Banat's University of Agricultural Sciences and Veterinary Medicine „King Mihai I of Romania" from Timisoara

Faculty of Food Processing Technology

HABILITATION THESIS

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TEZĂ DE ABILITARE

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Bioactive compounds in food technology, with a special focus on their contribution to antioxidant properties and color stability

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Teză de abilitare

Compuşi bioactivi în tehnologia produselor alimentare, cu un accent special pe contribuţia lor la proprietăţile antioxidante şi stabilitatea culorii

Acknowledgements

I tried to look in my memories for people who have contributed to my achievements and to sit them in order. It was impossible! Because there are so many people in my life who have a huge role to all my achievements. Actually, I'm the result of everything that I have lived. Words are so poor to express the thanks that I owe to my colleagues who I have worked with over the years and all who have contributed to my evolution: teachers, mentors, students. I'm sure that without their help my achievements wouldn't have existed. I would like to thank to my family for the absolute confidence in me over the years. They are my roots and the foundation of what I am today. I would like to express all my gratitude and warm thanks to my husband and my son for their understanding during these years. Sorry that I wasn't as I would have liked to be: a much better person.

Above all, I thank God for all the help he blessed me with, for his guidance without which I would never be able to write my habilitation thesis. For me, this work is like a puzzle and the pieces there are not the articles, books and so on, and rather, all the experiences that led to the defining of my professional identity. I always thought that the most important thing is to have a good direction and also to work for the fulfilment of the proposed objectives. For this reason, I think that this thesis is just a form of experience gained by me over the years, it's just a step in my career, it's the natural course of this journey. As for the future plans, I can say that they are hidden pieces in this game because the future can be unpredictable but I hope that I will have enough time, energy, power, vision and, not at least, chance to do a lot more in this life.

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Habilitation Thesis

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TEZĂ DE ABILITARE

Abstract

The present habilitation thesis consists of three main parts: **(I) *Scientific, academic and professional achievements,* (II) *Career evolution and development plans*** and **(III) *References*,** related to the content of the first two parts.

Part I (divided in two sections: ***Section I. Scientific achievements*** and ***Section II. Professional and academic achievements***) is the core of the thesis, in which are described the most important scientific results, proving the originality and relevance, published in ***10 selected papers*** (ISI quoted) and the main professional and academic achievements, all referring to the interval 2003-2013, which corresponds to the period after defending the PhD thesis (November 2002) and confirmed by the Ministry of Education and Research (April 2003).

In **Section I** are presented the main topics addressed in research activity during all this time, as follows: **(1) *The effect of bottle aging on chromatic and antioxidant properties of red wines*; (2) *The impact of processing and storage on antioxidant characteristic and color of fruit and gelled fruit products*; (3) *The capitalization of some by-products from food processing*; (4) *Use of some natural bioactive compounds for prevention and control of mycotoxins production in cereals*.**

Research activity in the field of red wine analysis has been directed towards the following topics: (i) red wine color analysis during aging using selective UV-VIS methods, including also the evaluation of indices expressing the wine "*chemical age*" and "*the degree of ionization of anthocyanins*"; (ii) assessment the contribution of copigmentation and polymeric pigments to the red wine color stabilization during aging; (iii) evaluating the changes of antioxidant properties in response to aging of bottled wines. On this subject, I published in 2008 the book entitled "*The analysis of red wine color" and* a book chapter entitled "*Phenolics compounds with antioxidant activity in grapes and wine*". Also, I have published 2 articles in ISI quoted journals, 8 articles in other national and international journals and 3 papers were presented at international conferences. 2 of these ISI quoted papers (***selected papers 1 and 2***) were presented in detail in **Section I/1.** Related to this direction I have taught 4 courses (2 of them to bachelor and 2 to Master). In this field, I wrote 3 course books and 2 practical work textbooks and also, I participated in 2 national programs related to antioxidant compounds in some various vegetal products which included also, grapes and wine.

In the field of fruit/gelled fruit products I have contributed with studies on the following topics: (i) impact of *Individual Quick Freezing* (IQF) and long-term storage of frozen fruit on their color stability and antioxidant properties; (ii) effect of thermal processing and storage on antioxidant characteristics and color quality of some low-sugar jam from various fruit rich in antocyanins; (iii) improving the color stability and increasing the amount of antioxidants retained in gelled products using different doses and types of pectin (high and low-esterified, amidated). The funding for this study was supported by a research project with the private sector, coordinated by me as director. In this field, I have published 5 articles in ISI quoted journals, 2 articles in other international journals and 2 papers were presented at international conferences. 4 of these papers (***selected papers 3-6***) were presented in detail in **Section I/2.** Also, I participated in 2 national reseach projects focused on the nutritional benefits offered by a diet rich in antioxidant compounds from vegetables and fruis.

In the field of by-products derived from food processing, I approached the following topics: (i) obtaining of crude freeze-dried extracts rich in polyphenolic compounds from pomace and grape seeds; (ii) assessment of inhibitory potential of freeze-dried grape seeds extract on

oxidative lipid degradation occurring in sunflower oil used in some food thermal applications; (iii) obtaining and characterization of some oils from by-products of fruits processing. On this topic, I have published 3 articles in ISI quoted journals, 2 papers in national journal included in international data basis and a paper was presented at an international conference. 2 of these papers (***selected papers 7 and 8***) were included in this thesis, **Section I/3.**

The interest for the fourth research direction, regarding the prevention and control of mycotoxins production in cereals using natural bioactive compounds has started since 2004 when I was involved in a national grant focused on reducing of fungal mycotoxin content from cereal products by food processing. Work on this topic has stagnated from 2006 to 2010, when I worked in a project funded by National Bank regarding the obtaining and characterization dietetic floury products (there are some notable achievements of us in this fild: 3 trademarks registered to OSIM, a book to which I'm co-author and a book in which I wrote a chapter). The research activity on this topic was resumed starting from 2010 when I participated in the team of an international project from Regional Program of Cooperation with South-East Europe (ReP-SEE). In the realisation of this project I have contributed with studies on the following topics: (i) assessing the mycotoxin contamination of cereals and medicinal herbs in west aria of Romania; (ii) investigating the inhibitory potential of some natural extracts and essential oils on mycotoxins production in cereals. On this topic, I was co-author for 2 chapters in a book published in English in partnership with teams from Serbia and Croatia. I have published 3 articles in ISI quoted journals and other 2 papers were presented at international conferences. 2 of these ISI quoted papers (***selected papers 9 and 10***) were detailed in **Section I/4.**

Apart from these key directions in the last two years I have performed studies concerning the use of Fourrier Transform Infrared (FT-IR) spectroscopy, as a rapid, non-destructive method, for detection of olive oil adulteration and degradation. This research topic has started since 2012 when I won a Bilateral Project Romania-Greece. This co-operation is focused on strengthening the relation between the two teams (from Romania and Greece) with complementary skills and establishing a framework for further collaboration. During this project, I organized 2 lectures with international participation, I have published 3 articles with international partnership and also, we performed mobilities in Greece.

Section II briefly presents the main professional and academic achievements after the Ph.D. Overall, in this period I published 23 articles in ISI quoted journals (*10* as *first author*, 1 as *corresponding author* and 12 as *co-author*), 7 books to CNCSIS recognized publishing houses, 4 book chapters and 2 practical work textbooks. Also, I coordinated as director 2 research projects (a bilateral project Romania-Greece, won by competition and a project funded by private sector). I participated as researcher in the team of 7 national projects, one international research project and I have been short-term expert, responsible for curriculum analysis, in a POSDRU project.

Part II shows ***the plans for career evolution and development***. For this purpose are presented the research topics that will be continue or will be developed. Also, are presented the main indicators to quantify the professional and academic development as well as the future actions that will be performed in order to fulfill the proposed objectives. Based on the activities developed so far, an extensive set of activities in my interest fields, both at national and international level, are expected. The results could be significantly enhanced if the research team will be consolidated by including of Master students and PhD students. It have to be underlined that my active role will continuously increase in the future and the main indicators to quantify my career evolution and development will be researches, lectures and applicative works developed in the mentioned directions.

Part III groups ***the bibliographic references*** associated to the content of the first two parts.

Rezumat

Teza de abilitare este alcătuită din trei părţi principale: **(I)** ***Realizări ştiinţifice, academice şi profesionale,*** **(II)** ***Planuri de evoluţie şi dezvoltare a carierei*** şi **(III)** ***Referinţe bibliografice*** asociate conţinutului primelor două părţi.

Partea I (împărţită în 2 secţiuni): ***Secţiunea 1. Realizări ştiinţifice*** şi ***Secţiunea 2. Realizări profesionale şi academice*** este nucleul tezei, în care sunt descrise cele mai importante rezultate ştiinţifice, probând originalitate şi relevanţă, publicate în ***10 lucrări selectate*** (cotate ISI) precum şi principalele realizări profesionale şi academice, toate referindu-se la intervalul 2003-2013, care corespunde cu perioada după susţinerea tezei de doctorat (noiembrie 2002) şi confirmată de Ministrul Educaţiei şi Cercetării (aprilie 2003).

În **Secţiunea I** sunt prezentate principalele direcţii de cercetare care au fost abordate în tot acest timp, după cum urmează: **(1)** ***Efectul învechirii în butelii asupra proprietăţilor cromatice şi antioxidante ale vinurilor roşii*****; (2)** ***Impactul procesării şi depozitării asupra caracteristicilor antioxidante şi culorii fructelor şi produselor gelificate din fructe*****; (3)** ***Valorificarea unor subproduse rezultate din procesarea alimentară*****; (4)** ***Utilizarea unor compuşi bioactivi naturali în prevenirea şi controlul producerii de micotoxine în cereale*****.**

Activitatea de cercetare în domeniul analizei vinului roşu a fost direcţionată spre următoarele subiecte: (i) analiza culorii vinului roşu utilizând metode UV-VIS selective, incluzând de asemenea, evaluarea indicilor care exprimă "*vârsta chimică*" a vinului şi "*gradul de ionizare a antocianilor*"; (ii) evaluarea contribuţiei copigmentării şi a pigmenţilor polimeri la stabilizarea culorii vinului roşu pe parcursul procesului de învechire; (iii) evaluarea modificărilor proprietăţilor antioxidante ca efect al învechirii vinului. Pe această tematică am publicat în 2008 o carte intitulată "*Analiza culorii vinului roşu*" şi un capitol intitulat "*Compuşi fenolici cu activitate antioxidantă în struguri şi vin*". De asemenea, am publicat 2 articole în reviste cotate ISI, 8 articole în alte reviste naţionale şi internaţionale iar 3 lucrări au fost prezentate la conferinţe internaţionale. 2 din aceste lucrări cotate ISI (***lucrările selectate 1 şi 2***) au fost prezentate în detaliu în **Secţiunea I/1.** Pe această direcţie, predau cursurile a 4 discipline (două dintre acestea la programe de licenţă şi 2 la programe de masterat). În acest domeniu am scris 3 cărţi de curs, 2 îndrumătoare de lucrări practice şi am participat la 2 proiecte de cercetare naţionale care au abordat aspecte referitoare la compuşii antioxidanţi din diverse produse vegetale printre care, strugurii şi vinul.

În domeniul fructelor, respectiv produselor gelificate din fructe am contribuit cu studii privind următoarele subiecte: (i) impactul congelării *Individual Quick Freezing* (IQF) şi al depozitării de lungă durată a fructelor congelate asupra stabilităţii culorii şi proprietăţiilor lor antioxidante; (ii) efectul procesării termice şi depozitării asupra caracteristicilor antioxidante şi calităţii culorii unor gemuri cu conţinut scăzut de zahăr obţinute din fructe bogate în compuşi antocianici; (iii) îmbunătăţirea stabilităţii culorii şi creşterea conţinutului de antioxidanţi reţinuţi în gem prin utilizarea unor diferite doze şi tipuri de pectină (înalt şi slab esterificată, amidată). Finanţarea pentru acest studiu a fost asigurată dintr-un proiect de cercetare cu sectorul privat pe care l-am coordonat în calitate de director. În acest domeniu, am publicat 5 lucrări în reviste cotate ISI, 2 articole în alte reviste internaţionale iar 2 lucrări au fost prezentate la conferinte internaţionale. 4 din aceste lucrări (***lucrările selectate 3-6***) au fost prezentate detaliat în **Secţinea I/2.** De asemenea, am participat la 2 proiecte naţionale de cercetare care au abordat subiecte referitoare la beneficiile nutriţionale oferite de o dietă bogată în compuşi antioxidanţi proveniţi din legume şi fructe.

În domeniul subproduselor rezultate din procesarea alimentară, am abordat următoarele subiecte: (i) obţinerea unor extracte brute liofilizate bogate în compuşi polifenolici din tescovină şi din seminţe de struguri; (ii) evaluarea efectului inhibitor al extractului liofilizat din seminţe de struguri asupra degradării oxidative a lipidelor din uleiul de floarea soarelui supus unor aplicaţii

termice specifice industriei alimentare; (iii) obţinerea şi caracterizarea unor uleiuri din subproduse rezultate la procesarea fructelor. Pe această temă am publicat 3 articole în reviste cotate ISI, 2 lucrări în jurnale incluse în baze de date internaţionale iar o lucrare a fost prezentată la o conferinţă internaţională. 2 din aceste lucrări (***lucrările selectate 7 şi 8***) au fost incluse în această teză, **Secţiunea I/3.**

Interesul pentru a patra direcţie de cercetare, privind prevenirea şi controlul producerii de micotoxine în cereale prin utilizarea unor compuşi bioactivi naturali a început încă din 2004 când am lucrat pentru un grant naţional axat pe reducerea conţinutului de micotoxine fungice din produsele cerealiere prin procesare alimentară. Cercetarea pe această direcţie a stagnat între 2006 şi 2010, fiind implicată într-un proiect finanţat de Banca Mondială referitor la obţinerea şi caracterizarea unor produse dietetice făinoase (există unele realizări notabile în acest domeniu: 3 mărci înregistrate la OSIM, o carte la care sunt coautor şi o carte în care am scris un capitol). Activitatea pe această temă a fost reluată din 2010 când am participat în echipa unui proiect internaţional din Programul de Cooperare regională cu sud-estul Europei (ReP-SEE). În realizarea acestui proiect am contribuit cu studii privind următoarele subiecte: (i) evaluarea contaminării cu micotoxine a cerealelor şi plantelor medicinale din zona de vest a României; (ii) investigarea potenţialului inhibitor al unor extracte naturale şi uleiuri esenţiale asupra producerii de micotoxine în cereale. Pe această temă de cercetare sunt coautorul a 2 capitole într-o carte publicată în limba engleză în parteneriat cu echipele din Serbia şi Croaţia. De asemenea, am publicat 3 lucrări în reviste cotate ISI iar alte 2 lucrări au fost prezentate la conferinţe internaţionale. Două din aceste lucrări ISI (***lucrările selectate 9 şi 10***) au fost prezentate în detaliu în **Secţiunea 1/4.**

Pe lângă aceste direcţii cheie, în ultimii 2 ani am realizat studii privind utilizarea spectroscopiei în infraroşu cu transformată Fourier (FT-IR), ca metodă rapidă, nedistructivă, pentru detectarea falsificării şi degradării uleiului de măsline. Acestă temă de cercetare a început din 2012 când am obţinut prin competiţie un proiect bilateral România-Grecia. Această cooperare s-a axat pe consolidarea relaţiilor între echipa de cercetare din România şi cea din Grecia, având competenţe complementare, şi stabilirea unui cadru pentru colaborări viitoare. În timpul derulării acestui proiect am organizat 2 prelegeri cu participare internaţională, am publicat 3 lucrări în parteneriat şi am efectuat mobilităţi în Grecia.

Secţiunea II prezintă pe scurt principalele realizări profesionale şi academice după obţinerea titlului de doctor. În ansamblu, in această perioadă am publicat 23 de articole în reviste cotate ISI (10 ca prim autor, 1 ca autor corespondent, 12 ca şi coautor), 7 cărţi în edituri recunoscute de CNCSIS, 4 capitole în cărţi şi 2 îndrumătoare de laborator. De asemenea, am coordonat în calitate de director 2 proiecte de cercetare (un proiect bilaterat Romania-Grecia, câştigat prin competiţie şi un proiect finanţat de sectorul privat). Am participat ca cercetător în echipa a 7 proiecte naţionale, un proiect de cercetare internaţional şi am fost expert pe termen scurt, responsabil cu analiza curiculară, într-un proiect POSDRU.

Partea a II-a prezintă ***planuri pentru evoluţia şi dezvoltarea carierei***. În acest scop sunt prezentate subiectele de cercetare care vor fi continuate precum şi cele care vor fi dezvoltate. De asemenea, sunt prezentaţi principalii indicatori utilizaţi pentru a cuantifica dezvoltarea mea profesională şi academică precum şi acţiunile viitoare care vor fi întreprinse pentru îndeplinirea obiectivelor propuse. Pe baza activităţilor desfăşurate până în prezent, se preconizează un set extins de activităţi în domeniile mele de interes, atât la nivel naţional cât şi internaţional. Rezultatele ar putea fi semnificativ îmbunătăţite în cazul în care echipa de cercetare va fi consolidată prin includerea de masteranzi şi doctoranzi. Trebuie subliniat faptul că rolul meu activ va creşte continuu în viitor iar principalii indicatori utilizaţi pentru a cuantifica evoluţia şi dezvoltarea carierei vor fi cercetările, prelegerile şi lucrările aplicative dezvoltate pe direcţiile menţionate.

Partea a III-a grupează ***referinţele bibliografice*** asociate conţinutului primelor două părţi.

List of abbreviations

A_{420}	The absorbance at wavelength 420 nm	LMAP	Low-methoxyl amidated pectin
A_{520}	The absorbance at wavelength 520 nm	LMP	Low-methoxyl pectin
A_{620}	The absorbance at wavelength 620 nm	LPP	Large polymeric pigments
a_w	Water activity	MA (%)	The contribution of monomeric anthocyanins to the total red wine color
ANOVA	Analysis of variance	NIR spectroscopy	Near-infrared spectroscopy
AU	Absorbance Units	O1	Essential oil from *Melissa officinalis* L.
BHA	Butylated hydroxianisole	O2	Essential oil from *Salvia officinalis* L.
BHT	Butylated hydroxytoluene	O3	Essential oil from *Coriandrum sativum* L.
C	Control, untreated sample	O4	Essential oil from *Thymus vulgaris* L.
CA	Copigmented anthocyanins	O5	Essential oil from *Mentha piperita* L.
CA (%)	The contribution of copigmented anthocyanins to the total red wine color	O6	Essential oil from *Cinnamomum zeylanicum* L.
CD	Color density	OTA	Ochratoxin A
CDs	Conjugated dienes	p-AV	P-anisidine value
CTs	Conjugated trienes	PV	Peroxide value
DA	Degree of amidation	PC	polymeric color
DE	Degree of esterification	PC (%)	Percentage of polymeric color
DON	Deoxynivalenol	PP	Polymeric pigments
DPPH	2,2-diphenyl-1-picrylhydrazyl	PP (%)	The contribution of polymeric pigments to the total red wine color
ELISA	Enzyme-linked immunosorbent assay	R	Pearson's correlation coefficient
F	Fischer's variance ratio	RP-HPLC	Reversed Phase High-Performance Liquid Chromatography
FRAP	Ferric reducing antioxidant power	SO_2	Sulfur dioxid
FT-IR spectroscopy	Fourier Transform Infrared spectroscopy	SO_2 – stable	"Stable" or not bleachable in the presence of the sulfite ions
FUMO	Fumonisin	SPP	Small polymeric pigments
FW	Fresh weight	T	Color tonality or hue
GAE	Gallic acid equivalent	TC	Total color of red wine
GPE	Grape pomace extract	TMA	Total monomeric anthocyanins
GSE	Grape seeds extract	TOTOX	Total oxidation value
HMP	High-methoxyl pectin	TP	Total phenolics
HPLC	High-Performance Liquid Chromatography	TSS	Total soluble solids
I1	The first index for expressing the "*chemical age*" of wine	ZON	Zearalenone
I2	The second index for expressing the "*chemical age*" of wine	α (%)	The "degree of ionization of anthocyanins"
IO (%)	Inhibition of oil oxidation	α−T	α−Tocopherol
IQF	Individual Quick Freezing	β−T	β−Tocopherol
K232	Specific extinction value at 232 nm	γ−T	γ−Tocopherol
K268	Specific extinction value at 268 nm	δ−T	δ−Tocopherol
L-AsAc	L-ascorbic acid		

PART I

Scientific, professional and academic achievements

Introduction

This habilitation thesis summarizes my activity performed after defending the PhD thesis (November 2002), and confirmed by the Ministry of Education and Research, on the basis of Order no. 3896, dated 24.04.2003, over a period of 10 years.

The research activity covers some topics specific to phenolics bioactive compounds involved in food technology, antioxidant properties and color stability.

The scientifical achievements presented here are developed in four main thematic directions illustrated in the following ***10 selected papers (P1-P10)***. The research directions I, III and IV are covered by 2 ISI quoted papers on each direction and the direction II includes 4 ISI papers, as follows:

I. *The effect of bottle aging on chromatic and antioxidant properties of red wines*

P1. **Poiana M.A.**, Dobrei A., Stoin D., Ghita A. *The influence of viticultural region and the ageing process on the color structure and antioxidant profile of Cabernet Sauvignon red wines*. Journal of Food, Agriculture and Environment. 2008, 6(3&4):104-108.
Additional information: ISSN 1459-0255, http://world-food.net/download/journals/2008-issue_3_4/f22.pdf.

P2. Dobrei A., **Poiana M.A.**, Sala F., Ghita A., Gergen I. *Changes in the chromatic properties of red wines from Vitis vinifera L. Cv. Merlot and Pinot Noir during the course of aging in bottle.* Journal of Food, Agriculture and Environment. 2010, 8(2): 20-24.
Additional information: ISSN 1459-0255, http://world-food.net/download/journals/2010-issue_2/f3.pdf.

II. *The impact of processing and storage on antioxidant characteristics and color of fruit and gelled fruit products*

P3. **Poiana M.A.,** Moigradean D., Raba D., Alda L., Popa M. *The effect of long-term frozen storage on the nutraceutical compounds, antioxidant properties and color indices of different kinds of berries*. Journal of Food, Agriculture and Environment. 2010, 8(1):54-58, ISSN 1459-0255.
Additional information: ISSN 1459-0255, http://world-food.net/download/journals/2010-issue_1/12.pdf.

P4. **Poiana M.A.**, Moigradean D., Dogaru D., Mateescu C., Raba D., Gergen I. *Processing and storage impact on the antioxidant properties and color quality of some low sugar fruit jams*. Romanian Biotechnological Letters. 2011, 16(5):6504-6512.
Additional information: ISSN 1224-5984, http://www.rombio.eu/rbl5vol16/6%20POIANA%20M.pdf.

P5. **Poiana M.A.**, Alexa E., Mateescu C. *Tracking antioxidant properties and color changes in low-sugar bilberry jam as effect of processing, storage and pectin concentration.* Chemistry Central Journal, 2012, 6:4.
Additional information: doi:10.1186/1752-153X-6-4, Published: 16 January 2012, ISSN 1752-153X, http://journal.chemistrycentral.com/content/6/1/4.

P6. **Poiana M.A.**, Munteanu M.F., Bordean D.M., Gligor R., Alexa E. *Assessing the effects of different pectins addition on color quality and antioxidant properties of blackberry jam*. Chemistry Central Journal 2013, 7:121.
Additional information: doi:10.1186/1752-153X-7-121, Published: 15 July 2013, ISSN 1752-153X, http://journal.chemistrycentral.com/content/7/1/121.

III. *The capitalization of some by-products from food processing*

P7. **Poiana M.A.** *Enhancing oxidative stability of sunflower oil during convective and microwave heating using grape seed extract***.** International Journal of Molecular Sciences. 2012, 13(7): 9240-9259.
Additional information: doi:10.3390/ijms13079240, ISSN: 1422-0067, http://www.mdpi.com/1422-0067/13/7/9240.

P8. Popa V.M., Bele C., **Poiana M.A.**, Dumbrava D., Raba D.N., Jianu C. *Evaluation of bioactive compounds and of antioxidant properties of some oils obtained from food industry by-products*. Romanian Biotechnological Letters, 2011, 16(3):6234-6241.
Additional information: ISSN 1224-5984, http://www.rombio.eu/rbl3vol16/12%20Mirela%20Popa.pdf.

IV. *The use of natural bioactive compounds for prevention and control of mycotoxins production in cereals*

P9. Alexa E., **Poiana M.A**., Sumalan R.M. *Mycoflora and ochratoxin A control in wheat grain using natural extracts obtained from wine industry by-products*. International Journal of Molecular Sciences. 2012, 13(4):4949-4967.
Additional information: doi:10.3390/ijms13044949, ISSN: 1422-0067, http://www.mdpi.com/1422-0067/13/4/4949.

P10. Sumalan R.M., Alexa E., **Poiana M.A.** *Assessment of inhibitory potential of essential oils on natural mycoflora and Fusarium mycotoxins production in wheat.* Chemistry Central Journal. 2013, 7:32.
Additional information: doi:10.1186/1752-153X-7-32, ISSN 1752-153X, http://journal.chemistrycentral.com/content/7/1/32.

"Bioactive compounds" are extranutritional constituents that usually are found in small amounts in foods. They are components of food that possess biological activity in addition to their nutritional value. Also, they have antioxidant properties and many works on this topic have demonstrated their beneficial health effects. These compounds widely can differ in their chemical structure and function. In the last decades, they were extensively studied to evaluate their effects on health. Therefore, it can be said, there is sufficient evidence to recommend consuming of food rich in bioactive compounds.

Phenolic compounds are bioactice compounds that have been studied detailed in fruits and vegetables. The first thing I notice about the phenolics bioactive components from natural sources or food products is that they can became inactive by reactions with oxygen or other food components, or as a result of processing methods or conditions. First of all, if food processing means all treatment of foodstuffs from harvest to consumption, more than 90% of our food may be considered as being processed. The processes and reactions occuring during food processing

and storage are complicated due to the complex chemical heterogeneity of foods and, accordingly, due to the complex reactions and processes that take place in this conditions. Many bioactive compounds are unstable during processing and storage. They undergo various chemical reactions such as oxidation, hydrolysis and thermal degradation resulting in a reduction in their bioactivity. In the same time, processing can generate new bioactive compounds that have been found to have a beneficial contribution on human health. But in mostly cases, food processing and storage lead to some reduction in the nutritional value of foods.

From a practical perspective, the reasons that led me to address these research directions are given by the following reasons:

- development the knowledge regarding the factors that affect the antioxidant properties and color stability during processing and storage;
- identification of some ways to improve the retention of color and bioactive compounds in thermally processed fruit products;
- exploiting the potential of some by-products as a source of bioactive compounds with potential applications to improve the nutritional and biological value of food;
- the need to investigate the use of natural bioactive compounds to control the mycotoxin production in cereals.

This work contains much information about current interests on the effect of processing on bioactive compounds, with a special focus on phenolic compounds, involved in red wine color and various fruit/pectin-gelled fruit products, as well as regarding some ways to exploit the potential of by-products resulted from food processing.

In order to provide a clear view and coherent flow of this document, as well as to facilitate the reading of habilitation thesis, I follow a similar structure during every reseach direction, namely: (i) *Background*, that shows a condensed state-of-knowledge on the research topic, other approaches addressing on the each topic and the research problem statement; (ii) *Our studies*, as solutions to the problem, having unitary structure: *aim*, *results* and *discussion*, *conclusions*; (iii) *Scientific contributions of the author to the actual state-of-knowledge***.**

Section I
Scientific achievements

1. Scientific achievements concerning the effect of bottle aging on chromatic and antioxidant properties of red wines

1.1. Background

Color is the most important attribute used, along with other variables, as an indicator to assess the red wine quality. This characteristic is directly dependent on the phenolics content and composition of the juice and the anthocyanins present in the grape skin (Wrolstad *et al*., 2005). Wine phenolic compounds consist of flavonoids and non flavonoids extracted from grape berries during winemaking. These compounds undergo several chemical transformations, which lead to change of organoleptic properties, particularly color, astringency, and bitterness during wine aging (Ribéreau-Gayon, 1983).

The polyphenolic contents of wine is strongly influenced by grape variety, viticultural and environmental factors (i.e. vineyard location, cultivation system, climate, and soil type, vine cultivation practices, harvesting time) and enological factors such as production process, and aging (Villano *et al*., 2006). The polyphenolic molecules have a functional role as antioxidants against the free radicals and increase the antioxidant capacity in the human body after red wine consumption. Also, moderate consumption of wine seems to reduce the risk of cardiovascular diseases and cancer (Perez *et al*., 2002).

The antioxidant capacity and free radical scavenging activity of wines has been proved in biological systems "*in vitro*" and "in *vivo*", being attributed to some bioactive compounds such as polyphenols (Roussis *et al*., 2005; Villano, *et al.*, 2005; Li *et al.*, 2009).

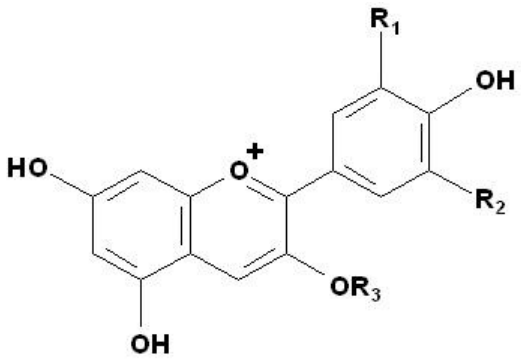
Wine color is a main parameter in red wine analysis. However, it has proven to be one of the most poorly understood. Although its color can be meaningfully measured easily by spectral techniques, the composition of the color is more difficult to determine because the red wine color is controlled by many factors such as the grape variety and the number of winemaking practices and environmental conditions. The red wine color is the result of a complex mixture of several components, including free monomeric anthocyanins, the enhancement of their color due to copigmentation with other noncolored phenolics (Boulton, 2001), and polymeric pigments (Somers and Evans, 1974). The color components of wine are the important parameters that contribute to the sensory characteristics (color and astringency) as well as the antioxidant properties of wine (Monagas *et al*., 2006).

Nowadays, there is the concept of red wine color analysis, very well set and implemented to the international level. This concept supposes a set of spectral determination based on which can be evaluated the contribution of all anthocyanins pigments categories that participates to the total red wine color.

In young red wines, free monomeric anthocyanins are the principal source of red color, but these compounds are not particularly stable. The red wine color continues to change during its

life, and can be strongly affected by anthocyanins content and composition as well as conditions of maturation and aging processes. During red wines aging, these free or monomeric anthocyanins are gradually incorporated into derived pigments (Poiana, 2008). Also, the formation of various anthocyanin-tannin complexes during aging process has been well investigated (Monagas *et al*., 2006; Versari *et al*., 2007), and it has also been proved that these compounds newly formed help to stabilize the red wine color and contribute to a progressive shift of the red-purple color of young red wines towards the more red-orange color which is specific to aged red wines (He *et al.*, 2012).

Usually, in the red wines obtained from *V. vinifera* grapes, the main monomeric anthocyanins are the 3-*O*-monoglucosides of six free anthocyanidins such as: pelargonidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, petunidin-3-*O*-glucoside and malvidin-3-*O*-glucoside (He *et al*., 2012). The structures of these monomeric anthocyanins are illustrated in *Figure 1.1*.



Names	R_1	R_2	R_3
Pelargonidin	H	H	H
Cyanidin	OH	H	H
Delphinidin	OH	OH	H
Peonidin	OCH_3	H	H
Petunidin	OCH_3	OH	H
Malvidin	OCH_3	OCH_3	H
Pelargonidin-3-O-glucoside	H	H	Glu
Cyanidin-3-O-glucoside	OH	H	Glu
Delphinidin-3-O-glucoside	OH	OH	Glu
Peonidin-3-O-glucoside	OCH_3	H	Glu
Petunidin-3-O-glucoside	OCH_3	OH	Glu
Malvidin-3-O-glucoside	OCH_3	OCH_3	Glu

Figure 1.1. Chemical structures of monomeric anthocyanins from *Vitis vinifera* wines and their corresponding anthocyanidins (He *et al*., 2012)

Among monomeric anthocyanins, malvidin-3-*O*-glucoside and its derivatives are the most abundant and also, they are the source of most of the red color of young red wines (He *et al*., 2012). The proportion, the type and the anthocyanins amount in red grapes depends in a great measure on the grape varieties, viticulture practices as well as the weather characteristics (González-Neves *et al*., 2007; He *et al*., 2012).

The anthocyanins composition in red wines depends not only on the original profile of anthocyanins in grapes, but also on the winemaking techniques (Gonzalez-San Jose *et al*., 1990). The content of total monomeric anthocyanins plays a significant role to the red color only in very young red wines (Monagas *et al*., 2005; Wrolstad *et al*., 2005). In these wines, the monomeric anthocyanins there are predominantly in a dynamic equilibrium among five major structural forms, including the bisulfite addition flavene compound (colorless), the quinoidal base (blue violet), the flavylium cation (orange to purple), the hemiketal or carbinol pseudobase (colorless) and the *cis*- and *trans*- forms of chalcone (weak or pale yellow), as it is shown in *Figure 1.2* (Lee *et. al*., 2005; He *et al*., 2012). The groups R_1 and R_2 are shown in *Figure 1.1*.

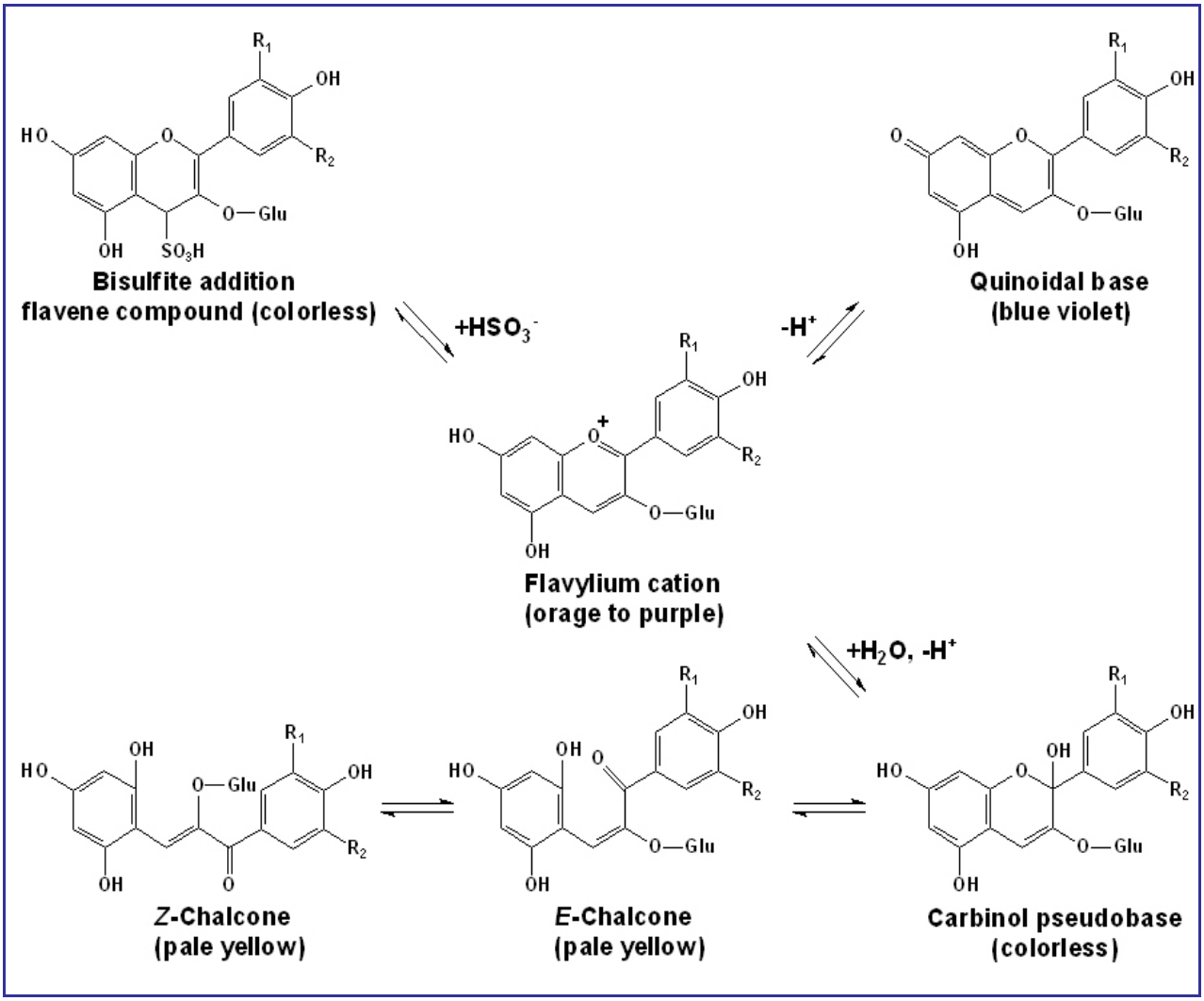


Figure 1.2. The equilibrium among major molecular forms of anthocyanins in red wines depending on pH (He *et al*. 2012)

The factors that influence the distribution of these structural forms and the color displayed in young red wines are the pH, temperature and the amount of free sulfur dioxide. The low pH leads to increase in the proportion of the flavylium cation form and also it delays the hydrolysis of anthocyanins. As the pH increases, the level of anthocyanins in the flavylium cation state and the color density quickly decline. At the usual red wine pH (3.3–3.5), the equilibrium is largely moved towards the hemiketal form, which is colorless. Additionally, the free anthocyanins in the reversed chalcones forms can offer a pale yellow color.

Thus, it can be say that the maximum absorption of young red wines at wavelength of 520 nm principally results from the flavylium ion and the quinoidal base forms (Brouillard *et al*., 2003; Lee *et. al*., 2005; He *et al*., 2012).

The monomeric anthocyanins in red wines are not particularly stable and decrease significantly during barrel maturation and bottle aging, with a significant increase in polymeric or condensed products (Monahas *et al*., 2005; He *et al*., 2012). Actually, during red wine evolution,

most of these free anthocyanins will react with other phenolic compounds to form more complex and stable pigments, while a small part of them is destroyed through oxidation or precipitation.

After several years of aging in bottle, although the wines' color is red, the monomeric anthocyanins are present in a very low amount. This fact is due to the polymerization or other reactions between monomeric anthocyanins and other compounds from red wines, as well as the breakdown reactions of a part of them (He *et al*., 2012).

The stability of free or monomeric anthocyanins in red wines depends on various factors, such as their chemical structure, pH value, the storage temperature and time, light exposure, the presence of sugars, sulfites, cofactors and different metallic ions (Monagas *et al*., 2005; Hillmann *et al*., 2011; He *at al*. 2012).

Anthocyanins are more stable at lower pH values than to higher pH. Also, the stability of anthocyanins is greater at lower temperatures and at higher concentrations (Bordignon-Luiz *et al*., 2007). The exposure of red wine to light promotes the degradation of anthocyanins (Bordignon-Luiz *et al*., 2007). Also, the presence of ascorbic acid, sugar and their degradation products contributes in a great extent to the decreasing of the anthocyanins stability (He *et al*., 2012).

In red wines can appear intramolecular copigmentation between anthocyanin molecules or between an anthocyanin and other colorless chemicals, named intermolecular copigmentation (Boulton, 2001). It can be argued that the copigmentation of anthocyanins in wines is a competitive equilibrium involving several anthocyanins and many cofactors. In young red wines, anthocyanins exist as weak complexes with themselves named self-associations, or with other compounds, named cofactors, resulting in the formation of copigmented anthocyanins (Boulton, 2001). Self-association is as a special form of copigmentation in which, the copigments are even anthocyanins. Contrary to the classical copigmentation between anthocyanins and other cofactors, self-association might produce a hypsochromic shift (the maximum absorption wavelength is shifted toward the lower values) (Miniati *et al.,* 1992; Boulton, 2001; González-Manzano *et al*., 2008). Therefore, compared with self-association, copigmentation process is more important concerning the color modification displayed in young red wines. Both of these associantions are formed by processes that involve stacked molecular aggregation by hydrophobic interaction (Boulton, 2001). As a result of these processes the color density of red wines can be significantly increased by hyperchromic effect, exhibited by a shift towards higher intensities and a bathochromic effect exhibited by an increase in the maximum absorption wavelength with 5 – 20 nm (Mirabel *at al*., 1999, Boulton, 2001, Gauche *et al*., 2010). In the same time, the color tonality may be affected because a bathochromic effect provides more purple hue to young red wines as a result of moving the anthocyanin equilibria towards their colored forms. This fact can explain many issues regarding the color expression in young red wines (Mirabel *at al*., 1999).

As stated by Boulton (2001), Cavalcanti *et al*. (2011), copigmentation is one of the most significant phenomenons with a significant impact on the red wines color. The understanding of this process that appears in very young red wines could help to predict the color properties of these red wines based on phenolic profiles of processed grapes. Copigmentation is of a great importance in understanding the relationship between grapes composition and wine color, the variation in color and pigments concentration between wines, and in all reactions involving anthocyanins during oak and bottle aging. Copigmented anthocyanins are the complexes that

result by reactions between anthocyanins and copigments molecules or cofactors. Cofactors are colorless compounds that when added to a solution containing anthocyanins will act to enhance the color of the solution. Thus, the copigmentation determines the pigments to exhibit a greater color than would be expected based on their concentration. The main cofactors in young red wines are expected to be the flavan-3-ols and flavanols, hydroxycinnamic acids and hydroxycinnamoyl derivatives, oligomeric proanthocyanidins and in the case of self-association even the antocyanins molecules can react as copigments (Mirabel *at al*., 1999; Boulton, 2001; Gauche *et al*., 2010).

The levels and ratios of the cofactors are considered one of the moust important factors that can affect the copigmentation phenomenon. The variation of the relative proportion of these cofactors among wines obtained from various grape varieties, vintages and winemaking techniques may result in red wines with different profiles of color (Schwarz *et al*., 2005; Soto *et al*., 2010). The sandwich configuration of the anthocyanins stacks occurring in the copigmentation complexes resulted in limiting of water access to the chromaphore of the anthocyanins, thus being limited the formation of chalcone or carbinol pseudobase which are colorless hydrated forms (Santos-Buelga, 2009). Therefore, copigmentation can result in a higher color intensity of anthocyanin solutions than could be expected from its anthocyanin level and the pH value.

The free anthocyanidins are more sensitive to the oxidative reactions resulting in irreversible losses of color and browning (Ribéreau-Gayon *et al*., 2005). From this point of view, the copigmentation plays a significant role in the protection of anthocyanins color. This phenomenon has the both results: wine color stabilization and enhancement.

Copigmentation has not previously been taken into account in traditional wine color measures, in the relationship between color and pigment analysis, or in spectrophotometric assays for anthocyanin content. Copigmentation is typical for young wines, which can account for 30 and 50% of their color, being primarily influenced by the levels of several specific, noncolored phenolic components or cofactors (Boulton, 1996; Mirabel *et al*., 1999; Boulton, 2001). Ther wines obtained from grapes rich in cofactors and/or with a high level of acylated forms of the non-malvidin pigments may have a higher level of copigmentation (Boulton, 2001). This is one of the reasons for the weak copigmentation in the Sangiovese wines, which are noticed a lack of the acylated pigments while red wines from Merlot and Cabernet Sauvignon grape varieties contain high levels of acylated anthocyanins.

There is an equilibrium that exists between the free anthocyanins and cofactors from grapes and the copigmented stacks. As the cofactors and the anthocyanins associate to form copigmented stacks, the equilibrium is shifted to favor extraction of both free anthocyanins and free cofactors. Thus, the copigmentation permits a greater extractability of anthocyanins and cofactors from the grape skins.

The copigmented stacks also act as a reservoir for free flavylium ion, and it can see a decrease in the contribution of copigmentation to red wine color over time. Once the red wine aging, the free anthocyanins react to form polymeric pigments, and this fact leads to shift the equilibrium to replenish free anthocyanins by releasing them from the copigmented

stacks. Therefore, in the aging time, the stacks tend to break-up and copigmentation decreases as a result of this equilibrium (Boulton, 1996; Boulton, 2001).

The structure and concentration of cofactors and pigments as well as pH, value are the main factors which influence the copigmentation process (Boulton, 2001). The pH suitable for copigmentation is around pH 3.5. Temperature has a crucial role in the development of copigmentation process. Fermentation at low levels of temperature can favor the copigmentation and also, can delay the dissociation of the colored complexes. High temperatures used for improving the color extraction in the case of thermovinification techniques cand obstruct the formation of self-associations or copigmentation process (He *et al*., 2012). The vinification technique can affect the copigmentation throught the amount of poyphenolics extracted from pomace. If the polyphenolic compounds are extracted in insufficient quantities, significant color losses can occur, due to both poor copigmentation and poor formation of polymeric pigments. Thus, in the case of young red wines with the same anthocyanin level, the wines with low cofactors content will show larger color losses than that would be expected based on their anthocyanins content due to the weak stability of the colored complexes. Therefore, it is an obvious need for more studies concerning the impact of maceration, wood and bottles aging on red wine color.

Bottle aging of red wine is the result of many chemical processes, mostly anaerobic, involving the copigmentation phenomenon and polymerisation of anthocyanins reaction, even though some oxygen is still present initially (Somers and Pocock, 1990). Over 25 years ago Somers and Evans (1986) observed that the aged red wines went through various changes in spectral characteristics. Malvidin-3-glucoside, the most abundant anthocyanin, principally responsible for wine's red color strongly decline over time (Harbertson *et al*., 2003; Monagas *et al*., 2006). The remaining colored compounds had unknown structures but were defined by their ability to resist against bleaching bisulfite and are known as polymeric pigments (PP). Many studies over the last 20 years which have tried to define the chemical structures of polymeric pigments (PP) have led to very few conclusive results. Some of these results have demonstrated that anthocyanins are not lost during wine aging; actually, the anthocyanins form covalent adducts with tannin, undergo derivatization by keto-acids, and are linked to tannins by acetaldehyde. During aging, the monomeric anthocyanins turn into polymeric anthocyanins with different molecular mass. In practice, the phenomenon of red wine color evolution is called *wine aging*. Polymeric pigments are known to have different characteristics than monomeric anthocyanins. They are resistant to bisulfite bleaching and are not as pH dependent as monomeric forms. Due to these two combined features it can be say that PP contribute to the "*stable color*" of red wines (Giusti and Wrolstad, 2005; Alcalde-Eon *et al*., 2006).

Somers and Evans (1986) estimated that PP retained more than 50% of their maximum color at wine pH, whereas monomeric or free anthocyanins only about 23% of their color. This detail demonstrates that at wine pH, a significant proportion of the red color is coming from PP. According to molecular mass, PP are classified in large polymeric pigments (LPP) and small polymeric pigments (SPP). It was proved that grapes contain very little LPP, while the corresponding wines have large amounts of LPP. As wine ages, the tannins continue to polymerize, and LPP are formed by the expense of SPP (Harbertson *et al*., 2003). In contrast, the

color due to SPP is mostly contributed by the grape berries, since the levels in the grape are nearly the same as in the finished wine. As regards the monomeric anthocyanins, the levels tend to be higher in grapes, than in the corresponding wines.

Objective measurement of the red wine color components is an essential part of the modern concept called in the modern winemaking "*red wine color management*". Standard spectroscopic method are useful in routine analysis of red wine for assessing the chromatic parameter such as color density (CD) and hue, or wine tonality (T) but not provide information regarding the contribution of different anthocyanin pigments to wine color. For solving this issue, different methods were developed by Somers and Evans method (1977), Boulton (1996) and Mercurrio (2007). These selective spectrophotometric assays have the ability to provide more data about in changes in red wine color as a result of different changes occurred in structure of antocyanins pigments. The spectrophotometric assays developed so far, are based on the assumption that PP are much less sensitive than the anthocyanins to sulfur dioxide (SO_2) as well as to the changes recorded in pH value. Based on understanding of the pH equilibrium and the different bleaching effect of SO_2 on monomeric and polymeric anthocyanins, as well as the preferential binding of SO_2 with acetaldehyde rather than anthocyanins, Somers and Evans (1974, 1977) have developed a set of spectrophotometric measures to determine CD, total monomeric anthocyanins (TMA), SO_2 resistant pigments called PP, "*chemical age*" and "*the degree of ionization of anthocyanins*" or "*the degree of pigment coloration*", α (%). Somers and Evans (1977) established a criterion for quantification of red wines "chemical age" based on the gradual conversion of monomeric anthocyanins to polymeric form. Thus, the "chemical age" is quantified by two indices (I1 and I2) and gives a measure of the extent to which polymeric pigments have replaced monomeric anthocyanins during wine aging. I1 represent the ratio of polymeric color to the color of polymeric pigments together with the color of free anthocyanins. I2 is calculated as ratio of polymeric color to the color of monomeric anthocyanins brought in the flavyllium form by addition of acid solution together with the color of polymeric pigments. These ratios are close to zero in very young red wine, but increase to about 1.0 and 0.9, respectively, for wines older than 10 years. The parameter "α" gives a measure of the amount of pigments in colored form. This parameter represents the percentage of free colored anthocyanins that can be decolorized by sulfur dioxide (Somers and Evans, 1977). As reported by Somers (1974) strong positive correlations have been made between wine color density and wine quality. The main shortcoming is that, this method is unable to assess the contribution of copigmented fraction to the wine color.

Other method described by Boulton (1996) and Mercurrio (2007) have the ability to provide information on the contribution of all types of pigments to the red wine color.

