SZENT ISTVÁN UNIVERSITY
FACULTY OF FOOD SCIENCE

COMPREHESIVE PROFILING OF POLYPHENOL ASSORTMENT IN APRICOTS (*Prunus armeniaca* L.) USING MASS SPECTROMETRY

ÁDÁM NAGY

Thesis of PhD dissertation

Supervisor:
László Abrankó, PhD

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Department of Applied Chemistry

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PhD School

Name: PhD School of Food Science

Field: Food science

Head **Prof. Gyula Vatai, DSc**
Szent István University
Faculty of Food Science
Department of Food Engineering

Supervisor: **Abrankó László, PhD**
associate professor until 31.01.2014
Corvinus University of Budapest
Faculty of Food Science
Department of Applied Chemistry

senior research fellow from 01.02.2014.
MS Proteomics Laboratory
Institute of Organic Chemistry
Research Centre for Natural Sciences
Hungarian Academy of Science

The applicant met the requirement of the PhD regulations of the Szent István University and the thesis is accepted for the defence process.

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Head of PhD School Supervisor

THE SIGNIFICANCE OF THE RESEACRH

The cultivation of apricot (*Prunus armeniaca* L.) has a deep tradition in Hungary. Apricot cannot be considered as an autochthonic species in the Carpathian basin. Hungary is situated on the northern border of the possible growing area of apricot therefore its cultivation is more difficult compared to the other indigenous fruit species. The genotypes adapted to the climate of Carpathian basin kept their Middle-Asian characteristics while enriched with new taste and flavour which make them unique (Surányi 2003).

In the last few years the domestic cultivation apricot was estimated for 15-40 thousand tonnes with which Hungary is the 32th among 67 apricot producer countries of the world (FAOSTAT 2013). In recent years its sales importance on the fresh market was increasing and in addition processed food products containing fruit is getting more significant. Generally the 25% of the total yield is merchandised freshly at the marketplace, 55% of it is processed by the industry and the 20% of it is exported (KSH 2013). The importance of the domestic apricot is indicated by that the EC declared „gönci magyar kajszi" as a product of specific origin with geographical denomination few year ago. It means that the product made from apricots cultivated in Gönc region can be indicated with protected geographical indication (PGI). The palinka of Gönc and Kecskemét has already gained the trademark of "Hungaricum".

Apricot is a good source of several valuable ingredients for human nutrition thereby the regular consumption of it is an important part of the healthy lifestyle. It has a very popular flavour due to its balanced acid and sugar content. It has high fibre and mineral content and contains also several bioactive microcomponents. The most important health promoting components of apricot are the polyphenols and the carotenoids. Epidemiologic studies have proved that long-term diet rich in polyphenols can significantly diminish the emergence of the "civilization diseases" (heart and vascular diseases and different types of cancers deriving from our current lifestyle (Feliciano et al. 2015; Yang and Kortesniemi 2015; Balasundram et al. 2006). Due to the diversity of their chemical structure their human physiological effects also show extreme diversity. Their health promoting effects largely depend on their bioavailability, absorption and metabolism (Crozier et al. 2010) which are influenced by many factors (size, solubility and structure of molecules and the matrix of source components, food processing method, compositing of our intestinal microbiota, etc.). Therefore nowadays in the case of nutritional science comprehensive investigation of polyphenols became more important.

However, the current knowledge about phenolics in apricot are quite limited which is especially true for the genotypes cultivated in Hungary. A typical example

for lack of knowledge about the apricot polyphenol assortment is that the most of the previous studies did not take into account the fact that the nutritional values and the physical parameters of fruit are constantly changing during the development and ripening. Therefore an accurate knowledge of the biochemical and metabolic processes in fruit including the change of the polyphenols is essential for the characterization of the polyphenol assortment and the determination of its suitability for different uses as well as its optimal harvest time following polyphenol characterization.

Aim of this study was to carry out a comprehensive analysis of polyphenols occurring in apricots cultivated in Hungary. Since significant attention is focused for that family of molecules due to their diversified health care properties.

AIMS

The apricot is very important in Hungarian fruit cultivation. Not only the fruit itself but all the food products made of it are very popular. Numerous products of these has already obtained the trademark of "Hungaricum". In the last decade the researches of health promoting compounds not only in the raw but also in the finished products are increased. Such studies in case of apricot are still very incomplete for example compared to grape.

Aim of my PhD study was to carry out a comprehensive analysis of polyphenols occurring in apricots cultivated in Hungary with which I can contribute to extending the current results of polyphenols not only in Hungary, but also worldwide. In order to achieve these goals the followings were aimed:

- Profiling flavonoids and hydroxycinnamoylquinic acids in such a representative assortment of apricots which covers the significant part of genotypes are cultivated in Hungary.
- Understanding the changes of polyphenol assortment in apricot during ripening and among successive vintages.

1. Analysis of vintage effect
   Qualitative and quantitative analysis of polyphenol in apricot genotypes grown in the same area and cultivated in the same way among successive vintages.

