reaction of oxidation of vitamin C with iodine is as follows:



acid ascorbic (Vitamina C)

Acid dehidroascorbic (forma oxidată)

The iodine required for the oxidation of vitamin C to dehydroascorbic acid results from the reaction between potassium iodate and potassium iodide in an acidic medium presented below:

$KIO_3 + 5KI + 6HCl \rightarrow 3\,I_2 + 6KCl + 3H_2O$

The reagents used are:

- 2% HCl solution,
-1% KI solution,
- 0.001 N KIO_3 solution,
- 1% starch solution

Working mode

In an Erlenmeyer flask, water-soluble vitamin C is extracted in 25 mL of distilled water from 5 g of dried fruit. Add 0.5 ml of 1% KI solution, 0.5 mL of 2% HCl and 2 ml of 1% starch to each sample. The sample thus prepared is immediately titrated with a solution of potassium iodate 0.001N until a blue color appears. In parallel, a control sample containing 1 mL of 2% HCl solution, 25 mL of distilled water, 2 mL of 1% KI and 2 mL of 1% starch is prepared. The control sample is titrated with potassium iodate solution 0.001 N. Two more samples were carried out in parallel and the average value was determined. For all these samples, the concentration of vitamin C expressed in mg% (mg vitamin C in 100 g of dried fruit) was calculated by performing the following calculations:

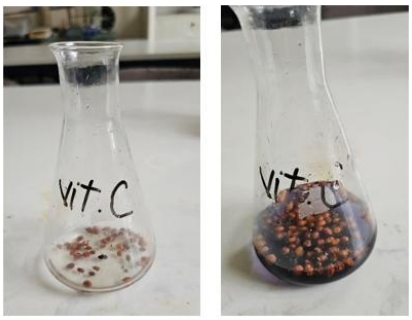


Figure 6. *Sea buckthorn dried fruit extracts before and after determination of vitamin c concentration by titration with KIO3 0.001 N in the presence of starch*

- Determination of the number of mL of potassium iodate used in the determination of vitamin C in the titrated sample: V_{iodate} (mL) = $V_P - V_M$

  where

V_P - represents the volume of the 0.001N potassium iodate solution used for sample titration (mL);

V_M - represents the volume of the 0.001N potassium iodate solution used for the titration of the control sample;

V_{iodate} - the volume of potassium iodate solution 0.001N corresponding to the concentration of vitamin C in the titrated sample (mL).

- Determine the mass of vitamin C (mg) from the sample of titrated dried fruits ($M_{vit,\,C}$ = 176 g and $Eg_{vit.\,C}$ = 88 g)
- It is known that at

  1 mol KIO_3 0.001 N.....3 moles I_2..... 3·176 g Vit C

- The number of moles of KIO_3 0.001 N is calculated with the relationship:

$$n_{KIO_3} = \frac{m_d}{M} = \frac{C_n \cdot (V_P - V_M) \cdot 10^{-3} \cdot E_g}{M}$$

$$= \frac{C_n \cdot (V_P - V_M) \cdot 10^{-3} \cdot \frac{M}{5}}{M}$$

$$= \frac{C_n \cdot (V_P - V_M) \cdot 10^{-3}}{5}$$

Where

c_N is the normal concentration of KIO_3 solution (0.001 N)

M is the molecular weight of the KIO_3 solution

Eg is the gram equivalent of the KIO_3 solution

The mass of Vitamin C corresponding to the number of moles of KIO_3 0.001 N determined above is:

n_{KIO_3} Moles.....

$3n_{KIO_3}\ moles\ I_2$ ……..$3 \cdot 176 \cdot \frac{C_n \cdot (V_P - V_M) \cdot 10^{-3}}{5}\ g\ Vitamin\ C$

The concentration of vitamin C expressed in mg per 100 g sample of dried fruits is determined by the ratio:

$3 \cdot 176 \cdot \frac{C_n \cdot (V_P - V_M)}{5}\ mg\ Vitamin\ C$ ……$m\ g\ sample$

$mg\ vitamin\ C$ ……100 $g\ dried\ fruits$

$$vitamin\ C\ (mg\%) = 3 \cdot 176 \cdot \frac{C_n \cdot (V_P - V_M)}{5 \cdot m} \cdot 100$$

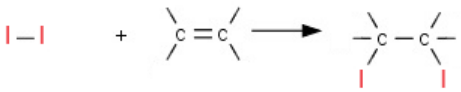
Where m is the sample mass of fruits from which the amount of vitamin C (g) was determined by titration with iodate

The concentration of vitamin C expressed in mg in 100 g of dried fruits is:

$$vitamin\ C\ (mg\%) = \frac{10{,}56(V_P - V_M)}{m}$$

II.5. Determination of iodine index

In the case of volatile sea buckthorn oil, we set out to determine the degree of unsaturation, knowing that many volatile substances can be from the terpene group, residues of vegetable fatty acids, compounds that have double bonds, in other words a degree of unsaturation. These substances can add iodine molecules to the double bond.



The number of grams of iodine that 100 mL of substance can fix is called the iodine index. The iodine index gives us an indication of the degree of unsaturation of the respective substance, varying between quite high limits.

After increasing the iodine index, there are three categories of vegetable oils:

- drying oils (flax, hemp, poppy, walnut etc.), with an iodine index greater than 120°.

