“AUREL VLAICU” UNIVERSITY OF ARAD
FACULTY OF FOOD ENGINEERING, TOURISM AND ENVIRONMENTAL PROTECTION
CHEMICAL AND TECHNOLOGICAL RESEARCH CENTER

Scientific and Technical Bulletin

Series CHEMISTRY, FOOD SCIENCE & ENGINEERING

Milk Analyses—Pages 24-27

Year XV, Vol. 14, 2017
ISSN 1582-1021

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ISSN 1582-1021

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EDITORIAL

WELCOME TO *SCIENTIFIC AND TECHNICAL BULLETIN, SERIES: CHEMISTRY, FOOD SCIENCE AND ENGINEERING*

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We are delighted to announce that ***Scientific and Technical Bulletin, Series: Chemistry, Food Science and Engineering (Scien. Tech. Bull-Chem. Food Sci. Eng.)***, an annual peer-reviewed, open access journal edited by Faculty of Food Engineering, Tourism and Environmental Protection, "Aurel Vlaicu" University from Arad, Romania, is currently publishing its fourteenth volume this year in a new form and Editorial Board.

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We are planning to address both ***research and education*** areas in chemistry, physics, biology, food science and engineering, environmental science and engineering, materials science and medicine, engeneering management, as we want to extend to a wider public, not only academic staff, but also students and teachers and people from our community and related others.

Our editors are recognized academic staff from wide areas of scientific researches: chemistry, physics, biology, food engineering, environmental science, etc. As well, we would like to thank to our collaborators which act as reviewers for their professional achievements.

In the present volume we present results obtained in (i) chemistry laboratories: synthesis and characterization of new compounds with copper, zinc, nickel; (ii) food science laboratories: old and new treatment methodologies applied to wines; freezing aromatic plants; beverages and milk analyses; computational simulations; (iii) educational science: impact of Erasmus programm on students.

We hope that you will enjoy this volume and you will propose us innovations, even in informal settings, to enhance the success of the journal.

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Scien. Tech. Bull-Chem. Food Sci. Eng., Vol. 14(XV), 2017, 4-8

POTASSIUM FERROCYANIDE WINE TREATMENT: A CONTROVERSIAL, YET NECESSARY OPERATION

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Abstract: *This paper presents the method of iron removal from wine by using a potassium ferrocyanide treatment, a method implemented in Romania by oenologist Ştefan Teodorescu, which became official in 1973. Potassium ferrocyanide treatment is a controversial operation that poses safety and environmental protection problems, but is absolutely necessary for the efficient removal of iron from wines rich in this cation. Without iron removal, wines with over 10 mg/l of iron become susceptible to fearsome chemical defects, such as turning whiter, darker or bluisher. The paper presents a case study for the white wine Fetească Regală, obtained in the Recaş vineyard, harvested in 2016, in order to establish the theoretical and practical doses of potassium ferrocyanide based on microprobes, to remove the iron up to the safety threshold (4 mg Fe total/l of wine). The conclusions of the paper include some "good practices" for the success of the treatment and the prevention of toxicity problems.*

Keywords: potassium ferrocyanide, wine, microprobes, iron removal.

INTRODUCTION

By mineral nutrition, the wine accrues small amounts of iron in grapes: 2-3 mg/kg. In addition to the mineral substances derived from grapes, wine is enriched with significant amounts of exogenous mineral substances coming from the tanks in which the wine is stored; the materials used to condition wines; the machines with which the wine comes in contact, especially the filters, pumps, hoses, metallic pipes (Ţârdea, 2007). Therefore, the iron in wine can be "biological iron" (coming from the vine), "agronomic iron" (coming from the ferruginous soil through the grapes), and "technological iron" (coming from metal machinery and equipment with which the wine comes in contact) (Cotea *et al.*, 2009).

Prior to stabilization, wine may contain larger quantities of mineral substances than the grapes from which it is made, reaching a level of 18-20 mg/l (Cotrău, 1983).

The prerequisites for iron-related defects (iron precipitations) occur due to increased iron content, aeration conditions, and low storage temperatures. Iron-related defects are physicochemical defects of wine that can take two forms: they can be ferrous-phosphate or discoloration defects, which occur in white wines allowed to breathe, and turning darker and bluisher in color, which occurs both in white and red wines (Gheorghiţă *et al.*, 2002).

In order to prevent the occurrence of these chemical defects and for a better stabilization of the wine, it is necessary to remove the iron from the wine. (Poiană, 2005)

Currently, in Romania, two different methods of iron removal from wine are used: the potassium ferrocyanide treatment, and the treatment with phytic acid and its salts (Mihalca, 2007).

Worldwide, in recent years, the research has begun to establish alternative methods (removal of iron from white wines through ion exchange techniques) that may enable the decrease od the metal content from white wine without altering its organoleptic characteristics (Benîtez *et al*., 2002).

The treatment of musts and/or wines with PVI-PVP for decreasing the levels of metals such as Fe, Cu, Zn and Al, has been under discussion at OIV by experts under both the "Wine Technology" and, also the "Food Security" groups.

In May 2006, the French Agency of Food Security commented in an advice (AFSSA 2006) that utilisation of PVI-PVP at a dosage of 80g/hl did not appear to present any risk to consumer health (Mira *et al*., 2007).

Potassium ferrocyanide treatment poses safety and environmental protection problems, but is absolutely necessary for the efficient removal of iron from wines rich in this cation. The alternative treatment with phytic acid/calcium phytate has the disadvantages that it only removes trivalent iron from the wine, requires oxidation that creates many uncertainties about the evolution of the wine, and last but not least, there remains important amounts of calcium that cause precipitation of calcium tartrate.

The objective of this study was to establish the theoretical and practical doses of potassium ferrocyanide for removing the iron up to reach the safety limit of 4 mg Fe total/l.

MATERIALS AND METHODS

Wine samples

White wine Feteasca Alba coming from Recaş vineyard (Timiş County), harvested in 2016, was used in this study. Wine samples are taken on the day of treatment, in duplicate, in 1 liter glass bottles, from each container (cask or tank). Samples that were not very clear were filtered.

The potassium ferrocyanide treatment to remove the iron from wine

Potassium ferrocyanide reacts with ferric iron to form a blue insoluble ferric ferrocyanide precipitate $Fe_4[Fe(CN)_6]_3$, easily separable by flocculation with gelatin or bentonite. Ferric iron in wine is mostly employed in complex combinations with very poorly dissociable organic ferritartrate and ferrimalate acids. When ferrocyanide is added to the wine, free Fe^{3+} ions are immediately made insoluble in the form of ferric ferrocyanide, a blue precipitate (Prussian or Berlin blue). The reaction that takes place is depicted in Scheme 1.

$$3K_4[(CN)_6Fe]+4Fe^{3+} \longrightarrow Fe_4[Fe(CN)_6]_3+12K^+$$

Potassium ferrocyanide   Blue ferric ferrocyanide

Scheme 1. The reaction that lead to the formation of ferric ferrocyanide.

The reaction of potassium ferrocyanide with ferrous ions (Fe^{2+}) is faster and more complete, compared to that with ferric ones (Fe^{3+}). Depending on the conditions, two compounds may be formed: ferro-potassium ferrocyanide $K_2Fe[Fe(CN)_6]$, which is white (Williamson white), and ferrous ferrocyanide $Fe_2[Fe(CN)_6]$, which is blue (Turnbull blue), or a mixture of these two salts according to the reactions from Scheme 2.

$$K_4[(CN)_6Fe]+Fe^{2+} \longrightarrow K_2Fe[Fe(CN)_6] + 2K^+$$

Ferro-potassium ferrocyanide (white)

$$K_4[(CN)_6Fe]+2Fe^{2+} \longrightarrow Fe_2[Fe(CN)_6] + 4K^+$$

Ferrous ferrocyanide (blue)

Scheme 2. The reaction that lead to the formation of ferrous derivatives.

The white and white-blue divalent iron salts are hard to detect in the dark blue ferric ferrocyanide precipitate formed in wine (Bosso and Castino, 1994).

The stoichiometric method of removing iron from wine (The Ştefan Teodorescu Method)

The Ştefan Teodorescu Method is based on the association of the principle of the classic German Von Der Haide method with Prilinger's stoichiometric calculation. The method consists in determining the amount of potassium ferrocyanide required to remove iron from wine, after the stoichiometric calculation and microprobing. A safety level of 4 mg of iron in total (2 mg Fe^{2+}, and 2 mg Fe^{3+}) is maintained in wine, to avoid the over-clarifying of wine with ferrocyanide (Teodorescu *et al.*, 1960).

The method has been experimented since 1956, and is the official method used in Romania to remove iron from white and rosé wines.

RESULTS AND DISCUSSIONS

The ferrous ion (Fe^{2+}) and ferric ion (Fe^{3+}) content is determined directly from wine by the colorimetric method by using potassium thiocyanate. Based on the results obtained, the stoichiometric calculation is performed to determine the theoretical quantities of ferrocyanide. A safety level of 4 mg total Fe/l of wine is maintained (2 mg Fe^{2+}, and 2 mg Fe^{3+}).

Case study for the white wine Fetească Regală

We determined the iron content of white wines obtained in the Recaş vineyard (Timiş County), harvested in 2016, and Fetească Regală variety had a content of 7 mg Fe^{3+}/l, and 9 mg Fe^{2+}/l, respectively.

As it is depicted in Scheme 1, 3 potassium ferrocyanide molecules react with 4 trivalent iron ions. By calculating the molecular weight of the ferrocyanide, and the atomic weight of the iron it follows:

422.2 x 3 = 1266.6 for ferrocyanide

55.84 x 4 = 223.4 for iron

The analysis of the figures obtained shows that 1266.6 mg of potassium ferrocyanide is combined with 223.4 mg of trivalent iron. Therefore, 5.6721 mg of ferrocyanide (specific consumption) is needed to precipitate 1 mg of trivalent iron,

- 7 mg Fe^{3+} - 2 mg = 5 mg/l ferric iron to be removed from the wine;
- 5 mg x 5.6721 mg ferrocyanide (specific consumption) = 28.360 mg ferrocyanide/l of wine, to remove Fe^{3+};

According with reactions presented in Scheme 2, it is found that 1 molecule of potassium ferrocyanide reacts with 1 bivalent iron ion.

Considering the molecular weight of the ferrocyanide (422.3 Da), and the atomic mass of the iron (55.84 Da), it results that 7.5643 mg of ferrocyanide (specific consumption) is needed for 1 mg of divalent iron.

- 9 mg Fe^{2+} - 2 mg = 7 mg/l ferrous iron to be removed from the wine;
- 7 mg x 7.5643 mg ferrocyanide (specific consumption) = 52.950 mg ferrocyanide/l of wine, to remove Fe^{2+}.

The theoretical dose of ferrocyanide used is: 28.360 + 52.950 = 81.310 mg/l ≅ 81 mg ferrocyanide/l of wine.

Initial test

We started from the theoretical dose of ferrocyanide of 81 mg/l, determined by the stoichiometric calculation. We used introduced 10 ml of wine in 5 glass tubes with the same sizes to which we added the potassium ferrocyanide solution. The calculation is depicted in Scheme 3.

81 mg ferrocyanide ....…. 1000 ml of wine
x ...………………………… 10 ml of wine

$x = \frac{81 \times 10}{1000}$ = 0.81 mg ferrocyanide for 10 ml of wine

100 ml solution .....………500 mg ferrocyanide
x…………………………0.81 mg ferrocyanide

$x = \frac{0.81 \times 100}{500}$ = 0.16 ml ferrocyanide solution for 10 ml of wine (theoretical dose)

Scheme 3. Calculation for initial test.

For the practical application of the treatment, the practical dose is calculated by compiling two series of microprobes according to Table 1.

Table 1. Microprobes for the treatment with potassium ferrocyanide

1st series	**Tube no.**	**1**	**2**	**3**	**4**	**5**
	Wine (ml)	10	10	10	10	10
	0.5% potassium ferrocyanide solution (ml)	0.13	0.16	0.19	0.22	0.25
2nd series	**Tube no.**	**1**	**2**	**3**	**4**	**5**
	Wine (ml)	20	20	20	20	20
	0.5% potassium ferrocyanide solution (ml)	0.26	0.32	0.38	0.44	0.50

In the tubes of both series we added 3-4 drops of gelatin solution, and 7-8 drops of tannin solution. The content of the tubes are shaken vigorously after each addition of reagents, and filtered at the end.

For the first series, we looked in the filtrate for the presence of iron remaining in the wine. To this end, we took 5 ml of the filtrate, added a drop of ferro-ferric mixed reagent, and 0.5 ml of HCl (1/1).

For the second series, we looked in the filtrate for potassium ferrocyanide excess, by adding one drop of alum solution (double iron and ammonium sulphate). The 5th test tube showed a blue-green coloration (strong ferrocyanide excess), and the 4th test tube showed a grayish color, therefore the practical ferrocyanide dose will be the one in the third tube.

0.19 ml of ferrocyanide solution x 5 = 95 mg of potassium ferrocyanide/l of wine is the practical ferrocyanide dose, higher than the theoretical dose of 81 mg/l.

Practical application of the ferrocyanide treatment

The amount of ferrocyanide determined for each individual container is dissolved in distilled water, 1:4 (v/v), at 20-25 °C (ferrocyanide is not dissolved directly in the wine because organic acids decompose it).

After 8-10 days following the application of the treatment, clear wine is drawn from the ferrous ferrocyanide deposit into another clean tank. The wine is then filtered through filters to retain fine ferric ferrocyanide suspensions. The efficiency of filtration is verified by passing the wine through a paper filter; if, after filtration and drying, the paper filter is colorless or has the color of the wine, the wine has been well filtered; if, after filtration and drying, the paper filter turns blue, then the wine was not well filtered, having a residual amount of ferrocyanide. In this case, it is recommended to repeat the filtration through filter plates with a lower porosity (0.45 μm).

Ferric ferrocyanide residue disposal

Ferric ferrocyanide residues have a high toxicity. Their management to avoid the pollution of surface waters and the environment remains an unresolved problem, cyanide ion having a long-lasting resistance over time. Presently, ferric ferrocyanide residues are collected in special containers, and deposited in deep caves (deserted salt mines) (Țârdea, 2007).

CONCLUSIONS

The results of this study have revealed that the potassium ferrocyanide treatment has good results if some conditions are fulfilled, as follows:

- it is a pretentious treatment to be applied only by authorized oenologists;
- microprobes should be taken for each batch of wine, and the theoretical and practical doses of potassium ferrocyanide should be rigorously calculated;
- longer contact (over 10 days) of wine with the ferrocyanide deposit should be avoided, as it decomposes under the action of organic acids in wine, releasing cyanide acid;
- particular importance should be given to filtration after the treatment, in order to separate fine ferric ferrocyanide suspensions;
- the ferric ferrocyanide residue must be disposed of in compliance with the environmental protection legislation;
- potassium ferrocyanide treatment does not apply to wines with an iron content lower than 5 mg/l; in order to obtain wines with low iron content, it is advisable to keep mash and wines in metallic containers protected with acid-resistant materials, so that the proportion of technological iron in wine is as low as possible.

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Scien. Tech. Bull-Chem. Food Sci. Eng., Vol. 14(XV), 2017, 9-16

NEW N^N LIGAND WITH HYDROPHYILIC SUBSTITUENTS FOR COORDINATION COMPLEXES BASED ON M(II) BIOMETAL IONS

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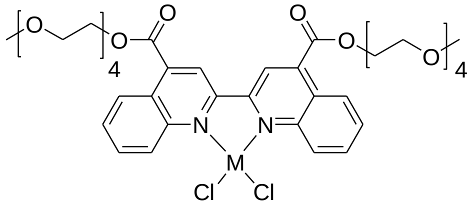
Abstract: *A new N^N chelating ligand based on 2,2'-bisquinoline functionalised with hydrophilic chains and its coordination complexes with Cu(II) and Zn(II) were synthesized and structurally characterized by IR and ^{1}H NMR spectroscopies, atomic absorption spectroscopy and elemental analysis. An accurate photophysical characterisation and conductivity measurements showed the presence of different species in polar solvents solution and in mixtures of solvent/water. The ligand and its Cu(II) and Zn(II) complexes showed no antioxidant activity.*

Keywords: *3d* transition metals; coordination complexes; photophysics; conductivity.

INTRODUCTION

Coordination complexes based on N^N chelating ligands with *3d* transition metals have intricate redox, magnetic and spectroscopic properties controlled by the coordination environment around the metal ion (Pettinari et al. 2010). The ability to tune the above mentioned properties by molecular engineering of the chemical structure allows one to design aesthetically attractive and functionally promising materials. In particular, coordination complexes based on biologically relevant metal ions like Cu(II) and Zn(II) are excellent candidates as metal-based therapeutic (Krajčiová et al. 2014, Starosta et al. 2013, García-Ramos et al. 2013, Liu et al. 2011), antimicrobial (Onawumi et al. 2013, Agwara et al. 2010) or anti-inflammatory agents (Sharma et al. 2009, Odisitse et al. 2007). Moreover, *3d* metal ions may expand the functional roles of inorganic elements applied to biology and make possible studies based on luminescence or magnetic resonance (Haas et al. 2009). However, the insufficient solubility in water of the final complexes can affect the bioavailability of the drugs (Mendiguchia et al. 2013, Jakupec et al. 2008).

The solubility of the coordination complexes, and, hence, their bioavailability may be improved, amongst other, by varying the ligand architecture (Wani et al. 2016). Herein, we report the synthesis and characterization of a new 2,2'-biquinoline ligand (*bq*) substituted in the 4,4'-position with hydrophilic chains of tetraethylene glycol (*TEG$_2$bq*). The reaction of the *bq* ligand with Cu(II) and Zn(II) chloride salts afforded the neutral metal coordination complexes with the general formula [(*TEG$_2$bq)*MCl$_2$]. The proposed chemical structure of the complexes is presented in Scheme 1.



[(*bq-TEG*)MCl$_2$]

Scheme 1. Proposed chemical structure of complexes [(*TEG$_2$bq*)MCl$_2$], where M = Cu or Zn.