2. Analysis of changes in polyphenol during ripeness in space and time
   Qualitative and quantitative analysis of polyphenol occuring in the peel and the flesh of two apricot genotypes.

3. Development of a HPLC-ESI-qToF-MS method which is capable of profiling hydroxycinnamoylquinic acids in plant extract.

4. Quantitation of major polyphenols considered by the result of profiling occuring in apricot. Development of a rapid, selective and efficient HPLC-ESI-QqQ-MS/MS method based on the obtained results.

MATERIALS AND METHODS

Assortment of apricot genotypes

Comprehensive analysis of polyphenol assortment in apricots cultivated in Hungary were carried out on fruits of seven different genotype. Namely *Prunus armeniaca* 'Ananasznij cjurpinszkij', 'Banaesa 4/11', 'Goldrich', 'Gönci magyarkajszi' as well as 1/15, 7/1 and Preventa hybrid (**Table 1.**). All apricot genotypes were grafted on *Myrobalan* rootstock and trees were maintained according to standard apricot orchard management procedure at germplasm collection of the Department of Genetics and Plant Breeding, SZIU (Szigetcsép, Central Hungary, 47° N latitude, 18° E longitude and 95 m altitude). One kg of fruit was harvested from each apricot genotypes at full ripeness stage based on skin colour measurement (CIELAB) in 2010 and 2011.

Table1. *Country of origin and pedigree of the apricot genotypes.*

Genotype	Origin		Pedigree
1/15 hybrid	Central Europe	Hungary	Unknown
7/1 hybrid	Central Europe	Hungary	Mamaia × 20/79/1
'Ananasznij cjurpinszkij'	Eastern Europe	Ukraine	Unknown
'Banaesa 4/11'	Eastern Europe	Romania	Unknown
'Goldrich'	North America	USA	Sunglo × Perfection
'Gönci magyarkajszi'	Central Europe	Hungary	Magyarkajszi clone (1960)
Preventa	Asia	Hungary	Unknown

Apricot ripening row

Changes of polyphenols occurring in apricot during ripening were investigated in cooperation with Faculty of Horticulture Science, Department of Genetics and Plant Breeding. Two apricot genotypes were selected for the analysis, a typical Hungarian apricot ('Gönci magyarkajszi') and a hybrid (Preventa) with ordinary and extraordinary polyphenol content, respectively. Fruits were collected at five different ripeness stage (**Figure 1.**) The flesh and the peel of apricot were separated in order to investigate the distribution of polyphenols in different parts of the fruit.