- semi-drying oils (cotton, rapeseed) with an iodine index between 100 - 120°.

- non-drying oils (olives, almonds) with an iodine index below 100°.

The volatile oil sample is treated with excess reagent, and the iodine remaining unfixed, is titrated with a solution of sodium thiosulfate, sodium thiosulfate reacts with iodine, according to the reaction:

$I_2 + 2\ Na_2S_2O_3 \rightarrow 2\ NaI + Na_2S_2O_6$

The end of the reaction is highlighted with a starch solution, with which iodine gives a blue color.

The substances used in this experiment were:

- solution I_2 0.1 N
- solution $Na_2S_2O_3$ 0,1N (F=1,000)
- potassium iodide (10%);
- n-hexane
- Starch 1%

Working mode

In an Erlenmeyer glass with a broken stopper, add 5 mL of volatile oil to 10 mL of n-hexane. Shake and add 5 mL of 0.1 N iodine solution. Close the bottle, shake and put in the dark to react for 15 minutes. Add 10 mL KI 10%- and 1-mL starch 1% to the sample and titrate with sodium thiosulfate solution 0.1 N. until the blue color disappears. The experiment was repeated two more times.

In parallel, a control sample is prepared in which 10 mL n-hexane is placed in which 5 mL of 0.1 N iodine solution, 10 mL of 10% KI and 1 mL of 1% starch are added and titrated with 0.1 N sodium thiosulfate solution.

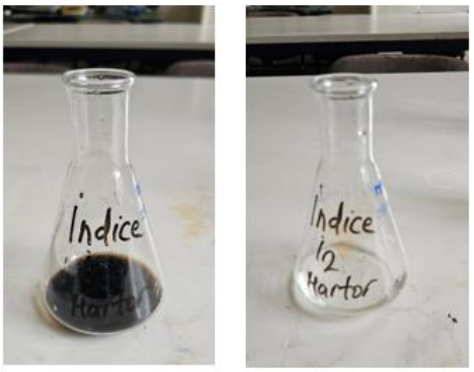


Figure 7. *Determination of Iodine Index in Sea Buckthorn Volatile Oil Sample by Na2S2O3 Titration*

From the difference between the titration of the control sample (V_M) and the volatile oil sample (V_P), the grams of iodine fixed by 100 g of oil and the iodine index are calculated

$$Iodine\ index = \frac{126{,}9(V_M - V_P)\cdot 10^{-3}\cdot c_{Na_2S_2O_3}\cdot F_{Na_2S_2O_3}\cdot 100}{V_{sample}} = \frac{12{,}69(V_M - V_P)\cdot c_{Na_2S_2O_3}\cdot F_{Na_2S_2O_3}}{V_{sample}}$$

Where

126.9 is the gram equivalent of iodine

V_M is the volume of sodium thiosulfate used in the titration of the control (mL)

V_P is the volume of sodium thiosulfate used in the titration of the volatile oil sample (mL)

V_{sample} is the sample volume of volatile oil at which the degree of unsaturation by

addition of iodine (mL) has been determined

In the case of our experiment, in which the volatile oil sample was 5 mL and the thiosulfate concentration was 0.1 N (F=1.000), the iodine index can be determined with the relationship:

:

$$Iodine\ index = 0{,}2538(V_M - V_P)$$

RESULTS AND DISCUSSIONS

III.1. Determination of acidity

The results obtained from making the determinations and calculations are listed in the following table

Table 1. Determination of Acidity of Sea Buckthorn Dried Fruits by Titration with NaOH 0.1 N

Sample No.	Sample mass (g)	V_{NaOH} 0.1 N (mL)	F_{NaOH} 0.1 N	Acidity (%)
1	10	6,3	0,9524	6,0001
2	10	6,2	0,9524	5,9049
3	10	6,1	0,9524	5,8096
Average	10	6,2	0,9524	5,9049

For sea buckthorn essential oil, which is insoluble in water, 2 mL of oil sample was measured, to which 20 mL of ethyl alcohol, 20 mL of distilled water and 2 drops of 1% phenolphthalein were added. The sample was also titrated with 0.1 N NaOH solution (F=0.9524).

In parallel, a control sample consisting of 20 mL of ethyl alcohol and 20 mL of distilled water was prepared, which was titrated with NaOH 0.1 N (F=0.9524) in the presence of phenolphthalein 1% to faint pink. The experiment was repeated two more times.

Figure 8. *Sea buckthorn samples prepared before the determination of acidity*

In this case, the calculations for determining the acidity expressed as the number of mmoli of NaOH 0.1 N consumed for the titration of 100 mL of volatile oil were performed with the ratio:

$$A\% = \frac{c_{NaOH} \cdot F_{NaOH} \cdot (V^{P}_{NaOH} - V^{M}_{NaOH}) \cdot 100}{V_{sample}}$$

Where

V^{P}_{NaOH} is the volume of 0.1 N NaOH used for sample titration (mL);

V^{M}_{NaOH} is the volume of 0.1 N NaOH used for control titration (mL);

V_{sample} is the volume of the essential oil sample expressed in mL.

The results obtained from making the determinations and calculations are recorded in the following table.