This method was developed based on chemical properties of anthocyanins, as follows:

- By bleaching a wine sample with an excess SO_2 (represented by potassium metabisulpite solution), the bisulphite ions react selective with free monomeric antocyanins and copigmented anthocyanins (CA) to form the colorless compounds (this property explains the lost of a part of the red wine color after addition of SO_2). The color displayed in red wine after bleaching with SO_2, due to SO_2 non-bleachable pigments is attributed to polymeric pigments (PP). The percentage of SO_2 non-bleachable pigments is a comparison of the wine color before and after addition of bisulfite solution. This method,

to measure the wine color after addition of excess bisulfite, enables the identification of the color provided by pigments that are stable to SO_2 bleaching (the color SO_2-stable).

- The bleaching effect of free SO_2 in a wine sample can be abolished by addition of acetaldehyde. This effect relies on the fact that SO_2 binds more strongly to acetaldehyde than of anthocyanins. Thus, by addition of acetaldehyde, the color measured at 520 nm represents the total wine color (TC);
- The copigmented anthocyanins are destroyed in a strong alcoholic medium, so the remained color is due to MA and PP. By subtracting the color corresponding to PP can be assessed the color of monomeric anthoxyanin (MA). The ratios between the color given by MA, CA, respectively PP and TC represent the contribution of monomeric anthocyanins, copigmented anthocyanins and polymeric pigments to the total red wine color: MA (%), CA (%) and PP (%). The percentage PP (%) measurement is an indicator of how much color is provided by SO_2 – stable pigments.
- The structural transformations of anthocyanins and the equilibrium among different forms are dependent on pH, *Figure 1.3.*

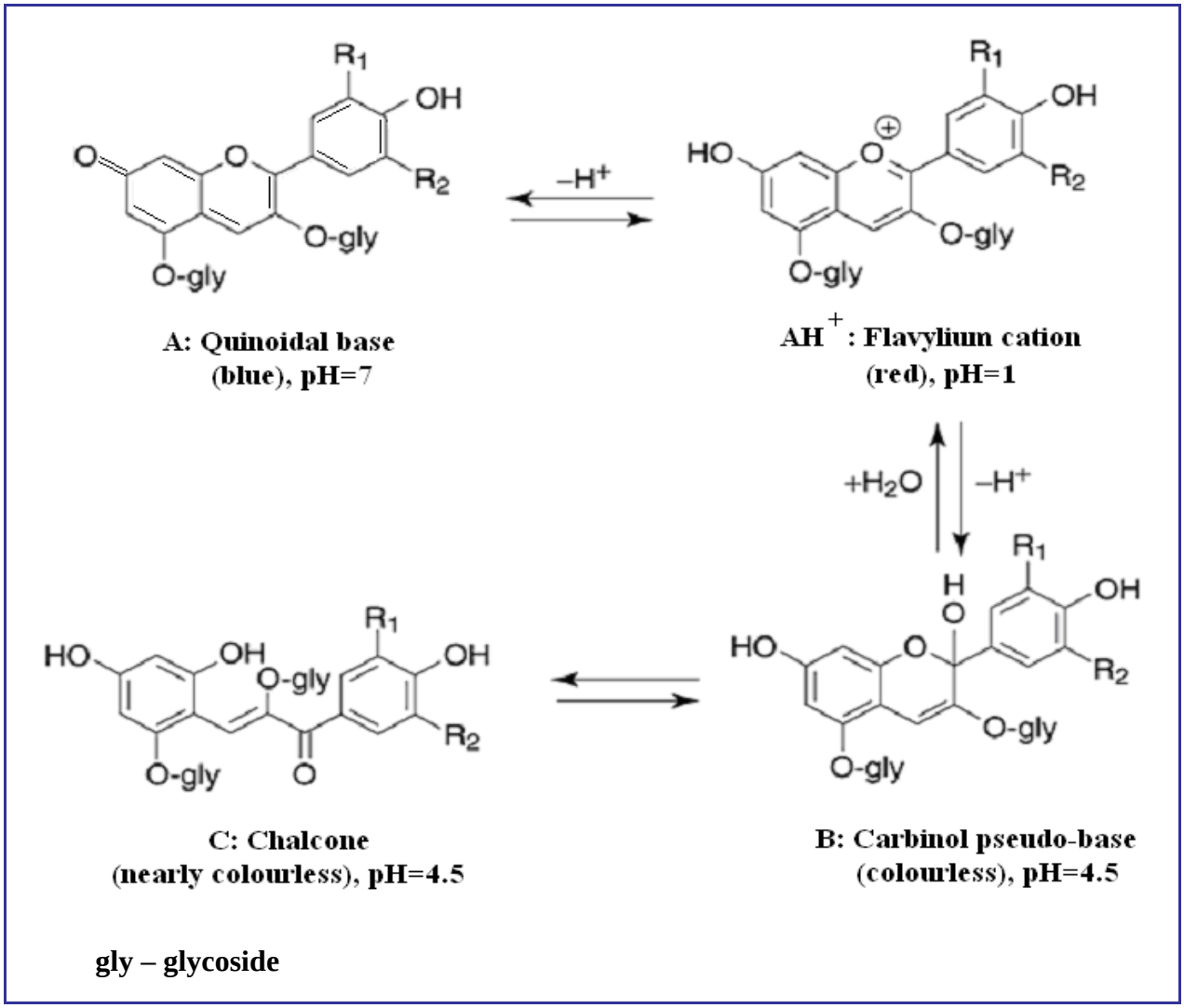


Figure 1.3. Structural transformations of anthocyanins depending on pH (Brouillard and Lang, 1990)

Nowadays, there is an obvious interest to quantify the changes occurring in red wine color over time in connection with red wines antioxidant characteristics because it is well documented that monomeric anthocyanins have a high antioxidant capacity due to their chemical structure specially adapted for this purpose. Also, it is known that the different anthocyanins pigments have not the same antioxidant properties. Thus, it is expected to be changes in antioxidant status of red wine as a result of dynamic changes in the content and profile of anthocyanin pigments.

The studies performed on this topic suggested that exists a strong correlation between color structure and antioxidant properties of red wine (Fernandez-Pachon *et al.*, 2004; De Beer *et al.*, 2005; Maletic *et al*., 2009). In agreement with study conducted by Tsai *et al.* (2004), the ferric reducing ability of plasma (FRAP) decreased during bottle aging of red wine and there was recorded a strong correlation between FRAP values and TMA content. Contrary, the radical scavenging ability of red wine, assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay increased and was highly correlated with the formation of polymeric pigments. Based on the results of these studies, it is easy to see that, during wine evolution, anthocyanin pigments and other polyphenolic compounds participate in many reactions that promote changes in the color with a great impact on antioxidant properties. Bottle aging is receiving a lot of attention today, unlike in the past because is a very important step in the red wine evolution and greatly affects its physico-chemical properties. For the wine industry, interest in high-quality products with a clear geographical origin is increasing. Nowadays, in this sector there is a growing focus for geographical identity preservation. The red wine color could be influenced by vineyard location, grape variety, age, but also by the proportion of anthocyanins and anthocyanins-derived pigments. The previously information highlighted the need for wine researchers and the wine industry to better understand the color properties of wine pigments during aging.

The effect of bottle aging on antioxidant activity of red wines and the relation between color changes and their antioxidant activity is not very well documented. Also, the level of monomeric anthocyanins during the aging of bottled red wines should receive a great attention to fully explain their contribution to the total color expression as well as to their antioxidant activity. More information is, however, needed regarding the effect of aging time on antioxidant properties of red wines prior to consumption. The red wines color stabilization during aging by polymeric pigments formation seems to be important in protection against loss of total antioxidant activity. As wines are not usually consumed immediately after production and some decreases in their antioxidant activity could occur even under favorable storage conditions (15-18°C) after one year, the use of total antioxidant activity values for analysis of market wines should be treated with a great careful. This is the main reason for that I have approached this research direction**.**

In line with the current concerns on this topic, the goal of the first study performed by Poiana et al. (2008) and presented in ***selected paper 1****, was to obtain correlated information about the changes occurred in the color of dry red wines originating from Recas and Minis vineyards related to the change in their antioxidant properties as a result to bottle aging for 30 months.*

*The second study, conducted by Dobrei et al. (2010) and presented in **selected paper 2**, was performed for assessing the impact of grape variety on the changes in the color structure of dry red wines Merlot and Pinot Noir from Recas vineyard, related to bottle aging for 24 months .*

The study presented in *selected paper 1* was designed and coordinated by me as first author, while in the research belonging to the *selected paper 2*, I was involved as co-author.

The information obtained from these studies provides a substantial basis for future researches on the red wine color topic. Also, they provide information about the stabilization of red wines color during bottle aging and the evolution of their antioxidant activity.

The objectives followed by this research direction are:

- Development of knowledge concerning the factors that contribute to red wine color change throughout its evolution;
- Identifying the causes and understanding the mechanisms that lead to changes in the antioxidant properties of red wine during its evolution;
- Obtaining of knowledge in order to predict how it will behave the wine color and its antioxidant profile during aging;
- Setting of some correlations between different categories of anthocyanin pigments and antioxidant capacity of wine.

1.2. The influence of aging time on color and antioxidant properties of Cabernet Sauvignon red wine

1.2.1. Aim

This study was an attempt to assess the changes occurred in color structure and antioxidant properties of dry red wines from *Vitis vinifera* L. cv. Cabernet Sauvignon (CS) grapes (2004 harvest year) from two viticultural regions of the Western part of Romania (Minis and Recas vineyards) during 30 months of bottle aging. For this purpose, young red wines (0-CS-R, 0-CS-M), as well as aged in bottles for 6, 12, 18, 24 and 30 months (6-CS-R, 6-CS-M; 12-CS-R, 12-CS-M; 18-CS-R, 18-CS-M; 24-CS-R, 24-CS-M; 30-CS-R, 30-CS-M) have been investigated. Bottles were kept in a dark storage room at 18°C horizontally on their side to moist the cork. This way, oxygen will have no chance of entering the bottle and the red wine will not oxidize. The wine samples were analysed in terms of color structure, expressed by contribution of monomeric, copigmented and polymeric pigments (MA, CA and PP) to the total wine color (TC) using the methods described by (Glories, 1984), as well as the content of total monomeric antocyanins (TMA), following the pH-differential method (Giusti and Wrolstad, 2005). Antioxidant profile of red wines was assessed on the base of total antioxidant activity using the FRAP assay (ferric reducing antioxidant power) as described by Benzie and Strain (1996) and free radical scavenger

activity determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Rivero-Perez et *al*., 2007). The analyses were presented in detail in ***selected paper 1***.

In performing of this research I worked closely with Prof. dr. Alin Dobrei [alin1969tmro@yahoo.com], Assoc. Prof. dr. Daniela Stoin [mucetedaniela@yahoo.com] and Lecturer dr. Alina Ghita [ghitaalina@yahoo.com].

1.2.2. Results and Discussion

This study was carried out for assessing the changes occurring in the color of red wine Cabernet Sauvignon related to aging time and vineyard of origin. Red wine color measurements were done after addition of acetaldehyde in order to abolish the bleaching effect of free SO_2 in red wine samples. The color measured at 520 nm at pH 3.6, represents the total wine color (TC) expressed in absorbance units (AU). TC is the color given by all pigments caterories such as: monomeric or free anthocyanins (MA), copigmented anthocyanins (CA) and polymeric pigments (PP). The color measured after addition of excess bisulfite solution was provided by pigments that are SO_2 – stable. The color of red wine remaining post-SO_2 bleaching is called "SO_2 - stable color" and is assigned to polymeric pigments (PP). Evaluating of SO_2 - stable wine color is of particular importance in aged wines as the content of monomeric anthocyanins is minimal. SO_2 - stable color is a measure of the anthocyanin-derived pigments that are stable to bleaching (Somers and Evans, 1977). The copigmented anthocyanins are destroyed in strong alcoholic medium and the remained color is assigned to MA and PP. Based on this measurements it was possible to quantified the contribution of MA(%), CA(%) and PP(%) to the total wine color. In *Table 1.1* are shown the values recorded for TC and the chromatic profile of red wines. Also, in *Table 1.1* are summarized the data obtained for TMA, "chemical age" indices and α.

The Recas and Minis vineyards, located in the Western part of Romania, possess high heliothermic resources, which favor the accumulation of important amounts of polyphenolic compounds in grapes. From this point of view, Recas and Minis are known as vineyards with a high degree of favorability for obtaining of red wines rich in anthocyanins. Based on data mentioned in ***selected paper 1*** it can be noted that Minis vineyard has a greater favourability for accumulation of anthocyanin pigments than Recas vineyard, reflected in TMA content recorded in young red wines (TMA was higher in 0-CS-M than in 0-CS-R).

From *Table 1.1* it is easy to see that TC decreased throughout the bottle aging. In regard to the color structure, expressed by contribution of MA, CA and PP to the total wine color, it was noticed that MA participate mostly in defining of young red wine color, while the anthocyanins in polymeric form have a relatively reduced contribution to the color of young wine. In respect to the contribution of CA to TC, it was registered higher values for wine samples originating from Minis than for similar samples from Recas. This difference, in favour of wine samples from Minis, partially explains the color more intense recorded in very young red wine of these wines. Here, the copigmentation phenomenon comes to explain the enhancement of color. Copigmentation is due to molecular associations between pigments and other, usually non-colored, organic molecules in solution. Due to these associations, the pigments exhibit a greater

color than it would be expected from their concentration Also, copigmentation phenomenon leads to both bathochromic and hyperchromic shift (Boulton, 2001).

For young red wines, copigmentation seems to lead to both a higher pigment concentration and an enhancement of the displayed color (Boulton, 1996; Landrault *et al.* 2001).

Table 1.1. Changes in TMA, color structure and "*chemical age*" during red wine aging

Wine sample	Total color (AU)	MA (%)	CA (%)	PP (%)	I1	I2	TMA ($mg \cdot L^{-1}$)
0-CS-R	7.91	75.89	12.18	11.93	0.18	0.12	167.33
6-CS-R	7.62	58.79	15.67	25.54	0.30	0.22	138.73
12-CS-R	7.14	48.25	11.31	40.44	0.40	0.38	122.16
18-CS-R	6.88	33.45	8.87	57.68	0.58	0.49	111.81
24-CS-R	6.51	25.1	8.51	66.39	0.66	0.61	104.33
30-CS-R	6.37	16.7	8.02	75.28	0.75	0.68	97.34
0-CS-M	9.08	72.03	18.83	9.14	0.11	0.08	221.16
6-CS-M	8.81	56.47	20.31	23.22	0.30	0.19	194.65
12-CS-M	8.51	44.85	14.52	40.63	0.42	0.38	162.73
18-CS- M	8.27	36.57	10.31	53.12	0.53	0.42	143.11
24-CS-M	8.04	30.4	8.77	60.83	0.61	0.46	137.14
30-CS-M	7.71	24.08	7.41	68.51	0.68	0.62	129.88

By aging, the contribution of MA (%) to TC decreases accompanied to the increases of PP (%). This phenomenon can be explained by polymerization or other reactions between monomeric anthocyanins and other compounds from red wines. PP are stable compounds responsible to the chromatic properties of wine. They are forming during wine making as well as in aging time through reactions between free anthocyanins and tannins. During aging, anthocyanins react with tannin to give rise to PP (LPP and SPP). These reactions can happen either directly, or indirectly through cross-linking of individual units-flavanols and anthocyanins-with acetaldehyde (Fernandez-Pachon *et al.*, 2004). The tannins continue to polymerize during bottle aging, and LPP are formed by consumption of SPP (Monagas *et al.*, 2006)**.**

In the first part of bottle aging (the first 6 months), it was noticed increases in contribution of CA (%) to the red wine color for both wine samples originating from Recas and Minis vineyards as a result of further copigmentation reactions. Further, the fraction of color corresponding to CA (%) decreased for both wines, throughout the aging process, *Table 1.1.*

The "*chemical age*" defines the relationship between polymeric pigments and wine anthocyanins. The "*chemical age*" assesses the "*variations in the aging characteristics*" of a red wine (Somers and Evans, 1977).

Generally, for wines older than 10 years, stable in respect to their chromatic profile, the reactions specific to the aging process are going very slowly or quite inexistent. For these wines, the values of indices expressing the "chemical age" are in the range 0.9-1.0 (Somers and Evans, 1977).

In this study, the "*chemical age*" of red wine was assessed on the basis of the two indices I1 and I2. I1 represent the ratio of polymeric color to the color of polymeric pigments together with the color of free anthocyanins. I1 is a measure of the color bleaching after addition of a bisulfite excess and the recovery of SO_2 bleached color due to the presence of bisulfite already in the wine by measuring the color absorbance after addition of acetaldehyde. This measurement is

based on the premise that the SO_2 bleaching of the anthocyanins color is reversed by adding excess of acetaldehyde.

Table 1.2. The impact of aging time on antioxidant profile of red wines

Wine sample	FRAP (mM $Fe^{2+} \cdot L^{-1}$)	DPPH (mM Trolox $\cdot L^{-1}$)
0-CS-R	30.87	9.28
6-CS-R	25.82	9.57
12-CS-R	20.16	11.12
18-CS-R	17.13	13.21
24-CS-R	15.83	13.46
30-CS-R	14.12	14.12
0-CS-M	39.64	10.16
6-CS-M	30.24	11.27
12-CS-M	26.16	12.83
18-CS- M	24.81	13.87
24-CS-M	20.05	15.17
30-CS-M	18.37	16.53

The "*chemical age*" index I2 is used to interpret the relationship between PP and wine anthocyanins measured in their flavilium form. I2 is an indication of how much of the total red pigments at low pH are provided by "SO_2 - stable wine pigments". I2 is calculated as ratio of polymeric color to the color of monomeric anthocyanins brought in the flavylium form by addition of acid solution together with the color of polymeric pigments. With aging, there was noted a decrease in anthocyanins concentration. Also, in response to the reactions between anthocyanins with other wine components there was noted a progressive increase in the "SO2 - stable pigments" particularly anthocyanin-tannin polymeric pigments. Along with aging of red wine, I2 increased accordingly.

It was noticed a significant evolution in "*chemical age*" once the evolution of color structure towards more stable forms regarding the chemical structure. The lowest values of I1 and I2 for both red wines were noticed for the youngest wines (0-CS–R and 0-CS–M).

By aging, TMA are gradually included in PP resulted in significant increases of "*chemical age*". At the end of aging, the highest values were recorded for 30-CS-R. These values prove that CS-R need shorter time for color stabilization than CS-M. This finding is strengthened by the fact that, the highest value for PP (%) was recorded at the end of aging in the same sample (30-CS-R).

Data shown in *Table 1.2* reveal a decrease of antioxidant capacity, expressed by FRAP values, with 53-54% reported to the initial value for both CS wines.

The linear correlations FRAP versus MA (%) obtained by applying of simple regression model are showed in *Figure 1.4*. From this chart it is obvious that, the decreases recorded in FRAP values in response to aging are strongly correlated with the fraction of color due to MA (correlation coefficients $R_1, R_2 > 0.98$).

During bottle aging, by decreasing of FRAP values it was noticed a significant increase in radical scavenging ability. Thus, at the end of aging, DPPH values were about 1.5-1.6 times reported to the initial value (0-CS). DPPH values were highly correlated with the fraction of color due to PP (%). The linear correlations DPPH versus PP (%) are shown in *Figure 1.5* (R_3, $R_4 > 0.985$).

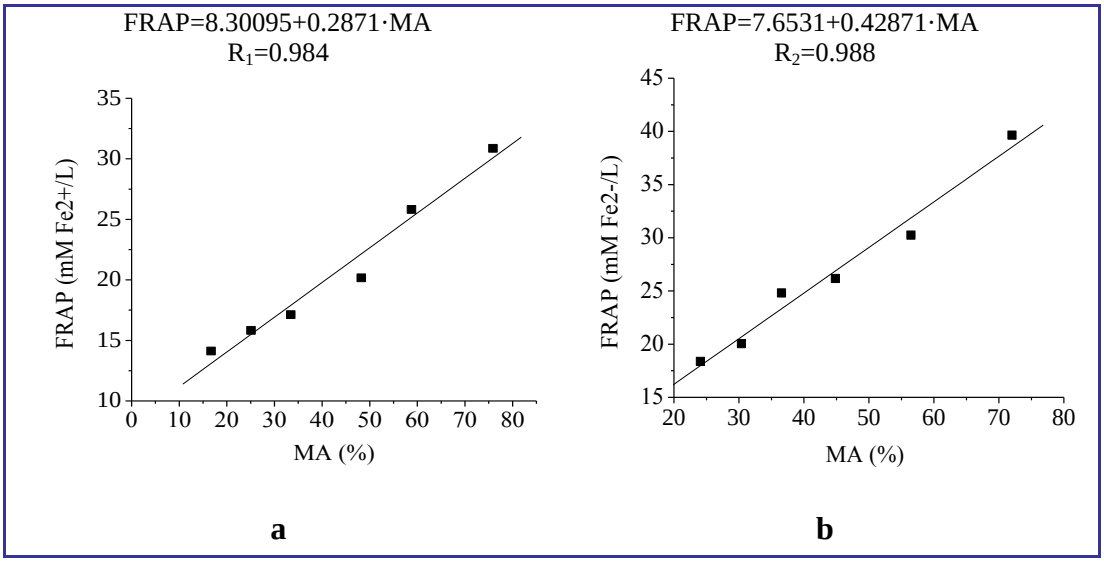


Figure 1.4. Linear correlation FRAP versus MA (**a**: CS-R; **b**: CS-M)

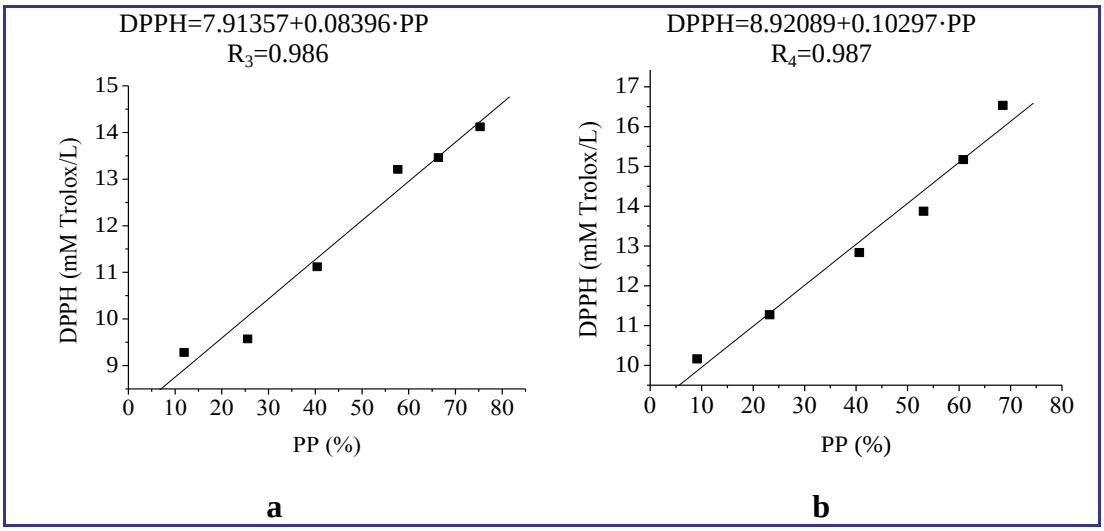


Figure 1.5. Linear correlation DPPH versus PP (**a**: CS-R; **b**: CS-M)

1.2.3. Conclusions

This study reveals that the aging time had a great impact on the color and antioxidant profile of red wine. The changes registered in color structure and antioxidant profile of the red wine subjected to ageing were strongly influenced by viticultural region and aging time. MA contributes in a highest measure to the red wine color. Contrary, the most part of color of aged wines is due to PP. For samples originating from both vineyards, SO_2 - stable color is a major contributor to the color of aged red wines. Additionally, during aging, the copigmentation process affected the color structure of red wines. The magnitude of copigmentation was more obvious in young red wines, in the first 6 months of aging, when it was recorded the highest contribution by CA to the red wine color. The FRAP values significantly decreased during aging being strongly correlated with MA (%). Contrary, the values recorded for DPPH increased by bottle aging, being highly correlated with PP (%).

Red wines vary in their aging characteristics: red wine originating from Recas vineyard appears to age faster, reaching a superior quality, compared to red wine from Minis vineyard. Based on our results, we can state that, the decreases recorded in TMA during bottle aging have affected not only the color profile but also the total antioxidant properties of red wines. Therefore, the setting of aging time could be critical for the color quality and antioxidant properties of red wines.

1.3. The effect of bottle aging on chromatic properties of Merlot and Pinot Noir red wines

1.3.1. Aim

The goal of the research presented in ***selected paper 2*** was to investigate the differences occurring in color of dry red wine during bottle aging for two years. The red wines have been obtained in Recas winery from Merlot (M) and Pinot Noir (PN) grape varieties (2005 harvest year) and investigated as young wines (0-M, 0-PN) and during aging for 4, 10, 18 and 24 months (4-M, 4-PN; 10-M, 10-PN; 18-M, 18-PN; 24-M, 24-PN) in terms of chromatic parameters (color density – CD; tonality – T; chromatic structure represented by the contribution of yellow or brown pigments, red pigments and blue pigments to the wine color) using the Glories methods (Glories, 1984), the color structure (MA%; CA% and PP%) by Boulton's method (1996), TMA content by pH-differential method (Giusti and Wrolstad, 2005), "chemical age" indices (I1 ans I2) and the "degree of ionization of anthocyanins" (α) by Somers and Evans method (1977).

The protocols of these investigations were detailed in *selected paper 2*. Red wines were kept in bottles at 18°C in dark because ultraviolet light cause the degradation of otherwise stable organic compounds from red wine. Also, the bottles were kept horizontally on their side to moist the cork. For this research I worked with Prof. dr. Alin Dobrei [alin1969tmro@yahoo.com], Prof. dr. Florin Sala [florin_sala@yahoo.com], Lecturer dr. Alina Ghita [ghitaalina@yahoo.com] and Prof. dr. Iosif Gergen [igergen@yahoo.com].

1.3.2. Results and Discussion

Standard spectroscopic method is commonly used in Romanian wineries and reseach laboratories to measure the red wine color. An additional method used worldwide to measure wine color has been developed by Glories (Glories, 1984). This analysis performed at natural wine pH, involves the absorbance measurements at three wavelengths, to assay the wine color. For simple and global characterization of the red wine color on the basis of absorbance recorded at characteristics wavelengths, we assessed different indices, out of which we recall: CD, T, chromatic structure based on the contribution of yellow or brown pigments, red pigments and blue pigments to the wine color.

Red wine color can be evaluated by summing of the contribution of three components: red, yellow or brown and blue. The yellow or brown pigments show absorbance at A_{420} assigned

to tannins and anthocyanins degradation products. The red pigments show absorbance at A_{520} being assigned to free anthocyanins under flavylium cations form and anthocyanins-tannins combinations in aged wines. The blue pigments show absorbance at A_{620} assigned to free anthocyanins under chinonic form or combinations between tannins and anthocyanins (Pascu, 2005).

Other methods that offer more information about red wine color were developed by Somers and Evans method (1977) as well as Boulton (1996). By addition the bisulfite solution in excess it was possible to measure the color provided by pigments that are stable to SO2 bleaching (polymeric pigments, PP). The percentage of SO2 non-bleachable pigments was found by comparing the wine color before and after addition of bisulfite solution. Also, the copigmented anthocyanins are destroyed in strong alcoholic medium and the remained color is assigned to MA and PP. Sommers and Evans method enables to assess the "*chemical age*" indices as well as "*the degree of ionization of anthocyanins*" or "*the degree of pigment coloration*", α (%).

From *Table 1.3* it can be seen the chromatic structure obtained by Glories method.

Table 1.3. The changes in chromatic parameters of red wines in response to aging

Wine sample	A_{420} (AU)	A_{520} (AU)	A_{620} (AU)	CD (AU)	T	Chromatic structure		
						(%) yellow or brown pigments	(%) red pigments	(%) blue pigments
0-M	3.117	4.898	0.693	8.708	0.64	35.79	56.25	7.96
4-M	3.184	4.476	0.705	8.365	0.71	38.06	53.51	8.43
10-M	3.352	3.973	0.713	8.038	0.84	41.70	49.43	8.87
18-M	3.449	3.831	0.724	8.004	0.90	43.09	47.86	9.05
24-M	3.528	3.671	0.742	7.941	0.96	44.43	46.23	9.34
0-PN	2.711	3.979	0.512	7.202	0.68	37.64	55.25	7.11
4-PN	2.749	3.647	0.519	6.915	0.75	39.75	52.74	7.51
10-PN	2.777	3.353	0.536	6.666	0.83	41.66	50.30	8.04
18-PN	2.832	3.278	0.548	6.658	0.86	42.54	49.23	8.23
24-PN	2.903	3.119	0.589	6.611	0.93	43.91	47.18	8.91

A closer look of data from *Table 1.3* reveals that during aging, the percentage of color due to yellow or brown pigments (flavanoids and tannins; some anthocyanins) increased and the fraction of color due to the red pigments (mostly anthocyanins) decreased, but the chromatic structure is more equilibrated in the aged red wines.

The blue pigments participated in a small measure to the investigated red wines. In the case of young red wines, the largest part of color is attributed to the red components while the yellow component has contributed with less than 40% to the red wine color.

It can be said that, during aging, the components of red color have recorded significant changes: the values registered for A520 decreased while A420 and A620 increased. The highest values of color intensity were registered for young red wines, particularly for the Merlot young red wine. The smallest values for CD were noticed for aged red wines.

The results presented in *Table 1.3* reveal the fact that the color intensity drops in the aging time, while the wine color hue, or tonality (T) intensified by aging. Following the same pattern, an increase in the hue value is expected for a red wine once it ages. This increase in the hue describes a shift from purple red via brick red to brown tones of the wine color. The hue values in the range 0.8-0.9 are specific for aged red wines, and the values in the range 0.5-0.6 for young

red wines.The decline recorded for CD is due to the consumption of monomeric anthocyanins, in the aging time. In this phase, due to the fact that A420 increased and A520 decreased, the color tonality is emphasizes, so that, it increased in response to bottle aging. The decrease of A520 was due to the precipitation of condensed tannins.

The decreasing of free anthocyanins content during aging is showed in the *Figure 1.6*. Based on these data we can see that, TMA content decreased from 179.44 to 122.29 mg·L^{-1} for Merlot wine and, from 132.81 to 85.72 mg·L^{-1} for Pinot Noir.

Data from *Table 1.4* reveal that, during aging, the fraction of color due to PP increased. Contrary, the color assigned to MA and CA decreased in response to aging. In this period, the monomeric anthocyanins turn into polymeric forms with different molecular mass. In practice, the phenomenon of red wine color evolutions is known as the wine aging (Somers and Evans, 1974). These changes are attributed to the stabilization of red wine color during bottle aging.

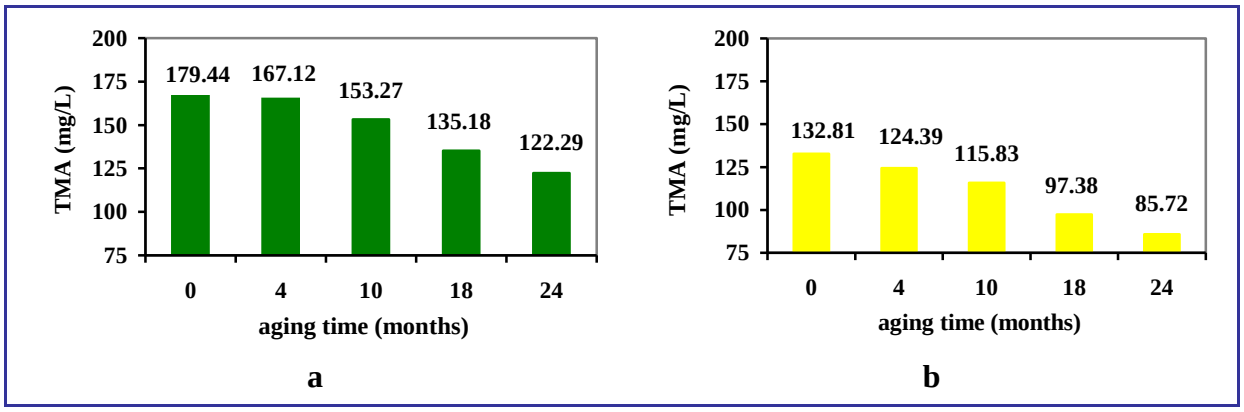


Figure 1.6. The changes in TMA content during red wines aging (**a**: M; **b**: PN)

The color stabilization can be attributed to the loss of a part from monomeric and copigmented forms of anthocyanins as a result of different combinations occurring between tannin and anthocyanins, formation of polymeric pigments and other intermolecular associations. The polymeric pigments are very stable compounds responsible for the color of aged red wine (Bakker *et al.*, 1986; Mazza *at al*., 1999; Ollala *et al*., 1996; Monagas *at al*.; 2006; Alcalde-Eon *et al*., 2006).

PP are present in a low measure in the young red wine Merlot and their contribution to the total red wine color increased with bottle aging. The red wines need different time for color stabilization depending on the grape variety, maturation and aging conditions (Monagas *et al*., 2006; Pascu, 2005).

The copigmented anthocyanins are the complexes that result by reaction between anthocyanins and copigments molecules. This phenomenon causes an enhancement in the color of young red wines that resulted in both a shift from reddish to bluish hue (bathochromic effect) and a increase in CD (hyperchromic effect). The small contribution of copigmented anthocyanins to the red wine color in the case of 0-PN is due the specifics of Pinot Noir grapes variety that contain a little amount of cofactors (especially flavan-3-ols and flavanols).

The effects of copigmentation are largely dependent on the molar ratio of cofactor to pigment in aged wines. Once the aging of red wines progresses, the level of free copigmentation

cofactors decreased (Mirabel *et al*., 1999; Boulton, 2001). The reducing of cofactor concentration over time could explain the weak copigmentation or lack of copigmentation in aged wines. The color exhibited by anthocyanins, when they are in copigmented complexes, can be several times higher than in the free form (Boulton, 2001)

Table 1.4. The evolution of red wines color structure during the course of bottle aging

Wine sample	PP (%)	MA (%)	CA (%)
0-M	10.33	54.18	35.49
4-M	18.71	48.92	32.37
10-M	27.96	43.88	28.16
18-M	44.55	32.68	22.77
24-M	53.99	26.82	19.19
0-PN	26.68	50.61	22.71
4-PN	34.39	46.16	19.45
10-PN	51.1	32.52	16.38
18-PN	53.8	34.17	12.03
24-PN	67.96	21.17	10.87

From data showed in the *Table 1.4* it can be observed that the copigmented anthocyanins are destroyed by aging. Copigmented anthocyanins also act as a reservoir for free flavylium ion, and it can be noted a decrease in the contribution of CA (%) to the red wine color over time. Therefore, by aging of red wine, the stacks tend to break-up and the copigmentation decreases to restore this equilibrium (Boulton, 1996).

Lower copigmentation identified for Pinot wines is due to the low concentration of cofactors of this grape variety (Boulton, 2001). The percentage of color assigned to copigmented anthocyanins decreases after 24 months of aging for both analyzed wines. From these data results that the color of Pinot Noir wine is more stable than color of Merlot wine. Thus, Merlot wine requires more aging time for color stabilization. This process could be extended during several months or even years.

From data presented in the *Tables 1.3* and *1.4* it can be seen that, the large decreases in color assigned to CA and MA were not resulted in important decreases in CD. We assumed that, the color of PP formed in response to aging compensated for a part of the color assigned to MA and CA that was lost over aging.

In *Figure 1.7* is presented the evolution of "*chemical age*" registered during bottle aging of red wine. The "*chemical age*" of wine was quantified by two indices, I_1 and I_2. The ratios are close to zero in new or very young wines, but increase to about 1.0 and 0.9, respectively, for wines older than 10 years (Somers and Evans, 1974). Based on the values of $I_{1\ it}$ can be appreciated the contribution of polymeric pigments to total red wine color.

It can be noticed that I1 and I2 had low values for both 0-M and 0-PN, while the value of these indices reached to 0.54, respectively 0.51 for 24-M and 0.68, respectively 0.64 for 24-PN.

These data show that after 24 months of aging, the color due to PP represents 54% from the total wine color for Merlot and 68% for Pinot Noir. The values recorded for I2 revealed that, the color due to PP represents 22-51% from the color of anthocyanins in flavylium form for Merlot, respectively 32-64% for Pinot Noir.

On the basis of these indices, it can be observed the gradual conversion of monomeric anthocyanins to polymeric form in relation to red wine aging. *Figure 1.8* shows the changes recorded for α in response to bottle aging. During aging time, α significantly increased. This parameter indicates the percentage of anthocyanins from red wine found in the flavylium or ionized form, being associated with the power of anthocyanins coloration.

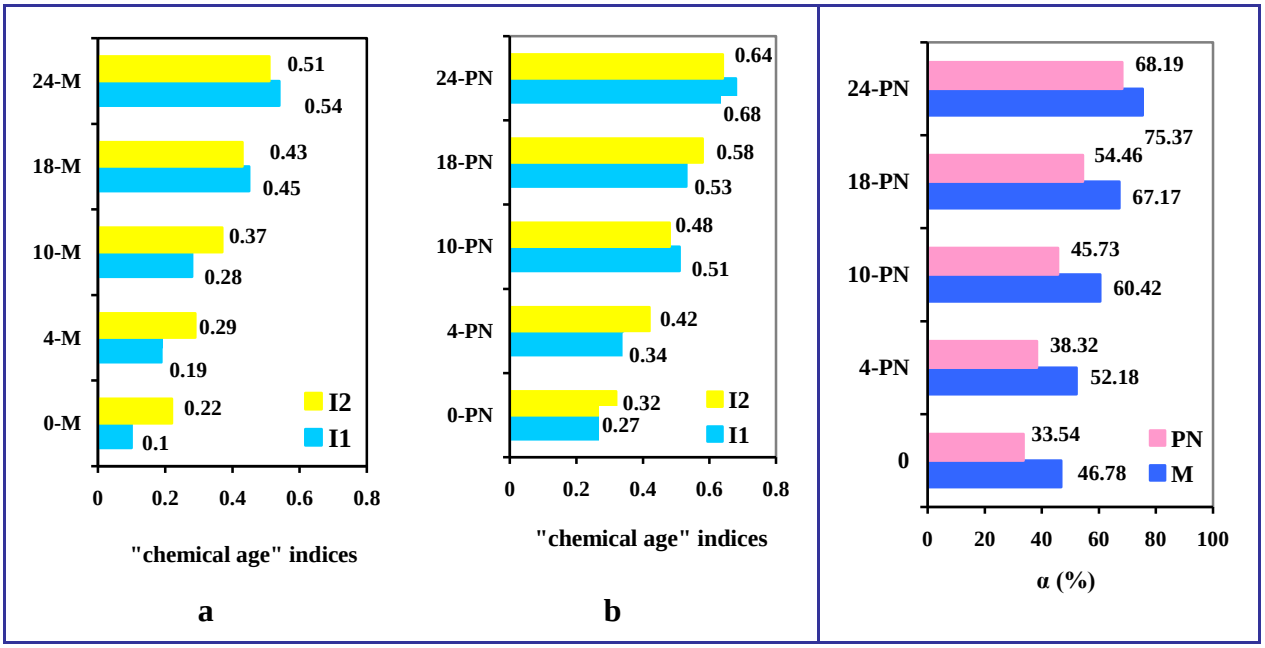


Figure 1.7. The changes recorded in "*chemical age*" during bottle aging (**a**: M; **b**: PN)

Figure 1.8. Changes in α value during bottle aging

1.3.3. Conclusions

The components of red wine color have passed through important changes during bottle aging highlighted by decreasing of color density in parallel with increasing of tonality. In addition, the percentage of color due to PP increased, while the contribution of MA and CA to the red wine color significantly decreased. PP, prevailing in aged red wines, are stable color compounds. MA participated in the highest measure to the color of young red wines and their contribution declined over time. Along the aging of red wine, it was noticed a significant increase in the contribution of yellow pigments to the total wine color, while the contribution of red pigments has recorded important decreases. The contribution of CA to the red wine color decreased in response to decrease of the cofactors content over time. The losses recorded in the contribution of CA to the red wine color in the aging time are related to the grape variety. The both indices I1 and I2 expressing the "chemical age" of wines, have recorded significant increases with the color evolution towards more stable form in terms of chemical structure. The red wines Pinot Noir and Merlot vary in their aging characteristics: Merlot wine requires more time of aging before reaching its optimum quality.

The obtained results support the assumption that, the grape variety and the aging time play a great role in the stabilization of red wine color.

1.4. Scientific contributions of the author to the actual state-of-knowledge

Regarding the subjects presented above and based on the studies done by the author and the obtained results on this topic, the personal contributions include:

- According to the candidate knowledge, the presented works done in the field of red wine color changes as result of aging process are the first studies conducted for red wines obtained in two famous wineries from Western Romania. Moreover, the two indices expressing the "*chemical age*" related to the grape variety, vineyard and aging time were for the first time evaluated in Romania;
- Meanwhile, the author being extremely interested in the red wine color analysis published in 2008 a book entitled "*The analysis of red wine color*" (*published in Romanian*) at EUROBIT Publishing House (ISBN 978-973-620-378-7, 181 pp.). This is a modest attempt to focus different aspects on this topic, such as: the opportunity to use some selective spectrophotometric methods for red wine color assessment, the influence of copigmentation on red wine color quality, the factors contributing to the change in red wine color over the time and development of some correlations between different anthocyanin pigments and antioxidant profile of red wine;
- Grape variety and the aging time are playing a great role in the red wine color stabilization. Also, the aging time has a great impact on the color and antioxidant profile of red wine. The red wine color passes through important changes during aging evidenced by decreasing of CD accompanied by increasing in its hue or tonality. An increase in the hue value is expected for a red wine as it ages. This increase describes a shift from purple red via brick red to brown tones of the wine color. The changes of chromatic parameters CD and T were strongly dependent on the viticultural region, aging time and grape variety;
- Wine pigments contribute with different amounts to wine color, depending not only on the age, grape variety, as well of the proportion and presence of anthocyanins and anthocyanins-derived pigments;
- The change in SO_2-stable color and the change in the percentage of SO_2-stable red wine pigments were related to the change in wine color during aging;
- The anthocyanins polymerization was prevailed among the reactions of anthocyanins occurring during aging. The stabilization of red wine color during aging involves the formation of PP on the base of free or monomeric anthocyanins consumption;
- TMA participated in a higher measure to the young red wine color as well as to their antioxidant properties. Contrary, the color of aged wines is due to PP that are stable color compounds, responsible for their antiradicalic properties. SO_2 - stable wine color is a major contributor to the color of aged red wine. Therefore, the color of PP may be the driving force which is behind the color density of aged wine;
- Once the aging of red wines progresses, the contribution of copigmented anthocyanins decreases. The weak copigmentation in aged wines could be explained by reducing in

cofactor concentration over time. The significance of copigmentation was still relevant after two years of bottle aging;

- Both indices I_1 and I_2 expressing the "*chemical age*" of wine, significantly increase with the color evolutions towards more stable form in terms of chemical structure;
- The chemical index I_2 values indicated that for young red wine, the major contributor to wine color were the pH-dependent wine pigments, while the SO_2-stable wine pigments provided only a minor contribution. For aged red wine, the situation was contrary;
- Red wines vary in their aging characteristics depending on the grape variety and vineyard: some wines appear to age faster, reaching a superior quality, while others require more time of aging before reaching their optimum quality;
- The selective UV-VIS methods used for red wine color measurements represent a valuable opportunity for winemakers that have not been considered in traditional wine color analysis; these methods offers some advantages over standard method used in the routine analysis of red wine color because they are able to provide more information about the red color structure, as well as concerning their aging characteristics. It may be advanced the idea that, the chromatic profile of red wines can be directed by setting of aging time.

The original elements of this research consits in the obtaining of a real image regarding the evolution of anthocyanic pigments and antioxidant profile of red wine during bottle aging, the extension of modern wine color analysis and interpretation of the wine color variations in relation to its antioxidant properties, the development of scientifically-based color profiles of red wines. Also, the obtained results are important in order to predict the evolution of red wine color during bottle aging.

Knowledge of the color pigments contribution to wine color will enable winemakers to manipulate various techniques and factors to achieve optimized color for their red wines.

2. Scientifical achievements concerning the impact of processing and storage on antioxidant characteristics and color of fruit and gelled fruit products

The studies on this direction were carried out for solving the objectives of research project no. 637/21.01.2009 with theme: *Studies regarding the impact of technological treatments on antioxidant characteristics of some products obtained from wild berries*, developed between Banat's University of Agricultural Sciences and Veterinary Medicine from Timisoara and SC Etco Europe Trade Company from Sebis (Arad County) in the period 2009-2011 and coordinated by me as director.

2.1. Background

As a result of increased attention paid by consumers to the health and nutritional aspects of fruit products, the significance of fruit phenolics as dietary antioxidants has recently been suggested by several research groups. Small fruits as different berries, sweet and sour cherries contain significant levels of phytochemicals with important biological properties (Moyer *et al*., 2002; Scalzo *et al*., 2005). Most people will associate the berries consumption with the idea of healthy food. In addition to being a delicious part of any diet, consumption of small fruits has been associated with diverse health benefits, these fruits are known for their bioactive properties such as antioxidant activity, cardiovascular protection, antidiabetic properties and inhibition of carcinogenesis, mutagenesis and other degenerative or age-related diseases (Bachgi *et al*., 2004; Schmidt *et al*., 2005). These beneficial effects could mostly be due to their high concentrations of natural antioxidants (Bachgi *et al*., 2004; Pantelidis *et al*., 2007) including phenolic compounds and ascorbic acid. Berries have a complex mixture of anthocyanins which may fortify blood vessel walls, induced increase in flexibility of the capillaries, improve blood flow and maintain good circulation (Kalt *et al.*, 2000; Zafra-Stone *et al*., 2007).

Blueberries, bilberries, raspberries, blackberries are very important natural resource possessing a high level of antioxidant properties which are closely linked to the levels of phenolic compounds such as ellagic acid, tannins, ellagitannins, quercetin, gallic acid, anthocyanins and cyanidins (Pantelidis *et al*., 2007). Thus, these berries are an excellent source of phytochemicals that are proven to have significant biological activity (Prior *et al*., 1998; Schmidt *et al*., 2005).
Due to the high contents of health-promoting compounds, these fruits have long been considered super foods, being often referred to as natural functional products (Joeph *et al.*, 2000). During the last decade, much interest has been focused on berries due to their high levels of anthocyanins and antioxidant capacity. Prior *et al.* (1998) reported a significant correlation between the antioxidant capacity and the total content of anthocyanins and phenolics among blueberries. Also, the results reported by Moyer *et al. (*2002) and Koca *et al.* (2008) are found significant differences in the anthocyanins, phenolics, and antioxidant capacity phenolic content among the different species of berries. Additionally, polyphenolic compounds including anthocyanins and proanthocyanidins are not completely stable. After harvest these compounds can change during food processing and storage, which may reduce related biological activity (Klopotek *et al*., 2005; Schmidt *et al*., 2005).

The berries harvest season in Romania as well as in the most part of Central Europe is short, lasting from July to September. Considering that berries are extremely perishable, only a small percentage of berries are marketed fresh, most berries end up frozen or canned. Frozen berries can be further processed into various shelf-life products such as jams, purees, jellies and juices available to consumers all year round (Lohachoompol *et al*., 2004; Schmidt *et al*., 2005).

Freezing has been successfully employed for the long-term preservation of many fruit, providing a significantly extended shelf life. It can be said that freezing and frozen storage is one of the best ways of preserving, resulting in increase the flexibility for consumers by extending the length of time in which fruits are available. Frozen fruit are available year round, and are often less expensive than their "fresh" counterparts. In frozen berries place changes in antioxidant content and color as a result of oxidation-reduction reactions occurring in fruits. These changes will be influenced by: the initial quality of berries, raw material processing prior to freezing, freezing methods, storage conditions (temperature and relative humidity), storage time of frozen berries and quality of container (Mullen *et al*., 2002; Scibisz and Mitek, 2007).

Due to the high antioxidant levels found in berries, fruit processors are seeking effective processing techniques such as IQF (*Individual Quick Freezing)* to further optimize the amount of antioxidants retained in the final product. Freezing of berries will increase flexibility for consumers by extending the length of time in which fruits are available. IQF is one of the simplest and least time-consuming ways to preserve berries, but the long-them frozen storage might affect anthocyanins, polyphenols, vitamin C, color quality and antioxidant effects of berries.

The literature provides several studies about the effects of freezing and frozen storage on the retention of antioxidants in different berries (Ancos *et al*., 2000; Kampuse *et al.*, 2002; Gonzalez *et al.* 2003; Lohachoompol *et al*., 2004; Mullen *et al*., 2002; Scibisz and Mitek, 2007). At some point, it was obvious that the content of bioactive compounds in frozen fruit is greatly affected by storage time. In this respect there are little information about the effect of long-term frozen storage on antioxidant properties, total phenolics, color indices and other bioactive compounds of different kind of berries. Considering that during frozen storage, the levels of antioxidants compounds from berries may be altered resulting in a change in antioxidant properties, *the goal of the first study on this research direction* performed by Poiana *et al.* (2010) *was to investigate how freezing and long-term storage can affect the retention of antioxidant properties and bioactive compounds in berries. This study is presented in* ***selected paper 3****.*

Recently, an increased interest in the identification of valuable possibilities for preserving the antioxidant properties of products obtained by thermal processing of fruits rich in bioactive compounds can be noticed. The increasing demand for food with antioxidant action has focused interest on fruit products as a good source of biologically active compounds with considerable antioxidant potential. Among various products for long-term preservation of fruits, one of the most popular, produced by both home canners and commercial processors is jam (Amakura *et al*., 2000; Savikin *et al*., 2009; Howard *et al*., 2010). The preservation of fruits by jam making is a major direction of the fruits processing but the antioxidant and sensorial characteristics of final products are strongly affected by various factors (Chaovanalikit and Wrolstad, 2004; Brownmiller *et al*., 2008). Raw material quality, products formulation, processing methods

varying in the number and type of unit operations, heating temperature, processing time and storage conditions can significantly affect the amount of bioactive compound preserved in fruit products and finally, their antioxidant properties (Patras *et al*., 2010; Rababah *et al*., 2011).

