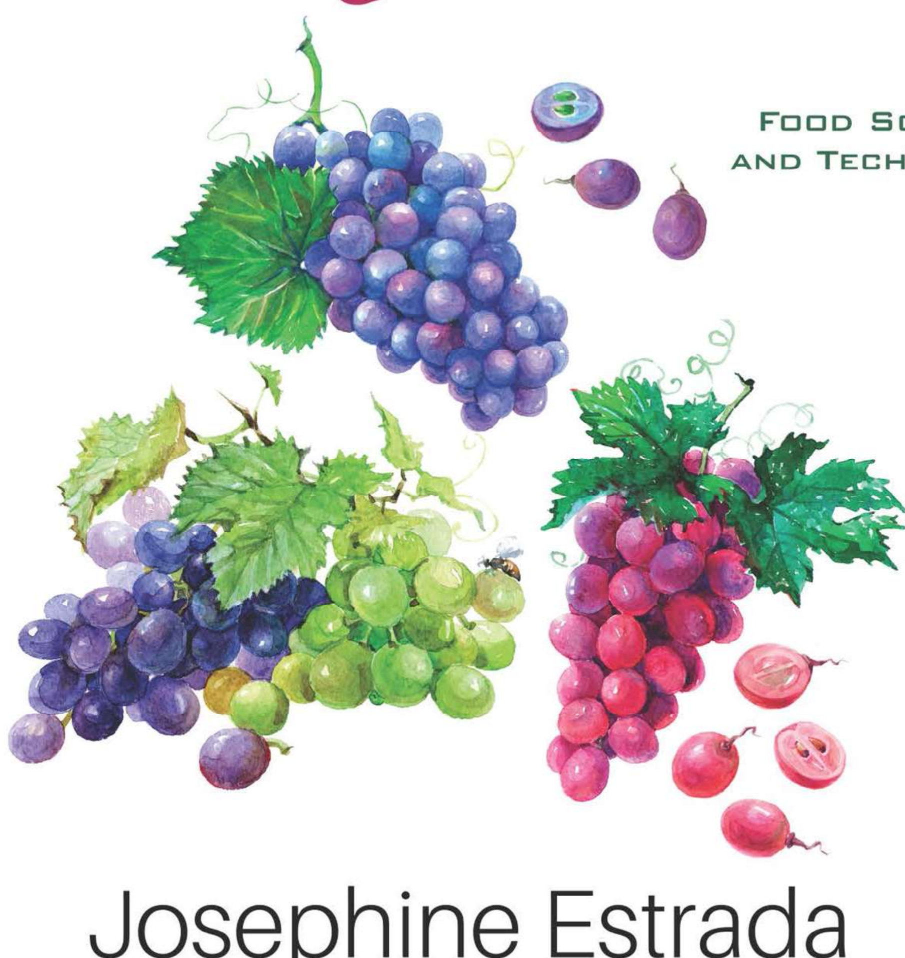
Grapevines at a Glance

Food Science and Technology



Josephine Estrada
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Chapter 1

CHARACTERIZATION OF GRAPEVINE VARIETIES INDIGENOUS TO THE BALKANS REGION

Dragana Č. Dabić Zagorac[1], Slavica R. Todić[2], Vera S. Rakonjac[2], Milica M. Sredojević[1] and Maja M. Natić[3]*

[1]Innovation center of the Faculty of Chemistry, Belgrade, Serbia
[2]Faculty of Agriculture-University of Belgrade, Serbia
[3]Faculty of Chemistry-University of Belgrade, Belgrade, Serbia

ABSTRACT

Central Balkan region is at the crossroads between Asia and Europe, on the line dividing various nations and civilizations of the East and the West. Due to its favorable climate and geological characteristics, it is an ancient wine growing region hosting a wide range of indigenous grapevine varieties, most of which are not internationally recognized.

* Corresponding Author's E-mail: mnatic@gmail.com.

Also, numerous traditional local varieties, present in this area for centuries, are out of cultivation. Therefore, preservation and characterization of grapevine germplasm is important not only for the breeding process, but also for the improvement of modern varieties and their preservation for the future generations. Also, indigenous varieties as valuable resource of gen donors could be helpful when facing the challenges of climate changes. Unfortunately, during the last decade grapevine germplasm is undergoing a process of rapid genetic erosion, and as a consequence we are facing with the loss of varieties which are traditionally related to different winegrowing regions. The only way to prevent the loss of this heritage is to locate them, evaluate, preserve and precisely characterize them. In this Chapter the results of morphological and genetic characterization, as well as chemical characterization of some of the most important indigenous grapevine varieties in the central Balkan, such as Vranac, Krstač, Smederevka, Prokupac, Žilavka, Plavac Mali, and Istrian Malvasia will be summarized. These old varieties have passed through the process of natural selection and adapted to particular environmental conditions. As such, they represent irreplaceable genetic value for each country, and for the entire region.

Keywords: autochthonous varieties, Balkans region, ampelographic characterization, genetic characterization, chemical characterization

1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the oldest cultivated plants in the world, with a long history of cultivation and utilization. Grapevine is considered to be one of the commercially most important horticultural species in the world.

The Balkan Peninsula is one of the three large peninsulas in Southern Europe and covers a surface area of 550,000 square km, including the islands. This area is interspersed with extensive mountain range systems and broad plains. It is characterized with a jagged coastline, a multitude of rivers, lakes, plains and gorges, all of which facilitated the initial settling of these areas, as well as successful cultivation of various plant crops. The Peninsula belongs to two clearly defined climatic zones: Mediterranean and Central European continental. Local climatic conditions are also affected by mountain ranges, plains and valleys, so there is a lot of variability between these two respective climatic zones. This climatic variability, combined with a wide variability in the soil types of Balkans, its geology, the use of various grapevine rootstocks, all contributed to a very rich specter of cultivated plants, including grapevines.

As the Balkans had been a crossroad of civilizations over millennia, and thanks to its favorable climate and geology, it is an ancient wine-growing region with a long tradition of viticulture [1]. Many grapevine varieties have been preserved and adopted in the Balkan region during this long history of grapevines migrating from Asia Minor to Europe. At the beginning of the 2nd millennium B.C. domesticated grapevines were found in the Southern Balkans [2].