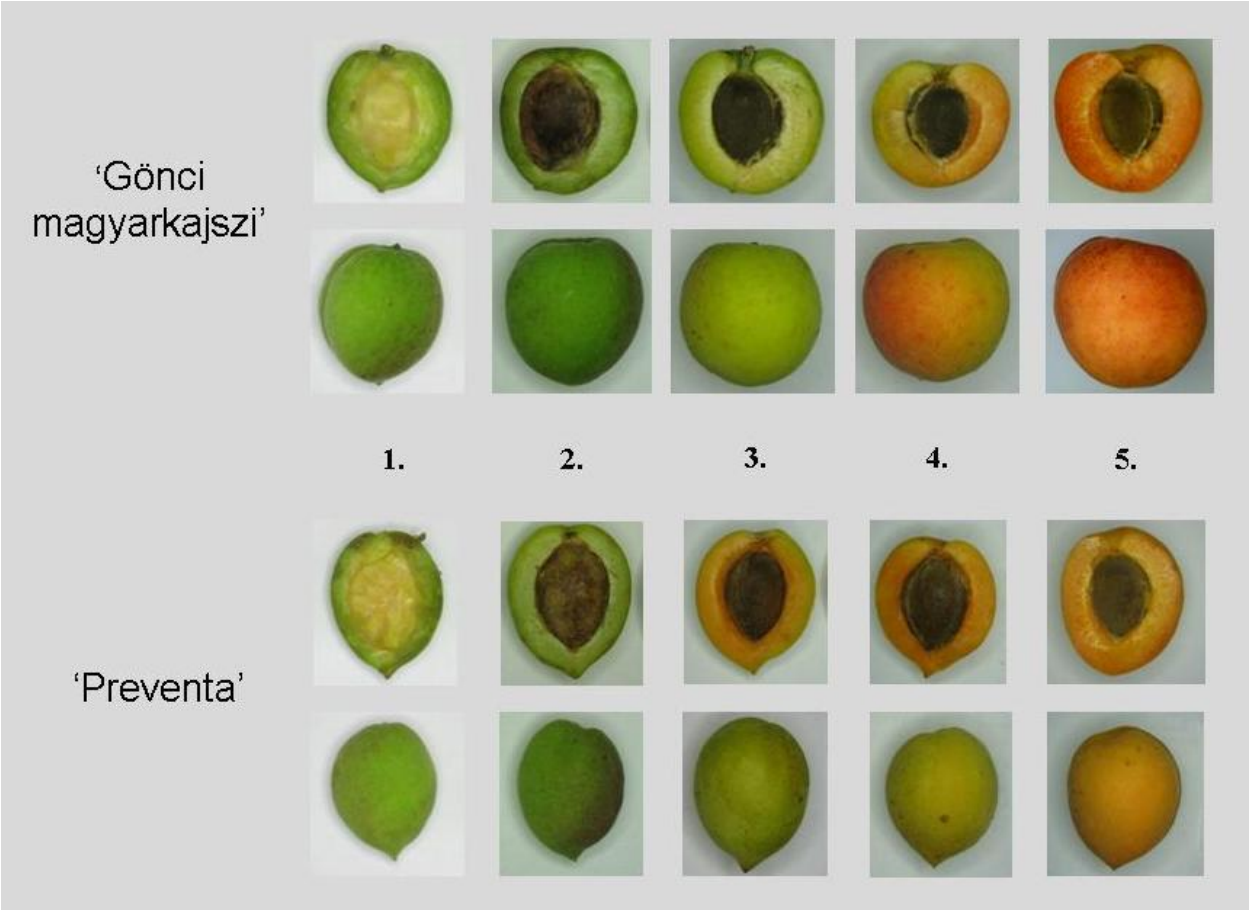

Figure1. *Fruits of 'Gönci magyarkajszi' and Preventa apricot genotypes at five ripeness stage (source: Pfeiffer, 2012).*

Sample preparation

Sample preparation procedure was adopted from Harnly et al. (2007) and was applied with slight modifications. Extracts were made from lyophilized and homogenized plant powders (apricot and green coffee bean) by a methanol/water/formic acid (60:39:1 v/v) extraction solution using ultrasonic bath at room temperature. In order to avoid the degradation of polyphenols the analysis of the diluted extracts were carried out in less than 24 hours.

Profiling HPLC-ESI-qToF-MS methods of polyphenols

Screening of flavonoid derivatives and hydroxycinnamoylquinic acids (HCQA) was carried out on an Agilent 1200 HPLC system (Agilent Technologies, Waldbronn, Germany) coupled to an Agilent 6350 Accurate-Mass Q-TOF LC/MS (quadruple – time-of-flight) hybrid tandem mass spectrometer (Agilent Technologies, Santa Clara, CA USA) equipped with a Dual-Spray ESI source. Chromatographic separation of flavonoids and HCQAs were carried out on a Phenomenex Kinetex C18 RP and Phenyl-hexyl RP 4.6 × 150 mm with 2.6 μm particle sized column (Phenomenex, Macclesfield, U.K.), respectively. Water containing formic acid (mobile phase A) and acetonitrile containing formic acid (mobile phase B) were used as solvents for the elution. During analysis high-resolution (more than 20,000 FWHM) and accurate mass spectra were recorded across the range of 50 - 1,100 *m/z*. The full-scan data recorded was processed with Agilent Mass Hunter software by Agilent MassHunter Software B.04.00 Build 4.0.497.0.

HPLC-ESI-QqQ-MS/MS method for quantitative determination of major polyphenol in apricot

The quantitative determination of the selected polyphenols were carried out by standard addition calibration technique on an Agilent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) connected to an Applied BioSystems 3200 Q TRAP LC/MS/MS (triple quadruple / linear ion trap) hybrid tandem mass spectrometer (Applied Biosystems, Framingham, MA, USA) equipped with Turbo-V IonSpray ESI source. Chromatographic separation was carried out on an Agilent Zorbax Rapid Resolution Eclipse XDB-C18 2.1 × 50 mm with 1.8 μm particle sized column (Agilent Technologies, Waldbronn, Germany). For the elution, 0.1% (*v/v*) formic acid in water (mobile phase A) and 0.1% (*v/v*) formic acid in acetonitrile (mobile phase B) were used as solvents. The MRM data acquisition and processing was processed with Analyst version 1.4.2. software.

RESULTS AND DISCUSSION

Aim of this study was to carry out a comprehensive analysis of polyphenols occurring in apricots cultivated in Hungary by state-of-the-art analytical techniques. Furthermore, my goal was to understand the qualitative and quantitative fluctuations experienced in polyphenol content of apricot among vintages or during ripening.

In order to achieve these goals, firstly a liquid chromatography - mass spectrometry method (HPLC-ESI-qTOF-MS) which is suitable for profiling hydroxycinnamoylquinic acids (HCQAs) from herbal extracts by high-resolution and accurate mass measurement was developed.

Method development for profiling method of HCQAs by HPLC-ESI-qToF-MS

The developed MS profiling method is based on the in-source collision induced dissociation (CID) fragmentation with which non-target screening can be feasible. During analysis it was successfully confirmed that HCQAs are cleaved asunder the bonds of their building blocks by in-source CID fragmentation. Therefore the original quinic acid conjugate can be built up from bottom by detecting these subunits. Such a database was asserted to discover these intact molecules which include all theoretically combination of quinic acid and hydroxycinnamic acids as well as their diagnostic fragments.