As a result of health benefits and medical restriction an increasing number of consumers are turning to fruit products with low-sugar content due to their high nutritional value (Moura *et al*., 2012). Low sugar jams were originally developed for diabetics and people with specific health problems. These products offer an important opportunity to create a healthy, seasonally independent and mixed diet. The food industry has been confronted with a new challenge for satisfying the consumers concretized in the development of low-calorie products with acceptable sensorial characteristics and competitive prices, by preferably employing the conventional processing equipment. One question that arises is whether the high quality low-sugar jams could represent a good source of bioactive compounds as fresh fruit does. In this regard, an extensive analysis is necessary in terms of thermal processed products behavior in relation to various factors. During jam processing, the fruits are subjected to a long heating at high temperature. A significant issue we face during jam processing in households, small-scale or industrial sectors is the negative impact of thermal treatment on the bioactive compounds and consequently, on the antioxidant properties displayed by the obtained products.

The researches conducted by Rommel *et al*. (1992), Patras *et al.* (2009, 2010), Srivastava *et al*., (2007), Brownmiller *et al.* (2008), Howard *et al*. (2010), Rababah *et al.* (2011), Syamaladevi *et al.* (2012) have shown that various processing methods of fruits cause serious alterations in their antioxidant properties due to the loss of anthocyanins and phenolic compounds**.** In fruit jams, anthocyanins represent both a source of natural antioxidants and a key parameter for color quality, affecting their acceptance by the consumers (Gimenez *et al*., 2001).

The anthocyanins content in fruit products derived from original fruits being much smaller than the original anthocyanin content in the raw material because the anthocyanins are highly unstable pigments, easily susceptible to degradation.

The manufacturing of berry products leads to deterioration of anthocyanins and the color of the final products. Moreover, during storage the color of berry products is degraded further. Thus, the choice of a processing method immensely affects the color quality of the food products. Besides its nutritional properties, the jam color is an important factor influencing consumer acceptability, thus minimizing of the anthocyanins losses during processing and storage is one of the primary concerns (Scibisz and Mitek, 2007). However, obtaining a strong and stable color of different fruit products is problematic during processing and storage. Anthocyanins content has a critical role in the color quality of many fresh and processed fruits. The color deterioration is associated with the loss of anthocyanin pigments or formation of brown pigments (Brownmiller *et al*., 2008).

The results reported by Sadilova *et al*. (2007) have revealed that during heating, the anthocyanins degradation generally cause the pigments discoloration having a great impact on color quality and also, on their in vitro antioxidant capacity. Pinto *et al.,* (2007) showed that anthocyanins are very sensitive to temperature, and a combined time/temperature process can greatly reduce the level of pigments in the obtained products. Anthocyanins losses are probably due to complexation with co-occurring compounds during jam processing.

During heating, degradation and polymerization usually lead to anthocyanins discoloration (Gimenez *et al*., 2001). Temperature, oxygen, pH, light illumination, water activity, presence of saccharides and their degradation products and activities of various enzymes are considered to be important factors influencing anthocyanins stability and the amount of bioactive compounds (Wrolstad *et al*., 2005; Howard *et al*., 2010). Generally, temperature and duration of boiling and pasteurization steps, jam recipe (sugar, citric acid content and pectin concentration), degree of fruit ripeness as well as storage conditions of products are the most important factors determining the antioxidant properties and color quality of berries jam (Kim and Padilla-Zakour, 2004; Scibisz and Mitek, 2007). The antioxidant activity during fruit thermal processing may also be affected by the loss of water-soluble antioxidants, such as phenolics, or interactions with non-phenolics compounds (Bursac Kovacevic *et al*., 2009).

Also, the storage of fruit products can induce additional losses in anthocyanins and antioxidant activity (Fracassetti *at al.,* 2013**).** Important losses in TMA content during storage of various fruit products were previously reported by other studies (Brownmiller *et al*., 2008; Hager *et al*., 2008; Holzwarth *et al*., 2012; Moura *et al*., 2012). Losses of anthocyanins and/or formation of brown compounds during storage of frui products have been attributed to many factors such as pH, phenolic compounds, sugars and sugar degradation products, oxygen, ascorbic acid, fruit maturity and thawing time. Other factors may have a significant role in the expression of color in fruit jams by copigmentation or some other physico-chemical processes (Lewis *et al*., 1995; Kopjar *et al*., 2007; Kopjar *et al*., 2009). In the storage time, oxidative reactions occur due to enzymatic activity exhibited by polyphenoloxidase, peroxidase and glucosidase. Moreover, natural light exposure, presence of saccharides and their degradation products will enhance the degree of pigments destruction (Wrolstad *et al*., 2005).

The main reason that drove us towards this study was the concern for finding solutions to improve the retention of bioactive compounds in fruit products. Recent studies have shown that some hydrocolloids, such as pectin, corn starch, and sodium alginate could improve the color stability in gel model systems which were mostly attributed to electrostatic interactions between the positively charged flavylium cations and the dissociated carboxylic groups of the pectin, while other hydrocolloids showed adverse effects or did not show any influence (Hubbermann *et al*., 2006; Buchweitz *et al*., 2012; Buchweitz *et al*., 2013). These studies highlighted the role of non-phenolic food components in stabilizing of anthocyanins in gelled fruit products.

Pectin is a high value functional food ingredient primarily used in food industry as a gelling agent for jellies, jams, spreads and other foods (El-Nawawi and Heinkel, 1997), *Figure 2.1*.

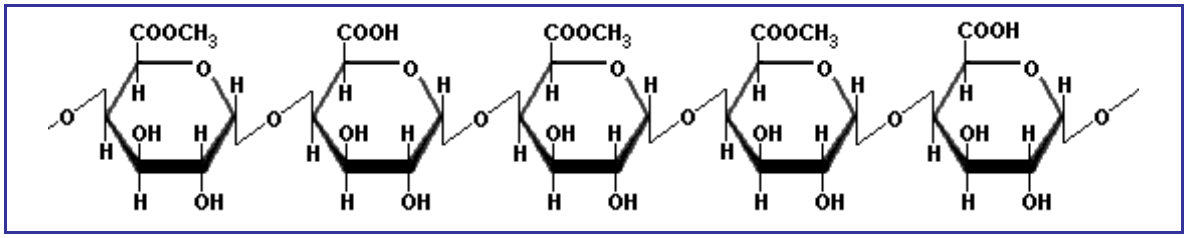


Figure 2.1. Chemical structure of pectin chain
(http://www.scientificpsychic.com/fitness/carbohydrates2.html)

Depending on the degree of esterification (DE), the pectins are divided into two classes: low methoxyl pectin (LMP) with DE<50%, and high methoxyl (HMP) with DE >50%. DE gives the ratio of esterified galacturonic acid units to total galacturonic acid units in the molecule.
The LML is obtained either enzymatically, in vivo, or by the controlled de-esterification of HMP in either acidic or alkaline conditions (Kopjar *et al*., 2009).

Ammonia is sometimes used in the process, introducing some amide groups into the molecule and yielding "amidated" pectin. The degree of amidation (DA) indicates the number of amidated carboxylic groups per 100 galacturonic acid residues.

The reduction of DE introduces dramatic changes in the functionality of HMP and LMP. During jam processing, gel formation involves the association of pectin chains that leads to the formation of three-dimensional networks. The ability of pectin to form gel depends on the molecular size and DE (Kopjar *et al*., 2009; Srivastava and Malviya, 2011).

The hydrogen bonds that occur between the pectin chains are the main factor responsible in the stabilization of a HMP network, *Figure 2.2*. In addition, hydrophobic interactions of the methyl ester groups are essential in gel formation (Oakenfull and Scott, 1985).

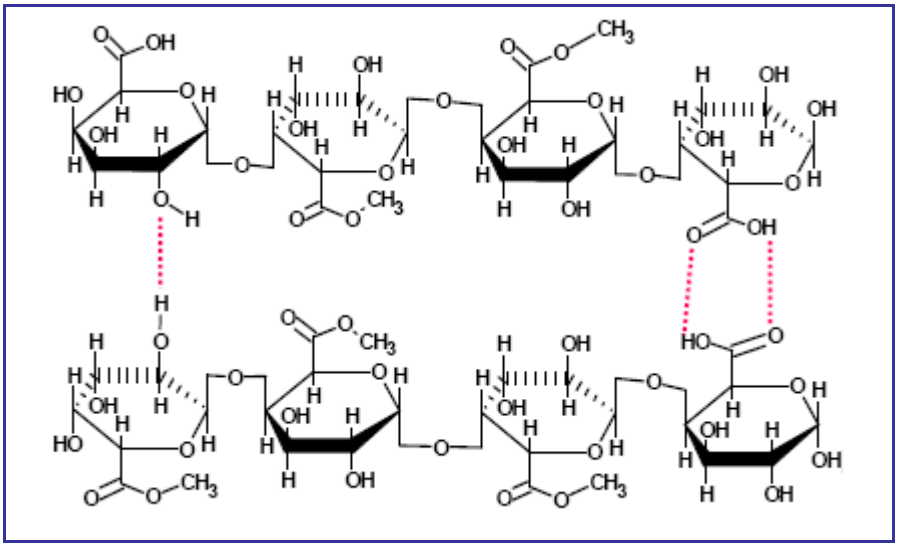


Figure 2.2. The binding mechanisms for connecting of HMP chains during gel formation (http://www.cfs.purdue.edu/class/f&n630/pdfs/pectin.pdf)

The gelling mechanism in jam obtained with LMP is based on the clustering of the pectin chains and occurring of some cavities between them as a result of bended shape of the pectin chains. These cavities will be occupied by carboxyl and hydroxyl groups.

The formation of these cavities as well as the carboxyl and hydroxyl groups promotes the association of pectin chains by calcium gelation, *Figure 2.3.*

Therefore, gelling mechanism involves the formation of a continuous network of ionic cross bindings via calcium bridges between the carboxyl groups belonging to two different chains located in close proximity (Kasapis, 2002; Kopjar *et al*., 2009).

In *Figure 2.4* is presented the arranged sequences in the pectin-calcium-gel "*egg box*" model.

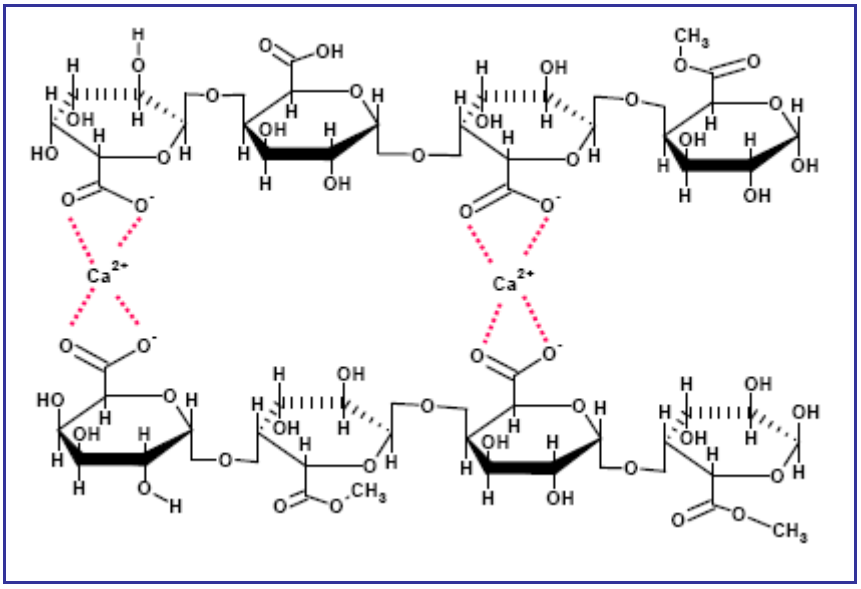


Figure 2.3. The binding mechanisms for connecting of LMP chains during gel formation (http://www.cfs.purdue.edu/class/f&n630/pdfs/pectin.pdf)

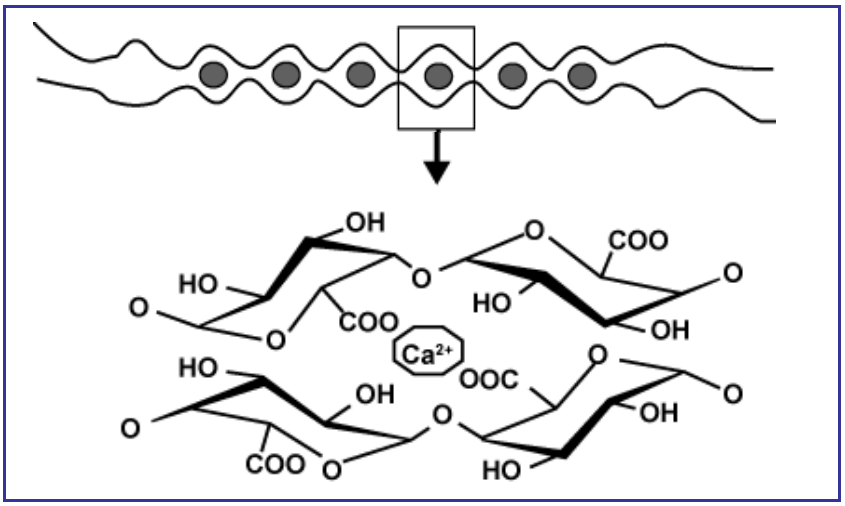


Figure 2.4. Arranged sequences in the pectin-calcium-gel "*egg box*" model (Axelos and Thibault, 1991)

In jam obtained with LMAP, supplementary links by hydrogen bonds occur as a result of the presence of amid groups. In this case, the clustering of pectin chains is more controlled than for LMP, because the network formation is due to the hydrogen bonds between the amid groups and occurs more slowly than the reaction of LMP chains with calcium ions (Walkinshaw and Arnott, 1981; Kopjar *et al.*, 2009). In *Figure 2.5* is shown the three binding mechanism for connecting of pectin chains during gel formation.

The results of the study performed by Holzwarth *et al.* (2013) regarding the influence of various pectins, process and storage conditions on anthocyanins and color of strawberry jams and spreads revealed that low-esterified pectins have proved better stabilizing effects on anthocyanins in fruit gelled products than high-esterified pectins.

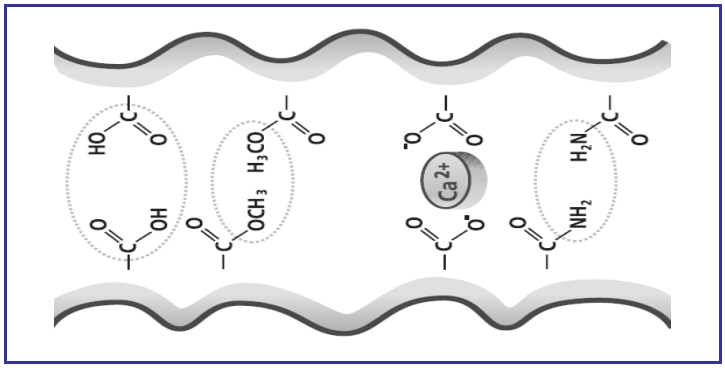


Figure 2.5. The binding mechanisms for connecting of pectin chains during gel formation (http://www.herbstreith-fox.de/fileadmin/tmpl/pdf/broschueren/Konfituere_englisch.pdf)

As a result of gel formation based on different types of chain associations, biologically active compounds from fruit jam could be protected against degradation by water attack, condensation reactions or thermal destroying (Kopjar *et al*., 2007; Kopjar *et al*., 2009). Lewis *et al*. (1995) suggested that pectin is involved in the color stabilization of gelled fruit products. The study carried out by Poiana *et al*. (2012) highlighted the impact of pectin dose on improving the antioxidant properties and color stability of bilberries jam. Also, the results reported by Kopjar *et al.* (2009) have revealed the effect of various pectins on the antioxidant activity of raspberry jam. Furthermore, recent studies on this topic conducted by Buchweitz *et al*. (2013) have shown the impact of the pectin type on the storage stability of black currant anthocyanin pigments in pectic model solutions.

Unfortunately, limited information is available concerning the effect of thermal processing and storage of jam obtained by varying the type and dose of pectin in the recipe on retention of antioxidant characteristics and color in final gelled products.

Since a high antioxidant capacity is a desirable characteristic for gelled-fruit products and the color is one of the most important attributes of their, that significantly decides over consumer preference, the successfully addressing these challenges it was possible by performing studies that will be presented at this point.

The purpose of the study shown in ***selected paper 4****, conducted* by Poiana *et al*. (2011) *was to assess the effect of thermal processing and storage period on antioxidant properties and color quality of some low-sugar jam obtained from strawberry, sweet and sour cherry*.

In line with the current concerns on this topic, *the goal of the the work presented in* ***selected paper 5*** *undertaken on this research direction* by Poiana *et al.* (2012) *was to determine the stability of total phenolics, anthocyanins, L-ascorbic acid, antioxidant capacity and color indices in low-sugar bilberry jams with different pectin concentrations following processing and storage at 20°C. Finally, the objective of the last study belonging to this research direction,* performing by Poiana *et al*. (2013) and slown in detail in ***selected paper 6****, was to explore the effects of pectin type (high and low-esterified, amidated) and dosage on color retention and antioxidant properties of blackberry jam after processing and storage at ambient temperature.*

The studies presented in detail in *selected papers 3-6* were designed and coordinated by me as first author. Based on the foregoing, the objectives of this research direction are:

- The obtaining of more information on the effect of IQF process on antioxidant properties of berries (raspberries, blueberries , blackberries);
- Expansion of knowledge regarding the effect of frozen storage period on the quality of color and antioxidant properties of berries;
- Assessing the impact of thermal processing and storage on antioxidant capacity and biologically active compounds of low-sugar jams made from strawberries, cherries and sour cherries;
- Evaluation the color stability of strawberry, cherries and sour cherries jam in the storage time;
- Assessing the effect of the pectin dose used in blueberry jam formulation in order to reduce the loss of bioactive compounds and antioxidant properties as a result of thermal processing and storage;
- The investigation of blueberry jam color stability during storage at 20°C related to the pectin doses;
- Improving the retention of antioxidant properties of blackberry jam in relation to the pectin type and dosage;
- Assessing the posibility to increasing the color stabiliy during thermal processing and storage by varying the pectin type and dosage.

By solving of these targets are brought substantial information regarding the effect of IQF process and long-term frozen storage on antioxidant characteristics and color stability of wild berries. These results contribute to the improvement of jam processing from various anthocyanin rich fruits, in order to limit the degradation of color and antioxidant characteristics occurring in response to thermal processing and storage.

This kind of data are needed for consumers, who wish to incorporate higher levels of bioactive compounds into their diet, and processors who desire to retain, or possibly to rise, the levels of bioactive compounds in their products. Also, these findings are needed to improve the quality of products obtained by thermal processing of fruits rich in anthocyanins.

2.2. Impact of freezing and long-term frozen storage on antioxidant properties, bioactive compounds and color indices of berries

2.2.1. Aim

The effects of Individual Quick Freezing (IQF) and long-term frozen storage at -18°C up to 10 months, on nutraceutical compounds, antioxidant properties and color indices of various

berries such as: blueberry (*Vaccinium myrtillus*), raspberry (*Rubus idaeus*) and blackberry (*Rubus fruticosus*) was the main goal of this research. Berries were harvested in Romania, at maturity stage. After harvesting berries were refrigerate (3-5°C for 24 h), then frozen by IQF techniques using a FRIGOSCANDIA freezing tunnel. The frozen berries were stored in polyethylene bags in freezing box at temperature –18°C for 10 months. Fresh and frozen berries were supplied by S.C. LEGOFRUCT S.R.L from Timisoara (the western part of Romania). According to the data specified in ***selected paper 3***, the samples were analyzed fresh (FR), immediately after freezing (0-F) and after 2, 4, 6, 8 and 10 months of frozen storage (2-F, 4-F, 6-F, 8-F and 10-F) in terms of total phenolics (TP) content using colorimetric method described by Singleton *et al*. (1999), L-ascorbic acid (L-AsAc) content using 2,6-dichlorophenolindophenol method described by AOAC (2000), antioxidant activity according to ferric-reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996), the content of total monomeric anthocyanins (TMA) and color indices using the method described by Giusti and Wrolstad (2005). Beside me, in this study were involved Assist dr. Diana Moigradean [dimoden@yahoo.com], Lecturer dr. Diana Raba [dianaraba@yahoo.com], Assist. dr. Mirela Popa [mirevio_gh@yahoo.com] and Assist. dr. Liana Alda [lianaalda@yahoo.com].

2.2.2. Results and Discussion

The values recorded for investigated parameters in initial state, after freezeng and during long-term storage were showed in *Table 2.1*. The first thing we can notice at a closer look of these data is that, there were no registered significant changes in L-AsAc content, TP, and FRAP values of investigated berry after IQF process. Only slight increases of TMA were found immediately after freezing. The long-term frozen storage of blueberries did not induce significant changes in TMA content. These findings are in agreement with the results obtained by Scibisz and Mitek (2007). It is most probable that the anthocyanins in frozen fruit become more easily extractable. This might be due to degradation of cell structures in berries. Also, an increase in TMA content in raspberry during freezing has been reported by Ancos *et al*. (2000).

Table 2.1. Effect of freezing and long-term frozen storage on TP, L-AsAc, TMA and FRAP values of different berries

Berries	FR	Frozen berries					
		0-F	2-F	4-F	6-F	8-F	10-F
L-AsAc (mg·100 g^{-1} FW)							
raspberry	31.55	31.41	29.91	27.15	26.22	25.15	22.13
blueberry	8.20	8.15	7.92	7.68	6.61	6.43	6.22
blackberry	6.63	6.46	5.81	5.46	5.28	4.39	3.97
TP (mg GAE·100 g^{-1} FW)							
raspberry	197.79	197.14	182.23	169.45	153.21	129.75	103.65
blueberry	641.53	640.11	611.43	589.31	550.4	511.22	458.54
blackberry	333.60	331.87	322.47	279.07	242.79	224.27	191.12
FRAP (mM Fe^{2+}·kg^{-1} FW)							
raspberry	40.16	39.21	37.89	35.72	31.38	28.37	24.84
blueberry	58.31	57.94	55.16	53.10	50.44	47.10	44.82
blackberry	49.64	48.73	46.02	43.17	38.46	37.32	32.29
TMA (mg·100 g^{-1} FW)							
raspberry	39.71	41.67	39.95	37.85	37.56	34.85	33.51
blueberry	205.48	207.12	205.14	202.67	198	185.12	180.31
blackberry	193.72	195.89	192.08	191.75	188.4	182.55	178.62

In *Figure 2.6* are depicted the losses of bioactive compounds and antioxidant activity during frozen storage of berries. Overall, long term frozen storage induced some losses in monitored parameters. In the first 4 months it was noticed a slow degradation of antioxidants. After long-term storage it was noticed a greater degradation of bioactive compounds. The level of recorded losses was dependent on the kind of berry and also, of the storage time. At the end of frozen storage the losses in TP content reached 28% from the values recorded immediately after freezing for blueberry, 42% for blackberry and the highest losses were reach for raspberry (47%). The long-term frozen storage affects the L-AsAc content: after 6 months the losses were 16-19% reported to the 0-F values, while after 10 months they ranged from 23 to 38%. The smallest loss was registered for blueberry. It was proved that storage for more than 8 months significantly affects the content of L-AsAc in frozen fruits (Noormets *et al*., 2006). Probably, the significant decrease recorded in monitored compounds was due to water content in non-frozen state. Activity and enzymatic reaction rate reached maximum values in the layers of liquid water in frozen fruits. Probably, this phenomenon contributes to the modification of chemical compounds, including biologically active substances.

In frozen products the enzymatic reactions are slow, but not completely blocked. The enzymatic activity in frozen berries is strongly linked to the presence of non-frozen water. At a temperature of -18°C, the water content in frozen berries represents approximately 89% of global water of berries. Thus, the liquid water percent of these products will be 11%. At a temperature of -30°C, the percent of frozen water in berries is 91% of total fruit water, and the liquid water represent 9%.

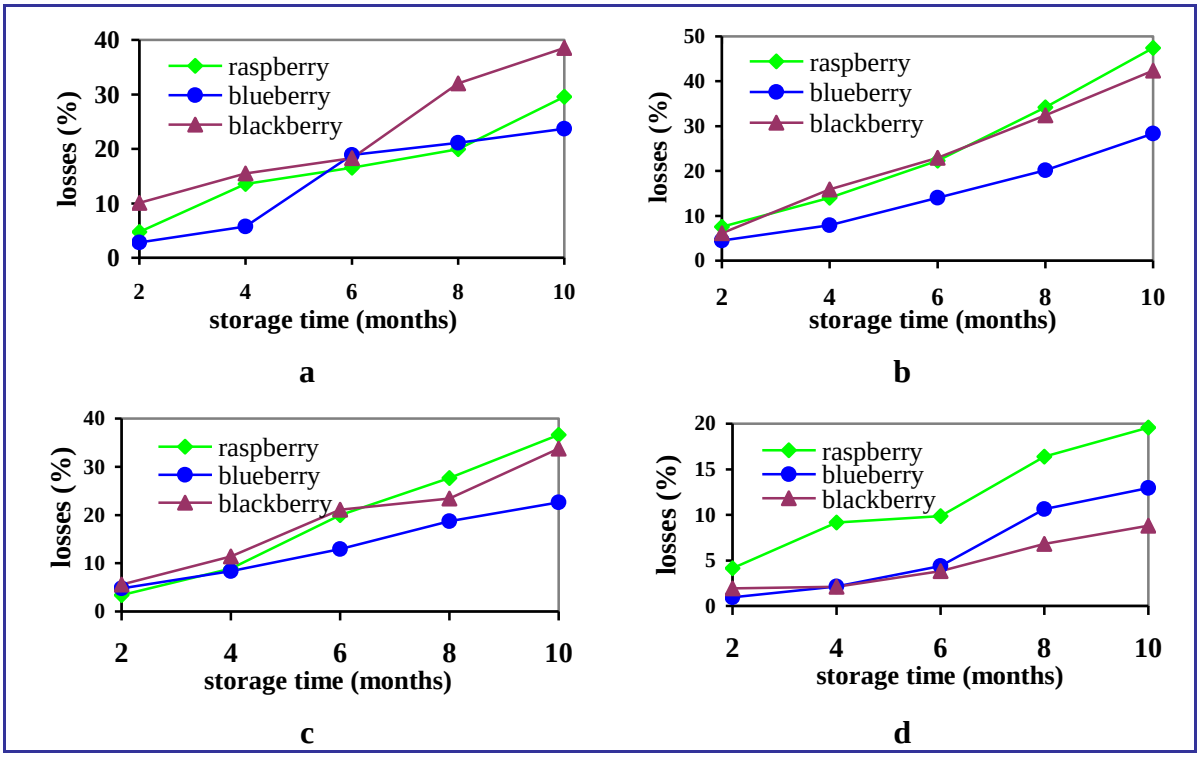


Figure 2.6. The losses of monitored parameters during long-term storage of frozen fruit (**a**: L-AsAc; **b**: TP; **c**: FRAP and **d**: TMA**)**

It was found that storage of frozen fruit for 6 months led to a slow TMA degradation. At the end of storage it was noticed increased alterations of this parameter. Thus, the relative losses of TMA were 9% for blackberry, 13% for blueberries and 20% for raspberries.

The results of study performed by Vollmannova *et al.* (2009) reveal a decrease around 17% in TMA content after 6 months of frozen storage at –18°C. The study conducted by Chaovanalikit and Wrolstad (2004) have reported dramatic losses, up to 88%, in TMA during 6 months of storage at –23°C of sweet cherries. Contrary, the results reported by Scibisz and Mitek (2007) claim that long-term frozen storage of blueberries did not induce significant losses in TMA content.

Data reported by Kmiecik *et al.* (1995) stated that the TMA content in frozen fruits depended on both, fruit species and the method of thawing. Thawing represent a crucial step for the quality of the frozen fruit because the compounds which under normal conditions are kept separate in the intact cell, can mix and react with each other. The highest TMA content was found in fruit thawed at 2-4°C, followed by those thawed at room temperature and at the end, the fruit thawed in a microwave oven (Kmiecik *et al*., 1995). Nevertheless, only in the case of fruit thawed by microwaves the content of anthocyanins was smaller, though maximum differences did not exceed 10%. In our study, all frozen fruit were thawed in refrigeration conditions (4h, 3-5°C). This information is important because TMA are responsible for about 25% of the total antioxidant capacity of berries (Beekwilder *et al*., 2005).

FRAP values decreased during frozen storage of berries. In the first 4 months it was noticed small decreases, followed by significant alterations in response to long-term storage. At the end of storage, the level of alterations increased to 23% of 0-F value for blueberry and 34-37% for both raspberry and blackberry. The correlations coefficients (R) obtained by applying of simple regression between FRAP values and the content of investigated bioactive compounds are presented in *Table 2.2.* It can be noted strong correlations FRAP *versus* TP, L-AsAc and TMA content. For all investigated berries, the highest correlation was recorded between FRAP and TP.

Table 2.2. Correlation coefficients obtained by simple linear regression applied to investigated parameters

Y=A+B • X	**R**		
	raspberry	**blueberry**	**blackberry**
FRAP=f(L-AsAc)	R=0.969	R=0.963	R=0.962
FRAP=f(TP)	R=0.992	R=0.992	R=0.991
FRAP=f(TMA)	R=0.968	R=0.967	R=0.963

Our results strengthen the findings pointed out by Gonzalez *et al*. (2003) regarding the documented relation between bioactive compounds and antioxidant activity of berries during 12 months of storage at –24°C. More than that, the changes of antioxidant activity of berries in close relation to their TMA and TP are confirmed by Pantelidis *et al*. (2007). In our study, a significant correlation was obtained not only for FRAP and TP but also for FRAP and TMA or L-AcAc.

The effect of freezing and frozen storage on berry color, quantified by color density (CD), polymeric color (PC) and polymeric color PC (%) is shown in *Table 2.3.* PC (%) provides information regarding the percentage of color represented by polymerized material (Rommel *et*

al., 1992; Giusti and Wrolstad, 2005). Thus, the polymeric color is the result of the anthocyanins polymerization (Rommel *et al.*, 1992; Yuksel and Koka, 2008).

Table 2.3. Effect of long-term frozen storage on the color indices of berries

Berries	FR	frozen berries					
		0-F	2-F	4-F	6-F	8-F	10-F
CD (AU)							
raspberry	7.14	7.09	6.9	6.71	6.05	5.27	5.04
blueberry	11.77	11.68	11.51	11.21	10.85	10.71	10.43
blackberry	12.28	12.21	12.15	11.96	11.8	11.53	11.58
PC (AU)							
raspberry	0.78	0.8	0.83	0.87	0.94	1.02	1.12
blueberry	1.05	1.1	1.17	1.23	1.36	1.44	1.5
blackberry	1.16	1.19	1.23	1.29	1.34	1.38	1.43
PC (%)							
raspberry	10.92	11.28	12.03	12.97	15.54	19.35	22.22
blueberry	8.92	9.42	10.17	10.97	12.53	13.45	14.38
blackberry	9.45	9.75	10.12	10.79	11.36	11.97	12.35

It was observed that IQF process not significantly affect the CD and PC (%). Based on data shown in *Table 2.3* it can be noted that the storage time affects the color quality of berries. CD decreased during frozen storage, maily as a result of alterations in TMA. This trend was the same over all storage time for all investigated fruit. Contrary, PC (%) recorded increases during long-term frozen storage in response to anthocyanins polymerization. The highest calues in PC (%) was reached for raspberry at the end of storage. Also, the best color stability was registered for blueberries.

2.2.3. Conclusions

The IQF process did not affect the amount of bioactive compounds of investigated berries. The frozen storage up to 4 months did not induced significantly alterations in investigated parameters while the relative losses of investigated parameters did not exceed 25% after 6 months of storage. At the end of storage, the recorded relative losses were as follows: TP (28-47%), TMA (9-20%) and L-AsAc (24-38%). With regard to the antioxidant characteristics, the investigated berries may be listed in the following order: blueberries>blackberries>raspberries. Thus, the smallest losses of FRAP values were recorded for blueberries and the highest for raspberries. It can be noticed a high correlations between FRAP and TP, L-AsAc and TMA. During storage time it was registered continue declines for TMA content and CD, while PC (%) increased in all this period. The color of raspberry was the most sensitive during long-term frozen storage, while the color of blueberry was the most stable in response to storage.

After performing of this study, we appreciate that IQF process could be used to retain various nutrients which are naturally found in berries. Considering that color and antioxidant properties of berries are appealing characteristics to consumers, we appreciate that the IQF process applied to various berries, followed by six months of frozen storage at -18°C is an attractive way to retain these characteristics.

2.3. Processing and storage impact on antioxidant properties and color of strawberry, sweet cherry and sour cherry jam

2.3.1. Aim

The objective of this research was to investigate the stability of color and the retention of antioxidant properties of low-sugar jam from strawberry, sweet cherry and sour cherry in response to thermal processing and storage at 20°C. Changes occurring in investigated parameters were compared for frozen fruit (as raw material for jam preparation), jam one day after processing and jam in storage for 1, respectively 3 months. Low-sugar jams were prepared in laboratory conditions by boiling in an open kettle under atmospheric pressure, with manual stirring according to a traditional procedure used in Romania as well as in other countries, for long-term preservation of different fruits. Fruits blended with largest part of sucrose and citric acid were mixed and thermally processed at 80°C. Pectin was mixed with part of sucrose and added at the final stage of jam processing. Citric acid was used for adjusting pH value for proper pectin gelatinisation (2.8-3.3). Total soluble solids content reached upon cooking was 45°Brix. According to data presented in ***selected paper 4***, jam samples were analyzed in terms of total phenolics content (TP) using the method presented by Singleton *et al.* (1999), L-ascorbic acid (L-AsAc) applying the protocol specified by AOAC (2000), antioxidant capacity by ferric reducing antioxidant power (FRAP) test (Benzie and Strain, 1996), total monomeric anthocyanins content (TMA) and color indices (color density: CD, polymeric color: PC, and percentage of polymeric color: %PC) using the method described by Giusti and Wrolstad (2005).

In this study were involved, beside me, my colleagues Assist dr. Diana Moigradean [dimoden@yahoo.com], Lecturer dr. Diana Dogaru, Prof. dr. Constantin Mateescu [c.mateescu@usab-tm.ro], Lecturer dr. Diana Raba [dianaraba@yahoo.com] and Prof. dr. Iosif Gergen [igergen@yahoo.com].

2.3.2. Results and Discussion

To provide a clear view on the changes occurring among the four stages of experiment (frozen fruit - jam one day after processing - jam in storage for 1 month - jam in storage for 3

months), the results of TP, L-AsAc, TMA content and FRAP values were processed by ANOVA test. Based on information obtained through statistical processing can be pointed the significance of alterations registered in monitored parameters in response to thermal processing relative to the values registered in frozen fruit, as control (C), *Table 2.4*.

Table 2.5 shows the statistical significance of changes recorded for FRAP values, TMA, TP and L-AsAc content after 1 and 3 months of storage at 20°C relative to the values registered in jam samples one day after processing, as control (C1), while *Figure 2.7* emphasizes the losses of measured parameters in response to jam storage.

Changes in L-AsAc content in response to fruit thermal processing and jam storage

Data presented in *Table 2.4* reveal that fruit thermal processing led to extremely significant alterations ($p<0.001$) in L-AsAc content in agreement with other previous results (Klopotek *et al*., 2005). Thus, the level of losses registered for L-AsAc content in response to thermal processing of strawberry was around 78% relative to the values recorded for frozen fruits, 70% for sour cherry and 54% for sweet cherry, *Table 2.4*.

Table 2.4. Alterations of measured parameters in response to thermal processing

Samples	**L-AsAc (mg·100 g^{-1} ds)**	
	frozen fruits (C)	**jam one day after processing**
strawberry	314.43±28.41 *(F=3.71)*	69.9±5.43***
sweet cherry	78.11±6.28 *(F=2.13)*	35.65±3.12***
sour cherry	172.93±14.61 *(F=2.13)*	51.12±4.29***
	TP (mM GAE·100 g^{-1} ds)	
	frozen fruits (C)	**jam one day after processing**
strawberry	17.79±1.56 *(F=2.00)*	10.24±0.81**
sweet cherry	19.37±1.64 *(F=1.14)*	13.32±1.06**
sour cherry	21.58±1.79 *(F=0.33)*	16.15±1.45*
	TMA (mg·100 g^{-1} ds)	
	frozen fruits (C)	**jam one day after processing**
strawberry	233.44±20.24 *(F=3.96)*	15.80±1.35***
sweet cherry	303.61±28.27 *(F=3.97)*	21.4±1.82***
sour cherry	547.46±40.11 *(F=3.95)*	42.55±3.12***
	FRAP (mM Fe^{2+}·100 g^{-1} ds)	
	frozen fruits (C)	**jam one day after processing**
strawberry	60.22±5.17 *(F=1.77)*	35.29±2.85**
sweet cherry	45.47±3.85 *(F=0.97)*	30.17±2.61**
sour cherry	72.99±6.47 *(F=0.84)*	50.60±4.52**

Data are shown as means ± standard deviation. Statistical differences are indicated relative to values recorded in frozen fruit (control, C), as follows: P<0.05=* (significant), P<0.01=** (highly significant) and P<0.001=*** (extremely significant). F – Fischer's variance ratio (F should be higher for the predictions to be significant).

From *Table 2.5* can be noticed that jam storage for 1 month at 20°C induced, for all investigated jam samples, non-significant alterations in L-AsAc content ($p>0.1$) relative to the values recorded in jam sample one day after processing.

Only after 3 months of storage it was found statistically significant differences in L-AsAc content relative to the values recorded one day after processing, as follows: significant ($P<0.05$) for sour cherry jam samples and highly significant ($p<0.01$) for strawberry and sweet cherry jam samples. Jam storage for 3 months led to relative decreases in L-AsAc content in the range 22-33%, *Figure 2.5a.*

Strawberry is the only one species among the three investigated that exhibited the highest loss of L-AsAc content in response to jam processing. In addition, after 3 months of storage, the highest loss of L-AsAc content was also recorded for strawberry jam. Based on these data, it we can say that strawberry jam shows the lowest tolerance to storage at 20°C.

Changes in TP content in response to fruit thermal processing and jam storage

Data from *Table 2.4* emphasizes that fruit thermal processing induced alterations of TP content, pointed out by statistical processing. During thermal processing it was noticed loss of 25-42% from TP content found in frozen fruit. These depreciations are highly significant ($p<0.01$) for strawberry and cherry jam samples and significant ($p<0.05$) for sour cherry jam samples. As regards the storage at 20°C, significant statistical differences were noticed after 3 months, *Table 2.5*.

According to data presented in *Figure 2.7b*, the relative losses of TP content reached 18-25% after 3 months of storage. The highest loss of TP content in response to thermal processing was recorded for strawberry jam and the lowest for sour cherry jam. The same trend was observed also, at the end of the experiment, suggesting that the polyphenolic compounds from sour cherry were the most stable in response to thermal processing and storage at 20°C.

Table 2.5 Alterations of measured parameters in response to jam storage at 20°C

Jam samples	L-AsAc (mg·100 g^{-1} ds)		
	1 day after processing (C1)	**after 1 month of storage**	**after 3 months of storage**
strawberry	69.9±5.43 *(F=0.27)*	60.13±5.79^{ns}	47.05±4.41**
sweet cherry	35.65±3.12 *(F=0.47)*	30.34±2.48^{ns}	25.43±2.24**
sour cherry	51.12±4.29 *(F=0.33)*	47.12±4.47^{ns}	39.78±3.30*
	TP (mM GAE·100 g^{-1} ds)		
	1 day after processing (C1)	**after 1 month of storage**	**after 3 months of storage**
strawberry	10.24±0.81 *(F=0.41)*	9.09±0.81^{ns}	7.64±0.58*
sweet cherry	13.32±1.06 *(F=0.28)*	12.04±0.86^{ns}	10.4±0.85*
sour cherry	16.15±1.45 *(F=0.53)*	14.7±0.97^{ns}	13.21±1.24^{ns}
	TMA (mg·100 g^{-1} ds)		
	1 day after processing (C1)	**after 1 month of storage**	**after 3 months of storage**
strawberry	15.80±1.35 *(F=0.48)*	14.10±1.27^{ns}	10.57±0.92**
sweet cherry	21.4±1.82 *(F=0.28)*	18.32±1.65^{ns}	15.49±1.37*
sour cherry	42.55±3.12 *(F=0.14)*	38.48±3.53^{ns}	33.37±2.95*
	FRAP (mM Fe^{2+}·100 g^{-1} ds)		
	1 day after processing (C1)	**after 1 month of storage**	**after 3 months of storage**
strawberry	35.29±2.85 *(F=0.09)*	32.88±3.07^{ns}	28.58±2.63^{ns}
sweet cherry	30.17±2.61 *(F=0.35)*	28.41±2.54^{ns}	25.73±1.92^{ns}
sour cherry	50.60±4.52 *(F=0.04)*	48.37±4.30^{ns}	45.22±4.07^{ns}

Data are shown as means ± standard deviation. Statistical differences are indicated relative to values recorded in jam one day after processing (control, C1), as follows: ns = non-significant ($P>0.1$), $P<0.05$=* (significant), $P<0.01$=** (highly significant). F – Fischer's variance ratio.

Changes in TMA content in response to fruit thermal processing and jam storage

From *Table 2.4* it could be noticed the massive losses of anthocyanins content in response to fruit thermal processing, highlighted based on the level of statistical significance of registered differences. Our data are in agreement with the results reported by other studies on this topic (Kim and Padilla-Zakour, 2004; Wrolstad *et al.*, 2005), which state that jam processing causes the

loss of about 90% of the TMA content found in processed fruit. Anthocyanins pigments are labile compounds; their stability is highly variable depending on their structure and the composition of the matrix in which they exist (Brownmiller *et al.*, 2008).

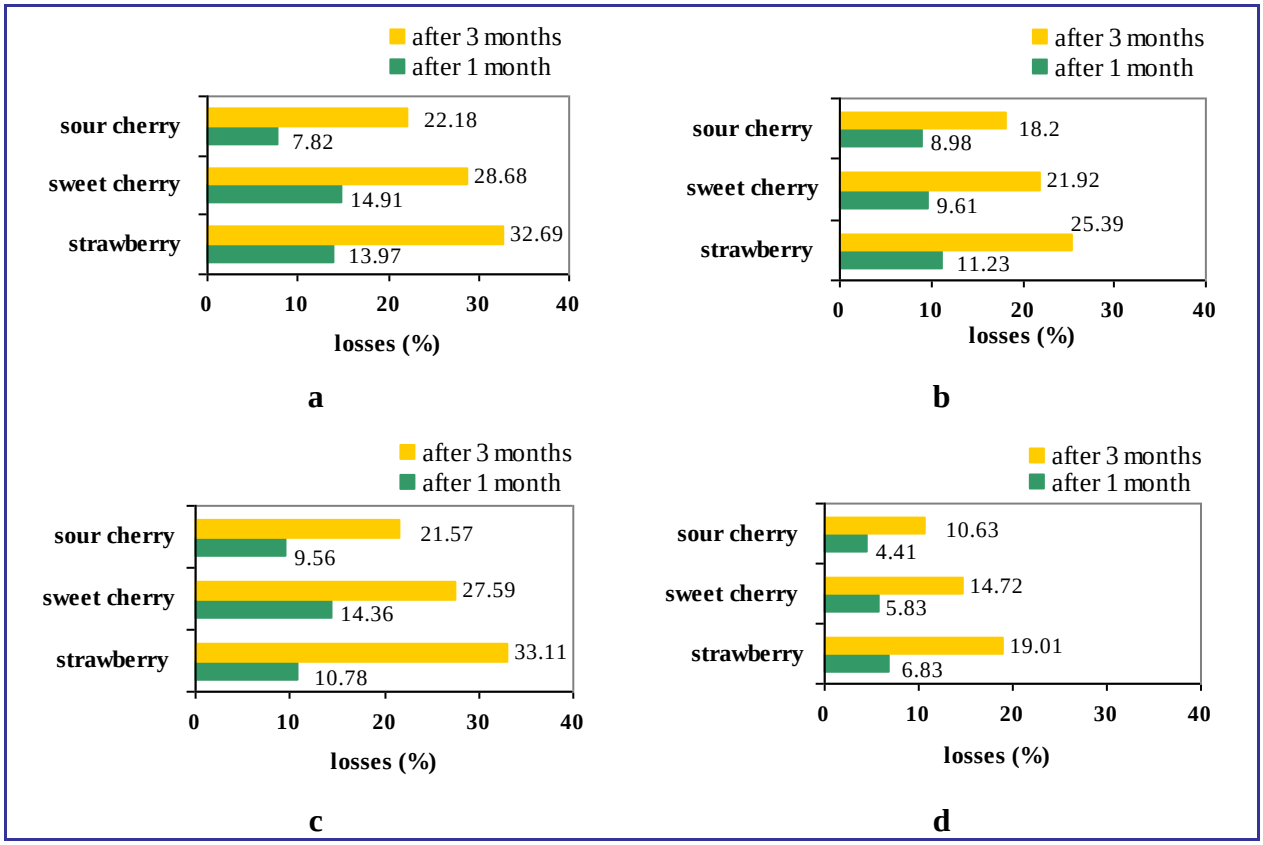


Figure 2.7. The losses of measured parameters in response to jam storage at 20°C (**a:** L-AsAc; **b:** TP; **c:** TMA; **d**: FRAP)

Anthocyanins losses are probably due to complex formation with other compounds during jam processing. The nature of the transformation products is unknown but there is obvious evidence regarding the participation of sugars and ascorbic acid as well as their thermal degradation products, hydrogen peroxide resulted from ascorbic acid and metal ions (Bursac Kovacevic et al., 2009). The losses of TMA and/or formation of brown compounds in jam during storage have been attributed to many factors such as pH and acidity, phenolic compounds, sugars and sugar degradation products, oxygen, ascorbic acid, fruit maturity and thawing time. Other factors may have a significant role in the expression of color in fruit jams by copigmentation or some other physico-chemical processes (Rommel *et al.*, 1992). The losses of TMA were most likely due to the formation of polymeric pigments (PP) during jam processing and storage (Wrolstad *et al.*, 2005). TMA content continued to decline during storage. At the end of 3 months of storage the registered losses reached 22-33% of the value recorded in jam samples one day after processing, *Figure 2.7c.* Jam storage for 1 month doesn't induce significant statistical differences in TMA content. After 3 months of storage the alterations of this parameter became significant ($p<0.05$) for cherry and sour cherry jam samples and highly significant ($p<0.01$) for strawberry jam samples, *Table 2.5.*

Changes in FRAP values in response to fruit thermal processing and jam storage

The decrease of bioactive compounds content such as: L-AcAc, TP, and TMA in response to fruit thermal processing led to a decreasing of antioxidant capacity recorded in jam samples investigated one day after processing. The alterations noted for FRAP values in response to fruit thermal processing are highly significant ($p<0.01$) for all jam samples, *Table 2.4.* Despite massive losses of TMA occurring during thermal processing, the FRAP values were affected to a lesser extent. Thus, fruit thermal processing led to the losses of antioxidant capacity in the range 30-41% of the values recorded for frozen fruit, as control (C). The most affected in response to thermal processing was the FRAP values recorded for strawberries and the most stable from this point of view was the FRAP values found in sour cherries jam samples. During storage, it was noticed alterations in FRAP values, in response to the losses of bioactive compounds, *Figure 2.7d*. Even though jam storage led to decrease of FRAP values, the noticed losses are not statistical significant even after 3 months of storage, *Table 2.5.*

Changes in color quality

In this study, the effect of storage at 20°C on jam samples color was quantified by measuring of CD, PC, and PC (%).

PC (%) is the ratio between PC and CD, widely used to determine the percentage of the color that is contributed by polymerized material (Rommel *et al*., 1992). In *Table 2.6* are presented the changes in jam color parameters as response of storage relative to the values registered in samples one day after processing (C1). It is important to mention that, even though significant alterations were noticed in anthocyanins content, only minor changes were found for CD of jam samples stored at 20°C. At the end of storage, the relative decreases recorded in CD were in the range 7-11%. It can be seen that 3 months of storage induce non-significant alterations in CD values ($p>0.1$).

Table 2.6. The effect of storage at 20°C on jam color quality

Jam samples	**CD (AU)**		
	one day after processing (C1)	**after 1 month of storage**	**after 3 months of storage**
strawberry	3.811±0.320 (*F=0.29*)	$3.524±0.360^{ns}$	$3.389±0.320^{ns}$
sweet cherry	4.703±0.390 (*F=0.29*)	$4.447±0.410^{ns}$	$4.311±0.390^{ns}$
sour cherry	5.503±0.470 (*F=0.02*)	$5.278±0.480^{ns}$	$5.102±0.450^{ns}$
	PC (AU)		
	one day after processing (C1)	**after 1 month of storage**	**after 3 months of storage**
strawberry	0.48±0.05 (*F=0.82*)	$0.52±0.035^{ns}$	$0.7±0.06^{**}$
sweet cherry	0.53±0.04 (*F=0.40*)	$0.57±0.037^{ns}$	$0.69±0.05^{**}$
sour cherry	0.55±0.06 (*F=5.38*)	$0.59±0.04^{ns}$	$0.71±0.08^{*}$
	PC(%)		
	one day after processing (C1)	**after 1 month of storage**	**after 3 months of storage**
strawberry	12.60±1.15 (*F=0.76*)	$14.76±1.35^{ns}$	$20.66±1.82^{**}$
sweet cherry	11.27±1.08 (*F=0.08*)	$12.82±1.14^{ns}$	$16.01±1.45^{**}$
sour cherry	9.99±0.88 (*F=0.44*)	$11.18±1.02^{ns}$	$13.92±1.24^{**}$

Data are shown as means ± standard deviation. Statistical differences are indicated relative to values recorded in jam one day after processing (control, C1), as follows: ns = non-significant ($P>0.1$), $P<0.05$=* (significant), $P<0.01$=** (highly significant). F – Fischer's variance ratio.