In recent decades, there has been an encouraging and growing resurgence of interest for indigenous grapevine varieties in the Balkans, and this trend has both the scientific and commercial aspects. The reason for this is that: i) these ancient varieties have passed through a long process of natural selection and adaptation, thus being ideally suited to the varied environmental conditions of the Balkan Peninsula; ii) globally, they represent a valuable source for future selection and hybridization, as they contain genes that could respond to the challenges of climate change; iii) wines produced from indigenous varieties are gaining attention in the world wine market, and through their authenticity they also contribute to the recognition of the regions of their origin.

The Balkan countries, such as Serbia, Bulgaria, North Macedonia, Montenegro, Bosnia and Herzegovina, Croatia, Greece, and Turkey have a very long tradition in viticulture and represent a very rich gene pool of grapevines. This gene pool has not been investigated into sufficient detail so far. Since grapevine varieties have been cultivated in the area for centuries, there were spontaneous hybridizations, and numerous mutations within the varieties as a result of adaptation to varying environmental conditions. Those varieties that have thrived in that geographical area have undergone a long process of natural selection and therefore represent an invaluable genetic resource for all the countries in the region.

There are many varieties with the same name in this area, and also there are varieties with different names but the same genotype, which to a great extent impedes the determination of the actual number of existing genotypes. In order to fully determine and evaluate the grapevine genetic resources in the Balkans, it is necessary to employ methods from ampelography, genetics and chemistry.

Therefore, the present Chapter provides an overview of ampelographic, genetic and chemical characteristics of certain commercially important indigenous varieties from Serbia, Croatia, Montenegro, and Bosnia and Herzegovina. All these countries from the Balkans possess a number of old indigenous grapevine varieties and these would by nature carry a multitude of mutations within themselves, reflecting the conditions and viticultural traditions of this geographic area.

2. Historical Overview of Grapevines

Using modern molecular analysis, a close genetic link has been determined between the local wild forms of grapevines and indigenous grapevine varieties cultivated in the Southern Anatolia [3]. This finding led to a precise determination of the location and the time where grapevines were first introduced into cultivation. Thus it is now thought that the ancient Mesopotamia, specifically the Tigris–Euphrates river system in the Taurus Mountains, represents the origin of viticulture [4].

Other research indicates that, in addition to this primary center of geographical origin, there are one or more secondary centers in the Mediterranean zone of Europe. This hypothesis of multiple centers of origin is based on the presence of significant morphological differences between the grapevine varieties of the Middle East and of the Western Europe [5]. Recent molecular research, as well as research looking into morphological characteristics of wild grapevine seeds and a number of varieties of cultivated grapevine, appears to support the hypothesis that there are multiple centers of introduction of grapevine into cultivation [6].

The Balkan Peninsula is an area where wild forms of grapevines occur naturally. Some regional archaeological findings from the Neolithic period contain grapevine seeds of the *spp. silvestris*, which was determined based on their morphological characteristics.

Cultivated grapevine was brought from the Middle East to the Balkans via the Greek and Thracian merchant routes. Ancient Greek merchants then further spread grapevines to various islands and all over the Adriatic coast (500 – 400 years B.C.), but it was the Romans who had the greatest role in spreading viticulture, as they had customarily planted grapevines besides their garrisons and villages. The first reported occurrence of *Vitis vinifera* in the Balkans dates to the Neolithic period, in the wild forms of grapevines [7]. In the beginning of the second millennium B.C. domesticated grapevines were found in the Southern Balkans [8].

The beginnings of viticulture and winemaking in the territory of the Balkans were determined thanks to the ancient wine vessels dating back to the Iron Age (approx. 400 B.C.), as well as the Bronze Age (approx. 200 B.C.). Based on the fossil remains found in the territories of Croatia, Bosnia and Herzegovina, and Serbia, it would appear that the grapevine has been independently domesticated in all these regions [7]. According to Ibid., historians Dio Cassius (40-110 AD) and Strabo (from 63-64 BC to about 24 AD) described the Illyrian and Celtic grapevines from the region of Pannonia (located in the present day Serbia, Croatia, Hungary and Romania).

The turbulent history of the Balkans and the influences of different cultures that came and went affected the ups and downs in the development of viticulture. During the Middle Ages, the Roman Empire promoted viticulture and spread the cultivation of grapevines throughout the Balkans. Upon the arrival of the Slavic peoples into the Balkans (600-900 AD), they discovered grapevines and took to viticulture. During the medieval period, viticulture progressed thanks to the feudal authorities and the Christian monasteries on whose properties the grapes were commonly grown. With time, wine gradually became a true national beverage among the common people.

After the Ottoman Empire expanded into the Balkans, wine production and consumption had significantly decreased, however the cultivation of table grapes was expanded, mostly involving varieties of the Middle Eastern origins, such as Afuz Ali, Drenak, Čauš and Sultana [7].

By the end of the 19th century, viticulture in the Balkans suffered the same horrendous fate as in the rest of Europe – the devastation of vineyards caused by the expansion of phylloxera, a sap-sucking insect native to North America. After this mass ruination of vineyards, a viticultural renewal commenced throughout Europe, including the Balkans.

After the World War II, there was a period of increased introduction and expansion of new grapevine varieties and rootstocks, in parallel with the intensification of viticulture in the Balkans [9]. This has affected the structure of cultivated varieties, whereby areas under minor local varieties were being reduced, while famous (mostly French) varieties gradually were replacing them. As a consequence of this, over the subsequent decades, varieties traditionally common in some regions of the Balkans almost became extinct.

3. Origin and Distribution of Economically and Historically Significant Grapevine Varieties Indigenous to the Balkan Peninsula

3.1. Prokupac

Synonyms: Kameničarka, Rskavac, Niševka, Crnka, Zarčin, Skopsko Crno

Prokupac is an old grapevine variety for used dry red wines, and considered to be autochthonous to Serbia. It belongs to Convar pontica, Convarietas balcanica [1].

Prokupac is the most significant Serbian indigenous grape variety, and is also historically important for the Serbian wine industry. A comparative DNA profiling of old grape varieties grown in Serbia showed that a sample of Prokupac collected in the south of Serbia perfectly matches the SSR profile of the Prokupac accession which is maintained in the DEU098 collection [1, 10]. Analyses have confirmed Prokupac to be the (male) parent of an old Turkish indigenous grapevine variety known as Papaskarasi [11]. Prokupac is common in all Serbian wine-growing districts, especially in southern Serbia (in the Toplica and Župa regions.) Prokupac is also grown in the neighboring North Macedonia and Bulgaria.