Identification is started with automatically seeking of diagnostic ions what is not based only on accurate mass measurement rather comparing chromatographic profiles (*i.e.* retention time, isotope distribution). The optimization of in-source fragmentation in which fragmentor voltage (FV) has key role was carried out by investigating fragmentation of five commercial reference HCQA materials. According to the obtained data the formation maximum of the parent ions are generally between -120 and -200 V, while the maximum of the QA ions observed between -200 and -280 V. For analysis -140 V and -240 V were chosen as a compromise value to the investigation of the parent ions (yielding more parent ions and fewer fragments) and the diagnostic fragments, respectively. The -240 V assists primary in qualitative study while the -140 V allows exploring the exact original intact form.

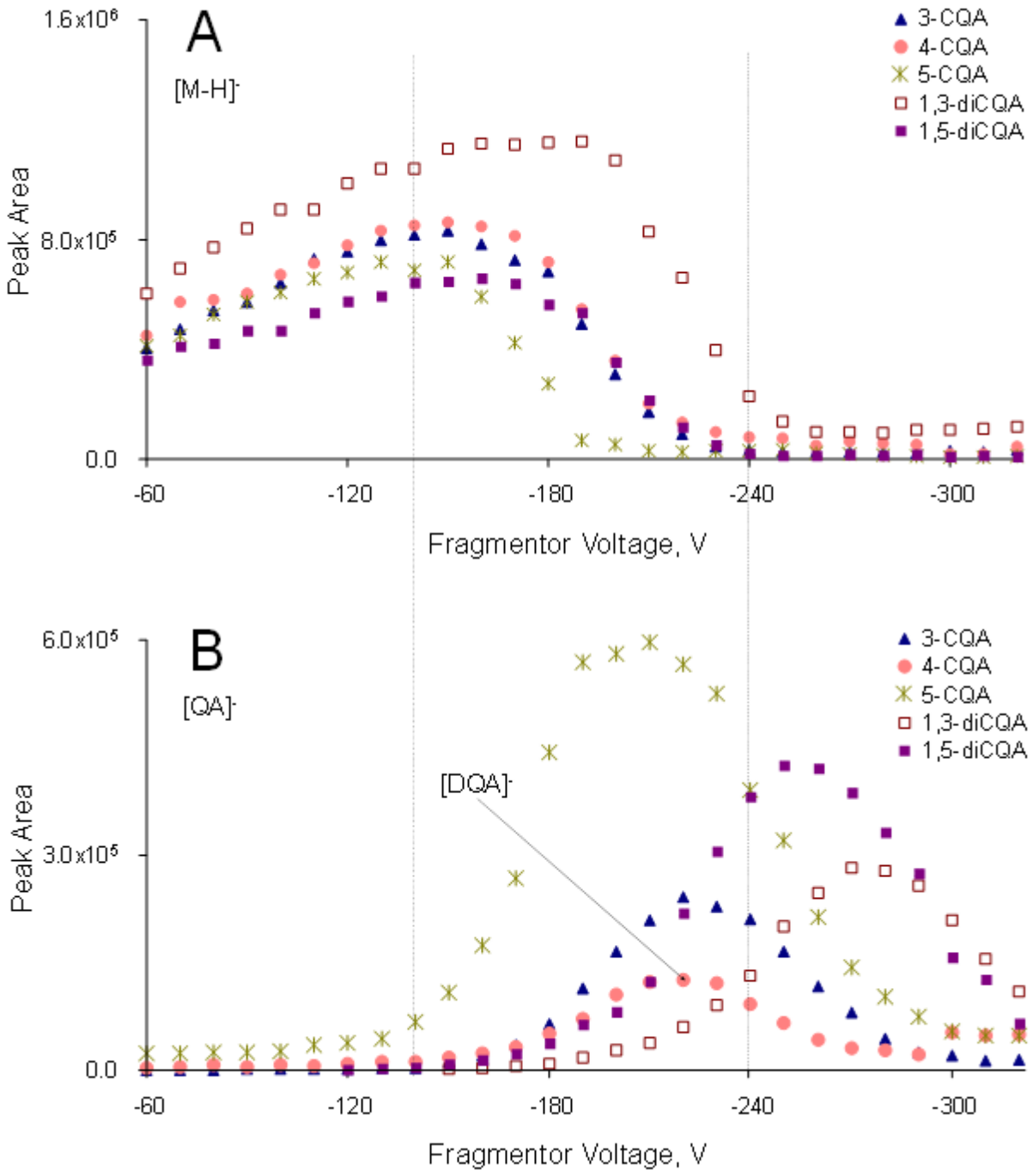

Figure 2. *Optimization of in-source CID fragmentation by fragmentor voltage.*

The method is capable for selective identification of the type of hydroxycinnamoyl moieties; however, it is unable to identify its exact binding location directly. Contingently there is a possibility to get partially structural information from the ratio of yielded fragments based on literature.