The compounds were characterized by FT-IR and ^{1}H NMR (for the diamagnetic species) spectroscopies, atomic absorption spectroscopy and elemental analysis. Moreover, their photophysical properties in solution and the antioxidant activity of the newly synthesized ligand (*TEG$_2$bq*) and its Cu(II) and Zn(II) complexes ([(*TEG$_2$bq)*MCl$_2$]) were also investigated.

MATERIALS AND METHODS

Materials and methods

2,2′-biquinoline-4,4′-dicarboxylic acid and tetraethyleneglycol monomethyl ether were purchased from SigmaAldrich; $CuCl_2 \cdot 2H_2O$ and $ZnCl_2$ were purchased from Merck. All reagents and solvents were of analytical grade and used without further purification. IR spectra were recorded on a Cary 630 FT-IR spectrophotometer, as KBr pellet, in the 400–4000 cm^{-1} range. ^1H NMR spectra were run on a Bruker Fourier 300 MHz spectrometer. To determine the metal content the samples were digested using Digesdahl® Digestion Apparatus Models 23130-20, -21 by HACH (USA). The concentration of copper and zinc ions was then measured using a Flame Atomic Absorption Spectrometer (SensAA, GBC Scientific Equipment, Australia) equipped with a copper/zinc hollow cathode lamp (detection limit: 1-5 μg/mL for copper and 0.4-1.5 μg/mL respectively, integration time 3s). The flame used was an air-acetylene mixture. For each complex, two determinations were made and the average absorbance value was further used. Molar electrical conductivities were measured with a Mettler Toledo FiveEasy plus (FP30) conductivity meter equipped with a Lab conductivity sensor LE740. Melting points were determined on an Olympus BM53X model microscope equipped with a Linkam heating and freezing stage. Absorption spectra were recorded using an Agilent Cary 60 spectrophotometer. Emission spectra were recorded on an UV-Vis Perkin Elmer LS 55 spectrometer. Elemental analyses of products were carried out using a Perkin Elmer 240C-CHN analyzer.

DPPH radical-scavenging activity: The DPPH radical scavenging activity of ligand (*bq*) and complexes ([(*TEG₂bq*)MCl_2]) was performed according to the method of Brand-Williams et al. (Brand-Williams et al. 1995).

Experimental Section

Synthesis of ligand (TEG₂bq)

Di(tetraethyleneglycol) 2,2’-biquinoline-4,4’-dicarboxylate (*TEG₂bq*): A mixture of 2,2′-biquinoline-4,4′-dicarboxylic acid (0.50 g, 1.49 mmol) and thionyl chloride (50 mL) were refluxed under nitrogen until a clear yellow solution was obtained. Excess thionyl chloride was removed and the residue was dried under vacuum for 2 h. The acid chloride was suspended in toluene (30 mL) and treated with a slight excess of tetraethyleneglycol monomethyl ether (0.70 mL, 3.49 mmol). The mixture was heated under reflux for 24 h. The solvent was evaporated before the addition of chloroform (40 mL) and the mixture was washed with a solution of saturated sodium hydrogen carbonate (40 mL), was washed with water (100 mL), dried over anhydrous sodium sulphate, filtered, and evaporated to dryness. The pure product was obtained by column chromatography by using the solvent mixture ethyl acetate/methanol (9:1) as elution medium. Evaporation of the solvent gave a waxy white solid in a 63% yield. M.p. 76-78ºC. IR (cm^{-1}, KBr): 3035 (ν (CH_3)), 2876 (ν (CH_2)), 1719 (ν (C=O)), 1587 (ν (C=N)), 1547, 1501, 1453 (ν (C=C)), 1270, 1233, 1189 (ν (C=O)-O), 1152, 1116, 1095 (ν_{as} (C-O-C)), 1040 (ν_s (C-O-C)). ^1H NMR (300 MHz, $CDCl_3$): δ = 9.33 (s, 2H, $H^{3,3'}$), 8.79 (d, ^3J = 8.5 Hz, 2H, $H^{5,5'}$), 8.32 (d, ^3J = 8.4 Hz, 2H, $H^{8,8'}$), 7.83 (t, ^3J = 8.3 Hz, 2H $H^{7,7'}$), 7.70 (t, ^3J = 8.4 Hz, 2H, $H^{6,6'}$), 4.69 (m, 4H, H^{COOCH2}), 3.98 (m, 4H, H^{CH2O}), 3.85 – 3.45 (overlapped peaks, 24H; $H^{CH2CH2O}$), 3.34 (s, 6H, H^{CH3}). Anal. calcd. for $C_{38}H_{48}N_2O_{12}$ (724.79 $g \cdot mol^{-1}$): C, 62.97; H, 6.68; N, 3.87; found: C, 63.14; H, 6.60; N, 3.99.

General procedure for the synthesis of metal complexes [(*TEG₂bq)*MCl_2]: A solution of ligand (*TEG₂bq*) (0.200 g, 0.276 mmol) in ethanol (20 mL) was added to a solution of metal salts (0.414 mmol) in ethanol (10 mL) and the reaction mixture was stirred for 4 hours at r.t. Then the solvent was evaporated, the residuum dissolved in chloroform, filtrated and recrystallized with hexane.

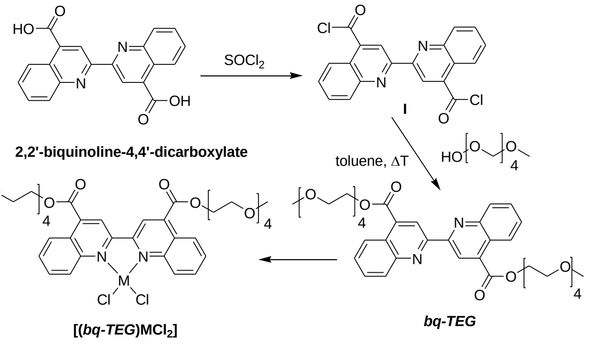
Complex [(*TEG₂bq)*$CuCl_2$]: brownish waxy solid 79% yield. M. p. 105-107ºC. IR (cm^{-1}, KBr): 3035 (ν (CH_3)), 2876 (ν (CH_2)), 1731 (ν (C=O)), 1589 (ν (C=N)), 1547, 1512, 1457 (ν (C=C)), 1261, 1239, 1210 (ν (C=O)-O), 1152, 1116, 1105 (ν_{as} (C-O-C)), 1027 (ν_s (C-O-C)). Anal. calcd. for $C_{38}H_{48}Cl_2CuN_2O_{12}$ (859.25 $g \cdot mol^{-1}$): C, 53.12; H, 5.63; N, 3.26; found: C, 52.91; H, 6.02; N, 3.19. AAS: Cu % calcd.: 7.24, found: 7.21.

Complex [(*TEG$_2$bq)*ZnCl$_2$]: yellowish waxy solid in a 95% yield. M. p. 107-109^{o}C. IR (cm^{-1}, KBr): 3083 (ν (CH$_3$)), 2894 (ν (CH$_2$)), 1733 (ν (C=O)), 1594 (ν (C=N)), 1545, 1513, 1459 (ν (C=C)), 1270, 1243, 1209 (ν (C=O)-O), 1152, 1104, 1095 (ν_{as} (C-O-C)), 1025 (ν_s (C-O-C)). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 9.11 (s, 2H, H$^{3,3'}$), 8.83 (overlapped peaks, 4H, H$^{5,5'}$, H$^{8,8'}$), 7.09 (t, ^{3}J = 8.3 Hz, 2H H$^{7,7'}$), 7.91 (t, ^{3}J = 8.4 Hz, 2H, H$^{6,6'}$), 4.78 (m, 4H, H$^{\mathrm{COO}CH2}$), 4.02 (m, 4H, H$^{CH2\mathrm{O}}$), 3.85 – 3.45 (overlapped peaks, 24H; H$^{\mathrm{CH2}CH2\mathrm{O}}$), 3.31 (s, 6H, H$^{\mathrm{CH3}}$). Anal. calcd. for $C_{38}H_{48}Cl_2N_2O_{12}Zn$ (861.09 g·mol^{-1}): C, 53.00; H, 5.62; N, 3.25; found: C, 52.81; H, 5.83; N, 3.01. AAS: Zn % calcd.: 7.59, found: 7.68.

RESULTS AND DISCUSSIONS

Synthesis and characterization

The ligand (*TEG$_2$bq*) was prepared adapting a reported method for similar derivatives (Pucci et al. 2011). In particular, the acid chloride of the 2,2'-biquinoline-4,4'-dicarboxylate (**I**) obtained by the reaction with thionyl chloride was coupled with tetraethyleneglycol monomethyl ether (Scheme 2). The pure ligand was obtained by column chromatography and its purity and chemical structure was determined by a combination of IR and ^{1}H NMR spectroscopies and elemental analysis.



Scheme 2. Synthesis of ligand *TEG$_2$bq* and complexes [(*TEG$_2$bq*)MCl$_2$], where M = Cu or Zn.

The synthesis of the complexes was performed according to the literature procedures (Pucci et al., 2012). In particular, the reaction between the ligand (*TEG$_2$bq*) and small excess of the corresponding metal chlorides in ethanol solution yielded the desired products in relatively high yields. The chemical structure of the complexes is proposed to be a tetracoordination (Figure 1), with one N^N chelating and two chloride ligands completing the coordination sphere of the metal, based on the molecular structure of the complexes having non-substituted biquinoline ligands reported in the literature (Muranishi et al. 2005). The analytical data of complexes [(*TEG$_2$bq)*MCl$_2$] (elemental analysis, atomic absorption spectroscopy analysis, IR and ^{1}H NMR spectroscopies - the latter for the diamagnetic Zn(II) complex) are consistent with the proposed structures (experimental section). In particular, the vibrational modes of *bq* ligand show the characteristic upward shifts for the coordinated ligand, with respect to the free molecule (Ozel et al. 2008).

Behavior in solution

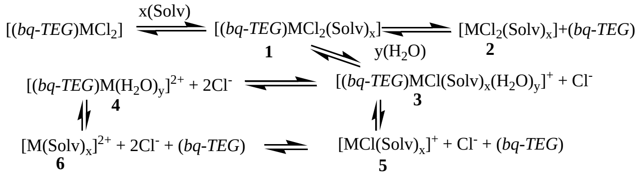
The behavior in solution for the ligand (*TEG$_2$bq*) and its Cu(II) and Zn(II) complexes was investigated by absorption and fluorescence spectroscopies and conductivity measurements.

The ligand and its Cu(II) and Zn(II) complexes are soluble in polar solvents and insoluble in water and nonpolar solvents. However, the addition of water to the polar solutions does not cause precipitation of the complexes even at high water content (95% volume).

Solutions of Cu(II) complex in different solvents are characterized by different colours: yellow in CH$_2$Cl$_2$, DMF and CH$_3$CN and blue in MeOH and EtOH, suggesting a different coordination environment around the metal centre. Moreover, the yellow colour of the CH$_2$Cl$_2$, DMF and CH$_3$CN solutions of complex [(*TEG$_2$bq)*CuCl$_2$] turns blue after addition of water. The solutions of [(*TEG$_2$bq)*ZnCl$_2$] are all colourless. After evaporating the solvents, always a brown solid is formed in the case of the Cu(II) complex and a yellow solid in the case of Zn(II) complex, that yield the same physico-chemical analysis data (AAS and elemental analysis) as the pristine [(*TEG$_2$bq*)MCl$_2$] complexes.

As documented in literature (Vatsadze et al. 2010), the dissolution of complexes of this type can occur in several steps, following different equilibrium reactions triggered by the competition of the coordinating solvent with the bidentate ligand (Scheme 3).

In particular, after the dissolution of the complex, a change of the geometry may be induced by coordinating solvents yielding penta- or hexacoordinated species (**1**, **3** and **4** in Scheme 3). Strong donor solvents may induce in steps the complete dissociation of the complex to form solvated metal chlorides (**2**, **5** and **6** in Scheme 3), as evidenced by spectrophotometric studies and ESI-mass spectrometric measurements (Vatsadze et al. 2010).



Scheme 3. Chemical species that may occur at the dissolution of complexes [(*TEG₂bq)*MCl₂] in different solvents (Solv).

Photophysical properties

In the absorption spectra of *TEG₂bq* recorded at room temperature in ethanol solution (Figure 1), two bands are detected (279 nm (ε = 31500 $M^{-1}\cdot cm^{-1}$) and 340 nm (ε = 15550 $M^{-1}\cdot cm^{-1}$)) originating from $\pi-\pi^*$ transitions on the aromatic rings of the 2,2'-biquinoline (Pucci et al., 2011). The absorption spectrum of the ligand does not change with varying the solvent. The ligand emits at 420 nm in the violet region. In Figure 1 the normalized absorption and emission spectra of the ligand in ethanol solution are presented.

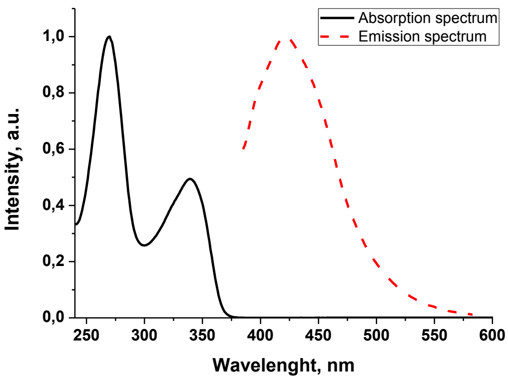


Figure 1. Absorption and emission spectra of ligand *TEG₂bq* in EtOH solution.

The UV-Vis data of complexes [(*TEG₂bq)*MCl₂] recorded in different solvents is summarized in Table 1.

Table 1. UV-Vis data for solutions of complexes [(*TEG₂bq)*MCl₂] in different solvents

Solvent	λ_{max}/nm (ε/$M^{-1}\cdot cm^{-1}$)	
	[(*TEG₂bq)*CuCl₂	[(*TEG₂bq)*ZnCl₂
CH_2Cl_2	278 (56950)	277 (46300)
	305 (11100)	310 (5800)
	376 (23070)	318 (5900)
	444 (1650)	371 (19050)
		380 (21550)
CH_3CN	272 (47080)	229 (26550)
	324 (13450)	272 (54400)
	347 (15730)	319 (10750)
	366 (12250)	357 (16050)
	445 (850)	377 (17000)
DMF	340 (23800)	340 (21850)
EtOH	272 (44400)	270 (41050)
	343 (18950)	340 (21250)
	368 (8850)	
	578 (1350)	
MeOH	270 (46050)	268 (40750)
	339 (21500)	340 (20450)
	576 (2450)	

In particular, the spectra in DMF of the Cu(II) complex is similar to that of the ligand, showing a hyperchromic effect only, whereas in CH_2Cl_2 and CH_3CN solution the lower energy $\pi-\pi^*$ transition is red shifted and a new band centred at 445 nm appears (Figure 2 up). The latter may be attributed to charge transfer transitions of tetrahedral Cu(II) complexes (Lever, 1968). Moreover, in MeOH and EtOH solutions transition bands centred at around 577 nm (Figure 2 down) suggest a change in the coordination environment around the metal centre.

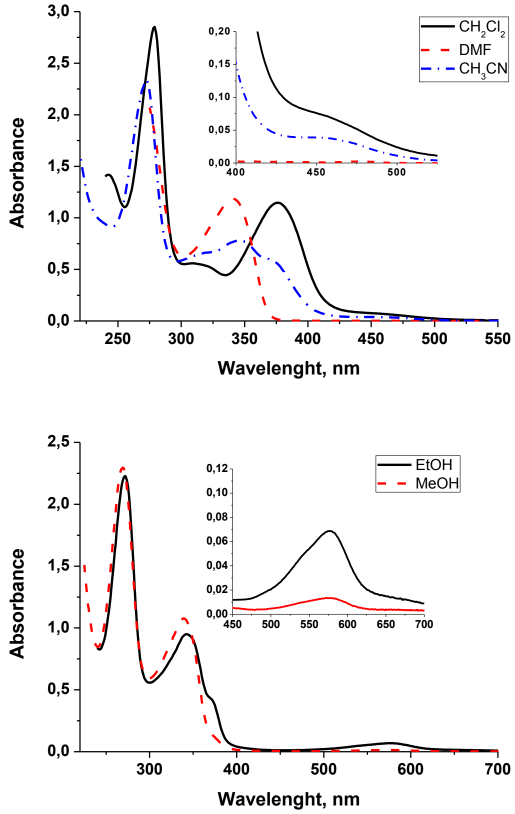


Figure 2. Absorption spectra of complex [(*TEG$_2$bq*)CuCl$_2$] in CH$_2$Cl$_2$, DMF and CH$_3$CN (up) and EtOH and MeOH (down) solutions.

In solvent/water mixture 5:95 volume, the absorption spectra of the [(*TEG$_2$bq*)CuCl$_2$] complex show similar features with the spectra of the ligand (Figure 3), but in all cases, a weak transition centred at 580 nm reveal the presence of octahedral species (Ozutsumi et al. 1991).

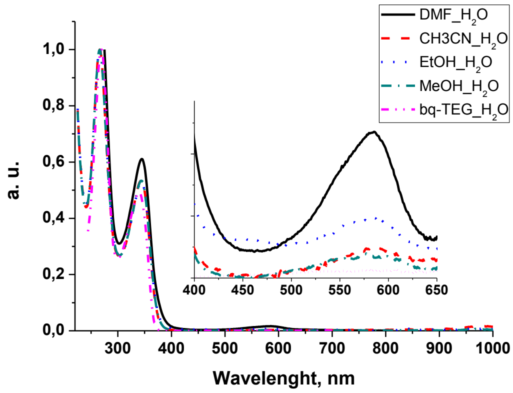


Figure 3. Absorption spectra of complex [(*TEG$_2$bq*)CuCl$_2$] in mixture of CH$_2$Cl$_2$, DMF, CH$_3$CN, EtOH and MeOH / water 5:95% volume solutions.

Regarding the Zn(II) complex, their spectra in solvents are similar with the spectra of the Cu(II) complexes, however lacking of the charge transfer bands (Figure 4).

In a mixture of solvent/water 5:95 volume, the absorption spectra of complex [(*TEG$_2$bq*)ZnCl$_2$] is similar with the absorption spectra of the ligand with small bathochromic or hypsochromic shifts.

