Babes-Bolyai University Cluj-Napoca

Faculty of Chemistry and Chemical Engineering

Ph.D. Thesis

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Contributions to the study of multiple hydrogen rearrangements in mass spectra of synthetic esters and to the investigation of the properties of some natural compounds with polyphenolic structures

Scientific Advisor,

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INTRODUCTION

Organic chemistry presented a remarkable development in the last decades, researches being more and more dedicated to the synthesis of compounds with complexes structures and important practical applications in the field of chemical and materials industry, medicine, pharmaceutical and food industry. The identification and structural characterization of these compounds demanded the development of modern and exhaustive analytical techniques.

The results of the present study are structured on two main directions and underline the importance of mass spectrometry and UV-VIS spectroscopy in structural analysis and properties investigations of some new synthetic esters and natural compounds with poyphenolic structure (especially anthocyanins).

The first part of the thesis presents the synthesis and mass spectrometric analyze of some new aromatic long-chain esters, in order to investigate and elucidate some new hydrogen rearrangements observed in the mass spectra of these compounds. The nature of different factors that influence the rearrangement reactions (the length of the alkyl chain, the presence and the position of a carbon-carbon multiple bond, the presence and the nature of the substituents on the aromatic ring) was also investigated.

The second part of the thesis is dedicated to the study of the degradation processes of anthocyanic pigments isolated from various native fruits by storage and processing in different temperature and pH conditions, with or without added food additives. In the last years, the interest on improving the isolation and identification techniques of anthocyanins from vegetal sources has strongly increased. This fact is due to their potential use of these compounds as natural colorants, especially in the food industry, where they represent a nontoxic alternative to the synthetic dyes.

Keywords: mass spectrometry, triple hydrogen rearrangements, long-chain benzoic acid esters, polyphenols, anthocyanins, degradation reactions, European cranberrybush fruits, cornelian cherry fruits

PART I

MULTIPLE HYDROGEN REARRANGEMENTS IN THE MASS SPECTRA OF NEW LONG-CHAIN AROMATIC ESTERS

Theoretical aspects

Mass spectrometry is physical analytical method, first used by Thomson, which in 1912 obtains the firs mass spectra and demonstrates the possibility of using these spectra in chemical compounds analysis. [Oprean, 1974**].**

Mass spectrometry is an analytical technique used successfully to determine the masses of particles and of molecules, to elucidate the chemical structures of molecules as well as to predict and study some reaction mechanisms, generating important chemical and structural information on organic molecules. Mass spectrometry works by separating in electrostatic /magnetic field the charged molecules or molecule fragments generated by ionization. The mass spectrometer separates the fragments according to their mass-to-charge ratio.

The formation of the molecular ions follows the Franck-Condon principle [Tate & Lozier, 1932], ionization being quite a vertical process. When an electronic transition is generated by an electronic or photonic beam, the time needed for this transition is extremely short, compared to the interatomic vibration period, so the structure of the molecule will not modify during the ionization.

As a result of the received energy, the generated charged molecules undergo fragmentation processes. The molecules can follow various fragmentation pathways, which can provide important structural information.

Fragmentations which occur in the mass spectrometer can be easily explained by the Quasi-Equilibrium theory (QET), at least when the impact energy is higher as the bonding energy. [Lester, 1963]. When the molecular ion has a sufficient amount of energy it undergoes fragmentation reactions. The obtained fragments may have sufficient energy to dissociate through a similar process or rearrangements of bonds may occur. [Rosenstock, Wallenstein, Wahrhaftig & Henry Eyring, 1952].

The EI fragmentation of the charged molecule generates a positive ion and a neutral fragment (molecule or radical). Typical EI fragmentations occur through a single bond cleavage (σ-bond) when a radical generates from the molecular ion via a homolytic or a heterolytic cleavage.

Even from the beginnings of the mass spectrometry of the organic compounds, the formation of some fragments could not have been explained only by the cleavage of one or more chemical bonds. Nowadays, a large number of fragmentations that generate such ions are known. These ions were designed as "rearrangement-ions". Rearrangement reactions make mass spectra more difficult to interpret, but in the same time provide important structural information.

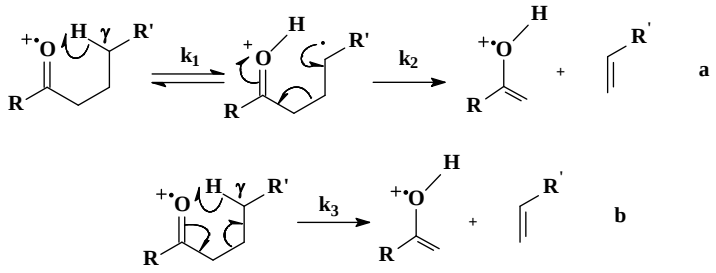
Depending on the rearrangement reactions that occur during the formation of these ions, one can differentiate fragmentations that involve the transfer of one, two or three hydrogen atoms (Hydrogen rearrangements) or rearrangements of other atoms or atoms groups from one part of the ion to another.

Not depending of the nature of the transferred atoms, all rearrangement reactions of the molecular ions are based on the following principles:

a) Rearrangement reactions take place through cyclic transitional states which are energetic favored
b) The process is accomplished by the cleavage of one neutral and very stable fragment (usually a molecule)
c) The resulted rearrangement ion is stable
d) The stereochemistry of the molecular ion allows the two parts of the molecule involved in the transfer to come as near as possible.

About 50 years ago, studding the mass spectra of different classes of organic compounds containing one double bond, McLafferty reveals the existence of a hydrogen rearrangement in the mass spectra of the aldehydes, that occurs by a hydrogen transfer towards the carbonyl double bond [McLafferty, 1959]. Using the deuterated analogs of the investigated compounds, he demonstrates that the hydrogen atom involved in the transfer is always bonded at a carbon atom in the γ position respecting the double bond (specific rearrangement) . In the same time, the cleavage of the C_α-C_β bond occurs and a cyclic transition state is formed. The mechanism of the *McLafferty* rearrangement was intensive investigated [Djerassi, von Mutzenbecher, Fajkos, Williams, & Budzikiewicz, 1965**;** Kingston, Bursey & Bursey, 1974]. Although it was originaly belived that this

rearrangement was a concertated process (Scheme 1 b) it is now belived to be a stepwise one (Scheme 1a) .



Scheme 1: Possible pathways of the McLafferty rearrangement

In the mass spectra of some organic compounds (especially esters), an intense peak "*McLafferty plus one*" was identified, corresponding to a double hydrogen rearrangement. These kind of rearrangement reactions were elucidated by deuterium marking of the compounds as well. The most hydrogen rearrangements take place through the effective gain or loss of one or more hydrogen atoms from the charged fragments (fragmentation cations) as well unidirectional [Kuck, 2005; Djerassi & Fenselau, 1965] as mutual[Eadon & Djerassi, 1969; Tökes, Jones & Djerassi, 1968]. These intramolecular migrations of two hydrogen atoms, double hydrogen rearrangemnts, can take place before or after the cleavage of a weak bond in the molecule.

Intramolecular transfer of three hydrogen atoms from one part of the charged fragment to another were very seldom observed in the mass spectra of organic compounds [Katoh, Jaeger &Djerassi, 1972; Kuck & Filges, 1988].

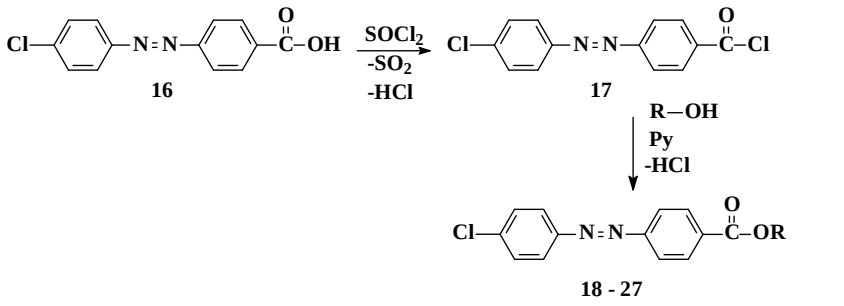
RESULTS AND DISCUSSION

As previously metioned, unidirectional double hydrogen rearrangement reactions are characteristic features in the mass spectra of many esters whereas unidirectional triple hydrogen migrations are extremely rare. The **aim** of the present study was the sinthesys of some new benzoic acid long chain esters as well as the investigation of the multiple hydrogen rearrangement reactions that take place during the EI-MS analysis of these compounds.

Esteri superiori ai acidului *p*-(4'-clorofenilazo)benzoic

In order to investigate the factors that influence the appearance and the abundance of the triple hydrogen rearrangement ions under electron impact, more than 40 *n*-alkyl, *n*-alkenyl, *n*-dienyl and *n*- alkynyl esters of p-(4'-chlorophenylazo) benzoic acid (CABE) were synthesized.

Scheme 2 presents the general reaction route for the synthesis of some new esters of p-(4'-chlorophenylazo) benzoic acid .



R = C_nH_{2n+1}; n = 1÷18

R = C_nH_{2n-1}; n = 10; 12; 13; 14; 16

R = C_nH_{2n-3}; n = 12; 14; 18 (R = dienyl)

R = C_nH_{2n-3}; n = 12 (R = alkynyl)

Scheme 2: Synthesis of p-(4'-chlorophenylazo) benzoic acid esters

The structures of the compounds obtained by this synthetic route are given in table 1.

Tabelul 1: Long chain esters of p-(4'-clorophenylazo)benzoic acid

Compound	R = alkyl	Compound	R = alkenyl
18a	CH_3	**19**	$C_{10}H_{19}$; Z 7*
18b	C_2H_5	**20a**	$C_{12}H_{23}$; Z 3
18c	C_3H_7	**20b**	$C_{12}H_{23}$; E 7
18d	C_4H_9	**20c**	$C_{12}H_{23}$; Z 7
18e	C_5H_{11}	**20d**	$C_{12}H_{23}$; Z 8
18f	C_6H_{13}	**20e**	$C_{12}H_{23}$; E 8
18g	C_7H_{15}	**20f**	$C_{12}H_{23}$; E 10
18h	C_8H_{17}	**21**	$C_{13}H_{25}$; Z 6
18i	C_9H_{19}	**22a**	$C_{14}H_{27}$; Z 5
18j	$C_{10}H_{21}$	**22b**	$C_{14}H_{27}$; E 7
18k	$C_{11}H_{23}$	**22c**	$C_{14}H_{27}$; Z 7
18l	$C_{12}H_{25}$	**22d**	$C_{14}H_{27}$; Z 8
18m	$C_{13}H_{27}$	**22e**	$C_{14}H_{27}$; Z 9
18n	$C_{14}H_{29}$	**22f**	$C_{14}H_{27}$; Z 11
18o	$C_{15}H_{31}$	**22g**	$C_{14}H_{27}$; E 11
18p	$C_{16}H_{33}$	**23a**	$C_{16}H_{31}$; Z 5
18q	$C_{17}H_{35}$	**23b**	$C_{16}H_{31}$; Z 7
18r	$C_{18}H_{37}$	**23c**	$C_{16}H_{31}$; Z 9
	R = dienyl	**23d**	$C_{16}H_{31}$; Z 11
24	$C_{12}H_{21}$; E 7, Z 9		
25	$C_{14}H_{25}$; Z 9, E 12		
26	$C_{18}H_{33}$; E 2, Z 13		
	R = alkynyl		
27	$C_{12}H_{21}$; Y 8**		

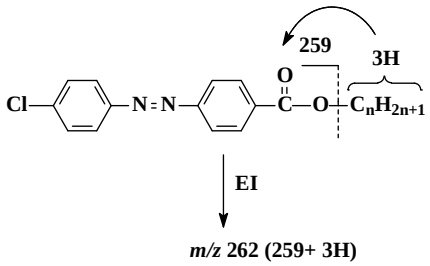
* geometry and position of the double bond ;

** position of the triple bond

The structure of the synthesized compounds was confirmed by NMR spectroscopy and mass spectrometry.

Unidirectional triple hydrogen rearrangements

The EI-MS analysis of long chain esters of *p*-(4'-chlorophenylazo)benzoic acid revealed the presence of a unidirectional hydrogen rearrangement reaction (scheme 3), generatig a fragmentation ion much more abbundant that in the cases previously mentioned.