Extract of green coffee beans in which HCQAs occur in most diversified and in highest amount, was used for discriminating the applicability of the profiling method since exact structure of its HCQAs has already been identified in the

literature (Clifford et al. 2003; Clifford et al. 2008; Clifford et al. 2005; Clifford et al. 2006; Marmet et al. 2014; Monteiro and Farah 2012).

Twenty-one HCQAs were identified by the developed method among which there is a caffeoyl-*p*-coumaroylquinic acid which has not been previously detected and described from green coffee bean by the author's knowledge. A typical ion chromatogram of green coffee bean is represented by overlapped extracted ion chromatograms (EIC) on **Figure 6.**

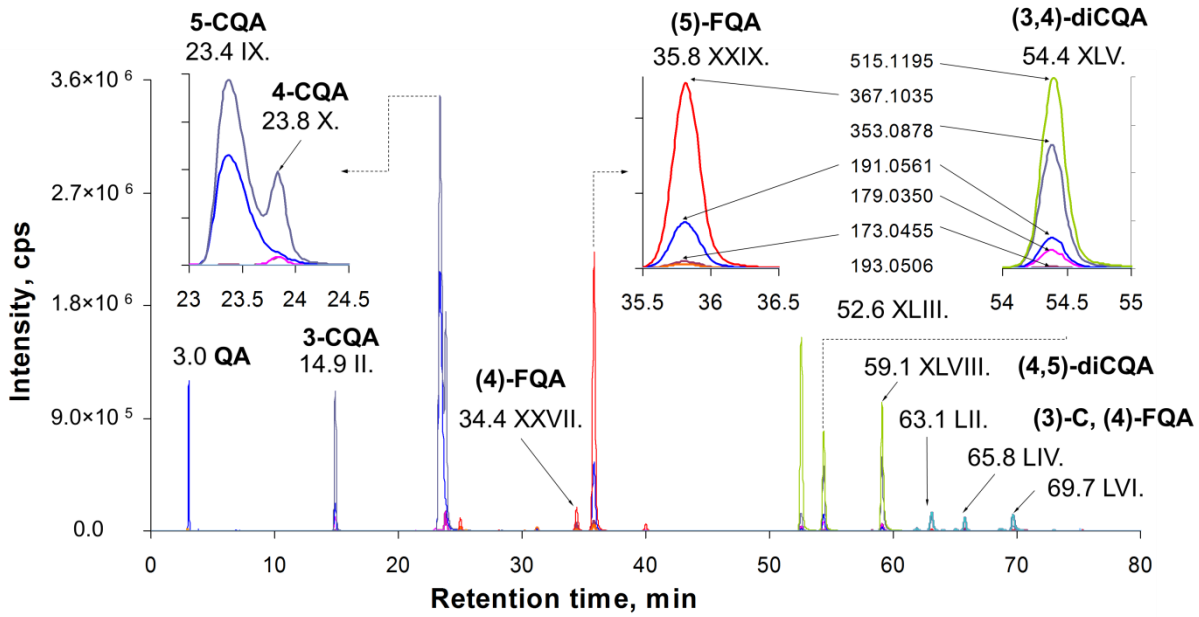

Figure 3. *Overlapped extracted ion chromatograms (EIC) of green coffe bean extract obtained from negative ion mode of HPLC-ESI-qToF-MS analysis.*

For were investigated by classic MS/MS fragmentation in qToF-MS/MS mode to proving the conformance of the method such investigation of the identified components was carried out by classic MS/MS fragmentation in qToF-MS/MS mode. The MS/MS analysis confirmed occurrence of each HCQA and could provide only approximately 30% further information compared to the profiling results. According to the results discussed so far the developed method is suitable for profiling HCQAs in plant extracts including apricot.

Analysis of polyphenol assortment of different apricot genotypes cultivated in Hungary

The major of phenolics occurring in apricot belongs to the family of flavonoid and hydroxycinnamic acid derivatives based on literature (Dragovic-Uzelac et al. 2007; Dragovic-Uzelac et al. 2005; Hegedűs et al. 2010; Ruiz et al. 2005). Fruits of seven apricot genotypes grown in the same area and cultivated in the same way in Hungary were harvested in two successive vintages. Two apricot genotypes which were harvested at five different maturity stage, were selected to examine the changes in polyphenol content during ripening.

Profiling analysis

Profiling of polyphenols occurring in apricot were carried out by two different HPLC-ESI-qToF-MS method. For profiling of hydroxycinnamoylquinic acid (HCQA) and flavonoid were utilized an own-developed method presented in this paper in detail above and a method developed by Abrankó et al (2011), respectively. Both method are based on in-source fragmentation and on accurate mass measurement.

28 different flavonoid derivatives were putatively identified by profiling analysis of flavonoid in apricot genotypes. Nine of these were structurally identified by commercially available reference materials: (+)-catechin, (-)-epicatechin, keracyanin, kuromanin, rutin, quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucoside and quercetin-3-*O*-glucosyl-6”-*O*-acetate. In case of the other components only the aglycone as well as formula of substituents (*i.e*. sugar, organic acid, *etc.*) were successfully identified.