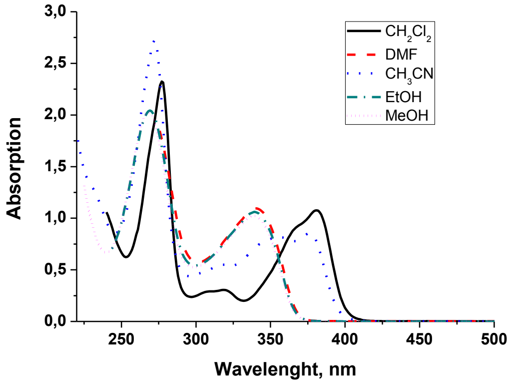


Figure 4. Absorption spectra of complex [(*TEG$_2$bq*)ZnCl$_2$] in CH$_2$Cl$_2$, DMF, CH$_3$CN, EtOH and MeOH solutions.

Regarding fluorescence, complex Zn(II) does not emit in all solvents, but in solvent/water mixture have similar emission as the ligand *TEG$_2$bq* with small blue shifts in CH$_3$CN and EtOH and red shift in MeOH (Figure 5). The change in the fluorescence spectra of Zn(II) complexes based on 2,2'-biquinoline ligand is due mainly to the conformational change of *TEG$_2$bq* ligand (Yagi et al. 1994).

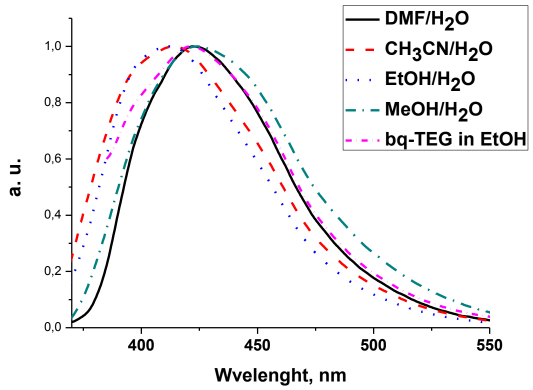


Figure 5. Emission spectra of complex [(*TEG$_2$bq*)ZnCl$_2$] in mixture of solvent/water, where solvent is CH$_2$Cl$_2$, DMF, CH$_3$CN, EtOH and MeOH.

Conductivity measurements

Conductivity measurements performed at a concentration of 10^{-3} mol·L^{-1} showed a neutral character in most of solvents, except for [(*TEG₂bq)*CuCl₂] in MeOH, where a value of 83 $\Omega^{-1}\cdot mol^{-1}\cdot cm^2$ reveal the presence of a monovalent specie (Geary, 1971). The results are presented in Table 2.

Table 2. Conductivity data for complexes [(*TEG₂bq)*MCl₂ in different solvents

Solvent	Conductivity ($\Omega^{-1}\cdot mol^{-1}\cdot cm^2$)	
	[(*TEG₂bq)*CuCl₂]	[(*TEG₂bq)*ZnCl₂]
CH_2Cl_2	< 10	< 10
DMF	34	< 10
CH_3CN	< 10	< 10
EtOH	< 10	< 10
MeOH	82	41

However, with the addition of water, monovalent or even bivalent species are formed, as showed in Figures 6 and 7.

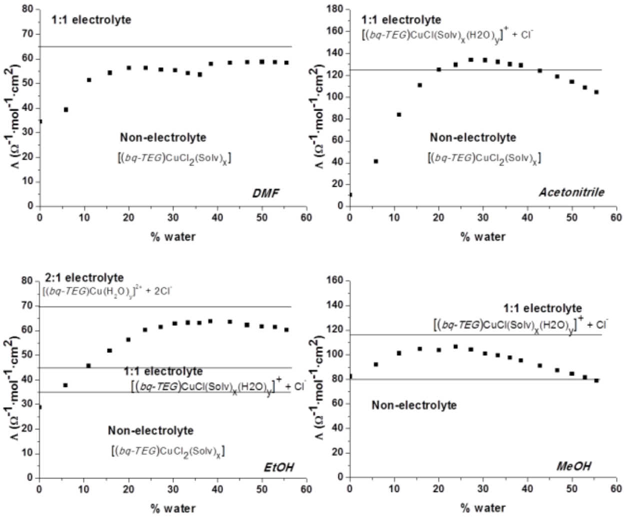


Figure 6. Conductivity measurements of complex [(*TEG₂bq)*CuCl₂] in mixture of solvent/water.

Based on the differences existent in the absorption spectra for complex [(*TEG₂bq)*CuCl₂] and in the emission spectra of complex [(*TEG₂bq)*ZnCl₂] in mixtures of solvent/water with respect to the absorption and emission spectra of the ligand, the monovalent and bivalent species are proposed to be the solvated species **3** and **4** from Scheme 3, probably in equilibrium with the solvated metal chlorides **5** and **6**.

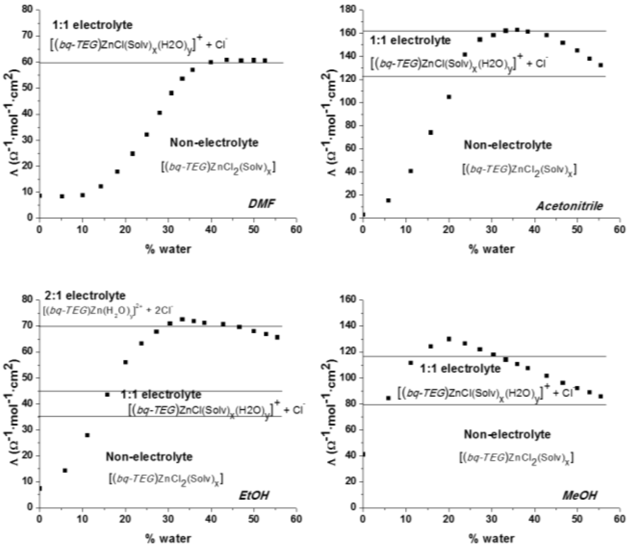


Figure 7. Conductivity measurements of complex [(*TEG₂bq)*ZnCl₂] in mixture of solvent/water.

Antioxidant activity

The antioxidant activity of the ligand *TEG₂bq* and its Cu(II) and Zn(II) complexes was tested using 1,1-diphenyl-2-picrylhydrazyl (DPPH) in ethanolic solution, according to the Brand-Williams method (Brand-Williams et al. 1995). However, no scavenging effect was evidenced for neither of compounds.

CONCLUSIONS

In search of highly soluble coordination complexes based on biometals for biological applications, we have prepared and characterized a new N^N donor ligand functionalized with hydrophilic chains. Its Cu(II) and Zn(II) complexes are highly soluble in polar solvents. The complexes are tetrahedral, with a chelating N^N ligand and two monodentate chlorine ligands completing their coordination sphere.

However, as demonstrated by an accurate photophysical investigation and conductivity measurements, in solution different species co-exist, depending on the coordinating strength of the solvent. Solutions of CH_2Cl_2, DMF and CH_3CN contains only neutral tetrahedral [(*TEG₂bq)*MCl₂] whereas in alcohols neutral octahedral species are present, formed by coordinating solvents. By addition of water to the polar solvents, ionic species are formed, the solvent displacing the chlorine ligands and/or the chelating N^N ligand.

Although the complexes did not show antioxidant activities in ethanolic solution, their use in the biological related fields may be relevant as carriers of active ligands, 2,2’-biquinoline being a well-known 1,4-diimine-type ligand, from the quinoline class, which are known to possess biological properties, such as antimicrobial, anti-inflammatory, etc. (Ingle et al. 2012).

ACKNOWLEDGEMENTS

This work was supported by the Program PNII of the MEN-UEFISCDI, project number 110/2014 and the Romanian Academy, Program 4.1.

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Scien. Tech. Bull-Chem. Food Sci. Eng., Vol. 14(XV), 2017, 17-23

FLUORESCENCE AND THERMAL STUDY OF 3D METAL COMPLEXES CONTAINING HEXADENTATE SCHIFF BASE LIGAND

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Abstract: *New binuclear $Zn_2(ZOPP\text{-}2H)(CH_3COO)_2$ complex (where ZOPP = N,N'-bis[(4-decyloxy-salicylideneamino)-propyl)-piperazine]) has been synthesized and characterized by elemental analysis, ^1H- NMR, FT-IR, UV-Vis and fluorescence spectroscopy. The non-electrolyte character of the complex was evidenced by conductivity measurement. The zinc ions are penta-coordinated by the N_2O donor sets namely one phenolate oxygen atom, one imine nitrogen and one piperazine nitrogen atoms of Schiff base and two oxygen atoms of the acetate group. The Zn(II) complex displayed fluorescence from the intra-ligand emission excited state in N, N-dimethylformamide (DMF) solution at room temperature. The thermal behaviour of Zn(II) complex and other Ni(ZOPP-2H) complex previously reported suggested a high stability of these compounds compared to ZOPP ligand.*

Keywords: 3d Schiff base complexes; fluorescence; thermal analysis

INTRODUCTION

Schiff base metal complexes have been extensively studied for years due to their selectivity and sensitivity towards the transition metal ions (Hernández-Molina et al. 2004, Andruh et al. 2005). Schiff bases derived from the salicylaldehydes and diamine are known as polydentate ligands with synthetic flexibility, which are often used as chelating ligands in the field of coordination chemistry leading to stable metal complexes (Kuma et al. 1980). Additionally, their 3d metal complexes turned out to be interesting in the fields of liquid crystals (Donnio et al. 1999, Paul et al. 2015), biological such as antimicrobial (Golcu et al. 2005), antibacterial (Chohan et al. 2010, Esmadi et al. 2016) and antifungal agents (Creaven et al. 2010), optoelectronic materials (Hai et al. 2011, Che et al. 2010) and sensors (Gebreyesus et al. 2015, Sakthinathan et al. 2017). Moreover, zinc(II) complexes containing Schiff base ligands are known as fluorescent material or candidates for biological use having intense emission at room temperature (Wen et al. 2012, Qin et al. 2009). Also, thermal behaviour of Schiff base metal complexes has been widely investigated (Ates et al. 2010, Lemos et al. 2004, Durmus et al. 2006, Bartyzel 2017), their metal oxides being used as photoconductors (Mishra et al. 2012), catalysts for a number of reactions like hydrogenation (Osawa et al. 2005), oxidation (Nichio et al. 1996, Abbasi et al. 2014) and decomposition (Deraz et al. 2009, Harraz et al. 2010).

Previously, we reported on the synthesis and characterisation of a new Schiff base ligand (ZOPP) and its coordination complexes with Cu(II) and Ni(II) metal centres (Cretu et al. 2008). In Chart 1 is presented the chemical structure of one of these metal complexes, based on Ni(II) metal centre.

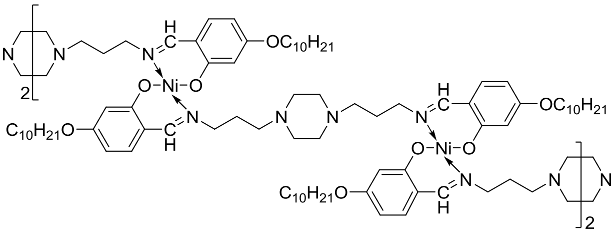


Chart 1. Chemical structure of complex **1**

In this paper we present the synthesis and physico-chemical characterization of a new binuclear zinc(II) complex of general formula $Zn_2(ZOPP\text{-}2H)(CH_3COO)_2$ based on the Schiff base ligand ZOPP. Also, the thermal stabilities of the previously reported Ni(ZOPP-2H) (**1**) and the herein synthesised $Zn_2(ZOPP\text{-}2H)(CH_3COO)_2$ (**2**), compared with ZOPP ligand are evaluated.

MATERIALS AND METHODS

Salicylaldehyde and 1,4-bis(3-aminopropyl)-piperazine, were purchased from Sigma Aldrich; zinc(II) and Ni acetate (II) was purchased from Merck. All reagents were of analytical grade and used without further purification. Elemental analysis was carried out on a Perkin Elmer model 240C elemental analyser for C, N, and H and a Flame Atomic Absorption GBC SENSAA Spectrometer. ^{1}H-NMR spectra were run on a Bruker Furier 300 MHz spectrometer. Electronic spectrum was recorded on an Agilent Cary 60 spectrometer. Electric molar conductivity was measured in dimethylformamide solution with a Mettler Toledo FiveEasy plus (FP30) conductivity meter equipped with a Lab. conductivity sensor LE740. Fluorescence spectrum was recorded on freshly prepared solution using a Perkin Elmer LS-55 spectrophotometer; the excitation slit was set at 15 and that of the emission at 5, while the scanning speed was 100 nm/min. IR spectrum was recorded on a Cary 630 FT-IR spectrophotometer, as KBr pellet, in the 400-4000 cm^{-1} range. Thermal analysis curves [thermogravimetric analysis (TGA) and differential thermal analysis (DTA)] were obtained using a TGA/SDTA 851-LF 1100 Mettler Toledo apparatus under the following conditions:

- 25-500 °C, N_2 dynamic atm. at a volumetric flow rate of 50 mL/min. and a heating rate of 10 °C/min.
- 500 °C, N_2 dynamic atm. at a volumetric flow rate of 50 mL/min. and an isothermal heating for 30 minutes under the same dynamic atmosphere.
- 500-900 °C air dynamic atm. at a volumetric flow rate of 50 mL/min. and a heating rate of 10 °C/min.

The air supplied by a compressor (4–5 bar) was passed over granular silica gel. The nitrogen was supplied from Linde gas cylinder (150 bar) of 4.6 purity class.

Synthesis

Schiff base (ZOPP) and Ni(ZOPP-2H) (**1**) were physico-chemical characterized and previously reported (Cretu et al. 2008). Here we discuss only their thermal behaviour in nitrogen – air conditions. The $Zn_2(ZOPP\text{-}2H)(CH_3COO)_2$ (**2**) complex was prepared according to the procedure described in our previous paper (Cretu et al. 2009) using a metal to ligand molar ratio of 2:1.

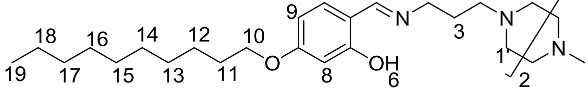
*Ni(ZOPP-2H) (**1**)*

TG/DTG/DTA (Mass loss %), solid residue of NiO: calcd. 9.60; found: 8.89.

*$Zn_2(ZOPP\text{-}2H)(CH_3COO)_2$ (**2**):* Yield: 78.4 %

Elemental analysis (%) calcd. for $C_{48}H_{76}N_4O_8Zn_2$ (967.91): C 59.56; H 7.91; N 5.79; Zn 13.51; exp. C 59.23; H 8.04; N 5.67; Zn 13.07;

Molar conductivity Λ_M, $\Omega^{-1}mol^{-1}cm^2$ (DMF, 10^{-3}M): 2.1;



^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm]: 7.95 (s, 1H, H^5); 6.90 (d, 1H, H^7); 6.31 (d, 1H, H^9); 6.17 (dd, 1H, H^8); 3.90 (t, 4H, $H^{10,4}$); 2.78-3.13 (m, 6H, $H^{1,1',2}$); 2.02 (s, 3H, $CH_{3acetate}$); 1.94 (m, 2H, H^{11}); 1.74 (m, 2H, H^3); 1.27–1.42(m, 14H, H^{12-18}); 0.88 (t, 3H, H^{19});

FT-IR (KBr) ν_{max} [cm^{-1}]: 2925, 2854 $\nu^{as,s}(CH_2)$, 1608 ν(C=N), 1528, 1436, 1400 $\nu^{as,s}(COO^-)$, 1211 ν(C-O), 683, 612 ν(Zn-O), 463 ν(Zn-N);

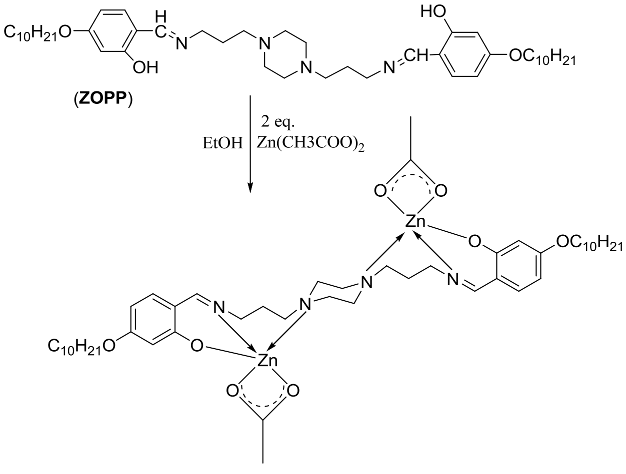
UV-Vis (DMF, 10^{-4}M) λ [nm] (ε, M^{-1}cm^{-1}): 287 (28500), 351 (21200);

Fluorescence (DMF): λex: 311 nm; λem: 424 nm.

TG/DTG/DTA (Mass loss %), solid residue of 2ZnO: calcd. 16.81; found: 16.39.

RESULTS AND DISCUSSIONS

The synthesis of the Zn(II) complex was done by reaction of stoichiometric amount of zinc (II) acetate dihydrate salt with ZOPP in ethanol as given in the Scheme 1.



$Zn_2(ZOPP\text{-}2H)(CH_3\text{-}COO)_2$

Scheme 1. The synthesis of complex **2**

The versatile nature of ZOPP allows it to act as bis-bidentate ligand for Ni(II) complex, forming a polymer compound (Chart 1) that usually is obtained when using a metal to ligand molar ratio 1:1, and as bis-tridentate ligand for Zn(II) complex, obtaining a binuclear compound for molar ratio metal to ligand 2:1. In both cases the piperazine bridge adopts a "chair" conformation. The Zn(II) complex structure is proposed by analogy with other similar complex earlier reported (Cretu et al. 2015).

The molar conductivity value at 2.1 $\Omega^{-1}mol^{-1}cm^2$ in DMF denotes non-electrolyte behaviour of **2** in this solvent (Geary 1971).

In the ^{1}H-NMR spectrum of **2** a shift of proton signals to lower values compared to its position in the free ligand was observed. Moreover, the signal at 13.92 ppm from the ligand spectrum corresponding to -OH proton is not present in the spectrum of **2**, being consistent with the coordination of the deprotonated ligand (ZOPP) to the metal centers. The methylene protons from acetate group are identified in the ^{1}H-NMR spectrum as sharp signal at 2.02 ppm.