Figure 9. *Sea buckthorn samples after determination of acidity with NaOH 0.1 N in the presence of phenolphthalein: aqueous extract of dried sea buckthorn fruits; extract in ethyl alcohol of volatile sea buckthorn oil; control sample of ethyl alcohol*

Table 2. Determination of Acidity of Sea Buckthorn Oil by Titration with NaOH 0.1 N

Sample No.	Sample volume (mL)	V^{P}_{NaOH} 0.1 N (mL)	V^{M}_{NaOH} 0.1 N (mL)	FNaOH 0.1 N	Acidity (%)
1	2	1,0	0,4	0,9524	6,4574
2	2	0,9	0,4	0,9524	5,3816
3	2	1,1	0,4	0,9524	7,5335
Average	2	1,0	0,4	0,9524	6,4574

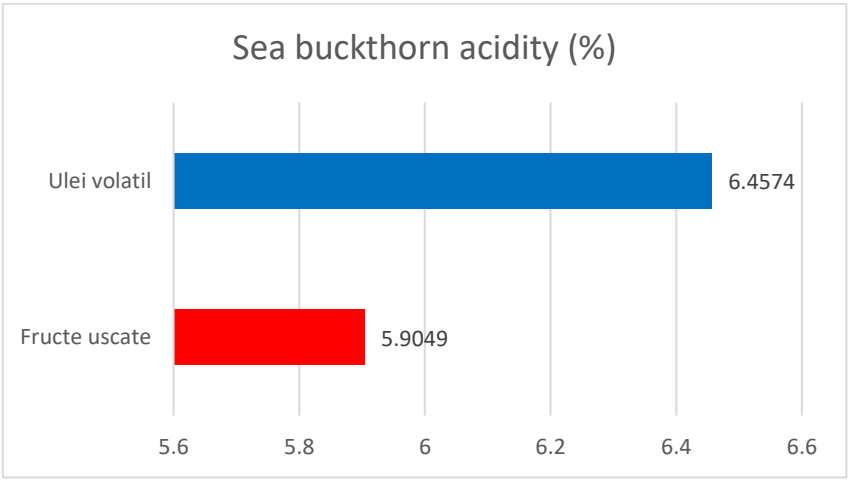


Figure 10. *Acidity of sea buckthorn fruits and volatile sea buckthorn oil*

From the data of the tables and graph above, it can be seen that the acidity value found was higher in sea buckthorn oil than in sea buckthorn fruits. The difference between the two values was calculated as a percentage with the relationship:

$$\Delta A\% = \frac{A\%_{ulei} - A\%_{fructe}}{A\%_{fructe}} \cdot 100$$

The results showed that the acidity is 9.4 % higher in volatile oil than in fruit, a difference that is not very large and may be caused by the different interpretation of the concentration, compared to 100 g of dry mass for fruit and 100 mL for the sample of sea buckthorn volatile oil, respectively. Another possible cause is related to the composition, i.e. a higher concentration of organic compounds with acidic properties in the volatile sea buckthorn oil.

III.2. Determination of cations concentration

The results obtained are recorded in the table 3.

III.3. Determination of calcium ion concentration

The results obtained are presented in table 4.

Table 3. Determination of cation concentration in 100 g of dried sea buckthorn fruit aqueous extract

Sample No.	Sample mass (g)	V_{EDTA} 0.01 M (F=1.0204) (mL)	Cations (mmols/100 g)
1	10	2,85	0,292
2	10	2,8	0,287
3	10	2,75	0,282
Average	10	2,8	0,287

Table 4. Determination of the concentration of calcium ions in aqueous extracts of sea buckthorn

Sample No.	Sample mass (g)	V_{EDTA} 0.01 M (F=1.0204) (mL)	Calcium	
			(mg/100 g)	(mmols/100 g)
1	10	2,1	8,59	0,214
2	10	1,9	7,77	0,194
3	10	2,05	8,38	0,209
Average	10	2,02	8,26	0,206

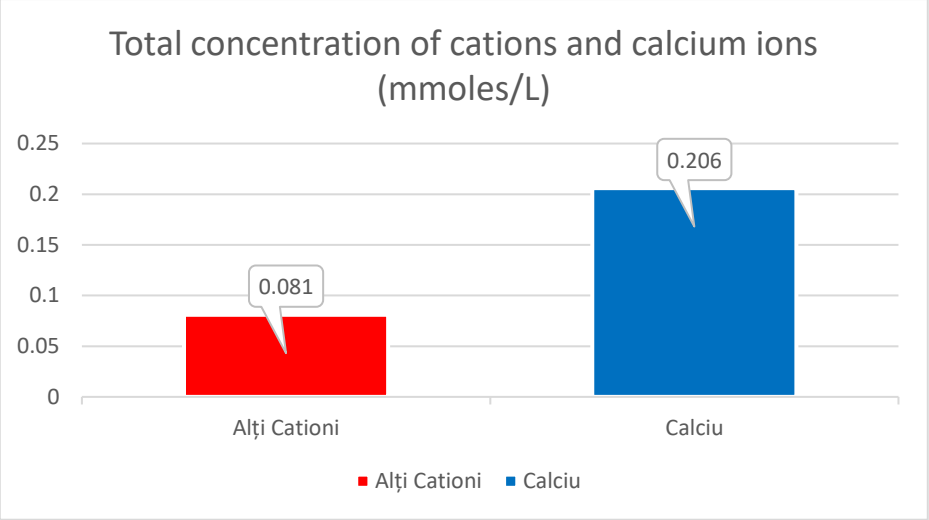


Figure 11. *Concentrations of cations and calcium in the aqueous extract of dried sea buckthorn fruits*

In order to determine how much the concentration of calcium ions in all cations found in dried sea buckthorn fruits can be determined by complexonometric titration, the following relationship is used:

$$\%Ca = \frac{\text{Calcium (mmoles/100 g)}}{\text{Cations (mmoles/100 g)}} \cdot 100$$

The calculations showed that calcium ions account for 71.78%, and this percentage is represented in the graph below, where it can be seen that sea buckthorn fruits are very rich in calcium and are an important source of vegetable calcium (fig.12).