3.2. Smederevka

Synonyms: Dimyat, Belina, Belina Krupna, Zoumiatiko, Galan, Szemendriai Zold, Dertonia

Smederevka is an old grapevine variety from the Balkans. Smederevka belongs to Convar pontica, Convarietas balcanica [1]. Serbian variety Smederevka and the Bulgarian Dimyat were suggested as synonyms on the basis of their morphological descriptors [12]. It was reported that Zoumiatiko (with its synonyms Dimyat and Smederevka) is a grapevine variety considered to originate from the Egyptian city Dimyat (Dammieta), hence its name [13, 14]. The Greek name for this variety, Zoumiatiko, has its origin in the Greek word "ζουμίν", which suggests a high sugar concentration of its berries. This variety is grown in almost all Serbian wine regions, and its local name originates from Smederevo, the town around which it has been grown since the times of the Roman Empire (third century B.C. [15]. Smederevka is a natural cross of Coarna Alba and Heunisch Weiss. It is grown in Serbia, Bulgaria, North Macedonia, Greece, Russia, Moldavia and Turkey. It is mainly used for the production of crisp white wines with a non-specific aroma and a high acid content [16].

3.3. Krstač

Synonyms: Krstača Bijela, Bijeli Krstač, Bijela Vinogradarska

Krstač is an autochthonous white grape variety of Montenegro and it is dominant among the local white wine varieties. Its name comes from the appearance of the bunch which resembles a cross. Krstač was believed to be autochthonous of Montenegro and probably originated from Beri (near Podgorica) [17]. Through international research projects which utilized methods of ampelography and DNA profiling, these local origins of 'Krstač' were confirmed [18].

3.4. Vranac

Synonyms: Vranec, Vranac Prhljavac, Vranac Crmnički

Vranac is an autochthonous red wine grape variety of Montenegro. It produces a dry red wine of a dark ruby color. The oldest historical document mentioning Vranac, among other autochthonous varieties, was the Medieval Statute of Budva, published in the 15th century, as cited in [19]. Previously, it was thought that Vranac has a parent-offspring relationship with Kratošija. Recent research involving pedigree and parent-offspring analyses was conducted in order to examine the genetic relationship between the two varieties and their origin and history. This research determined that Vranac was the progeny of Kratošija (the male parent) and Duljenga (the female parent). Vranac shares the same chlorotype (D) with Duljenga. This pedigree was confirmed using 25 micro-satellite markers [20].

Nowadays Vranac is widely planted in North Macedonia and Montenegro, where it is the main red wine grape variety. It is also grown in Serbia, Bosnia and Herzegovina, and to a lesser extent in Croatia. Although Vranac is the most commonly planted variety in North Macedonia, genetic studies have not shown a close relationship between this and other indigenous varieties grown in that region [21, 22].

Montenegro's viticulture and wine production were based, and still are today, on growing indigenous varieties, and wines from these varieties (Kratošija, Vranac, Krstač) have become recognizable national brands of Montenegro.

3.5. Plavac Mali

Synonyms: Babić, Crljenak, Kaštelanski, Kasteljanac, Pagedebit Mali, Pagedebit Crni, Kastelka, Plavac Mali Kaštelanski, Zelenac

Plavac Mali is an autochthonous Croatian (Dalmatian) grapevine variety. It is known for its rich, flavorsome wines. Well known Plavac Mali wines are Dingač and Postup from the Pelješac peninsula and the islands of Hvar and Brač. In the early 19th century, Dalmatia had as many as 400 grape varieties [23] that thrived in its favorable climate, and its viticulture benefited from the geographic location, the long maritime tradition and the well-developed trade with surrounding countries. Around 200 varieties were described in Dalmatia[24], most of which were considered to be autochthonous [25]. In spite of significant genotype losses, a considerable number of indigenous Dalmatian grapevine varieties had been preserved [26]. Plavac Mali is among the most widely planted and venerated autochthonous varieties from this region. This variety was first described by [27].

Comparative DNA analysis had shown that Plavac Mali is the progeny of Zinfandel and Dobricic [28]. Plavac Mali is mostly planted in the Middle and Southern Dalmatia sub-regions, and is also found on the island of Krk, and the Northern Dalmatia and Dalmatinska Zagora sub-regions.

3.6. Malvazjia Istarska

Synonyms: Malvazija, Istarska Malvazija Malvazija Istarska Bijela, Vrbić, Vrbić Bijeli, Polijšakica Drnovk, Malvasia, White Malvasia Istria, Malvoisie d'Istrie Blanche, Malvasia Friulana, Malvasia Nostrale, Istrijanka, Borgonja Bela

Malvazija Istarska is an autochthonous Istrian variety and the most frequently planted white grape in Istria. Although it is widely accepted that Malvasia is a name of Greek origin (from the medieval town Monemvasia) and that wines named Malvasia had been brought into Europe from the Peloponnese region, published studies have not determined a strong link between the modern-day Greek varieties and those grown in Croatia and Italy. There is one exception to this, and this is one variety that exists in Greece, Italy and Croatia under synonyms, respectively: Pavlos, Malvasia Lunga (Malvasia del Chianti) and Maraština [29]. Current understanding is that Malvazija istarska represents a unique genotype from the Istrian peninsula and the north-east Italy.

3.7. Žilavka

Synonyms: Žilavka Mostarska

Žilavka is considered to be an autochthonous grape variety originating from Herzegovina, and is nowadays the most economically significant white wine grape in that region. Žilavka has been in cultivation in the region of Herzegovina for longer than 600 years, being first mentioned in the 14th century: the medieval Bosnian King Tvrtko was described as drinking a Žilavka wine [30]. In the 19th century, due to the famed grape quality and its resistance to bunch rot (*Botrytis cinerea*), the Austro-Hungarians used this variety for the production of a special dessert wine of the Malaga type [31]. The parentage analysis performed by [11] showed that among the progenitors of Žilavka were Furmint, Alba Imputotato and Goher. It was reported that the DNA profiling could suggest a genetic relationship between Žilavka and Teran Bijeli (Prosecco) grown in Istria [32].

Results in [33] suggested that Žilavka might be the offspring of an old female Romanian progenitor named Alba imputotato and another Bosnia and Herzegovina accession Dobrogostina (synonym Stara Žilavka).