Progressive increases of PC (%) and the corresponding losses of TMA in response to storage were most likely due to extensively polymerization phenomena (Wrolstad *et al.*, 2000). After 1 month of storage the relative increases in PC (%) were marked as non-significant ($P>0.1$), while 3 months of storage led to highly significant increases ($P<0.01$), located in the range 39-63%. The most relevant increases of PC (%) were obtained for strawberries jam. Thereby, no significant differences were noticed for PC (%) of sweet and sour cherries jam samples. Even though highly significant increases of PC (%) were noticed, there were not observed significant alterations in CD. This fact proves the stability of jam color throughout 3 months of storage.

Although TP and L-AsAc are the major potential candidates as a selection criterion for antioxidant properties of fruit jams, antioxidant activity is not limited just to them (Klopotek *et al*., 2005; Bursac Kovacevic *et al*., 2009). Previous data reporded by Tsai *et al.* (2004) and Brownmiller *et al*. (2008) pointed out that PP show antioxidant properties, which compensate for the loss of a part of FRAP value assigned to TMA affected by storage. Also, it has been proven that some degradation products of anthocyanins have antioxidant capacity (Tsai and Huang, 2004). The obtained data revealed that, after 3 months of storage at 20°C, the FRAP values of fruit jam samples recorded lower depreciations than the content of investigated bioactive compounds. This is confirmed by the results of statistical test, which prove that, at the end of 3 months of storage, the changes recorded in FRAP values were not statistically significant.

2.3.3. Conclusions

Thermal processing of fruits led to statistical significant alterations for all measured parameters. The most important losses, reported to the values corresponding to frozen fruit, were recorded for TMA (92-93%), L-AsAc (54-78%), FRAP values (30-41%) and TP (25-43%). Jam storage induced additionally alterations of measured parameters. One month of jam storage at 20°C resulted in not statistically significant alterations, while three months of storage led to significant and highly significant alterations. At the end of storage, it was noticed significant increases in PC (%) in the range of 39-63% relative to the values recorded one day after processing, while the alterations recorded for CD didn't show any statistical significance. The best retention of antioxidant properties and color was recorded for sour cherry jam.

Our data suggest that investigated low-sugar jams may still represent an excellent source of compounds with antioxidant potential.

2.4. The effect of processing and storage on antioxidant properties and color of low-sugar bilberry jam with different pectin concentrations

2.4.1. Aim

The purpose of the present work was to assess the effect of processing and storage at 20°C on antioxidant properties and color quality of low-sugar bilberry (*Vaccinium myrtillus* L.) jam with different low-methoxyl pectin (LMP) doses. Low-sugar bilberry jams were prepared in laboratory conditions, according to a traditional procedure, by boiling in an open kettle under atmospheric pressure, with manual stirring. The final soluble solids content reached upon cooking was 45°Brix. Commercial low-methoxyl pectin (LM40, Danisco Ingredients, Denmark) was added at four different concentrations, 0.3, 0.5, 0.7 and 1% (m/V) at the final stage of the jam cooking. Citric acid was added towards the end of cooking for adjusting pH values for proper LMP gelatinization (2.8-3.3). Jam samples were analyzed one day after processing (0) and after 1, 3, 5 and 7 months of storage at 20°C in terms of TMA, L-AsAc, TP content, FRAP values and color indices. These parameters were estimated using the methods presented in ***selected paper 5***. Also, correlations between investigated parameters were established by regression analysis. Significant statistical differences of investigated parameters were determined by Fisher's least significant differences (LSD) test at $P<0.05$, after analysis of variance (ANOVA) of a two-factor experiment in an factorial designs with four LMP doses, five storage periods and three replicates as sources of variation.

For performing of this study I had a close cooperation with Prof. dr. Ersilia Alexa [alexa.ersilia@yahoo.ro] and Prof. dr. Constantin Mateescu [c.mateescu@usab-tm.ro]. The contribution of each author is also specified in ***selected paper 5***.

2.4.2. Result and Discussion

Chemical parameters of fresh bilberries were reported in the *Table 2.7.* They are important to estimate the magnitude of alterations due to fruit thermal processing.

Low-sugar jams with different LMP concentrations were analyzed one day after processing (0) as well as after 1, 3, 5 and 7 months of storage at 20°C, in terms of TMA, TP, L-AsAc, CD, PC (%) and FRAP values.

Table 2.7. Chemical characteristics of fresh bilberries

Component (Units)	Values
TP (mg GAE·100 g^{-1} fresh bilberries)	683.88±25.52
TMA (mg·100 g^{-1} fresh bilberries)	238.51±18.73
FRAP (mM Fe^{2+}·100 g^{-1} fresh bilberries)	5.53±0.38
L-AcAc (mg·100 g^{-1} fresh bilberries)	17.09±1.22
CD (AU)	12.31±0.94
PC (AU)	0.38±0.025
PC (%)	3.09±0.27

The obtained results were processed by two-way ANOVA test in order to provide a clear view on the significance of changes occurring in investigated parameters in response to pectin doses used in jam recipe and storage time.

Changes in TMA content and color indices in response to jam processing and storage

Based on the amount of fruit needed to obtain 100 g jam was determined the theoretical content of TMA in bilberry jam. Since jams contained about 69 g of fresh fruit per 100 g, it was to be expected that TMA content would be approximately 69% from the value registered for fresh fruit. Contrary, the real content of TMA in bilberry jam was much lower than in the corresponding fresh fruit. The difference between theoretical and real content of TMA recorded in bilberry jam was due to thermal processing. It can be seen the massive decrease of TMA content due to thermal processing (*Tables 2.7* and *2.8*). Thus, jam preparation caused a decrease of total anthocyanins content by 81-84% reported to the value corresponding to fresh fruit. Our data are in agreement with the results reported by Savikin *et al*. (2009), when the relative reduction of TMA in response to jam processing was 85%

The changes of TMA content in jam samples in response to pectin concentration, as well as storage time are shown in *Table 2.8.* In regard to the TMA content registered in jam one day after processing, it can be seen that the highest content was recorded in sample with 1% LMP. By increasing of LMP dose in the jam recipe it was noticed an increase in the amount of retained anthocyanins. Thus, by increasing of LMP concentration from 0.3 to 1% there was noticed an increase in TMA content of 13%. Since pectin is polyuronic acid, their stabilizing effect on the jam color might base on electrostatic interactions between the flavylium cation of anthocyanin and the dissociated carboxyl groups of pectin. Due to these associations, anthocyanins may be protected against water attack, which leads to color stabilization (Hubbermann *et al*., 2006).

Table 2.8. Alterations of TMA, TP, L-AsAc and FRAP in jam as effect of LMP dose and storage time

Samples	TMA (mg·100 g^{-1} jam)				
	storage time (months)				
	0	**1**	**3**	**5**	**7**
1.0% LMP	30.74±1.86^{a,A}	27.63±1.54^{ab,A}	23.56±1.93^{b,A}	18.45±1.64^{c,A}	13.04±0.97^{d,A}
0.7% LMP	30.01±1.80^{a,A}	24.77±1.86^{b,A}	21.60±1.65^{b,A}	16.68±1.22^{c,A}	10.46±0.91^{d,B}
0.5% LMP	28.12±1.81^{a,A}	22.75±1.75^{b,AB}	19.80±1.61^{b,A}	15.37±1.13^{c,A}	9.26±0.75^{d,BC}
0.3% LMP	26.63±2.06^{a,A}	20.95±1.50^{b,AB}	18.25±1.45^{b,AB}	13.74±1.27^{c,AB}	7.42±0.58^{d,C}
	TP (mg GAE·100 g^{-1} jam)				
1.0% LMP	275.41±18.73^{a,A}	261.83±13.64^{a,A}	244.80±18.74^{a,A}	219.33±17.0^{ab,A}	163.19±11.89^{b,A}
0.7% LMP	260.11±17.0^{a,A}	239.71±15.33^{a,A}	219.32±15.30^{ab,A}	193.78±15.35^{b,A}	141.09±8.56^{c,A}
0.5% LMP	248.22±13.61^{a,A}	224.43±11.91^{a,A}	205.71±11.92^{ab,AB}	176.81±11.91^{b,AB}	122.43±6.84^{c,AB}
0.3% LMP	231.23±18.71^{a,A}	204.03±17.3^{a,AB}	181.93±13.61^{ab,AB}	158.1±11.94^{b,AB}	98.59±8.51^{c,AB}
	L-AsAc (mg·100 g^{-1} jam)				
1.0% LMP	5.51±0.35^{a,A}	5.08±0.22^{a,A}	4.74±0.31^{ab,A}	4.20±0.21^{b,A}	3.27±0.24^{c,A}
0.7% LMP	5.37±0.25^{a,A}	4.91±0.30^{a,A}	4.43±0.36^{ab,A}	3.79±0.28^{b,A}	2.85±0.18^{c,A}
0.5% LMP	5.26±0.35^{a,A}	4.74±0.26^{a,A}	4.24±0.37^{ab,A}	3.53±0.29^{b,AB}	2.66±0.19^{c,AB}
0.3% LMP	4.91±0.37^{a,A}	4.32±0.30^{a,A}	3.85±0.35^{ab,A}	3.15±0.21^{b,AB}	2.30±0.15^{c,AB}
	FRAP (mM Fe^{2+}·100 g^{-1} jam)				
1.0% LMP	2.45±0.18^{a,A}	2.30±0.14^{a,A}	2.18±0.16^{a,A}	1.99±0.13^{ab,A}	1.64±0.12^{b,A}
0.7% LMP	2.35±0.17^{a,A}	2.18±0.16^{a,A}	2.03±0.15^{a,A}	1.82±0.09^{ab,A}	1.46±0.09^{b,A}
0.5% LMP	2.14±0.17^{a,A}	1.97±0.14^{a,A}	1.80±0.13^{a,A}	1.59±0.15^{ab,AB}	1.27±0.09^{b,AB}
0.3% LMP	2.02±0.14^{a,A}	1.80±0.12^{a,Ab}	1.62±0.15^{ab,AB}	1.47±0.09^{b,B}	1.08±0.10^{c,B}

Means in a row (a-d across storage time) followed by the same letter are not significantly different (P<0.05). Means in a column (A-C across LMP concentration) followed by the same letter are not significantly different (P<0.05).

By increasing the storage time may be affected the hydrolysis of compounds, which resulted in a gradual reduction in anthocyanins content. The level of TMA gradually decreases throughout storage. At the end of storage, it was noticed losses in the range 58-72% reported to the values recorded one day after processing, *Figure 2.8a*. The level of losses was related to the pectin dose used in jam recipe: the losses were more pronounced in samples with low dose of pectin. These findings are consistent with data previously reported by Kopjar *et al*., (2007).

Polyphenoloxidase, peroxidase, and glycosidase enzymes can have a devastating effect on anthocyanins. Light exposure will promote the pigments destruction while a reduced water activity will enhance their stability (Wrolstad *et al*., 2005). It may be assumed that the oxidative reaction proceeds in jams during storage, even if the jams were hot-packed into glass jars. A strong decline of TMA content during storage was also reported for various processed blueberry products (Brownmiller *et al*., 2008).

From the statistical test it could be seen that, the storage time affected in a greater extent the stability of TMA than the pectin concentration, ($P<0.05$). At any level of LMP the decrease registered for TMA content in response to storage has statistically significance at $P<0.05$. Contrary, the decreases recorded for TMA in jam samples one day after processing in response to decreasing of LMP level had not any statistical significance at $P<0.05$.

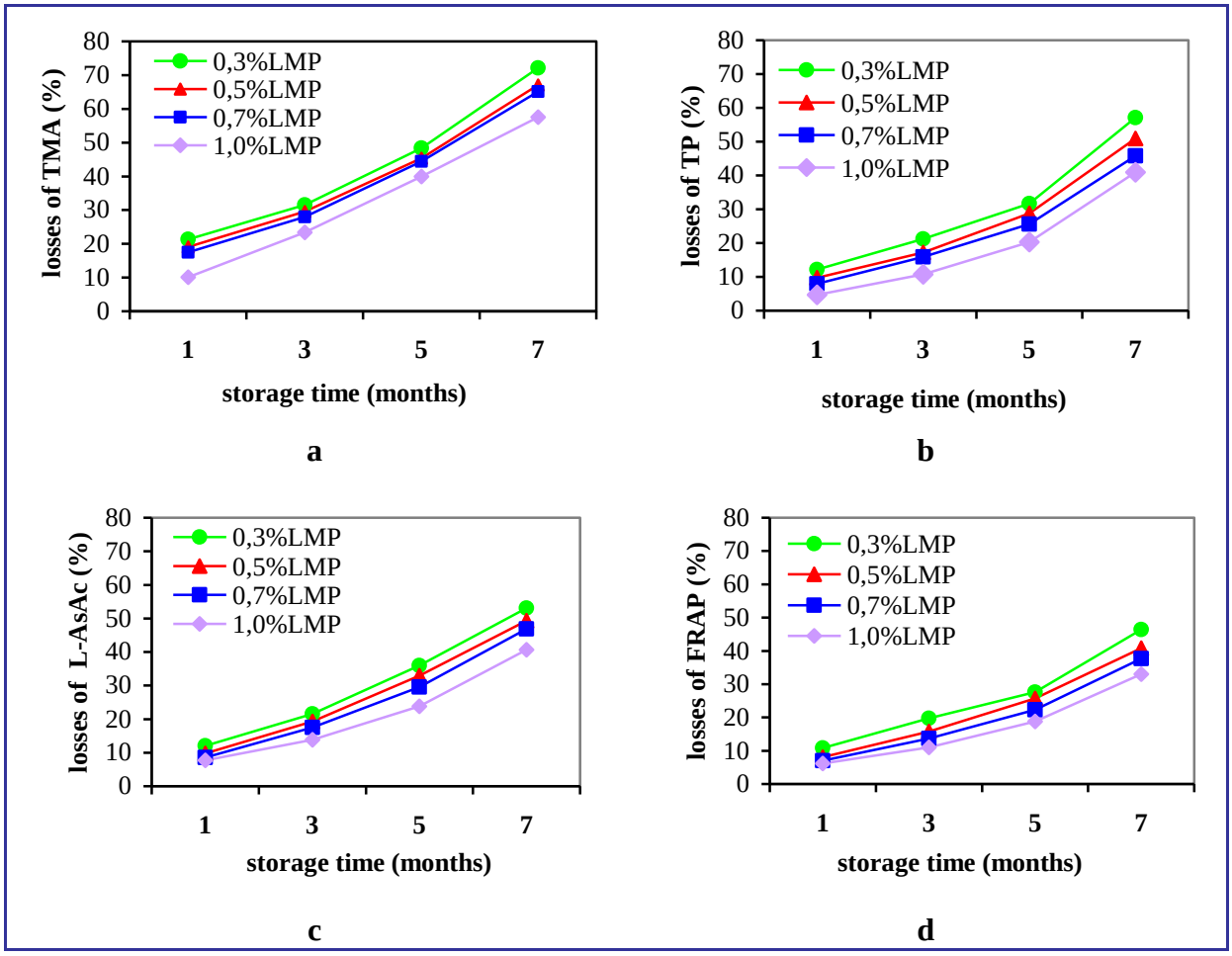


Figure 2.8. The relative losses of investigated parameters in response to lam storage at 20°C (**a:** TMA; **b**: TP; **c:** L-AsAc; **d**: FRAP)

The effect of processing, LMP concentration and storage for 7 months at 20°C on bilberry jam color was quantified by measuring the following color indices: CD, PC and PC (%).

The changes recorded in color parameters in response to LMP concentration and storage time were presented in *Table 2.9.* It can be noticed that PC (%) increased in response to jam processing depending on LMP level. The increases recorded for PC (%) were consistent with losses of TMA content registered in result of processing. In addition to the formation of PP, the losses of TMA occurring in response to processing may be associated with enzymatic and thermal degradation (Brownmiller *et al*., 2008; Yuksel and Koka, 2008). Exposure of berries to elevated temperatures during jam processing and pasteurization most likely contributed to TMA losses, because the anthocyanins degradation is time and temperature dependent (Rommel et al., 1992; Brownmiller et al., 2008). It has been demonstrated that the level of polymeric pigments increases with storage time, this fact having a great impact on color stability in juices and red wines (Wrolstad *et al*., 2005).

It was noted that the rate of loss recorded for CD is much slower than the rate of TMA degradation. For major losses of TMA only minor changes were found for CD, proving the stability of the jam color during long-term storage. This fact proves the stability of PP formed in response to storage. These compounds are a source of "*stable color*" (Wrolstad *et al*., 2005; Yuksel and Koka, 2008), they compensated for the loss of color due to the significant degradation of TMA during jam storage.

Pectin acts differently on the variety of anthocyanins from berries. In some cases, pectin acts as a copigment, thus increasing the color (Lewis *et al*., 1995; Kopjar *et al*., 2009). Thus, the effect of pectin level on jam color is still not accurately known.

Table 2.9. Changes of jam color as effect of LMP concentration and storage time

Samples	storage time (months)				
	0	**1**	**3**	**5**	**7**
			CD (AU)		
1.0% LMP	$11.82\pm0.88^{a,A}$	$11.64\pm0.62^{a,A}$	$11.21\pm0.77^{a,A}$	$10.53\pm0.86^{a,A}$	$10.07\pm0.77^{a,A}$
0.7% LMP	$11.68\pm0.83^{a,A}$	$11.28\pm0.78^{a,A}$	$10.92\pm0.80^{a,A}$	$10.24\pm0.78^{a,A}$	$9.81\pm0.78^{a,A}$
0.5% LMP	$11.47\pm0.64^{a,A}$	$11.03\pm0.68^{a,A}$	$10.37\pm0.62^{a,A}$	$9.95\pm0.70^{a,A}$	$9.55\pm0.82^{a,A}$
0.3% LMP	$11.25\pm0.81^{a,A}$	$10.51\pm0.86^{a,A}$	$10.08\pm0.83^{a,A}$	$9.52\pm0.65^{a,A}$	$9.31\pm0.73^{a,A}$
Samples			**PC (AU)**		
1.0% LMP	$1.18\pm0.09^{a,A}$	$1.27\pm0.10^{a,A}$	$1.46\pm0.12^{a,A}$	$1.79\pm0.15^{b,A}$	$2.23\pm0.17^{c,A}$
0.7% LMP	$1.25\pm0.10^{a,A}$	$1.36\pm0.12^{a,A}$	$1.57\pm0.11^{a,A}$	$2.14\pm0.16^{b,A}$	$2.38\pm0.15^{c,A}$
0.5% LMP	$1.37\pm0.11^{a,A}$	$1.51\pm0.12^{a,A}$	$1.73\pm0.14^{a,A}$	$2.37\pm0.16^{b,AB}$	$2.71\pm0.18^{c,AB}$
0.3% LMP	$1.55\pm0.13^{a,B}$	$1.73\pm0.14^{a,B}$	$1.97\pm0.12^{a,B}$	$2.71\pm0.20^{b,B}$	$3.12\pm0.24^{c,B}$
Samples			**PC (%)**		
1.0% LMP	$9.98\pm0.65^{a,A}$	$10.91\pm0.65^{a,A}$	$13.02\pm0.71^{b,A}$	$17.00\pm0.78^{c,A}$	$22.14\pm1.21^{d,A}$
0.7% LMP	$10.70\pm0.85^{a,A}$	$12.07\pm0.72^{a,A}$	$14.38\pm0.64^{b,A}$	$20.90\pm1.15^{c,B}$	$24.26\pm1.12^{d,A}$
0.5% LMP	$11.94\pm0.87^{a,A}$	$13.69\pm0.70^{a,B}$	$16.68\pm0.78^{b,B}$	$23.82\pm0.98^{c,C}$	$28.38\pm1.33^{d,B}$
0.3% LMP	$13.78\pm1.11^{a,B}$	$16.46\pm0.86^{a,C}$	$19.54\pm0.93^{b,C}$	$28.47\pm1.44^{c,D}$	$33.51\pm1.77^{d,C}$

Means in a row (a-d across storage time) followed by the same letter are not significantly different ($P<0.05$). Means in a column (A-D across LMP concentration) followed by the same letter are not significantly different ($P<0.05$).

The decrease of LMP concentration from 1 to 0.3% resulted in increase of PC (%) in samples analysed one day after processing. For jam samples obtained with the same dose of pectin, PC (%) progressively increased during storage. At the end of storage, the highest values

of PC (%) were obtained for jam samples with the lowest LMP doses. During storage it was noted a tendency towards slowing of increase in PC (%) values by increasing of LMP dose in the jam recipe. This fact could be due to associantions formed as a result of interactions between the flavilium cation of anthocyanins and the dissociated carboxilic grups of pectin. Due to this stabilising effect, anthocyanins may be protected of condensation reactions occurring among anthocyanins and procyanidins. The crosslinks formed between anthocyanins and procyanidins are no more stable than those existing between anthocyanins and pectin.

From the results of statistical test it may be noted that the LMP dose did not exert a major impact on PC (%) recorded immediately after jam processing, but its effect has become statistically significant by increasing of storage time ($P<0.05$). CD decreased as effect of fruit thermal processing. It can be observed that CD undergoes minor changes by increasing the LMP dose from 0.3 to 1%. CD registered slight decreases in the storage time. The results of statistical processing pointed out that, the LMP level and storage time induced non-significant changes in CD (*Table 2.9*), proving that this parameter was not stable only in response to processing but were also stable during long-term storage.

Changes in TP content in response to jam processing and storage

By analysis of data reported in *Tables 2.7* and *2.8*, regarding the content of TP, it can be noted that thermal processing of wild bilberries led to major alterations of TP content located in the range 42-51% reported to the value corresponding to fresh fruit. Previous studies on this topic reported significant losses in TP content in response to thermal processing of various berries (Schmidt *et al*., 2005; Savikin *et al*., 2009; Howard *et al*., 2010). The largest loss in TP content in response to fruit thermal processing was noted in jam sample with 0.3% pectin, and the highest in jam with 1% pectin.

In addition to the losses occurring as a result of thermal processing, the storage for 7 months at 20°C induced significant alterations of TP registered in jam samples ($P<0.05$). At the end of storage the losses of TP reached 41-47% of the values recorded one day after jam processing, *Figure 2.8b*. Our data suggest that, the polyphenolic compounds had a better stability in jam samples with high doses of LMP than in those obtained with low pectin doses.

Changes in L-AsAc content in response to jam processing and storage

At a closer look of data shown in *Tables 2.7 and 2.8* related to the content of L-AsAc could be observed that fruit thermal processing induced significant alterations of this parameter, located in the range 53-58% reported to the value corresponding to fresh fruit. The highest degree of L-AsAc alteration in response to fruit thermal processing was noted in jam sample with the lowest dose of pectin. Contrary, the highest level of pectin provided the best protection of L-AsAc content in response to jam processing.

Storage at room temperature also influenced the amount of L-AsAc and the effect was more expressed in jam with 0.3% LMP than in jams with 0.7-1% LMP. It was found that 7 months of storage resulted in significant alterations ($p<0.05$) in L-AsAc content. At the end of storage period, the recorded losses reached 41-53% reported to the values recorded one day after

processing, *Figure 2.8c.* L-AsAc was more stable in jam samples with high LMP doses in response of both processing and long-term storage at 20°C.

Changes in FRAP value in response to jam processing and storage

Data from *Tables 2.7* and *2.8* related to FRAP values show that 36-47% of the antioxidant capacity corresponding to fresh fruit was lost during jam processing. Our data are consistent with the results reported by Schmidt *et al.* (2005), Savikin *et al.* (2009), Howard *et al.* (2010). The alterations noted in FRAP values can be attributed to the decrease in the content of TMA, TP, L-AsAc, as well as other bioactive compounds in response to thermal treatment. The thermal treatment has the lowest negative impact on FRAP value recorded in jam sample with the highest concentration of pectin. The FRAP values declined by decreasing the LMP dose in jam formulation, *Table 2.8.* In terms of storage impact on FRAP values, it was found that the increase of storage time resulted in depreciations of antioxidant activity of jam samples, depending on LMP concentration. The losses noted in FRAP values at the end of storage period were located in the range 33-46% reported to the value recorded one day after processing, *Figure 2.8d*. The results of statisitical processing highlighted that, after 7 months of storage at 20°C, the alterations of FRAP values were statistically significant ($p<0.05$) for all LMP levels. Also, the alterations of this parameter have increased by decreasing of pectin dose in the jam recipe as well as by extending of storage time.

Correlations among investigated parameters

A high positive correlation "FRAP values *versus* TP content" it was noted during storage (R>0.99), *Table 2.10.* In addition, FRAP was highly correlated with TMA content (R>0.98) and L-AsAc content (R>0.99). Also, a positive correlation "TP *versus* TMA content" (R>0.97) was observed, which confirm once again that the anthocyanins are the most important phenolic compounds present in bilberry (Moyer *et al*., 2002). A significant negative correlation was found between TMA and PC (%), which means that, more polymeric pigments will be accumulated in jam samples in response to TMA degradation. Also, our results indicate that FRAP and PC (%) were negatively correlated (R>0.96). In jam obtained from various berries, wherein phenolic compounds and, more specifically, anthocyanins, have a major contribution to the total antioxidant activity, simple colorimetric tests such as Folin-Ciocalteu test (Singleton *et al*., 1999) for phenolics measurement or the protocol described by Giusti and Wrolstad for TMA determination (2005) can be very useful to estimate the changes in antioxidant activity occurring during jam storage.

Table 2.10. Correlation coefficients obtained by simple regression applied to investigated parameters

Y=A+B·X	**R**			
	1% LMP	**0.7% LMP**	**0.5% LMP**	**0.3% LMP**
FRAP=f(TP)	0.996	0.999	0.999	0.998
FRAP=f(TMA)	0.989	0.994	0.995	0.995
FRAP=f(L-AsAc)	1.00	0.999	0.999	0.994
TP=f(TMA)	0.977	0.989	0.997	0.990
TMA=f(%PC)	-0.985	-0,973	-0,974	-0,974
FRAP= f(%PC)	-0,992	-0,976	-0,985	-0,966

Prior *et al.* (1998) noted that phytochemicals responsible for the antioxidant activity in berries are most likely to be phenolic acids, anthocyanins, and other flavonoid compounds.

TP and L-AsAc have a major contribution to the antioxidant characteristics of bilberry jams but in the expression of antioxidant activity are involved also, other compounds (Klopotek *et al*., 2005; Bursac Kovacevic *et al*., 2009). Previous results reported by Tsai *et al.* (2004) and Brownmiller *et al.* (2008) proved that PP display antioxidant activity, which compensates for the loss of a part of antioxidant activity as a result of monomeric anthocyanins degradation. Also, it has been proven that some degradation products of anthocyanins have the antioxidant properties (Tsai and Huang, 2004). After 7 months of storage it was noticed lower depreciation in FRAP values than the content of investigated bioactive compounds. This is confirmed by the results of ANOVA test. More studies are required for assessing the contribution of polymeric pigments to the antioxidant activity of bilberry jam during storage.

2.4.3. Conclusions

Based on aforementioned results, it can be concluded that thermal processing of wild bilberries into low-sugar jams resulted in significant losses of investigated parameters, reported to the values corresponding to fresh fruit, as follows: TMA: (81-84%), L-AsAc: (53-58%, TP: 42-51% and FRAP values: 36-47%. Moreover, jam storage at 20°C induced additional alterations of investigated compounds. Thus, jam storage for 7 months resulted in severe relative losses, as follows: TMA: 58-72%; L-AsAc: 40-53%: TP: 41-57% and FRAP: 33-46%. LMP dose used in jam recipe affected the antioxidant properties as well as the color stability of bilberry jams. By increasing of LMP dose from 0.3 to 1% it was recorded an increase in retained bioactive compounds and FRAP values. Also, the increases in PC (%) during storage were higher in jam samples processed with low pectin level. The increases recorded for PC (%) were consistent with losses of TMA content registered in result of processing. Our data suggest that, both antioxidant properties as well as the color had a better stability in jam samples with high doses of LMP than in those obtained with low pectin doses. Overall, the obtained results indicated that the bilberry jams are still excellent sources of nutritional substances with antioxidant potential, although compared to the fresh fruit, important losses seem to occur.

2.5. The impact of pectin type and dose on color quality and antioxidant properties of blackberry jam

2.5.1. Aim

In the last years pectin and other hydrocolloids were tested for improving the color stability and the retention of bioactive compounds in gelled fruit products. In line with these concerns, the study shown in ***selected paper 6*** has been directed to quantify the changes in antioxidant status and color indices of blackberry jam obtained with various types of pectin (degree of esterification: DE; degree of amidation: DA) and doses in response to processing and storage for 1, 3 and 6 months at 20°C. Blackberry jam was obtained by a traditional procedure used in households or small-scale systems with various types of commercial pectins (HMP: high-methoxyl pectin, LMP: low-methoxyl pectin and LMAP: low-methoxyl amidated pectin) added to three concentrations (0.3, 0.7 and 1.0%). The pectins were separately added under continuous stirring at the final stage of the jam cooking. The pH of mixture was adjusted with citric acid to 2.90±0.05 (for jams with HMP) and 3.3±0.05 (for jams with LMAP and LMP). Additionally, calcium chloride dihydrate was added in jam formulations with LMP and LMAP. The calcium ions dose/g pectin was established according to manufacturer's recommendations depending on the pectin type. Jam samples were investigated, according to the protocols specified in *selected paper 6*, in terms of TMA, FRAP values, TP, CD and PC (%).

In performing of this research I worked closely together with Lecturer dr. Melania-Florina Munteanu [anaionescuro@yahoo.com], Lecturer dr. Despina-Maria Bordean [despina.bordean@gmail.com], Lecturer dr. Ramona Gligor [ramona_gligor@yahoo.com] and Prof. dr. Ersilia Alexa [alexa.ersilia@yahoo.ro]. The contribution of each author is shown in ***selected paper 6***.

2.5.2. Results and Discussion

Blackberry jam obtained with various types of pectin (DE, DA) applied at three levels were analyzed one day post-processing (0) and after 1, 3 and 6 months of storage at 20°C in terms of TMA, CD, PC (%), TP and FRAP values. In *Table 2.11* are shown the main chemical parameters of fresh fruit used for jam preparation. The content of TMA, TP and FRAP values recorded in jam after processing were assimilated with the real values of these parameters.

Table 2.11. Chemical characteristics of fresh blackberries

Component (Units)	Values
TP (mg GAE·100 g^{-1} FW)	521.8±12.8
TMA (mg·100 g^{-1} FW)	190.1±8.1
FRAP (mM Fe^{2+}·100 g^{-1} FW)	4.4±0.3
TSS (°Brix)	14.0±0.6
CD (AU)	9.9±0.7
PC (%)	6.0±0.4

Data resulted from mass balance performed to jam processing revealed that about 68 g fresh fruit were needed to obtain 100 g jam with 45°Bx. This information is useful to evaluate the theoretical content of investigated compounds in obtained jams. We assumed that the differences between theoretical and real content of investigated parameters were caused by fruit thermal treatment. The obtained results were processed by one-way ANOVA test to highlight the

significance of changes occuring in assessed parameters in response to storage, reported to the values recorded one day post-processing (as control, C), Also, to represent the variation of the studied parameters during storage we have used star charts which plot the values of each category along a separate axis that starts in the center of the chart and ends on the outer ring. Star charts are a useful way to display multivariate observations with an arbitrary number of variables (Chambers *et al*., 1983). Neighbor-Joining Cluster analysis was performed by using Past Software Packages (Hammer *et al*., 2001) for clustering of jam samples based on TMA, TP and FRAP to identify the best methods for jam processing in order to maintain the highest levels of antioxidant parameters. Usually, this analysis is used as a clustering method for the creation of phenograms, but it can also be used as a classification method to identify the best methods from a set of multiple procedures involving multiple variables (Saitou and Nei, 1987).

Changes recorded in TMA content

In *Table 2.12* is presented the TMA content from blackberry jam after processing and storage. Considering the values reported in *Tables 2.11* and *2.12* related to TMA content, can be assessed the losses registered in response to fruit thermal processing.

Table 2.12. The impact of storage at 20°C on TMA content of blackberry jam

Jam samples	TMA (mg·100 g^{-1} jam)			
	1 day (0)	1 month	3 months	6 months
LMP1	36.8±0.9	34.1±1.3^{ns}	29.6±1.1**	22.4±1.0***
LMP2	34.0±1.2	31.2±0.9*	26.8±1.2**	20.0±1.0***
LMP3	31.9±0.9	26.9±0.5*	23.9±0.8**	17.3±0.8***
LMAP4	40.7±1.6	38.8±1.3^{ns}	34.2±1.3**	28.2±0.9***
LMAP5	39.1±1.3	36.6±1.2^{ns}	32.5±1.4*	25.6±1.2***
LMAP6	35.8±0.7	31.2±1.1*	28.0±1.3**	20.9±0.8***
HMP7	28.0±0.9	24.7±1.1*	20.1±1.1**	15.2±0.9***
HMP8	26.2±0.9	22.41±0.8*	19.8±0.7**	13.3±0.8***
HMP9	23.3±0.9	19.0±0.9*	15.4±0.7**	10.2±0.8***

Statistical differences are indicated as follows: ns – non-significant, P>0.1; * – significant, P<0.05; ** – highly significant, P<0.01 and *** – extremely significant, P<0.001.

Legend:
LMP1: LMP 1%; LMP2: LMP 0.7%; LMP3: LMP 0.3%; LMAP4: LMAP 1%; LMAP5: LMAP 0.7%; LMAP6: LMAP 0.3%; HMP7: HMP 1%; HMP8: HMP 0.7% and HMP9: HMP 0.3%.

Our data revealed that thermal processing of fresh fruit induced significant losses in TMA content, in the range 69-82% reported to the values corresponding to fresh fruit. The TMA content in jam samples one day after processing revealed that this parameter was affected by the pectin type as well as by the dosage used in jam recipe. These results are consistent with other that reported losses in TMA content during jam processing from various wild berries in the range 70-85% (Amakura *et al*., 2000; Savikin *et al*., 2009, Poiana *et al*., 2012). Anthocyanins exhibit a high sensitivity to temperature (Gimenez *et al*., 2001). Thermal treatments of fruit, especially those involving prolonged exposure at high temperature, cause dramatic alterations of TMA due to oxidation, cleavage of covalent bonds or enhanced oxidation reactions (Zhang *et al*., 2012). Also, thermal processing leads to complexation reactions between anthocyanins and other compounds resulted in response to high temperature exposure. Apart from these, the losses of

TMA could be due to formation of anthocyanin polymers or condensation between anthocyanins and procyanidins or other phenolic compounds (Patras *et al*., 2010; Moura *et al*., 2012).

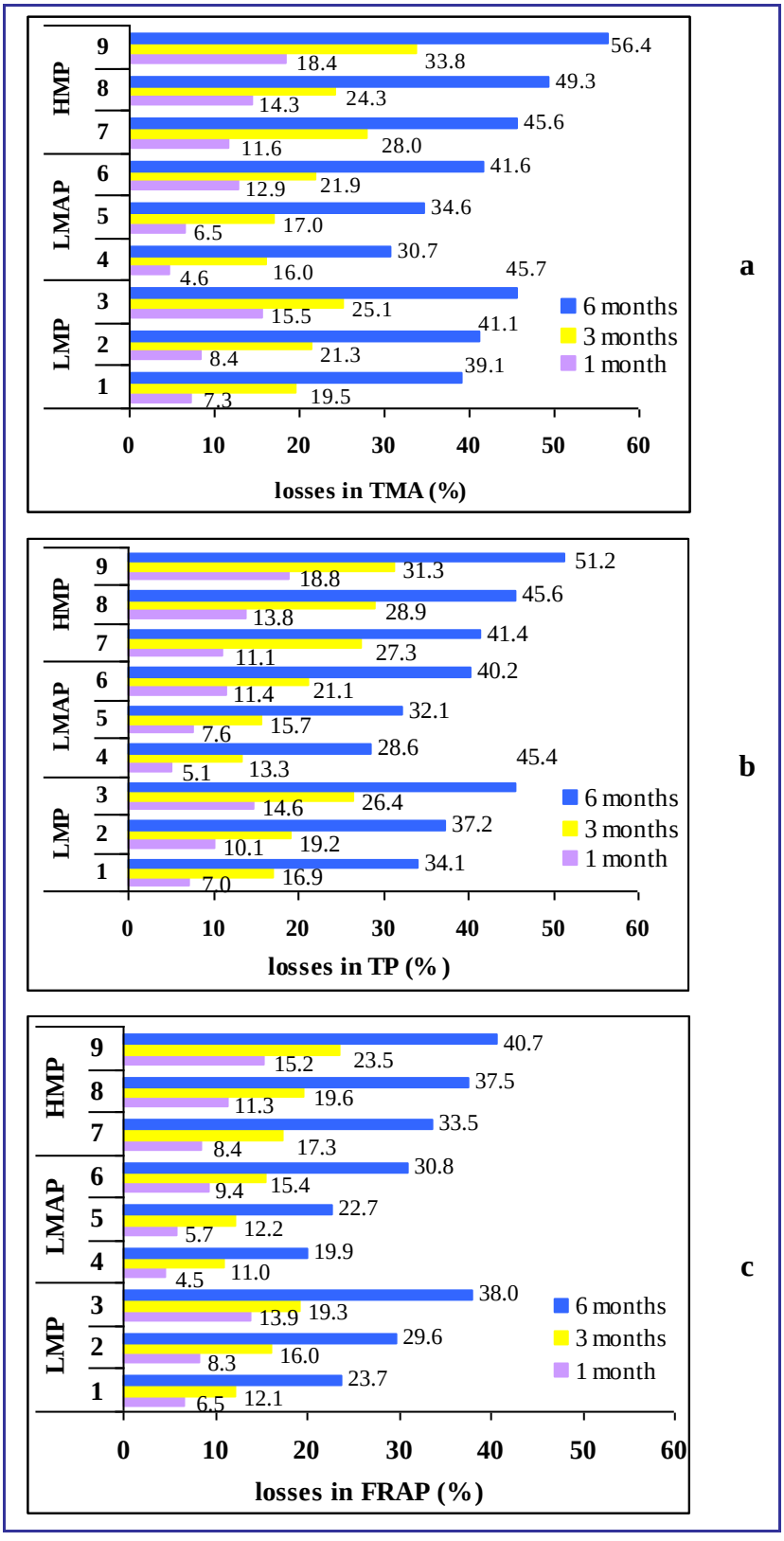
By using of LMAP, LMA and HMP the losses recorded in TMA content were in the ranges: 71-75%, 69-71%, 78-82% reported to the values corresponding to fresh fruit. These data revealed that anthocyanin pigments were better retained in jam samples obtained with low-esterified pectin than in samples with high-esterified pectin. Among the jams obtained with pectins having similar DE, the best retention was noticed by using of amidated pectin. Moreover, the increasing of pectin dose resulted in improvement of TMA retention in jam. This fact could be explained by interactions between anthocyanins and pectin chains.

Holzwarth *et al.* (2013), Kopjar *et al.* (2007) and Buchweitz *et al.* (2013) reported that the pectin type has a great impact on its functionality. The mechanism of gel formation during jam processing is of a great importance to explain our results. The different types of associations that occur between the pectin chains are determined by its type (DE, DA) (Hubbermann *et al*., 2006; Kopjar *et al*., 2007; Buchweitz *et al*., 2013). LMP and LMAP probably interact more easily with anthocyanins because they have fewer methoxyl groups than HMP (Hubbermann *et al*., Kopjar *et al*., 2009). The improved stability of pigments in jams prepared with LMAP might be due to the formation of additional hydrogen bonds between the hydroxyl groups of anthocyanins and the amide groups of pectin (Holzwarth *et al*., 2013). In result, anthocyanins can be protected against water attack or condensation reactions among anthocyanins and procyanidins (Hubbermann *et al*., 2006). Thus, it can be suggest that it is possible to control the content of TMA in gelled fruit products by pectin type and dose. As presented in *Table 2.12*, TMA content significantly decreased during jam storage. At the end of storage the relative losses in TMA content were in the range 31-56%, *Figure 2.9a*. Anthocyanins stability during storage strongly depended on the pectin type and dosage used in jam formulation. After 6 months of storage, the best retention of TMA was noticed in samples with LMAP and the lowest in jams with HMP. Among the jams prepared with low-esterefied pectin, anthocyanins stability was better in samples obtained with pectin having similar DE and amidation. More researches are needed to study individual anthocyanins to assess if there are any differences in their degradation pattern and stability during jam processing and storage in relation with pectin type and its dosage.

In *Figure 2.10a* is shown the variation of TMA content in response to storage. The highest value of TMA corresponds to LMAP4.0 and the lowest to HMP9.6. Statistical analysis reveals that the changes of TMA content were greatly affected by storage period. After 6 months, the changes in TMA content became extremely significant for all jam samples ($P<0.001$).

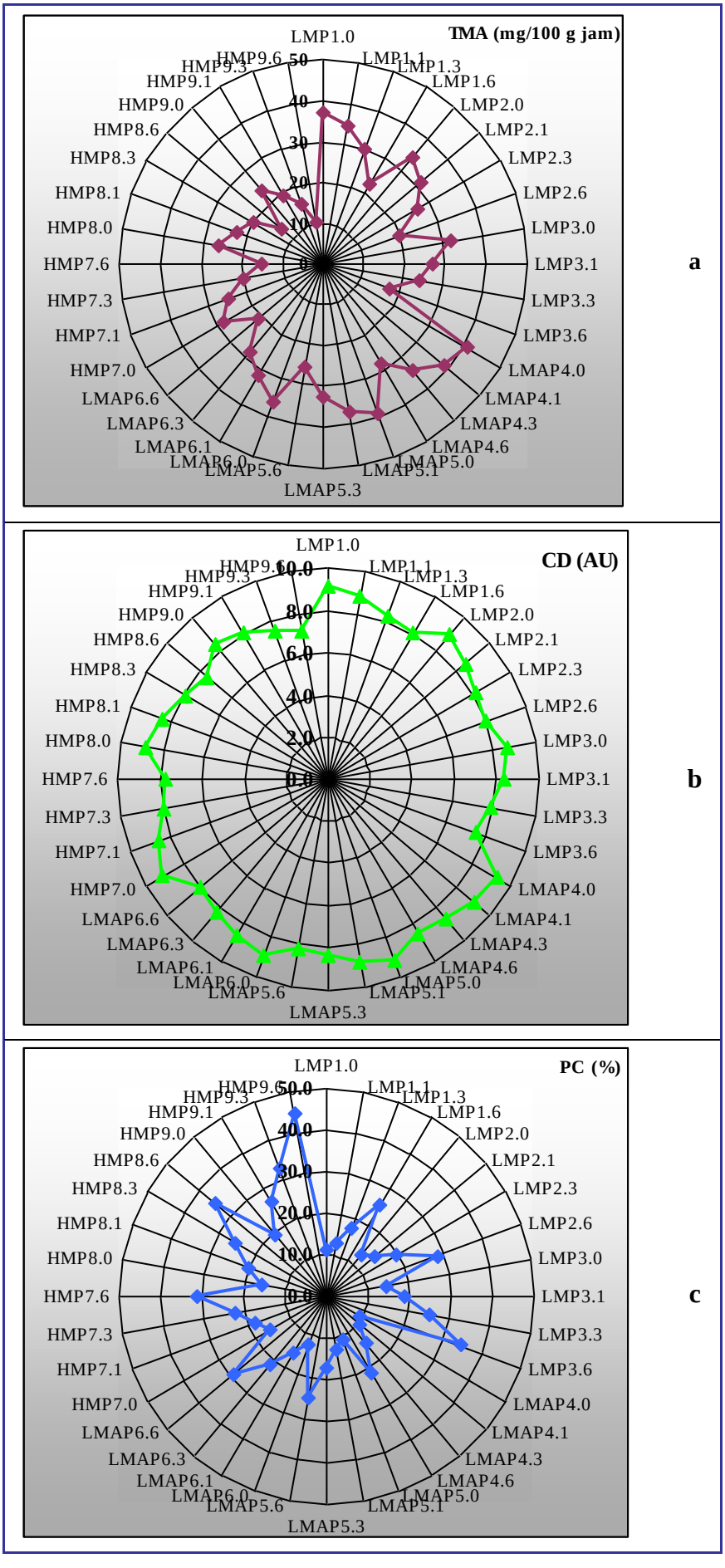
Changes recorded in color indices

The color quality was quantified by CD and PC (%). It was noticed a certain sensibility of CD to pectin type and dose used for jam preparation. Thus, one day post-processing, jam samples prepared with various types or different doses of pectin presented different values of CD, *Figure 2.11a*. Samples with HMP had lower values of CD than samples with LMP or LMAP. Also, it can be seen that samples with LMAP had slightly higher values of CD than samples with LMP. This tendency remained during 6 months of storage. It could be noticed that CD exhibited a good stability in response to long-term storage.



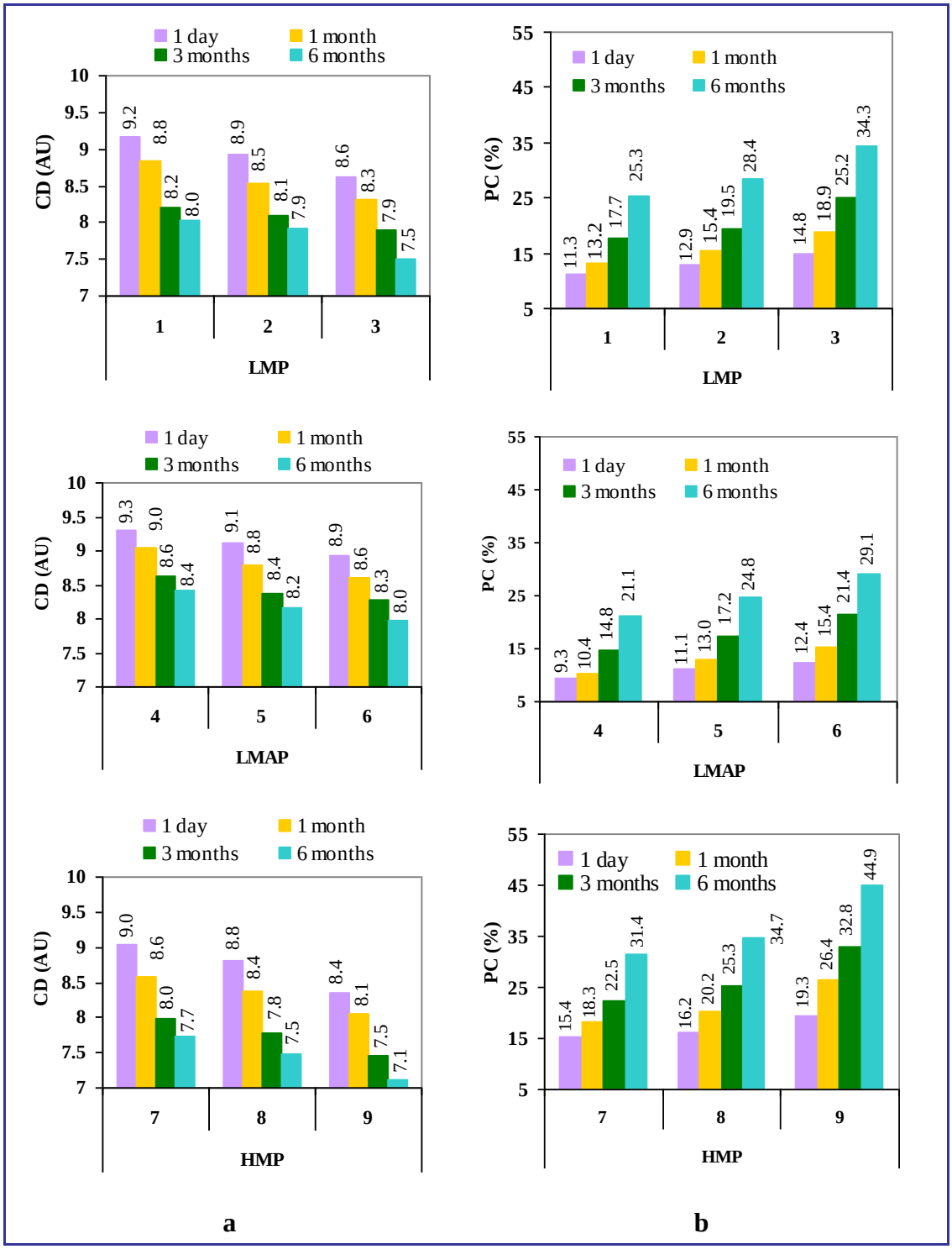
Legend:
LMP1: LMP 1%; LMP2: LMP 0.7%; LMP3: LMP 0.3%; LMAP4: LMAP 1%; LMAP5: LMAP 0.7%; LMAP6: LMAP 0.3%; HMP7: HMP 1%; HMP8: HMP 0.7% and HMP9: HMP 0.3%.