In the FT-IR spectrum of **2** some bands appear shifted compared with the free ligand and some of them disappear as a result of complexation process. Thus, the strong band at 1626 cm^{-1} attributed to azomethine vibration mode $\upsilon(C{=}N)$ in the spectrum of free ligand is shifted to lower frequency at 1608 cm^{-1} in the spectrum of complex **2**. The absorption bands at 2925 cm^{-1} and 2854 cm^{-1} are assigned to aliphatic $\nu^{as}(CH_2)$ and $\nu^{s}(CH_2)$ group, respectively. The phenolic C-O stretching vibration at 1228 cm^{-1} in ZOPP is shifted at 1211 cm^{-1} for the complex **2** as a result of deprotonation and coordination of phenolic oxygen (Sundararajan et al. 2014). The piperazine >N-CH_2 bands disappeared in the spectrum of complex **2**, phenomenon observed when nitrogen atoms from piperazine are coordinated to metal ion. The medium bands at 1528, 1438 and 1400 cm^{-1} in the spectrum of the Zn(II) complex corresponding to $\nu^{as}(COO^-)$ and $\nu^{s}(COO^-)$ of acetate group indicates a bidentate mode of coordination, presenting Δ values significantly less than the ionic values (Nakamoto 1963). Moreover, the vibrational mode $\delta(OCO)$ from acetate group have been identified at 682 cm^{-1} for Zn(II) complex. Also, new bands at 612 cm^{-1} and 463 cm^{-1} attributed to $\nu(M–O)$ and $\nu(M–N)$, respectively were observed.

The absorption spectral data for **2** was obtained in freshly prepared DMF solution and compared with ZOPP spectrum (Figure 1). The complex spectrum shows two absorption peaks at 287 nm and 351 nm that may be assigned to $\pi - \pi^*$ intra-ligand transition.

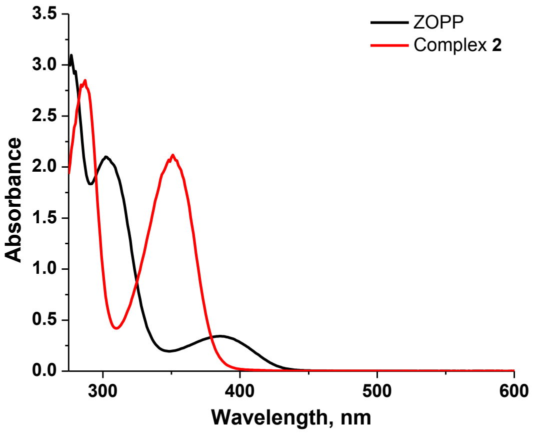


Figure 1. UV-Vis spectra of ZOPP and complex **2** in DMF (10^{-4}M)

The fluorescence spectrum of **2** recorded in DMF solution at room temperature (Figure 2) shows intense fluorescence band usually observed in similar Schiff-base Zn(II) complexes (Pucci et al. 2009), with the emission maximum wavelength at 424 nm attributed to $\pi–\pi^*$ singlet ligand-centered excited state. The emission enhancement of

Zn(II) complex is probably due to the chelation effect created through complexation process resulting in increased rigidity of the ligand and thus minimize the loss of energy (Yu et al. 2008).

A blue shift of complex emission compared to ZOPP (λem= 436 nm) was highlighted in the normalized spectrum from Figure 3.

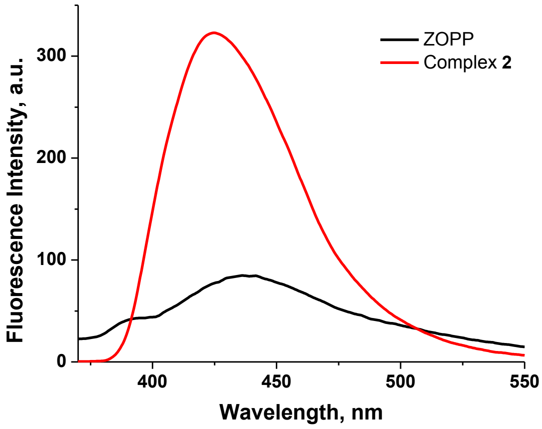


Figure 2. Emission spectra of free ligand and complex **2** in DMF solution

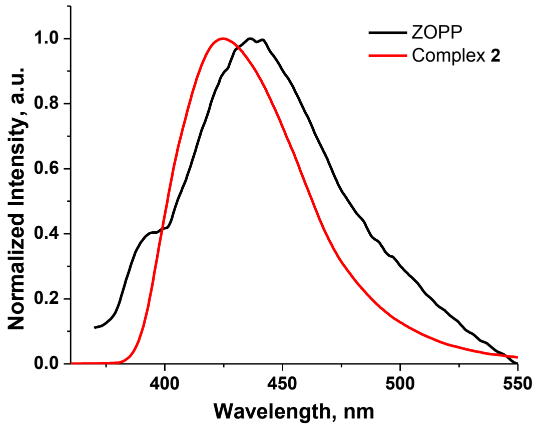
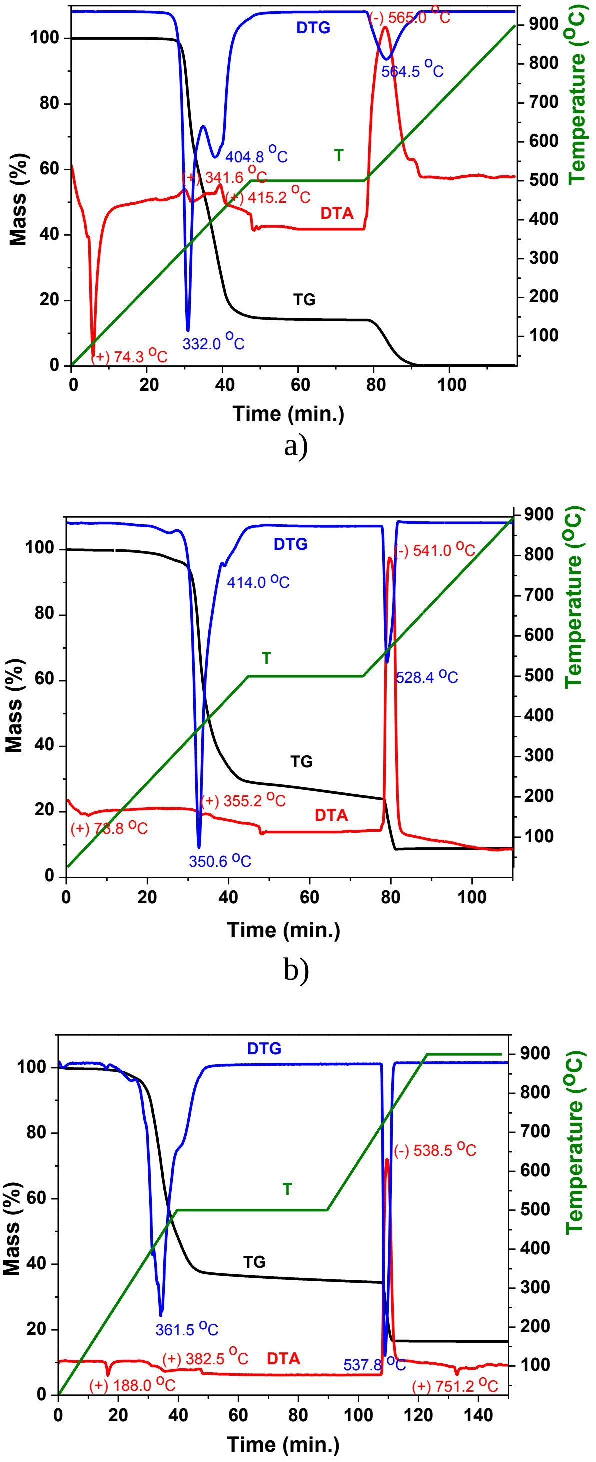


Figure 3. Normalized photoluminescence spectra of free ligand and complex **2**

Thermal behaviour of the complexes (**1** and **2)** and also of their ligand was followed up to 900 ºC under dynamic atmosphere of nitrogen or air and presented characteristic pathways of thermal decomposition, as can be seen from the TG/DTG and DTA curves (Figure 4).

The stages of decomposition, temperature range, decomposition product and weight loss percentage of ZOPP and its complexes are given in Table 1.

Thermal decomposition of the complexes is produced in four steps: two major steps highlighted on DTG curve by intense and well delimited peaks and the other two marked as shoulders. These steps have endo- and exothermic effects on DTA curve.



a)

b)

c)

Figure 4. Thermal decomposition of: a) the ligand (ZOPP), b) Ni(ZOPP-2H) and c) Zn_2(ZOPP-2H)$(CH_3COO)_2$ in nitrogen/air condition.

Both **1** and **2** complexes melt at 74 ºC (**1**) and 188 ºC (**2**) then begin to decompose. The first stage of decomposition is in the range 250-300 ºC, the weight loss corresponding to the loss of one or two water from phenolic–OH groups. The second stage of decomposition occurs in the range 300-420 ºC, indicating the loss of piperazine and an organic moiety of the complex with one endothermic DTA peak at 355 ºC (**1**) and 382 ºC (**2**), respectively. The third stage of decomposition in the range 420-500 ºC, is due to loss of two decyl chains of both complexes and two acetate molecules of **2**.

Table 1. The thermal decomposition data for the ligand and its complexes

Comp.	Temp. range (T°C)	Mass loss %		Assignment
		Calcd.	Found	
ZOPP	250-370	46.32^{a}	46.43^{a}	$2H_2O$ from OH, Piperazine, $6CH_2$, $C_7H_2N_2O$
	370-500	39.38^{a}	39.53^{a}	2 decyl
	500-900	14.14^{b}	13.78^{b}	C_7H_2O
	Solid residue	0	0	-
1	250-290	2.31^{a}	2.32^{a}	$1H_2O$ from OH
	290-370	36.50^{a}	36.88^{a}	Piperazine, $6CH_2$, C_7H_2NO
	370-500	36.50^{a}	36.54^{a}	2decyl
	500-900	17.10^{b}	15.37^{b}	$C_7H_3NO_2$
	Solid residue	9.60^{b}	8.89^{b}	NiO
2	0-300	3.71^{a}	3.21^{a}	$2H_2O$ from OH
	300-420	49.64^{a}	49.56^{a}	Piperazine, $6CH_2$, 2decyl, N_2
	420-500	14.88^{a}	12.80^{a}	2Acetate, 2CH
	500-900	18.82^{b}	18.04^{b}	$C_{12}H_2O_2$
	Solid residue	16.81^{b}	16.39^{b}	2ZnO

[a] - in nitrogen; [b] - in air

Further, for a better understanding of the decomposition process, the temperature was maintained at 500°C for 30 min. then the system was supplied with air for complete oxidation of organic residues. A continuous mass loss was observed for **1** and **2** compared with ZOPP where the sample reached constant mass (Figure 4a). The fourth step which occurs at more than 500 ºC is due to loss of other organic moiety and the resulted product were NiO and ZnO with an extremely exothermic DTA peak at 541 ºC and 538 ºC, respectively. A very interesting phenomenon was the melting point at 751 ºC observed in the Figure 5c for complex **2** that is characteristic for ZnO nanorods, greatly reduced comparing with the bulk form (Su et al. 2006). These results of decomposition sub-steps are also identified by IR spectra at 340 and 500 ºC. It is noteworthy that unlike the ligand, mass loss is significantly lower for complexes **1** and **2** in the temperature range of 25-500 °C. This behaviour suggests a high stability of the complexes, the representative mass loss taking place after the temperature of 500 ºC.

CONCLUSIONS

New Zn(II) complex was obtained by direct metal-ligand reaction using 2:1 molar ratio and fully characterized. As results of physico-chemical measurements a discrete binuclear structure was proposed for this complex with a penta-coordinate environment of Zn(II) ions described by the N_2O donor set of Schiff base and two oxygen atoms belonging to the bidentate acetate, the piperazine bridge adopting a “*chair*” conformation. This complex exhibits intense intra-ligand (π–π*) fluorescence in solution. Thermal decomposition of nickel (II) and zinc (II) complexes show four characteristic pathways and higher thermal stability compared with free ligand. The result products in both cases were metal oxides.

ACKNOWLEDGEMENTS

This work was supported by the Romanian Academy, Project 4.1.

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Scien. Tech. Bull-Chem. Food Sci. Eng., Vol. 14(XV), 2017, 24-27

NEW METHOD FOR TESTING MILK ACIDITY BASED ON VOLATILE ORGANIC COMPOUNDS EMISSION

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Abstract: *Milk storage is one of the most important condition for people access to healthy food. The volatile organic compounds pattern could be a great tool for determination of fermentation stage. The present paper has been shown that 2-butanone concentration decreased over time of storage while the acetic acid concentration depends not only by time but also by storage temperature. The emission of acetic acid could be used as a marker of fermentation degree and its concentration has been correlated with classical approach of Thorner degrees measurements.*

Keywords: milk fermentation, volatile organic compounds, milk acidity

INTRODUCTION

Milk is one of the basic foods and the way it is stored is very important for its usage by the consumers. The milk taste depends by many factors including cows feeding systems as grazing or conserved forages and concentrates (Aizaki et al., 2013; Ueda et al., 2016). Milk exposed to light and temperature can cause the development of flavour characterised as being *burnt protein, oxidised, cabbage and/or mushroom-like* (Zardin et al., 2016). Even more, other flavour as *cardboard-like, metallic or rancid* taste could be developed (Hedegaard et al., 2006). Many studies have been examined the volatile organic compounds emission from dairy products mainly regarding to terpenes used as tracers of highland milk (see for examples (Tornambe et al., 2006; Ueda et al., 2016; Viallon et al., 2000)). Furthermore, the feeding system or geographical source of milk could be identify using the pattern of volatile organic compounds (Coppa et al., 2011). The pattern of the volatiles has been used to study as well other milk products as Grana Trentino, Grana Padano, Parmigiano Reggiano cheeses (Boscaini et al., 2003), mozzarella cheeses (Gasperi et al., 2001) and even butter from different origin (van Ruth et al., 2008). The volatiles compounds emitted by the milk stored in light have been shown the presence of aldehydes such as formaldehyde acetaldehyde, pentanal, hexanal, heptanal, that increased over time, while the concentration of methanethiol increased rapidly (Zardin et al., 2016).

The aims of this study are to find a volatile organic compound marker for milk freshness and to identify the pattern of the volatile milk emission during storage.

MATERIALS AND METHODS

Milk was taken from a cow farm in Arad County. The milk collection process was carried out with a milking machine under perfect hygienic conditions. Collection of the research material was done in flasks with lid. The sample transportation was carried out in half an hour, which did not affect the physical and chemical properties of milk.

The milk has been divided in 3 parts: one has been kept in refrigerator at +4 ℃ (and used as a control sample), the second one at room temperature +25 ℃ and the third one at +40 ℃ in the full sun. Samples have been collected every 30 minutes for 7 hours. All experiments have been run in triplicate.

For sample collection of volatile organic compounds, 10 mL of milk was placed in a 50 mL plastic tube and a special pump with constant debit (SKC Inc., Houston, TX, USA) has been used to suck the air in the multibead tubes filled with carbotrap (Kannaste et al., 2014). The determinations have been made at +25 ºC. All tubes have been desorbed and analysed using Shimadzu TD20 automated cartridge desorber and Shimadzu 2010 plus GC-

MS instrument (Shimadzu Corporation, Kyoto, Japan). The chromatographic method has been described in (Copolovici et al., 2009).

The acidity of the milk (in Thorner degrees) was determined using classical method by titration with a solution of NaOH in the presence of phenolphthalein.

RESULTS AND DISCUSSIONS

Volatile organic compounds emission

There are some volatile organic compounds which have been detected from fresh milk, so even before fermentation (Figure 1).

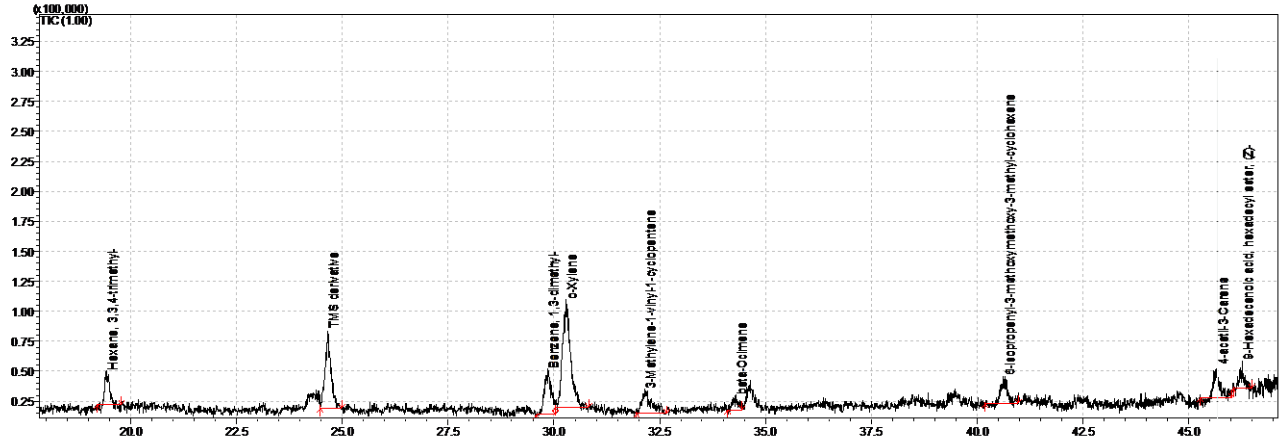

Figure 1. Volatile organic compounds profile of the milk

In the case of the milk which has been kept at +25 °C the concentration of 2-butanone decreased after 5 hours, while for the milk kept at +40 °C after 1 hour (Figure 2). The same pattern has been observed for 2-pentanone (data no show).