Scheme 3: Genesis of the triple hydrogen rearrangement ion in p-(4'-clorophenylazo)benzoates

The origin of the transferred hydrogen atoms was investigated by deuterium labeling of each position in the alkyl chain, but as previously mentioned for the case of trimellitic anhydride, one can not specify the exact position of the hydrogen atoms involved in the triple rearrangement reaction, the process not presenting a high specificity. In no case, the transfer of the hydrogen atoms from the ending C-atom of the chain was observed, fact that was in agreement with the difficulty of removing a H-atom from one primary C-atom, due to the instability of the resulting radical.

Unidirectional triple hydrogen rearrangements of n-alkyl esters of p-(4'-clorophenylazo)benzoic acid

Figures 1 and 2 show the EI mass spectra (70 eV) of *n*-butyl (C_4-CABE) and *n*-tetradecyl *p*-(4'-chlorophenylazo)benzoate (C_{14}-CABE), respectively.

From Figure 1 it is apparent that, in the case of the *n*-butyl *p*-(4'-chlorophenylazo) benzoate (C_4-CABE) **18d** , the most abundant ions are generated by alternative C-N cleavage of phenylazo group (*m/z*: 111, 139, 177, 205).

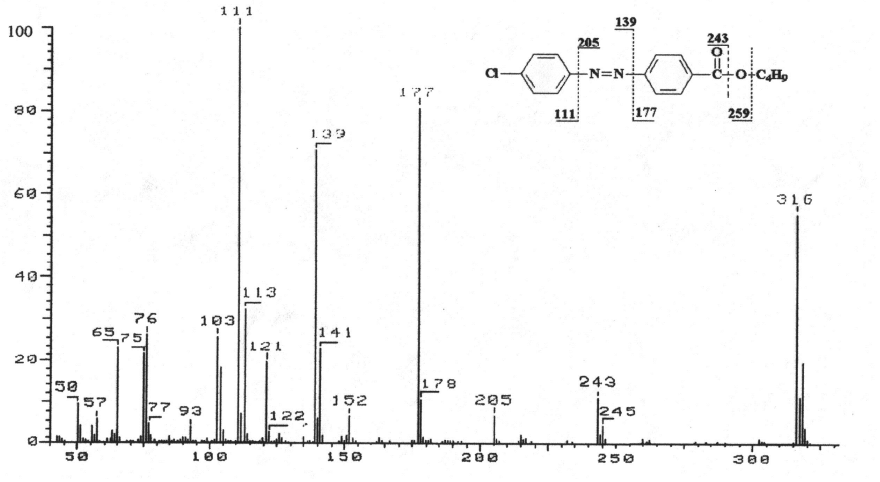


*Figure 1: Mass spectrum of n-butyl-p-(4'-chlorophenylazo)benzoate (C_4-CABE)**18d***

Cleavage of the acyl-oxygen bond generates the m/z ion 243 (10%), while an oxygen-alkyl cleavage with concurrent transfer of one or two hydrogen atoms (*m/z* 260,

respectively *m/z* 261) characteristic for *n*-butyl benzoate [Djerassi & Fenselau, 1965] has a minimal importance (I<1%).

On the contrary, it was found from Fig.2 that, in the case of *n*-tetradecyl *p*-(4'-chlorophenylazo) benzoate (C_{14}-CABE) the peak of *m/z* ion 261 (28%) corresponding to the oxygen-alkyl cleavage with transfer of two hydrogens is accompanied by a more abundant peak at *m/z* 262 (53%) corresponding to a triple hydrogen transfer. Interesting was to remark that no C-C cleavage in the long chain of the ester was observed in contrast to the other esters [Meyerson, Kuhn, Puskas, Fields, Leitch & Sullivan, 1983].

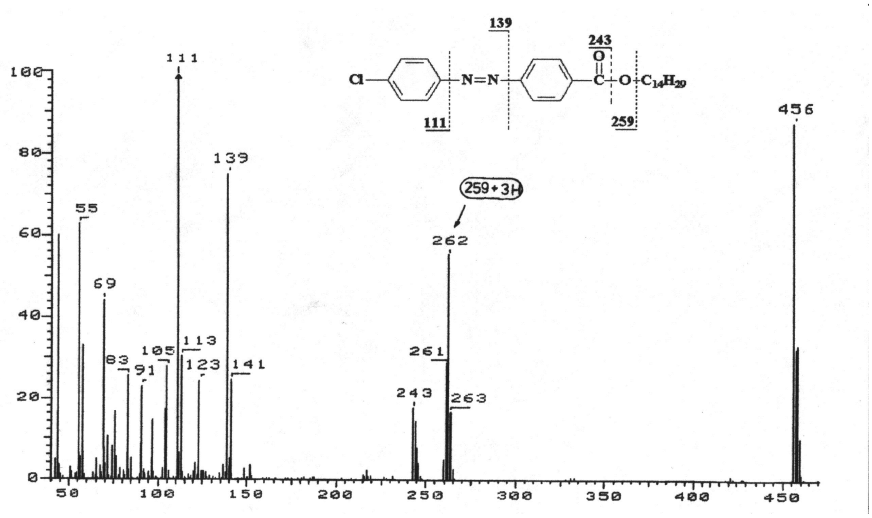


*Figure 2: Mass spectrum of n-tetradecyl-p-(4'-chlorophenylazo)benzoate **18n***

Partial spectra of C_1-C_{18} alkyl *p*-(4'-chlorophenylazo)benzoates are shown in the Table 2.

Analysing the results, is to be observed that, compounds **18a-18d** (C_1-C_4 CABE) show very similar spectrato that obtained for the *n*-butyl-*p*-(4'-chlorophenylazo)benzoate (C_4-CABE) presented in figure 1. In the spectra of **18e-18g** (C_5-C_7 CABE) the peak *m/z* 260 (1H transfer) becomes more intensive and appears a new peak at *m/z* 261 (2H transfer). The peak *m/z* 262 corresponding to a triple hydrogen transfer (3H) proceeds in the compounds **18h-18r** to the extent of 0,5-70% of the base peak of spectrum.

The abundance of this ion exhibits an increase with the increase of chain's length. The same chain length dependence shows the abundance of the ions *m/z* 261 (2H transfer) and *m/z* 244 (*m/z* 262-H_2O).

*Tabelul 2: Partial EI-MS spectra of n-alkyl p-(4'-chlorphenylazo)benzoates **18** (R=alkyl) (C_1-C_{18} CABE)*

Compound	R	Relative intensity of ions (%) (base peak=100)	
		261* (2H)	262* (3H)
18a	CH_3	-	-
18b	C_2H_5	-	-
18c	C_3H_7	-	-
18d	C_4H_9	-	-
18e	C_5H_{11}	0,6	-
18f	C_6H_{13}	1,0	-
18g	C_7H_{15}	1,7	-
18h	C_8H_{17}	3,4	0,5
18i	C_9H_{19}	6,0	8,0
18j	$C_{10}H_{21}$	7,2	7,0
18k	$C_{11}H_{23}$	14,0	35,0
18l	$C_{12}H_{25}$	19,2	30,0
18m	$C_{13}H_{27}$	27,0	60,0
18n	$C_{14}H_{29}$	27,0	53,0
18o	$C_{15}H_{31}$	41,0	92,0
18p	$C_{16}H_{33}$	35,0	70,0
18q	$C_{17}H_{35}$	31,0	62,0
18r	$C_{18}H_{37}$	42,0	87,0

* corrected for naturally occurring heavy isotopic contributions

From figure 3 we can observe that this dependence is more accentuated in the case of triple hydrogen transfer ions (m/z 262).

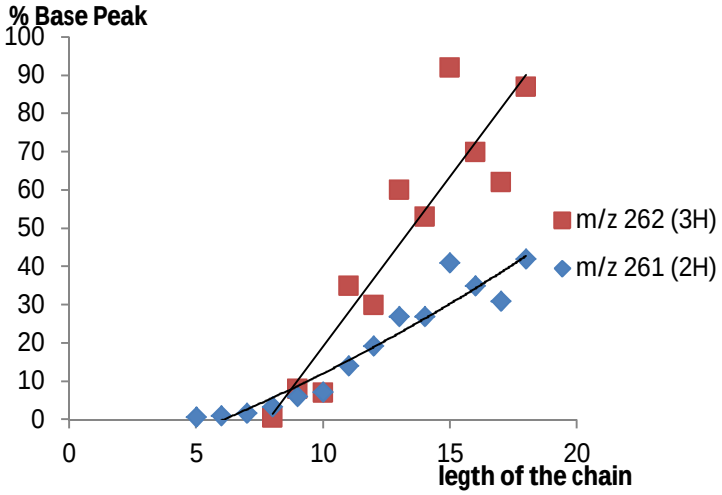
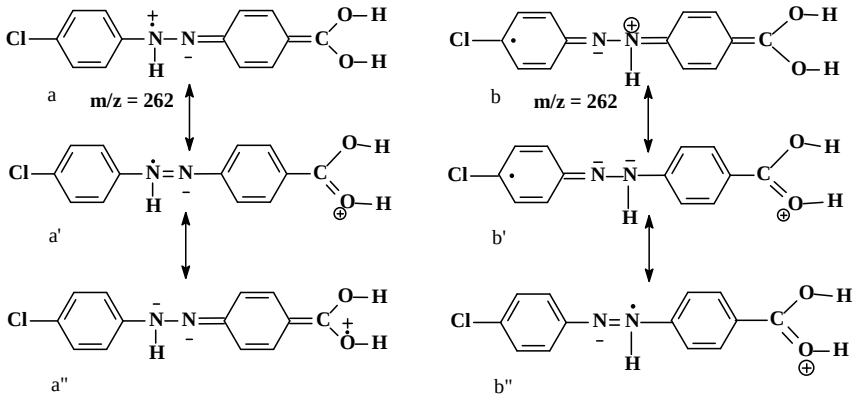


Figura 3: Effect of chain length on relative intensities of rearranged ions in C_1-C_{18} CABE

The genesis of the ion at *m/z* 262 (3H) is possible to explain through a long-range intramolecular interaction between the alkyl chain and the nitrogen atoms of the azo group, accompanied by the expected transfer of two hydrogens typical for carboxylic acid esters, in a similar manner with hydrogen migration in long chain esters of trimellitic anhydride[Cable & Djerassi, 1971]

We proposed a mechanism for the triple hydrogen rearrangement, given in scheme 4.



Schema 4: Mechanism of the genesis of triple hydrogen rearrangement ions

The multiple resonance formula of this ions (a, a', a" or b, b' ,b") explain, probably, its great abundance.

The obtained results suggested that the hydrogen atoms transfer occurs from the hydrocarbonated chain towards the azo and carbonyl groups of the molecule, a sufficiently long alkyl chain being needed in order that the two groups come close enough for the hydrogen migration. [Oprean, **Moldovan** & Oprean, 2003a; Oprean, **Moldovan** & Oprean, R., 2003b].

Transpoziţii unidirecţionale triple de hidrogen în moleculele esterilor *n*-alchenilici, *n*-alchinilici şi *n*-alcadienilici ai CABE

The mass spectra of 19 long chain unsaturated esters of p-(4'-chlorophenylazo) benzoic acid were studied, in order to confirm the triple hydrogen rearrangement process observed for the saturated analogs. Another aim of the study was to investigate the influence of the double bond position and geometry on the triple hydrogen rearrangement reations [Oprean, **Moldovan** & Oprean, R., 2003a].

From table 3 is to observe that the general feature of the spectra is similar to the spectra of the alkyl analogs. All the unsaturated compounds show a molecular peak less intensive as the saturated ester with the same chain length. Furthermore, all compounds exhibit a remarkable peak at *m/z* 262 corresponding to a triple hydrogen migration.

The intensities of this peaks are practically not influenced by the double bond geometry.