Figure 2.9. The relative losses of investigated parameters during jam storage (**a**: TMA; **b**: TP; **c**: FRAP)



Legend:
LMP1: LMP 1%; LMP2: LMP 0.7%; LMP3: LMP 0.3%; LMAP4: LMAP 1%; LMAP5: LMAP 0.7%; LMAP6: LMAP 0.3%; HMP7: HMP 1%; HMP8: HMP 0.7% and HMP9: HMP 0.3%.
In samples labeled as LMP1.0, LMP1.6 and so on, the number after point represents the storage time (e.g. LMP1.0: LMP1 one day post-processing; LMP1.6: LMP1 after 6 months of storage).

Figure 2.10. Star representation of TMA (a), CD (b) and PC % (c) variation during jam storage



Legend: LMP1: LMP 1%; LMP2: LMP 0.7%; LMP3: LMP 0.3%; LMAP4: LMAP 1%; LMAP5: LMAP 0.7%; LMAP6: LMAP 0.3%; HMP7: HMP 1%; HMP8: HMP 0.7% and HMP9: HMP 0.3%.

Figure 2.11. The impact of storage on the color indices of blackberry jam (**a**: CD; **b**: PC %)

The results reported by Mazzaracchio *et al.* (2004) suggest that pectin could induce a slight increase in color displayed by flavilium cation that is in equilibrium with the pseudobase at the same pH. Also, a weak hydrophobic interaction between methoxyl groups of anthocyanin aglycons and methoxyl groups of pectin chains could occur, resulting in a weak copigmentation

effect (Mazzaracchio *et al*., 2004). The differences recorded in CD in relation with pectin type might be explain by the fact that low-esterified pectins interact more easily with anthocyanins because they have fewer methoxyl groups than high-esterified pectins.

Figure 2.10b presents the variation of CD during jam storage. This chart reveals that the highest value of CD corresponds to sample LMAP4.0 and the lowest to HMP9.6. Also, the variation registered for CD during jam storage is very low.

Thermal processing led to the formation of polymeric pigments (PP) revealed by increasing of PC (%), *Figure 2.11b*. PP formed in response to storage represent an important part of "*stable color*". (Tsai and Huang, 2004; Hager *et al*., 2008).

Significant increases in PC (%) have also been noticed for other thermally treated, shelf-stable products from blackberries (juices, canned products, and purees) during storage at 25°C (Hager *et al*., 2008). During jam processing, the fruit are exposed to thermal treatment around 80-100°C, therefore, sugar degradation products is expected to be formed. In addition, sugar degradation products may be formed during storage and this is known to promote anthocyanins degradation and also may reduce the stabilizing effect on color caused by decreasing of water activity (Kopjar *et al*., 2009).

PC (%) markedly increased during storage and this fact plays an important role on the color stabilization. From *Figure 2.11* it can be seen that by occurrence of PP during storage, only minor changes were found for CD, proving that the color provided by PP compensates for a part of the color lost due to the degradation of TMA during storage.

At the end of storage, the lowest values of PC (%), in the range 21-29%, were noticed in jams with LMAP and the highest, in the range 31-45%, for jams with HMP. The lowest increase of PC (%) was observed in jam sample prepared with LMAP to a level of 1%. Also, the increase in PC (%) has been dose-dependent.

The variation of PC (%) during jam storage can be seen in star chart from *Figure 2.10c*. It can be noted that the highest value of PC (%) corresponds to HMP9.6 and the lowest to LMAP4.0.

Figures 2.9a and *2.11b* reveal an obvious connection between the increasing of PC (%) and decreasing of TMA. In line with the findings of Brownmiller *et al*. (2008), Hager *et al*. (2008) and Poiana *et al.* (2012), we assumed that, the increases in PC (%) are due to the gradual inclusion of TMA in PP matrix. The best stabilization of jam color during 6 months of storage was achieved by LMAP followed by LMP and HMP. It is likely that the cross links formed in response to reactions of anthocyanins polymerization or condensation among anthocyanins and procyanidins are no more stable than those occurring between TMA and pectin.

Changes recorded in TP content

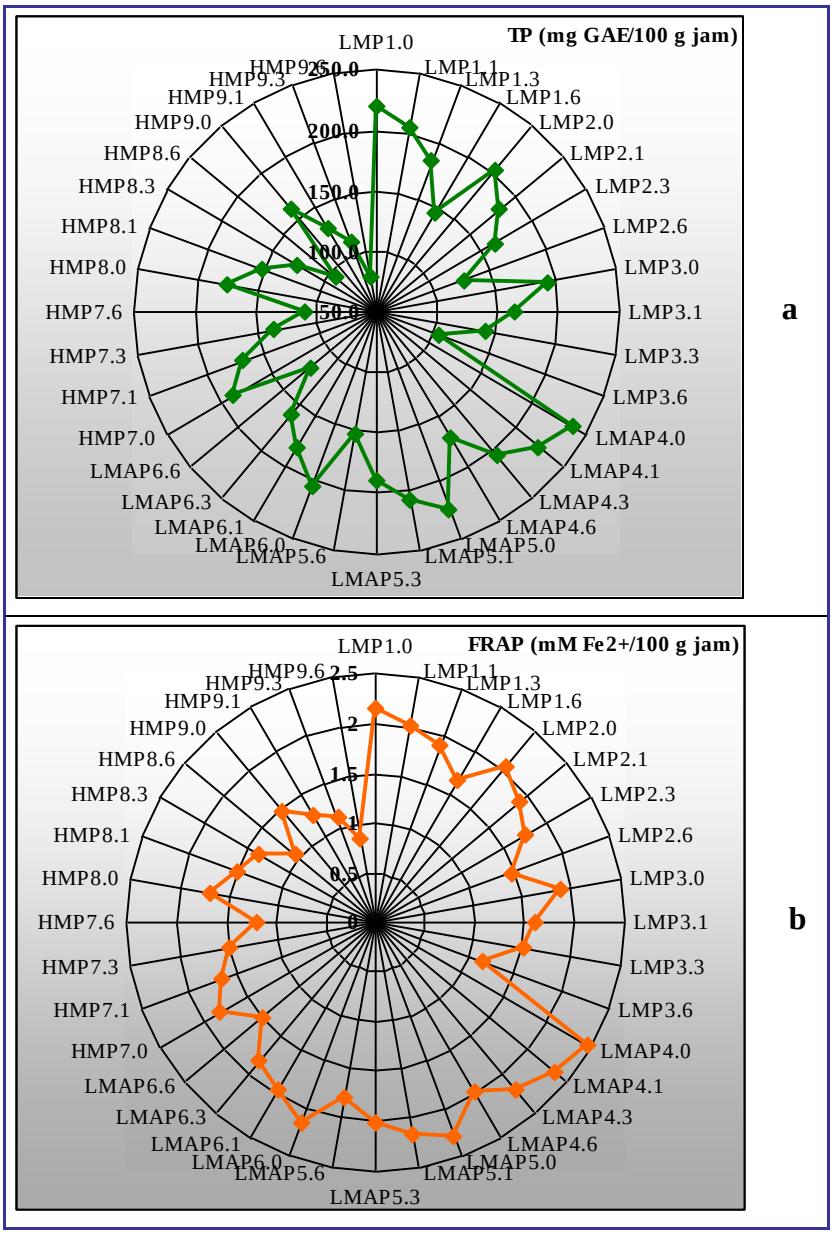
Considering the data presented in *Tables 2.11* and *2.13* related to the TP content, we can estimate the losses occurring in this parameter in response to fruit thermal processing. Thus, it can be seen that blackberry thermal processing during jam processing induced significant depreciations in TP content of jam samples obtained with LMAP, LMA and HMP, as follows: 38-46%, 33-43% and 47-55% reported to the values corresponding to fresh fruit.

Table 2.13. The impact of storage at 20°C on TP content of blackberry jam

Jam samples	TP (mg GAE·100 g^{-1} jam)			
	1 day (0)	1 month	3 months	6 months
LMP1	219.5±9.8	204.1±9.0^{ns}	182.3±7.2*	144.7±7.3***
LMP2	201.2±10.8	181.0±10.3^{ns}	162.6±9.1*	126.4±7.3***
LMP3	192.1±10.7	164.1±8.5*	141.3±8.4*	104.8±7.0***
LMAP4	237.2±8.9	225.1±9.8^{ns}	205.6±11.1*	169.3±8.0***
LMAP5	224.3±10.9	207.3±11.3^{ns}	189.2±10.3*	152.2±8.8***
LMAP6	203.2±13.3	180.1±11.1^{ns}	160.3±8.7*	121.6±7.5***
HMP7	187.3±11.3	166.5±10.5^{ns}	136.2±7.2**	109.8±6.0***
HMP8	175.4±10.6	151.2±7.4*	124.8±7.4**	95.5±6.1***
HMP9	160.1±10.7	130.1±5.8*	110.1±6.3**	78.1±5.6***

Statistical differences are indicated as follows: ns – non-significant, P>0.1; * – significant, P<0.05; ** – highly significant, P<0.01 and *** – extremely significant, P<0.001.

Legend:
LMP1: LMP 1%; LMP2: LMP 0.7%; LMP3: LMP 0.3%; LMAP4: LMAP 1%; LMAP5: LMAP 0.7%; LMAP6: LMAP 0.3%; HMP7: HMP 1%; HMP8: HMP 0.7% and HMP9: HMP 0.3%.



a

b

Legend:
LMP1: LMP 1%; LMP2: LMP 0.7%; LMP3: LMP 0.3%; LMAP4: LMAP 1%; LMAP5: LMAP 0.7%; LMAP6: LMAP 0.3%; HMP7: HMP 1%; HMP8: HMP 0.7% and HMP9: HMP 0.3%.
In samples labeled as LMP1.0, LMP1.6 and so on, the number after point represents the storage time.

Figure 2.12. Star chart of TP (**a**) and FRAP (**b**) variation during jam storage

The most pronounced losses were noticed in jam with HMP and the lowest in jam samples obtained with LMAP. Therefore, by choosing of pectin with low DE and amidated groups could be improved the retention of TP compounds in jam. Moreover, by increasing of pectin dose in jam recipe it was noted increases in TP content.

The research done on this topic pointed out that the losses of TP content in response to thermal processing of various berries were dependent on the processing conditions, quality of fresh fruit as well as the jam formulation (Amakura *et al*., 2000; Kopjar *et al*., 2007; Savikin *et al*., 2009).

The effect of jam storage on TP content is shown in *Figure 2.10b*. At the end of storage, the highest relative loss in TP content was recorded in jam sample with HMP to a dose of 0.3% and the lowest in jam with LMAP to a level of 1%. These findings revealed that the highest stability of TP in the blackberry jam throughout storage period was achieved by LMAP, followed by LMP and HMP. Also, it was proved that the highest TP content in jam samples was provided by the highest dose of pectin.

The star chart representation of TP variation during jam storage highlights that the highest value of TP it was found in sample LMAP4.0 and the lowest in HMP9.6, *Figure 2.12a*.

The results of statistical processing revealed that at the end of storage, the differences in TP content registered among investigated jam samples have become extremely significant $P<0.001$).

Changes recorded in FRAP value

Data from *Tables 2.11* and *2.14* regarding the FRAP values reveal the losses in this parameter as result of thermal processing. Thus, in jam samples with LMAP, LMA and HMP, the losses recorded in FRAP values were in the ranges: 28-37%, 18-28%, 40-52% reported to the value corresponding to fresh fruit.

The best retention of antioxidant activity was registered in jam samples obtained with LMAP. Also, the FRAP values have been dependent on the pectin dose. Our results are consistent to other previously reported by Hager *et al*. (2008), Patras *et al. (*2009), Rababah *et al*. (2011) and could be explained by destruction of polyphenols or any other biologically active compounds which are relatively unstable to thermal treatment.

TP compounds but especially anthocyanin pigments greatly contribute to the antioxidant activity of blackberries and corresponding jams (Hager *et al*., 2008; Bowen-Forbes *et al*., 2010). Also, PP resulted in response to processing and storage exhibited antioxidant activity (Tsai *et al.*, 2005; Brownmiller *et al.*, 2008; Hager *et al*., 2008). In addition, some degradation products of TMA resulting in response to thermal treatment displayed antioxidant activity (Kopjar *et al*., 2009).

Figure 2.9c provides information regarding the relative losses of FRAP values in response to storage. At the end of storage, the lowest relative losses in FRAP values, in the range 20-34%, were noticed in samples with 1% pectin and the highest (31-41%) in jam samples with 0.3% pectin. Also, the highest FRAP values were registered in jams with LMAP followed by samples with LMP and HMP.

Our data suggest that small changes in the composition of jam matrix, such as pectin type or its dosage, could affect the antioxidant properties of jam, probably due to the changes occurred in the interactions between food matrix ingredients.

Figure 2.12b shows the FPAP variation in response to storage time. The highest value of FRAP corresponds to LMAP4.0 and the lowest to HMP9.6. The sample LMAP4 followed by LMAP5 present the smallest losses of FRAP values at the end of storage.

At the end of storage, the losses recorded for FRAP were lower than those registered for TP or TMA. Thus, PP (Tsai and Huang, 2004; Tsai *et al*., 2005) and other compounds (Hager *et al*., 2008; Savikin *et al*., 2009) formed as a result of heating and storage could compensate a part of antioxidant activity lost in response to TMA degradation.

Table 2.14. The impact of storage at 20°C on FRAP values of blackberry jam

Jam samples	FRAP (mM Fe^{2+}·100 g^{-1} jam)			
	1 day (0)	1 month	3 months	6 months
LMP1	2.2±0.2	2.0±0.1^{ns}	1.9±0.1^{ns}	1.6±0.1**
LMP2	2.1±0.1	1.9±0.1^{ns}	1.7±0.1*	1.5±0.1**
LMP3	1.9±0.6	1.6±0.1^{ns}	1.5±0.1*	1.2±0.1***
LMAP4	2.5±0.2	2.4±0.2^{ns}	2.1±0.2^{ns}	2.0±0.2*
LMAP5	2.3±0.2	2.2±0.2^{ns}	2.0±0.2^{ns}	1.8±0.1*
LMAP6	2.1±0.2	1.9±0.2^{ns}	1.8±0.1^{ns}	1.5±0.1**
HMP7	1.8±0.1	1.6±0.1^{ns}	1.5±0.1*	1.2±0.1**
HMP8	1.7±0.2	1.5±0.1^{ns}	1.4±0.1*	1.1±0.1***
HMP9	1.5±0.1	1.2±0.1^{ns}	1.1±0.1*	0.9±0.1***

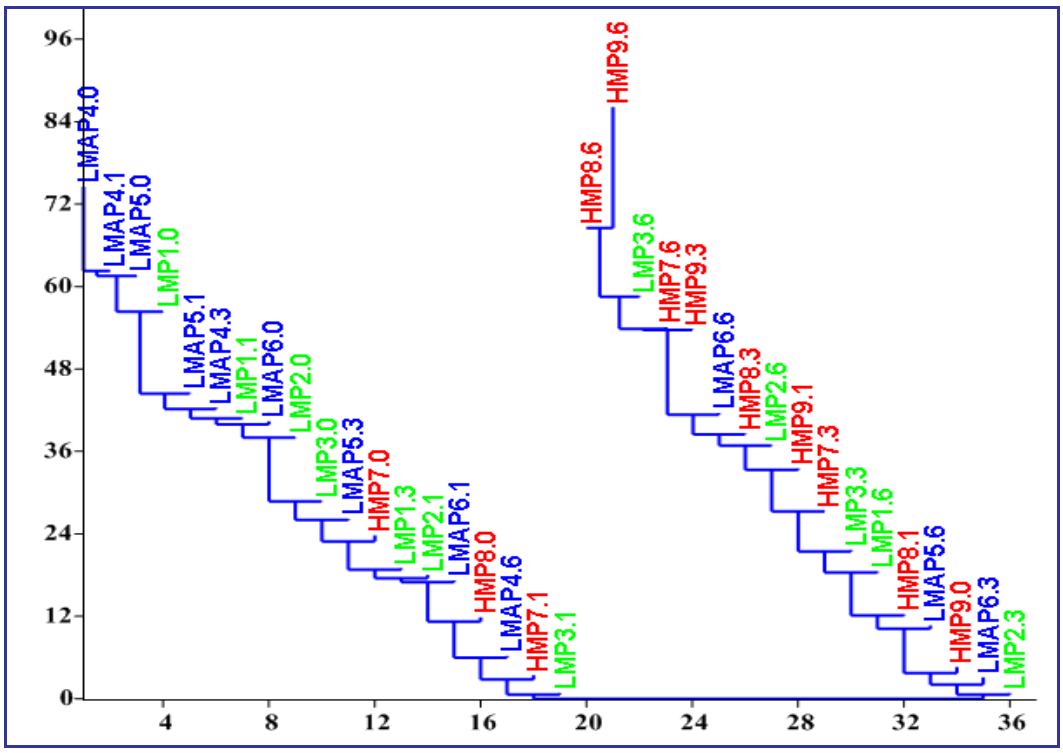
Statistical differences are indicated as follows: ns – non-significant, P>0.1; * – significant, P<0.05; ** – highly significant, P<0.01 and *** – extremely significant, P<0.001.

Legend:
LMP1: LMP 1%; LMP2: LMP 0.7%; LMP3: LMP 0.3%; LMAP4: LMAP 1%; LMAP5: LMAP 0.7%; LMAP6: LMAP 0.3%; HMP7: HMP 1%; HMP8: HMP 0.7% and HMP9: HMP 0.3%.

Based on the results of statistical analysis it can be observed that after 6 months of storage the alterations of FRAP have become significant (P<0.05) and highly significant (P<0.01) for jam samples with LMAP and highly significant (P<0.01) and extremely significant (P<0.001) for samples obtained with LMP or HMP. These findings suggest that the antioxidant capacity was best protected in jam samples with LMAP in response to long-term storage.

From *Figures 2.10* and *2.12*, it can be seen that the highest variation recorded in the storage time is given by TP, followed by TMA and PC (%), while FRAP and CD displayed lower variations.

From Neighbor-Joining Cluster analysis based on TMA, TP and FRAP it can be noted two clusters: Cluster I joining the jam formulations that maintain the highest levels of antioxidant parameters and Cluster II revealing the formulations with largest losses of antioxidant properties, *Figure 2.13*. According to the results of this analysis,we can recommend the following types and doses of pectin related to the storage period for processing of blackberry jam with high levels of antioxidant parameters: LMAP 1% (0 to 6 months), LMAP 0.7% (1 to 3 months), LMP 1% (0 to 3 months), LMAP0.3% (0 to 3 months), LMP0.7% (0 to 1 month) and LMP0.3% (0 to 1 month).



Legend:
LMP1: LMP 1%; LMP2: LMP 0.7%; LMP3: LMP 0.3%; LMAP4: LMAP 1%; LMAP5: LMAP 0.7%; LMAP6: LMAP 0.3%; HMP7: HMP 1%; HMP8: HMP 0.7% and HMP9: HMP 0.3%. In samples labeled as LMP1.0, LMP1.6 and so on, the number after point represents the storage time.

Cluster I
LMAP4.0>LMAP5.0>LMAP4.1>LMP1.0>LMAP5.1>LMAP6.0>LMAP4.3>LMP1.1>LMP2.0>LMAP5.3> LMP3.0>LMAP6.1>LMP2.1>LMP1.3>LMAP4.6>LMAP6.3>HMP7.0>LMP3.1

Cluster II
LMP2.3>HMP8.0>LMAP5.6>HMP7.1>LMP3.3>HMP9.0>HMP8.1>LMP1.6>LMAP6.6> HMP7.3> LMP2.6> HMP8.3>HMP9.1>LMP3.6>HMP9.3>HMP7.6>HMP8.6>HMP9.6

Figure 2.13. Representation of Neighbor-Joining Cluster analysis of jams based on TMA, TP and FRAP

2.5.3. Conclusions

The extent of losses for analysed parameters recorded in response to jam processing and storage were closely related to the pectin type and dosage. The losses recorded in response to processing, reported to the values corresponding to fresh fruit were as follows: TMA (69-82%), TP (33-55%) and FRAP (18-52%). Biologically active compounds and color were best retained in jams with LMAP followed by samples with LMP and HMP. Storage for 6 months brings along additional dramatic losses reported to the values recorded one day post-processing, as follows: TMA (31-56%), TP (29-51%) and FRAP (20-41%). Both processing and storage resulted in significant increases in PC (%). Over 6 months of storage, the best color retention and the highest TMA, TP and FRAP were achieved by LMAP, followed by LMP and HMP. In addition, a high level of bioactive compounds in jam could be related to a high dose of pectin. Our results suggest that the retention of bioactive compounds and jam color stability were strongly dependent on pectin type and dosage. We can conclude that LMAP to a level of 1% is the most indicated for processing of blackberry jam with the highest antioxidant properties and color stability.

2.6. Scientific contributions of the author to the actual state-of-knowledge

As respects the subjects presented above and based on the results obtained by the author as a result of four studies done on this topic, the following remarks could be considered that bring some contributions to the actual state-of-knowledge:

Regarding the effect of IQF process and long term frozen storage

- The IQF process did not affect the bioactive compounds amount of investigated wild berries. Contrary, the long-term frozen storage had a great impact on nutraceutical compounds and color stability of berries;
- The relative losses of TMA, TP, L-AsAc did not exceed 25% over six months of frozen storage. After 10 month of frozen storage, the smallest losses of antioxidant activity were recorded for blueberries and the largest for raspberrie;
- According to their antioxidant characteristics, the analyzed berries may be listed in the following order: blueberries>blackberries>raspberries;
- The color of raspberries was the most sensitive to long-term frozen storage while the color of blueberry and blackberry was more stable in response to freezing and long-term frozen storage;
- 6 months of storage at -18°C of berries packed in polyethylene bags or plastic boxes is reasonable for keeping the antioxidant properties and color of frozen fruit to a high level.

Regarding the effect of jam processing and storage

- Fruit thermal processing led to pronounced deterioration of L-AsAc, TP and FRAP values. Additionally, jam storage at 20°C brings along dramatic alterations of antioxidant properties. Anthocyanin pigments from berries were massive degraded in response to thermal processing and storage with a great impact on colour quality and antioxidant properties.
- The extent of losses recorded in response to fruit thermal processing and jam storage was closely related to the fruit species and jam formulation (pectin type and dosage);
- Among strawberry, cherry and sour cherry, the first one exhibited the highest losses of bioactive compounds in response to jam processing. Moreover, strawberry jam showed the lowest tolerance to storage conditions in terms of investigated properties. The best retention of antioxidant properties and color in response to jam processing and storage was recorded for sour cherry jam;
- TMA and CD decreased with increasing of storage time whereas the percent of polymeric color increased. The same trends were observed in all investigates jam samples;
- There is an obvious connection between the increasing of PC(%) and the decreasing of TMA due to their gradual inclusion in polymeric pigments matrix during jam storage;
- It is remarkable that the rate of the color loss is much slower than the rate of TMA degradation suggesting that the polymeric pigments occurring in response to storage compensated for a part of the loss of color due to anthocyanins degradation;
- Although TP and L-AsAc are the major potential candidates as a selection criterion for antioxidant properties of fruits jams, antioxidant activity is not limited to these. We

suppose that PP show antioxidant properties, which compensate a part of antioxidant capacity assigned to monomeric anthocyanins lost in response to storage. Although this research does not completely confirm the antioxidant properties of PP, it can be used as a basis for further studies required to clarify this effect;

- Jam formulation is very important considering that the composition of the matrix strongly affects its antioxidant properties due to the changes occurred in interactions between matrix constituents. The type and dosage of pectin are very influential factors for limiting the alterations occurring in response to thermal processing and storage;
- The mechanism of gel formation during jam processing is important in explaining of our results. The different types of associations that occur between chains are determined by the pectin type (DE, DA). LMP and LMAP probably interact with anthocyanins more easily because they have fewer methoxyl groups than HMP. The improving of anthocyanins stability in jam might be explained by the fact that pectins are polyuronic acids and their ability to retain anthocyanins is attributed to electrostatic interactions between the dissociated carboxylic groups of pectin and the flavylium cations of the pigments. The improved stability of pigments in blackberry jam prepared with amidated pectin might be due to the formation of additional hydrogen bonds between the hydroxyl groups of the anthocyanins and the amide groups of pectin. Due to these associations, anthocyanins can be protected against water attack or condensation reactions among anthocyanins and procyanidins. Based on the aforementioned remarks it might be suggested that is possible to control the content of TMA retained in gelled fruit products by pectin type and dose;
- Small changes in the jam matrix composition, such as pectin type or its dosage, greatly affect the jam quality;
- The retention of bioactive compounds and jam color stability were strongly dependent on the pectin type and dosage. A high level of bioactive compounds in jam could be related to a high dose of pectin. By a proper selection of pectin type and dose in the formulation could be improved the degree of bioactive compounds retention in the fruit-gelled products, thus, being limited the losses recorded in response to processing and storage. LMAP to a level of 1% is the most indicated for processing of bilberry and blackberry jam with the highest antioxidant properties and color stability;
- Fruit jams are still an excellent source of nutritional substances with antioxidant potential, although compared to the fresh fruit, important losses seem to occur;
- The derived knowledge will be very useful to optimize the processing of pectin-gelled fruit products and storage conditions, to adopt new concepts and technologies that offer advantages over conventional systems for improving the health promoting properties of products. These findings will be useful to fruit processors wishing to improve the final content of polyphenolic compounds, color retention and antioxidant capacity in their products. From the aforementioned, it is logical for fruit processing industry to reevaluate the existing thermal treatments based on studies that demonstrate a dramatic degradation of polyphenolic compounds, especially anthocyanin pigments.

3. Scientific achievements concerning the capitalization of some by-products from food processing

3.1. Background

Food industry is marked by the high volume of produced waste. Nowadays, the management of agro-food industry by-products for capitalizing their potential is an important issue for the economics. The processing of fruits results in high amounts of waste materials such as peels, seeds, stones and oilseed meals representing a great problem for industries due to their large production, year after year, and, in the same time, agricultural wastes have a limited exploitation. Recently, there is a pressing need for obtaining of supplements with nutritional value and in the same time with more benefits on health. Based on these reasons, the reutilization of agro-food industry by-products as sources of bioactive compounds represents nowadays an inexpensive, efficient and environmentally friendly means for their capitalization as natural additives for food industry, cosmetics or pharmaceuticals. Thus, the possibility to use these wastes as by-products for further exploitation in order to obtain potential food additives or supplements with high nutritional value have gained an increasing interest because these are high-value products and their recovery is economically attractive.

Agro-industrial wastes obtained by fruit processing contain some quantities of valuable compounds (Shrikhande, 2000) whose extraction conditions and antioxidant properties have been the subject of several works (Moure *et. al*., 2001).

This research direction discusses the potential of the most important by-products of wine industry and fruit processing as a source of valuable compounds. While the wine industry by-products can create great environmental problems, the concentrated waste could be more easily introduced into the food cycle in form of natural additives and ingredients. Recently there is a considerable interest in the development and evaluation of natural antioxidants from plant materials that are rich in flavanoids and other polyphenolic compounds (Burns *et al*., 2001).

Grapes are one of the most popular fruit in the world. About 80% of the total grapes are used in wine making (Mazza & Miniati, 1993) and pomace represents approx. 20% of the weight of the raw processed grapes. Grape pomace represents the skin, pulp and seed remaining from wine industry after grapes processing. Thus, the wine industry generates, every year, huge amounts of grape pomace. Nowadays, grape pomace is considered, rightly, a great source of different compounds such as polyphenols, pigments, sugars, tartrate, fibers, oils and ethanol (Nerantzis and Tataridis, 2005).

Grape skins and seeds represent about 13% of the amount of processed berries (Torres and Bobet, 2001) and constitute a rich source of health-promoting polyphenols with high antioxidant properties that may have applications as food additives with nutritional benefits (Torres and Bobet, 2001; Lapornik *et al*., 2005). The improving of the grape seeds utilization has a major importance in order to be use as a source of natural food additives, ingredients, and supplements (Soong and Barlow, 2004). Currently, grape pomace represents a valuable low-cost raw material for the extraction of value-added compounds such as polyphenols especially flavonoids (catechin, epicatechin), anthocyanins and procyanidins, phenolic acids that include

gallic acid and ellagic acid and stilbenes such as resveratrol and piceid (Yilmaz and Toledo, 2006) with potential as food additives or nutraceuticals. These compounds collectively are referred to as phenolic compounds, possess antibacterial, antiviral, anti-inflammatory, anti-cancerogenic properties and can prevent cardiovascular diseases (Shrikhande, 2000). Also, the phenolic compounds of these extracts are responsible for their antioxidant activity and have been reported to possess biological properties such as anti-carcinogenic, anti-mutagenic, anti-inflammatory and antimicrobial properties (Jayaprakasha 2003; Yilmaz and Toledo, 2004).

Resveratrol, which is found in grape skins, has been proven to possess many functions in modulating physiological and pathological reactions of the body, such as anti-cancer, anti-mutagenesis, and cardioprotection (Yilmaz and Toledo, 2004).

In this regard, grape pomace has become an ideal candidate as a cost-effective product with natural and high value-added polyphenolic phytochemicals (Guendez *et al*., 2005).
Increasing knowledge about the health promoting impact of antioxidants in everyday foods, together with the assumption that a number of common synthetic preservatives may have hazardous effects, led to considerate grape pomace as an economical source of demanded compounds. Grape pomace would be beneficial for the use as a source of natural food additives, ingredients, and supplements (Soong and Barlow, 2004).

Based on these statements, different approaches were exposed and investigated in this thesis, in order to assess the potential applications of natural extracts obtained from grape seed and grape pomace. Also, the characteristics and differences among the extracts from two grape varieties were studied and compared to define their ability as a source of bioactive compounds.

Many studies have addressed the application of natural extracts as potential natural antioxidants to improve the oxidative stability of edible oils subjected to various thermal treatments. It is known that deep frying is widely used for the preparation of many types of foods. Frying in vegetable oils involves the maintaining of oil at a high level of temperature, in the range 170-220°C (Silva *et al*., 2010). The high temperatures reached during frying lead to a complex series of reactions that result in severe changes including thermo-oxidation, cis/trans-isomerization, cyclization, polymerization and hydrolysis due to the high temperature of the process (Gertz *et al*., 2000). Lipid oxidation is the main deterioration process that occurs during edible oils heating containing lipid molecules with polyunsaturation (Gertz *et al*., 2000; El Anany, 2007).

The thermal treatment of oil induces compositional changes by decomposition of polyunsaturated, monounsaturated and saturated fatty acids. The lipids degradation process in edible oils has generally been established as being a free radical mechanism that has as result the occurring of primary oxidation products called hydroperoxides. They are odorless and colorless, but are labile species that can undergo both enzymatic and non-enzymatic degradation to produce a complex array of secondary oxidation products (aliphatic aldehydes, ketones, lactones, alcohols, hydrocarbons, acids and epoxides) which are more stable during heating process. The instability of peroxides may explain the decrease in peroxide values (PV) during advanced stages of rancidity, so that, the breakdown into smaller molecules compounds associated with oxidation of lipids would be expected to occur. The secondary products have the potential to affect flavour, aroma, taste, nutritional value and overall quality of foods. Additionally, certain oxidation

products are potentially toxic at relatively low concentrations (Silva *et al*., 2010). Therefore, oxidation of oil use as cooking medium is very important in terms of palatability, toxicity as well as nutritional quality of the fried products.

The oxidative stability of oils is an important indicator of performance and shelf-life and also for ensuring that oils show a good resistance during exposing at high temperature (Choe and Min, 2006). The chemical changes occurring in oils in response to frying have been extensively reported by Silva *et al*. (2010).

In addition to convective ovens, where heating occurs by forcing hot air to flow around the food, microwave ovens are used in recent times more often for heating, reheating or cooking but the effect of microwave heating on the edible oil can significantly differ from those produced by convective heating. Exposure to microwave determines the increase of free fatty acids level, possible isomerization of the double bonds of fatty acids and oxidation of polyunsaturated fatty acids. As a result, free radicals can be formed in high amounts (Dostalova *et al.,* 2005). Although, there are many data regarding the consequences of microwave heating on the composition and nutritional quality of food, little has been published about the changes occurring in oxidative stability of edible oils during microwave exposure in response to supplementation by natural extracts. On this topic, there were controversies regarding the free radical formation when oils and fatty food are subjected to microwave treatment (Dostalova *et al*., 2005; Erkan *et al*., 2009; Megahed *et al.,* 2011).

The synthetic antioxidants, i.e. butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are very cost-effective given a high stability. The addition of synthetic antioxidants for improving oxidative stability of edible oils is discouraged due to their suspected action as promoters of carcinogenesis, as well for the general consumer rejection of synthetic food additives (Nyam *et al*., 2013).

Nowadays, there is a proeminent interest in finding of phytochemicals as an alternative to synthetic additives, commonly used in the food, pharmaceutical and cosmetic industry. Thus, due to toxicological issues regarding the synthetic antioxidants, in the last years it has been seen an increasing concern in identifying of potential natural sources such as agro-food by-products, spices and other plant materials in order to obtain natural antioxidants used to minimize or delay the lipid oxidation in fat-containing food products.

Many studies have dealt with evaluation of different crude extracts as natural antioxidants in comparation with synthetic antioxidant (BHT) on the stability and quality of edible oils during thermal treatments requiring elevated temperature (Yanishlieva and Marinova, 2001; Kalantzakis and Blekas, 2006; Zhang *et al*., 2010).

Some components isolated from have been proven in model systems, being effective as natural antioxidants (El Anany, 2007; Rehab, 2010) As such, nowadays there is a great interest in the use of natural antioxidants derived from plant extracts and is expected to rise enormously in the foreseeable future. Plant extract offers a unique range of applications for health. Secondary metabolites such as phenolic compounds from plant sources are highly valuable for their therapeutic attributes as antioxidants (Nyam *et al*., 2013).

Grape seed extract (GSE) contains large amounts of phenolic compounds and antioxidants (Rababah *et al*., 2008). GSE is rich in proanthocyanidins and the mechanism of its antioxidant

action consists in its potential of radical scavenging, metal chelation, and synergism with other bioactive compounds. Also, GSE exhibited high antioxidant activity, and may be used for food preservation and health supplements (Jayaprakasha *et al.*, 2001). Antioxidant activity of GSE has been confirmed by β-carotene linoleate and linoleic acid peroxidation methods (Lafka *et al.*, 2007) as well as by DPPH and phosphomolybdenum complex methods (Yilmaz and Toledo, 2006).

Many attemps have been made to clarify the inhibitory potential of GSE on lipid opidation developed in food systems. Most of the studies regarding the GSE effect on lipid oxidation were conducted on meat (Mielnik et al., 2006; Brannan and Mah, 2007). Rababah *et al.*, (2011) proved that GSE is an effective antioxidant to minimize lipid oxidation in corn chips during storage. Shaker *et al.* (2006) reported that GSE (200 ppm) exhibited reasonable antioxidant activity during the first day of sunflower oil heating at 60°C but showed pro-oxidative effect with prolonged treatment.

Currently, the literature information on the effect of GSE on lipid oxidation of sunflower oil during food applications which require heating to frying temperature seems to be limited. This is the reason that drove me towards the study presented in ***selected paper 7***.

Considering the concern for the lipid oxidation and its implications on food quality and human health, the objective of the study performed by Poiana (2012) and presented in ***selected paper 7****, was to assess the inhibitory potential of freeze-dried grape seeds extract (GSE) against lipid oxidation development in refined sunflower oil subjected to thermal treatments at elevated temperatures. In this study, the oxidative stability of sunflower oils supplemented with GSE to various doses was comparative investigated with the synthetic antioxidant (BHT) in order to highlight the applicability of GSE as potential natural antioxidant in edible oils subjected to some thermal applications specific to the food industry.* This study was designed and performed by me as single author.

Another valuable by-product that has retained our attention is represented by the fruit kernels resulted from fruit processing. Seeds of apricot, peach and plum, belonging to the *Rosaceae* family, are produced as by-products in large quantities from fruit canning industry. Huge amounts of kernels of peach, apricot and plum result every year as a result of processing of jams, jellies, and other sweet preserves of fruit, but their oils have not yet been fully studied. These kernels are considered as potential non-traditional resources that can be exploited for oil extraction (Hassanein, 1999) because these kinds of oil are considered a valuable source of unsaturated fats due to their high content of oleic and linoleic acid.

Nevertheless, plum and apricot seeds are used worldwide only to small-scale for vegetable oils industry due to the difficulty to break the shell covering the kernel. In addition to the lipid fraction, these oils contain different bioactive compounds such as β-carotene (provitamin A) and tocopherols. Tocopherols together with phytosterols and squalene are components present in the unsaponifiable lipid fraction of fruit kernels oil. Tocopherols are fat soluble antioxidants that protect the lipids and other membrane components because they act in quenching singlet oxygen. Also, tocopherols have demonstrated the ability to inhibit lipid peroxidation, protecting

the stability of oils and fats. Also, the tocopherols may protect against atherogenesis by blocking oxidation of low-density lipoprotein cholesterol and by favorably influencing plaque stability, vasomotor function, and tendency for thrombosis (Min and Boff, 2002).

Antioxidants from seeds and fruit kernels oil are able to neutralize free radicals and have a potential role in preventing the onset of some chronic diseases such as cardiovascular diseases, some neurological disorders or certain inflammatory processes. These natural antioxidants are important lipid oxidation inhibitors in food and biological systems and are found in oil seeds in four vitamin E congeners called α–tocopherol (α–T), β–tocopherol (β–T), γ–tocopherol (γ–T), and δ–tocopherol (δ–T) (Medina-Juarez *et al*.. 2000; Bele *et al*., 2013). α-T has three methyl groups, β and γ forms have two methyl groups and the δ has one methyl group. The most active form of vitamin E is α–T which it seems to protect the body against degenerative disorders such as cancer and cardiovascular diseases. γ–T has been reported to be more potent than α–T in decreasing the platelet aggregation and delaying LDL oxidation diseases (Hak *et al*., 2009).

20 years ago, apricot kernels oil has been studied for their fatty acids whereas peach kernels oil had been investigated for both their sterols and fatty acids composition (Saadany *et al*., 1993). Also, the study conducted by Hassanein (1999) reported some data about plum, peach and apricot kernel oils in terms of fatty acid composition, sterols and tocopherol pattern. In the last years, the study performed by Turan *et al*. (2007) has investigated the fatty acid, triacylglycerol, phytosterol, and tocopherol variations in different varieties of apricot kernel oil. Also, the research conducted by Ozcan *et al.* (2010) offers some information concerning the properties of apricot kernel oils. According to the results of these studies, kernels oils contained appreciable amounts of oleic and linoleic acids, but linolenic acid was found in negligible amounts. These studies prove the concern for a more detailed characterization of these oils.

However, there are limited information concerning the antioxidant properties and the existing bioactives compounds besides the lipidic fraction of these oils. Moreover, there is a lack of information related to the plum kernel oil composition. Phenolic compounds from crude fruit kernels oil have an important role on the oxidative stability of the polyunsaturated fatty acids of these oils. This protective role is due to their antioxidant properties (Arranz *et al*., 2008).

Peach and apricot kernel oils have been used as adulterants for some expensive oils such as almond oil (Hassanein, 1999). Kernel oils could be utilized into various food products and cosmetics offering health benefits due to their nutritional qualities as well as their composition (Amaral *et al*., 2008). Oil composition depends on the fruit variety, origin place, harvest year and agro-technical measures (Zhang *et al*., 2009).

In light of the aforementioned, *the main objectives of the study* performed by Popa *et al*. (2011) *and presented in* ***selected paper 8****, was to describe a potential way to capitalise the fruit kernels resulted as by-products in fruit canning industry and also to bring more information about antioxidant properties, β-carotene, total phenolics content and tocopherol pattern of apricot and plum kernel oils.*

The obtained data are very useful to characterize these kernel oils and to facilitate their differentiation from the other vegetable oils. In this study, I was involved as co-author.

By centralizing of the foregoing, the targets of this research direction are:

- Assessing the possibility to exploit some by-products from wine industry in order to obtain natural extract rich in polyphenolic compounds and their antioxidant properties evaluation;
- Improving the oxidative stability of sunflower oil used in food thermal applications by supplementation with grape seed extract;
- Assesment the possibility to exploit the plum and apricot kernels obtained as by-products in fruit processing industry in order to obtain crude oil;
- Investigation concerning the antioxidant properties and some bioactive compounds in raw oil extracted from plum and apricot kernels.

3.2. Obtaining and antioxidant properties investigation of some natural extracts from wine industry by-products

The increasing knowledge about the health promoting impact of antioxidants in foods, together with the assumption that a number of common synthetic preservatives may have dramatic effects, led to considerate the grape pomace a valuable source of bioactive ompounds. In a first part, grape seed and pomace extracts were obtained and their antioxidant properties were investigated in order to compare and define their ability as a source of bioactive compounds.

In the study concerning the phenolic antioxidants from various plant materials, solvent extraction has mostly been used to obtain the phenolic extracts due to both, its simplicity and low cost. Organic solvents commonly used for extraction include absolute methanol, ethanol, ethanol and acetone, ethyl acetate. The mixtures of these organic solvents with water were also widely used (Tananuwong and Tewaruth, 2010).

The extractability of phenolic compounds and their antioxidant activities in the crude extract depends on many factors such as polarity and pH of solvents, extraction time and temperature, as well as the chemical structure of phenolic compounds (Perez-Jimenez and Saura-Calixto, 2006). In the view of bioactive compounds extraction from grape seeds were taken into account previous results on this topic reported by Yilmaz and Toledo (2006), Lafka *et al.* (2007) and Spigno and Faveri (2007).

Processing of GSE and GPE

Pressed pomace obtained from Cabernet and Merlot (*Vitis vinifera* L.) grape varieties (western part of Romania, Recas winery - vintage 2010). Grape pomace resulted to Cabernet

Sauvignon wine processing was divided into two parts. The first part was used as whole pomace in order to obtain grape pomace extract (GPE). From the second part, grape seeds were manually removed from the skin and pulp and then they were used for obtaining of grape seed extract (GSE). From pomace resulted to Merlot wine processing it was used only grape seeds in order to obtain GSE. Both, grape pomace and separated seeds were subjected to drying, grinding, defatting and than extraction with ethanol 70% (v/v) under shaking, filtration and centrifugation, according to protocols specified in selected papers 7 and 9. The supernatants were concentrated using a rotary evaporator and then, they were freeze-dried. The freeze-dried crude extracts (GPE, respectively GSE) were kept at −18°C until the analyses were performed.

Evaluation of antioxidant properties of freeze-dried extracts

The antioxidant characteristics of freeze-dried crude extract GPE and GSE were reported in the *Table 3.1*. Grape skins and seeds are valuable sources of health-promoting polyphenolic compounds. They contain flavonoids such as catechin, epicatechin, procyanidins and anthocyanins. They also contain phenolic acids that include gallic acid, cydroxicinnamic acid and ellagic acid and stilbenes such as resveratrol (Shrikhande, 2000; Torres and Bobet, 2001; Yilmaz and Toledo, 2006). In grape seeds, the two most abundant phenolic compounds were catechin and epicatechin. Ellagic acid, hydroxycinnamic acid, flavanols, flavonol glycosides, anthocyanins, resveratrol, myricetin, quercetin, and kaempferol were found in skins and gallic acid was found as one of the phenolic compounds present in grape seeds (Pastrana-Bonilla *et al*., 2003).

Table 3.1. Antioxidant characteristics of GSE and GPE

Sample	FRAP value (μmol $Fe^{2+}\cdot g^{-1}$)	Total phenolics (μmol $GAE\cdot g^{-1}$)
GSE (Merlot)	1231.56 ± 17.29	1019.83 ± 15.68
GSE (Cabernet Sauvignon)	1042.38±38.69	795.83±32.18
GPE (Cabernet Sauvignon)	804.17±29.54	561.28±26.41

Our data reveal that TP content of GSE from Merlot grape variety was higher than in GSE from Cabernet Sauvignon grape variety. Many studies focused on antioxidant activity and phenolic antioxidants of grape seeds have reported variable TP content of GSE, ranged from 580–3930 μmol $GAE\cdot g^{-1}$, possibly due to differences in grape varieties and/or in extraction methods and conditions (Kelen and Tepe, 2007; Lafka *et al*., 2007; Yemis *et al*., 2008; Al-Habib *et al*., 2010). Among two dried-freeze extracts (GSE and GPE) obtained from Cabernet Sauvignon pomace, the content of TP recorded for GSE was higher than in GPE. These results were consistent with data previously reported by Negro *et al.* (2003). According to Pastrana-Bonilla *et al.* (2003), TP content in grape parts were, on average, 5 times more concentrated in seeds than in skin and 80 times more than in the pulp. In regard to the FRAP values of these extracts, it was noticed the following order: GSE (Merlot)>GSE (Cabernet Sauvignon)>GPE (Cabernet Sauvignon).

GSE obtained from Merlot grape variety was used in the study detailed in ***selected paper 7*** and other two natural extracts, GSE and GPE obtained from Cabernet Sauvignon grape variety were used in the study presented in ***selected paper 9***.

3.3. Assessment of inhibitory effect of grape seeds extract on lipid oxidation occurring in sunflower oil during some thermal applications

3.3.1. Aim

The research presented in ***selected paper 7*** deals with an efficient application of freeze-dried crude grape seed extract (GSE) derived from Merlot grapes variety, as potential additive for improving the oxidative stability of sunflower oil used in some food thermal applications. Thus, this paper work was performed in order to exploit the potential of GSE, as natural antioxidant, compared to synthetic antioxidant butylated hydroxytoluene (BHT) to inhibit the lipid oxidation developed in sunflower oil subjected to convective and microwave heating up to 240 min under simulated frying conditions. For this purpose, the lipid oxidation that occurs in response to heating was analyzed as a function of time, antioxidants (BHT, GSE) as well as antioxidant levels. The progress of lipid oxidation was monitored by chemical indices: peroxide value (PV), p-anisidine value (p-AV), conjugated dienes and trienes (CDs, CTs), inhibition of oil oxidation (IO) and TOTOX value. The peroxide value (PV) was determined iodometrically according to standard methods for the oils analysis (AOCS, 1998). The p-anisidine value (p-AV) was estimated by the standard method according to AOCS (1998). CDs and CTs were measured according to the method reported by Kim and Labella (1987). IO and TOTOX value were calculated according the formulas specified in ***selected paper 7***. In addition, total phenolic content (TP) was evaluated in oil samples before and after heating using the method described by Singleton *et al*. (1999) for assessing the changes of these compounds relative to the extent of lipid oxidation Also, for samples supplemented by GSE at the 1000 ppm level, TP in the heating time was monitored, relative to the progress of the oxidative lipid degradation.

3.3.2. Results and Discussion

The present study was carried out in refined sunflower oil, free of additives, supplemented by five concentrations of freeze-dried crude extract GSE (*i.e.*, 200, 400, 600, 800 and 1000 ppm) and one level of BHT (200 ppm). Oil samples were subjected to convective and microwave heating for 10, 20, 30, 60, 120 and 240 min under simulated frying conditions, at comparable temperatures. Convective heating was carried out in an electrical oven (Esmach, Italy, 1200W, 50Hz) regulated at 200°C. Microwave heating was conducted in a microwave oven for home appliances (Candy, Model CMG 2394DS, 50 Hz, microwave frequency 2450 MHz, maximum

power 900 W). The samples were heated at the input of 80% power (720 W). Both heating treatments were performed at comparable temperatures: after 30 min from the starting treatment, the oil temperature remained at 185±7°C during the whole monitored period. The doses of GSE were chosen in agreement with previous studies that have proved that the inhibitory effect on lipid oxidation increased with the antioxidants concentration (Mielnik *et al*., 2006; Brannan and Mah, 2007; Rababah *et al*., 2011).

In order to highlight the significance of changes occurring in the monitored indices of oil samples in the heating time in response to supplementation by BHT and GSE, the obtained results were processed by one-way ANOVA test.

The reducing power of BHT and GSE is a reliable indicator of their antioxidant activity, indicating that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process (Jayaprakasha *et al*., 2001; Jayaprakasha *et al*., 2003). In this study, FRAP value recorded for BHT was 1339.14 μmol $Fe^{2+}\cdot g^{-1}$. As it can be noticed from the *Table 3.1*, GSE presented lower antioxidant activity than BHT. These results are consistent to those reported by Bonilla *et al.*, (1999). The profile of phenolic compounds is likely to be more important than the antioxidant activity (Shaker, 2006; Kelen and Tepe, 2007). Phenolic compounds are known to act as antioxidants not only due to their ability to donate hydrogen or electron, but also attributed to their stable radical intermediates, which prevent the oxidation of various food ingredients particularly fatty acids and oil (Zhang *et al*., 2010). The antioxidant activity may vary widely depending on the lipid substrate. Hydrophilic antioxidants are more effective in lipid systems, whereas lipophilic antioxidants work better in emulsions where more water is present. In lipophilic environment, hydrophilic antioxidants are oriented to oil-air interface, providing better protection against lipid oxidation than in hydrophilic environment where hydrophilic antioxidants prefer to dilute and thus act poorly against lipid oxidation (Frankel and Meyer, 2000). Contrary, lipophilic antioxidants are diluted in lipid environment and are not suitably oriented to the oil-air interface to inhibit the oxidation (Frankel *et al*., 1994).

Impact of supplementation with GSE and BHT on oil quality in the heating time

Changes in PV and IO in response to oil heating

PV and IO were used as indicators for the primary oxidation of sunflower oil. Determination of peroxides can be used as oxidation index for the early stages of lipid oxidation (Rebab *et al*., 2010; Zhang *et al*., 2010). Measuring the content of primary oxidation products is limited due to the transitory nature of peroxides, but their presence may indicate a potential for later formation of sensorial objectionable compounds. PV increases only when the rate of peroxides formation exceeds that of its destruction.