III.4. Quantitative determination of vitamin C

The results obtained from the determinations are noted in the table 5:

III.5. Determination of the iodine index

The results obtained are recorded in the table 6.

The iodine index in the case of sea buckthorn volatile oil has a very low value (0.500), which shows that this volatile oil has a very low content of unsaturated compounds with double bonds.

CONCLUSIONS

Following the analyses carried out on sea buckthorn fruits and oil, the acidity value found was higher in sea buckthorn oil than in sea buckthorn fruits. The acidity was 9.4 % higher in the volatile oil than in the fruit, a difference which is not very large and may be due to the different interpretation of the concentration in relation to 100 g of dry fruit mass and 100 mL of sea buckthorn volatile oil sample, respectively.

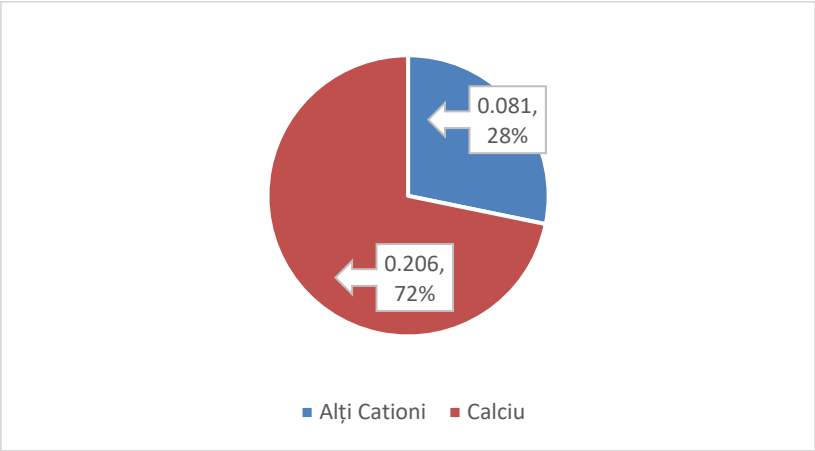


Figure 12. *Percentage distribution of cations in dried sea buckthorn fruits*

Table 5. Determination of vitamin C concentration (mg/100 g dried fruit)

Sample No.	Sample mass (g)	V_P (mL)	V_M (mL)	V_{iodate} (mL)	$Vitamin\ C$ % (mg%)
1	5	12,1	0	12,1	25,55
2	5	12,0	0	12,0	25,34
3	5	12,0	0	12,0	25,34
Average	5	12,03	0	12,03	25,41

Table 6. Determination of iodine index in volatile sea buckthorn oil

Sample No.	V_{sample} (mL)	V_M (mL)	V_P (mL)	Iodine Index
1	5	5	2,0	0,7614
2	5	5	1,9	0,4822
3	5	5	2,0	0,7614
Average	5	5	1,96	0,500

Sea buckthorn is a fruit rich in vitamins, a significant amount representing vitamin C – about 25.41%, which is a powerful oxidant of the whole body. Following the laboratory analyzes carried out, we found out that the oxidase activity in sea buckthorn fruits is 76% higher than in volatile oil. The oxidase of the aqueous extract from dried fruits and the alcoholic extract from volatile oil was determined.

Sea buckthorn fruits are rich in vitamin C, organic acids, flavonoids,

phenols and catechins, studies have shown that this component contributes to the treatment of several diseases, such as cancer, cardiovascular diseases, gastrointestinal diseases, has an antioxidant effect on the whole body, hepatoprotective, immunostimulating, radioprotective, anti-inflammatory effect. They also contain a significant amount of calcium, so the calcium ions we determined represent 71.78%, which is why sea buckthorn fruits are a rich natural source of calcium.

Sea buckthorn oil has a relatively low degree of saturation, compared to other volatile oils, which is evidenced by the iodine index value that we found to have a value of 0.500, which demonstrates that this oil has a very low content of unsaturated compounds with double bonds.