Morphological analyses of varieties grown in Herzegovina indicated a large degree of similarity between the varieties Žilavka and Krkošija [34]. These two varieties are commonly grown together in the same vineyards [35].

Žilavka produces popular and economically important wines in Bosnia and Herzegovina, but also in Croatia, North Macedonia, Montenegro and Serbia, where it is also grown [35].

4. Ampelographic Description

Morphological characterization is an essential first step when describing grapevine varieties, and is also performed in varietal collection plantation and when testing the accessions within a collection [25]. Although there has been a substantial advancement in the biochemical and genetic identification of varieties, an ampelographic description of varieties using morphological features is still essential when researching grapevine species, varieties or clones [36, 37]. The application of ampelographic descriptors is also quite simple, inexpensive and can be used either in the field or in a laboratory setting [37]. In Table 1 ampelographic description of Balkans autochthonous varieties: Prokupac, Smederevka, Vranac, Krstač, Plavac Mali and Malvazija Istarska is shown. Description and measurements were done according to given instruction for each descriptive code (OIV: OIV descriptor list for grape varieties and *Vitis* species, 2009).

Table 1. Ampelographic description of some grapevine varieties that are autochthonous to the Balkan region using OIV codes

Trait		OIV code	Prokupac	Vranac	Krstač	Smederevka	Žilavka	Plavac Mali	Malvazija Istarska
Young shoot	Intensity of anthocyanin coloration on prostrate hairs of the shoot tips	3	5	3	3	5	1	3	1-3
	Density of prostrate hairs on the shoot tip	4	5-7	5	5	5-7	5	5	1
Young leaf	Color upper side of blade (fourth leaf)	51	2	3	3	4	2	1	2
	Density of prostrate hairs between main veins	53	7	5	5	7	5	5	1
Mature leaf	Shape of blade	67	3	2	2	3	4	3	3
	Number of lobes	68	2	3	2	2	3	3	2
	Area of anthocyanin coloration of main veins on the upper side of blade	70	5	1	1	3-5	1	/	1
	Shape of teeth	76	3	2	2	2	3	3	4
	Degree of opening/overlapping of petiole sinus	79	3	7	9	7	7	4	3
	Shape of base of petiole sinus	80	2	1	1	2	2	2	1
	Density of prostrate hairs between main veins on lower side of blade	84	5	5	5	5	5	5	1
	Density of erect hairs between main veins on lower side of blade	87	7	3	1	3	3	3	1
Flower	Sexual organs	151	3	3	3	3	3	3	3
Bunch	Length (peduncle excluded)	202	5	7	9	5	5	5	5
	Density	204	5-7	7	9	3-5	7	5	5
	Length of peduncle of primary bunch	206	3	1	1	5-7	1	/	5

Trait		OIV code	Prokupac	Vranac	Krstač	Smederevka	Žilavka	Plavac Mali	Malvazija Istarska
	Shape	208	/	2	1	/	3	2	/
	Length	220	5	3-5	5	5	5	/	5
Berry	Shape	223	2	2	3	4	2	2	2
	Color of skin	225	6	6	1	1	1	6	1
	Sugar content of must (OE)	505	5	9	7	5	4	7	5
	Total acidity of must	506	7	3	3	7	7	3	3
Source			[1]	[19]	[19]	[38]	[34]	[28]	*Database Radmilovac

* The reported description data were reprinted from the ampelographic varietal description database maintained in the Experimental Station "Radmilovac" (the printed version).

5. Molecular Caracterisation of Balkans Autochthonous Grapevine Varieties

Following domestication, thousands of grapevine cultivars derived from spontaneous or controlled crosses, but also from somatic variation, have been selected and spread by vegetative propagation throughout the world. Traditionally, identification of grape cultivars has been based on ampelography, which is the analysis and comparison of morphological characteristics of leaves, shoot tips, fruit clusters, and berries [38], following descriptor lists prepared by international organizations (IPGRI, UPOV, OIV). The expression of these traits is influenced by environmental factors, individual plant biology, and life history and in relation to that some genetically related cultivars are morphologically very similar and difficult to differentiate by visual comparison. To overcome these limitations, molecular markers have been used for differentiation, characterization, and identification of grapevine accessions.

Types of molecular markers differ from each other with respect to important features such as genomic abundance, level of polymorphism detected, locus specificity, reproducibility, technical requirements and cost. Also, each type of marker system has advantages and disadvantages and it is necessary to evaluate the usefulness of each marker before its application [39].

The first molecular marker used was isozymes [40]. About 20 polymorphic isoenzyme systems have been identified in grapevine and two of them (GPI and PGM, OIV 701 and OIV 702, respectively) are recommended for accession descriptions. Later, DNA-based markers like RFLPs [41], RAPDs [42] and AFLPs [43] have proved useful for identifying grapevine varieties. Genetic profiling of individuals is nowadays based on SSR (Simple Sequence Repeat) markers, which have a number of positive features that make them superior to any other type of molecular marker. Their popularity is related to their monolocus and multiallelic features, codominant inheritance, and high reproducibility.

The relatively small size of the nuclear grapevine genome (475 - 500 Mbp and 2n=38 chromosomes) has enabled significant progress in genomics of this species, especially after 2007 when the whole-genome sequence of the inbred line PN40024 was published [44]. Microsatellite repeats are spread all over the whole grapevine genome. This has allowed the identification of hundreds of them and the design of primers for their analysis so that many SSR probes can be currently found at the different database [45]. The use of SSRs in germplasm characterization provided a broader estimation of genetic diversity in collections studied and found a high degree of clonal relationships synonyms, homonyms, and curation errors.

Among the large number of SSR markers described, the consortium of GrapeGen06 project selected 9 SSR loci as a reference tool to grapevine genotyping [46]. The names of these loci and primer sequences are shown in Table 2.