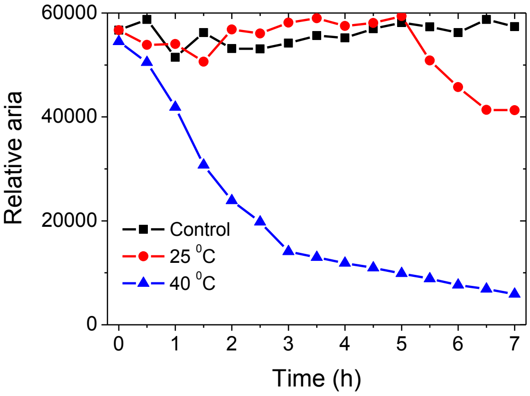

Figure 2. The evolution of 2-butanone emission

In contrast, in the same time, the emission of acetic acid is increasing (Figure 3). The emission of acetic acid is correlated with the *oxidised* taste. Such increase over time could be as well due to transformation of acetaldehyde through oxidation reaction in light (Zardin et al., 2016).

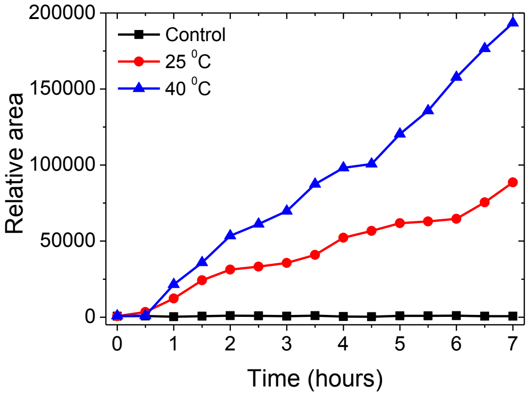

Figure 3. The evolution of acetic acid emission

While hexanal is associated with the oxidation of linoleic acid (Nukuntornprakit et al., 2015) the emission of Strecker aldehydes formed in Maillard reactions is due to thermal treatments (Makhoul et al., 2016). The formation of acetic acid in milk due to lactic acid bacteria (as *Streptococcus thermophilus* or *Lactobacillus delbrueckii ssp. bulgaricus*) has been shown for all milk products (Dan et al., 2017).

In Figure 4 it has been shown the 3 D trend of the emission of acetic acid. The trend of concentration emission is increasing with the time and storage temperature.

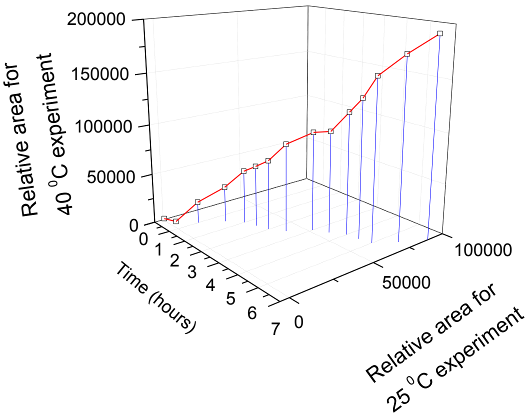

Figure 4. The 3D plot of acetic acid emission function of time

Correlation between acetic acid emission and milk acidity

A very good correlation between acetic acid emissions with the milk acidity (in Thorner degrees) has been found for both storage temperatures (Figure 5).

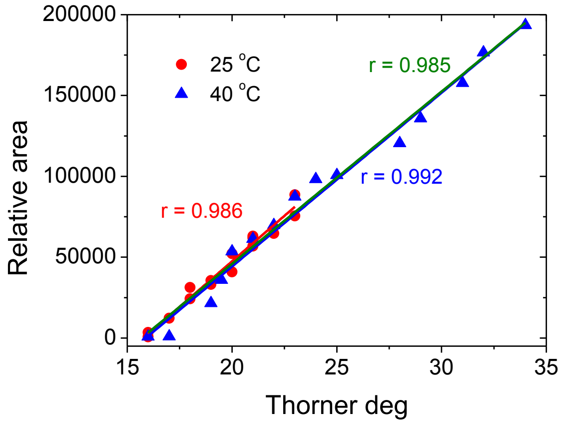

Figure 5. The correlation between acetic acid emission and Thorner degree

The slopes for both storage temperatures are statistically equal with $p < 0.05$ (11247 ± 530 for +25 °C compared with 10741 ± 378 for +40 °C). Even more, concatenated linear fitting (the green line in figure 5) has been shown a very good correlation (r = 0.985).

CONCLUSIONS

Different volatile organic compounds have been identified from milk during storage at two different temperatures. One candidate as possible marker of milk fermentation, acetic acid, has been detected and its concentration has been correlated with the classical method of determination in Thorner degrees. More work is necessary to identify more stress markers and difference in the pattern of volatile organic compounds emitted by milk during storage time.

ACKNOWLEDGEMENTS

This work was supported by the POSCCE project nr. 621/2014.

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Scien. Tech. Bull-Chem. Food Sci. Eng., Vol. 14(XV), 2017, 28-32

QUANTITATIVE STRUCTURE-FRAGMENTATION RELATIONSHIP TECHNIQUE APPLIED FOR DISCRIMINATION OF *cis*-β-OCIMENE AND *trans*-β-OCIMENE FROM LAVENDER OIL

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Abstract: *Elucidation by mass spectrometry of isomeric structures only by using a library search presents difficulties due to the high similarity of the spectra. So, the GC-MS analysis of trans-β-ocimene and cis-β-ocimene, biologically active compounds from lavender oil, offers contradictory results when are used mass spectral libraries and retention indeces database. Under these circumstances, supplementation of analysis with an independent path is absolutely necessary to clarify the configuration of these compounds. Quantitative structure-fragmentation relationships (QSFR) techniques offer such a possibility. These techniques use thermochemical data obtained through quantum chemical calculation (QCC) for structures which should be discriminated against. In our paper we opted for an ordering algorithm (ORD) which gives good results in the case of high similarity spectra. ORD uses the inverse sorting of the relative intensities' row of the main isobaric ions with the corresponding enthalpies' row. The result thus obtained validates the structures achieved by retention indices. One of the advantages of this approach is that the use of these three analytical variants can provide high accuracy of analysis without the direct use of chemical standards.*

Keywords: quantitative structure-fragmentation relationship, quantum chemical calculation, differential mass spectrometry, lavender oil, ocimene

INTRODUCTION

The limitations of search library in mass spectrometry regarding elucidation of isomeric structures with similar mass spectra are well documented (Stein 2012). In these cases, the discrimination between isomers through the interpretation of fragmentation patterns is very difficult to achieve, because the mass spectra of these isomeric analytes contain the same signals. The use of spectral libraries can give erroneous results because of (i) the low sensitivity of the search algorithm in the case of spectra with high similarity or (ii) the absence of the analyte's spectrum from the database. We have circumvented this problem by additional use of analytical information contained in the intensity of ionic currents of, for example in the case of tetrachlorinated biphenyls (Dinca et al. 2012). In this case, an additional search for the analyte is done in a database calculated for the group of possible structures provided by library search. It includes the formation enthalpies for primary fragmentations of each molecular structure considered. Because no unified enthalpies databases exist, they were calculated by us using quantum chemical methods, usually semi-empirical, which easily provide values with good relativity, on ordinary computers (Dinca et al. 2012). The ORD algorithm compares the experimental and calculated data (e.g., the difference in the relative intensity of ionic current with the corresponding fragmentation enthalpy difference) for some main ions in all possible variants of structural assignments. The result of chemical structure identification (CSI) is presented as a list of the decrease in the probability of these variants (Bettendorf *et al.* 2007). Therefore, the aim and the novelty of our work consists of: (i) the calculation of a database containing the fragmentation enthalpies involved in chemical structure identification (CSI) of *trans* β-ocimene and *cis*-β-ocimene, two biologically active compounds from lavender oil, and (ii) the use of this database for the discrimination with ORD algorithm of the above stereoisomers.

MATERIALS AND METHODS

Lavender essential oil was obtained by steam distillation using classic Clevenger equipment. The separation and identification of different compounds had been done using a GC-MS system Shimadzu 2010 Plus gas chromatography apparatus (Shimadzu, Kyoto, Japan) and triple quadrupole mass spectrometer (TQ 8040). The column used was a capillary column DB-1 (30 m length; 0.25 mm i.d.; 0.25 µm film thickness) with helium as gas carrier at 0.93 L·min^{-1}. The oven temperature setting was 70 °C for 11 min, then 5 °C/min to 190 °C and 20 °C/min to 240 °C, and then left at 240 °C for 5 min runs. The injector temperature and MS source were maintained at 250 °C and 200 °C, respectively. The ionization energy was 70 eV.

The strategy of $\Delta_f H$ *database calculation.* The heats of formation ($\Delta_f H$) were calculated with the semi-empirical method RM1 using the *HyperChem* 8.0.10 software. The geometries of the ocimenes molecules and radicals were optimized with the MM+ force field and re-optimized, using the RHF operators for molecules or ions and UHF for radicals (Rocha *et al.* 2006), the molecule being considered in vacuum. The fragmentation enthalpies ($\Delta_f H$(frag)) were calculated according to eqn. (1):

$$\Delta_f H(\text{frag}) = \Delta_f H(\text{ion}) + \Sigma\Delta_f H(\text{F}) - \Delta_f H(\text{M}) \qquad (1)$$

where $\Delta_f H$(ion) is the formation enthalpy of the main ion, $\Sigma\Delta_f H$(F) is the sum of the formation enthalpies of secondary fragments (radicals and molecules) and $\Delta_f H$(M) the formation enthalpy of the molecule.

We calculated the mass spectra similarities and the probabilities using the CSI-Diff-MS 3.1.1 software (for the ORD algorithm). Chemical structure identification was conducted by strictly adhering to the protocols for ORD as reported previously (Dinca *et al*. 2012).

RESULTS AND DISCUSSIONS

The partial chromatogram with the most volatile compounds of lavender oil is shown in Figure 1.

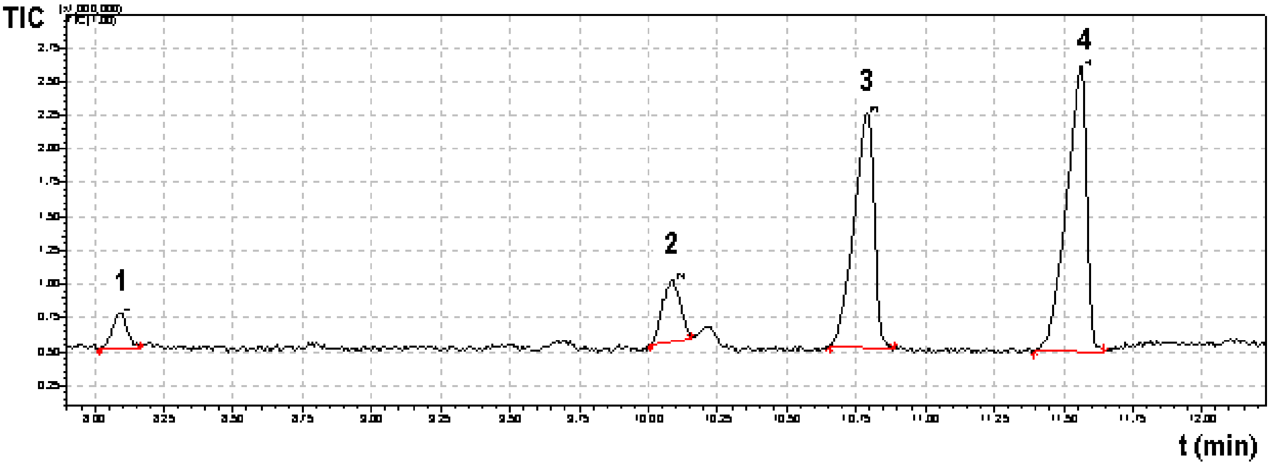


Figure 1. The first four peaks of GC-MS chromatogram of lavender oil

The search in mass spectral reference library identified the gas chromatographic peaks 3 and 4 as belonging *trans*-β-ocimene and *cis*-β-ocimene respectively (Table 1). This result is in contradiction with the structure search in retention indices (RI) database of targeted ocimenes because the retention index of *trans*-β-ocimene is greater than that of *cis*-β-ocimene (http://webbook.nist.gov). This mismatch is explicable due to the high similarity of mass spectra and very close retention indices of these compounds (Kovats RI: 1029 for *cis*-β-ocimene and 1036 for *trans*-β-ocimene). It should also be mentioned that only ocimen's formula ($C_{10}H_{16}$) corresponds to more than 240 known species of whom about 60 are isoprenoids (http://webbook.nist.gov/cgi/formula/). Under these circumstances, an additional analysis path was necessary to clarify the configurations.

Table 1. The spectral library search results

GC Peak	Retention time (min)	Compound	Molecular formula	MW
1	8.09	β-Myrcene	$C_{10}H_{16}$	136.24
2	10.07	Eucalyptol	$C_{10}H_{18}O$	154.25
3	10.78	*trans*-β-Ocimene	$C_{10}H_{16}$	136.24
4	11.54	*cis*-β-Ocimene	$C_{10}H_{16}$	136.24

The correlation between fragmentation enthalpies and differential mass spectrum provides a different analytical way that can confirm one or other of results obtained by the above mentioned methods: (i) the structure search in mass spectral reference library and (ii) in retention indices database.

Figure 2. The mirrored mass spectra of GC peak 4 (above) and GC peak 3 (down)

The mass spectra (70 eV) of *cis*-β-Ocimene and *trans*-β-Ocimene (Figure 2) are mirrored very well due to their high similarity (91%). It is obvious why the two diastereomers cannot be distinguished using only the spectral libraries. The differential mass spectrum (ΔMS) of GC *peak 4 versus* GC *peak 3* (Figure 3) clearly highlight the intensification of the allyl fragmentations: $[M-C_4H_7^{\bullet}]^+$ at m/z 81, $[M-C_5H_9^{\bullet}]^+$ at m/z 67, and the loss of methyl at m/z 121 (Scheme 1), in the diastereomer with higher energy, *trans*-β-ocimene.

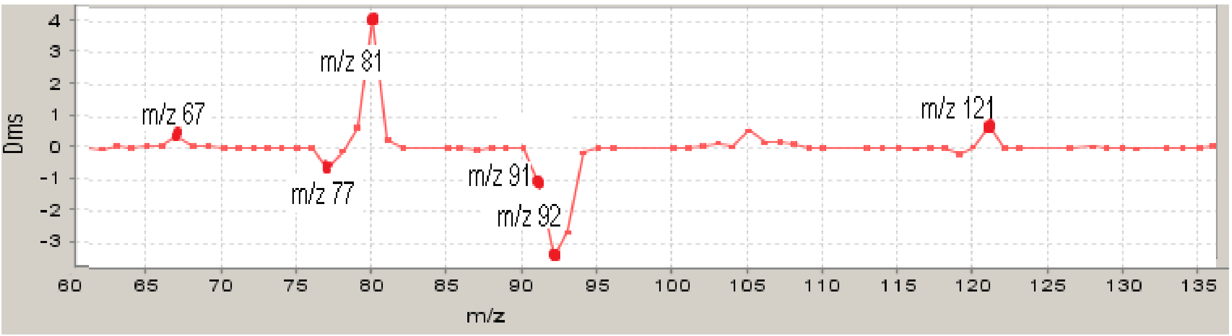
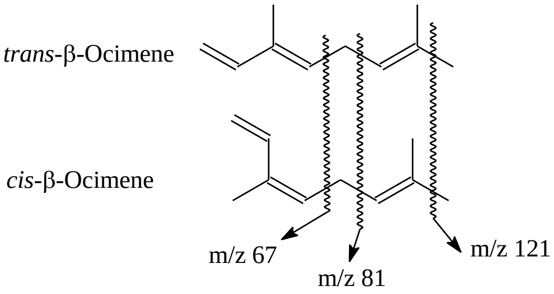


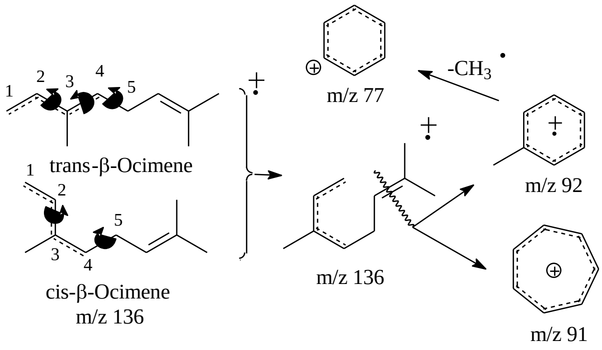
Figure 3. The differential mass spectrum (ΔMS) corresponding to the chromatogram peak 4 versus peak 3.

At the same time, one can notice a decrease in the intensity of the resulting ions by cyclization and fragmentation, m/z 92, m/z 91, and m/z 77 (Scheme 2). The decreasing of the cyclization rate of *trans*-β-ocimene is due to an additional (C3-C4) rotation which he has to do compared to *cis*-β-ocimene (Scheme 2). Its energy barrier is 11 kcal/mol (Fig. 4).



Scheme 1

Even only the common rotation C2-C3 (0.6 kcal/mol) consumes the energy excess (0.63 kcal/mol) of *trans*-β-ocimene. Values used in the ORD database (Table 2) are obtained by differential accounting (Dinca *et al.* 2012) of rotation and fragmentation energies for m/z 92, m/z 91, and m/z 77 ions of the two isomers.



Scheme 2

For the algorithm ORD applied to the isobaric ions, a good relativity of these values is sufficient even if they could have systematic errors.

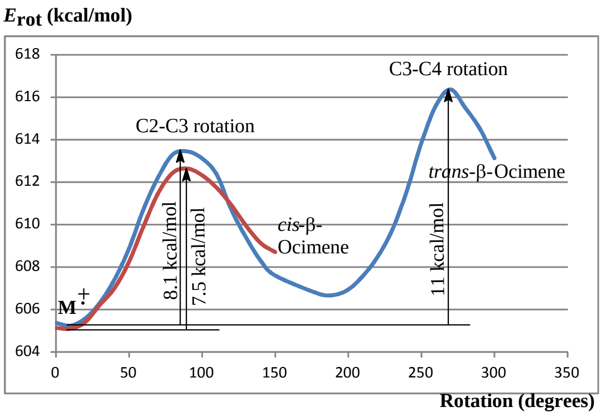
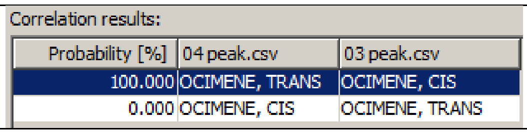


Figure 4. The rotation energy (E_{rot}) diagram of the ocimenes calculated from $\Delta_f H$ of conformers resulted by C2-C3 and C3-C4 rotations in $M^{+\cdot}$ (Scheme 2).