REFERENCES

1. Enescu, C.M., 2014. Sea-buckthorn: a species with a variety of uses, especially in land reclamation. *Dendrobiology*, (72).
2. Swati Pundir, Prakrati Garg, Ananya Dviwedi, Aaliya Ali, V.K. Kapoor, Deepak Kapoor, Saurabh Kulshrestha, Uma Ranjan Lal, Poonam Negi, Ethnomedicinal uses, phytochemistry and dermatological effects of Hippophae rhamnoides L.: A review, Journal of Ethnopharmacology, Volume 266, 2021, 113434, ISSN 0378-8741, https://doi.org/10.1016/j.jep.2020.113434.
3. Kubczak, M., Khassenova, A.B., Skalski, B. *et al. Hippophae rhamnoides* L. leaf and twig extracts as rich sources of nutrients and bioactive compounds with antioxidant activity. *Sci Rep* **12**, 1095 (2022). https://doi.org/10.1038/s41598-022-05104-2
4. https://www.ugal.ro/files/doctorat/sustineri/2021/CORBU_Alexandru_REZUMAT_TEZA_DOCTORAT_210219.pdf accessed at 25.06.2024
5. Ren R, Li N, Su C, Wang Y, Zhao X, Yang L, Li Y, Zhang B, Chen J, Ma X. The bioactive components as well as the nutritional and health effects of sea buckthorn. RSC Adv. 2020 Dec 17;10(73):44654-44671. Doi: 10.1039/d0ra06488b. PMID: 35516250; PMCID: PMC9058667.
6. Olas B. Sea buckthorn as a source of important bioactive compounds in cardiovascular diseases. Food Chem Toxicol. 2016 Nov; 97:199-204. doi: 10.1016/j.fct.2016.09.008. Epub 2016 Sep 9. PMID: 27616182.

7. Kubczak, M., Khassenova, A.B., Skalski, B. *et al. Hippophae rhamnoides* L. leaf and twig extracts as rich sources of nutrients and bioactive compounds with antioxidant activity. *Sci Rep* **12**, 1095 (2022). https://doi.org/10.1038/s41598-022-05104-2
8. https://www.ellemental.ro/435-ulei-de-catina.html?ssa_query=c%C4%83tin accessed at 30.05.2024
9. https://vegis.ro/blog/wp-content/uploads/2019/04/ulei-de-catina-beneficii-1-410x1024.png accessed at 30.05.2024
10. Wang Y, Zhang ZZ, Wu Y, Zhan J, He XH, Wang YL. Honokiol protects rat hearts against myocardial ischemia reperfusion injury by reducing oxidative stress and inflammation. Exp Ther Med. 2013 Jan;5(1):315-319. doi: 10.3892/etm.2012.766.
11. Zielińska, A. and Nowak, I., 2017. Abundance of active ingredients in sea-buckthorn oil. *Lipids in health and disease*, *16*, pp.1-11.
12. Bal, Lalit & Meda, Venkatesh & Naik, Satya & Satya, Santosh. (2011). Sea buckthorn berries: A potential source of valuable nutrients for nutraceuticals and cosmoceuticals. Food Research International. 44. 1718-1727. 10.1016/j.foodres.2011.03.002.
13. Baoru Yang, Heikki Kallio, Composition and physiological effects of sea buckthorn (Hippophaë) lipids, Trends in Food Science & Technology, Volume 13, Issue 5, 2002, Pages 160-167, ISSN 0924-2244, https://doi.org/10.1016/S0924-2244(02)00136-X.
14. https://www.google.com/search?q=imaginii+catina+alba&sxsrf=APwXEdcRJA2G995URqQZbMQH96i3TKXmXQ:1684162117467&source=lnms&tbm=isch&sa=X&ved=2ah accesed în 30.05.2024
15. Zeb A. 2004. Chemical and nutritional constituents of sea buckthorn juice. Pakistan Journal of Nutrition 3: 99–106. http://dx.doi.org/10.3923/ pjn.2004.99.106
16. Leskinen H.M., Suomela J.P., Yang B., Kallio H.P. 2010. Regioisomer compositions of vaccenic and oleic acid containing triacyglycerols in sea buckthorn (Hippophaë rhamnoides) pulp oils: influence of origin and weather conditions. Journal of Agriculture and Food Chemistry 58: 537–545. http:// dx.doi.org/10.1021/jf902679v
17. Petre, Alina. Sea Buckthorn Oil: Top 11 Health Benefits. *Healthline*, Healthline Media, 27 Apr. 2022, www.healthline.com/nutrition/sea-buckthorn-oil, accessed at 19.02.2023.

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ROSEMARY – STUDY OF ANTIOXIDANT ACTIVITY

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Abstract: *In this study, we aimed to qualitatively and quantitatively determine certain flavonoids from rosemary. The analysis of rosemary was performed using an alcoholic extract obtained through a Soxhlet extraction process and an ultrasound-assisted method. The extracts were then analyzed using spectrophotometric methods.*

Key words: Rosmarinus Officinalis, DPPH, Folin – Ciocâlteu, polyphenols

INTRODUCTION

The global interest in the use of medicinal plants has grown, with their beneficial effects being rediscovered for the development of new drugs. Based on their extensive ethnopharmacological applications, which have inspired current drug discovery research, natural products can provide new and significant leads against various pharmacological targets.

Rosmarinus officinalis L. is a medicinal plant belonging to the Lamiaceae family, commonly known as rosemary. Beyond its culinary uses due to its distinctive aroma, this plant is widely utilized by indigenous populations where it grows wild. [1] Extracts obtained from rosemary are used as natural antioxidants, improving the shelf life of perishable foods. The European Union has approved rosemary extract (E392) as a safe and effective natural antioxidant for long-term food preservation.

Given the growing interest in the medicinal properties of *R. officinalis* L., it is of great importance to review previous studies and establish professional connections between companies, governments, major pharmaceutical corporations, and academic Institution[1].

Fig 1. Rosmarinus Officinalis [2].

Rosemary belongs to the *Lamiaceae* family, which is one of the largest and most distinguished plant families, comprising approximately 236 genera and 6,900–7,200 species worldwide. The original name of the family is *Labiatae*, as the flowers

typically have petals resembling upper and lower lips. However, most botanists now use the name *Lamiaceae*.