Table 2. Locus name, linkage group and primer sequence of standard set of 9 SSR markers

Locus	Linkage group	Nucleotide sequence 5′→ 3′	
		Forward primer	Reverse primer
VVS2	11	CAGCCCGTAAATGTATCCATC	AAATTCAAAATTCTAATTCAACTGG
VVMD5	16	CTAGAGCTACGCCAATCCAA	TATACCAAAAATCATATTCCTAAA
VVMD7	7	AGAGTTGCG GAG AAC AGG AT	CGAACCTTCACACGCTTGAT
VVMD25	11	TTCCGTTAAAGCAAAAGAAAAAGG	TTGGATTTGAAATTTATTGAGGGG
VVMD27	5	GTACCAGATCTGAATACATCCGTAAGT	ACGGGTATAGAGCAAACGGTGT
VVMD28	3	AACAATTCAATGAAAAGAGAGAGAGAGA	TCATCAATTTCGTATCTCTATTTGCTG
VVMD32	4	TATGATTTTTTAGGGGGGTGAGG	GCAAAGATGGGATGACTCGC
VrZAG62	7	GGTGAAATGGGCACCGAACACACGC	CCATGTCTCTCCTCAGCTTCTCAGC
VrZAG79	5	AGATTGTGGAGGAGGGAACAAACCG	TGCCCCCATTTTCAAACTCCCTTCC

Genetic profile of seven Balkan variety and four standard cultivars for nine SSR loci are presented in Table 3 (data were taken from http://www.eu-vitis.de/index.php). Each variety is characterized by a unique genotype, which confirms their authenticity. This is expected given that these varieties have a long tradition of growing and represent economically the most important indigenous varieties in this region. Varieties Prokupac, Vranac, Krstač, Žilavka, Plavac Mali, and Istrian Malvasia are internationally recognized and under these names are included in the VIVC catalogue and many collections of grapevine. The unique DNA profile and authenticity of these varieties, both in the set of international varieties [45, 47 - 51] and using other SSR markers [1, 22, 26, 32, 33, 52, 53] has been confirmed.

Serbian variety Smederevka is considered as a synonym for Bulgarian variety Dimyat on the basis of morphological descriptors and SSR markers. On the contrary to that [22] found differences among these two hypothetical synonyms at locus VVS2. Two allele sizes were reported (140:142 bp) for cultivar Dimyat, while Smederevka had only one allele present (142 bp).

In grapevine variety studied all nine SSR loci are polymorphic (Table 3) with the number of alleles per locus ranging from 3 (VVMD7) to 9 (VVMD28). As such, these cultivars represent irreplaceable genetic value for this region, and beyond. A high degree of heterozygousness in relation to these SSR loci for Prokupac, Krstač, Smederevka Žilavka i Plavac Mali indicates that these varieties are old, have long been present in these areas and can be considered as indigenous varieties of the Balkans as such. Vranac, although homozygous for 5 out of 9 loci, is considered autochthonous because genetic analysis suggested a first degree of relationship between this cultivar and the old Balkan cultivar Kratošija [18]. A high degree of homozygosity is characteristic of the Malvasia Istriana, but its origin is still unknown.

In many studies of grapevine germplasm stand out group which are classified as Balkan cultivars. Cultivars from this group by Negrul classification belong to convar. Pontica subconvar. Balcanica. According to [51] Balkans grapevine geographic group contains equal proportion of non-admixed and admixed genotypes. This group mainly composed of wine variety was characterized by a large proportion of genotypes belonging to one STRUCTURE group only, probably corresponding to separate regional grapevine cultivar development and selection. Also, the rather well differentiated cluster containing wine cultivars from the Balkan region obtained using SNP markers [45].

The Balkan varieties represent a unique genepool of grape and were established by [33]. In a set of 196 samples of grapevine cultivars collected in vineyards from countries of ex-Yugoslavia, these authors found that most of the cultivars belonging to different geographic areas appeared to be intermixed and no subpopulation could be recognized. Distance-based clustering of cultivars showed the presence of high variability within and among the obtained groups, although no significant groups of cultivars related to either the region were obtained. A model-based approach showed that Slovenian are most distant from Macedonian and Montenegrin genotypes and are representatives of different clusters. The genotypes with the highest proportion of admixed genetic structure are Serbian, while the highest level of original genetic structure was found in BIH genotypes.

Previous experience shows that molecular markers shown high potential for cultivar fingerprinting but do not have sufficient resolution to identify genetic differences within cultivars. Intravarietal clones can differ considerably in phenotype even though they have identical DNA profiles. But genetic diversity within cultivars is of interest because of the potential to improve traditional cultivars. There are a number of traits in grapevines that are changed by different types of mutation. These polymorphisms include very important agronomic traits such as berry colour, ripening time, cluster density, pests and diseases resistance and productivity. Possible explanations of this variability may be the polyclonal origin of cultivars or accumulation of genetic mutations in their genotypes [54]. Clonal selection is based on genetic variability within cultivars. As a result of clonal selection, a large number of clones of Prokupac [55], Vranac [56], Žilavka [35] and Plavac Mali [36] was separated.

Table 3. SSR profiles of seven native Balkans cultivars and four reference cultivars at 9 core set microsatellite loci. Allele length given in base pairs.

Var	VVS2	VVMD5	VVMD7	VVMD25	VVMD27	VVMD28	VVMD32	VrZAG62	VrZAG79
Prokupac	143:145	228:230	249:249	241:245	182:186	246:260	272:272	194:200	243:251
Vranac	133:133	228:228	247:249	239:241	182:182	236:248	256:256	194:200	259:259
Krstač	133:139	234:242	239:239	239:239	186:186	244:258	240:256	188:196	251:259
Smederevka (Dymiat)	143:143	242:248	239:249	249:255	180:182	234:246	250:264	188:204	237:259
Žilavka	133:153	228:240	239:239	239:241	180:195	248:254	252:264	188:188	249:249
Plavac Mali	143:145	228:230	247:249	239:241	180:180	248:258	252:256	190:204	237:259
Malvasia Istriana	143:143	224:242	239:239	255:255	180:180	254:278	256:272	188:188	237:255
Cabernet Sauvignon	139:151	234:242	239:239	239:249	176:190	234:236	240:240	188:194	247:247
Chardonnay	137:143	236:240	239:243	239:255	182:190	218:228	240:272	188:196	243:245
Muscat A Petits Grains Blancs	133:133	230:238	233:249	241:249	180:195	246:268	264:272	186:196	251:255
Pinot Noir	137:151	230:240	239:243	239:249	186:190	218:236	240:272	188:194	239:245

Source: Data from http://www.eu-vitis.de/index.php.

This genetic variation within grapevine cultivar raises some concern about how to identify such variants. The more general problem of accurately discriminating among clonal variants requires different approaches. One option could be the use of high throughput techniques, such as arrayed SNPs; another could consider the short reads produced with new sequencing technologies. One might also consider variations due to transposable elements [49].