Data from *Table 3.2* express the changes of PV in the heating time in response to oil supplementation with BHT and GSE. It can be noted that thermal treatments promoted oxidation in sunflower oil leading to a significant increase in PV but this effect was markedly reduced by supplementation with GSE and BHT. At any time of convective and microwave heating, significant differences ($p<0.05$) in PV were observed between the control sample and oil samples with BHT (200 ppm) or supplemented by various doses of GSE.

The inhibitory effect of GSE against primary oxidation of lipid was dose-dependent. At the end of convective heating, PV of samples with BHT decreased by approx. 32% relative to the control, while in samples with GSE, PV decreased in the range 19–48%. These results are consistent with data reported by Brannan and Mah (2007), Rababah *et al.* (2011), Shaker *et al*. (2006) and show that the antioxidant compounds of GSE have a great role in inhibiting of free radical formations during the initiation step of oxidation, interruption of the propagation of the free radical chain reactions by acting as an electron donor, or scavengers of free radicals.

Table 3.2. Effect of GSE and BHT on peroxide value (PV) during sunflower oil heating

Time (min)	PV (meq/kg oil)						
	Control	BHT 200 ppm	GSE (ppm)				
			200	400	600	800	1000
convective heating							
0	1.77 ± 0.09 ^{a}	1.77 ± 0.09 ^{a}	1.77 ± 0.09 ^{a}	1.77 ± 0.09 ^{a}	1.77 ± 0.09 ^{a}	1.77 ± 0.09 ^{a}	1.77 ± 0.09 ^{a}
10	4.14 ± 0.12 ^{a}	3.09 ± 0.10 ^{c}	3.78 ± 0.11 ^{b}	3.45 ± 0.18 ^{b}	3.13 ± 0.14 ^{c}	2.72 ± 0.13 ^{e}	2.34 ± 0.12 ^{f}
20	4.60 ± 0.16 ^{a}	3.47 ± 0.18 ^{c}	4.23 ± 0.18 ^{a}	3.89 ± 0.17 ^{b}	3.53 ± 0.12 ^{c}	3.18 ± 0.11 ^{d}	2.46 ± 0.08 ^{e}
30	5.37 ± 0.16 ^{a}	4.28 ± 0.13 ^{c}	5.08 ± 0.21 ^{a}	4.61 ± 0.23 ^{b}	4.37 ± 0.24 ^{c}	3.47 ± 0.17 ^{d}	2.69 ± 0.18 ^{e}
60	8.89 ± 0.35 ^{a}	6.30 ± 0.24 ^{c}	8.09 ± 0.19 ^{b}	7.34 ± 0.27 ^{b}	6.13 ± 0.23 ^{c}	5.38 ± 0.26 ^{d}	4.47 ± 0.33 ^{e}
120	10.01 ± 0.41 ^{a}	7.48 ± 0.26 ^{c}	9.12 ± 0.25 ^{b}	8.25 ± 0.23 ^{b}	7.31 ± 0.33 ^{d}	6.24 ± 0.24 ^{e}	5.19 ± 0.32 ^{f}
240	12.05 ± 0.76 ^{a}	8.24 ± 0.31 ^{c}	9.81 ± 0.50 ^{b}	9.43 ± 0.59 ^{b}	8.29 ± 0.23 ^{c}	7.31 ± 0.27 ^{d}	6.22 ± 0.32 ^{e}
nicrowave heating							
0	1.77 ± 0.09 ^{a}	1.77 ± 0.09 ^{a}	1.77 ± 0.09 ^{a}	1.77 ± 0.09 ^{a}	1.77 ± 0.09 ^{a}	1.77 ± 0.09 ^{a}	1.77 ± 0.09 ^{a}
10	9.69 ± 0.45 ^{a}	6.27 ± 0.37 ^{d}	9.00 ± 0.43 ^{a}	8.13 ± 0.46 ^{b}	7.12 ± 0.55 ^{c}	6.27 ± 0.35 ^{d}	4.79 ± 0.31 ^{e}
20	14.73 ± 0.40 ^{a}	10.08 ± 0.47 ^{d}	13.5 ± 0.39 ^{b}	12.3 ± 0.35 ^{c}	10.96 ± 0.37 ^{d}	9.61 ± 0.20 ^{e}	7.09 ± 0.34 ^{f}
30	19.14 ± 0.61 ^{a}	14.71 ± 0.42 ^{d}	17.59 ± 0.61 ^{b}	16.1 ± 0.34 ^{c}	14.57 ± 0.36 ^{d}	12.36 ± 0.45 ^{e}	9.88 ± 0.39 ^{f}
60	15.02 ± 0.55 ^{a}	7.59 ± 0.46 ^{d}	12.52 ± 0.41 ^{b}	10.04 ± 0.69 ^{c}	9.38 ± 0.43 ^{c}	7.88 ± 0.53 ^{d}	5.79 ± 0.45 ^{e}
120	18.81 ± 0.37 ^{a}	14.02 ± 0.38 ^{d}	17.07 ± 0.49 ^{b}	15.7 ± 0.51 ^{c}	15.09 ± 0.50 ^{c}	12.83 ± 0.55 ^{e}	12.24 ± 0.34 ^{f}
240	16.21 ± 0.38 ^{a}	12.13 ± 0.34 ^{c}	15.56 ± 0.40 ^{a}	14.17 ± 0.58 ^{b}	13.15 ± 0.33 ^{b}	11.97 ± 0.42 ^{c}	11.28 ± 0.17 ^{d}

Means in a row (a-f across GSE level) followed by the same letter are not significantly different ($p < 0.05$).

PV showed significant changes ($p<0.05$) during microwave heating up to 240 min, but the values did not steadily increase in the heating time, *Table 3.2.* Che Man *et al*. (1999) reported a decrease in PV of oil samples after an initial increase. A significant decrease of PV after an initial increase confirms that peroxides formed in the early stages of oxidation are unstable and highly susceptible to further changes that result in the formation of secondary products of oxidation (Farhoosh and Moosavi, 2009). PV still tends to increase during the early stages of oxidation, when the rate of hydroperoxides formation is higher than the rate of their decomposition. However, a low PV represents either an incipient oxidative process or an advanced oxidation. At the end of heating in microwave oven, PV for samples with BHT decreased by approx. 25% relative to the control and in the range 4–30% relative to the control, for samples with GSE.

Figure 3.1 provides information about the inhibitory power of GSE and BHT on primary lipid oxidation in the heating time. Based on statistical test, it could be concluded that at any time of heating treatments the increasing of GSE dose resulted in significant increases of IO ($p<0.05$).

These data show that GSE to a level of 200–400 ppm had an inhibition power lower than BHT during both treatments. During convective heating GSE to 600 ppm had an inhibitory effect of primary lipid oxidation comparable to BHT, while in the microwave heating, the effect of BHT was similar to that of GSE to 800 ppm. GSE to 1000 ppm showed an inhibition power

higher than BHT in both treatments. GSE did not show pro-oxidative effect during treatments up to 240 min. According to Shaker *et al.* (2006) the pro-oxidative effect had been proven by increasing the amount of oxidized products with prolonged heating, when additives were added.

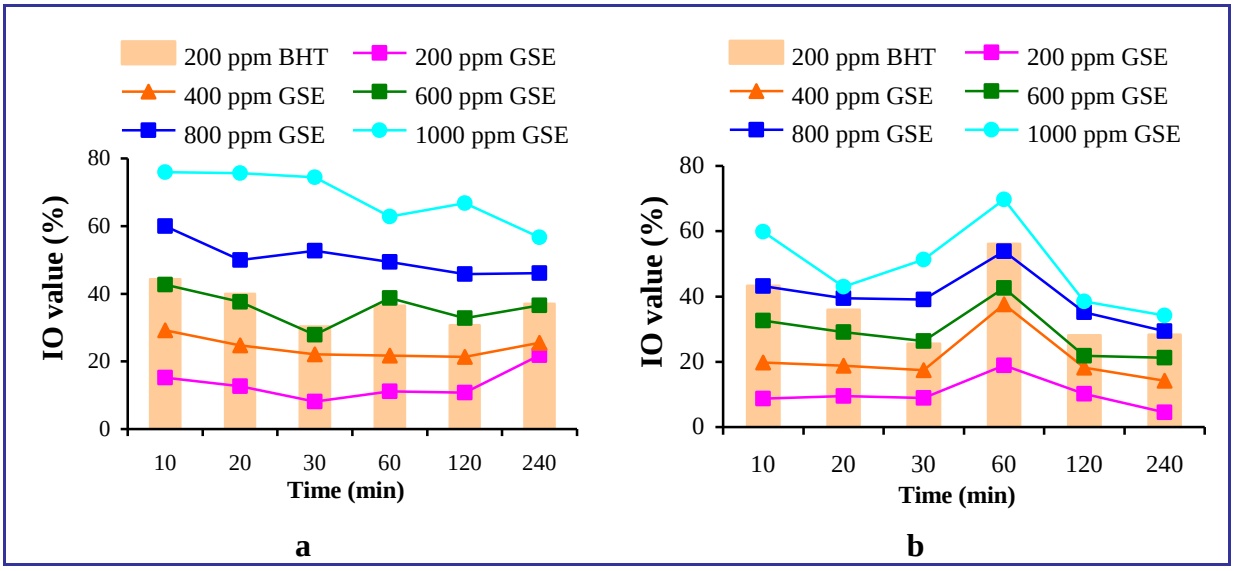


Figure 3.1. Inhibitory effect of GSE and BHT on primary lipid oxidation during oil heating (**a:** convective heating; **b:** microwave heating)

Changes in p-AV in response to oil heating

During lipid oxidation, hydroperoxides, the primary reaction products, decompose to produce secondary oxidation products which are more stable during the heating process, responsible for off-flavors and off-odors of edible oils. In order to ensure a better monitoring of lipid oxidation in the heating time, the simultaneous detection of primary and secondary lipid oxidation products is necessary. p-AV is a reliable indicator for amount of secondary oxidation products (De Abreu *et al*., 2010; Zhang *et al*., 2010).

Table 3.3 shows the changes recorded in p-AV in the heating time in response to supplementation with GSE and BHT. It can be observed that both treatments promoted fast transformation towards secondary products which contributes to the off-flavors of oil. The addition of BHT and GSE resulted in significant decrease in p-AV ($p<0.05$) relative to the control.

The highest level of GSE provides the best protection against secondary oxidation of oil samples subjected to heating. These data are in agreement with those reported by Kalantzakis and Blekas (2006) which highlight that the natural extracts showed a significant inhibitory effect against thermal oxidation of refined oils heated at 180°C.

At the end of convective heating, p-AV of samples with BHT decreased by approx. 16% relative to the control, while addition of GSE resulted in decrease of p-AV in the range 10–29% relative to the control. Also, data presented in *Table 3.3* revealed that after 240 min of microwave heating, p-AV of samples mixed with BHT decreased by approx. 26% relative to the control and in the range 10–40% relative to the control for oil samples with various doses of GSE.

The results of statistical test pointed out that the extent of secondary lipid oxidation was significantly decreased by increasing of GSE dose, for both treatments ($p<0.05$). At the end of

heating, there were no significant differences ($p>0.05$) between oil samples with BHT or GSE to 600 ppm. GSE to a level of 600 ppm provided protection against secondary lipid oxidation in a similar manner to BHT, GSE to 800 ppm showed an inhibitory effect higher than BHT, while GSE to a level of 200–400 ppm displayed a lower inhibitory potential than BHT.

Table 3.3. Effect of GSE and BHT on p-AV during sunflower oil heating

Time (min)	p-AV						
	Control	BHT 200 ppm	GSE (ppm)				
			200	400	600	800	1000
convective heating							
0	2.28 ± 0.16 ^{a}	2.28 ± 0.16 ^{a}	2.28 ± 0.16 ^{a}	2.28 ± 0.16 ^{a}	2.28 ± 0.16 ^{a}	2.28 ± 0.16 ^{a}	2.28 ± 0.16 ^{a}
10	5.27 ± 0.43 ^{a}	3.68 ± 0.31 ^{c}	4.95 ± 0.38 ^{a}	4.56 ± 0.31 ^{a}	4.08 ± 0.36 ^{b}	3.77 ± 0.32 ^{c}	2.81 ± 0.26 ^{d}
20	9.91 ± 0.54^{a}	7.47 ± 0.52 ^{c}	9.48 ± 0.61 ^{a}	8.34 ± 0.69 ^{b}	7.85 ± 0.51 ^{c}	7.39 ± 0.56 ^{c}	5.24 ± 0.41 ^{d}
30	13.3 ± 0.45^{a}	11.23 ± 0.71 ^{b}	12.79 ± 0.71 ^{a}	12.27 ± 0.79 ^{a}	11.51 ± 0.65 ^{a}	10.24 ± 0.70 ^{c}	8.03 ± 0.50 ^{d}
60	24.95 ± 1.07 ^{a}	20.81 ± 1.01 ^{b}	23.01 ± 1.04 ^{a}	21.7 ± 1.29 ^{b}	19.79 ± 1.14 ^{c}	17.79 ± 1.08 ^{d}	15.89 ± 1.06 ^{e}
120	36.24 ± 1.22 ^{a}	31.82 ± 1.36 ^{b}	34.01 ± 1.21 ^{a}	33.28 ± 1.13 ^{a}	31.39 ± 1.33 ^{b}	28.48 ± 1.64 ^{c}	26.04 ± 1.66 ^{d}
240	50.03 ± 2.01 ^{a}	42.16 ± 1.67 ^{c}	45.25 ± 1.86 ^{b}	43.9 ± 2.14 ^{c}	41.73 ± 1.81 ^{c}	39.18 ± 1.62 ^{d}	35.75 ± 1.44 ^{f}
nicrowave heating							
0	2.28 ± 0.16 ^{a}	2.28 ± 0.16 ^{a}	2.28 ± 0.16 ^{a}	2.28 ± 0.16 ^{a}	2.28 ± 0.16 ^{a}	2.28 ± 0.16 ^{a}	2.28 ± 0.16 ^{a}
10	6.55 ± 0.55 ^{a}	4.82 ± 0.39 ^{b}	5.62 ± 0.41 ^{a}	5.27 ± 0.45 ^{b}	5.03 ± 0.39 ^{b}	4.70 ± 0.41 ^{b}	4.36 ± 0.31 ^{c}
20	11.64 ± 0.89 ^{a}	8.78 ± 0.70 ^{b}	10.27 ± 0.82 ^{a}	9.46 ± 0.77 ^{b}	9.05 ± 0.74 ^{b}	8.69 ± 0.63 ^{b}	8.02 ± 0.57 ^{c}
30	19.10 ± 1.68 ^{a}	16.23 ± 1.28 ^{a}	17.66 ± 1.25 ^{a}	16.22 ± 0.88 ^{a}	16.46 ± 1.04 ^{a}	15.96 ± 1.20 ^{a}	14.10 ± 1.06 ^{b}
60	32.92 ± 2.21 ^{a}	29.67 ± 1.59 ^{a}	31.26 ± 1.28 ^{a}	30.86 ± 1.71 ^{a}	30.35 ± 2.10 ^{a}	29.21 ± 1.82 ^{a}	28.02 ± 1.24 ^{b}
120	47.93 ± 2.50 ^{a}	43.07 ± 2.03 ^{a}	46.35 ± 2.10 ^{a}	43. 5 ± 2.44 ^{a}	40.13 ± 2.33 ^{b}	38.52 ± 2.09 ^{b}	37.24 ± 1.84 ^{c}
240	68.80 ± 2.33 ^{a}	50.70 ± 2.89 ^{d}	62.15 ± 3.31 ^{b}	59.69 ± 3.68 ^{c}	50.8 ± 3.46 ^{d}	43.49 ± 2.20 ^{e}	41.36 ± 1.78 ^{f}

Means in a row (a–f across GSE level) followed by the same letter are not significantly different ($p < 0.05$).

Change in TOTOX value in response to oil heating

TOTOX value allows a mathematical prediction of oxidative stability based on values: PV and p-AV. Moreover, TOTOX value provides a comprehensive overview of the oxidation process in oil samples. It represents an indicator of overall oxidative stability being correlated with the extent of oil deterioration (De Abreu *et al.,* 2010).

TOTOX values for samples mixed with BHT and GSE were significantly lower than the value registered for control, *Figure 3.2*. At the end of convective heating, the addition of GSE to oil samples resulted in decrease of TOTOX value in the range 13–35% relative to the control while exposure to microwave resulted in decline of TOTOX value in the range 8–37%. At any stage of both treatments, the lowest TOTOX values were recorded by supplementation with GSE to 1000 ppm. This means that the highest level of GSE had the best inhibitory effect on oil oxidation. GSE to a level of 600 ppm inhibited the lipid oxidation in a similar manner to BHT.

Based on *Figure 3.2*, it can be seen that oxidative degradation was greater in the microwave heating than in the convective heating. Yhese data are in agreement with other results reported by Dostalova *et al.* (2005), Megahed (2011), Erkan *et al.* (2009) that revealed that, even a short period of microwave heating accelerates the formation of some undesirable and harmful compounds (e.g. oxidation products, transformed pigments) due to interactions between electromagnetic field with the chemical constituents of oil. These results prove that GSE could limit the lipid oxidation in sunflower oil subjected to heating. The effect was dose-dependent. Probably, the addition of natural extract created an oil system surrounded by antioxidants that

were able to prevent oxidation because phenolic compounds were located on the interface of the lipid system.

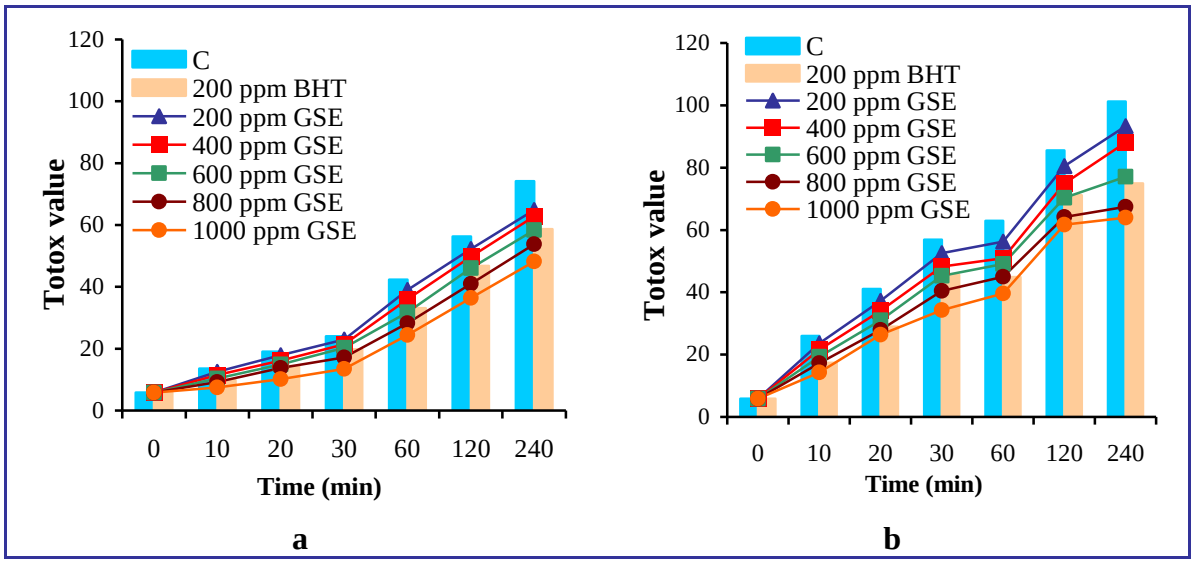


Figure 3.2. Impact of GSE and BHT on TOTOX value during sunflower oil heating (**a:** convective heating; **b:** microwave heating)

Changes in CDs and CTs in response to oil heating

The polyunsaturated fatty acids oxidation occurs with the formation of hydroperoxides. Further, the non-conjugated double bonds present in natural unsaturated lipids suffer a rearrangement generating conjugated dienes (CDs), which absorb at 232 nm (Gertz *et al.*, 2000). When polyunsaturated fatty acids containing three or more double bonds (e.g., linolenic acid) are subjected to oxidation, the conjugation can be extended to include another double bond resulting in the formation of conjugated trienes (CTs) which absorb at 268 nm. The measurement of CDs and CTs provides a better view on lipid oxidation because these compounds remain in the frying oil (Sulieman *et al.*, 2006). Thus, the changes in UV absorbance at 232 and 268 nm, quantified by K232 and K268 have been used as a relative measure of oxidation. The increase in K232 and K268 is dependent on the uptake of oxygen and formation of peroxides during the early stages of oxidation as well as with the degradation rate of linoleic acid (Che Man *et al.*, 1999; Sulieman *et al.*, 2006). The results presented in *Figures 3.3* and *3.4* highlight that both heating processes caused positional rearrangement of the double bonds in oil samples and, consequently, a part of the non-conjugated system was converted to conjugated diene and triene double bonds. Accordingly, the absorbance values at 232 and 268 nm were gradually increased in the heating time. It can be noted that the rate of CDs formation was higher than the decomposition rate, leading to their accumulation in oil, *Figure 3.3* The values recorded for K232 represent a measure of lipid alterations due to double bonds conjugation in response to primary oxidation.

The changes of K268, associated with CTs accumulated during heating are shown in *Figure 3.4*. These changes reflect the formation of oxidation by-products such as unsaturated α- and β-diketones and β-ketones, typical for oils in the process of going rancid (Che Man *et al.*, 1999). As in the previous case, CTs level increased during treatments. By oil supplementation

with BHT and GSE, the accumulation of CDs and CTs decreased. The inhibitory effect of GSE on CDs and CTs formation was dose-dependent. The oil samples with the highest dose of GSE had the lowest amounts of CDs and CTs at any stage of heating.

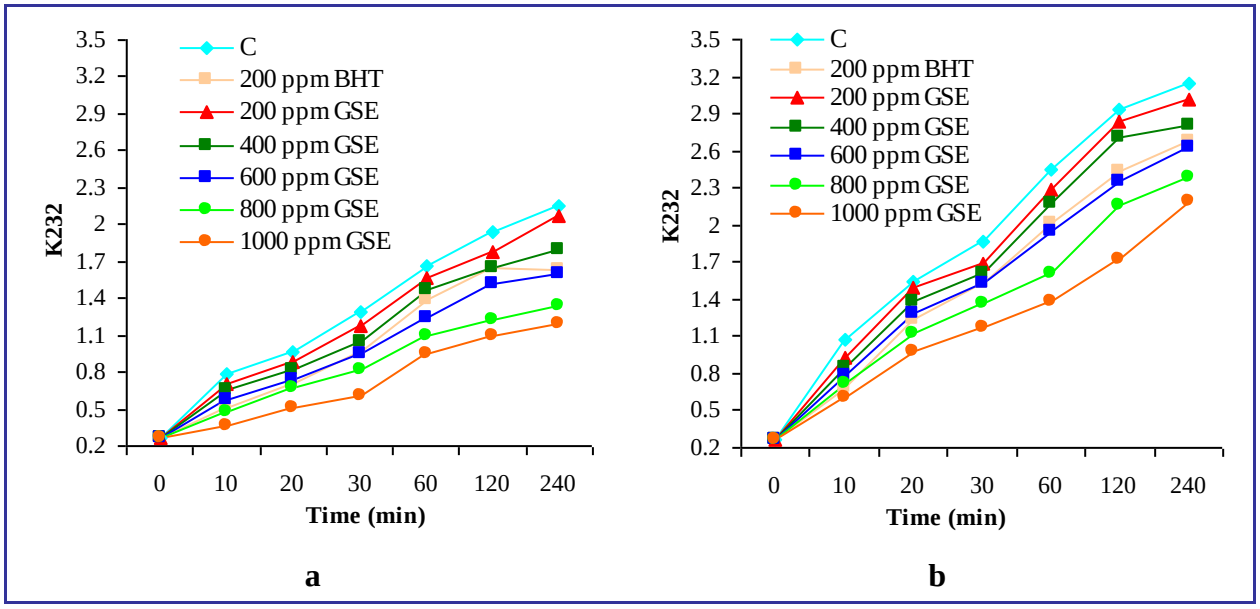


Figure 3.3. Effect of supplementation with GSE and BHT on K232 during oil heating (**a:** convective heating; **b:** microwave heating)

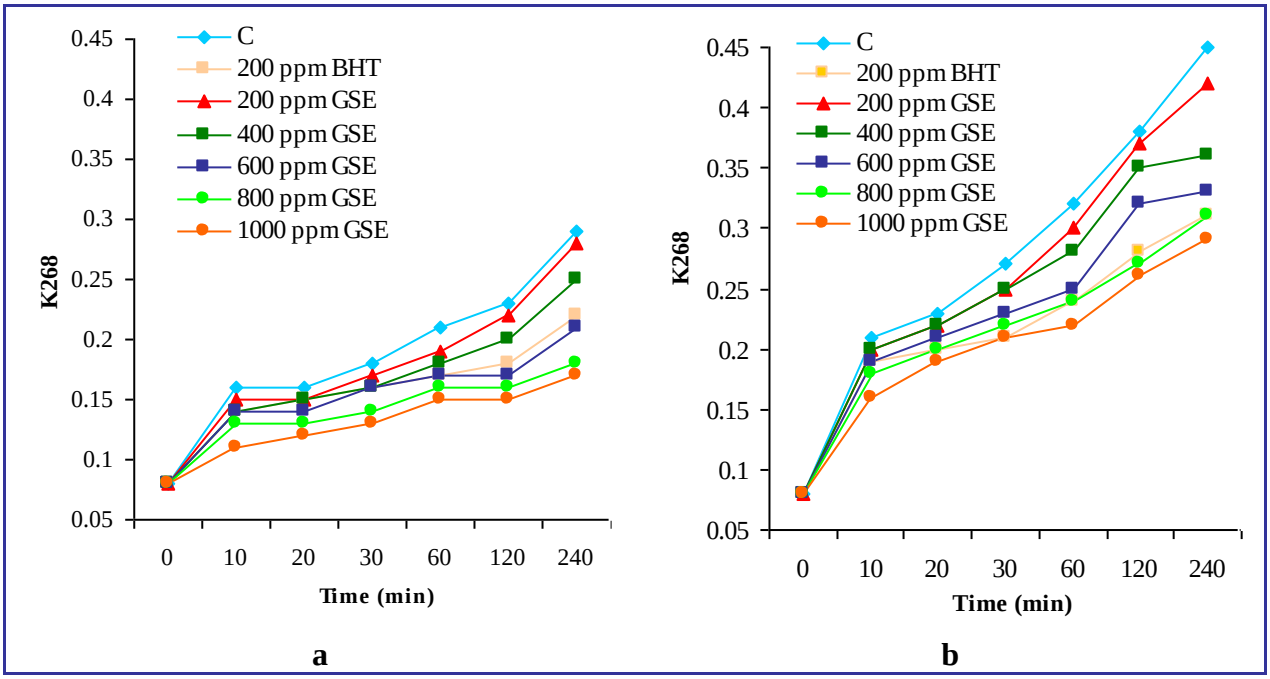


Figure 3.4. Effect of supplementation with GSE and BHT on K268 during oil heating (**a:** convective heating; **b:** microwave heating)

The inhibition of CDs and CTs by addition of GSE is important in the early stages of lipid oxidation to prevent the formation of reactive lipid radicals. The ability of GSE to reduced CDs and CTs accumulation was higher in the convective heating than in the microwave treatment. At

the end of treatment, GSE at level of 1000 ppm reduced the accumulation of CDs, respectively CTs by about 45%, respectively 41% relative to the control in the convective heating and by 30%, respectively 36% in the microwave treatment. In both treatments, GSE at 600 ppm showed the same potential to reduce the formation of CDs as BHT. Also, BHT inhibited the formation of CTs similar to GSE at 600 ppm during convective heating. Less efficient against CT formation in oil samples was GSE during microwave heating. Only a level of 800 ppm GSE provided a similar protection as BHT. These results are consistent with those reported by El Anany (2007) and Rehab (2010) which revealed that the addition of natural extracts to sunflower oil heated at 180°C induced a strong antioxidant activity and at a level of 800 ppm provided a better inhibitory effect on lipid degradation than BHT.

Correlations among indices of lipid oxidation and TP in oil samples during heating

Figure 3.5 offers information about TP content recorded in oil samples supplemented by GSE at the beginning and also, at the end of heating. From this chart, it can be noted the alterations of TP in response to heating.

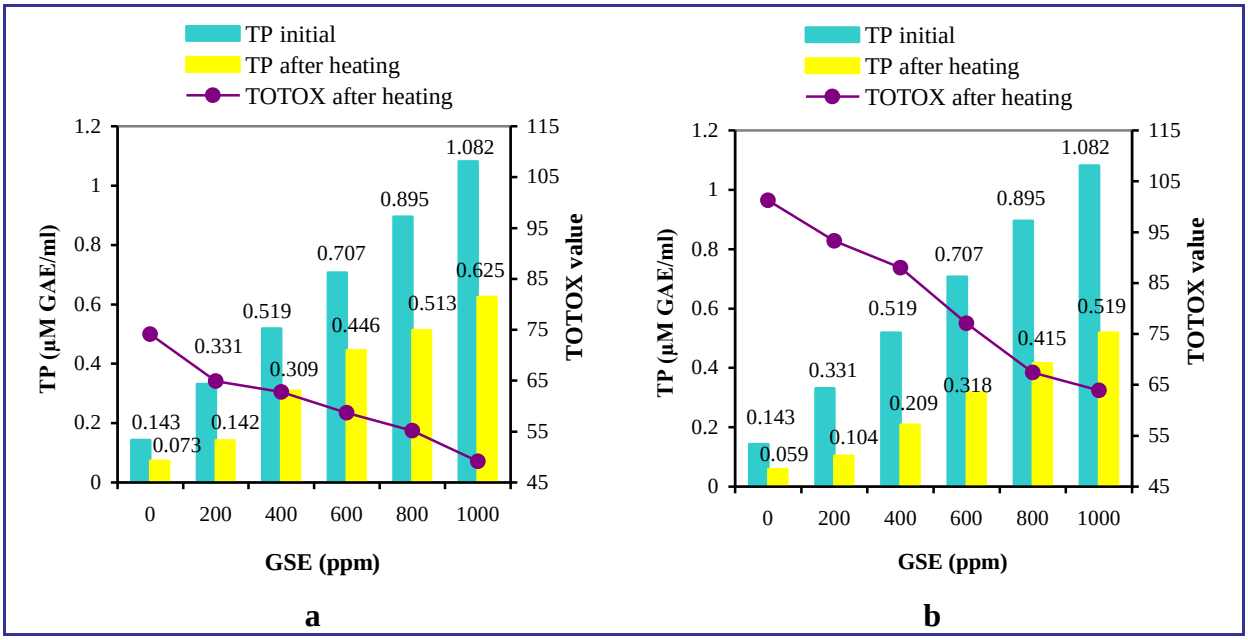


Figure 3.5. Impact of heating on TP content in oil samples with GSE related to TOTOX value (**a:** convective heating; **b:** microwave heating)

At the end of convective heating, the relative losses recorded in TP content were in the range 42–57%, while in the case of microwave exposure the losses were located in the range 52–69%. The aforementioned results pointed out that the extent of lipid oxidation was greater in samples heated in microwave oven than in convective heating; consequently, to inhibit the lipid oxidation higher amounts of TP were required in oil samples exposed to microwave than in those subjected to convective heating. Based on TOTOX value, it can be seen that the lowest extent of lipid oxidation at the end of heating was noted in oil samples supplemented by GSE to 1000 ppm.

These data highlight that TP significantly contributed to antioxidant activity of GSE in the heating time. These results are in agreement with the findings of other authors Mielnik *et al.*,

(2006) who reported strong linear correlations between the amount of antioxidants and the ability of GSE to prevent lipid oxidation.

Figure 3.6 shows the changes in TP content of oil samples supplemented by GSE to 1000 ppm in response to time, related to TOTOX value. The extent of TP degradation increased with heating time. The lowest content of TP were found in oil samples with the highest extent of lipid oxidation, expressed by the highest TOTOX value. A high negative correlation was detected between TOTOX value and TP consumed in the heating time, *Table 3.4.* This fact could be attributed to the protective action of TP against thermo-oxidative degradation. Data obtained in this study are consistent with results reported by Chantzos and Georgiou (2007) and support the idea that total antioxidant capacity of oil samples is inversely related to the extent of lipid oxidation, expressed by TOTOX value.

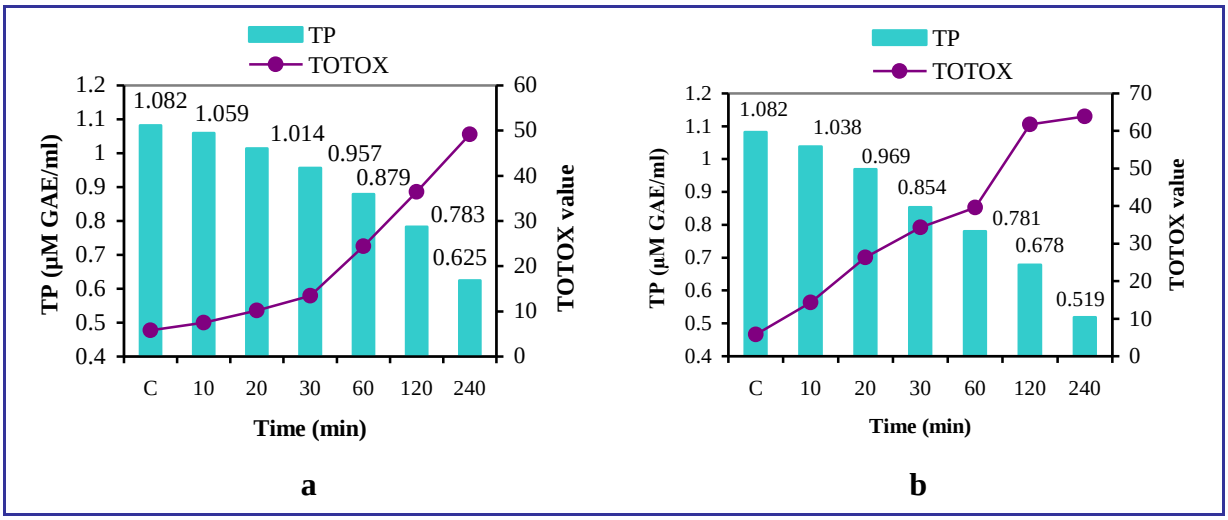


Figure 3.6. Alterations of TP in oil with GSE (1000 ppm) related to TOTOX value during heating (**a:** convective heating; **b:** microwave heating)

Table 3.4. Correlation coefficients obtained by linear regression

Y = f(X)	R	
	convective heating	**microwave heating**
TOTOX = f(TP)	−0.985	−0.986
CDs = f(TP)	−0.966	−0.985
CTs = f(TP)	−0.953	−0.981
PV = f(TP)	−0.972	−0.983
p-AV = f(TP)	−0.991	−0.986
IO = f(TP)	0.976	0.994

Also, a high positive correlation was detected between IO values and TP content consumed in response to oxidative degradation developed in oil samples during heating, *Table 3.4.* Also, high negative correlations were found between TP consumed in the heating time and PV, p-AV, CDs and CTs, demonstrating once again that the ability of GSE to inhibit the lipid oxidation was concentration-dependent. The profile of TP compounds is more important than the TP content (Kelen and Tepe, 2007). Further research is needed to obtain more results regarding the chemical composition of GSE and to determine the compounds contributing to the inhibitory effect of GSE on lipid oxidation.

3.3.3. Conclusions

The exposure of sunflower oil to convective and microwave heating led to the formation of hydroperoxides and secondary oxidation products resulting in significant alterations of oil quality. Supplementation with GSE and BHT prior to heating significantly improved oxidative stability of sunflower oil. GSE showed a significantly inhibitory effect on lipid oxidation during both treatments, although to a different extent. This ability was dose-dependent in the studied range (200–1000 ppm); therefore, the extent of lipid oxidation was inversely related to GSE level. Convective heating, respective microwave exposure for 240 min of samples supplemented by GSE to a level of 1000 ppm, resulted in significant decreases of investigated indices relative to the control values as follows: PV (48%; 30%), p–AV (29%; 40%), CD (45%; 30%), CT (41%; 36%), TOTOX (35%; 37%). Oil supplementation with GSE to a level in the range 600–800 ppm inhibited the lipid oxidation in a similar manner to BHT, while a level over 800 ppm limits thermo-oxidative degradation of sunflower oil more than BHT. These results prove that TP content of samples could be correlated to oxidative deterioration and support the idea that total antioxidant capacity of oil samples is inversely related to the extent of lipid oxidation, expressed by TOTOX value. These data prove the potential of natural antioxidants derived from grape seeds in slowing down lipid degradation and increasing the oxidative stability of oil even when exposed to high temperatures, suggesting that GSE may be used as potential source of natural antioxidants in the application of food industry to prevent lipid oxidation. The introducing of natural antioxidants during the production and/or processing is a valuable option for manufacturers who want to meet the requirements of the consumers for safe and functional food.

3.4. Assessing the antioxidant properties and some bioactive compounds of fruit kernel oils obtained from fruit processing by-products

3.4.1. Aim

The objective of the study presented in ***selected paper 8*** was to investigate the possibility to exploit the potential of apricot and plum kernels, resulted as by-products in fruit canning industry, by obtaining of crude oils and their analysing in terms of antioxidant capacity and some bioactive compounds content such as: β-carotene, tocopherols and total phenolics. Plum *(Prunus domestica) and* apricot *(Prunus armeniaca)* kernels were purchased at a small-scale fruit canning factory (season 2004, 2005 and 2006) from Western Romania. The β-carotene content was determined using the spectrophotometric assay described by Tamas and Neamtu (1986). The

quantification of α–, β–, γ– and δ–tocopherols (α–T, β–T, γ–T, δ–T) in order to obtain tocopherol pattern of plum and apricot kernel oil was performed by reversed phase high performance liquid chromatography (RP-HPLC) with fluorescence detector at 290 nm excitation wavelength and 325 nm emission wavelength. The content of total phenolics (TP) was evaluated by Folin-Ciocalteu colorimetric method (Singleton *et al*., 1999) and the antioxidant activity was measured using the FRAP assay acccording to Benzie and Strain (1986).

In performing of this study the research team was formed by Assist. Dr. Mirela Popa [mirevio_gh@yahoo.com], Lecturer dr. Delia Dumbrava [delia_dumbrava@yahoo.com], Lecturer dr. Diana Raba[dianaraba@yahoo.com] and Assoc Prof. dr. Calin Jianu[calin.jianu@gmail.com] from Banat's University of Agricultural Sciences and Veterinary Medicine (Timisoara) and Prof. dr. Constantin Bele[Cbele2002@yahoo.com] to University of Agricultural Sciences and Veterinary Medicine from Cluj-Napoca.

3.4.2. Results and discussion

Fruit kernels obtained by manual processing from plums and apricots stones were dried at 70°C for 10 h and then, they were ground and extracted, to obtain crude oil, with petroleum eter (1:5, m/v) for 3 h. The oil content related to dry basis was 48.73% for plum kernels and 42.09% for apricot kernels.

Evaluation of β–carotene content from analyzed oil

Results obtained for β–carotene content of investigated oil samples are reported in the *Table 3.5.* These data show that β–carotene content depends on the harvest year and fruit species. It can be observed that the plum kernels oil is richer in β-carotene than the apricot kernels oil.

Table 3.5. β-Caroten content of fruit kernel oil

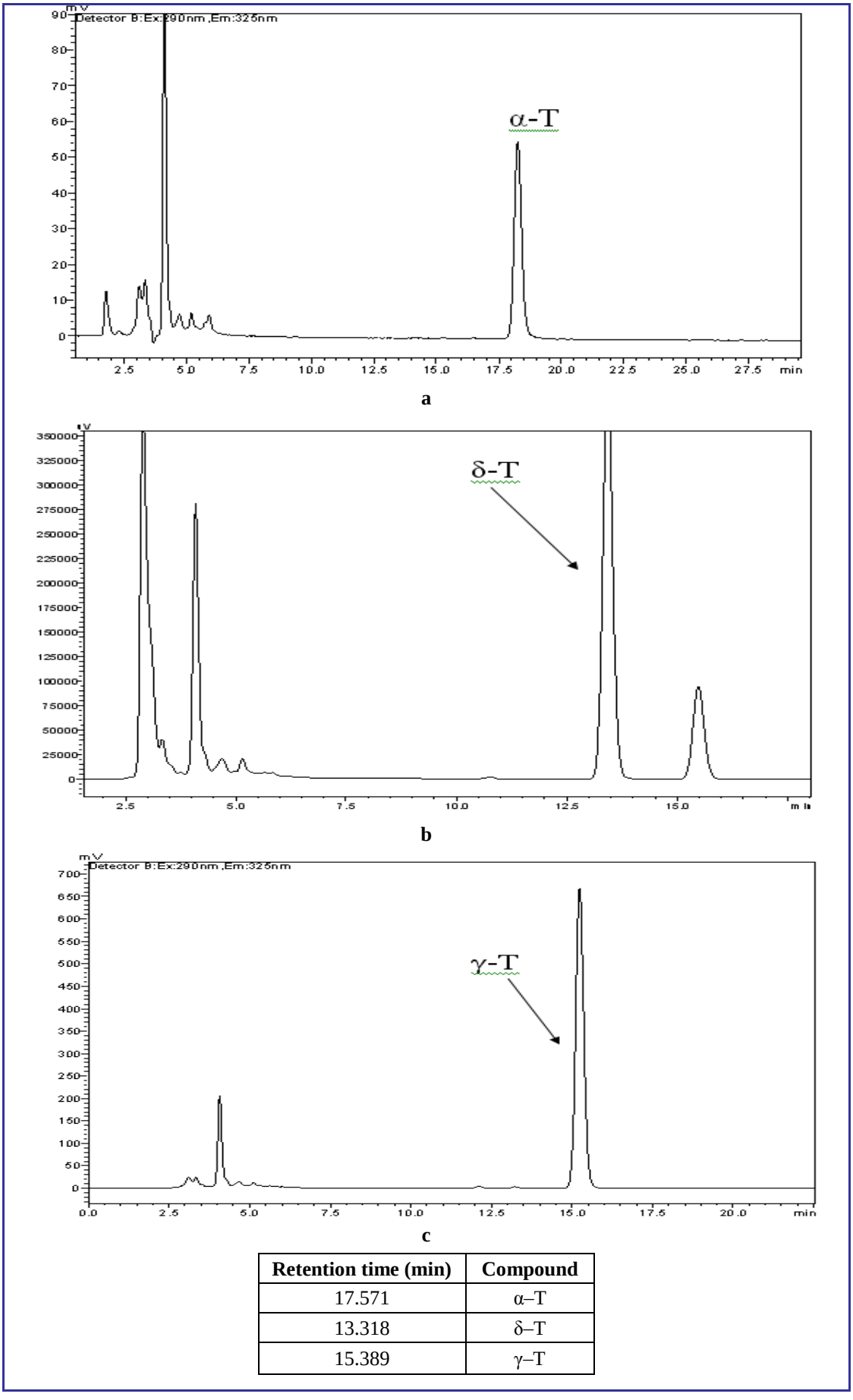
Harvest year	β-caroten content of sample ($\mu g \cdot g^{-1}$)*	
	plum kernel oil	apricot kernel oil
2004	188.65±2.03	61.05±2.08
2005	184.95±1.84	58.35±1.51
2006	191.21±2.13	62.46±2.16

*Each value is expressed as mean ± standard deviation (n = 3).

Tocopherol HPLC pattern of fruit kernel oil

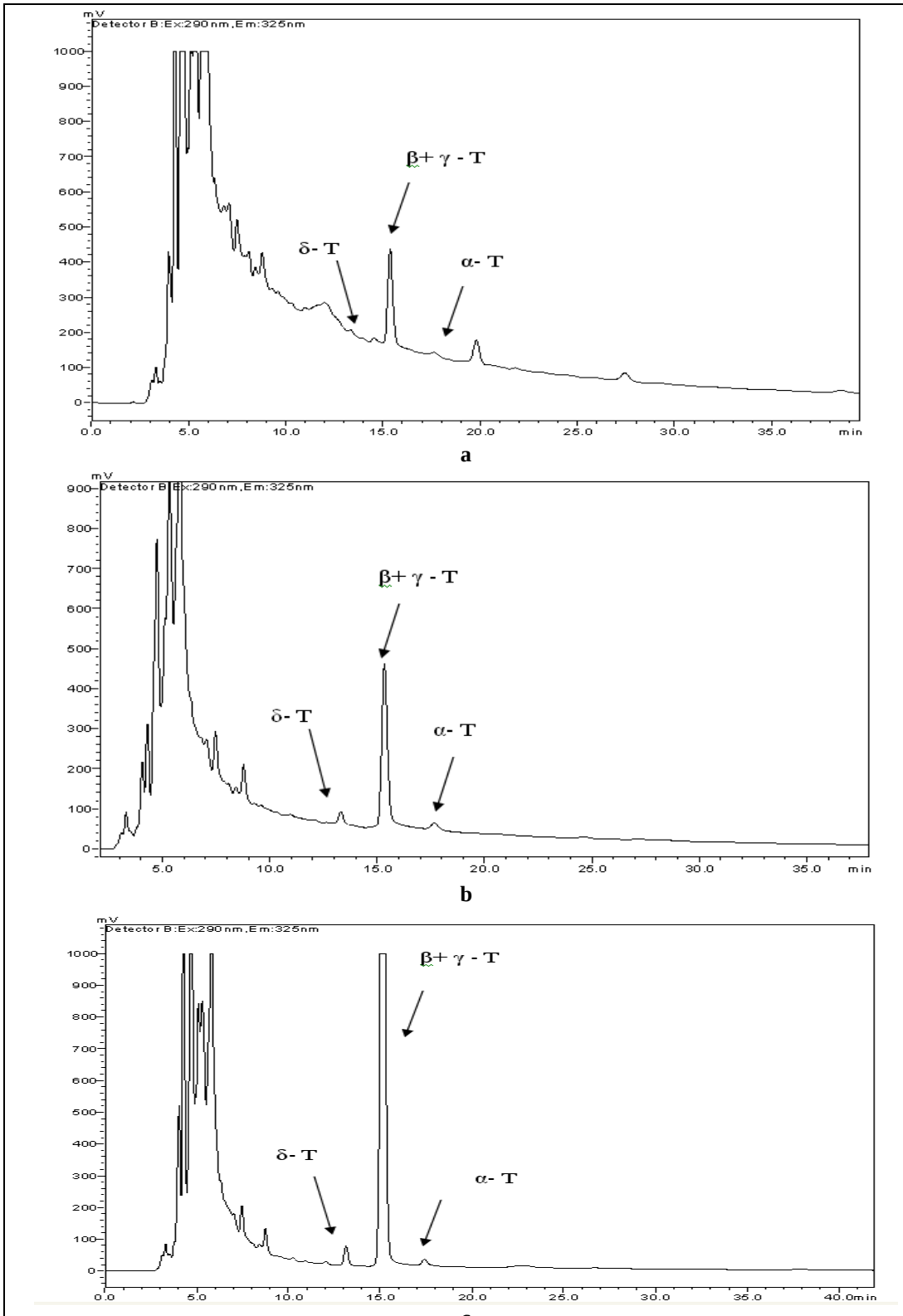
Figure 3.7 shows the HPLC chromatograms of standard α–T (a), δ–T (b) and γ–T (c). The investigated fruit kernel oils revealed the presence of significant amounts of tocopherols, *Figures 3.8* and *3.9*.

Concerning the HPLC analysis of tocopherols in vegetable oils, this can be performed either normal or reversed phase columns, using fluorescent, electrochemical and UV detector. The normal phase columns provide separation of all tocopherol isomers, while reversed phase columns (usually C18) are unable to separate the β– and γ– tocopherols (Andres *et al.*, 2011; Bele e*t al*, 2013). The RP-HPLC method used in our study for tocophenols analysis does not distinguish between β– and γ–isomers of tocopherol. Thus, the sum of these isomers is shown throughout this work as β+γ–T.