Table 3: Partial EI-MS spectra of n-alkenyl p-(4'-chlorophenylazo)benzoates

Compound	R	% Relative intensity of ions (base peak = 100)	
		261* (2H)	262* (3H)
19	Z 7**-10***	27	19
20a	Z 3-12	6	1
20b	E 7-12	33	36
20c	Z 7-12	27	26
20e	E 8-12	42	47
20d	Z 8-12	32	39
20f	E10-12	50	52
21	Z 6-13	44	55
22a	Z 5-14	11	13
22b	E 7-14	31	39
22c	Z 7-14	33	34
22d	Z 8-14	38	50
22e	Z 9-14	36	47
22g	E11-14	60	78
22f	Z11-14	56	73
23a	Z 5-16	18	27
23b	Z 7-16	35	49
23c	Z 9-16	45	61
23d	Z11-16	51	72

* corrected for naturally occurring heavy isotopic contributions

** geometry and position of the double bond

*** chain length

It is from interest to study the influence of the double bond position on the peak intensities concerning the hydrogen transfer ions. Therefor we studied the relative intensity of each peak concerning hydrogen transfer: *m/z* 260 (1H), *m/z* 261 (2H) , *m/z* 262 (3H) in the C_{12} , C_{14} and C_{16} alkenyl esters of p-(4'-chlorophenylazo)benzoic acid . It is to observe that, in all 3 series, the relative intensity of the peaks *m/z* 260 (1H) decrease with the increase of the distance between carbonyl function and double bond position in the long chain esters . On the contrary, the relative intensities of the peaks *m/z* 261 (2H) and *m/z* 262 (3H) increase with the increase of the distance between the double bond

position and the carbonyl function in the long chain esters. In all instances, the abundance of the ions *m/z* 262 (3H) is greater than the abundance of the ions *m/z* 261 (3H).

A cumulative effect of the chain length and double bond position upon the magnitude of triple hydrogen migration ions *m/z* 262 in the spectra of p-(4'-chlorophenylazo)benzoates was studied. The relative intensities of ions *m/z* 262 (3H) in the spectra of alkenyl esters are always smaller when compared with the intensities of this ion by the corresponding saturated esters.

The same feature, more accentuated, was observed in the mass spectra of some analog alkynyl and dienyl esters (Table 4)

Table 4: Partial spectra of some n-alkynyl and n-dienyl of p-(4'-chlorophenylazo) benzoates

Comp.	R	% Relative intensity of ions (base peak = 100)	
		261* (2H)	262* (3H)
24	E 7, Z 9-12**	32	37
27	Y 8-12***	20	20
25	Z 9, E 12-14	47	50
26	E 2, Z 13-18	100	19

* corrected for naturally occurring heavy isotopic contributions

** geometry and position of the double bond

*** Y-abbreviation for triple bond

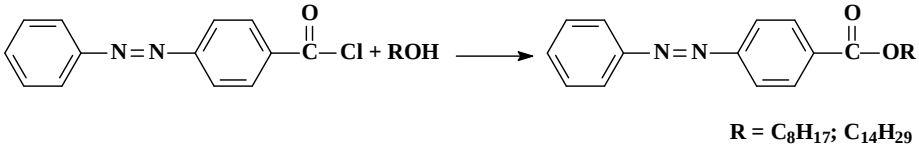
A possible explanation of this feature consists in a reduced flexibility of randomly orientated hydrocarbon chain, caused by a rigid double or triple bond.

Long chain benzoic acid esters

The aim of the present study was to determine if the chlorine atom, the azo- group or the aromatic ring have an influence on the occurrence and the abundance of the triple hydrogen rearrangement ion. Therefore, we synthesized a series of *n*-alkyl esters of phenyldiazobenzoic acid without the chlorine atom in the *p'*-position as well as series with modified diazo-group and other functional groups.

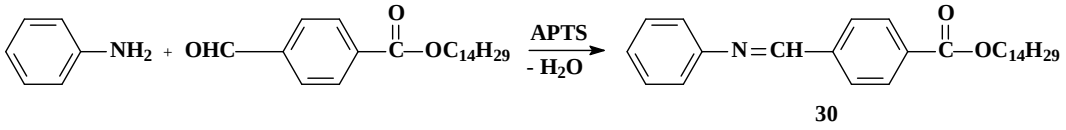
Synthesis

The *n*-alkyl esters of phenyldiazobenzoic acid were obtained by an esterification reaction between phenyldiazobenzoyl chloride and the corresponding alcohol (Scheme 5):



Scheme 5: Synthesis of phenylazobenzoates

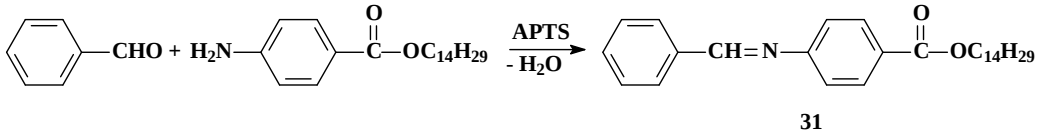
Esters with similar structure, but containing a modified diazo group were prepared by a condensation reactions. The tetradecyl ester of 4-(phenylimino)methyl-benzoic acid **30** was prepared from aniline and the tetradecyl ester of 4-formyl benzoic acid, in the presence of *p*-toluenesulfonic acid (scheme 6) [Johnston, Smart & Smith, 1984].



*Schema 6: Synthesis of the tetradecyl ester of 4-(phenylimino)methyl-benzoic acid **30***

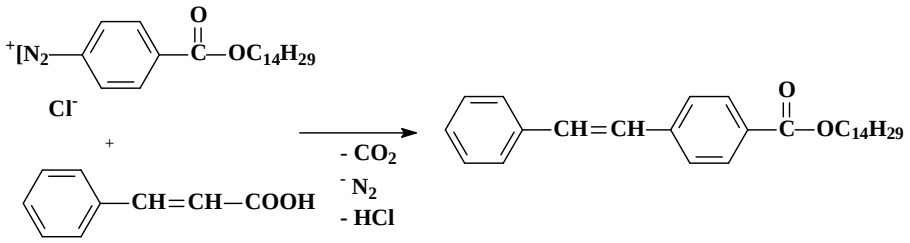
The structure of the compounds was confirmate by NMR-spectroscopy and mass spectrometry.

In order to obtain the similar derivative **31,** a condensation reaction of benzaldehyde with the tetradecyl ester of *p*-aminobenzoic acid was performed (scheme 7).



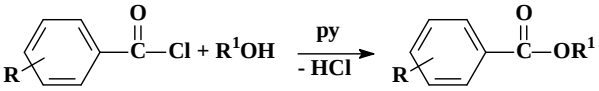
Scheme 7: Synthesis of tetradecyl ester of 4-amino-(N-benzylidene) benzoic acid

The condensation reaction of the diazonium salt of tetradecyl *p*-aminobenzoate with cinnamic acid leaded to the corresponding ester of 4-carboxy stilbene (Scheme 8) [Merweein, Büchner & vanEmster, 1939]:



Scheme 8: Synthesis of tetradecyl ester of 4-carboxy stilbene

Esterification of some acyl chlorides with fatty alcohols was used for the synthesis of a series of different substituted long chain esters of benzoic acid (scheme 9)

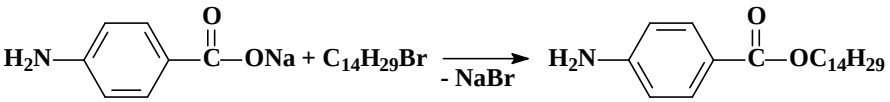


R = *m*-NO_2, *p*-NO_2, *p*-CN, *p*-CHO

R^1 = C_8H_{17}, $C_{14}H_{27}$, $C_{14}H_{29}$

Scheme 9: Sinthesis of long chain benzoic acid esters

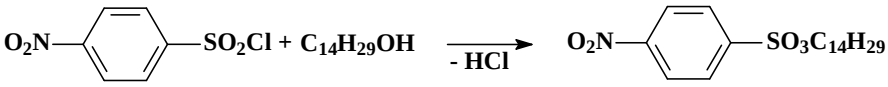
The synthesis of the similar derivative **33** bearing a NH2 group in the *para* position of the aromatig ringwas performed using the reaction from the diazonium salt of *p*-amino benzoic acid and myrrystyl bromide (scheme 10) in dymethylformamide [Winnik & Kwong, 1976]:



33

Scheme 10: Synthesis of tetradecyl–p-aminobenzoate

Compound **40** was obtained from *p*-nitrobenzenesulfochloride and myrystic alcohol [Shirley, Smith, Brown & Reedy, 1952]:



40

Scheme 11: Synthesi sof tetradecyl–p-nitrobenzenesulfonate

The structure of all synthesized compounds was investigated by NMR spectroscopy and mass spectrometry.

Unidirectional triple hydrogen rearrangements in mass spectra of some long chain benzoic esters

In Table 5 are presented some selected data from the mass spectra of different aromatic long chain esters [**Moldovan**, Oprean & Oprean, 2003].

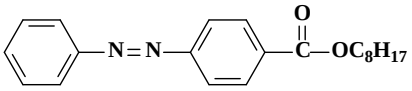
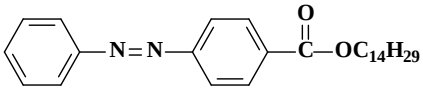
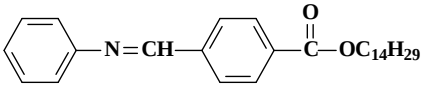
From the obtained results it is to conclude that the presence of the chlorine atom in the phenylazobenzoates is not necessary, but it is useful for fragments identification .

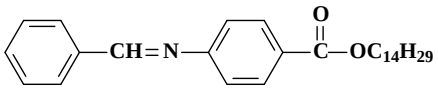
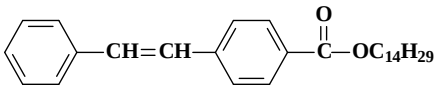
By alternative replacement of one nitrogen atom from the N=N group of the long chain esters of phenylazobenzoic acid with a CH group, the abundance of the ion m/z 227 corresponding to the triple hydrogen rearrangement decreases (compounds **30** and **31**). By replacement of both nitrogen atoms with CH=CH group the triple hydrogen transfer ion disappears from the mass spectra of compound **32.**

This fact suggests that the substituent from the *para*-position of the benzoica acid esters may influence the appearance of the triple rearranged ion., in a similar wa tat hese substituents inluence the genesis of the single and double rearrangement ions[Tobita, Tajima & Tsuchiya, 1984]. To elucidate this supposition we studied the mass spectra of some benzoate long chain esters with different substituents on the aromatic ring. (Table 5)

The mass spectra of unsubstituted, *p*- and *m*-methoxy- as well as *p*-amino long chain long chain benzoates showed no triple hydrogen rearrangement ions [Oprean, **Moldovan** & Oprean, 2003b], [**Moldovan**, & Oprean, 2006].

Table 5: The occurrence of hydrogen transfer ions in mass spectra of some long chain benzoates

Compound	Structure	Relative intensity of ions* (%)		
		[1H]	2[H]	3[H]
28		1.5	1	0.7
29		4	24	33
30		24	100	2

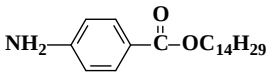
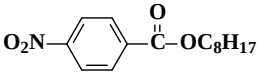
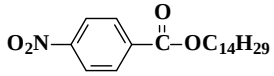
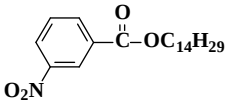
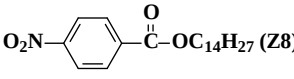
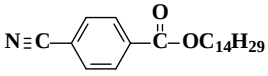
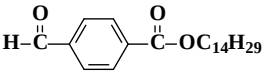
31		27	42	13
32		1	0.5	-

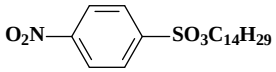
* corrected for naturally heavy occurring isotopic contributions

The presence of a NO_2 , CN, or CHO group in the *p*- or *m*- position of the aromatic ring in the long chain benzoates appears to be essential for the triple hydrogen transfer (Table 6).

The results indicated that electron-releasing substituents favor the appearance of $[M\text{-}C_nH_{2n}]^{+\bullet}$ fragment, generated by transfer of one hydrogen atom (*McLafferty* rearrangement), while electron-withdrawing substituents determine the appearance of the $[M\text{-}C_nH_{2n-1}]^{+}$ ion obtained by a double hydrogen rearrangement as well as the genesis of the $[M\text{-}C_nH_{2n-2}]^{+\bullet}$ fragment corresponding to a triple hydrogen rearrangement.

Table 6: The occurrence of hydrogen transfer ions in mass spectra of some long chain benzoates

Comp.	Structure	Relative intensity of ions* (%)		
		[1H]	2[H]	3[H]
33		100	8	-
34		1	34	17
35		1	37	81
36		4	8	23
37		1	29	76
38		2	93	22
39		2	100	57

40		-	5	3

* corrected for naturally heavy occurring isotopic contributions

This substituent effect on the triple hydrogen transfer is in good agreement with Hammet's σ_p values.