Table 2. The ORD probability calculation is based on the rule that the more stable ion has the higher relative intensity. The window represents the probabilities list obtained by the ordering algorithm of CSI-Diff-MS 3.1.1 software.

m/z	ΔMS (GC peaks 4 vs.3)	$\Delta_f H$(frag) difference (*cis*- vs. *trans*-)	$\Delta_f H$(frag) difference (*trans*- vs. *cis*-)
67	0.413	0.63	-0.63
77	-0.630	-2.87	2.87
81	0.250	0.45	-0.45
91	-1.079	-2.87	2.87
92	-3.409	-2.87	2.87
121	0.718	0.51	-0.51
ORD probability	 Correlation results: Probability [%] | 04 peak.csv | 03 peak.csv 100.000 | OCIMENE, TRANS | OCIMENE, CIS 0.000 | OCIMENE, CIS | OCIMENE, TRANS	0 %	100 %

The window of probabilities list resulted by software (Table 2) shows that the GC peak 3 is *cis*-β-ocimene and the GC peak 4, *trans*-β-ocimene (100% probability). This result is consistent with the retention indices of the two isomers.

CONCLUSIONS

In the computational simulations made for diastereomeric discrimination between of *trans*-β-ocimene and *cis*-β-ocimene, ORD algorithm based on QSFR strategy, invalidate the configuration achieved by the search in library spectra but it was consistent with the retention indices of the two diastereomers.

ACKNOWLEDGEMENTS

Part of this work was supported by the POSCCE project nr. 621/2014.

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Scien. Tech. Bull-Chem. Food Sci. Eng., Vol. 14(XV), 2017, 33-37

DETERMINATION OF ASCORBIC ACID IN FRUIT JUICES

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Abstract: *The present study has in attention the determination of ascorbic acid in beverages. This presents important interests as the amount of C vitamin content in certain food products, especially in beverages, has to be known by the consumers. Moreover, the food industry should not add more than 20% than the amount of vitamin C that is declared on the label. We propose an electrochemical method for the determination of ascorbic acid using the standard addition method. It was found that in most of the cases, the declared concentration of C vitamin is much lower than the ones found through electrochemical detection.*

Keywords: ascorbic acid, glassy carbon, electrochemistry

INTRODUCTION

Antioxidants in food are the most debated issue. Although the body has its own antioxidant defence system that usually reacts in case of exposure to radiation and chemical pollutants, a nutrition rich in antioxidants helps to potentiate the assimilation and metabolism of antioxidants. A diet rich in antioxidants does not increase the body concentration of these substances. Their levels remain within physiological limits due to homeostasis regulatory systems, which act for most endogenous compounds (Borut, P. et al. 2013).

As the knowledge of the various aspects of free radicals has become more extensive, the importance of antioxidants in diet has grown, becoming a subject not only in the biochemistry field, but also in nutrition, pharmacology and medicine (Ramalingum, N. et al. 2014).

Of particular importance are vitamins with antioxidant action (A, E, C and bioflavonoids) that have the most powerful effect of preventing membrane lipid peroxidation and accumulation of compounds that affect the integrity of the vascular wall. Ascorbic acid participates in the metabolism of collagen, intervening in unaltered maintenance of the vascular walls structure. Additionally, it prevents the transformations of mucopolysaccharides from the heart, avoiding alteration of selective permeability and ordered spatial disruption of the fundamental substance (Flora, S. J., 2009). Between ascorbate and cholesterol is a significant negative correlation, probably due to the vitamin's participation in the formation of 7-dehydro-cholesterol and/or other catabolism products (Pinto, J. T. et al. 2014.

The controversial roles of vitamin C could be partly explained by both the influence of concentration (by accepting diametrically opposite effects in many processes) and the interaction with other antioxidants. Thus, the interactions of vitamins C and E can be compared. In fact, although they act in different environments, lipophilic and hydrophilic membranes, their reciprocal regeneration is possible especially at interfaces of cell membranes and low density lipoproteins (LDL) particles. Unlike vitamin E that acts just at the site of peroxidation of polyunsaturated fatty acids interrupting the chain of oxidation, ascorbic acid is located in heterogeneous environments, varied and as regeneration possibilities, benefiting from the intake of several antioxidants, such as the reduced form of nicotinamide adenine dinucleotide (NADH) by the catalyzed reaction of semidehydroascorbate reductase from the mitochondria (Krajčovičová-Kudláčková, M et al. 2004).

In this sense, vitamin C is more likely to be regenerated, so oxidation stops to the dehydroascorbic acid stage that can be easily regenerated by reacting with an H donor (Traber, M. G. et al. 2011).

Although it has been discovered since the 17th century, the exact role of this vitamin in human biology and health is still a mystery in terms of benefits (Naidu, K. A. 2003).

The determination of C vitamin from pharmaceutical or food samples can be performed through different analytical methods, such as spectrophotometry (Güçlü, K. et al. 2005, Tabata, M. et al., 1997), chromatography (Dennison, D.B. et al., 1981, Lykkesfeldt, J., 2000) flow injection (Grudpan, K. et al. 1999) chemiluminescence (Alwarthan, A.A. 1993), mass spectrometry (Conley, J.M., et al. 2008) and electrochemical methods (Baghizadeh, A. et al. 2015, Bijad, M., et al. 2013, Karimi-Maleh, H., et al., 2014, Sun, W., et al., 2007, and Wang, X., et al., 2013).

The electrochemical methods have several advantages in comparison to the other analytical methods, which reside in their high sensitivity, lower cost, good accuracy, high dynamic range and simplicity.

In this paper we present a rapid and simple method for the determination of ascorbic acid from different juices using the standard addition method and electrochemical detection.

MATERIALS AND METHODS

All electrochemical experiments were performed using a Voltalab 40 (PGZ 301) potentiostat (Radiometer Analytical, France) controlled by a Voltamaster 4 electrochemical software (version 7.08).

The working, reference and counter electrode electrodes were: glassy carbon electrode (working area 0.07 cm^2), AgCl|AgCl electrode filled with 3 M KCl (BAS, Bioanalytical Systems, West Lafayette, IN, USA), and a coiled platinum electrode (23 cm). The used electrolyte was 0.02 M sodium acetate buffer, pH 4.0. Other solutions used in the experiments were the fruit juices chosen to be tested.

The glassy carbon electrodes were polished successively on alumina slurries with diameter of 5; 1; 0.3 and 0.05 μm (Buehler Ltd, USA) and then carefully rinsed with distilled water.

The working electrode prepared in this way was placed in an electrochemical cell (Ag|AgCl reference electrode (3 M KCl), and platinum wire as an auxiliary electrode). The electrodes were connected to the potentiostat.

The operating temperature of the entire system was maintained constant at 20 °C.

Reagents were prepared with distilled water. The standard solution of 1% ascorbic acid was prepared using solid ascorbic acid and 0.02 M sodium acetate buffer, pH 4.0 to limit ascorbic acid losses to less than 5%.

The fresh fruit juices were prepared at the time of use and used without further dilution.

The purpose of this experiment is to determine the concentration of ascorbic acid in commercial and home-made juices using analytical voltammetry and the standard addition method.

To the cell were initially added 10 mL of the analyte, then two successive additions of standard solution of 1 ml each were made, and each time the resulting current was measured.

RESULTS AND DISCUSSIONS

The standard addition method is used to determine the amount of ascorbic acid in juices. The basic analytical relationship is:

$$i_{aa} = k \cdot c_{aa} \quad (1),$$

where: i_{aa} is the oxidation current.

c_{aa} is the concentration of ascorbic acid.

k is a constant which depends on the type of electrodes, the number of electrons and on the dissolution capacity of ascorbic acid.

Since the value of k is not known for the juice samples, the standard addition method is used. This involves the addition of a known amount of the standard with the concentration, c_s, then the current i_{an} is measured. The value of the current i_{an} can be related to the amount of standard addition:

$$i_{an} = k \cdot c_{an} = k \frac{c_{aa} \cdot V_0 + c_s \cdot \Delta V}{V_0 + \Delta V} \quad (2)$$

Where:

n is 2 or 3

V_0 = the initial volume of the solution to be analyzed (10 mL).

ΔV = the volume of the added standard solution of C vitamin.

From the relationships (1) and (2) the concentration of ascorbic acid can be determined).

$$c_{aa} = \frac{i_{aa} \cdot c_s \cdot \Delta V}{i_{an}\left(V_0 + \Delta V\right) - i_{aa} \cdot V_0} \qquad (3)$$

The results obtained during the experiments are presented in the following tables according to the registered voltammograms (data not shown).

Table 1. The measured and calculated parameters for the first standard addition

	i_{aa}, μA	i_{a2}, μA	c_{aa}, **mg/mL**	
			determined	**label declared**
Commercial Orange Juice	7.72	14.00	100.52	15
Commercial tomatoes juice	6.11	31.04	21.79	15
Homemade tomato juice	2.41	38.39	6.05	19
Commercial grapefruit juice	4.55	13.21	45.59	35
Commercial lemon juice	3.43	17.53	21.64	9
Fresh Orange juice	11.85	23.56	84.25	53
Fresh Grapefruit juice	5.76	19.51	36.69	34
Fresh Lemon juice	7.26	29.91	28.31	45

As it can be observed, the determined values of C vitamin present in the analyzed juices are higher than the label declared values indicated by the producer (the case of commercially juices). This can be explained taking into account that on the label is stated just the amount of C vitamin intentionally added to the juice, without taking into account the amount of C vitamin naturally contained by the fruit (Rodríguez-Comesana et al. 2002).

Table 2. The measured and calculated parameters for the second standard addition

	i_{aa}, μA	i_{a3}, μA	c_{aa}, **mg/mL**	
			determined	**label declared**
Commercial Orange Juice	7.72	26.9	62.87	15
Commercial tomatoes juice	6.11	48.06	23.70	15
Homemade tomato juice	2.41	51.47	8.12	19
Commercial grapefruit juice	4.55	18.02	53.30	35
Commercial lemon juice	3.43	28.14	22.61	9
Fresh Orange juice	11.85	27.53	111.87	53
Fresh Grapefruit juice	5.76	29.93	38.20	34
Fresh Lemon juice	7.26	35.12	41.62	45

Another explaination can be attributed to the fact that the amount indicated on the label must be present in the product on the last day of its validity. The amount of ascorbic acid introduced into the product is higher and is calculated taking into account the degradation rate with temperature (Johnson, O. R, et al, 2013).

It is believed that the ascorbic acid losses of juice packaged in Tetra Pack are about 50% each month at room temperature.

CONCLUSIONS

The differences found between the values on commercially purchased juice packs and the measured ones can be attributed either to the loss of ascorbic acid due to the storage conditions or to the fact that the producer does not take into account the initial concentration of vitamin C that exists initially un the fruits juice. Another possible explanation might be attributed to the complexity of the juice matrix that can be change during the spiking of the sample with C vitamin prepared in 0.02 M acetate buffer pH 4.0. Further experiments will be necessary to validate the electrochemical method purposed in this study and to check the influence of the buffers on the matrix of the studied juices.

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Scien. Tech. Bull-Chem. Food Sci. Eng., Vol. 14(XV), 2017, 38-44

THE EFFECT OF PULSED ELECTRIC FIELD AND WINE AGING ON TOTAL PHENOL CONTENT AND COLOUR OF RED WINES

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Abstract: *Pulsed Electric Field (PEF) is a non-thermal emerging technology that is applied as pre-treatment to the extraction of bioactive compounds from grapes in order to obtain high quality wine. In the present work, we applied PEF treatment (with different parameters) to two varieties of red grapes (Pinot Noir and Merlot) and recorded the total phenols and chromatic properties (RGB, CIE L*a*b*, CIE L*C*h*, wine colour index (CI), and wine tint (Tint)) of fresh (November 2016) and aged wines (6 months, April 2017). Our results showed that the efficiency of PEF treatment depends on the variety of grapes. The best extractivity of total phenols was obtained for Pinot Noir grapes, compared to Merlot (2.5 times, and 1.4 times, respectively, compared to the untreated sample). All PEF treatments, for both Pinot Noir and Merlot wines, produce an increase in wine quality by positive higher levels of chromatic properties, wine colour properties. These properties are changing with the aging of wine, but differently for Pinot Noir and Merlot wines. Our results show the PEF treatment can be used in the winemaking process in order to increase the phenol content and consequently colour intensity in the red wine.*

Keywords: Pulsed Electric Field, red wine aging, total phenols, colour

INTRODUCTION

The studies presented in the scientific literature show that the use of various non-thermal methods in the food industry resulted in the inactivation of harmful microorganisms by destroying the cell membranes. For this reason, these methods have been used for preserving food. In recent years, non-thermal methods (especially pulsed electric field, PEF) are used in the wine industry to extract phenolic compounds (anthocyanins) as efficiently as possible in order to obtain high quality wines (López, et.al., 2008).

The use of the pulsed electric field (PEF) contributes to the increase in the extraction of bioactive compounds. This method involves low temperatures, low power consumption, and reduced processing times. Boussetta, et al., (2009), Grimi, et. al., (2009), and Puértolas, et. al. (2010) used pulsed electric field (PEF) treatment to loosen the cell membrane in vegetables and fruits. PEF treatment involves the application of short-lasting electrical field pulses at high intensity to liquid or semi-liquid foods, usually located in a room containing two electrodes (Clodoveo et al., 2016).

PEF treatment used for phenol extraction during maceration and fermentation of red grapes has been also studied by Donsi, et.al. (2010), Delsart et.al. (2012, 2014), Luengo et al. (2014), and Leong, et.al. (2016). They analyzed the extraction of anthocyanins and tannins obtained by this method. They showed that an increase in the pulsed electric field (PEF) from 1 to 7 kV/cm causes an increase in the anthocyanin and total phenol extraction rate for the three varieties investigated, namely Merlot, Syrah, and Cabernet Sauvignon.

From the polyphenol class, flavonoids are the most abundant in wine, representing over 85% of total phenols (Garcia-Martin and Sun, 2013). The flavonoid class includes colourless flavan-3-ols (catechin, epicatechin, and their procyanidins and condensed tannins). Along with these compounds, in red wines are also present anthocyanins and flavonols.

Anthocyanins are the compounds responsible for the color of grapes and wines,

being extracted from the skin of grapes along with tannins at the beginning of fermentation. During maturation, aging, and storage of wine, phenols play an important role in the colour and taste of the wine. During storage, phenols are subject to a number of reactions, resulting in colour and sensory changes. Generally, anthocyanins react with flavan-3-ols, undergo polymerization reactions and reactions that lead to the formation of pyranoanthocyanins (Ivanova et al., 2012; Remy et al, 2000).
Besides the role of phenolic compounds in viticulture and oenology, they present a broad spectrum of beneficial effects including anti-inflammatory, antimicrobial, anti-carcinogenic, antioxidant etc. (Xia et al., 2010).

The aim this of study was to highlight the influence of applying PEF treatment to two varieties of red grapes (Pinot Noir and Merlot) on total phenols and chromatic properties (RGB, CIE L*a*b*, CIE L*C*h*, wine colour index (CI) and wine tint (Tint)) of fresh (November 2016) and aged wines (6 months, April 2017).

MATERIALS AND METHODS

Samples and the process of obtaining wine

The study was performed on two varieties of red grapes: Merlot (MT) and Pinot Noir (PN) harvested in the Crişana-Sântimreu vineyard (47°14′55″N 22°2′42″E47°14′55″N 22°2′42″E) in Romania, in 2016. After declustering and crushing the grapes, the samples were PEF-treated (PN_PEF and MT_PEF). The originality of this study consists in the use of a two-conductor drum system that rotates in opposite directions through which the sample is applied.

A sample of each variety, representing the control sample (PN_CTRL and MT_CTRL), was not PEF-treated. The steps taken to obtain the wine are shown in Figure 1. The parameters used for PEF treatments and sample coding are shown in Table 1.

The method applied in our study involves the use of a voltage of 7 kV and 8 kV, respectively, a frequency of 178 Hz and 344Hz, respectively, and a processing time between 150 and 300 s (Table 1).

Table1. Parameters used for PEF-treatment

Sample no.	Distance between the drums [mm]	Voltage[kV]	Pulse Duration[s]	Frequency[Hz]
PN_CTRL & MT_CTRL	-	-	-	-
PN_PEF_13 & MT-PEF_23	2.5	7	150	178
PN_PEF_14 & MT-PEF_24	2.5	8	300	344

The untreated mash and PEF treated mash were maintained at room temperature for some days, followed by the pressing process to obtain the must and seeding with selected yeasts (*Sacharomyces cerevisiae*, EC1118) in order to reach the fermentation process. The residual sugar concentration was monitored daily and fermentation was considered finalized when the concentration of sugar was under 3g/L. At the end of the fermentation, the yeast was separated by open decanting, obtaining the clear wines, that were placed into glass bottles of 750 ml and kept in a refrigerator at 4 °C. The total phenol content, and colour parameters were determined in fresh wine and wine aged at 6 months.

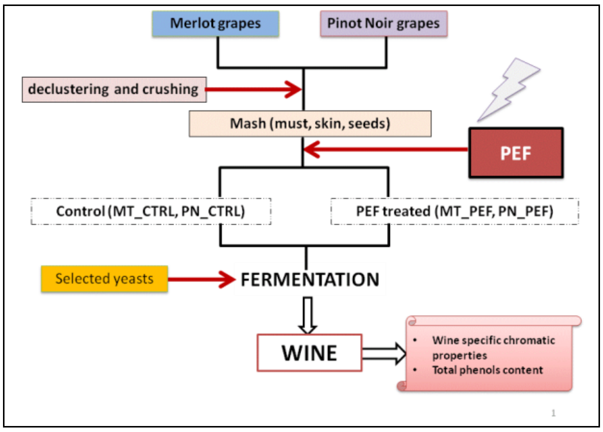

Figure 1. Flow chart of the wine-making process, after treatment with PEF of two varieties of red grapes (Pinot Noir and Merlot)

Total phenol analysis (TPh)

Total phenolic compounds from both wines (young and 6 months aged) were evaluated by the Folin-Ciocalteu method (Singleton, et al., 1999). The total polyphenol content of the samples was expressed as mg gallic acid equivalents (GAE)/L.