The *Lamiaceae* family is well-known for its biologically active essential oils, which are common to many members of this family, including ornamental and culinary herbs such as basil, lavender, mint, rosemary, sage, and thyme.

Several studies report the presence of a wide range of compounds in their structure, such as terpenes, iridoids, flavonoids, and phenolic compounds found in plants of the *Lamiaceae* family. This family includes plant species rich in phenolic acids, such as rosmarinic acid, which possess antibacterial, antiviral, antioxidant, and anti-inflammatory properties. [1]

Multiple medicinal applications have been identified for *Rosmarinus officinalis*, including treatments for disorders related to the nervous, cardiovascular, gastrointestinal, genitourinary, menstrual, hepatic, reproductive, respiratory, and skin systems. [21] Due to its diverse properties, many biomolecules in rosemary have been recognized as responsible for the biological effects of its essential oil and crude extract. However, the specific compounds causing these effects have rarely been identified; these effects are largely attributed to the synergistic actions of multiple metabolites present in rosemary. [22]

Rosemary has garnered attention due to its abundance of secondary metabolites with therapeutic potential, such as carnosol, carnosic acid, rosmarinic acid, ursolic acid, and oleanolic acid. These compounds have been studied and shown to exhibit anti-inflammatory effects, wound-healing potential, tissue survival benefits, skin anti-cancer properties, antifungal activity, and UV protection.

In traditional medicine, rosemary is known for its therapeutic properties in alleviating abdominal pain and treating inflammatory respiratory diseases, such as bronchial asthma. Some experimental studies have reported the anti-inflammatory and analgesic activities of its essential oil and biologically active terpenes, including carnosic acid, carnosol, ursolic acid, betulinic acid, rosmarinic acid, rosmanol, and oleanolic acid. Other studies have documented their antinociceptive activity, with individual triterpenes demonstrating potency comparable to ketorolac, a non-steroidal anti-inflammatory drug. [4]

MATERIALS & METHODS

- Salvia Rosmarinus
- Ethanol
- Distilled Water
- Sodium Carbonate

- Folin–Ciocalteu Reagent
- DPPH (2,2-diphenyl-1-picrylhydrazyl)
- Ultrasound Bath
- Soxhlet Extraction Apparatus
- Atomic Absorption Spectrophotometer

RESULTS & DISCUSSIONS

The experimental part of the study involves the qualitative and quantitative determination of flavonoids from rosemary. The analysis was carried out using an alcoholic extract obtained through Soxhlet extraction and ultrasound-assisted extraction methods. After the extraction process, the obtained sample was analyzed by spectrophotometry.

For Soxhlet extraction, 5 grams of rosemary (*Salvia Rosmarinus*) were weighed, and the extraction was performed using 88 mL of ethanol and 50 mL of distilled water (Fig. 7). The same quantities were subjected to ultrasound-assisted extraction at a temperature of 30°C for 30 minutes (Fig. 8a and b).

Figure 7. Rosemary extraction by the Soxhlet method

The obtained extracts were analyzed using spectrophotometric methods to determine their polyphenol content and antioxidant activity.

Figure 8. a) Ultrasound bath extraction

Figure 8. b) Filtration of the rosemary extract

Determination of Total Polyphenol Content

The Folin–Ciocalteu method is one of the most commonly used techniques for determining the total polyphenol content in various extracts.

Preparation of the Calibration Curve

In Erlenmeyer flasks, the following are mixed:

- 8 mL of distilled water,
- 1 mL of working standard solutions, and
- 1 mL of Folin–Ciocalteu reagent.

The mixture is left to rest for 1 minute, after which 2 mL of prepared sodium carbonate is added. The solutions turn blue in color. The mixture is then placed in an oven at 40°C for one hour to complete the reaction, after which absorbance is measured at a wavelength of $\lambda = 765$ nm.

Reference Sample Preparation

A reference sample is prepared simultaneously by mixing:

- 8 mL of distilled water,
- 1 mL of the solution prepared in the previous step,
- 1 mL of Folin–Ciocalteu reagent, and
- 2 mL of sodium carbonate solution prepared earlier.

This mixture is also placed in the oven at 40°C.

The results obtained are presented in Table 1. Using these values, the calibration curve is constructed, showing the dependence of solution absorbance on the concentration of gallic acid solution.

Table 1. Values for determining the calibration curve

No.	Concentration mg/ml	Absorbance
1	0,05	0,3688
2	0,075	0,4876
3	0,1	0,6101
4	0,15	0,8661
5	0,2	1,0346

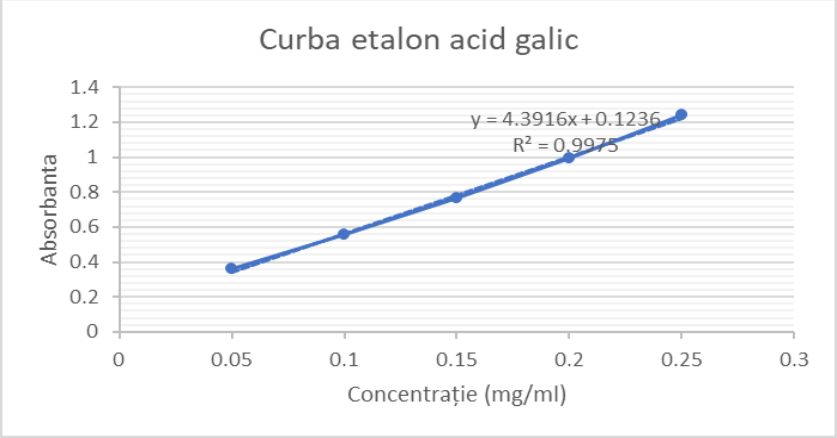

Figura 10. Calibration curb

In the concentration range of 0.05 – 2.0 mg/mL (gallic acid), a linear relationship between concentration and absorbance is obtained. The equation of the resulting line is y = 4.3916x + 0.1236, with a correlation coefficient R^2 = 0.9975, showing a good correlation of the results.