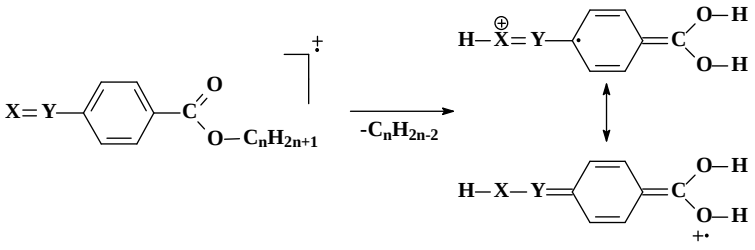
The correlation between the effect of the substituents in the *para* position on the triple rearrangement ion abundance relative to the double rearrangement ion observed in the mass spectra of higher esters of benzoic acid with Hammett constant σ_p values is shown in Table 7.

From the presented data it can be concluded that electron-withdrawing substituents on the aromatic ring of long chain aqlkylbenzoates favor the formation of triple hydrogen rearrangement ions. The same phenomenon was observed for long chain alkylbenzosulfonates.

Tabelul 7: The abundance p-X-$C_6H_4COOH_3$/ p-X-$C_6H_4COOH_2$ ratio and the σ_p values for several alkylbenzoates

Substitent	**[3H]/[2H]**	**σ_p**
NH_2	0	-0,66
OCH_3	0	-0,27
H	0	0
CN	0,24	0,66
CHO	0,57	0,22
N=N-C_6H_5	1,37	0,64
NO_2	2,19	1,27

The proposed mechanism of the triple hydrogen rearrangement of the molecular ion of these compounds is shown in Scheme 12



Scheme 12: Mechanism of the triple hydrogen rearrangement

CONCLUSIONS

- Unidirectional triple hydrogen rearrangements are very rarely seen in mass spectrometry of organic compounds. For the first time, the triple hydrogen rearrangement has been revealed in the series of synthesized long chain alkyl-aryl esters. It was also proposed as a mechanism that generalizes the fragmentation of the molecular ion with triple hydrogen rearrangement.
- In order to prove this phenomenon and to elucidate the mechanism of the triple hydrogen rearrangement, a homologous series of **18** saturated esters of *p*-(4'-chlorophenylazo) benzoic acid, containing n-alkyl C_1-C_{18} rests, of which **15** are new compounds, has been synthesized and structurally characterized. In the case of saturated alkyl benzoates, the triple hydrogen rearrangement ion occurs only in the mass spectra of compounds with more than 4 chain atoms, the intensity of this ion increased with increasing number of carbon atoms in the molecule.
- The transfer of the hydrogen atoms occurs from the alkyl chain towards the azo and carbonyl groups, nevertheless requiring a chain of sufficient length for the two groups to achieve the minimum distance required to complete the transfer.
- The study was extended to unsaturated long chain esters, in order to investigate double bond influence on this phenomenon. To this end, a series of **23** new unsaturated esters of *p*-(4'-chlorophenylazo) benzoic acid has been synthesized and structurally characterized, finding that if the unsaturation is located closer than 3 atoms to the ester group, the triple rearrangement ion does not occur due to the lack of flexibility imposed by the rigidity of the chain double bond.
- It has also been investigated the influence of the substituents in *meta* and *para* positions of the aromatic ring on the occurrence and intensity of the triple rearrangement ion. To this end, 5 new long chain esters with modified azo group and 8 new long chain benzoates have been synthesized and structurally characterized. Analyzing the mass spectra of the products, it was found that the electron- releasing substituents favor McLafferty rearrangement ions, while the electron-withdrawing substituents favor the formation of triple hydrogen rearrangement ions.

- A correlation between triple and double hydrogen rearrangement ions intensities ratio and the values of the Hammett constants σ_p of the substituents of the aromatic ring has been established.

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PART II

INVESTIGATION OF THE PROPERTIES OF SOME NATURAL POLYPHENOLIC COMPOUNDS

THEORETICAL ASPECTS

Anthocyanins are a group of naturally occurring phenolic compounds, which play an important role in the color quality of many flowers, fruits, vegetables and related products derived from them. Their color may vary from pink, through red mauve, purple and violet to dark blue. They represent the largest class of water-soluble pigments in the vegetal kingdom, although the greater part of them occurs rarely.

The term anthocyanin was initially coined to indicate the substance responsible for the color of cornflower: it derives from the Greek term *anthos*=flower, and *cyanos* =blue.

Up to now, more than 540 anthocyanins and 23 anthocyanidins were reported [Andersen & Jordheim, 2006] of which only six are the most common in vascular plants. During the last years the scientific interest in these pigments has exponentially increased, interest which can be explained by the potential use of anthocyanins as health beneficial compounds, especially in food industry, as a nontoxic alternative to the synthetic dyes. Anthocyanins, like other polyphenolic compounds found in fruits, are known for their antioxidant activity. This plays a vital role in the prevention of cancer, diabetes, cardiovascular and neuronal illnesses. Anthocyanins from fruits and vegetables are also reported as responsible for their antimicrobial properties [Lule & Xia, 2005; Konczak & Zhang, 2004; Kong, Chia, Goh, Chia & Brouillard, 2003].

Structuraly, anthocyanins represent one of the twelve main classes of flavonoides.

The anthocyanins are glycosides of anthocyanidines, aglycones which structural are flavylium cation derivatives [Konczak & Zhang, 2004]. All anthocyanidines contain hydroxyl groups, mostly in the 3, 5, 7 positions of the benzopyrylium ring, respectively in the 3', 4', 5' positions of the phenolic moiety (figure 1).

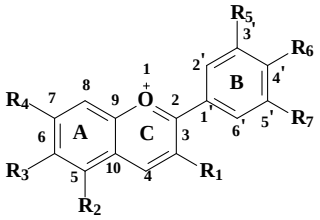


Figure 1: General structure of anthocyanins

Up to know, about 30 different antocyanidins were identified [Kong, Chia, Goh, Chia & Brouillard, 2003], the most common being presented in table 1.

About 90 percent of the anthocyanins contain as aglycone one of the six most widespread anthocyanidins, which are named according to the plants of which they were firs isolated: pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin, which only differ by the hydroxylation and methoxylation pattern on their B-rings (figure 1) [Clifford, 2000].

Table 1: Structure of the most common anthocyanidins occurring in nature

Anthocyanidin	**Structure**							
	3	5	6	7	3'	4'	5'	**Colour**
Carajurin	H	H	OH	OH	H	OCH_3	OCH_3	-
Arrabidin	H	H	OH	OH	H	OH	OCH_3	-
3'-Hydroxy-arrabidin	H	H	OH	OH	OH	OH	OCH_3	-
Apigeninidin	H	OH	H	OH	H	OH	H	**orange**
Luteolin	H	OH	H	OH	OH	OH	H	**orange**
Tricetinidin	H	OH	H	OH	OH	OH	OH	**red**
Pelargonidin	OH	OH	H	OH	H	OH	H	**orange**
Aurantinidin	OH	OH	OH	OH	H	OH	H	**orange**
Cyanidin	OH	OH	H	OH	OH	OH	H	**red-orange**
5-Methyl -cyanidin	OH	OCH_3	H	OH	OH	OH	H	**red-orange**
Peonidin	OH	OH	H	OH	OCH_3	OH	H	**red**
Rosinidin	OH	OH	H	OCH_3	OCH_3	OH	H	**red**
6-Hydroxy-cyanidin	OH	OH	OH	OH	OH	OH	H	**red**
6-Hydroxy-delphinidin	OH	OH	OH	OH	OH	OH	OH	**violet**
Delphinidin	OH	OH	H	OH	OH	OH	OH	**violet**
Petunidin	OH	OH	H	OH	OCH_3	OH	OH	**violet**
Malvidin	OH	OH	H	OH	OCH_3	OH	OCH_3	**violet**
Pulchelidin	OH	OCH_3	H	OH	OH	OH	OH	**violet**
Europinidin	OH	OCH_3	H	OH	OCH_3	OH	OH	**violet**
Capensinidin	OH	OCH_3	H	OH	OCH_3	OH	OCH_3	**violet**
Hirsutidin	OH	OH	H	OCH_3	OCH_3	OH	OCH_3	**violet**
Riccionidin A	OH	H	OH	OH	H	OH	H	-

The most commonly found in nature are the glycoside derivatives of the three non-methylated anthocyanidins: cyanidin (50 %), delphinidin (12%) and pelargonidin (12%), being found in 80% of pigmented leaves, 69% in fruits and 50% in flowers [Dey & Harborne, 1993], The most rare pigments are the malvidin and petunidin derivatives (7% each). [Kong, Chia, Goh, Chia & Brouillard, 2003].

Anthocyanins were mainly found in fresh berries, fruits and some vegetables, the most important source of these pigments being the red grapes from *Vitis vinifera* species. Other anthocyanins rich varietal cultivars are those from *Rosaceae* and *Ericaceae* families such as *Fragraria* (strawberries), *Rubus* (raspberries) *Vaccinium* (blueberries and cranberries) and *Ribes* (blackberries).

Anthocyanins are very unstable and susceptible to degradation. Due to their high reactivity, isolated anthocyanins easily convert to colorless or undesirable brown compounds. Among many factors that can influence anthocyanin stability, the most significant is temperature. Anthocyanins present a very high thermal sensitivity. Beside the temperature, pH, light, oxygen, enzymes, ascorbic acid, sugars and hydrogen peroxide also affect the stability of anthocyanins. [Iacobucci & Sweeny, 1983; Francis, 1989; Cabrita, 1999**]**.Thus, measurement of anthocyanin content and investigation of their degradation is useful for the food industry [Hernandez-Herrero & Frutos, 2011; Kirka, Özkan & Cemeroğlu, 2007; Pliszka, Huszcza-Ciołkowska, Mieleszko & Czaplicki, 2009].

RESULTS AND DISCUSSION

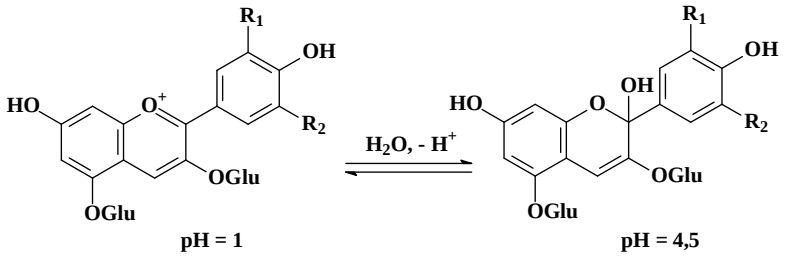
In order to predict the quality changes of anthocyanins during their storage and processing, the accurate determination of the degradation kinetic parameters is a matter of a great concern. The **purpose** of this study was to determine the storage stability in different conditions (pH, temperature, solvent, added food additives) of anthocyanins isolated from several sources and to establish the optimal storage and processing conditions for their use as food colorants.

The aim of this study was to determine the degradation kinetic parameters of sour cherry fruits anthocyanins in ethanolic extract during storage at room temperature (25^{o}C).

Investigation of anthocyanins stability

The present study was based on the investigation of some natural extracts obtained from fresh or frozen anthocyanins rich fruits, such as : sour cherries, cornelian cherries, European cranberrybush fruits as well as from some dried fruits mixtures such as commercialy available fruit teas.

Substantial quantitative and qualitative information can be obtained from the spectral characteristics of anthocyanins. Anthocyanin pigments undergo reversible structure transformation as a function of pH, which can be measured using optical spectroscopy. The colored flavilium cation form predominates at pH = 1, while, at pH = 4.5 the colorless hemiketal form is the most stable (Scheme 1).



Scheme 1: Structural forms of anthocyanins at pH = 1, respective pH = 4.5

The pH differential method is a rapid and easy procedure to determine the total anthocyanins content from various sources. The method is based on the reaction depicted in Scheme 1 and permits an accurate measurement even in the presence of other interfering compounds. [Giusti & Wrolstad, 2001].

The red to purple oxonium form predominates at pH = 1, while at pH = 4.5, the colorless hemiketal form is the major structural form. The difference in absorbance of the anthocyanins solutions between these two pH values, permits an accurate and rapid determination of total monomeric anthocyanins content in the sample matrix.