Chromatic analysis of wines

The chromatic properties of wine samples were determined by UV-VIS absorbance spectrum measurement using a Shimadzu PharmaSpec UV-1700 (Shimadzu Corporation, Kyoto 604-8511, Japan) with a 10 mm path-length quartz cuvette.

Wine colour intensity index (CI) was calculated by summation of the absorbance at 420nm, 520 nm, and 620 nm. Tint or shade was calculated as the percentage ratio between the 420 nm and 520 nm absorbance. Yellow colour (%Yellow), red colour (%Red), and blue colour (%Blue) percentages were determined as ratio between 420 nm, 520 nm, and 620 nm, respectively, and colour intensity [Puértolas, et al., 2011]. The evaluation of the chromatic parameters starts from the visible transmittance spectrum of wine extract between 380 and 780 nm.

The spectrum is convoluted with the colour matching functions specific for the human eyes. The convoluted functions are integrated and the results are the XYZ standard chromatic coordinates.

The transformation of XYZ coordinates in RGB coordinates allows us to optically display the rendered colour. The CIEL*a*b*and CIE L*C*h* parameters (L*, a*, b*, C*, and h*) were also calculated from XYZ chromatic coordinates.

The colour differences were generated from CIE L*a*b* coordinates: $\Delta E_{12} = [(L^*_1 - L^*_2)^2 + (a^*_1 - a^*_2)^2 + (b^*_1 - b^*_2)^2]^{1/2}$, where L* is lightness, **a*** is the chromatic axis from green(-) to red(+), **b*** is the chromatic axis from blue(-) to yellow(+) and the numeral indices are provided from two different colours.

Statistical analysis

The obtained results represent the mean value ± standard deviation and were processed by one-way analysis of variance (ANOVA). Mean value differences were analyzed with Tukey-s test ($p \leq 0.05$), using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA.

RESULTS AND DISCUSSIONS

Effect of PEF treatments on total phenol content of young and 6-month old wines

In this study, PEF treatment was used to extract as effectively as possible phenolic compounds from the skin of two varieties of grapes. The results regarding the extraction of phenols present in wine are shown in Figure 2.

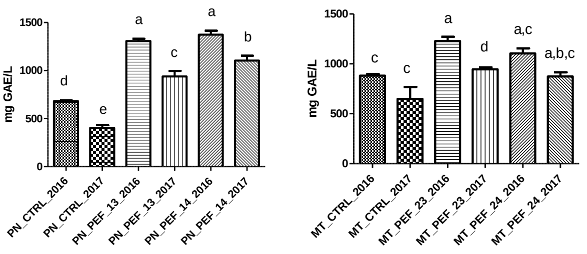

Figure 2. The effect of PEF on total phenol content (mg GAE/L) of young (2016) and 6-month old wines (2017) (Pinot Noir, PN and Merlot, MT). Different letters prescribe statistical significant differences between the samples ($p \leq 0.05$).

PEF treatment, regardless of the parameters used (voltage, frequency, and pulse duration) resulted in a significantly higher extraction compared to the control sample. PEF treatment led to an extraction of more effective total phenols in the case of PN grapes compared to MT (2.5 times and 1.4, respectively, compared with the control sample).

Puértolas et al. (2010) following red grapes treatment with PEF identified increased concentrations of (+) -catechins and (-)-epicatechins. El Darra et al., (2016) have determined that the most appropriate stage for PEF treatment to obtain red wines is during cold maceration.

Effect of PEF treatments on chromatic properties of young and 6-month old wines

Fresh and 6-month old wines obtained by treating PN and MT grapes with PEF have also been analyzed from the point of view of chromatic properties. The results obtained are presented in Tables 2 and 3.

Table 2. Chromatic properties of young Pinot Noir and Merlot wines (2016). Untreated samples (PN_CTRL, MT_CTRL) and PEF treated samples (PN_PEF_13, PN_PEF_14, MT_PEF_23, MT_PEF_24)

2016	R	G	B	L*	a*	b*	C*	h*	Colour
PN_CTRL	246	139	112	68.91	37.84	32.06	49.59	40.27	🟧
PN_PEF_13	198	59	46	45.96	54.21	39.52	67.08	36.10	🟥
PN_PEF_14	194	81	53	49.03	43.60	38.64	58.26	41.55	🟥

2016	R	G	B	L*	a*	b*	C*	h*	Colour
MT_CTRL	239	95	87	59.12	55.11	33.44	64.47	31.25	🟥
MT_PEF_23	186	1	17	38.65	63.19	46.66	78.55	36.44	🟥
MT_PEF_24	183	5	9	38.29	62.06	49.18	79.18	38.40	🟥

Table 3. Chromatic properties of 6-month old Pinot Noir and Merlot wines (2017). Untreated samples (PN_CTRL, MT_CTRL) and PEF treated samples (PN_PEF_13, PN_PEF_14, MT_PEF_23, MT_PEF_24)

2017	R	G	B	L*	a*	b*	C*	h*	Colour
PN_CTRL	248	132	102	67.61	41.66	36.09	55.12	40.90	🟧
PN_PEF_13	206	50	32	46.27	59.33	47.18	75.80	38.49	🟥
PN_PEF_14	225	93	57	56.31	49.34	45.71	67.26	42.81	🟧

2017	R	G	B	L*	a*	b*	C*	h*	Colour
MT_CTRL	240	74	64	55.88	62.55	42.51	75.63	34.20	🟥
MT_PEF_23	189	0	1	38.47	65.89	52.74	84.39	38.67	🟥
MT_PEF_24	190	0	0	39.07	65.68	55.56	86.03	40.23	🟥

At first the colour differences with the control sample as reference for each wine age was calculated (Figure 3 and 4).

All the investigated Pinot Noir PEF treatments for both fresh and aged wines present positive colour differences (ΔE). The PEF treatment variant 13 produces the highest colour difference. Also this treatment generates the highest content of anthocyanins. Thus, the PEF treatment variant 13 has the best electroporation efficiency compared with the other treatment for both young and 6-month old Pinot Noir wines.

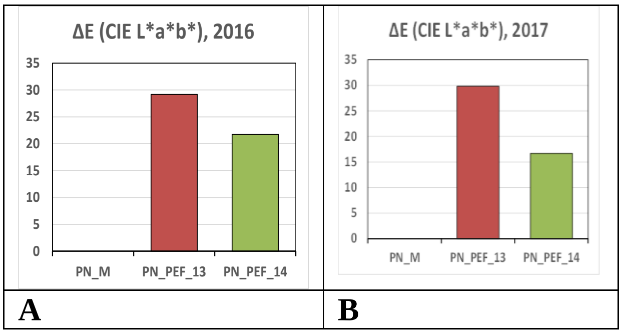

Figure 3. The colour differences with the control sample as reference for young, 2016 (A) and 6 months aged, 2017 (B) Pinot Noir wines. Untreated sample (PN_M) and PEF treated samples (PN_PEF_13, PN_PEF_14)

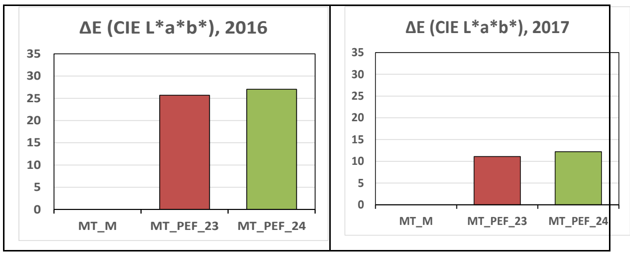

Figure 4. The colour differences with the control sample as reference for young, 2016 (A) and 6 months aged, 2017 (B) Merlot wines. Untreated sample (MT_M) and PEF treated samples (MT_PEF_23, MT_PEF_24)

The comparisons between the same treatments but for different wine ages show that the colour differences are higher for PEF treated wine samples (Figure 5). This fact suggest that, after 6 months, the Pinot Noir PEF wines present higher biochemical "activity" and are not as stable as the untreated wine sample.

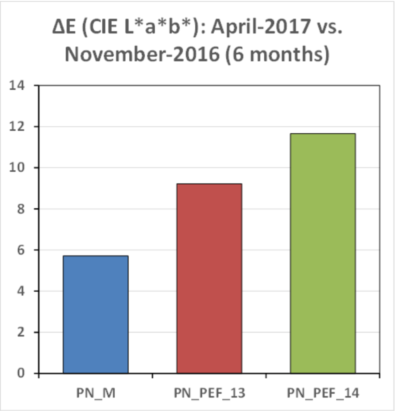

Figure 5. The colour differences between the same treatment but for different ages of Pinot Noir wines. Untreated sample (PN_M) and PEF treated samples (PN_PEF_13, PN_PEF_14)

All the investigated PEF treatments for both Merlot wine ages present positive colour differences (ΔE). Both PEF treatment variants 23 and 24 produce the higher colour difference compared with the untreated wine sample. This fact is the effect of the PEF electroporation treatment.

In order to compare the wine chromatic properties and MAP (monomeric anthocyanin pigment) content, we calculated the relative differences expressed in percentage with the control wine sample as reference (Figure 6).

MAP and CI show an increase with PEF treatment. The highest gains for MAP and CI are present for PEF variant 13, as prescribed by colour differences. This result is valid in both Pinot Noir studied wine ages.

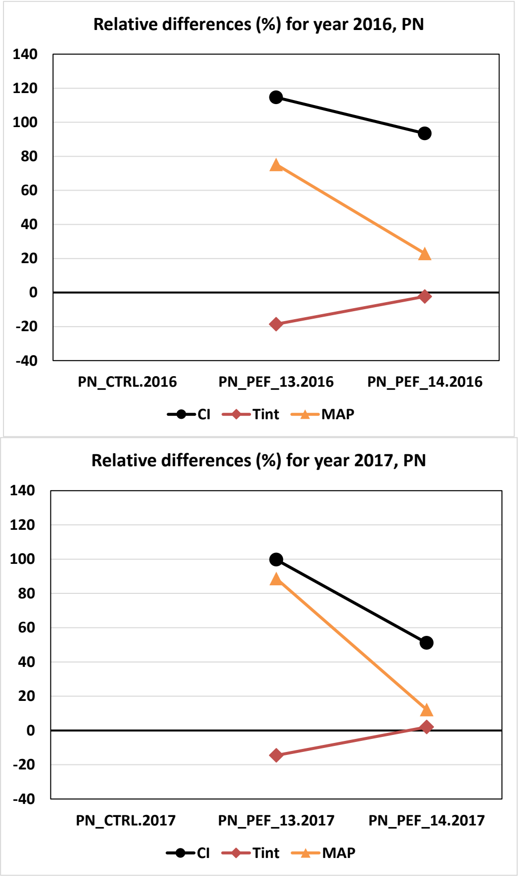

Figure 6. Relative differences of CI, Tint, and MAP expressed in percentage with the control Pinot Noir wine sample as reference. Untreated sample (PN_CTRL) and PEF treated samples (PN_PEF_13, PN_PEF_14)

Relative differences between the same PEF but different Pinot Noir wine ages show that for this wine the PEF treatment comes with biochemical instability over the aging process.

The MAP and CI parameters show positive increases with PEF treatment for the Merlot wines (Figure 7). Due to PEF electroporation, both PEF variants 23 and 24 produces higher levels of MAP and CI, compared with the untreated wine sample.

Due to relative constant relative differences between the same PEF but different Merlot wine ages, show that for this wine, the PEF treatment comes with good biochemical stability over the aging process.

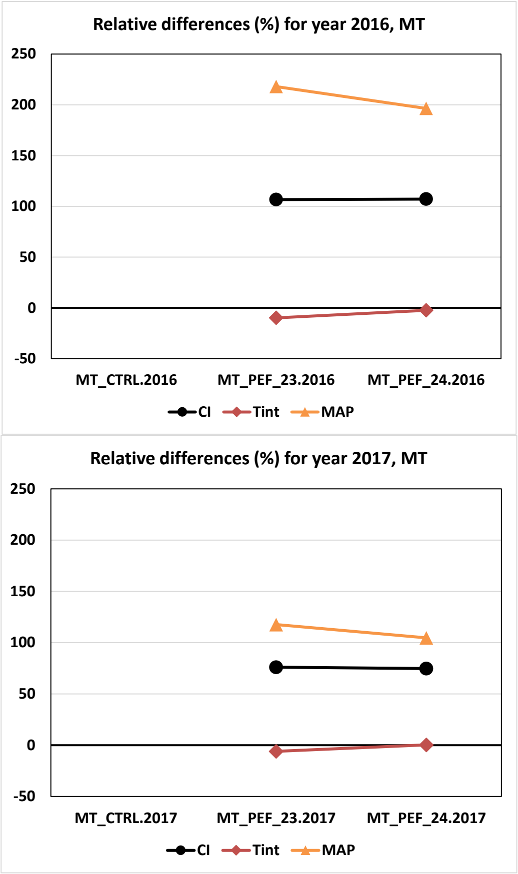

Figure 7. Relative differences of CI, Tint, and MAP expressed in percentage with the control Merlot wine sample as reference. Untreated sample (MT_CTRL) and PEF treated samples (MT_PEF_23, MT_PEF_24)

CONCLUSIONS

All PEF treatments, for both Pinot Noir and Merlot wines, produce an increase in wine quality by positive higher levels of chromatic properties, wine colour properties, and total phenols and MAP content. These properties are changing with the aging of wine, but differently for Pinot Noir and Merlot wines.

For the PEF-treated Pinot Noir wines there is biochemical instability (higher property differences between the aged PEF compared with the control wines). For the PEF Merlot wines there is better biochemical stability (lower property differences between the aged PEF compared with the control wines). Despite the wine aging process, the PEF electroporation effect is still the same after 6 months and the wines quality is the same, higher than that of the untreated wines.

ACKNOWLEDGEMENTS

This work was performed through the Partnerships Program in priority areas - PN II, developed with the support of MEN - UEFISCDI, project PN-II-PT-PCCA-2013-4-2225, No. 170/2014 “Electromagnetic methods to improve wine processes”.

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Scien. Tech. Bull-Chem. Food Sci. Eng., Vol. 14(XV), 2017, 45-48

CASE STUDIES OF FORMER ROMANIAN ERASMUS STUDENTS

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Abstract: *Student mobility, particularly Erasmus is a programme developed by European Union in 1987 aiming to increase the educational, cultural, and social interactions between young people from different universities. Many Romanian students were involved in this programme. The present study evaluated the motivation and the impact of the study stage abroad of two Romanian former students including their human development and career evolution.*

Keywords: mobility, Erasmus, motivations, capabilities

INTRODUCTION

One of the educational exchange schemes, started by European Union in 1987, is represented by the student mobility programme Erasmus (European Region Action Scheme for the Mobility of University Students). This is an international programme developed to support the mobility of young students throughout European universities and successfully had an impact not only in the participant's education but also helped to democratize, stabilize and reconciliate the relations between local societies (Amendola and Restaino, 2017; Aramburu, 2017; Beerkens et al., 2016; Dvir and Yemini, 2017; Klemencic et al., 2017; Martinez-Usarralde et al., 2017; Stapleton et al., 2016; Tuzun and Mede, 2016). Nowadays, Erasmus continues with Erasmus+ programme, and in the last 30 years 9 million people were involved in this programme mainly for education and training (https://ec.europa.eu/programmes/erasmus-plus/anniversary/erasmusplus-9-million). Many questionnaires were used by researchers to evaluate the participation into a short or long study stage abroad of the students. The barriers and drivers of the students to accept or to decline the participation in this programme is similar for students from different countries. The non-participant students from Europe indicated that they are involved in family responsabilities or work commitment, therefore it is hard for them to accept even a short study abroad (Beerkens et al., 2016). The participants in the programme indicated their initial motivation to study abroad based on: career perspectives, inter-cultural experience, availability of the Erasmus grant, available administrative support, and a good fit of the programme (Beerkens et al., 2016; Gabaudan, 2016). Amendola et al. reported that the Erasmus experience is regarded by italian students as very important in most of the cases, because they socialized with new cultures, increased skills and competencies, learnt foreign languages or improved their knowledge, but in some cases it was negatively appreciated because it could delay the graduation (Amendola and Restaino, 2017). The main obstacle faced by students is financial, therefore the need for extra budget for the study stage abroad is emphasized (Sin et al., 2017). (Suciu et al., 2017)

This study evaluated the motivation to study abroad in Erasmus programme and its impact to the human and career development of two students from Romania.

MATERIALS AND METHODS

This work focused on a case study where interviewed two formally Erasmus female students: DC and DP in order to make a descriptive analysis related to Erasmus stage period that comprise the personal experiences and achievements. This exploratory study was performed to evaluate the impact of educational programme from a foreign university during a stage of 5 months on two Romanian students in two different periods: DC 31.03.2004-31.08.2008 at Institute für Anorganische und Physikalische Chemie, Bremen University, Germany; and DP 27.01.2016-02.07.2016, Universidade do Algarve, Portugal. DC was mainly involved in research as she was PhD student at Faculty of Chemistry and Chemical Engineering, “Babes-Bolyai” University from Cluj-Napoca, while DP attended the second semester courses: Spirit drinks from Algarve production and analysis, Industrial microbiology, Valorisation of by-products in Angro-Food Industries, Water and environment, Traceability and food safety, Seminar, Portuguese course as she is a master student at Faculty of Food Engineering, Tourism and Environmental Protection from “Aurel Vlaicu” University. Also we want to focus on the evolution of capabilities (possibilities) of the subjects for real life projects after the Erasmus stage.

RESULTS AND DISCUSSIONS

The main reasons of the students for choosing to study abroad is to increase the opportunities to progress in their field and to live new experiences. The young students are firstly facing with the daily life problems in a new surrounding: new accommodation, university classrooms and laboratories, professors, colleagues, etc. This process is made easily and smoothly for the students to adapt as there are people in host universities that help Erasmus students to integrate and to meet with each other’s. The language classes are mandatory to be attended, therefore the vocabulary and your skills in the host country language are increased (Borghetti and Beaven, 2017). Also there are many trips organized in order to visit the host country, to learn about the culture and civilisation, but nevertheless to meet people.