Based on the obtained results, the formula for calculating the total phenol content is:

Conc (mg gallic acid/mL) = (A – 0.1236)/4.3916

Where:

- A is the absorbance,
- Conc is the polyphenol concentration.

Results:

The absorbance measured for the rosemary sample was:

- 1.1850 by the Soxhlet extraction method (S),
- 0.3341 by the ultrasound-assisted extraction method (UUS).

For Soxhlet extraction:

Conc (mg gallic acid/mL) = (1.1850 – 0.1236)/4.3916 = 1.1568 mg gallic acid/mL (S)

For ultrasound-assisted extraction:

Conc (mg gallic acid/mL) = (0.3341 – 0.1236)/4.3916 = 0.305 mg gallic acid/mL (UUS)

The study also determined the free radical scavenging capacity using DPPH as a reagent. The free radical scavenging capacity correlates with the antioxidant activity of the sample under analysis. Molecular UV-VIS absorption spectrophotometry was used for the analysis, with measurements taken at a wavelength of 517 nm. Absorbance was measured at the initial time, A_1 (immediately after adding the DPPH solution), and after 60 minutes, A_2 (a time during which the free radical is neutralized by the antioxidants in the samples kept in the dark for 60 minutes).

The results obtained for rosemary are:

Tabelul 2. Valori absorbanțe – A_1 – la timpul initial; A_2 – după 60 minute

No.	Rosemary	A_1	A_2
1	S	0,6894	0,8141
2	UUS	0,1918	0,2285

A_0 – the reference absorbance value is 1.3499; the reference solution consists of 2 mL water + 1 mL DPPH + 0.5 mL extract.

The free radical scavenging capacity was determined using the following formula:

$$C\ (\%) = (A_0 - A_1) \times 100 / A_0$$

Soxhlet Method:

- **C_1 = (1.3499 – 0.6894) × 100 / 1.3499 = 48.92% (initial)**
- **C_2 = (1.3499 – 0.8141) × 100 / 1.3499 = 39.69% (after 60 minutes)**

Ultrasound Method:

- **C_1 = (1.3499 – 0.1918) × 100 / 1.3499 = 85.79% (initial)**

- **C_2 = (1.3499 – 0.2285) × 100 / 1.3499 = 83.07% (after 60 minutes)**

CONCLUSIONS

As a result of the analyses performed, a relatively high content of polyphenols and a good free radical scavenging capacity were observed.

In a future study, we will attempt to determine other flavonoids from the rosemary extract. It is recommended to continue the research for quantitative analysis as well as for identifying other elution systems.

Therefore, rosemary represents an exceptionally rich source of various bioactive compounds.

For this reason, the objective of this study was to investigate the composition of rosemary leaves harvested in Oradea, Romania, to explore the presence of bioactive substances. In this regard, the present study demonstrates that extracts from this plant could be used as natural sources of several bioactive compounds, particularly carnosol, carnosic acid, and triterpenes, which could be useful ingredients in complementary alternative medicine, dietary supplements, as well as natural antioxidants for food preservation.

REFERENCES

1. Joana M Andrade, Celia Faustino, Catarina Garcia, Diogo Ladeiras, Catarina Reis, Patricia Rijo. Rosmarinus officinalis L.: an update review of its phytochemistry and biological activity.
2. Enciclopaedia Britanica, Inc (2004).
3. Johnson, J.J. Carnosol: A promising anti-cancer and anti-inflammatory agent. Cancer Lett. 2011, 305, 1–7.
4. Altinier, G.; Sosa, S.; Aquino, R.P.; Mencherini, T.; Loggia, R.D.; Tubaro, A. Characterization of topical antiinflammatory compounds in Rosmarinus officinalis L. J. Agric. Food Chem. 2007, 55, 1718–1723.
5. Del Campo, J.; Amiot, M.; Nguyen-The, C. Antimicrobial effect of rosemary extracts. J. Food Prot. 2000, 63, 1359–1368.
6. Bozin, B.; Mimica-Dukic, N.; Samojlik, I.; Jovin, E. Antimicrobial and antioxidant properties of rosemary and sage (Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae) essential oils. J. Agric. Food Chem. 2007, 55, 7879–7885.