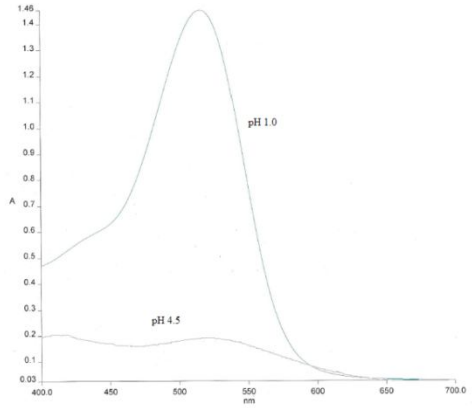


Figure 2. UV-VIS spectra of anthocyanins in buffer solutions at pH = 1 and pH = 4.5

The total monomeric anthocyanin content (expressed as cyanidin-3-glucoside equivalents) can be calculated using the following equation (Giusti & Wrolstad, 2001):

$$TA = \frac{A \cdot MW \cdot DF \cdot 1000}{\varepsilon \cdot l} \qquad (1)$$

where: TA = total anthocyanin content ($mg \cdot l^{-1}$) ;

A = absorbance, calculated as: (eq. 2)

$$A = (A_{\lambda vis\text{-}max} - A_{700})_{pH=1} - (A_{\lambda vis\text{-}max} - A_{700})_{pH=4,5} \qquad (2)$$

MW = molecular weight;

DF = dilution factor;

l = pathlenght;

ε = molar extinction coefficient;

Stabilty of anthocyanis from Sour cherries extracts

Sour cherries, dark-red, acidic and fleshy drupes, are the fruits of *Prunus cerasus L.*, fruit tree of the genus *Prunus,* native to much of Europe and southwest Asia. Raw sour cherries contain sugars, tannins, proteins, pectin, organic acids, minerals, flavonoids and they also are an excellent source of vitamin C and vitamin A.

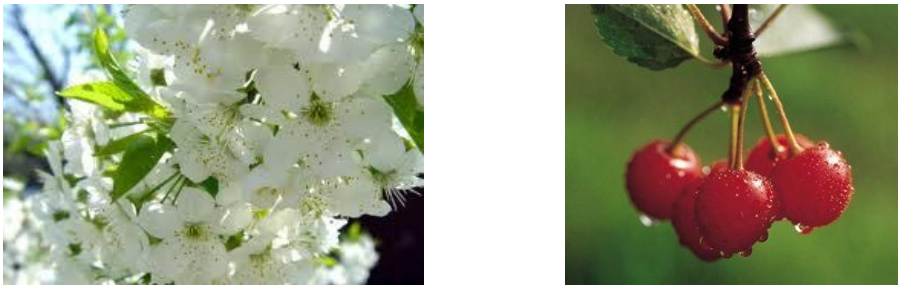


Figure 3: Flowers and fruits of Sour Cherry (Prunus cerasus L.)

Sour cherries are also a good source of anthocyanins. The anthocyanin content of sour cherries varies largely with genotipe. The most abundant anthocyanins found in the fruits of *Prunus cerasu*s are the glucoside forms of cyanidin (Cy) and peonidin (Pn) Cy-3-glucoside, Cy-3-glucosylrutinoside, Cy-3-sophoroside, Cy-3-rutinoside, Cy-3-xylosylrutinoside, Pn-3-glucoside, Pn-3-rutinoside, Cy-3-gentobioside.

The aim of this study was to determine the degradation kinetic parameters of sour cherry fruits anthocyanins in aqueous and ethanolic extract during storage at room temperature (25^{o}C) and at a pH value of 1.9 [**Moldovan**, David, Donca & Chişbora, 2011].

The anthocyanin content during storage at 25^{0}C was plotted as a function of time (figure 4). The linear dependence of ln [A] = f(t) demonstrates that the degradation process of monomeric anthocyanins follows first order reaction kinetics, results which are in agreement with those from the previous studies, reporting a first order reaction model for the degradation of these flavonoidic compounds from various sources [Kirka, Özkan & Cemeroğlu, 2007; Wang & Xu, 2007].

The kinetic parameters were calculated using the following equations:

$$\ln[TA] = \ln[TA_0] - kt \qquad (3)$$

$$t_{1/2} = \frac{0.693}{k} \qquad (4)$$

where:

[TA] = total anthocyanin content (mg/l) at time t

$[TA_0]$ = initial total anthocyanin content (mg/l)

k = reaction rate constant (h^{-1})

t = reaction time (h)

$t_{1/2}$ = half-life (h)

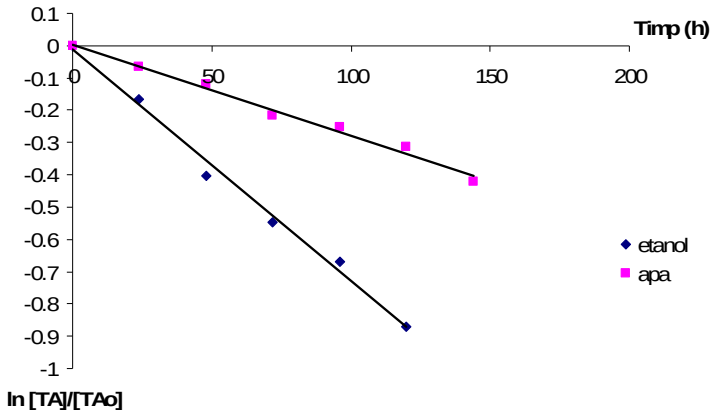


Figure 4: Degradation of anthocyanins in sour cherries aqueous and ethanolic extract during storage at 25^0C

Table 2: Kinetic parameters of sour cherries anthocyanins degradation

Solvent	**k (h^{-1})**	**$t_{1/2}$ (h)**	**Determination coefficient (R^2)**
Water	$2.8x10^{-3}$	247.5	0.9879
Ethanol	$7.2x10^{-3}$	96.25	0.9927

The determined value for the kinetic rate constants half-life values are given in table 2. First order reaction kinetics for the degradation process of anthocyanins in sour cherry concentrates with a 38 days value for $t_{1/2}$ at 20^0C was reported [Cemeroğlu, Velioğlu & Işik, 1994]. Comparing to this, the alcoholic and aqueous extracts of anthocyanins from sour cherries are significantly less stable ($t_{1/2\ water}$ = ~ 10 days, $t_{1/2\ ethanol}$ = ~ 4 days).

Although sour-cherries are a considerable rich in anthocyanins, the raw fruits present high nutritional and economical values, fact that does not recommend them as an industrial source for anthocyanin extraction. For the above mentioned reason, we considered that finding other sources, less consumed as raw fruits, for isolation of these pigments is a matter of a great concern.

Poliphenolic compounds from European crannberrybush fruits

European cranberrybush (*Viburnum opulus L.*—also known as Snowball tree, Guelder rose or Crampbark) belongs to the *Caprifoliaceae* family. This plant is widely distributed especially in the Eastern Europe countries, Turkey, North Asia and North Africa and it is generally used as an ornamental plant. The cranberrybush has a red fruit, ripened in August–September, which remains through the winter. The berries are edible, although seldom consumed directly as food as they are bitter and highly astringent [Jordheim, Giske & Andersen, 2007]. Among the dietary usages, the juice from the berries is the best known product; however, the berries can also be cooked into preserves like jams, jellies, marmalades or fermented to make an alcoholic drink. The berries are also well known for their biological properties, traditionally being used for the treatment of menstrual, stomach and kidney cramps, duodenal ulcers, high blood pressure, heart troubles, coughs and colds [Altun, Citoğlu, Yilmaz & Özbek, 2009; Dennehy, 2006; Velioğlu, Ekici & Poyrazoglu, 2006].

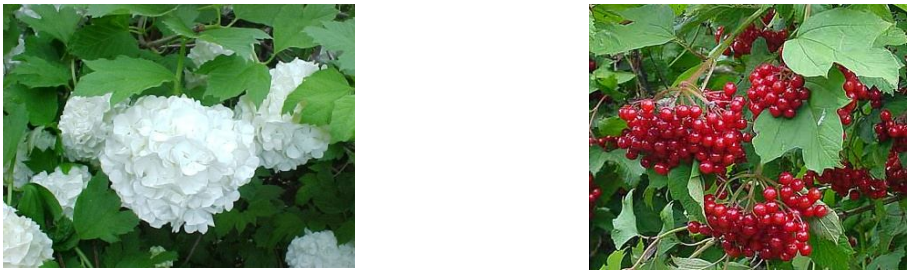


Figure 5: Flowers and fruits of European Cranberrybush (Viburnum opulus L.)

The anthocyanins extract from European cranberrybush fruits was successfully used for the synthesis of new hybrid biomaterials based on metallic nanoparticles (Au and Ag) with remarcable applications in cutaneous inflamatory processes [Crisan, David, **Moldovan**, et al., 2013].

Reported studies on cranberrybush fruits and their properties are limited. Given their known health benefits, the content in anthocyanin for berries of some *Caprifoliaceae* species has lately received attention. Thus, measurement of anthocyanin content and investigation of their degradation is useful for the food industry. However, to date, no information is available in the literature on the degradation kinetics of anthocyanins found in European cranberrybush fruits.

In order to predict the quality changes of anthocyanins during their storage and processing, the accurate determination of the degradation kinetic parameters is a matter of a great concern. The purpose of this study was to determine the storage and thermal stability of the anthocyanins from European cranberrybush fruits extracts in different storage solvents at different temperatures and pH values. Accurate knowledge of the degradation kinetics for the anthocyanins is essential for predicting changes that may occur either during storage in various conditions or/and during thermal processing of food products (especially fruit juices) containing these anthocyanins. The conditions investigated here assure a high compatibility with storage (refrigerated and room temperature storage) and processing techniques generally applied in the food industry (for example, 75 °C representing juice pasteurization temperature).

The content of anthocyanins in European cranberrybush fruits was calculated with the easy and convenient pH differential method, and found to be 0.356 ± 0.014 g/kg (frozen fruit), expressed as cyanidin-3-glucoside.

Thermal degradation of aqueous and ethanolic extracts of anthocyanins from European cranberrybush fruits was studied at various pH values (3 and 7) and at different temperatures (2 °C, 37 °C and 75 °C).

The degradation of monomeric anthocyanins from *Viburnum opulus* fruits was studied in aqueous and ethanolic extracts during storage at 2 °C, 37 °C and 75 °C. Table 3 presents the variation of the anthocyanin content from the aqueous extract by storage in the investigated conditions.

Table 3 : Total anthocyanin content of european cranberries aqueous extract

	Total monomeric anthocyanin content (mg/l)					
Time (h)	**pH = 7 t = 37°C**	**pH = 3 t = 37°C**	**pH = 7 t = 2°C**	**pH = 3 t = 2°C**	**pH = 7 t = 75°C**	**pH = 3 t = 75°C**
0	42.72	42.72	42.72	42.72	42.72	42.72
2	nd	nd	nd	nd	29.74	42.18
4	nd	nd	nd	nd	12.62	38.16
6	nd	nd	nd	nd	3.81	34.51
12	nd	nd	nd	nd	1.21	25.23
24	19.39	30.05	38.39	41.53	nd	12.96
36	nd	nd	nd	nd	nd	nd
48	11.45	27.53	34.52	40.95	nd	5.59
72	5.36	25.21	33.02	40.66	nd	1.49
97	2.08	20.20	29.60	40.30	nd	nd
122	1.20	19.44	29.21	40.13	nd	nd
145	nd	16.05	28.45	38.78	nd	nd
167	nd	12.75	25.33	38.23	nd	nd

The content of aqueous anthocyanins from *Viburnum opulus (L.)* fruits during storage was plotted as a function of time (figure 6). The linear regression of the total anthocyanins content during storage confirmed that degradation of aqueous anthocyanins from European cranberrybush (as observed in figure 6) followed first order reaction kinetics. The obtained results are in agreement with those from the previous studies which showed that storage degradation of anthocyanins from various sources is described by first order reaction kinetics [Kirka, Özkan & Cemeroğlu, 2007; Wang & Xu, 2007; **Moldovan**, David, Donca, Chişbora, 2011] .

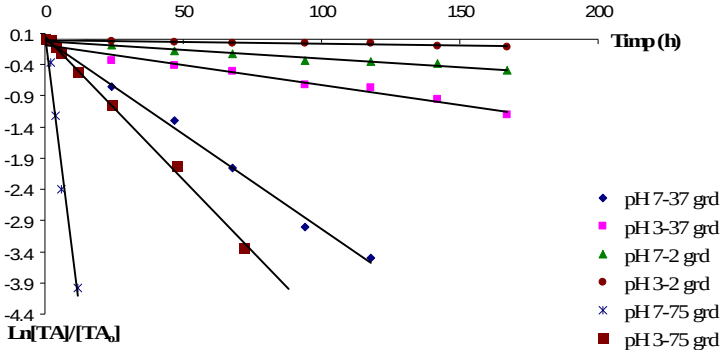


Figure 6: Degradation of anthocyanins in cranberrybush fruits aqueous extract during storage at different temperature and pH values

The kinetics for this reaction type can be expressed by the equations (3) and (4). The determined values for the kinetic rate constants and the half-life values are summarized in Table 4.