In addition to germplasm characterization and cultivar fingerprinting, in grapevine molecular markers have been also used for construction of linkage maps, identification and mapping of quantitative trait loci (QTLs), marker assisted selection (MAS) and gene cloning.

6. Chemical Characteristics

The chemical composition of grapes and wines, and especially the content of polyphenols, is the subject of numerous researches [57, 58]. Commercial wines of both international and autochthonous varieties were often subject of investigations as evidenced by numerous publications [59 - 63]. Indigenous varieties were examined mainly in countries with a long tradition of growing grape varieties such as Italy, Spain and Greece [64 - 67]. The importance of cultivating autochthonous grape varieties was also recognized by the experts who are making efforts to preserve diversity among wine varieties.

Current research in the authenticity testing is based on the establishment of a link between the chemical composition, on the one hand, and geographical origin, botanical and varietal affiliation, on the other hand [68]. Elemental profile is mentioned in publications as a potential chemical marker of the geographical origin of grape vines and wines, volatile compounds as markers of varietal affiliation, while the polyphenolic profile can provide both data [69, 70]. Also, the polyphenol "fingerprint" can be used for the analysis of grape variety, while some primary metabolites, such as amino acids and biogenic amines, are considered as suitable markers of wine production technology [71]. Some of the published papers indicate the possibility of applying thin layer chromatography in such investigations [72].

Although the native grapevine cultivars play an important role in Balkan viticulture, rapid erosion of autochthonous cultivars occurred, and this is the reason for limited data on chemical compositions of Balkan region indigenous grapevine varieties (Prokupac, Vranac, Plavac Mali, Smederevka, Žilavka, Krstač, and Istrian Malvasia). Therefore, in this section a unique overview from a detailed search of literature related to the characterization of autochthonous grape varieties of the Balkans is given, with the exception of chemical characterization of Prokupac as this grapevine variety is presented in detail as a separate Chapter in this book.

The characteristic phenolic compounds and data related to the total composition of some of the classes of phenolics, such as anthocyanins, flavonoids, and flavan-3-ols, together with antioxidant activity assays are summarized in Tables 4-6. The results of total phenolic (TPC), total anthocyanin (TAC), total flavonoid (TF), and total flavan-3-ol (TF3-ols) contents determined in seed, skin, and pulp extracts of grape varieties Vranac and Smederevka are presented in Table 4. The results show that the highest contents of total phenolic and flavonoid were found in seed extracts, while the lowest were determined in pulp extracts which is accordance with literature data [73]. Similarly, the highest radical scavenging activity was in seed extracts (82.2%), follow by skin extracts (68.1%) and pulp extracts (35.6%) [74]. Antioxidant properties of skin extracts of grape variety Vranac were determined in [75] and the values of antioxidant capacity were 156 mg gallic acid equivalent (GAE) L^{-1}, 475 mg GAE L^{-1}, 15.3 mM TE, and 87.4% for DPPH radical-scavenging activity IC_{50}, Fe (II) chelating ability, ferric reducing/antioxidant power (FRAP), and antioxidant activity in the b-carotene–linoleic acid emulsion system (C_{AA}), respectively. The levels of TPC, TF, and TF3-ols obtained in the red grape variety Vranac [74 - 77] was higher when compared with that of white grape variety Smederevka [76]. A literature survey showed no data of TPC, TF, TAC, and TF3-ols values of grape varieties Plavac Mali, Žilavka, Krstač, and Istrian Malvasia.

Table 4. The content (mg g^{-1}) of total polyphenols, anthocyanins, flavonoids, and flavan-3-ols of Balkan region indigenous grapevine varieties

Grape varieties	Part of berry	TPC	TAC	TF	TF3-ols	Extraction	Reference
Vranac	seed	139.0	-	52.0	16.7	acetone/water (80/20, v/v) + 0.1% (v/v) HCl (conc.)	[76][a]
		267.2	-	n.i.	n.i.	methanol/acetone/water/acetic acid (30/42/27.5/0.5)	[74][b]
	skin	48.3	8.4	10.20	2.8	acetone/water (80/20, v/v) + 0.1% (v/v) HCl (conc.)	[76][a]
		n.i.	2.1	n.i.	2.5	hydro-alcoholic buffer (pH 3.2) + 2 gL^{-1} $Na_2S_2O_5$ and 12% of ethanol	[77][a]
		104.0	48.5	n.i.	n.i.	methanol/acetone/water/acetic acid (30/42/27.5/0.5, v/v/v/v)	[74][b]
		2.3	0.74	1.4	0.10	ethanol/water (80/20, v/v)	[75][a]
	pulp	2.2	0.2	0.4	0.05	acetone/water (80/20, v/v) + 0.1% (v/v) HCl (conc.)	[76][a]
		37.8	-	n.i.	n.i.	methanol/acetone/water/acetic acid (30/42/27.5/0.5, v/v/v/v)	[74][b]
Smederevka	seed	108.0	-	49.4	24.5	acetone/water (80/20, v/v) + 0.1% (v/v) HCl (conc.)	[76][a]
	skin	29.9	-	10.8	0.5	acetone/water (80/20, v/v) + 0.1% (v/v) HCl (conc.)	[76][a]
	pulp	1.5	-	0.2	0.04	acetone/water (80/20, v/v) + 0.1% (v/v) HCl (conc.)	[76][a]

[a] Results are expressed as mg g^{-1} fresh mass; [b] Results are expressed as mg g^{-1} dry mass.