7. Visanji, J.M.; Thompson, D.G.; Padfield, P.J. Induction of G2/M phase cell cycle arrest by carnosol and carnosic acid is associated with alteration of cyclin A and cyclin B1 levels. Cancer Lett. 2006, 237, 130–136.
8. Takaki I, Bersani-Amado LE, Vendruscolo A et al. Anti-inflammatory and antinociceptive effects of Rosmarinus officinalis L. essential oil in experimental animal models. J. Med. Food. 11(4), 741–746 (2008).
9. Luqman S, Dwivedi GR, Darokar MP, Kalra A, Khanuja SPS. Potential of rosemary oil to be used in drug-resistant infections. Altern. Ther. Health Med. 13(5), 54–59 (2007).
10. Shin H-B, Choi M-S, Ryu B et al. Antiviral activity of carnosic acid against respiratory syncytial virus. Virol. J. 10(1), 303 (2013).
11. Wang W, Li N, Luo M, Zu Y, Efferth T. Antibacterial activity and anticancer activity of Rosmarinus officinalis L. essential oil compared to that of its main components. Molecules 17(3), 2704–2713 (2012).
12. Klancnik A, Guzej B, Kolar MH, Abramovic H, Mozina SS. In vitro antimicrobial and antioxidant activity of commercial rosemary extract formulations. J. Food Prot. 72(8), 1744–1752 (2009).
13. Sarac, N. and A. Ugur, 2007. Antimicrobial activities and usage in folkloric medicine of some Lamiaceae species growing in Mugla, Turkey. EurAsia J. Bio. Sci., 1: 28-34.
14. Giuliani, C. And L. Maleci Bini, 2008. Insight into the structure and chemistry of glandular trichomes of Labiatae, with emphasis on subfamily Lamioideae. Plant Systematic and Evolution, 276: 199-208.
15. Z. Jamzad Research Prof., Research Institute of Forests and Rangelands, Tehran, Iran. A survey of Lamiaceae in the flora of Iran (2013).
16. R. Amber, M. Adnan, A. Tariq, S. Mussarat, A review on antiviral activity of the Himalayan medicinalplants traditionally used to treat bronchitis and related symptoms, J. Pharm. Pharmacol. 69 (2) (2017) 109-122.
17. S. Jarić, M. Mitrović, P. Pavlović, Review of ethnobotanical, phytochemical, and
18. pharmacological study of Thymus serpyllum L., Evid. Based Complement. Alternat. Med. 2015.
19. A. Begum, S. Sandhya, S. Shaffath Ali, K.R. Vinod, S. Reddy, D. Banji, An in-depth review on the medicinal flora Rosmarinus officinalis (Lamiaceae), Acta Sci. Pol. Technol. Aliment. 12 (1) (2013).
20. UniProt. Taxonomy—Rosmarinus Officinalis (Rosemary) (Salvia Rosmarinus). https: //www.uniprot.org/taxonomy/39367.

21. Begum, A.; Sandhya, S.; Vinod, K.R.; Reddy, S.; Banji, D. An in-depth review on the medicinal flora Rosmarinus officinalis (Lamiaceae). Acta Sci. Pol. Technol. Aliment. 2013, 12, 61–74.
22. Hyun, H.B.; Shrestha, S.; Boo, K.H.; Cho, S.K. Evaluation of antioxidant potential of ethyl acetate fraction of Rosmarinus officinalis L. and its major components. J. Korean Soc. Appl. Biol. Chem. 2015, 58, 715–722.
23. C. Munteanu, T. Zaharescu, V. Tecuceanu, E. Oprea, Antioxidant activity of rosemary extracts in solution and embedded in polymeric systems (2015).
24. Chang, S.S.; Ostric-Matijasevic, B.; Hosieh, O.A.L.; Huang, C.L. Natural antioxidants from rosemary and sage. J. Food Sci. 1977, 42, 1102–1106.
25. Bracco, U.; Loliger, J.; Viret, J.-L. Production and use of natural antioxidants. J. Am. Oil Chem. Soc. 1981, 58, 686–690.
26. Jiang, Y.; Wu, N.; Fu, Y.-J.; Wang, W.; Luo, M.; Zhao, C.J.; Zu, Y.G.; Liu, X.L. Chemical composition and antimicrobial activity of the essential oil of Rosemary. Environ. Toxicol. Pharmacol. 2011, 32, 63–68.
27. Del baño, M.J.; Lorente, J.; Castillo, J.; Benavente-Garcia, O.; Marín, P.; Del Río, J.A.; Ortuó, A.; Ibarra, I. Flavoid distribution during the development of leaves flowers, stems and roots of Rosmarinus officinalis postulation of the Biosynthetic pathway. J. Agric. Food Chem. 2004, 52, 4987–4992.
28. Gordon, M.H. The mechanism of antioxidant action in vitro. In Food Antioxidants; Hudson, B.J.F., Ed.; Elsevier Science Publishing: New York, NY, USA, 1990; pp. 1–18.
29. Inatani, R.; Nakatani, N.; Fuwa, H. Antioxidative effect of the constituents of Rosemary (Rosmarinus officinalis L.) and their derivatives. Agric. Biol. Chem. 1983, 47, 521–528.
30. Munné Bosch, S.; Alegre, L. Subcellular compartmentation of the diterpene carnosic acid and its dervivatives in the leaves of rosemary. Plant Physiol. 2001, 125, 1094–1102.

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[2] Carter, B., and Connell, C. (1980). Moa Point Wastewater Treatment Plant and Outfall Study, Report for the Wellington City Council, Wellington, pp. 31.

(Book)

[3] Grady, C.P.L., and Lim, H.(1980). Biological Wastewater Treatment Theory and Application, Marcel Dekker, New York, pp. 375.