Table 4: Kinetic parameters of degradation of anthocyanins from Cranberrybush fruits in aqueous extract

pH	**Temp. (°C)**	**k x10^3 (h^{-1})**	**$t_{1/2}$ (h)**	**Determination coefficient R^2**
3	2	0.6	1155	0.9503
3	37	6.4	108.28	0.9740
3	75	46.1	15.03	0.9971
7	2	2.8	247.5	0.9680
7	37	30.5	22.72	0.9944
7	75	348.8	1.98	0.9762

The rate constants and the half-life values (Table 4) indicate a significant influence of the pH of the storage solution on the stability of anthocyanins which correlate to the dependence of their structure on pH. In agreement with other studies showing the influence of the pH value on the stability of anthocyanins [Kirka, Özkan & Cemeroğlu, 2007], we observed that acidic media increased the stability of anthocyanins from aqueous *Viburnum opulus (L.)* fruits extract, as indicated by higher $t_{1/2}$ values.

By comparing the half-life values, one can conclude that, at lower temperatures, European cranberrybush anthocyanins in aqueous extract are ~4.7 times less susceptible to degradation than they are at lower pH values ($t_{1/2,\ pH\ =\ 3}/t_{1/2,\ pH\ =\ 7} \approx 4.7$), the highest stabilities being observed at pH = 3, regardless of storage temperature. At higher temperatures, such as 75 °C, the effect of pH on the degradation process becames more significant, the stability of anthocyanins at pH = 3 being 7.6 fold higher than at pH = 7.

The thermal stability of the extracts was also evaluated. As expected, the degradation rate of anthocyanins increased with the increase of temperature. Regardless of the pH value, storage at 37 °C resulted in a 10.7 times faster degradation as compared to degradation at refrigerated storage (at 2 °C). At pH = 7, the effect of temperature on the degradation process is more important. At lower pH = 3, the degradation process at 75 °C occurs 76.8 times faster than at 2 °C, while at higher pH (pH = 7), the ratio $t_{1/2,\ 2\ °C}/t_{1/2,\ 75\ °C}$ is 125 in aqueous extract.

The degradation of monomeric anthocyanins from European cranberrybush fruits in ethanolic extract was also investigated at the same pH and temperature values as the aqueous one. The decrease of the anthocyanin content during storage is given in table 5.

Table 5 : Total monomeric anthocyanin content in ethanolic extract

	Total monomeric anthocyanin content (mg/l)					
Timp (ore)	**pH = 7 t = 37°C**	**pH = 3 t = 37°C**	**pH = 7 t = 2°C**	**pH = 3 t = 2°C**	**pH = 7 t = 75°C**	**pH = 3 t = 75°C**
0	58.14	58.14	58.14	58.14	58.14	58.14
2	nd	nd	nd	nd	43.60	57.40
4	nd	nd	nd	nd	27.23	54.40
6	nd	nd	nd	nd	16.56	45.72
12	39.54	nd	nd	nd	7.62	32.47
14	nd	nd	nd	nd	3.54	nd
24	24.62	37.79	54.07	54.12	nd	17.13
36	13.36	nd	nd	nd	nd	nd
48	10.46	29.07	46.55	55	nd	6.31
72	3.48	25.58	45.02	53.48	nd	nd
97	1.1	20.19	42.99	50.87	nd	nd
122	nd	11.04	41.49	50.11	nd	nd
145	nd	6.97	39.98	47.65	nd	nd
167	nd	nd	nd	45.68	nd	nd

The total content of anthocyanins from ethanolic extract of *Viburnum opulus (L.)* fruits during storage was plotted as a function of time (Figure 7). In all investigated cases, the degradation was fitted to a first order reaction model.

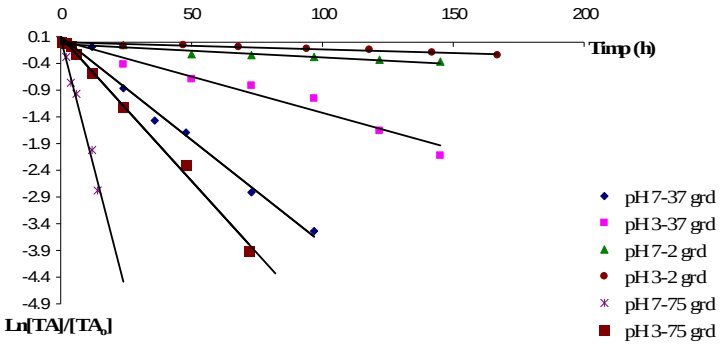


Figure 7: Degradation of anthocyanins in cranberrybush fruits ethanolic extract during storage at different temperature and pH values

The determination of the kinetic parameters of the degradation process of the anthocyanic pigments from european cranberries by storage in ethanol conducted to the results presented in table 6.

Table 6: Kinetic parameters of european cranberrybush fruits anthocyanins degradation in ethanolic extract

pH	**Temp. (°C)**	**k x10^3 (h^{-1})**	**$t_{1/2}$ (h)**	**Determination coefficient (R^2)**
3	2	1.3	533.07	0.9575
3	37	13.6	50.95	0.9602
3	75	53.9	12.86	0.9948
7	2	2.5	277.2	0.9298
7	37	38.5	18.0	0.9867
7	75	189.3	3.66	0.9824

As observed in the case of aqueous solutions, the European cranberrybush anthocyanins showed two distinct stability profiles: at higher pHs and at lower pHs, with the highest stability being observed in acidic media. The higher pH value decreased the ethanolic anthocyanins storage stability, the half-life ratio values being influenced by the temperature (at 2 °C $t_{1/2,\ pH\ =\ 3}/t_{1/2,\ pH\ =\ 7}$ = 1.9 while at 37 °C $t_{1/2,\ pH\ =\ 3}/t_{1/2,\ pH\ =\ 7}$ = 2.8 and at 75 °C $t_{1/2,\ pH\ =\ 3}/t_{1/2,\ pH\ =\ 7}$ = 3.5).

The storage temperature has a strong influence on the degradation rate of anthocyanins; storage at 37 °C resulted in a faster degradation compared to refrigerated storage at 2 °C, the ratio between the half-life values depending on the pH values (at pH=3, $t_{1/2,\ 2\ °C}/t_{1/2,\ 37\ °C}$ = 10.5, while at pH = 7 the value of this ratio is 15.4). As expected, the increase of the temperature at 75 °C resulted in a more accelerated degradation of the anthocyanins (at pH = 3, $t_{1/2,\ 2\ °C}/t_{1/2,\ 75\ °C}$ = 41.4, while at pH = 7 the value of this ratio is 75.7).

According to data summarized in Tables 5 and 6, at pH = 7, the determined values for the kinetic rate constants and the half-life values during storage at 2 °C and 37 °C are similar to those obtained in the same conditions in the aqueous extract, hence the influence of the storage solvent in these conditions is less important. In contrast, at higher temperatures (such as 75 °C), the solvent manifests a greater influence on the stability of anthocyanin extracts, the degradation rate being 1.8 fold higher in water as in ethanol.

At pH = 3 and lower temperatures (2 °C and 37 °C), the degradation of anthocyanins present in the ethanolic extract proceeds at twice the rate observed in the aqueous extract. At 75 °C, a slightly faster degradation process of anthocyanins from *Viburnum opulus (L.)* fruits was observed in the ethanolic extract, compared to the aqueous one, so one can conclude that in acidic environments and at higher temperatures, the influence of the storage solvent on the stability of monomeric anthocyanins from *Viburnum opulus (L.)* is less important.

To determine the effect of temperature on the kinetics of the degradation process, the constants obtained from Equations (3) and (4) were fitted to an Arrhenius type equation:

$$k = K_o e^{-Ea/RT} \quad (5)$$

where E_a = the activation energy (kJ/mol);

K_o = frequency factor (h^{-1});

R = the universal gas constant (8.314 J/mol·K);

T = absolute temperature (K).

The anthocyanin degradation rate constants obtained for each extract were plotted as a function of temperature (Figure 8).

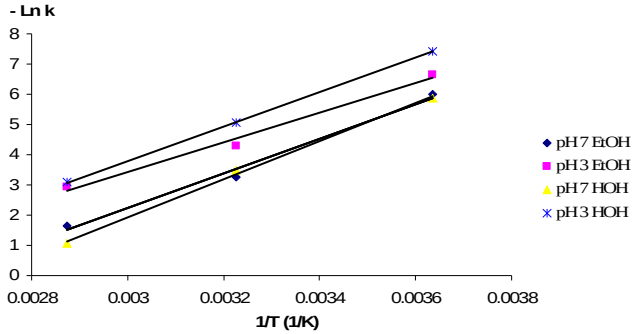


Figure 8: The Arrhenius plots for degradation of anthocyanins in cranberrybush fruits extracts.

The calculated activation energies are given in Table 7.

High activation energy implies that the anthocyanins in the extracts are more susceptible to degradation by exposure to elevated temperatures. The highest influence of

the temperature on the degradation process (the highest value of E_a) was observed for the anthocyanins stored in water at pH = 7, while the lowest value of the E_a (lower susceptibility to thermal degradation) was obtained for storage in ethanol at pH = 3. The cross influence of the pH and solvent on the degradation process of the anthocyanins extracted from *Viburnum opulus (L.)* fruits seems to be more significant in case of storage in water at pH = 3 and ethanol at pH = 7 (where the activation energy values are practically identical).

Table 7: Effect of temperature on the degradation of anthocyanins from cranberrybush fruits extracts

pH	Solvent	E_a (kJ/mol)	R^2	K_o (h^{-1})	Q_{10}	
					2-37 °C	37-75 °C
3	Water	47.39	0.9886	$5.94x10^5$	1.018	1.681
7		52.47	0.9975	$2.43x10^7$	1.978	1.898
3	Ethanol/water	40.79	0.9890	$8.08x10^4$	1.956	1.436
7		47.34	0.9999	$2.84x10^6$	2.184	1.52

The dependence of degradation rate on temperature was also evaluated by calculating the temperature coefficient Q_{10}, according to Equation (6):

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{10/(T_2 - T_1)} \quad (6)$$

where Q_{10} = the temperature coefficient (K^{-1});

$k_{1,2}$ = rate constant (h^{-1}) at temperature $T_{1,2}$ (K).

Almost the same Q_{10} values were obtained for the degradation of anthocyanins in water at pH = 7, proving that the influence of the increase of the temperature on the stability of the anthocyanins is the same for both studied temperature intervals (2–37 °C and 37–75 °C). The lowest temperature coefficient value (1.018 K^{-1} at 2–37 °C) was obtained in water at pH = 3 indicating that low storage temperatures and acidic media are needed to inhibit degradation of anthocyanins from European cranberrybush fruits extracts.

European cranberrybush fruits are also reported to be a good source of other flavonoids. Their composition and the high content of substances having antioxidant activity make the fruits of European cranberry bush unique [Rop et al., 2010].

The purpose of this study was also to monitor the changes in the phenolic content and the antioxidant capacity of the Cranberrybush fruit ethanolic extract during refrigerated storage.

The method employed for the evaluation of the total phenol content (TPC) of studied extracts based on their reduction properties. Conventional Folin-Ciocalteu method reports different responses to different phenolic compounds, depending on chemical structures, being used for the measurement of total reducing capacity of samples [Singleton, Orthifer & Lamuela-Raventos, 1999].

The total phenol content of the extracts was spectrophotometricaly evaluated, by measuring the absorbance at 765 nm. The results were expressed as gallic acid equivalents (GAE) / l extract, using a calibration curve of the standard.

Our measurements revealed high contents of poliphenolics in *Viburnum opulus* fruits. The determined content was 4.22 g gallic acid equivalents / kg fruit, higher as the value determined from Akbulut et al. who obtained 3.25 g gallic acid equivalents / kg fruit. [Akbulut, Causir, Marakoglu & Coklar, 2008].