According to the results a naringenin-hexoside belonging to flavanon glycosides can be detected in the majority of the apricot genotypes. Moreover, several procyanidins was also detectable from the fruits. Procyanidins or condensed tannins are developed from flavan-3-ols (*e.g.* (+)-catechin, (-)-epicatechin) which were not originally aim of my researches, however, due to data evaluation it comes upon that the profiling method is also suitable for profiling the group of these compounds.

For the most part high purity (one component) reference material for qualitative identification of HCQAs occurring in apricots was not available therefore green coffee bean which HQCAs are well known in literature can be used perfectly and practically as quasi-reference material. Thus it was utilized as derived standard during the analysis of HCQAs in apricot.

Sample preparation was prepared by spiking with zero (blank), 25 mg and 50 mg lyophilised green coffee bean powder to weighing 150 mg of lyophilised apricot powders with the same extraction manner detailed above earlier. It should be noticed that this kind of approach is only capable of qualitative identification since the HCQA content in extract of green coffee beans is unknown. Components parity was successfully confirmed by addition experiments of green coffee bean thus it has been possible to identify the accurate and presumably structure of the found and confirmed components.

Fourteen different HCQAs were successfully identified by profiling analysis in the fruits of apricot genotypes in which there are a dicaffeoylquinic acid (diCQA) as well as four *cis*-hydroxycinnamoylquinic acids which have not been publicised yet. Clifford et al. (2008) exposed own-synthesized HCQAs to UV radiation which caused partially formation of *cis*-HCQAs from *trans*-HCQAs. In case of green coffee beans which might be exposed to UV radiation during processing, transport and sales, the occurrence of *cis*-HCQAs is awaited not at all or in only very small amount. In spite of this, apricot, especially its peel is continuously exposed to UV radiation that is why the possible occurrence of *cis*-HCQAs is easily explicable in multiple and higher volume. According to my knowledge nobody has been publicised yet about occurrence of *cis*-HCQAs forming naturally. By the way MS/MS analysis also confirmed the occurrence of these four *cis*-HCQAs.

Quantity of polyphenol in apricot

In addition to profiling my goals included determining accurate amount of polyphenols occurred in fruit of apricot. Therefore a rapid and selective HPLC-ESI-QqQ-MS/MS method was developed for quantitative determination of major polyphenols occurring in apricot. According to literature and own profiling results this targeted method was carried out by quantitation of following eight polyphenol: neochlorogenic and chlorogenic acid (HCQAs) as well as (+)-catechin, (-)-epicatechin, rutin, quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucoside and quercetin-3-*O*-glucosyl-6”-*O*-acetate (flavonoid derivatives). The selective MS/MS method was carried out by multiple reaction monitoring (MRM) scan. Enhanced product ion scan was utilized across the range of 50 - 620 *m/z* for the establishment of transitions. Two most abundant fragment ion characteristic only for given compound obtained from EPI spectra were selected for the transitions of the developed MRM method.

Based on results it can be stated that the quantity of the eight polyphenol are particulary diverse in the analysed apricot genotypes. They can be divided into three

groups by polyphenol content: low (‘Ananasznij cjurpinszkij’, ‘Gönci magyarkajszi’, 1/15 hybrid), mid (‘Banaesa 4/11’ ‘Goldrich’, 7/1 hybrid) and high (Preventa). Neochlorogenic acid is dominant in apricots. Chlorogenic acid, (+)-catechin and (-)-epicatechin as well as rutin considered to be major polyphenols which hierarchy was differing among genotypes. Quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucoside and quercetin-3-*O*-glucosyl-6”-*O*-acetate considered to be minor polyphenol which rather are formed in the peel of apricot.

The successfully identified polyphenols from apricot are summarised in **Table 2.**

Qualitative and quantitative fluctuation of polyphenol between vintages

According to the results of two vintages (2010 and 2011) qualitatively only negligible differences were observed in the polyphenol compositing of apricot since same polyphenols were synthetized in the majority of apricot genotypes in both vintage. Although an extremely high (50-112%) variability was observed in the quantities of the measured compounds. The smallest and the largest deviations were observed in case of chlorogenic acid and (-)-epicatechin, respectively.

The means of polyphenol content derived from both years were compared to data observed in the literature (Phenol-Explorer 2004) and were summarized in **Table 3.** The phenolic content of apricot genotypes cultivated in Hungary despite the great variability so far fit well with published results. Generally it can be said that the individually polyphenol content of apricot genotypes cultivated in Hungary are rather close to the upper limits.