Table 5. The content of flavan-3-ols of Balkan region indigenous grapevine varieties

Grape variety	Part of berry	C	EC	ECG	B1	B2	B3	B4	C1	B2G	Extraction	Reference
Vranac	seed	1.13	7.45	0.013	n.i.	2.33	n.i.	n.i	n.i.	n.i.	methanol/acetone/water/ acetic acid (30/42/27.5/0.5, v/v/v/v)	[74][a]
		0.57-1.51	0.62-1.10	n.i.	0.08-0.19	0.14-0.26	0.16-0.26	0.08-0.14	n.i.	n.i.	/	[75][c]
	skin	n.d.	0.018	n.d.	n.i.	0.027	n.i.	n.i.	n.i.	n.i.	methanol/acetone/water/ acetic acid (30/42/27.5/0.5, v/v/v/v)	[74][a]
		0.002	n.d.	0.003	0.003	0.003	n.i.	n.i.	n.i.	n.i.	ethanol/water (80/20, v/v)	[75][b]
Plavac Mali	seed	2.88-5.04	1.84-3.36	n.i.	0.29-0.57	0.38-0.95	0.34-0.53	0.29-0.43	n.i.	n.i.	/	[75][c]
		0.040-0.064	0.025-0.028	0.004-0.005	0.009-0.012	0.008-0.009	0.006-0.009	0.006-0.008	0.004-0.005	0.008-0.013	acetone/water (80:20) + methanol/water (60:40)	[87][b]
	skin	0.0006-0.0008	0.0002	n.d.	0.001-0.003	0.0002	0.0002-0.0003	n.d.	0.0003	0.0004-0.0011	acetone/water (80:20) + methanol/water (60:40)	[87][b]
Smederevka	seed	0.068	0.392	0.011	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	50% methanol	[88][a]
Istrian Malvasia	skin	0.008	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	methanol/formic acid/2,6-di-tert-butyl-4-methylphenol (BHT)	[119][c]

C: catechin; EC: epicatechin; ECG: epicatechingallate; B1: procyanidin B1; B2: procyanidin B2; B3: procyanidin B3; B4: procyanidin B4; C1: procyanidin trimer C1; B2G: procyanidin trimer B2G.

[a] The contents of individual flavan-3-ols are expressed as mg g^{-1} dry weight; [b] The contents of individual flavan-3-ols are expressed as mg g^{-1} fresh weight; [c] The contents of individual flavan-3-ols are expressed as mg g^{-1} frozen weight.

Table 6. The content of anthocyanins in skin extracts of Balkan region indigenous grapevine varieties

Grape variety	DpGl	CyGl	PtGl	PnGl	MvGl	Sum Ac + Qum	Extraction	Refe-rence
Vranac	3.280[a]	0.980[a]	3.600[a]	2.410[a]	7.090[a]	3.170[a]	methanol/acetone/water/acetic acid (30/42/27.5/0.5)	[74]
Plavac Mali	12.8-13.8[b]	2.7-2.8[b]	10.1-11.4[b]	7.0-7.6[b]	43.8-45.5[b]	21.2-21.3[b]	methanol/water/perchloric acid 80/15/5, v/v/v	[94]
	0.026-0.036[c]	0.016-0.020[c]	0.031-0.037[c]	0.034-0.052[c]	0.166-0.195[c]	0.099-0.140[c]	acidified methanol (0.1% HCl)	[87]

DpGl: delphinidin 3 glucoside; CyGl: cyanidin 3-glucoside; PtGl: petunidin 3-glucoside; PnGl: peonidin 3-glucoside; MvGl: malvidin 3-glucoside; Sum Ac + Qum: sum of acetylated and *p*-coumarylated derivatives of anthocyanins.

[a] The amounts of individual anthocyanins are expressed as mg g^{-1} dry mass of grape skins. The level of delphinidin-3-glucoside is expressed as cyanidin-3-glucoside equivalent. The levels of petunidin-3-glucoside and peonidin-3-glucoside are expressed as malvidin-3-glucoside equivalents.

[b] The amounts of individual anthocyanins areexpressed as percentage as the % total anthocyanin concentration.

[c] The amounts of individual anthocyanins are expressed as mg g^{-1}fresh weight of grape skins.

Previous trials of the polyphenol profile of individual parts of berries have shown that flavanols are the most abundant in seeds and that in terms of their content there are significant differences among varieties [78, 79]. Mesocarp mainly contains phenolic acids, monomeric flavanols and flavonols [80]. The grape skin is rich in anthocyanins, tannins, stilbenes, and in smaller quantities can be found flavan-3-ols and flavonols [81]. In addition, miricetin is cited in the literature as a compound characteristic of skins of black varieties and therefore for red wines [82]. Among the anthocyanins, in the varieties *V. vinifera* L. the most commonly are 3-O-monoglucoside derivatives, and especially malvidine 3-O-glucoside and its acetyl- and cumaroyl derivatives [83].

In study of phenolic composition of seed and skin extracts of varieties Vranac and Smederevka, 31 phenolics were quantified [76]. Flavan-3-ol monomers, (+)-catechin (C) and (−)-epicatechin (EC), together with flavan-3-ol dimers (procyanidins B1, B2, B3, and B4) were detected in the skins and seeds of the both grape varieties. Results in [74] showed that the seed extracts of grape variety Vranac were rich in flavan-3-ols. Three flavan-3-ol monomers, (+)-catechin, (-)-epicatechin, and (-)-epicatechingallate (ECG) and dimer procyanidin B2, were quantified in the grape seeds of the Vranac variety (Table 5). The most abundant flavan-3-ol monomer in seed extracts was (-)-epicatechin, while (-)-epicatechin gallate was present in trace amounts which is consistent with data previously reported [84, 85]. Similar levels of flavan-3-ols were determined in a study of seed extracts of grape variety Plavac Mali [86], while notably lower contents of quantified flavanols in seed extracts of variety Vranac were also shown. The highest number of flavan-3-ols in seed extracts was reported in [87] where concentrations of monomeric (C, EC, EGC) and oligomeric flavan-3-ols (dimers B1, B2, B3, B4; trimer C1 and B2G) were determined in seed extract of variety Plavac Mali. This investigation showed that the flavanol fingerprints of seed extracts were cultivar dependent and can be used in the control of the origin of grape. In study of seed extracts of variety Smederevka a total of ten flavan-3-ols (C, EC, ECG, five proanthocyanidin dimers and two proanthocyanidin dimers) were quantified [88]. The grape skin extracts had significantly lower contents of flavan-3-ols (Table 5) which is in agreement with reported data [85, 89, 90] but were rich in flavonols and skin extracts of red grape varieties have high contents of anthocyanins.