During 8 days of storage at 4^0C, fluctuations in the total phenolic content were observed, as shown in Figure 9:

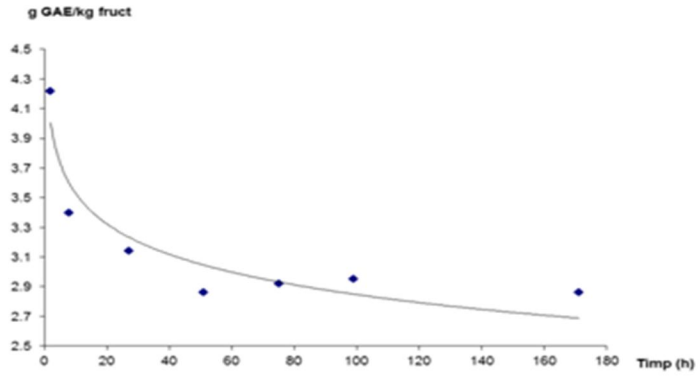


Figure 9: Total phenol content of ethanolic extract from european cranberrybush fruits as function of time, by refrigerated storage.

In the first 51 hours of refrigerated storage, a significant decrease of total phenolic content of the ethanolic fruits extract was observed. The initial rapid decrease in the phenol content of our sample was followed by a slightly increase of the value of total phenolics in the next 48 hours. After approximately 3 days of refrigerated storage, the phenolic content of the European cranberrybush fruits extract remained constant.

The free radical scavenging capacity of the ethanolic extract of cranberrybush fruits using the free radical ABTS reaction was also evaluated by measuring the absorbance of the sample at 734 nm. The antioxidant activity was calculated as a decrease in the absorbance value using the formula (7):

$$A = A_0 - A_1 \qquad (7)$$

where: A = the resulting absorbance

A_0 = absorbance of the ABTS solution without sample

A_1 = absorbance of the mixture containing the sample

The resulting absorbance values are converted in ascorbic acid equivalents (AAE) using a calibration curve of the standard.

The evolution of the antioxidant activity in cranberrybush fruits extract during refrigerated storage is shown in Figure 10. The initial radical scavenging capacity was 7.05 g AAE / kg frozen fruits, according to literature data [Rop & al., 2010].

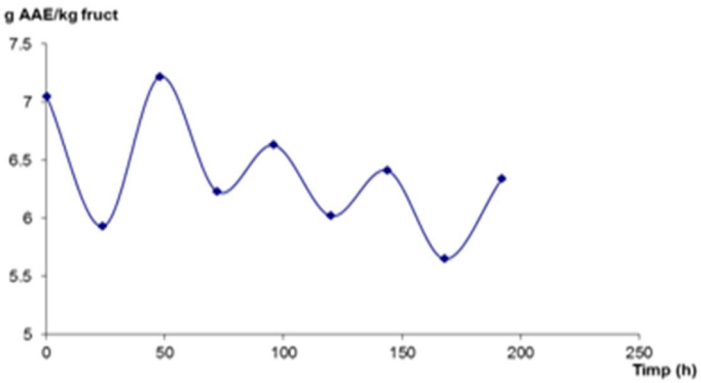


Figure 10: Variation of the antioxidant activity during storage

In the first 24 hours of refrigerated storage, a 16% decrease of the antioxidant capacity was observed, followed by a 22% increase in the next 24 hours. This pattern in the variation of the antioxidant capacity (decrease – increase) can be observed during the

whole storage period. Increases in the antioxidant activity for grape extracts during storage have been previously reported [Pinelo, Manzocco, Nunez & Nicoli, 2004].

According Pinello & all the increasing activity may be explained by strong tendency of poliphenols to undergo polymerization reactions, the resulting oligomers possessing larger areas for charge delocalization. The decrease in the antiradical scavenging capacity results when the degree of polymerization exceeds a critical value, due to the increase of a molecular complexity and steric hindrance which reduce the capacity of hydroxyl groups to react with the radicals.

A slightly tendency of decreasing the antioxidant activity of cranberrybush fruits ethanolic extract can be observed, the value remaining rather stable at 4^0C.

Anthocyanins from cornelian cherries

Cornelian cherry (*Cornus mas L.*) is a species of dogwood native to Southern Europe and Southwest Asia. The fruit is an oblong, red drupe, 2-3 cm long, containing a single seed, edible, but when unripe astringent. Fresh cornelian cherry fruits contain twice as much ascorbic acid (vitamin C) as oranges, being also rich in sugar, organic acids and tannins [Tural & Koca, 2008].

Figure 11: Flowers and fruits of Cornelian Cherry (Cornus mas L.)

Cornelian cherry fruits also contain significant amounts of anthocyanins which are known to possess antioxidant and anti-inflammatory effects and can be used as natural food pigments. However, to date, no information is available in the literature on the degradation kinetics of Cornelian cherry fruits anthocyanins. On these premises, the purpose of this study was to determine the stability of the anthocyanins from Cornelian cherry fruits extracts during storage. The accurate determination of the degradation kinetics for these compounds during storage or during thermal processing is essential for

predicting changes that may occur in food products containing anthocyanins. The investigated conditions (temperature and nature of an added preservative) assure a high compatibility with processing techniques often applied in the food industry. Sodium benzoate and potassium sorbate are used as food preservatives, due to their antimicrobial properties. They are widely used in foods such as soft drinks, jams and fruit juices, dairy products and many others.

The influence of temperature and food preserving agents on the stability of anthocyanins from the Cornelian cherry fruits extract during storage was investigated. The determined values for the kinetic parameters (kinetic rate constants and the half-life values) are summarized in Table 8.

The content of anthocyanins from Cornelian cherries aqueous extract during refrigerated storage (2 ^{0}C) was plotted as a function of time (figure 12). The linear regression of the total anthocyanins content of Cornelian cherry fruits extracts during storage confirmed that the degradation process of these pigments followed first order reaction.

The values of the kinetic parameters (table 8) illustrate the high stability of the anthocyanis from cornelian cherries by storage at 2ºC in aqueous solution, presenting a half-life value of about 58 days, superior to that determined for the degradation of the anthocyanins from european cranberrrybush fruits (~ 48 days) [**Moldovan**, David, Chișbora & Cimpoiu, 2012].

By comparing the rate constants, one can conclude that, the presence of food preservatives displayed a slightly destabilizing effect on the anthocyanins from the investigated extracts. However, the difference between the two added food preservatives was not significant, the degradation process being 1.14 fold faster in the presence of potassium sorbate as compared to sodium benzoate.

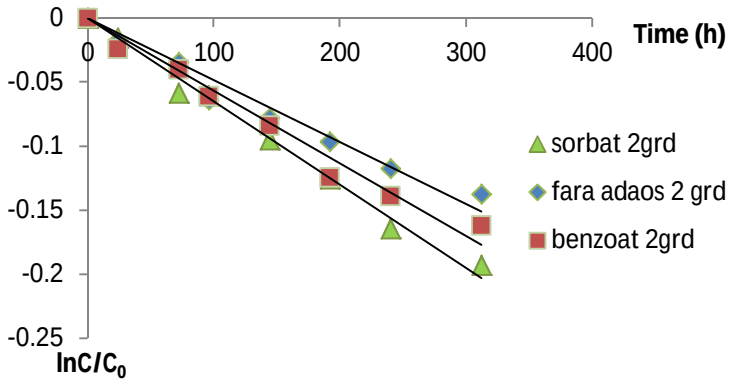


Figure 12: Influence of different food preservatives on the anthocyanin stability during storage at 2 ^0C

Table 8: Kinetic parameters of the cornellian cherries anthocyanins degradation by storage in different conditions

Sample	**Temp. (^{o}C)**	**k.10^{-3} (h^{-1})**	**$t_{1/2}$ (h)**	**R^2**
Crude extract	2	0.5	1386	0.9568
Extract+sodium benzoate (0.1%)	2	0.6	1155	0.969
Extract + potasium sorbate (0.1%)	2	0.7	990	0.9893
Crude extract	25	0.9	770	0.9474
Extract+sodium benzoate (0.1%)	25	0.9	770	0.9684
Extract + potasium sorbate (0.1%)	25	1.1	630	0.9629
Extract+vitamin C (0.1%)	25	7.7	90	0.9039
Crude extract	75	82.8	8.3	0.9923
Extract+sodium benzoate (0.1%)	75	79.1	8.7	0.9911
Extract + potasium sorbate (0.1%)	75	77.6	8.9	0.9917
Extract+ vitamin C (0.1%)	75	125.3	5.5	0.9982

As observed in the case of refrigerated storage, the Cornelian cherry anthocyanins stored at 22 ^{0}C showed the same degradation profile (Figure 13). In this case, storage of the extracts was strongly influenced by the added food additive so storage in the presence of potassium sorbate resulted in a faster degradation compared to storage of sodium benzoate added extract, the half-life ratio value being 1.21.

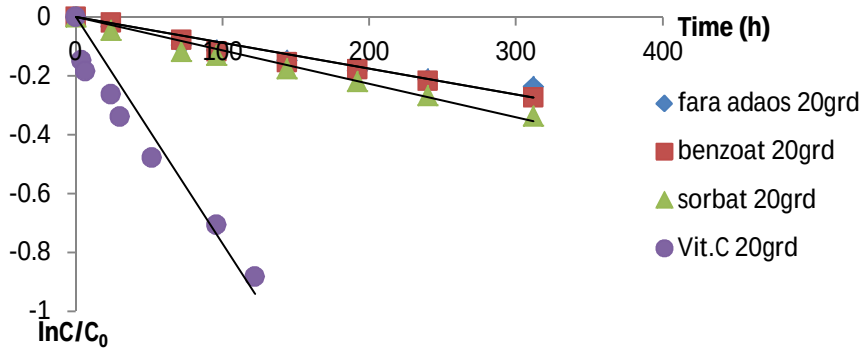


Figure 13. Degradation of anthocyanins from cornellian cherries aqueous extract by storage at 25°C

As expected, the increase of temperature at 75 ^{0}C resulted in an accelerated degradation of anthocyanins (figure 14). During high temperature storage, as applied at 75 ^{0}C, the destabilizing effect of sodium benzoate and potassium sorbate on the anthocyanic pigments (observed at lower storage temperatures) did however not maintain. In contrast, the added food preserving agents slightly increased the stability of these pigments the half-life values being practically the same for all the investigated extracts (8.4 ÷ 8.95 h).

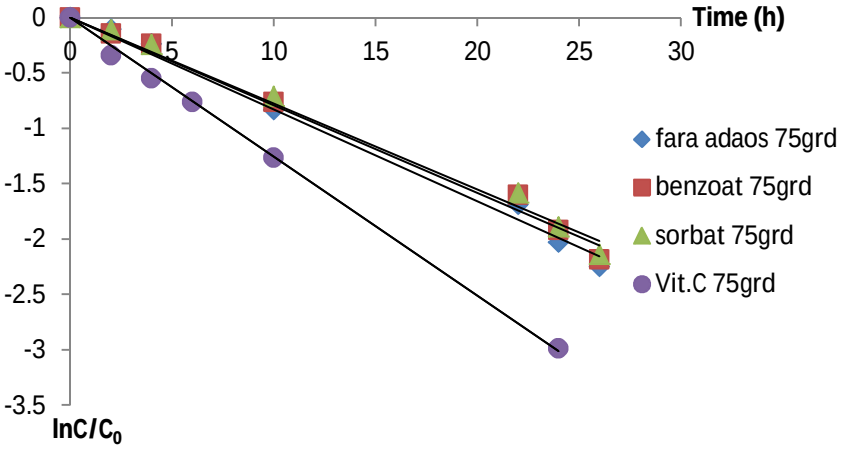


Figure 14. Degradation of anthocyanins from cornellian cherries aqueous extract by storage at 75 ℃

The effect of temperature on the kinetics of the degradation process was determined by fitting the rate constants to an Arrhenius type equation.

The anthocyanin degradation rate constants obtained for each extract were plotted as a function of temperature (figure 15).