Table 2. *List of polyphenols occurring in apricot.*

Polyphenol	Class	Component	Substitution pattern	Formula	Theroretical monoisotopic mass
Flavonoids	Flavan-3-ols	**(+)-catechin**	**3, 5, 7, 3', 4'-OH**	**$C_{15}H_{14}O_6$**	**290.0790**
		(-)-epicatechin	**3, 5, 7, 4', 5'-OH**	**$C_{15}H_{14}O_6$**	**290.0790**
	Procyanidins	procyanidin dimer		$C_{30}H_{26}O_{12}$	578.1424
		procyanidin trimer		$C_{45}H_{38}O_{18}$	866.2058
	Flavonol glycosides	quercetin-deoxyhexoside	3, 5, 7, 4′-OH; *O*-hexoside	$C_{21}H_{20}O_{11}$	448.1006
		quercetin-3-*O*-glucoside	**5, 7, 3'-OH; 3-*O*-glucoside**	**$C_{21}H_{20}O_{12}$**	**464.0955**
		quercetin-3-*O*-glucosyl-6”-*O*-acetate	**5, 7, 3'-OH; 3-*O*-glucoside; 6"-*O*-acetate**	**$C_{23}H_{22}O_{13}$**	**506.1060**
		quercetin-hexosyl-acetate	3, 5, 7, 3'-OH; *O*-hexosyl-acetate	$C_{23}H_{22}O_{13}$	506.1060
		quercetin-hexosyl-malonate	3, 5, 7, 3'-OH; *O*-hexosyl-malonate	$C_{23}H_{22}O_{15}$	538.0959
		quercetin-dihexoside	3, 5, 7, 3'-OH; *O*-dihexoside	$C_{27}H_{30}O_{17}$	626.1483
		kaempferol-3-*O*-glucoside	**5, 7, 4′-OH; 3-*O*-glucoside**	**$C_{21}H_{20}O_{11}$**	**448.1006**
		kaempferol-3-*O*-rutinoside	**5, 7, 4′-OH; 3-*O*-rutinoside**	**$C_{27}H_{30}O_{15}$**	**594.1585**
		quercetin-deoxyhexosyl-hexoside	3, 5, 7, 3',4'-OH; *O*-deoxyhexosyl-hexoside	$C_{27}H_{30}O_{16}$	610.1534
		rutin (quercetin-3-*O*-rutinoside)	**5, 7, 3',4'-OH; 3-*O*-rutinoside**	**$C_{27}H_{30}O_{16}$**	**610.1534**
		kaempferol-deoxyhexosyl-dihexoside	3, 5, 7, 4'-OH; *O*-deoxyhexosyl-dihexoside	**$C_{33}H_{41}O_{20}$**	757.2191
	Flavanon glycosides	naringenin-hexoside	5, 7, 4'–OH; *O*-hexoside	$C_{21}H_{22}O_{10}$	434.1213
	Anthocyanins	**kuromanin (cyanindin-3-*O*-glucoside)**	**5, 7, 4'-OH; 3', 5'-OCH_3; 3-*O*-glucoside**	**$C_{21}H_{21}O_{11}^+$**	**449.1084**
		keracyanin (cyanindin-3-*O*-rutinoside)	**5, 7, 4'-OH; 3', 5'-OCH_3; 3-*O*-rutinoside**	**$C_{27}H_{31}O_{15}^+$**	**595.1663**
Hydroxycinnamoyl-quinic acids (HCQAs)		*p*-coumroylquinic acid	1, 3, 4, 5-OH; *O*-*p*-coumaroyl	$C_{16}H_{18}O_8$	338.1002
		necholorogenic acid (3-*O*-caffeoylquinic acid)	**1, 4, 5-OH; 3-*O*- caffeoyl**	**$C_{16}H_{18}O_9$**	**354.0951**
		kriptocholorogenic acid (4-*O*-caffeoylquinic acid)	**1, 3, 5-OH; 4-*O*- caffeoyl**	**$C_{16}H_{18}O_9$**	**354.0951**
		cholorogenic acid (5-*O*-caffeoylquinic acid)	**1, 3, 4-OH; 5-*O*-caffeoyl**	**$C_{16}H_{18}O_9$**	**354.0951**
		feruloylquinic acid	1, 4, 5-OH; *O*-feruloyl	$C_{17}H_{20}O_9$	368.1107
		dicaffeoylqunic acid	1, 3, 4, 5-OH; di-*O*-caffeoyl	$C_{25}H_{24}O_{12}$	516.1268

Table 3. *Comparing polyphenol contents among apricot cultivated in Hungary and publicised in literature (source: Phenol-Explorer).*