DP: “What I really enjoyed was the fact that excursions were organized both inside Portugal and outside. The students were keen to participate in these excursions, and the students from the Students league organized everything up to the slightest detail. Some of the places I've visited are Lagos, Sagres, Silves, Albufeira, Cabo da Rocca, Sintra, Cascais, and Lisbon.”

These experiences are leading to attaining multicultural skills such as open-mindness, social initiative, emotional stability, flexibility and cultural empathy (Stapleton et al., 2016) and the interview contained, in the first part, questions from the Multicultural Personality Questionnaire developped by van der Zeen and van Oudenhoven (van der Zee et al., 2013; Van der Zee and Van Oudenhoven, 2000). So that, the following items were increased: attention to the other’s needs, listening, appreciation of other people’s stories, empathy and sympathy with others, communication, work according to a plan, work according to rules, accepting and liking routine, to try out various approaches, to find solutions, to seek people from different backgrounds, to broaden the range of interests and hobbies, to start new life easily, trendsetter in social development.

Besides the multicultural skills, the professional ones are exhibited also, as these are necessary for the job requirements, as are presented in Figure 1.

DP: “I want to tell you that homework was much more difficult than the work at the institute, as master student. After school hours, you went to the library to get the books you needed. You arrived home and you had to read and prepare the materials for the next day. Sometimes I had the impression that every day you have to work enormously so you can cope with the day ahead. All lab experiments were done individually or at most two, so you had to prepare yourself intensely. What impressed me very much is that some of the students present at the institute never complained about the high volume of work or the multitude of hours spent in the lab. My Brazilian colleagues said that what we were doing was just a small part of what they had to work in their native country”.

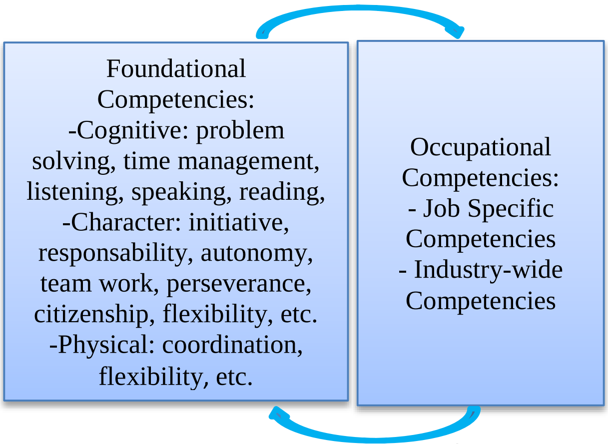

Figure 1. Competencies needed for a job requirements.

The multicultural skills are very important to be acquired during formal educational training as these will augment the person's capabilities to adapt in an international environment within a job and in a society (Figure 1). For example, DC continued to apply for other international scholarships and post-doctoral projects, meanwhile DP started to work in an international company. The students are not afraid to accept new challenges by accepting jobs in other country as well as to take into consideration the trans- and inter- disciplinarily skills needed for actual jobs.

DC: "All people, regardless of their culture or society, are the same and different, that is, the same human typologies you can meet in any international group. The probability of making friends here is much higher than at home, especially as all students find themselves in a similar situation: they are far from family and friends, they miss home, the university and all that is related to it are new, misunderstandings may arise due to language skills, etc."

Erasmus stage influenced the professional carrier of all the students, mainly in a positive pathway (Figure 1), e.g. Tiago Brandão Rodriguez, the Portuguese Minister of Education; Simona Bisboaca, a Romanian entrepreneur that develop and manufacture anti-oxidant supplements; Tania Habimana, which has a TV show in UK, etc. (https://ec.europa.eu/programmes/erasmus-plus/anniversary/).

DP: "First of all, the Erasmus stage has enriched my resume considerably. It is very important to have internships abroad because many employers appreciate and take this into account. Second, after completing this experience, your knowledge baggage is at a much higher level."

DP: "Erasmus stage has influenced my education more than positive, it has changed my perception of education first and foremost. Education is not only about civilized behavior in society, but also about accepting that we live in a world with multiple cultural varieties and that we must accept and respect everything that surrounds us, whether we like or not. We have benefited from all forms of education, both professionally and personally. When you return to your native country, you have the impression that everything is different, you wonder how you can stay in one place when the world is so big. Once you start to know, you are increasingly keen to visit and discover other places. Everywhere you get to change your perception of life and you cannot stay immune to things that are constantly changing around you."

DP: "Go at least once in such a mobility, regardless of the country, because the perception of life will change radically and I'm sure you will never regret this unique experience. Take the Erasmus experience as a perfect blend of hard work, fun, education, responsibility, etc."

Finally, we can state that the Romanian students appreciated the Erasmus experience, besides the attainment of multicultural skills, as well the achievement of professional capabilities, similar with that reported by Martinez-Usarralde et al. (Martinez-Usarralde et al., 2017). We name here several adaptive capabilities mentioned by these students: respect, mind-opening, efficiency, tolerance, independence, fair-play, responsibility, teamwork, empathy, problem solution, capacity to learn, negotiation, autonomy, courage, better adaptation in different environment /multidisciplinary groups, entrepreneurship, elevate resource management, use of technological competences, better communication both in native and foreign languages, etc.

CONCLUSIONS

Erasmus programme is a successful educational platform that had involved during the last 30 years 9 million people. The present case study confirmed, once again, the beneficial impact of the study stage of the Romanian participants from two different universities, in diverse decades, on the future life evolution. The programme contributed to the human development and to a higher empowering of the participants, making them better prepared for the labour market.

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Scien. Tech. Bull-Chem. Food Sci. Eng., Vol. 14(XV), 2017, 49-52

THE INFLUENCE OF FREEZING ON THE CONTENT OF VITAMIN C, CHLOROPHYLLS AND CAROTENOIDS IN CHIVES (*ALLIUM SCHOENOPRASUM* L.)

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Abstract: *In this paper we analyzed the influence of freezing at -18°C for 4 months, for blanched and non-blanched chives (Allium schoenoprasum* L.*) on the content of ascorbic acid, chlorophylls and carotenoids. In the case of fresh chives, the vitamin C content (determined by iodometric method) was 56.48 mg/100g; for frozen non-blanched chives, were determined losses of 49.91% and for frozen blanched chives, losses of 15.88%. The total chlorophylls concentration (determined by spectrophotometry) in fresh chives was 268.15 µg/g, of which 205.00 µg/g chlorophyll "a" and 63.15 µg/g chlorophyll "b", respectively. After the freezing treatment on the fresh chives there were determined losses of 18.95% from total chlorophylls, 18.27% chlorophyll "a" and 21.14% chlorophyll "b". Freezing of the blanched chives has caused less loss of chlorophylls (9.58% loss of total chlorophylls) compared with the same treatment applied to fresh chives. The content of carotenoids determined by using spectrophotometric methods, revealed that fresh chives had 36.41 µg/g, and losses were of 31.86% for frozen non-blanched chives and of 16.97% for frozen blanched chives.*

Keywords: chives, freezing, vitamin C, chlorophylls, carotenoids.

INTRODUCTION

Chives (*Allium schoenoprasum* L.) *Alliaceae* family, is a perennial aromatic plant, widespread in nature mostly in Europe, Asia and North America. This plant has been used for centuries to flavor food, for medicinal qualities, and has been used in some parts of the world for some religious rituals (Parvu *et al*., 2014; Rattanachaikunsopon and Phumkhachorn, 2008; Stajner *et al*., 2011).

Chives contains vitamins A, B as well as iron, sulfur, phosphorus, potassium, iodine, sodium, etc. It also contains chlorophylls, carotenoids and a lot of vitamin C that helps to absorb and fix the iron in the body, so the anemic can consume it with confidence at every meal. It is a safe source of vitamin B6, E, magnesium, iron, calcium, selenium, zinc, protein, but also water, carbohydrates, fibers and fats (Poulsen, 1990). Chives has been found to have similar medicinal properties to garlic, but weaker. The plant is used to reduce blood pressure, but also as antimicrobial and antifungal agent (Rattanachaikunsopon and Phumkhachorn, 2008). Chives are used in many dishes. Only the leaves are of interest for consumption, the bulbs being too small.

Industrially, chives leaves are processed by freezing, by hot air drying or by freeze-drying (Poulsen, 1990). Freezing is one of the most widespread methods of food preservation. As a result of the development of freezing technologies, nowadays we can get frozen products that keep almost all the qualities of fresh products (Evans, 2008) from the market.

The purpose of this paper is to study the influence of freezing with and without preliminary blanching on the content of ascorbic acid, chlorophylls and carotenoids from the chives.

MATERIALS AND METHODS

Leaves of chives (*Allium schoenoprasum* L.) were purchased fresh from the local market in Timisoara (supermarket) in May, 2016.

From the raw material were used both fresh samples as well as samples after freezing, with or without preliminary blanching treatment. In both cases, freezing was achieved at -18°C for 4 months. Blanching was done on steam for 5 minutes.

Determination of vitamin C content in fresh and frozen samples was performed according to the methodology presented by Dumbravă *et al*. (2016). The chlorophylls and carotenoids pigments levels were assessed spectrophotometrically (Dumbravă *et al*., 2012), using an UV-VIS Perkin Elmer - Lambda 25 spectrophotometer. All determinations were made in triplicates, their mean being calculating.

RESULTS AND DISCUSSIONS

Vitamin C content

The ascorbic acid content (mg/100g fresh weight) of the fresh and frozen chives samples is shown in Figure 1.

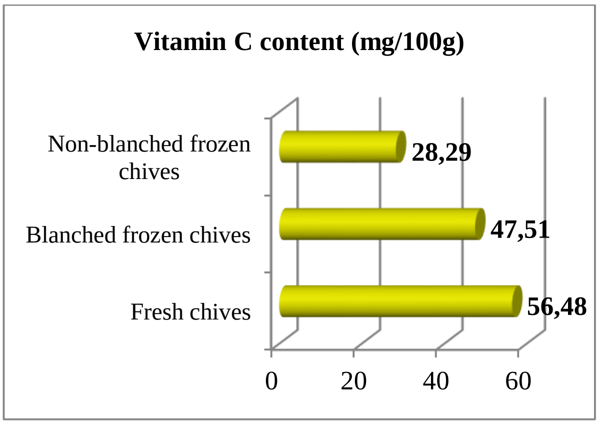

Figure 1. Ascorbic acid content in fresh and frozen chives samples.

Experimental data show that freezing causes drops in ascorbic acid concentrations in chives. In frozen chives without prior blanching, vitamin C content dropped to nearly half (28.29 mg/100g) of the value found for fresh chives leaves (56.48 mg/100g), whereas in chives blanched on steam and then frozen, the content of ascorbic acid was closer (47.51 mg/100g) to fresh chives.

The loss (%) of vitamin C in samples of non-blanched frozen chives were 49.91%, while in the case of blanched frozen chives, the losses were only 15.88%.

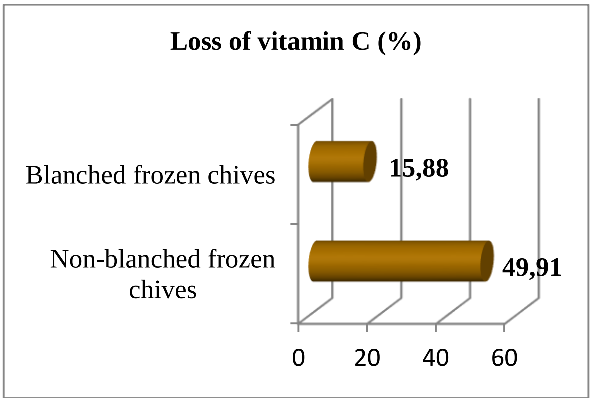

Figure 2. Loss of vitamin C as a result of chives freezing.

Chlorophyll content

Calculated values for chlorophyll "a", chlorophyll "b" and total chlorophylls (µg/g fresh weight) of the analyzed samples are shown in Figure 3.

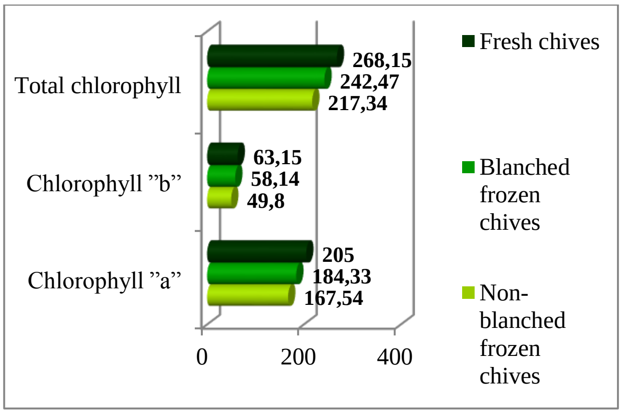

Figure 3. Chlorophyll content (µg/g fresh weight) of fresh and frozen chives.

It can be noticed that freezing causes a decrease in chlorophylls concentration compared to fresh chives, the most significant decrease being in samples of frozen chives without preliminary blanching. For non-blanched frozen chives, the biggest losses of chlorophylls were in the case of chlorophyll “b” (21.14% loss of chlorophyll “a” being of 18.27%), while for blanched frozen chives, the highest losses were in the case of chlorophyll “a” (10.08%, loss of chlorophyl “b” being of 7.93%), as is depicted in Figure 4.

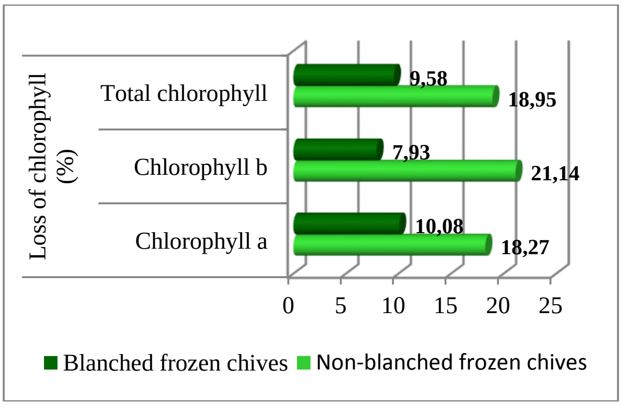

Figure 4. Chlorophyll loss in frozen chives.

Figure 5 shows the ratio of the chlorophyll "a" and chlorophyll "b" content in fresh and frozen chives.

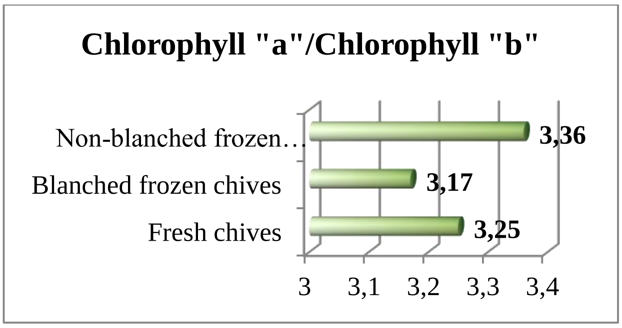

Figure 5. The ratio of chlorophyll "a" and Chlorophyll "b" in fresh and frozen chives.

Analyzing the ratio of chlorophyll "a" and chlorophyll "b", were observed close values for the analyzed samples. Compared to fresh chives, the ratio is slightly higher in the case of non-blanched frozen chives, and in blanched frozen chives the ratio is slightly lower.

Carotenoids content

The content of carotenoids (carotenes and xanthophylls) from fresh and frozen chives, determined spectrophotometrically, is shown in Figure 6.

Also with regard to carotenoids, the freezing preservation of chives leaves led to a decrease in the concentration of these compounds, the most important decrease being in the case of non-blanched frozen chives leaves.

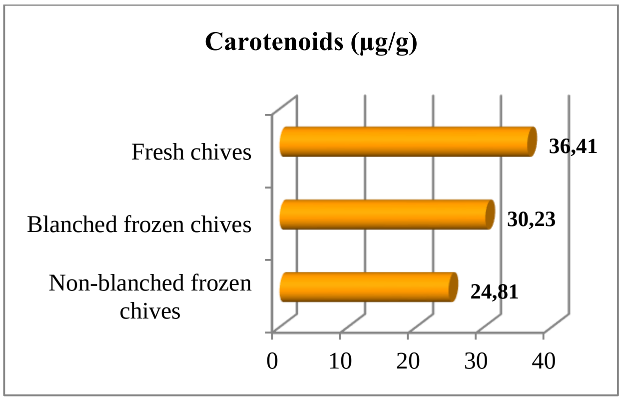

Figure 6. Carotenoids content in fresh and frozen chives (µg/g fresh weight)

For blanched frozen chives the carotenoids losses were almost two times lower (16.97%) than for non-blanched frozen ones (31.86%) (Figure 7.).

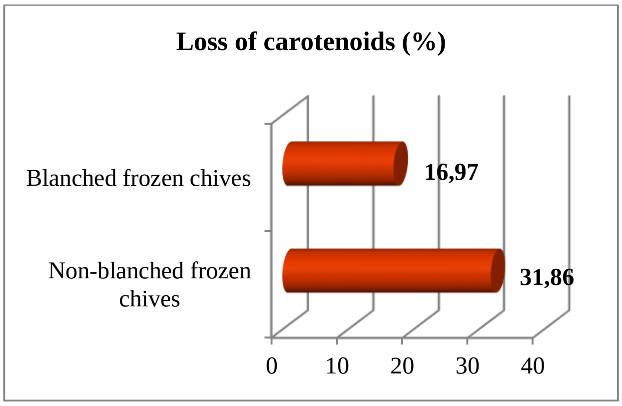

Figure 7. Carotenoids loss in frozen chives.

Kmiecik and Lisiewska (1999) also found that in chives blanched and then frozen, vitamin C, chlorophylls and carotenoids are better preserved than in non- blanched frozen chives.

CONCLUSIONS

Fresh chives are a rich source of vitamin C, chlorophylls and carotenoids, compounds that can be largely conserved by freezing chives at -18°C for 4 months, preceded by 5 minutes of blanching on steam. If the chives are frozen without pre-blanching, the losses of these compounds are higher: in this case, the highest losses were recorded for vitamin C (49.91%), then for carotenoids compounds (31.86%). More studies are needed to evaluate how other conditions and times of freezing affect the content of bioactive principles in chives leaves.

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ISSN 1582-1021

Edited by “AUREL VLAICU” University
Arad, 2017