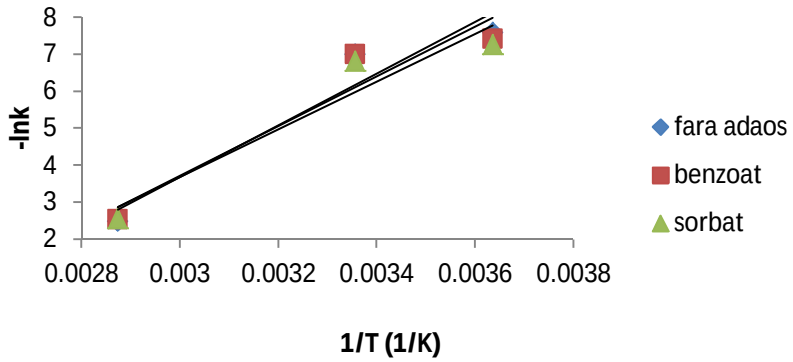


Figure 15: The Arrhenius plots for degradation of anthocyanins in Cornelian cherries extracts

The calculated activation energies are given in Table 9. Since high activation energy reactions are more sensitive to temperature, the anthocyanins in the extract proved to be more susceptible to degradation by exposure to elevated temperatures. The calculated E_a values ranged from 53.72 to 58.11 kJ· mol^{-1}. The highest influence of the temperature on the stability of the investigated compounds (the highest value of E_a) was observed for the anthocyanins stored in crude extract, while the pigments stored in the presence of potassium sorbate exhibited lower susceptibility to thermal degradation, presenting the lowest value of the E_a.

Tabelul 9: Effect of temperature on the degradation of anthocyanins from Cornelian cherry fruits extracts

Extract	**E_a (kJ/mol)**	**R^2**	**K_o (h^{-1})**	**Q_{10}**	
				2-25°C	**25-75°C**
Crude extract	58.11	0.9304	$3.46x10^7$	1.290	2.47
Extract+sodium benzoate (0.1%)	55.82	0.9142	$1.45x10^7$	1.192	2.447
Extract + potasium sorbate (0.1%)	53.72	0.9209	$7.06x10^6$	1.216	2.342

In order to evaluate the dependence of degradation rate on temperature, the temperature coefficient Q_{10}, the change of degradation rate upon a temperature increase of 10 K was calculated.

Higher Q_{10} values for storage temperatures of 22-75 ^{0}C were obtained, indicating that anthocyanins are more sensitive to temperature elevations at high storage

temperatures compared to low storage temperatures (2-22 ^{0}C) where the Q_{10} values were ranged from 1.277 to 1.346, whereas the differences were insignificant. Almost the same Q_{10} values were obtained for the degradation of anthocyanins stored at 2-22 ^{0}C for all the investigated extracts (crude or with added preservative), proving that the influence of the added food preserving agents was not significant. Storage at 22-75 ^{0}C also resulted in almost the same Q_{10} values for all the studied extracts. The lowest temperature coefficient value (1.277 at 2-22 ^{0}C) was obtained for the anthocyanins stored in the presence of sodium benzoate, indicating that low storage temperatures are needed to inhibit the degradation process of these pigments from Cornelian cherries extracts [**Moldovan** & David, 2013].

Polyphenols extracted from dried fruits (fruit teas)

In the last years, there is a growing interest in consumption of functional food due to their important health-beneficial effects that are related with the phytochemical compounds. Beverages such as fruits, teas and wines are important source of polyphenols and anthocyanins in the human diet. Also, intensified efforts are getting to find new sources of natural antioxidants.

Next to water, tea is one of the most consumed beverages all around the world. Although tea produced from the *Camelia sinensis L.* leaves (green, black, oolong or white) are the most popular, various types of herbal and fruit infusions are widely consumed due to their positive health effects, their fruity flavor and their caffeine lower content. Fruit tea infusions support the human diet with some important sources of antioxidants like phenolic compounds, vitamins (C and E), carotenoids [Belščak, Bukovac & Piljac-Žegarac, 2011]. The antioxidant capacity and polyphenolic content of fruit tea infusions were less investigated although these are widely consumed products and provide a dietary source of biologically active compounds.

Considering these facts, the aim of our study was to determine the total phenolic and anthocyanins contents and the antioxidant capacity of 12 commercially available fruit teas and of dried calyces of hibiscus flowers (hibiscus being found in all investigated teas) (table 10).

The method employed for the evaluation of the total phenol content (TPC) of studied tea infusions is based on their reduction properties. Conventional Folin-Ciocalteu method reports different responses to different phenolic compounds, depending on chemical structures, being used for the measurement of total reducing capacity of samples.

Table 10: Type and composition of analyzed teas

No	Type of fruit tea[a]	Producer/Series	Contained fruits
1	Raspberry	1	Raspberry, hibiscus, blackcurrant, blueberry
2	Raspberry	2	Raspberry, blackcurrant, hibiscus
3	Blueberry	1	Blueberry, hibiscus, blackcurrant
4	Blueberry	2	Blueberry, blackcurrant, strawberry, hibiscus
5	Rosehip	1	Rosehip, hibiscus, blackcurrant
6	Rosehip	2	Rosehip, blackcurrant, hibiscus
7	Strawberry	2	Strawberry, blackcurrant, hibiscus
8	Strawberry	3	Hibiscus, rose hip, strawberry, raspberry, cherry, wild strawberry
9	Wild berry	1	Blueberry, blackcurrant, blackberry, hibiscus
10	Wild berry	1	Blackberry, raspberry, wild strawberry, blueberry, rose hip, hibiscus
11	Wild berry	2	Blackberry, blackcurrant, raspberry, strawberry, hibiscus
12	Wild berry	3	Hibiscus, rose hip, raspberry, blueberry, blackberry, strawberry, cherry
13	Hibiscus		

The TPC determined by Folin-Ciocalteu method, for three series (from three different producers) of fruit tea infusions is reported in Table 11. For the teas from producer 1, the highest TPC was found for the blueberry tea (23.229 mg GAE/g fruit tea), the lowest value being exhibited by the raspberry tea (15.844 mg GAE/g fruit tea). The teas obtained from producer 2 presented the highest TPC value for the rose hip infusion (28.179 mg GAE/g fruit tea) while the lowest value was obtained for the raspberry tea (12.531mg GAE/g fruit tea). In this series, there is a 2.2-fold difference between the highest and the lowest TPC values of the teas. The TPC value for the investigated hibiscus tea (19.054 mg GAE/g fruit tea) was found to be closer to the highest TPC value found in the series 1. The highest TPC value of all investigated teas was found for the strawberry tea made by producer 3, probably due to its high content of rose hip fruit.

Table 11: TAC, TPC and AA for investigated teas

Tea No.	Total anthocyanin content (TAC) mg Cy-3-glu/g tea	Total phenolic content (TPC) mg GAE/g tea	Antioxidant activity (AA) mg AAE/g tea
1	4.486±0.286	15.844±0.218	12.948±1.619
2	3.646±0.092	12.531±0.154	15.194±2.503
3	5.572±0.516	23.229±0.419	18.268±0.165
4	4.394±0.206	13.892±0.199	12.961±1.621
5	5.264±0.013	18.605±0.221	15.408±0.332
6	2.844±0.116	28.179±0.399	10.96±0.418
7	4.58±0.463	15.537±0.066	13.134±1.957
8	4.161±0.089	29.326±0.198	19.022±0.22
9	5.138±0.076	18.222±0.110	13.416±0.295
10	2.624±0.335	16.358±0.463	11.243±0.036
11	3.607±0.206	13.275±0.166	10.871±2.508
12	4.039±0.332	14.127±0.132	19.060±1.955
13	3.892±0.006	19.054±0.6185	12.672±0.276

The results obtained by Folin-Ciocalteu method varied widely in the studied types of tea (obtained from different fruits) as well as from one producer to the other (for the same fruit). This fact can be explained by the different composition of the fruits teas as well as of the infusion times which vary from producer to producer and one might be too short for a sufficient extraction of water-soluble polyphenols.

The determined TPC values were in the range of 12.531 to 29.326 mg GAE/g fruit tea (126.07 to 295.02 mg GAE/L infusion), in accordance to previously reported literature data [Belščak, Bukovac & Piljac-Žegarac, 2011] which mentioned TPC values between 323.4 - 1549.1 mg GAE/L infusion, for a twice concentrate infusion (2 g tea / 100 ml water) prepared from similar fruit teas. As expected, these values are much lower as the TPC of fruit juices - blueberry 179.5; strawberry 1302; blackcurrant 1919 mg GAE/L [Piljac-Žegarac, Valek, Martinez & Belščak, 2009], due to the fact that fruit tea manufacturing involves processing procedures (such as drying, grinding) that may determine the degradation of the phenolic compounds.

Apart of other polyphenols, all investigated teas contain also anthocyanins, which play an important role in the color of these teas, but being also important for their biological activities.

The TAC of the fruit tea infusions was investigated using the pH differential method. The results obtained from the spectrophotometric determinations are reported in Table 11 and were expressed as mg Cy-3-glu/g tea.

The anthocyanin levels were similar in all fruit infusions from producer 1 (in the range of 4.486 - 5.572 mg Cy-3-glu/g fruit tea), except for wild berry tea no 10 which had the lowest value for the TAC (2.624 mg Cy-3-glu/g fruit tea). Furthermore, by analyzing the TAC and the TPC data, a statistically significant correlation was found between these results (r^2=0.8223 and p=0.093). For series 2, except the rose hip tea no 6 (2.844 mg Cy-3-glu/g fruit tea), similar TAC values were found for the investigated fruit infusions (in the range of 3.607 - 4.58 mg Cy-3-glu/g fruit tea). As in the previous case, the TAC values followed the same trend as the TPC values, being statistically correlated (r^2=0.77 and p=0.12). The determined TAC values for the fruit tea infusions of producer 3 are almost the same (~ 4 mg Cy-3-glu/g fruit tea).

The TAC value for the investigated hibiscus tea (3.892±0.006 mg Cy-3-glu/g fruit tea) was found to be closer to the highest TAC value found in the series 2.

The lowest anthocyanins content was found for the rosehip fruit tea no 6 (producer 2), being 1.85 fold lower as the TAC determined for the rosehip fruit tea no 5 (producer 1). By comparing these values, it is important to take into account the fruit content of analyzed teas. This result may be due to the fact that tea no 6 contains a higher quantity of rosehip fruits, opposite to the tested tea no 5, that contains higher amounts of blackcurrant (berries that possess a TAC higher than rosehip fruits). These differences may also explain the lower than expected TPC value determined in tea no 5.

To the best of our knowledge, there are no published data characterizing the anthocyanins from infusions prepared from bagged fruit teas.

Antioxidant activities of 12 fruit tea infusions were determined using the ABTS assay. The ABTS measured the hydrogen and electron-donating abilities of primary antioxidants respectively.

Significant differences were observed among various tea infusions. The highest ABTS radical cation scavenging activity was exhibited by the blueberry tea from producer 1 (18.268 mg AAE/g fruit tea) and by the raspberry tea (15.194 mg AAE/g fruit tea) from producer 2, respectively. The lowest antioxidant activity values were found for

the wild berry tea no 10 in series 1 (11.243 mg AAE/g fruit tea) and for the wild berry tea no 11 (10.871 mg AAE/g fruit tea) in series 2. There is a 1.4-fold difference between the lowest and the highest antioxidant activity values of the investigated teas in series 1, the same as for the TPC values. A statistically significant relationship between TPC and AA was observed (r^2=0.908 and p=0.046), indicating that the concentration of phenolic compound may be a good indicator of the antioxidant activity of the investigated fruit tea infusions. Surprisingly, for the producer 2, no correlation between TPC and AA was observed. The determined antioxidant activity for the fruit tea infusions of producer 3 is almost the same (~ 19 mg AAE/g fruit tea), and was the highest of the all investigated teas.

As expected, the studied tea infusions exhibit an antioxidant capacity (in the range of 10.9 – 15.4 mg AAE/100 mL infusion) significantly lower as in infusions of conventional teas of *Camelia Sinensis (L.)* (green, black and oolong).

CONCLUSIONS

- The stability of the anthocyanic pigments extracted from sour cherries, European cranberrybush fruits and cornelian cherries by storage in different conditions was for the first time investigated. In the same storage conditions (temperature and pH) the most stable pigments were found to be those separated from cornelian cherries. The study of the influence of the storage solvent revealed the higher stability of anthocyanins stored in water, compared to storage in ethanol.
- In the case of European cranberrybush extracts, the variation of the total phenol content and the antioxidant activity by room temperature storage was investigated. The studies proved a notable decrease of the total phenol content of the extracts in the first 4 days of storage, followed by a stabilization of this content. The antioxidant activity of the extract varied according to a sine curve, fact the indicated the presence of some possible equilibrium reactions.
- The total phenol content, the antioxidant activity and the anthocyanin content of 12 fruit teas from 3 different producers was also investigated. Although a good correlation between TAC and TPC values of the infusions obtained from the teas of the same producers was observed, no correlation between TPC and AA values could be established.

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