The anthocyanin fingerprints are primarily determined by genotype and can be used to discriminate red grapes according to botanical origin [91, 92]. A total of 15 anthocyanins, such as the 3-monoglucosides, 3-acetylglucosides and 3-p-coumaroylglucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin, were identified in the skin of the grape variety Vranac [76]. The most abundant anthocyanins found in the skin extract of Vranac were anthocyanin-monoglucoside, which is in accordance with data reported in [93]. The relative amount of malvidin-3-glucoside was significantly higher when compared with the other identified anthocyanin-3-monoglucoside (malvidin, peonidin, petunidin, and delphinidin). The anthocyanin profile of Vranac skin investigated by Andjelkovic et al. [74] showed that the order of abundance of quantified anthocyanin glucosides was as follows: malvidin>petunidin>delphinidin> peonidin>cyanidin. Similarly, 15 different anthocyanins in skin extract of grape variety Plavac Mali were identified and amounts of 3-monoglucosides and sum of acetylated and *p*-coumaroylated derivatives of anthocyanin in [87, 94], as presented in Table 6. The most abundant anthocyanin in all presented skin extracts was malvidin-3-glucoside representing about 40% of overall content, which has already been shown in other *V. vinifera* cultivars[95, 96].

Phenolic acids and their derivatives are the most abundant phenolic compounds in pulp extracts of white grapevine varieties [97]. A total of six hydroxycinnamic acids (caffeic, *p*-coumaric, ferulic, *trans*-caftaric, *trans*-coutaric, and *trans*-fertaric) were quantified in juice of Istrian Malvasia grape [97]. The most abundant hydroxycinnamic acid was *trans*-caftaric, which amount was 9.93 mg L^{-1}. Hydroxybenzoic acids are present in grape juce of variety Istrian Malvasia in lower concentrations than hydroxycinnamic acids. Among five identified hydroxybenzoic acids, the highest level was obtained for gallic acid (12.61 mg L^{-1}). Phenolic acids in the seed and skin extracts of grape variety Vranac were detected as gallic acid, *t*-coutaric acid, caffeic acid and *p*-coumaric acid [74]. The phenolic acid with the highest amount was gallic acid (0.850 mg g^{-1} dry weight), while the most abundant phenolic acid in skin extract was caffeic acid (0.085 mg g^{-1} dry weight).

Flavonol profile of skin extract of grape variety Vranac showed that the predominant flavonol hexosides are the glucosides of myricetin, quercetin, laricitrin, and syringetin, while only quercetin-3-O-glucoside and quercetin-3-O-glucuronide were detected in the skin of the white grape Smederevka [76]. Similarly, investigation of the composition of individual flavonols in the skin extract of variety Vranac at optimal harvest time showed that glucosides of quercetin, myricetin, kaempferol and luteolin were predominant [74]. Among six quantified flavonols rutin was found to be the more abundant (0.440 mg g^{-1} dry weight).

Although the amounts of stilbens in grape skin extracts are low when compared with the overall polyphenols, the great interest that has been devoted to these biologically-active compounds is the main reason to investigate stilben profiles. In study [75] a special attention was given to stilbene compounds, when *cis*-resveratrol (0.93 mg kg^{-1} FW), *trans*-

resveratrol (0.78 mg kg^{-1} FW), and astringin (0.75 mg kg^{-1} FW) were found in the skin extract of grape variety Vranac. The amounts of quantified stilbens were higher in comparison to the results published in [98].

The organic acid profile has major contribution on the color, organoleptic characteristics and stability of wine [99]. The most abundant organic acids in wine are tartaric, malic and citric acids [100] The amounts of organic acids quantified in juice of grape berries of variety Plavac Mali were in range 5.08-6.47g L^{-1}, 1.36-2.07 g L^{-1}, and 110-325 mg L^{-1} for tartaric, malic, and citric acid, respectively [101] Similar results were obtained in study of [97] where the amounts of tartaric, malic, and citric acid in grape juice of Istrian Malvasia were 2.91, 2.45, and 0.27 g L^{-1}, respectively.

Krstač is dominant grape variety among autochthonous white varieties in Montenegro. However, data of the chemical characterization of this variety is missing. The impact of yield on TPC, TAC, and antioxidant potential in wines produced from Montenegrin autochthonous grape varieties was studied in [102]. TPC value obtained for Krstač wine (253.9 mg GAE L^{-1}) was similar to the content of total phenols found in the investigated white wine Žižak (275.1 mg GAE L^{-1}) and several times lower when compared to the content of total phenols determined for red wine produced from variety Vranac [104]. Similarly results were obtained for antioxidan capacity expressed as reducing power and DPPH radical-scavenging activity. On the other hand, contents of tartaric acid were similar for wine produced from varieties Krstač, Vranac and Žižak.

To the extent of our knowledge, there are no data about chemical characterization of grape of variety Žilavka. In research of [103] total phenol contents and antioxidant activities of eighteen commercially available white wines made from autochthonous grape varieties Žilavka were determined. TPC values of investigated white wines were ranged from 175.0 to 801.9 mg GAE L^{-1}. The antioxidant capacity determined by $DPPH^{\cdot}$ method ranged from 28.8% to 70.2%. Reports found in literature show the existence of relationship between total phenolic content and antioxidant capacity, as individual phenolics are considered to contribute the most to the scavenging ability ([73, 104, 105], and this was also confirmed in [103]. Nevertheless, to assess chemical profiles it is necessary to use hyphenated chromatographic techniques that enable analyzing on both quantitative and semiquantitative data [106 - 108].

In addition to a limited number of publications dealing with the examination of the chemical composition of individual parts of grape beeries, a certain number of studies reports on the composition of the wine [109 – 116] and leaves [107, 118] of autochthonous varieties from the Balkans. In the future larger number of investigations dealing with the grapes of indigenous varieties could be expected, mainly due to the fact that the chemical composition of the berries directly affects the characteristics of the wine.

7. Future Perspectives

Autochthonous grapevine varieties described herein are a part of the biodiversity of the Balkans cultural heritage. Identification and preservation of these varieties require their detailed characterization – ampelographic, genetic and chemical. Production of authentic wines made from autochthonous varieties can help Balkans countries find its place in the demanding and crowded world wine market.

Further genetic research will be continued in order to fully characterize the germplasm of Balkans region grapevine varieties and to understand the genetic links between these varieties and the genetic diversity of autochthonous varieties of this region.

Finally, studies show that the chemical properties can be related to the geographical origin and the varietal origin of the wine. In addition to the fact that such works have a significant scientific contribution to the characterization of grapes and wines from the territory of Serbia, the results also provide useful information for improving the conditions of cultivation and preserving the genetic resources of the grapevine, but also in terms of verification of their origin. Due to a complex composition of grape, the development and application of procedures for the assessment of authenticity is of particular importance not only for consumers, but also for the producers and exporters, especially bearing in mind that illegal practices in the food industry can have serious repercussions on human health.

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