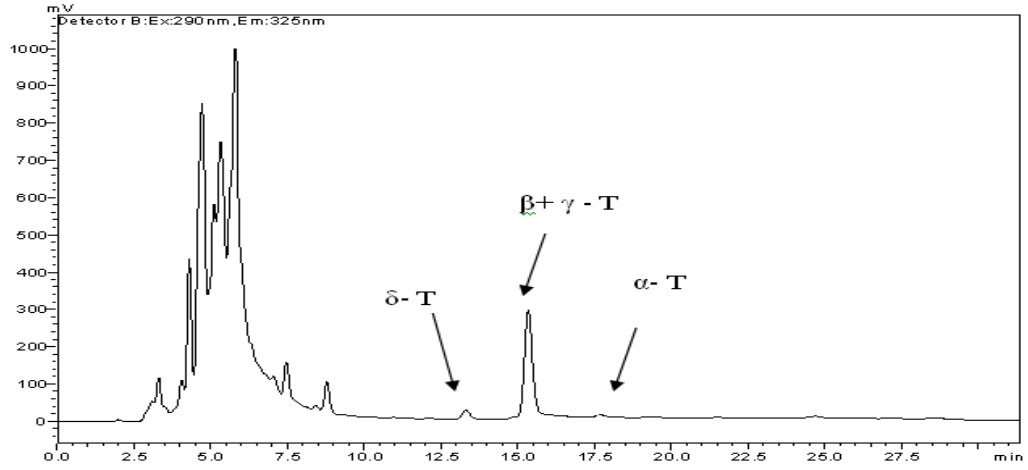
Retention time (min)	Compound
17.571	α–T
13.318	δ–T
15.389	γ–T

Figure 3.7. HPLC chromatograms corresponding to standards (**a**: α–T; **b**: δ–T; **c**: γ–T)

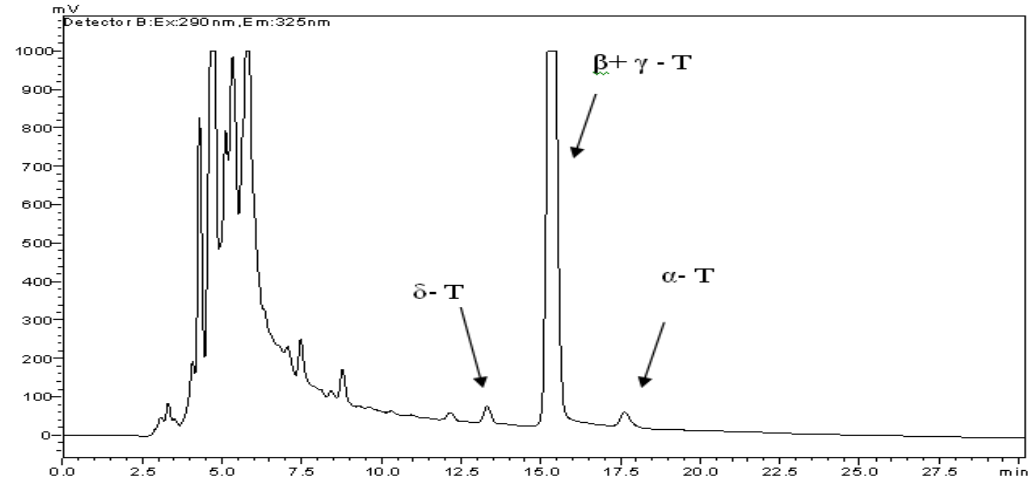


No. crt.	Tocopherols	Retention time (min)	Content (μg·100 g^{-1})		
			2004	2005	2006
1	α–T	17.571	n.d.	42.40	43.54
2	β+γ–T	15.381	152.00	207.00	1259.40
3	δ–T	13.318	9.04	32.80	60.00

Figure 3.8. Tocopherol HPLC profile of apricot kernel oil
(**a**: 2004; **b**: 2005; **c**: 2006)



a



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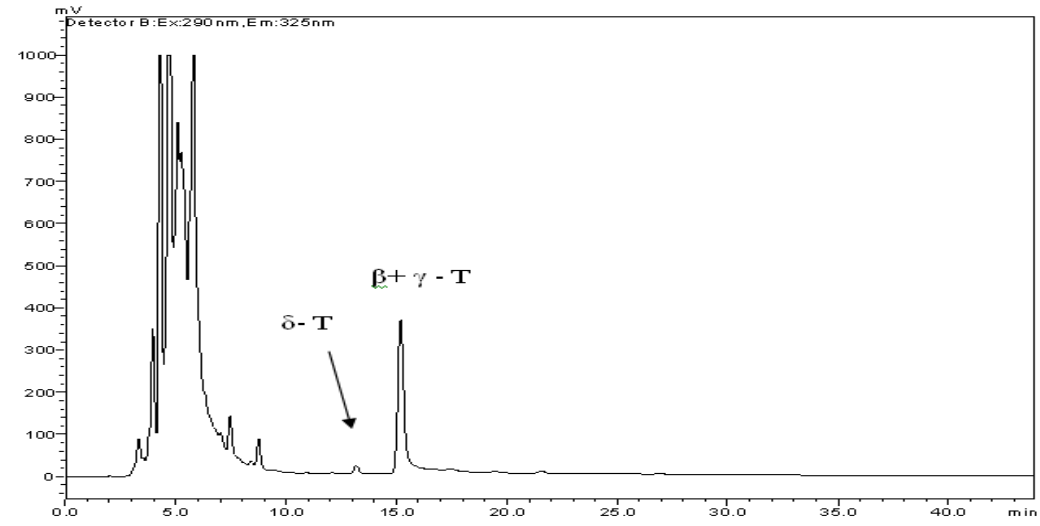


c

No. crt.	Tocopherols	Retention time (min)	Content (µg·100g^{-1})		
			2004	2005	2006
1	α–T	17.571	n.d. (<0.1)	122.80	n.d. (<0.1)
2	β+γ–T	15.381	164.00	1057.20	162.20
3	δ–T	13.318	27.00	44.00	20.20

Figure 3.9. Tocopherol HPLC profile of plum kernel oil
(**a**: 2004; **b**: 2005; **c**: 2006)

According to the study performed by Bele e*t al.* (2013), RP-HPLC is preferred when the separation of β– and γ–T is not the main point of analysis due to the reproducibility of retention times, fast equilibration, and robustness of reversed-phase columns. Also, fluorescence detection permits to get lower detection limits.

Lack of separation of β– and γ–T by RP-HPLC did not introduce significant error in the determination of γ-T because the vegetable oils contain only small quantities of β–T compared to γ–T (Bele *et al*., 2013). The tocopherol values reported in this paper are lower than the values obtained in a similar study conducted by Medina-Juarez *et al.* (2000). One reason for these lower values of tocopherols content could be that the analysis was performed after three months of oil extraction. During this time tocopherols content has undergone some alterations because tocopherols are very light sensitive.

The obtained data show that the content of tocopherol isomers depends on the harvest year and fruit species. The isomers β+γ–T and δ–T were identified in all investigated oil samples, while α–T was not detected in apricot kernel oil (2004 harvest year) and plum kernel oil (harvest years 2004 and 2006).

The major tocopherol isomer in both oil types was β+γ–T. For apricot kernel oil, β+γ–T accounted between 73.4 and 94.4% of the total tocopherols content while in the case of plum kernel oil, the sum of isomers β+γ was located between 85.9 and 88.9% reported to the total tocopherols content. These results prove that the investigated oils are rich in β+γ–T. Contrary, α–T and δ–T were detected only in minor amounts.

Taking into account that vegetable oils contain only small quantities of β–T compared to γ–T (Bele *et al*., 2013), we can state that these oils contain high amonts of γ–T. Both kernel oils show very characteristic tocopherol pattern in which the sum of isomers β+γ–T is the predominating one. Based on the tocopherol pattern of kernel oils, it can be noted that these oils are expected to be highly resistant to autoxidation due to the presence of γ–T in high amonts. The later exhibits a high antioxidant activity (Hassanein, 1999).

Evaluation of antioxidant properties

Antioxidant propertiese of kernel oil samples was expressed by FRAP values and TP content, *Table 3.6*. These results prove that the fruit kernel oil possesses significant antioxidant properties, strongly dependent on the fruit species and the harvest year.

Table 3.6. Total polyphenols and total antioxidant capacity values for fruit kernel oil

Samples	FRAP (mM $Fe^{2+}\cdot L^{-1}$)			TP (mM GAE$\cdot L^{-1}$)		
	2004	**2005**	**2006**	**2004**	**2005**	**2006**
apricot kernel oil	1.29±0.11	1.33±0.12	0.86±0.07	1.28±0.14	1.30±0.15	0.88±0.09
plum kernel oil	1.78±0.15	0.42±0.03	1.90±0.16	1.79±0.17	0.61±0.05	2.85±0.24

* Each value is expressed as mean ± standard deviation (n = 3).

The correlation "FRAP *versus* TP content" reveal a high dependence of these parameters(R=0.899), *Figure 3.10*. It may be noted that TP content is a potential candidate as a selection criterion for antioxidant activity of fruit kernel oil, but antioxidant activity of these oils is not limited only to phenolics compounds.

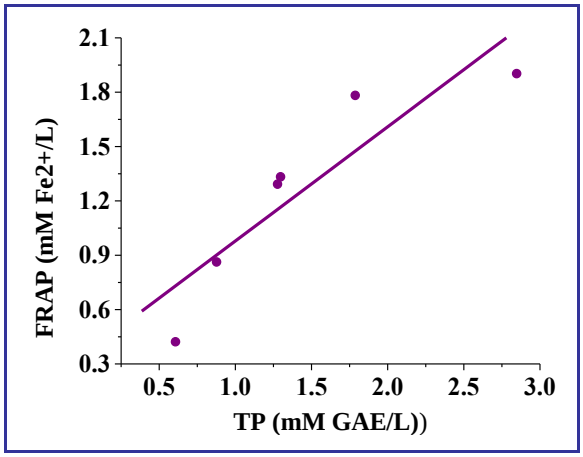


Figure 3.10. Correlation between FRAP and polyphenols content from fruit kernel oil

3.4.3. Conclusions

The results of this study highlight that plum kernel oil is a richer in β-carotene than apricot kernel oil. The content of isomeric forms of tocopherols depends on the harvest year and fruit species. The prevalent tocopherol fraction in all investigated kernel oils was represented by the sum of isomers β+γ. α–T and δ-T were detected in minor amounts in investigated kernel oils. Lack of separation of β- and γ–T using RP–HPLC method, did not introduce major error in the quantification of these isomers because vegetable oils contain small quantities of β–T as compared to γ–T. This method can be successfully used for the usual analysis of α–, β+γ–T and δ–T in different vegetable oils.

Based on the tocopherol pattern of the investigated kernel oils, it can be denoted that these oils are highly resistant to autoxidation due to the presence of β+γ–T to a high level. Additionaly, the fruit kernel oils possesses significant antioxidant properties that strongly depend on the fruit species and the harvest year. Our results pointed out a positive linear correlation between FRAP and TP. These results highlight that the apricot and plum kernels are a potential source of valuable oil which might be used for edible and other industrial applications.

3.5. Scientific contributions of the author to the actual state-of-knowledge

Regarding the subjects presented above and based on the two studies done by the author on this topic, the following points of view, ideas, conclusions and remarks contribute to the actual state-of-knowledge:

Regarding the possibility to exploit the potential of wine industry by-products

- The freeze dried extracts GSE and GPE obtained by capitalisation of wine industry by-products are rich sources of health-promoting polyphenols with significant antioxidant activity;
- Grape variety determines difference in antioxidant properties of obtained extracts;

- GSE derived from Merlot variety possess higher antioxidant properties in terms of TP and FRAP value than GSE from Cabernet Sauvignon grape variety.

Regarding the possibility to exploit the potential of GSE as natural antioxidant for edible oil industry

- GSE at various levels exhibited very strong antioxidant activity. Probably, the addition of natural extract created an oil system surrounded by antioxidants that were able to prevent oxidation because phenolic compounds were located on the interface of the lipid system;
- The potential of GSE to enhance the oxidative stability of sunflower oil during thermal applications was dose-dependent in the studied range: 200–1000 ppm. The highest oxidative stability of sunflower oil subjected to convective or microwave heating for 4 h at 180°C was reached in oil samples supplemented by GSE to a level of 1000 ppm;
- GSE did not show pro-oxidative effect during treatments up to 240 min;
- Both convective heating and microwave exposure caused positional rearrangement of the double bonds in oil samples and, consequently, a part of the non-conjugated system was converted to conjugated diene and triene double bonds;
- The ability of GSE to reduce the accumulation of primary and secondary products of lipid oxidation was higher in the convective heating than in the microwave treatment;
- Oil supplementation with GSE to a level in the range 600–800 ppm inhibited the lipid oxidation in a similar manner to BHT, while a level of GSE over 800 ppm limits the thermo-oxidative degradation of sunflower oil more than BHT;
- TP content of oil samples significantly contributed to antioxidant activity of GSE in the heating time. Thus, TP content of oil samples could be related to the lipid oxidative deterioration;
- The extent of lipid oxidation was greater in samples heated in microwave oven than in convective heating; consequently, to inhibit the lipid oxidation higher amounts of TP were required in oil samples exposed to microwave than in those subjected to convective heating. Based on TOTOX value, it can be seen that the lowest extent of lipid oxidation at the end of heating was noted in oil samples supplemented by GSE to a level of 1000 ppm;
- Thus, these results support and strengthen the idea according to which, the total antioxidant capacity is inversely related to the extent of lipid oxidation in the investigated conditions;
- GSE is a very effective inhibitor against lipid oxidation in food thermal applications requiring the oil heating at high temperatures and can be recommended as a potential natural antioxidant for edible oils industry.

Regarding the possibility to exploit the potential of fruit processing industry by-products

- The plum and apricot kernels are important non-traditional sources with a high content of potential edible oil.

- The recovery of plum kernels seems to be more appropriate in approaching of some possibilities for oil recovery due to the higher oil content in plum kernels than in apricot kernels. In comparison with apricot seeds, the plums seeds resulted annually in considerable quantities as by-products from fruit canning industry as well as from natural distilled beverages industry.

Regarding the plum and apricot kernel oil quality

- Fruit kernel oils contain considerable amounts of tocopherols, β-carotene and phenolics compounds strongly dependent on the harvest year and fruit species;.
- The correlation between FRAP and TP content reveal a high dependence of these parameters;
- TP content is a potential candidate as a selection criterion for antioxidant activity of fruit kernel oil, but antioxidant activity of these oils is not limited only to the phenolics compounds;
- Both kernel oils showed very characteristic tocopherol pattern in which β+γ–T is the predominating one. α–T and δ–T were detected in minor amounts in both kernel oils.
- Lack of separation of β– and γ–T using RP–HPLC method, did not introduce major error in the quantification of these isomers because vegetable oils contain small quantities of β–T as compared to γ–T.
- Although the chemical structure of these oils make them more susceptible to turning rancid from lipid peroxidation, the presence of natural antioxidants, such as tocopherols, help to offset decomposition and extend their shelf life.
- The apricot and plum kernels are a potential source of valuable oil which might be used for edible and other industrial applications. These oils could be also used as dietary supplements because they are excellent sources of essential fatty acids and antioxidants.

4. Scientific achievements concerning the use of some natural bioactive compounds for prevention and control of mycotoxin production in cereals

The studies presented in this part of thesis were performed for achieving the objectives of the project SEE-ERA.NET PLUS, ERA 139/01[http://www.cereals-mycotoxins.ro], implemented in the period 2010-2012, with theme: "*Systems to reduce mycotoxin contamination of cereals and medicinal plants in order to preserve native species and traditional products in Romania-Serbia-Croatia*" in which I was involved as researcher.

4.1. Background

Mycotoxins are toxic chemical products formed as secondary metabolites by a few fungal species that colonize crops and contaminate them with toxins in the field or after harvest (Moss, 1996). They are produced during growth and multiplication of fungus when micro ecological conditions are favorable (Alexa *et al*., 2011).

Mycotoxins usually enter in the body through ingestion of contaminated food, but also inhalation of toxic spore's and direct dermal contact are also important ways of penetrating. In food and fodder naturally contaminated with fungi are found in high concentrations only seven mycotoxins: *aflatoxin, ochratoxin A, patulin, zearalenone, trichothecene, citrinin and penicilic acid.*

In *Figure 4.1* is presented the mycotoxins distribution in the food chain. Practically, there are no known areas in the world without mycotoxins and it is estimated that 25-60% of the world's grains contaminated with mycotoxins are produced mainly by fungus of the genera *Aspergillus, Fusarium, Penicillium* (Alexa *et al*., 2013).

The contamination of cereals products with mycotoxins has been a serious problem in Balkan communities. Cereals and cereal products are significant human food resources and livestock feeds in the whole world. Each year, a large number of crops are susceptible to fungal attack either in the field or during storage, leading to considerable financial losses and damage the health of humans and animals (Jajic *et al*., 2008).

Several researches on the mycotoxins' role in endemic kidney disease were geographically limited to the Balkan region (Puntaric *et al*., 2001). Balkan endemic nephropathy (BEN) is found in certain rural areas of the Balkans and affects at least 25 000 inhabitants. A number of descriptive studies have suggested a correlation between the exposure to ochratoxin A (OTA), Balkan endemic nephropathy and the mortality caused by urothelial urinary tract tumors (Peraica *et al*., 2008).

Mycotoxins can be produced in pre-harvest and post-harvest, during food and feed production. Grains are exposed to fungal contamination in the field, before harvest, but especially during storage for longer periods in improper conditions, being favourable environments for molds development. Among them, representatives of the genera *Alternaria, Cladosporium, Fusarium, Aspergillus* and *Penicillium* are known to have negative impact on the preservation of grains determining quantitative and qualitative losses (Rasooli *et al*., 2006).

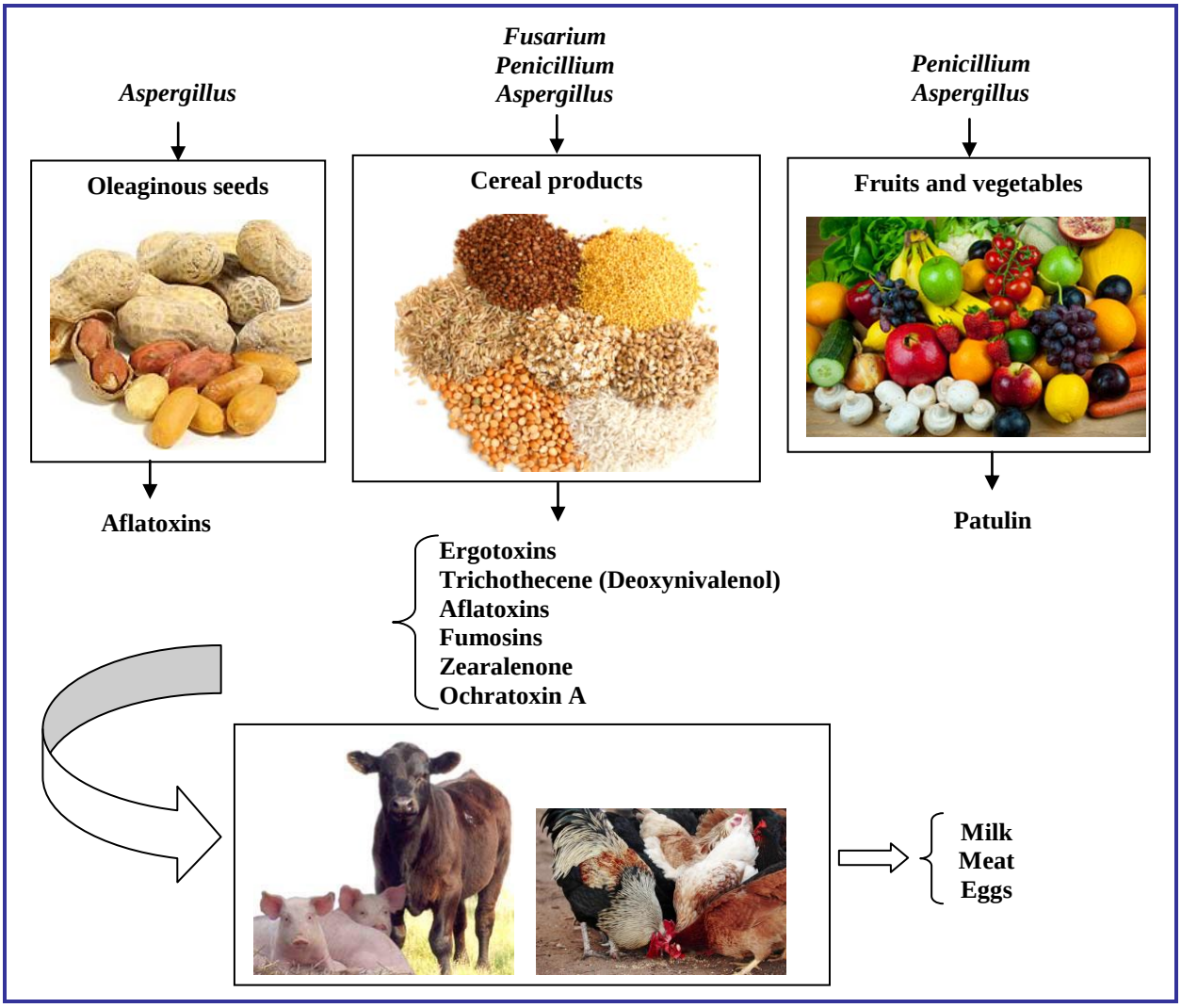


Figure 4.1 . The mycotoxins distribution in the food chain

The most important groups of mycotoxins that often occur in cereals destined for food and feed consumption are: aflatoxins, ochratoxins, trichothecenes (deoxynivalenol, nivalenol), zearalenone and fumonisins (Moss, 1996).

Ochratoxins are the first major group of mycotoxins identified after the discovery of aflatoxins. Ochratoxin A (OTA), a toxin produced by *Aspergillus ochraceus*, *Aspergillus carbonarius* and *Penicillium verrucosum*, is one of the most abundant food-contaminating mycotoxins in the world, that occurs in vegetal products especially in cereals (Van der Merwe *et al.*, 1965). OTA has been considered as a possible cause of the human disease known as *Balkan Endemic Nephropathy*. OTA acts as a nephrotoxin for all studied animal species but it's also toxic for humans, having the longest period of elimination from the body. OTA is also a carcinogenic, teratogenic and immunotoxic compound, affecting both humoral and cell-mediated immunity (Dehelean *et al.*, 2011; Alexa *et al*., 2013).

Another species of fungi responsible for the production of mycotoxins called trichothecenes are *Fusarium species* (Kuiper-Goodman, 1995).

Deoxynivalenol (DON) is the most frequent trichotecene contaminants of agricultural crops throughout the world and it is produced by species such as *Fusarium graminearum* and *Fusarium culmorum.* Extensive survey data indicate the occurrence of this mycotoxin, particularly in wheat and corn (Mankeviciene *et al*., 2011). DON is a potent antifeedant, inducing in animals, especially in swine, feed refusal and vomiting and can also affect the immune system. In human body, DON causes vomiting, headache, fever and nausea (Richard, 2007).

Zearalenone (ZON) is a fungal metabolite, mainly produced by *Fusarium graminearum* and *Fusarium culmorum*, which are known to colonize maize, barley, wheat, oats and sorghum (Krska, 1999). ZON and its related compounds can cause hyperestrogenism and severe reproductive and infertility problems in animals, especially in swine (Kuiper-Goodman *et al*., 1987). Regarding the rate of incidence and concentration levels in cereals, maize and oats were the most frequently contaminated (Kumar *et al*., 2008).

Fumonisins (FB1 and FB2) represent a group of mycotoxins produced by *Fusarium verticillioides.* Fumonisins are cancer-promoting metabolites of *Fusarium proliferatum* and *Fusarium verticillioides* that have a long-chain hydrocarbon unit with role in their toxicity. Consumption of food contaminated by fumonisins has been associated with elevated human oesophageal cancer incidence. The total intake of FB1 in the European diet has been estimated at 1.4 μg/kg of body weight per week (Soriano and Dragacci, 2004). *Fusarium* moulds have become nowadays a serious problem because they produce a range of toxic metabolites (mycotoxins) which imperil the health of both humans and animals. Although *Fusarium* species are predominantly considered as field fungi, it has been reported that FUMO production can occur post-harvest when storage conditions are inadequate (Marin *et al*., 2011).

Prevention of fungal infection during plant growth, harvest, storage and distribution as well as the measures that must be taken for decontamination represent a current issue for the *European Commission* (Commission regulation EC No. 1126/2007).

Romania is a major regional producer of wheat, ranking third in Central Europe behind Serbia and Hungary (Jajic *et al*., 2008). Wheat dominates in the west part of Romania as a primary crop and represents an important part in human and animal feed. The presence of mycotoxins in cereals is potentially hazardous to human and animal's health.

The study carried out by Alexa *et al*., (2013), in which I participated as co-author, reported the mycotoxins incidence and their co-occurrence in wheat harvested in Western Romania during two consecutively harvest years (2010 and 2011). Also, in this study was evaluated the histopathological impact caused by consumption of grains contaminated with mycotoxins. It was determined that although none of the analyzed samples exceeded the stipulated maximum limits for cereals used as feed, a high incidence of mycotoxins produced by *Fusarium species,* DON and ZON, has been recorded in wheat samples harvested in Western Romania. Also, it was pointed out that the incidence of mycotoxins in cereals was influenced by seasonal weather conditions. DON was the mycotoxin with the highest incidence in wheat samples due to agro-climatic conditions typical for the west part of Romania. Regarding the co-occurrence of *Fusarium* mycotoxins, the results proved that ZON was found as a co-contaminant together with DON, especially when climatic conditions for development of fungus are favorable (high relative humidity of air). Considering all these factors, it can be concluded that measures to control the mycotoxins content in cereals are necessary. Also, the development of some strategies concerning the reducing of mycotoxins contamination in the affected areas is for a great importance. With regard to the histopathological investigations, it was noticed that the most toxic compounds after a short time of feeding with natural contaminated wheat were FUMO and DON. They produced significant tissue lesions in liver and kidney of rats and reduced or determined the absence of vascular endothelial growth factor expression which indicates no possibility for recovery on these areas.

The prevention is the best method to control the contamination with fungi and mycotoxins. Storage in adequate conditions (moisture, temperature) and the addition of antifungal agents may diminish the fungal growth but can not detoxify the contaminated samples. If mycotoxins contamination occurs, the risk associated with the toxin must be removed if the products are going to be used for food or feed.

Quality assurance and safety of cereals has determined the identification of new alternative ways to preserve the nutritional value of grains. The main techniques used to reduce the mycotoxins contamination of cereals refers to the physical (Magan and Alfred, 2007), chemical (Bullerman and Bianchini, 207), microbiological (Reddy *et al*., 2010) and biotechnological methods (Bozoglu, 2009), as shown in *Table 4.1*.

Nowadays is revealed the need to prevent fungal spoilage and mycotoxins accumulation by using of natural substances with fungicidal effects. Substances which do not directly interact with mycotoxins, such as antioxidant agents, immunostimulatory agents, may be very efficient for decreasing the toxicity of mycotoxins. It seems, that some antioxidants such as vitamins A/E and BHT can influence the activity of mycotoxins *in vivo* by modulating their bioavailability, their bioactivation, their metabolization etc, and, hence, they are able to decrease the harmfull effects produced by some mycotoxins (Kennedy *et al.*, 1990).

Substances with preservative action are often added to cereals, especially those for animal feed. Propionic, acetic and formic acid, butylated hydroxianisole (BHA), butylated hydroxytoluene (BHT) and propyl paraben were applied singly or in combination to assess their effectiveness in preventing of moulds growth (Magan *et al.*, 2010).

Previous studies were performed for assess the effect of food grade antioxidants such as propyl paraben (PP), butylated hydroxyanisole (BHA) and butylated hydroxytoluen (BHT) to control the *Fusarium* species and mycotoxins production (Etcheverry *et al*., 2002). These compounds were effective to control the growth of *Aspergillus, Penicillium* and *Fusarium* populations as well as the synthesis of aflatoxin and fumonisin (Farnochi *et al*., 2005; Nesci *et al*., 2008). BHA and propyl paraben inhibited the production of deoxynivalenol and nivalenol in wheat grain (Fanelli *et al*., 2003; Hope *et al*., 2005).

Table 4.1. The main techniques used for reducing the mycotoxins contamination of cereals

Physical methods	Eliminating of altered fractions	Separation Grinding, sanding
	Distorting of toxins	Thermal distortion Irradiation
Chemical methods	Acid-base distortion	Ammonification Nixtamalization
	Distortion using oxidizing and reducing agents	
Adsorption of toxins	On clay On active carbon On resin	
Microbial inactivation and fermentation		

The synthetic phenolic antioxidants (e.g. BHT, BHA) are preferred as fungicides due to their protective effect on health.

In the last few years some alternatives to synthetic compounds used in prevention of mycotoxins accumulation have been developed. The natural antioxidants have been proven some effects on fungal growth and mycotoxin production (Fanelli *et al*., 2003). Plant extracts could be a valuable alternative to chemical products for fungal prevention, because they are biodegradable, are natural products and their use don't contaminate the environment. Some plant extracts contain antioxidant compounds as polyphenols (flavonoids and phenolic acids, etc.) and others, such as terpenes, known for their effect on human health. These compounds could be the basis for the antimicrobial effects exhibited of plant extracts. The mechanism of action of phenolic compounds includes the inhibition of enzyme by the oxidized compounds which affect the integrity of membrane, pH homeostasis and equilibrium of inorganic ions (Dambolena *et al.*, 2010).

The treatment with synthetic resveratrol on maize grain led to the reduction of ZON production by *Fusarium graminearum* (Marin *et al*., 2006). The study performed by Fanelli *et al*., (2003) concluded that the resveratrol exhibited a particularly wide spectrum of mycotoxin control, although nowadays, this is an expensive product. Resveratrol is able to inhibit OTA production by *Penicillium verrucosum* and *Aspergillus westerdijkiae* in naturally contaminated wheat grain and is more effective in fungus control than the essential oils (Aldred *et al*., 2008).

One of the most valuable natural sourse of resveratrol is grape pomace. The effect of synthetic trans-resveratrol and natural extracts obtained from wine industry by-products on *Fusarium* species and mycotoxins production was evaluated by Marin *et al.* (2006), (2011). No difference was found when it was used synthetic trans-resveratrol or natural extracts, suggesting

that, the by-products from wine industry are a cheaper source of resveratrol than the synthetic one.

In line with these concerns, the objective of the study presented ***in selected paper 9*** *published by Alexa et al. (2012) was to assess the potential of two freeze-dried natural extracts obtained from grape pomace and grape seeds (GPE and GSE) compared to synthetic antioxidant (BHT) in order to control the ochratoxin A (OTA) production in naturally contaminated wheat grain. The processing and characterization of freeze-dried extracts (GPE and GSE) used in this study was done in Section I/3/3.2.* In performing of this study, I was involved as principal author (marked as corresponding author).

In the last years, essential oils and natural formulas with antioxidant activity were tested as potential inhibitors of fungal development and mycotoxin production (Hope *et al*., 2005). Moreover, the essential oils from different herbs and aromatic plants were used in the prevention of fungi and mycotoxins accumulation in cereals as potential inhibitors of fungal development and mycotoxin production (Hope *et al*., 2005; Aldred *et al*., 2008).

Essential oils, also known as volatile oils, are complex mixtures of volatile constituents biosynthesized by plants, which mainly include terpenes, terpenoids, aromatic and aliphatic constituents, all characterized by low molecular weight (Bassole and Juliani, 2012). They are a valuable source of antioxidants and biologically active compounds. Natural essential oils are expected to be more advantageous than the synthetic agents. Due to their bioactivity in the vapors phase, essential oils could be used as fumigants for the protection of stored cereals (Naeini *et al*., 2010).

The inhibitory mechanism of some essential oils against moulds is due to the modification in cytoplasm, inhibiting some of its functions, cytoplasmatic membrane rupture as well as the inactivation and/or inhibition of intracellular synthesis of enzymes. Also, the antifungal effect of essential oils could be explained by the modifications induced on the fungal morphogenesis and fungus growth through the interference of their components with the enzymes responsible for wall cell synthesis leading to changes in the hyphae integrity, plasma membrane disruption and mitochondrial destruction (Rasooli *et al*., 2006). These effects can occur simultaneously or alone resulted in the inhibition of spore germination. For this reason, plant extracts or essential oils with antimicrobial properties can replace the use of synthetic chemicals, in order to control mycotoxicogenic moulds in raw materials and foods.

The most attractive aspect derived from using of essential oils and/or their constituents as crop protectants is due to their biodegradability and non-toxicity (Isman, 2000).

Several researches have reported the preservation of grains by using of essential oils (Soliman and Badeaa, 2002) and their impact on FUMO production by *Fusarium verticillioides* (Dambolena *et al*., 2010; Menniti *et al*., 2010) or by *Fusarium proliferatum* (Velluti *et al*., 2003).

The results reported by Bluma *et al.* (2008) have pointed that the antifungal activity was strongly associated with the presence of monoterpenic phenols, especially thymol, carvacrol and eugenol in essential oils. These studies have suggested that only a few essential oils such as cinnamon and clove leaf oil have the capacity for control the mycotoxigenic *Fusarium* species,

Penicillium verrucosum, Aspergillus ochraceus and DON and OTA production depending on the environmental conditions. Thus, it can be notice that many studies have been carried using essential oils in microbiological media, but only few were conducted *in vivo* for assessing the antifungal effect of essential oils on opportunistic fungi of cereals (Magan *et al*., 2010).

In this regard, the study conducted by Sumalan et al. (2013), reported in ***selected paper 10****, was focused on investigating the inhibitory potential of some essential oils derived from aromatic herbs and spices (Mentha piperita, Melissa officinalis, Salvia officinalis, Coriandrum sativum, Thymus vulgaris and Cinnamomum zeylanicum) against Fusarium mycotoxins production in wheat seeds in relation with their antioxidant properties.*

The originality of this research is supported by the fact that, the antifungal and fungicidal effect of essential oils was investigated in vivo. In this study I was involved as co-author.

Therefore, the goal of this research direction was to investigate the possibility to prevent or control the mycotoxin production in cereal grains by using the natural extracts rich in polyphenolic compounds obtained from winery by-products as well as, by applying the treatments with some essential oils from aromatic herbs and spices.

4.2. Impact of treatment with natural extracts from wine industry by-products on ochratoxin A production in wheat grain

4.2.1. Aim

The aim of the research detailed in ***selected paper 9*** was to evaluate the potential of two freeze-dried crude extracts obtained from wine industry by-products (grape pomace extract: GPE and grape seeds extract: GSE derived from Cabernet Sauvignon grapes variety, Recas winery, harvest year 2010)) compared to a synthetic food antioxidant (BHT), in order to control ochratoxin A (OTA) production in naturally contaminated wheat. This study was carried out directly in naturally contaminated wheat. For this purpose, first, the wheat grains were chemically sterilized with dilute hypochlorite for inactivation of opportunistic mycoflora. Then, the wheat samples were separately treated with different concentrations of GPE, GSE and BHT (500, 1000, 2500 ppm) and kept in storage conditions (temperature 20°C, aw =0.85). After 7, 14, 21 and 28 days the samples were analyzed in terms of fungal population and level of OTA. OTA content

was determined by enzyme-linked immunosorbent assay (ELISA) according to Turner *et al*. (2009), using ELISA-RIDASCREEN tests. The summary of validation data of ELISA method is shown in *selected paper 9*. The analysis of antioxidant properties for GSE, GPE and BHT was shown in *Section I/3/3.2*. To provide a clear view on the changes occurred for the investigated parameters as a result of different types of antioxidant in wheat grain samples, the obtained data were processed by ANOVA one-way test. Based on information obtained by statistical processing, the significance of changes occurring in ochratoxin A content, as response to extracts type and level were pointed out.

For performing of this study I worked closely with Prof. dr. Ersilia Alexa [alexa.ersilia@yahoo.ro] and Assoc. Prof. dr. Renata-Maria Sumalan [srenata-maria@ahoo.com]. The contribution of each author is shown in ***selected paper 9***.

4.2.2. Results and Discussion

The impact of treatment with natural extracts and BHT on OTA accumulation

In *Table 4.2* was presented the changes recorded in OTA content of wheat grain samples during storage as effect of treatment with natural extracts and BHT. Also, *Figure 4.2* provides information on the OTA decrease registered in response to the treatments with natural extracts or BHT during the storage time relative to control sample. The different antioxidant levels were chosen in agreement with previous studies that have proved that the inhibition of fungus and mycotoxins production increased with the dose used for treatment (Marin *et al*., 2006).

Table 4.2. Changes in OTA content in wheat grain in response to treatment with natural extracts and BHT

Sample	OTA (ppb)				
	period (days)				
	0	**7**	**14**	**21**	**28**
Control	12.93±0.17	13.15±0.35^{ns}	13.32±0.26^{ns}	13.67±0.25*	14.12±0.32***
500 ppm GSE	12.93±0.17	13.41±0.29^{ns}	12.28±0.34*	11.07±0.32***	10.28±0.47***
1000 ppm GSE	12.93±0.17	12.08±0.29*	11.29±0.36*	10.90±0.34**	10.38±0.37***
2500 ppm GSE	12.93±0.17	11.68±0.46*	10.62±0.49*	11.09±0.39*	9.42±0.41***
500 ppm GPE	12.93±0.17	12.78±0.37^{ns}	11.68±0.35**	10.83±0.44**	8.89±0.48***
1000 ppm GPE	12.93±0.17	14.49±0.43*	11.21±0.50**	10.98±0.54**	9.00±0.44***
2500 ppm GPE	12.93±0.17	12.27±0.57^{ns}	11.96±0.52^{ns}	10.45±0.34***	9.01±0.32***
500 ppm BHT	12.93±0.17	11.98±0.33*	10.79±0.36**	10.33±0.45**	10.17±0.37**
1000 ppm BHT	12.93±0.17	10.48±0.38*	9.55±0.46**	9.43±0.32**	9.32±0.27**
2500 ppm BHT	12.93±0.17	15.09±0.43*	10.74±0.45**	9.86±0.48**	9.12±0.33***

Data are shown as means, relative to control (C) response recorded in the wheat grain in initial time (0). Statistical differences are indicated as: ns=non-significant (P>0.1), P<0.05=* (significant), P<0.01=** (highly significant) and P<0.001=*** (extremely significant).

With regard to the antioxidant properties, the FPAP value recorded for BHT was 1328.14 μmol $Fe^{2+}\cdot g^{-1}$. Also, on the basis of data presented in *Section I/3/3.2* (*Table 3.1*), it can be seen that BHT had the maximal FRAP value followed by GSE (1042.38 μmol $Fe^{2+}\cdot g^{-1}$) and GPE (804.17 μmol $Fe^{2+}\cdot g^{-1}$). The content of TP for GSE was higher than for GPE. These results are

consistent with those reported by Negro *et al*. (2003). Pastrana-Bonilla *et al.* (2003) stated that TP were five times more concentrated in grape seeds than in the skin and 80 times more than in the grape pulp.

The initial concentration of OTA in control sample was 12.93 ppb while after treatments with natural extracts and synthetic antioxidants, the OTA content was located in the range 9.00-15.09 ppb, depending on the nature of the antioxidant, dose and the time from the start of treatment. The previous studies regarding the use of synthetic antioxidants in control of mycotoxins synthesis during storage showed that BHT and BHA, alone or in combination with another antioxidants, are effective to control the toxin production in maize and wheat grain in different experimental conditions (concentration, water activity- a_w and temperature) (Etcheverry *et al*., 2002; Lafka *et al*., 2007). On the one hand, our results pointed out that at the end of 28 days from the start of treatment with BHT, OTA content was in the range 9.12-10.17 ppb. During this period, OTA content increased from 12.93 to 14.12 ppb in the control sample. On the other hand, by increasing of BHT dose from 500 to 2500 ppm it was not much affected the OTA level in wheat samples. After 28 days from the start of treatment with BHT to a level of 2500 ppm, OTA production decreased from 12.93 to 9.12 ppb. Also, the treatment with BHT to a level of 1000 ppm induced a similar decrease in OTA accumulation during the same period. These results demonstrate that the use of high concentrations of BHT similar to those suggested by the producing companies (0.2-0.25%), is not justifiable from this point of view.

The results from *Figure 4.2* show that, the level of losses registered in OTA content in response to treatments increased compared to the control sample, except the first 7 days from the start of treatment.

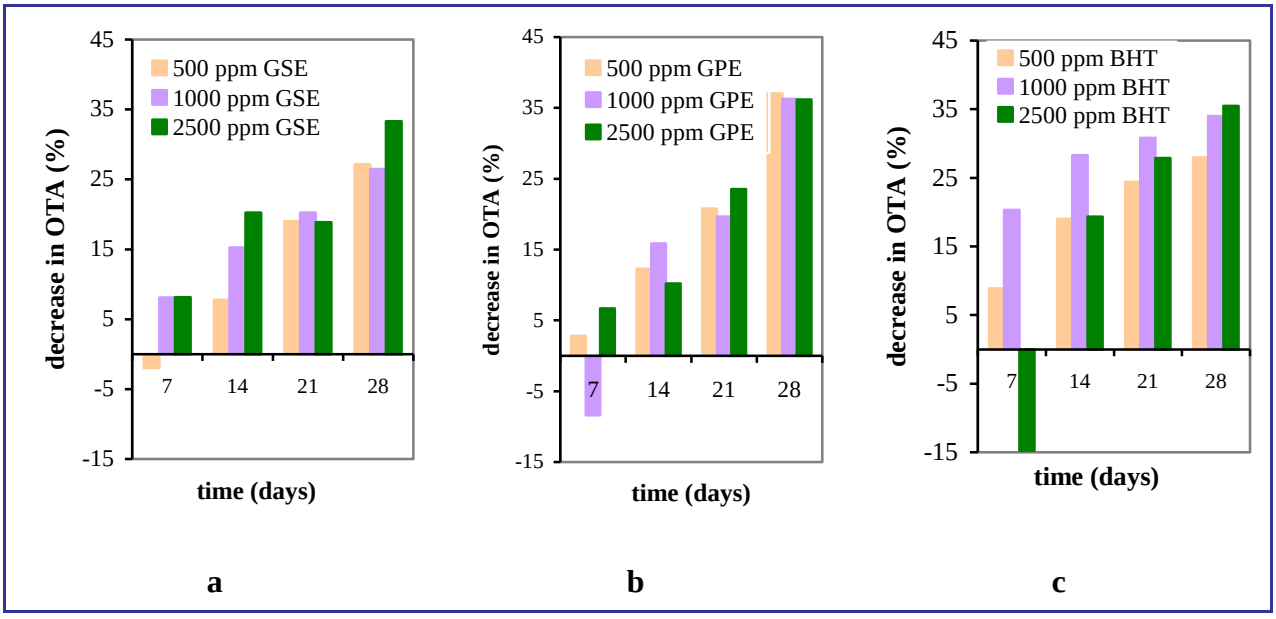


Figure 4.2. The decline of OTA content in response to treatment with natural extracts and BHT (**a:** GSE; **b:** GPE; **c**: BHT)

After 14, respectively 21 days from the start of treatment, it can be noticed that the efficiency of BHT treatment quantified by decrease in OTA content reported to the values

recorded in control sample, were higher at a level of 1000 ppm than those recorded at 500 ppm and 2500 ppm. After 28 days from the start of treatment with BHT to a level of 1000 and 2500 ppm it was recorded decreases in OTA production about 35% relative to the control.

The results presented in *Table 4.2* showed that the treatments with natural extracts (GPE and GSE) were efficient in decreasing on OTA accumulation, having at least similar effect with BHT.

The addition of GSE at 500 ppm level was induced a slow increase of OTA content after 7 days, followed by a decrease of OTA concentration after 28 days from the start of treatment. By increasing the dose of GSE to a level of 1000 ppm it was recorded moderate relative decreases in OTA content (10-12%). These results were in agreement with those reporded by Fanelli *et al.* (2003) which revealed that resveratrol isolated from grapes and used for treating of wheat and corn seeds led to a sharp reduction of OTA production (Lafka *et al*., 2007).

The treatment with GPE also led to the inhibition of OTA synthesis compared to control sample. Previous researches on this topic proved that resveratrol from grapes was able to completely inhibit the OTA production to a level of at 500 ppm, proving to be more effective than essential oils to control the OTA synthesis (Lafka *et al*., 2007; Aldred *et al.*, 2008). The treatment with GPE to high concentration (1000 and 2500 ppm) had similar effects on OTA accumulation suggesting no advantage in using of high dose. Comparable results were also obtained by Reynoso *et al.* (2002) when synthetic antioxidants were used to control the *Fusarium* speciesl.

From the *Figure 4.2* it can be noted that, after 14 days from the start of treatment with GSE and GPE it was recorded decreases in OTA production in the range 8-28% relative to the control sample. After 28 days from the start of treatment, the recorded decreases were in the range 26-37% relative to the control sample. The highest decrease in OTA production was obtained for treatment with GPE to a level of 500 ppm.

Our data are in agreement with those reported by Aldred *et al.* (2008) concerning the effect of resveratrol (at 200 ppm level) on OTA production by *Penicillium verrucosum* in stored wheat grain for 28 days at 25°C, when the losses registered in OTA production were between 27 an 71% relative to control, depending on a_w.

The registered results pointed out that, there are no major differences in OTA production among treatments with natural extracts to levels of 500, 1000 and 2500 ppm, proving that the inhibition of OTA production is not dependent on the dose of antioxidant agent (Marin et al., 2006).

Some stimulation of OTA production was observed with 500 ppm GSE, 1000 ppm GPE and 2500 ppm BHT, after 7 days of treatment. These findings could indicate that, in response to antioxidants stress, the fungus species produce more mycotoxins as a survival mechanism, (Reynoso *et al*., 2002).

After 14 days from the start of treatment, the OTA accumulation decreased compared to the control sample, proving the inhibitory potential of both synthetic antioxidant and natural extracts on OTA production in wheat grain. The results showed that after 28 days of starting treatment the most efficient on OTA decreasing was GPE followed by BHT and GSE. Data presented in *Section I/3/3.2* revealed that GPE does not have the highest TP content, i.e.

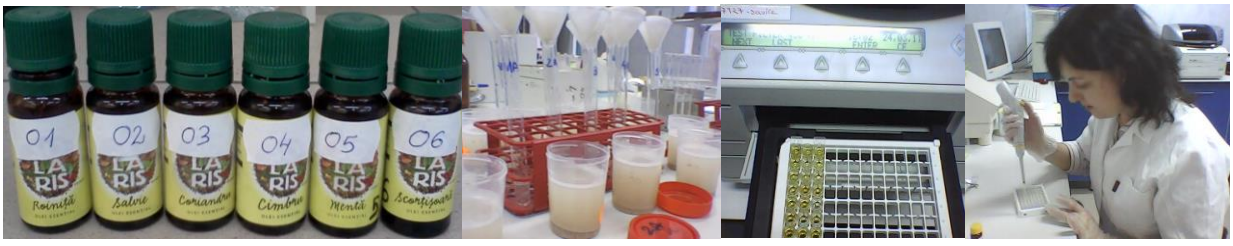
antioxidant capacity. Thus, the antifungal activity of natural extracts could be determined by their polyphenolic compounds profile. Literature studies indicate that resveratrol, that has proved to be an effective agent to control the OTA accumulation in cereals, is found in larger amounts in grape skin than in seeds (Lafka *et al*., 2007). GPE was obtained from the whole pomace and, probably contains more amounts of resveratrol compared with GSE. Starting from these assumptions, more studies are required to prove the mechanisms involved in the inhibition of OTA synthesis by treatment with natural extracts obtained from wine industry by-products.

From statistical analysis it can be noted that after 7 days from the start of treatment with GSE (500 ppm) and GPE (500 ppm, 2500 ppm) it was induced non-significant changes ($p>0.1$) in OTA production. After 14 days were recorded statistical significant differences in OTA accumulation: significant ($P<0.05$) for GSE and highly significant ($p<0.01$) for GPE at 500 and 1000 ppm, but non-significant ($p>0.1$) at 2500 ppm. After 28 days, for all treatments, highly significant ($p<0.01$) and extremely significant ($P<0.001$) differences were recorded. BHT induced significant differences in OTA production ($p<0.05$) after 7 days of treatment and highly significant ($p<0.01$) after 14, respectively 21 and 28 days, excepting the sample treated with 2500 ppm, when after 28 days, the difference related to control was extremely significant ($P<0.001$).

4.2.3. Conclusions

OTA production was significantly inhibited by addition of natural extracts obtained from wine-industry by-products. The best results concerning the potential of natural extracts to control OTA synthesis were obtained for treatment with GPE. This data support the idea according to which, the antifungal activity of natural extracts depends not only on the level of antioxidant agents used for treatment or the amont of polyphenolic compounds of extract, but also of their polyphenolic compounds profile. GPE and GSE are able to provide fungicidal and fungistatic protection and also to control the OTA accumulation in wheat grain samples at least similarly to BHT. The proved potential of these extracts to prevent or control the fungal development and OTA accumulation in wheat grain, highly recommends them as additives in antifungal treatments applied to cereals destined for human consumption or feed.

4.3. The effect of treatment with essential oils on *Fusarium* mycotoxins production in wheat grain



4.3.1. Aim

The goal of the study shown in ***selected paper 10*** was to investigate the inhibitory effect of some essential oils: *Melissa officinalis* (O1), *Salvia officinalis* (O2), *Coriandrum sativum* (O3), *Thymus vulgaris* (O4) *Mentha piperita* (O5) and *Cinnamomum zeylanicum* (O6) against *Fusarium* mycotoxins production in relation with their antioxidants properties. In this paper work, total phenolic content (TP) of essential oils was determined using the Folin-Ciocalteu colorimetric method (Singleton *et al.*, 1999). The antioxidant activity of essential oils was measured using the ferric reducing antioxidant power (FRAP) test (Benzie and Strain, 1996). The mycotoxins were analyzed by enzyme-linked immunosorbent assay (ELISA) according to Turner et al. (2009), using ELISA-RIDASCREEN tests. The decreases recorded in mycotoxin production in response to applied treatments with essential oils were expressed as a percentage related to the content of mycotoxin registered in control sample.

In performing of this research I worked closely with my colleagues Prof. dr. Ersilia Alexa [alexa.ersilia@yahoo.ro] and Assoc. Prof. dr. Renata-Maria Sumalan [srenata-maria@ahoo.com]. The contribution of each author is shown in ***selected paper 10***.

4.3.2. Results and Discussion

Impact of essential oils on FUMO and DON production

Figure 4.3 provides information on the decrease in FUMO content registered in response to treatment with essential oils relative to the control sample, after 22 days of treatment. The results proved that the treatment with essential oils resulted in decreasing of *Fusarium* mycotoxin accumulation in wheat seeds.