	Polyphenols	Apricot			1/15 hybrid	7/1 hybrid	'Ananasznij cjurpinszkij'	'Banaesa 4/11'	'Goldrich'	'Gönci magyarkajszi'	Preventa
		Min	Max	**Mean**	mg/100 g fresh weight						
Flavanols	(+)-catechin	0.31	4.95	**2.96**	1.40	8.60	3.61	9.85	3.83	4.37	52.45
	(-)-epicatechin	0.02	6.06	**3.47**	0.66	6.45	7.01	18.07	7.82	10.32	5.16
	Procyanindin dimer B1	0.09	0.09	**0.09**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Procyanindin dimer B3	0.05	0.05	**0.05**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Procyanindin dimer B7	0.01	0.01	**0.01**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Procyanindin trimer EEC	0.01	0.01	**0.01**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Flavonols	Kaempferol-3-*O*-rutinoside	0.01	0.56	**0.12**	0.11	0.25	0.36	0.37	0.18	0.22	0.41
	Rutin	0.24	2.27	**0.83**	4.03	7.69	8.06	9.06	5.01	5.07	4.38
	Quercetin-3-*O*-glucoside	-	-	-	0.47	0.59	0.42	0.36	0.13	0.28	0.19
	Quercetin-3-*O*-glucosyl-6"-*O*-acetate	-	-	-	0.57	0.26	0.75	0.73	0.30	0.35	0.05
HCQAs	Necholorogenic acid	2.60	7.80	**5.38**	22.31	25.74	9.53	59.50	23.24	18.19	180.54
	3-*O*-feruloylquinic acid	0.40	1.20	**0.60**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	3-*O*-*p*-coumaroylquinic acid	0.20	0.70	**0.38**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Cholorogenic acid	0.30	10.30	**3.58**	2.49	11.45	4.32	9.48	7.49	4.71	28.13
	5-*O*-feruloylquinic acid	0.00	0.20	**0.04**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	5-*O*-*p*-coumaroylquinic acid	0.00	0.30	**0.06**	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.a.: nont analysed

Changes of polyphenols in apricot during ripening

According to profiling and quantification results of polyphenols in peel and flesh derived from the five maturity stage of the two apricot genotypes, the phenolics content are initially increased during the ripening then started to decrease as ripening progressed to full ripeness stage, however, this change has different profile in the case each of polyphenol groups.

Based on these cannot be determined such stage of maturity in which maximum of total polyphenol can be interpretable therefore can not speak about a generally and uniformly interpreted "maximum phenolic maturity" in case of apricot. Concept of maximum phenolic maturity makes sense only in case of polyphenol groups which maximum maturity is close to each other. Furthermore it can be stated that stage is considered full ripeness by colour parameters does not meet the stages in which the different phenolics group reach their quantity maxima.

Prominent apricot genotype

Preventa hybrid is a unique apricot genotype in several aspects. It has prominent polyphenol content which is mostly due to the content of its neochlorogenic acid. It contains neochlorogenic acid in 3-19 times higher amount compared to other apricot genotypes and (+)-catechin and chlorogenic in significant quantity. These can be explained with the fact that flesh of Preventa contains these polyphenols also in very large quantity compared to the other apricot.

Furthermore it is nameable that among the analysed apricot genotypes fluctuations in its polyphenol contents were proved to be the lowest between 2010 and 2011.

NOVEL SCIENTIFIC RESULTS

1) I developed a liquid chromatographic-mass spectrometric method based on high resolution and accurate mass for the selective identification of hydroxycinnamoylquinic acids. I used for the first time green coffee bean extracts as reference materials for confirmation and identification of hydroxycinnamoylquinic acids occurred in apricot.

Identification is started with automatically seeking of diagnostic ions what is not based only on accurate mass measurement rather measurement comparing chromatographic profiles (*i.e.* retention time, isotope distribution). The method is capable for selective identification of the type of hydroxycinnamoyl (HCA) moieties; however, it is unable to identify their exact binding location directly.

2) I carried out the profiling of flavonoids and hydroxycinnamoyl quinic acids in the most significant apricot genotypes cultivated in Hungary and determined quantity of their major polyphenols.

Based on the profiling analysis, 28 different flavonoid derivatives and 14 hydroxycinnamoylquinic acids were detected. Eleven of these were structurally identified by commercially available reference materials.

3) According to the results of two vintages (2010 and 2011), the quantity of polyphenols in studied apricots showed an extremely high (50-112%) variability for most polyphenols.

Among the studied compounds, the smallest and the largest deviations were observed in case of chlorogenic acid and (-)-epicatechin, respectively.

4) **I confirmed that the amount of phenolics in the peel and flesh of apricot fruit is initially increasing during the ripening, then starts decreasing as ripening progressed to full ripeness stage, however, this change has different profiles in the case of each polyphenol group. I concluded that no particular maturity stage in which polyphenols generally and uniformly reach their maxima cannot be deteremined in case of apricot. Moreover, the maturity stage, which is considered full ripeness based on colour parameters, does not meet the stages in which the different phenolics group reach their maxima.**

5) **I confirmed that Preventa hybrid has prominent polyphenol content, which is mostly due to its outstanding neochlorogenic acid content, which compound is also characteristic for the other apricot genotypes.**

Preventa contains neochlorogenic acid at 3-19 times higher quantity compared to other apricot genotypes and (+)-catechin and chlorogenic acid are also present at outstanding levels. These can be explained with the fact that flesh of Preventa contains these polyphenols also in very large quantities compared to the other studied apricots.

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LIST OF PUBLICATION RELATED TO THE DISSERTETION

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