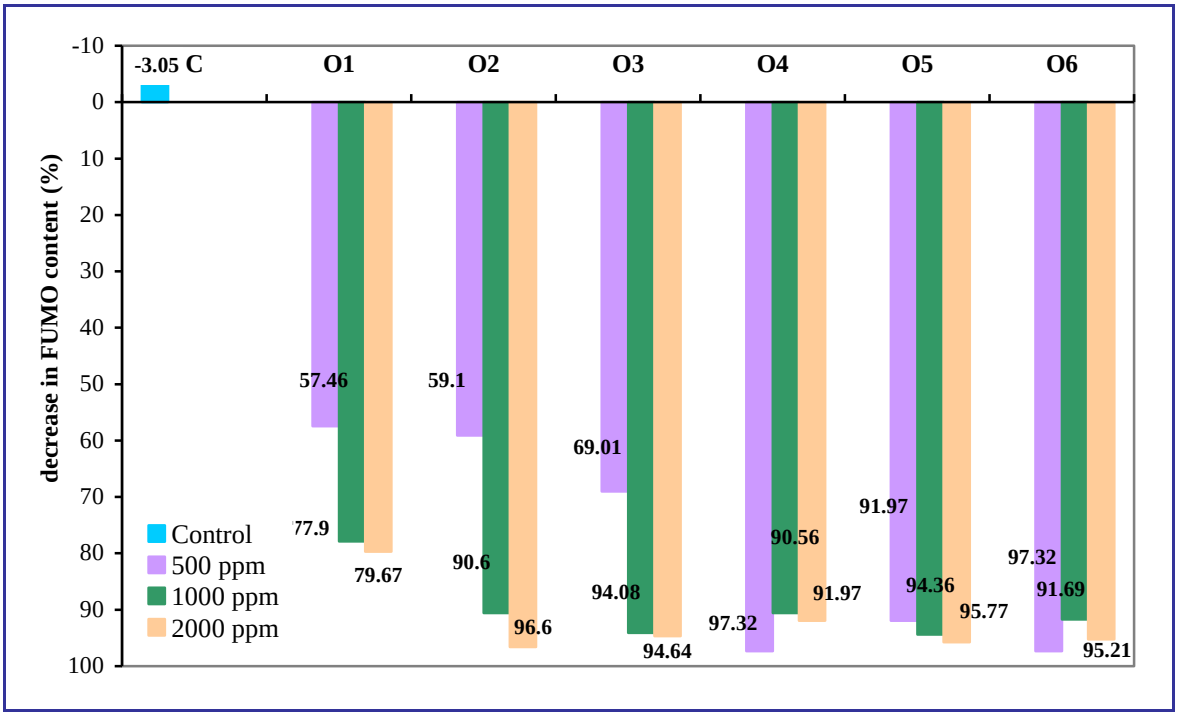


Figure 4.3. The declines registered in FUMO content by treatment with essential oils

At the beginning of the experiment, it was recorded a content of 0.689 ppm for FUMO and 0.420 ppm for DON.

The declines registered in FUMO production after 22 days of treatment with essential oils were in the range 57-97% related to the initial value, depending on the applied treatment (type and dose of essential oil). The best control on FUMO production, expressed by decreasing greater than 90% reported to the control value, was recorded for all treatments with O4, O5 and O6. These results are in agreement with the study conducted by Soliman and Badeaa (2002) which revealed that, the effect of treatment with essential oils on FUMO production control was as follows: O4>O6>O5.

The treatments with O1, O2 and O3 applied to the lowest level (500 ppm) resulted in a moderate inhibitory effect. Our results shown that the relative decreases in FUMO production recorded in wheat samples in response to treatment with O1 were in the range 57-80%. Similar results were also noticed for treatments with O2 and O3 applied to a level of 500 ppm, while the treatment with these essential oils in doses of 1000 and 2000 ppm resulted in substantial decreases in FUMO production, in the range 91-97%.

The results reported by Velluti *et al.*, (2003) proved that a_w, temperature, dose and type of essential oil as well as some of their interactions had a significant effect on FUMO production by *Fusarium proliferatum*. The mycotoxins production is affected by the treatment conditions (temperature and the humidity of grain). The penetration of essential oils into the internal parts of the grain is improved in the presence of water.

In our study, the constant conditions, in terms of a_w (0.900) and temperature (25±2°C), resulted in decreasing of DON and FUMO production in wheat grain samples after 22 days from the start of treatment.

In regard to the effect of essential oil composition on mycotoxin synthesis, on the one hand a few studies have reported high inhibitory activity exhibited by phenolic compounds. The mechanism of action of phenolic compounds supposes the involvement of these compounds in enzyme inhibition, possibly through reaction with sulfhydryl groups or through interactions with proteins (Dambolena *et al*., 2008). On the other hand, it has reported that the relative antifungal activity of the essential oils can not be correlated with any individual component, but only with the mixture of compounds from these oils (Hashem et al., 2010).

The inhibition of fungal development as well as the toxins production not always can be observed together (Magan *et al*., 2010). For example, previous studies with *Fusarium culmorum* and *Fusarium graminearum* pointed out that growth of fungi was significantly inhibited by cinnamon essential oil, but toxin production was enhanced (Dambolena *et al*., 2010). Also, Magan *et al.* (2010) found that the suboptimal levels of fungicides stimulated DON production by *Fusarium culmorum* in wheat grain. The additional stress of the fungicidal agents combined with water stress could stimulate the mycotoxin production (Aldred *et al*., 2008).

After 22 days from the start of treatment with O4-O6 it was noted high FUMO inhibition, but the most fungicidal effect was recorded for O2 to a level of 2000 ppm.

According to data reported by Dambolena *et al*. (2008), the inhibitory effect of terpenes on *Fusarium* growth and FUMO production followed the sequence: limonene>thymol>menthol>menthone. O4 contains high amounts of thymol, as previously

reported Dambolena *et al.* (2008). Thus, the treatment with O2 to a level of 500 ppm induced a significant inhibitory effect on FUMO biosynthesis, reported to the control value. After 22 days of treatment with essential oils, DON was undetectable in all wheat grain samples. Similar effect of essential oils regarding the accummulation of DON produced by *Fusarium* species was reported by Velluti *et al.* (2003). The inhibition of DON production in control sample can be explained by the maintaining of a_w to a value of 0.900 during the entire period of incubation. Other previous studies proved that the minimum value of a_w for DON production by *Fusarium* species seems to be limited about 0.93 at 25°C (Hope *et al*., 2005).

Antioxidants properties of essential oils

TP and FRAP value were used for screening of antioxidant properties of essential oils tested in this paper. In *Table 4.3* are presented the values of these papameters for all essential oils used in this study.

Table 4.3. Antioxidant characteristics of essential oils

Essential oils	TP (μM GAE·g^{-1})	FRAP (μM Fe^{2+}·g^{-1})
O1	33.01±2.52	246.23±9.37
O2	18.52±1.06	55.48±3.81
O3	16.71±0.93	40.41±2.73
O4	473.44±11.27	650.48±14.29
O5	22.48±1.63	100.85±5.21
O6	30.17±2.41	230.03±8.12

The inhibitory effect on fungal growth and mycotoxins production was associated with antioxidant properties of investigated essential oils. O4 exhibited the highest FRAP value followed by O1 and O6.

The high antioxidant activity of these essential oils could be attributed to phenolic components (mainly, carvacrol and thymol) and their hydrogen donating ability by which they are considered powerful free radical scavengers (Chia-Wen *et al*., 2009; Chrpova *et al*., 2010). Carvacrol and thymol are phenolic compounds with similar structures isolated from many aromatic plants, and have been demonstrated to exert multiple pharmacological effects.

O2 and O3 showed lower values recorded for FRAP than other investigated essential oils. Our findings are in agreement with the results reported by Hussain *et al.* (2009), who noted that the antioxidant activity of essential oil from *Salvia officinalis* displayed less radical scavenging activity than the essential oils obtained from other *Lamiaceae* species. Contrary to data reported by Chia-Wen *et al.* (2009), in our study O1 exhibited a higher antioxidant activity.

According to our results, the highest TP content was noticed for O4 while the values recorded for other investigated essential oils were in the range 16.71-33.01 μM GAE·g^{-1}. Many studies have reported variable phenolics content in essential oils (Chia-Wen *et al*., 2009; Chrpova *et al*., 2010). Geographical area and culture conditions can influence the chemical composition as well as and the antioxidant properties of aromatic herbs, resulting in differences in data reported by different authors. According to data shown in *Table 4.4*, the antioxidant properties of essential oils were as follows: O4>O1>O6>O5>O2>O3.

Correlations

Table 4.4 presents the values of Pearson's correlation coefficients (R) obtained in response to linear regression between: FRAP and FUMO and TP and FUMO registered after 22 days from the start of treatment.

The Pearson's correlation coefficient (R) represents a quantitative measure to describe the strength of the linear relationship between investigated parameters. Based on regression analysis between antioxidant properties of essential oils and FUMO content recorded in wheat grain samples after 22 days it can be noted that the correlation coefficients did not exceed the value of 0.84 for all essentials oils.

Table 4.4. Correlation coefficients obtained by linear regression applied to investigated parameters

Correlation	**R**					
	22 days after treatment					
Y=A+BX	**O1**	**O2**	**O3**	**O4**	**O5**	**O6**
FRAP = f(FUMO)	-0.78	-0.84	-0.81	-0.65	-0.69	-0.68
TP = f(FUMO)	-0.81	-0.84	-0.81	-0.65	-0.71	-0.68

This fact highlight that, a high antimycotoxin activity of the essential oils could be related to the presence of other components, major and minor, or could be due to their synergistic action, as suggested by Rota *et al.* (2008), Velluti *et al.* (2003) and Prakash *et al.* (2012).

Although, the essential oils were not as efficiently as some organic preservatives, they are recommended in food technologies due to the absence of toxic effects.

Regarding the correlation between *Fusarium* mycotoxins production expressed by FUMO content and antioxidant activity of essential oils, there was not recorded a high correlation. This fact could suggest that TP and FRAP have not a crucial role in expression of antimycotoxin properties of these essential oils. Although it has been found a strong positive correlation between FRAP and TP of investigated essential oils (R=0.94), besides polyphenolic compounds there are others compounds in essential oils that might be involved in the expression of their inhibitory potential on *Fusarium* mycotoxins production.

4.3.3. Conclusions

Essential oils from cinnamon and lemon balm exhibited a significant antifungal activity. The highest inhibition of fungal growth was registered after 5 days of treatment and decreased after 22 days, probably due to the high volatility of essential oils.

In regard to the impact of essential oils on mycotoxin production, at the end of treatment it was recorded the inhibition of DON and FUMO production. The best control on FUMO production was noted in samples treated with O6 followed by those treated with O5 and O4. It was not recorded a good correlation between FRAP/TP and FUMO content, suggesting that, the antioxidant properties of essential oils have not a crucial role in expression of antimycotoxin effect. As a result of this study, the essential oils may be recommended as natural preservatives applied during cereals storage.

4.4. Scientific contributions of the author to the actual state-of-knowledge

Regarding the aforementioned subjects and based on the two studies done by the author for assessing the effect of some extracts obtained from wine industry by-products as well concerning the inhibitory potential of essential oils on natural mycoflora and mycotoxins production in naturally contaminated wheat, the following remarks contribute to the actual state-of-knowledge on this topic:

- The effect of natural freeze-dried extract (GSE and GPE) and BHT on OTA production was not the same during the whole treatment. After 7 days of treatment, some stimulation of OTA production was observed. These remarks could indicate that, in response to antioxidants stress, the fungus species produce more mycotoxins quantity, as a survival mechanism. After 14 days from the start of treatment, the OTA accumulation decreased reported to the control sample, proving the inhibitory potential of BHT and natural freeze-dried extracts on OTA production in wheat grain. After 28 days of treatment the most efficient regarding the inhibition of OTA production was GPE followed by BHT and GSE;
- GPE had a greater effect to control OTA synthesis than GSE, although GPE does not have the highest polyphenols content, i.e. antioxidant capacity. We advanced the idea that the antifungal activity of natural extracts could be related not only to the level of antioxidant agents, but also the profile of their polyphenolic compounds;
- GPE and GSE are able to provide fungicidal and fungistatic protection and control of OTA production in wheat grain at least similar to BHT;
- The efficiency of these extracts to control the fungal development and OTA production in wheat grain, highly recommends them as natural additives in antifungal treatments applied to cereals for human consumption or feed;
- These extracts could be a valuable alternative to conventional methods used for control of OTA production in stored cereals;
- In regard to the inhibitory effect of above mentioned essential oils obtained from aromatic herbs and spices, we can say that the treatment with these oils led to the inhibition of *Fusarium* mycotoxins production in wheat grain;
- The essential oils with the best antifungal properties was not the most effective inhibitor in *Fusarium* mycotoxins production;
- There was not recorded a good correlation correlation between *Fusarium* mycotoxins production expressed by FUMO content and antioxidant activity of essential oils suggesting that, the antioxidant properties of essential oils have not a crucial role in expression of antimycotoxin effect;
- Although it has been found a strong positive correlation between FRAP and TP of investigated essential oils, in addition to polyphenolic compounds from essential oils, there are other compounds involved in the expression of their inhibitory potential on *Fusarium* mycotoxins production.
- Considering the proven inhibitory potential of investigated essential oils on *Fusarium* mycotoxins production, we strongly recommend them as natural preservative agents that could be applied during cereals storage.

Section II
Academic and professional achievements

This part of ***Habilitation Thesis*** summarises the main academic and professional achievements of the candidate in the last 10 years, in the period **2003-2013**, after defending the PhD Thesis and confirmed by The Ministry of Education and Research, on the basis of Order no. 3896, dated 24.04.2003.

In terms of professional and academic achievements, the period after defending the PhD thesis is divided into two parts.

In the **first part,** between years 2003-2007**,** I paid a particular attention to the study disciplines taught, especially at bachelor level. As a lecturer, I taught *Fermentative and extractive technologies* and *Vegetal food technologies* to the students from the Faculty of Food Processing Technology.

In this direction, I reviewed the laboratory works; also, I introduced new applications and technological calculations which contributed to the understanding of technological issues in the field of above mentioned subjects. Also, the courses for my teaching classes was completed and organized in a form easily accessible for students. Thus, I have published to CNCSIS recognized publishing houses 2 books and a practical work textbook, as follows:

- Poiana Mariana-Atena, *Fermentative and extractive technologies* (*published in Romanian*), EUROBIT Publishing House, Timisoara, ISBN 973-620-126-0, 438 pp., 2004.
- Poiana Mariana-Atena, *Vegetal food technologies* (*published in Romanian*), EUROBIT Publishing House, Timisoara, ISBN 973-620-180-5, 297 pp., 2005.
- Poiana Mariana-Atena, *Vegetal food technologies. Methods of analysis, applications and technological calculations* (*published in Romanian*), EUROBIT Publishing House, Timisoara, ISBN 973-620-129-5, 242 pp., 2004.

Along with my professional evolution, the thematic of subjects taught was updated, so in 2007, I published 2 books as follows:

- Poiana Mariana-Atena, *Extractive technologies* (*published in Romanian*), SOLNESS Publishing House, Timisoara, ISBN 978-973-729-106-6, 276 pp., 2007.
- Poiana Mariana-Atena, *Fermentative products technologies* (*published in Romanian*), EUROBIT Publishing House, Timisoara, ISBN 978-973-620-287-2, 397 pp., 2007.

At the same time, during this period I was involved in many research topics that address aspects of nutrition in order to protect health through nutritional intervention with antioxidant functional foods. Thus, we performed screening of quality for some matrices of functional food and studies concerning the bioavailability of polyphenols and vitamins in various natural extracts intended to obtain functional food.

Below are presented the national research projects that I attended in this period:

- Grant AT, Theme no. 3/2003, no. 33556/1.07.2003, theme: *Research on the isolation, purification and characterization of some active principles from phytoncides class*, 2003-2005, project director Mucete Daniela
- Grant AT, theme no. 1, code CNCSIS 4, no. 33370/29.06.2004, theme: *Food processing - means for reducing of cereal fungal contamination,* 2004-2005, project director Alexa Ersilia
- Project CEEX 10/2005, theme: S*tudy of synergistic bioactivity of antioxidant functional food in reversible metabolic syndrome* (*MET-ANTIOX)*, 2005–2007, project director Dragan Simona
- Project CEEX, 44/2006, theme: "*The impact of multicomponent functional foods to combat obesity and atherosclerosis ANTIATERO-ALIM*", 2006-2008, project director Dragan Simona
- Project no. 3941/2007, PNCDI2/Module IV/Partnerships in priority areas, no. 7368/06.11.2007, theme: *Performant piezoelectric sensor based on new structure alpha-quartz type, sensors for food quality and safety* (SENZ-ALIM), project director Miclau Marinela

Also, during this period I coordinated two research themes with the economic environment (Contract 7139/06.11.2007 between USAMVB Timisoara and S.C. LEGOFRUCT SRL from Timisoara and Contract 5737/14.09.2007 between USAMVB Timisoara and S.C. PADURE FRUCTE PROD SRL from Caransebes) focused on the influence of processing techniques on sensory and physico-chemical characteristics of some fruits and vegetables from conventional and organic farming as well as on the assessment of anthocyanin pigments in various berries grown under protected conditions (greenhouse).

During this time I began the first studies on the analysis of red wine color and antioxidant properties. Actually, it is the time when I started an extensive documentation on this theme, I began to put into practice the studied aspects, I tried to adapt some methods for determination of total antioxidant capacity and phenolics content, I applied some selective methods for wine color analysis. It was a period of massive theoretical and practical accumulations, it was the proper time to learn some techniques of analysis and analytical issues that I applied in my further studies. This period has been a journey with many questions, searches, replies discovered only after a long study, it was my road towards some independent research directions, it was my beginning in this field, the basis for my professional defining. During this period I started to publish my first results in this area. The work from this period has materialized by publishing of 4 articles in ISI quoted journals, 19 articles in other journals included in international data basis and also, I participated with 4 papers to international conferences.

In the second part of my activity, since 2008 till 2013, I scored the most professional and academic achievements. During this time I worked, but I also initiated numerous research topics and, as a result of this work I have completed several publications (books, book chapters, articles) with a great importance in my professional evolution.

In 2008, as a result of theoretical and practical studies undertaken in the previous stage, I finished the book "*The analysis of red wine color* (*published in Romanian*)", author: Poiana

Mariana-Atena, EUROBIT Publishing House, Timisoara, ISBN 978-973-620-378-7, 181 pp. Also, in 2008 I wrote a book chapter (*Chapter 2.3.*: "*Phenolic compounds with antioxidant activity from grapes and wine",* pp. 217-272) in book: "*Functionally alimentation with natural bioactive components in metabolic syndrome"* (*published in Romanian*), coordinators: Dragan Simona, Gergen Iosif, Socaciu Carmen, EUROSTAMPA Publishing House, Timisoara, ISBN 978-973-687-761-2, 2008. Some of these materials are useful in performing courses and practical works for **"***Advanced technologies for obtaining of vegetal products*" at Master program "*Advanced technologies for agricultural raw materials processing"* (Faculty of Food Processing Technology), respectively **"***Special techniques for obtaining of different types of wines*" at Master program "*Quality of viti-vinicole products and by-products* (Faculty of Horticulture and Forestry).

In 2009 I published the book "*Techniques for minimal processing of food products* (*published in Romanian*)" [Poiana Mariana-Atena, Editura SOLNESS, Timisoara, ISBN 978-973-729-165-3, 222 pp.]. Currently, this material is useful for course "*Advanced food processing techniques*" taught at Master program titled "*Food. Human Nutrition*".

In 2010 I published the practical textbook ***"****Fermentative technologies. Methods of analysis, applications and technological calculations* (published in Romanian)**"** [Poiana Mariana-Atena, Diana Moigradean, SOLNESS Publishing House, Timisoara, ISBN 978-973-729-239-1, 231 pp.] useful for performing laboratory works to bachelor and also, to Master programs.

During this period I was involved in 5 research projects (2 international and 3 national) and a POSDRU project. Of these, I coordinated as director 2 and I participated as researcher in 3 projects, as follows:

Project Director

- Bilateral Project Romania-Greece, Program Capacities/Module III, no. 565/01.06.2012, theme: *Rapid Spectroscopic Methods for assessment of olive oil quality and adulteration (SPECTRAOIL)*, 2012-2014, value 21710 lei, project director: **Poiana Mariana-Atena** [http://uefiscdi.gov.ro/userfiles/file/CAPACITATI/Bilaterale/RO-GR/Lista%20proiecte%20bilaterale%20Romania%20Grecia-de%20contractat.pdf].
- Research Project no. 637/21.01.2009 between USAMVB Timisoara and S.C. ETCO EUROPE TRADE COMPANY SRL from SEBIS, ARAD County, theme: *Studies regarding the impact of some technological treatments on antioxidant characteristics of some wild berries based products*, 2009-2011, value 45 000 lei, project director: **Poiana Mariana-Atena**.

Researcher

- Project from Regional Program for Cooperation with South-East Europe (ReP-SEE), [http://plus.see-era.net], Reference number: ERA 139/01, theme: *Systems to reduce mycotoxin contamination of cereals and medicinal plants in order to preserve the native species and traditional products in Romania-Serbia-Croatia*, 2010-2012, [http://www.cereals-mycotoxins.ro].
- Project from MAKIS Program funded by The World Bank, no. 141529/2008, AG no. 142.004/02.10.2008, theme: *The implementation of modern technological systems to obtain dietary floury food*, 2008-2011, [http://www.alimente-dietetice-fainoase.ro/index.html].

- Project no. 52157/2008, PNCDI2/Module IV/Partnerships in priority areas, no. 6324/23.09.2008, theme: "*Interdisciplinary research on the soil-plant correlations, establishment of some transfer factors for areas with historical anthropogenic pollution*", 2008-2011, total value 2000000 lei/for BUASVM 300000 lei, [www.ubm.ro/sites/CISPPA_2008/cisppa_2008.html].

The Bilateral Project Romania-Greece is focused on strengthening the relation between the two teams (from Romania and Greece) with complementary skills and establishing a framework for further collaborations. The food security has become a domain of highest priority and, I strongly believe that this collaboration has allowed the development of complementary methods for detection of olive oil adulteration and degradation. In the frame of this project I set a close cooperation with Prof. Dr. Georgiou Constantinos from *Agricultural University of Athens, Chemistry Laboratory* and Senior researcher Dr. George Mousdis *from National Hellenic Research Foundation, Theoretical and Physical Chemistry Institute* in order to develop fast and low-cost spectroscopic methods for detection of olive oil adulteration and evaluation of olive oils quality in response to thermal and/or UV treatments. To achieve this objective was performed a comparative study between two spectroscopic techniques: synchronous scanning fluorescence spectroscopy (SSF) and FT-IR spectroscopy combined with chemometric analysis of spectral obtained data for analysis of adulterated olive oils with low cost oils (e.g. sunflower, soybean, corn germ oil) or thermally degraded olive oils. During this project, I together with other 3 researchers from project team performed mobilities in Greece (Athens) to National Hellenic Research Foundation, Theoretical and Physical Chemistry Institute and Agricultural University of Athens, Chemistry Laboratory (17-22 September 2013). Also, I organized two lectures (on November 2012 and 2013) at Faculty of Food Processing Technology (Banat's University of Agricultural Sciences and Veterinary Medicine from Timisoara), and our partners from Atena held lectures about "*Use of florescence spectroscopy for detection of oil adulteration and degradation*", "*Toxicity assessment of carbonyl compounds during edible oil thermal stress" and "Food Authentication: Analysis, Regulation & Consumers"*.

The purpose intended in the frame of research project with economical environment coordinated by me as director (contract no. 637/21.01.2009 between USAMVB Timisoara and S.C. ETCO EUROPE TRADE COMPANY SRL from SEBIS, ARAD County) was to develop simple ways to improve the antioxidant properties and color stability of gelled fruit products. For this purpose, I have contributed with studies on the following concerns:

- Assessing the impact of freezing and frozen storage on antioxidant properties and color stability of some wild berries;
- Evaluation the impact of fruit thermal treatment applied for jam processing as well as the effect of jam storage on antioxidant properties and color indices of resulted gelled fruit products;
- Providing of some viable solutions in order to improve the antioxidant properties and color quality of finished products.
- Developing of some recipes for low-sugar jams with improved antioxidant properties.

The aim of research performed for solving of MAKIS Project was to obtain and characterise some dietary floury food for both people with various diseases (diabetes, gluten intolerance, errors of metabolism) and healthy people, but with specific food needs (infants, pregnant women, athletes, overweight people) [http://www.alimente-dietetice-fainoase.ro/index.html].

In the realisation of this project I have contributed with studies on the obtaining and characterization of dietary floury products, as follows: gluten free products (based on premixes, bakery products, biscuits) for people intolerant to gluten (celiac disease); aproteic products (premixes, bakery products, biscuits), products for people with errors of metabolism (phenylketonuria); hypoglucidic products for people with diabetes; infant products and baby food based on cereal, with or without addition of fruits and vegetables; iron fortified products specifically designed for people with anemia; floury products for elderly.

As a result of our activity, it was registered ***3 Trademarks to OSIM,*** as follows:

- Certificate of Trademark Registration to OSIM no. 112438, for Trademark: TPA DIET HIPOGLUCIDICBISC, deposit number M 2010 05684, C1:30: Biscuits (*hypoglucidic biscuits with chickpeas for people with diabetes, except for medical use*).
- Certificate of Trademark Registration to OSIM no 112402, for Trademark: TPA DIET COZOHIPOGLUC, deposit number M 2010 05685, C1:30: Pastry product (*hypoglucidic cake with fruit jelly for people with diabetes, except for medical use*).
- Certificate of Trademark Registration to OSIM no. 112403, for Trademark: TPA DIET Fe NUTRIPREMIX, deposit number M 2010 05686, C1:30: Gris (*Nutritive premix, enriched in iron, based on semolina wheat, lentils and apricots*).

  Trademarks owners: Alexa Ersilia Calina, Trasca Teodor Ioan, **Poiana Mariana-Atena**, Pop Georgeta, Stoin Daniela, Negrea Monica, Cocan Ileana

Among these ones, the dietaty product TPA DIET – COZOHIPOGLUC won:

- *gold medal* at European Exhibition of Creativity and Innovation (EURO INVENT, 11 May 2013, Iasi, Romania);
- gold medal and diploma of excellence at the International Exhibition of Inventions (PROINVENT, the XI Edition, 19-22 March, 2013, Cluj-Napoca)

In the frame of this project I participated in the organization of workshop [http://www.alimente-dietetice-fainoase.ro/index.html] to Faculty of Food Processing Technology (in 2010, September 2). Also, I was lecturer for two sections: (i) *Physico-chemical and nutritional characterization of dietary floury food. Theoretical and practical aspects*; (ii) *The importance of germinared cereals in processing of dietary floury food*" in the specialization course "*Dietary food - characterization, processing technology and health impact*" organized from the funds of this project at Banat's University of Agricultural Sciences and Veterinary Medicine, Faculty of Food Processing Technology between 5-21 May, 2011[http://www.alimente-dietetice-fainoase.ro/index.html]. The material presented by me at this course was published as a chapter entitled **"***The importance of germinated cereals in processing of dietary floury food"* in the course support.

Some of the results obtained in this project have been published in the book *"Dietary floury foods testing and their impact on consumer*" (*published in Romanian*), authors: Ersilia

Alexa, Mariana-Atena Poiana, Monica Negrea, SOLNESS Publishing House, Timisoara, ISBN 978-973-729-242-1, 83 pp., 2010.

In the frame of project ERA 139/01[http://plus.see-era.net], from Regional Program of Cooperation with South-East Europe (ReP-SEE), I was involved in the solving of following objectives: (i) monitoring the content of mycotoxins in cereal grains and medicinal plants from west part of Romania (ii) the possibility to control the mycotoxin production in cereals and medicinal herbs by using of bioactive compounds. For this purpose, I attended the training stage performed by DIAMEDIX IMPEX S.A (Bucuresti) for quantitative determination of mycotoxins in accordance with the legislation using ELISA-RIDASCREEN tests. As a result of work in the frame of this project, we have published 2 chapters: (i) *Chapter VI: The occurence of fungal and mycotoxins in cereals from west Romania* (published in English*)*, pp. 144-164, authors: Ersilia Alexa, Mariana-Atena Poiana, Renata-Maria Sumalan, Monica Negrea and (ii) *Chapter VII*: *Strategies to reduce fungal and mycotoxins contamination of cereals and medicinal plants* (*published in English*), pp. 165-185, authors: Ersilia Alexa Mariana-Atena Poiana, Renata-Maria Sumalan, Monica Negrea in the book "*Occurence of fungi and mycotoxins in cereals and medicinal plants from Romania-Serbia-Croatia area"*, coordinators: Ersilia Alexa, Biljana Avramovic, Jasenka Cosic, EUROBIT Publishing House, Timisoara, 2012, ISBN 978-973-620-935-2.

Also, I was involved in the publication of a booklet entitled **"***Strategies for prevention and control of mycotoxin contamination in cereals and medicinal herbs*" (*published in English*), authors: Ersilia Alexa, Biljana Abramovic, Jasenka Cosic, Georgeta Pop, Mariana-Atena Poiana, Calin Jianu, Monica Negrea, EUROBIT Publishing House, Timisoara, ISBN 978-973-620-919-2, 63 pp. 2012. In addition, during the implementation of this project, I performed mobilities in Croatia (Osijec) to University of Osijek.

In last years I attempted to publish the results of my studies in ISI quoted journals, considering that such publications give international visibility and prestige to those who are involved in the field of education and research. Therefore, in the period 2008-2013, I have published 19 articles in international ISI quoted journals (9 as first author, 1 as corresponding author, 9 as co-author). 10 of these articles were presented in detail in this thesis (Part I/Section I). Also, 11 ISI quoted papers were awarded by UEFISCDI/Program - *Human Resources/Awards for research results/Articles.* Also, I have published 17 articles in journals included in international data basis (7 as first author, 10 as co-author, 3 with international partnership), and 20 articles were presented at international conferences.

Since 2008, I have coordinated the Master program "*Advanced technologies for agricultural raw materials processing***"** at Faculty of Food Processing Technology from our university. In this quality, I have dealt with curriculum development by introducing of new courses, the diversification of optional study packages, the updating of curriculum to requirements of jobs market.

In the frame of Project POSDRU 86 "University for future", DMI 1.2 "Quality in Higher Education", with theme: "*Improving Master programs in the agrofood field by promoting*

innovation and quality assurance, according with qualifications requirements of the Romanian and European Union" (CALIMAS)[http://calimas.usamvcluj.ro/]. I have been short-term expert, responsible for curriculum analysis. For this purpose I have dealt with: (i) the analysis of the content and compatibility of Master programs in the field of food science that run in the universities from Romania and EU countries; (ii) development of some Master Programs Framework in the field of food science; (iii) defining of key concepts, specific and generic descriptors, knowing and functional skills as well as correlations between skills - areas of content - study courses - number of credits for the Master Programs Framework.

In addition to the aforementioned achievements, I was member in the scientific committee for "*The 4th International Conference on Food Chemistry, Engineering & Technology*" (May 30–31, 2013, Timişoara, Romania[http://www.usab-tm.ro/utilizatori/tpa/file/manifestari/Invitation_2013_TPA_Timisoara.pdf]. Also, I'm member in Editorial Advisory Board of *Banat's Journal of Biotechnology* [http://www.bjbabe.ro/editorial-advisory-board/].

I'm member in 3 professional Associations as follows: *Association of Food Industry Specialists from Romania - from Education, Research and Production* (no. 190); *Chemical Society from Romania* (ID 1793) and *General Association of Engineers from Romania* (no. 60732). Also, I'm expert evaluator for Romanian Agency for Quality Assurance in Higher Education.

My professional experience has been enhanced through participation as reviewer in peer-review process for ISI journals such as: *Food Chemistry*; *Food Science and Biotechnology*; *Chemistry Central Journal*, as evaluator for research project such as: *Partnership*, *Human Resources*, *Ideas* and as a member in PhD Juries to the following thesis on my interest topics:

- Member in the PhD Jury of Riron Ramona Cristina, theme: "*Research concerning the antioxidant activity of propolis extract from west part of Romania*", Banat,s University of Agricultural Sciences and Veterinary Medicine, Faculty of Food Processing Technology, November 2006.
- Member in the PhD Jury of BUTA Nadina Ibolya, theme: "*Use of natural extracts in order to improve the oxidative stability of some vegetable oils*", Banat,s University of Agricultural Sciences and Veterinary Medicine, Faculty of Food Processing Technology, September 2013.
- Member in the PhD Jury of ROMAN Lucian-Alexandru, theme: "*Contributions to the study of antioxidant and chromatic properties of red wines from west part of Romania*", Banat,s University of Agricultural Sciences and Veterinary Medicine, Faculty of Food Processing Technology, September 2013.

PART II

Career evolution and development plans

1. Plans for scientific evolution and development

The scientific development plans in my interest field is heading towards the same issues previously mentioned. Considering the results obtained till now, I will continue the work related to bioactive compounds - antioxidant properties of red wine and fruit products for a better assessment of some aspects concerning the impact of different factors, treatments, processing methods on these characteristics. For this purpose, the plan is structured on several interrelated activities in my field of interest that fully complement each other and aims to develop the knowledge in the above mentioned topics. These researches will complement the already described studies.

Also, in the next years, I plan to grow my research on several key directions. My future research will be focused on the study of possibilities to retain the active principles from different vegetable matrices in food products and their influencing factors, to investigate the possibilities to use non-destructive techniques such as NIR, FT-IR spectroscopy to detect the changes and transformations occurring in foods in response to various techniques of processing.

For this purpose the following research topics will be continued or will be developed:

(i) Studies concerning the possibility to enhance the color stability of fruit products by different copigments or cofactors addition. The results may be used for improving the color quality of different berry products as well as for development of various foods with anthocyanin-rich ingredients.

(ii) The identification of factors influencing the level of polyphenolic compounds and polymeric pigments in red wines. The obtained results could be useful to gained more information about wine pigments, especially the polymeric pigments which are the main responsible for the permanent color of red wines. In parallel, will be assessed the impact of enological practices (enzyme treatments, use of commercial tannins, use of alternative oak sources, micro-oxygenation) on red wine antioxidant properties. Also, I will try to assess the antioxidant capacity of red wines by using of multiple assays that could give an overall picture about the antioxidant profile of red wines;

(iii) Assessing the impact of different pre-treatments and techniques used to obtain berries juice on their polyphenolic compounds. These findings will be useful to processors for improving the final content of polyphenolics compounds and antioxidant properties in their products.

(iv) Evaluation the effect of pre-treatments and drying methods on anthocyanins from various berries;

(v) Studies on the optimization of natural extracts rich in bioactive compounds obtaining from different agro wastes by using of advanced techniques that could provide an innovative approach to increase the production of specific compounds used as nutraceuticals or ingredients in the design of functional foods;

(vi) The use of FT-IT spectroscopy for monitoring the lipid oxidation during thermal processing and storage of vegetable oils. The information gained by performing of this

study will be useful in oil quality assessing, promoting the FT-IR spectroscopy as a valuable tool with advantages in terms of speed and expense per analysis.

(i) A research direction that I've been thinking in recent years and I plan to approach in the future is refers to the study of possibility to improve the stability of anthocyanins from different berry products by different copigments or cofactors addition. Scientific research on the chemistry of colors have become of a great significance for improving the color of different fruit products. In berry products the color is an important quality parameter, which influences the consumer's behavior. This parameter could be improved and stabilized by copigmentation. I agree with the opinion of many researchers according to which, copigmentation can be considered a natural, valuable tool for improving the color of food products rich in anthocyanins. Copigmentation reactions developed in different berry products and storage effects on the copigmentation phenomenon are not fully studied until now. Therefore, more studies are required concerning the copigmentation occurring in food products rich in anthocyanins. For this purpose will be studied the factors which stabilize and enhance the anthocyanins color. I intent to test as copigments both pure substances such as flavonoinds, phenolic acids as well as natural extracts rich in cofactors. The overall objective of this direction is to study the factors that enhance and stabilize the color of both pure anthocyanins and different berry juice as a material rich in anthocyanins. The obtained results will contribute to the better understanding of the chemical behavior of anthocyanins in different natural matrix.

The results reported by Wilska-Jeszka and Korzuchowska (1996) highlighted that copigmentation is more intense in berry juices than when it was used the purified anthocyanin molecules. This fact indicates that, there are several other components in the juice that play an important role in the copigmentation phenomenon than just an added copigment molecule. Till now, the most studied copigments were the flavonoids (flavones, flavonols, flavanones, and flavanols). Also, phenolic acids such as caffeic acid, ferulic acid, gallic acid, chlorogenic acid, rosmarinic acid have an important effect on the enhancement and stabilization of anthocyanins, but these compounds have not been studied as extensively as flavonoids (Darias-Martin *et al*., 2002; Talcott *et al*., 2003). Berries do not contain free phenolic acids in high amounts. There are different plant materials that can be used as copigments. A potential source of copigments could be the natural extracts obtained from wine industry by-products (Poiana, 2012) or the extracts obtained from herbs belonging to the *Lamiaceae* family (Khomdram and Singh, 2011). Thus, these extracts could be natural color enhancers, end they could be tested for this purpose. The copigmentation reactions will be monitored using HPLC analysis to assess the changes in anthocyanins, flavonoids and phenolics acid content, but in the same time for identifying the compounds responsible for color enhancement. By using the spectrometry it will be possible to notice the hyperchromic effect and bathochromic shift as a result of copigmentation phenomenon. By color analysis it will be possible to quantify the main color parameters in order to follow the color stability.

(ii) The main objectives of following research direction, which I consider important for my career development, are to investigate the factors influencing the level of polyphenolic

substances, the formation polymeric pigments as well as the antioxidant profile during red wines processing and aging. For solving of some problems, I will performe detailed studies concerning the influence of commercial tannins addition, enzyme treatments, fermentation variables, alternative oak sources, micro-oxygenation, fining agents (bentonite, gelatine), storage temperature and storage time on color and antioxidant profile of red wines. Polymeric pigments occurring in red wines during fermentation and wine aging are stable color compounds. They are less affected by pH and SO_2 than monomeric anthocyanin forms, and their color is usually stable over storage time. Tannin concentrations seem to have more effect on the final concentrations of polymeric anthocyanin than the content of monomeric anthocyanis. During aging, anthocyanins react with tannins to form polymeric pigments or pigmented tannins which are considered to have different protein-binding properties than tannin, and thus, may contribute to the reduction of wine astringency (Remy *et al.*, 2000). Polymerization of anthocyanins occurs most rapidly during fermentation and maceration, but the process may continue throughout the life of red wine. In the wine aging, a greater proportion of their anthocyanins content is polymerized. Like the other wine polymers, they may also be removed due to precipitation. As a result, fining agents that remove tannin may also remove polymeric anthocyanins and reduce the red wine color. It is important to determine if the pre-fermentation treatments (enzyme treatments or the addition of commercial tannins) affect the level of polymeric pigments before fermentation of grape must. In this stage, phenolic compounds are extracted from skin and seeds, and their extraction is influenced by winemaking procedures. The use of pectolytic enzymes have beneficial impact on increasing the anthocyanins content in wines, being a common practice used in oenology. Bautista-Ortin *et al.* (2005) reported that the macerating enzymes may help the extraction of phenolics compounds. However, their addition may modify the color, stability, taste and structure of red wines, because not only anthocyanins are released from skins, but also tannins bound to the cell walls. Also is necessary to study the effect of fermentation variables on extraction of SPP and formation of LPP during winemaking, with a special attention on temperature: by increasing the maximum temperature, will increase the amount of tannin and SPP extracted from grapes as well as the amount of LPP formed during fermentation.

Barrel aging affects the wine color and other sensory characteristic such as astringency due to declining in amount of tannin and dramatically increasing in amount of LPP and SPP. It will be interesting to see the red wine color behavior through maturation using of alternative oak sources.

Another factor that could affect the wine color is micro-oxygenation. The results reported by Castellari *et al*. (2000) have shown that oxygen supply has an essential role in improving of red wine color, because the modification of phenolic compounds in response to oxidation result in more colored and less astringent products. The oxygenation enhanced the content in LPP but decreased the content of caffeic and ferulic acid, catechin, epicatechin and trans-resveratrol compared to the control. On the one hand, oxygen treatment seems to have a negative impact on antioxidant capacity of wine as a result of decreasing the content of low molecular weight phenolics, but on the other hand is helpful for stabilizing the wine color.

Fining agents used in wine industry, such as bentonite, gelatine, casein, egg albumin can lead to considerable decreases in some phenolic compounds (Stankovic *et al*., 2004; Castillo-

Sanchez *et al*., 2008). Thus, I intend to investigate the effects of fining agents on the structure of red wines color as well as on their antioxidant properties.

Based on the knowledge derived from the above-mentioned research may be provided solutions for improving the stability of the red wine color, as well as the enhancing its antioxidant properties.

Regarding the antioxidant capacity of wine, it was mentioned by Rivero-Perez *et al*., (2007) the need to use more determination methods to have a wider picture of their multiple effects. Previous studies on this topic usually reported data obtained from small group of wines. Furthermore, the available researches were done using a reduced number of methods. Sometimes, the results seem to be contradictory, inducing erroneous conclusions and some confusion about the real antioxidant value of wines. For that reason, it is necessary to perform more studies using a large numbers of samples, and different methodologies that could give an overall picture about the antioxidant profile of red wines, which will be very useful for clarifying this confusing situation. For this purpose can be applied different methods for assessing the total antioxidant capacity such as: ORAC or oxygen radical absorbance capacity, ABTS or 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid), DPPH or 2,2-diphenyl-1-picrylhydrazyl, N,N-dimethyl-p-phenylenediamine dihydrochloride, FRAP or ferric reducing antioxidant power, hydroxyl and superoxide radical scavenger activities.

(iii) The next research direction with a great impact on my further evolution, is refer to the evaluation of different pre-treatments and processing methods on the content and profile of polyphenolic compounds in berry juice. In the juice processing technology, significant amounts of health promoting compounds are left in the press cake. The polyphenol compounds contents and their profiles in the processed juice differ from polyphenols in fresh berries matrix. The lost of polyphenols may be significant during processing but it can be often reduced by choosing of a proper processing technique. The objective of this study is to determine the effectiveness of different pectolytic enzymes addition, initial heating, sulfur dioxide treatments, various clarification treatments (by addition of pectinases, gelatine, silica gel, bentonite, cold storage of the juice) and pasteurization to improve the color quality and antioxidant properties of berry juice. The content and profile of anthocyanins, flavonols, and procyanidins, as well as the color parameters and antioxidant properties will be determined throughout processing. Based on obtained results it could be possible to determine the most appropriate pre-treatments and processing method available to produce berry juice with a stable color and high antioxidant properties.

(iv) This research direction is a consequence of the fact that the pre-treatments and drying methods applied to different fruit rich in anthocyanins (blueberries, black and red currants, strawberries, cherries) lead to a significant declines in anthocyanins content, phenolics and antioxidant activity. Also, different degradation products of anthocyanins were identified in samples. Since these fruit are seasonal and their life in fresh state is limited, they have to be frozen or processed. Dried fruit are required in many breakfast cereals, cereal energy bars and health bars. The freeze-drying treatment is an expensive option, thus, there is an increasing

interest in designing cost effective preservation methods able to reduce the losses of biologically active compounds in dried fruit. For this purpose, various drying treatments (air-drying at high and low temperatures, microwave drying, freeze-drying) will be assessed regarding the drying parameters (time, temperature for air-drying, power and time for microwave drying) and quality of the dried product in terms of anthocyanins, phenolic compounds as well as antioxidant activiy. Prior to drying, the fruit will be dehydrated by osmosis using different solute and concentration of osmotic solution in order to reduce the drying time and implicit, for minimizing the losses in bioactive compounds.

(v) Vegetable wastes continue to be a rich and promising source of bioactive compounds. In the next research direction I will try to exploit the potential of different agro wastes, such as fruit seeds and peels resulted in fruit processing sector, to generate natural extracts having a high level of bioactive components with particular advantages, by using of advanced techniques. These extracts could be potentially used as nutraceuticals, ingredients in cosmetics, pharmaceuticals, or in the design of functional foods. The main disadvantages of conventional methods currently used for phenolic compounds extraction (maceration and Soxhlet extraction) are low extraction efficiency and toxic solvent residues in the extracts because they use organic solvent (methanol, ethanol, ethyl acetate, acetronitride) for extraction. The technological advances and the development of new methods (*Pressurized liquid extraction, Subcritical fluid extraction, Supercritical extraction, Microwave-assisted extraction*) for extraction of bioactive compounds provide the opportunity to obtain natural extracts rich in active principles. These methods are highly applicable in obtaining of extracts enriched in bioactive compounds from natural products with several advantages over traditional extraction techniques, such as shorter extraction time, lower cost of the solvent, higher quality of the extraction and environment friendly.

(vi) Concerning the use of FT-IR spectroscopy for assessing the quality of edible oil, I intend to deal with monitoring the oxidative processes occurring during thermall processing or storage of edible oils. My concern for this topic has begun since 2012, in the frame of bilateral project Romania-Greece conducted by me, when I started to use the FT-IR spectroscopy for assessing the olive oil adulteration and degradation on the base of spectral changes at specific wavenumbers. By oxidative degradation of lipids in response to thermal processing or storage, substantial changes are taking place throughout the IR spectrum but most obviously are in the OH region reflecting the formation of alcohols (~3544 cm^{-1}) and hydroperoxides (~3425 cm^{-1}), in the single cis double bonds region (~3005 cm^{-1}), around 1740 cm^{-1} (specific to carbonylic compounds resulted from the hydroperoxide decompositions) and in the fingerprint region (1500−900 cm^{-1}) including the isolated trans portion (967 cm^{-1}). Also, the changes in region (700–725 cm^{-1}) belong to the cis double bonds in unsaturated fatty acids. In addition, I am considering improving the oxidative stability of edible oils by adding natural extracts as potential additives with antioxidant properties. FT-IR spectroscopy could be a useful tool to assess the oxidative stability of oils in a simple and fast way by detecting and quantifying the functional groups arising during the oxidative degradation of lipids.

2. Plans for professional and academic evolution and development

Expanding both the research limits and capabilities to offer support in the field of the bioactive compounds in food processing will be a continuous preoccupation. The experience gained through the research concerning the impact of processes, techniques and storage conditions on bioactive compound in vegetal food, in relation with their antioxidant properties was partially included in the lectures at the graduate and undergraduate levels. Moreover, in some of the laboratory works, some of these aspects are treated. I intend to constantly improve the lectures with the new findings in the areas described above.

Based on the activities developed so far, an extensive set of activities in my interest fields, both at national and international level, are expected. A successful activity for this purpose is not possible without a solid team. The consolidation of research team will be one of the main objectives for the next years. The results could be significantly enhanced if the interdisciplinary research team will be enlarged with Master students and PhD students coordinated as a result of the Habilitation Thesis.

Improving the cooperation with researchers and professors from different research centres and universities both from EU countries and Romania is a priority of the research group. The research activity will be funded by the national and European programs as well as by establishing some contracts with private sector. The results are planned to be valorized in the scientific community, but also to be oriented towards the public interested in the subjects of the research activity.

With respect to the teaching activity, the course "*Advanced food processing techniques*" from Master program "*Food. Human Nutrition*", will be improved by adding new information from the literature, as well as derived from my research activity. Also, the curriculum of the course "*Advanced technologies of plant origin food*" taught at Master program "*Advanced technologies for processing of agricultural raw materials*", will be updated according to my professional development.

In my opinion, university has the role to integrate research and education, and in the same time to disseminate the knowledge towards social and economic environment. Moreover, I consider that the sustainable development of food industry could be helped by addressing the research themes related to immediately needs of this industry. The solving of these issues will involve forms and methods of study whose main reason is to preserve as much as possible the natural potential of raw material to offer foods rich in nutrients and biologically active compounds for consumers.

The strategies applied for my future career development are considering the increase of our university visibility in relationship to the European research centres of similar interest. It will be another objective for the next years and I believe it will be possible by involving in common research projects, exchange of Master or PhD students, exchange of researchers and by publishing of some scientific materials. As a feed-back, these actions have the role to improving the quality of scientific research. As a feed-back, these actions have the role to improving the quality of scientific research.

As a form of exploitation of the results obtained in the research activity, I intend to prepare a teaching program closely related to the needs and motivation of learners. I'm fully aware that the main goal of the teaching/learning process is to provide to the learners a set of knowledge and skills that can be used by them to meet their needs of knowledge and communication. Therefore, I intend to adopt a dynamic form of teaching that can meet multiple objectives and that can be easily adapted to the teaching needs. I think that teaching should be flexible and dynamic to suit the learner's talent and ability while the teacher should be more imaginative, creative and to persist in ensuring that all students receive the necessary knowledge and skills. I will be focused to enhance the teaching qualities and also, I will try to give to my students the opportunity to get more involved in the activities which could develop their interests.

Finally, it have to be underlined that my active role will continuously increase in the future and the main indicators to quantify my professional and academic development as well as evolution will be researches, lectures, and applicative works developed in the mentioned directions.

In order to fulfill the ones previously mentioned, the following future actions will be taken:

- Applying the project proposals in research and teaching directions, both at national and international level;
- Including the results obtaining from research in the teaching programs, mainly for Master and PhD;
- The consolidation of research team by including of Master students and PhD students;
- Creation of sustainable collaborative mechanisms with national and international partners who work in the same or related research and teaching fields
- Publishing the books and articles in specialized journals (especially ISI quoted) together with other researchers and professors on the topics in our field of interest;
- Participation with new research topics to international conferences;
- Improving the cooperation with the economic field, especially in applicative research direction.

The above described foreseen research directions and their results are strongly interesting with respect to the general progress of the knowledge in the field of food technology with direct applications on improving the content of bioactive compounds and antioxidant properties of foods. Starting to the saying "*We are what we eat*", the changing and opening of young generation perception towards the improving of nutritional value and antioxidant properties of food products, the knowledge of the influential factors regarding these characteristics, exploiting the natural potential of raw material, applying of new methods, environment friendly, to obtain foods with added nutritional and biological values, has to be a permanent concern. This can be done by knowledge and education and the teachers, by their responsible actions, have a great role for this purpose. Therefore, it is of a great importance to be performed a continuous action by improving of curriculum for students, by publishing articles in newspapers, through organization informative workshops and seminars.

Part III

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