Composition of Bulgarian Varieties of Grape Fruit Seeds and the Changes in Their Composition during Development

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ABSTRACT: *General composition of seeds isolated from four Bulgarian grape varieties and their changes during growing was studied. The total content of the main compounds in the seeds was: glyceride oil: 11.6-16.5%; proteins:6.3-8.9%, carbohydrates:65.5-70.9%, ash:2.1-2.8%. The water soluble saccharides were found to be 3.6-6.3%. The ratio of mono-, di- and trisaccharides was 13.5-19.4%, 70.2-73.8% and 7.1-16.3% respectively. In monosaccharide fraction the main components were fructose (16.2-33.9%), glucose (38.2-44.4%) and galactose (17.6-39.2%). Xylose, rhamnose and arabinose were detected too. In the disaccharide fraction predominated iso-maltose (82.1-91.5%), followed by saccharose and maltose.The changes of the content and composition of soluble carbohydrates in grape seeds during growing were also examined.The content of saccharides decreased relatively from 6.9% in the first to 5.1% in the third stage. The percentage of trisaccharidesrised from 7.1% to 16.3% at the expense of the decrease of the quantity of monosaccharides from19.4% to 13.5% and of the disaccharides from 73.5% to 70.2%. The content of fructose increased from 23.7% to 33.9% at the expense the galactose level which dropped from 32.7% to 17.6%. The increase of the content iso-maltosefrom 55.9% to82.1% at the expense of all other disaccharides was detectedin the disaccharide fraction.*

Keywords:*grape seeds, development, glyceride oil, proteins, water soluble saccharides.*

1. INTRODUCTION

The wine making is one of the important branches of the Bulgarian agricultural industry. Wine production generates significant amounts of grape pomaces as a by-product.Sincepomaces consist mainly of grape seeds, which are rich in carbohydrates, proteins, glyceride oil, soluble saccharides, mineral compounds, they can be used as inexpensive sourcefor obtaining of valuable products - mainly glyceride oil [1], [2], [3], [4], [5]. The rest can be utilized as animal food rich in proteins; while carbohydrates and polyphenols are used as antioxidant compounds [6], [7]. According Campos *et al.* [8] the seeds contain about 40% fibres, 11% proteins, 7% phenoliccompounds, as well as sugar and minerals. Kamel*et al.*, [9] reported for about 8.2% crude proteins. Mironeasa*et al.*, [10] announced 28% cellulose, 4-6% tannins, 10-25% glyceride oil, 2-4% minerals. Licev*et al.* [11] established about 10-11% cellulose, 4-6% tannins, 20% glyceride oil. Razuvaev[12] indicates 8-10 oil, 44-57% cellulose, 3.7% tannins and 1-2% mineral substances.

Besides the unsolublecarbohydrates, the seeds content soluble saccharide fraction including mainly monosaccharides as fructose, ramnose, arabinose etc., as well as di- and trisaccharides [13]. Sabir*et al.* [14] investigated the distribution of sugars at different stages of berry development and established a slight reduction of glucose and fructose levels in the berries.

However there are nodata about accumulation of saccharides in the seeds during growing of the grape.

In this connection, the aim of the study is to investigate the content and general composition of the grape seeds separated from several grape-wine cultivars and their changes during, development especially the accumulation of soluble saccharides in the seeds and determining the variation of their individual composition.

2. MATERIALS AND METHODS

All solvents and reagents were of analytical grade and were used without additional purification.

2.1 Samples

The seeds were separated from the grape fruits during growing at the experimental stationof the Agrocultural University, Plovdiv region, crop 2012. Four Bulgarian varieties - two white dessert – *Super ran bolgar* and *Bolgar* and two red wine - *Mavroud* and *Shirokamelnishkaloza-* were investigated. The changes of composition of *Super ran bolgar* were studied during growing in June-August period. The seeds were separated from the grapefruits on the 50^{th}, 80^{th} and 110^{th} day after flowering.

2.2 Determination of the moisture

The content of moisture in the seeds was established according toISO 665 [15]. The seeds were dried at 105^{0}C up to constant weight.

2.3 Isolation of glyceride oil and determination of oil content.

The air-dried seeds (50 g sample) were milled and the oil was extracted with n-hexanein Soxhlet unit for 8 h [16]. The solvent was partly removed in a rotary vacuum evaporator, the residue was transferred in pre-weight glass vessels and the rest of the solvent was removed under a stream of nitrogen to a constant weight in order to determine the oil content.

2.4Determination of protein content

Total proteins were established by Kjeldhal method according to ISO 20483 [17] using VELP scientificaapparature including DK 6 Heating digester, JP water aspirator and UDK 127 Distillation unit. The percentage of proteins was calculated using Factor 6.25.

2.5 Determination of ash

The ash wasquantited after mineralization of the seeds at 600^{0}C [18].

2.6 Determination of carbohydrate composition

Total content of carbohydrates was determined by difference from total content of other constituents (100 - amounts of glyceride oil, proteins, ash and moisture) [19].

The water soluble carbohydrates were separated by extraction with hot water (90^{0}C)for 15 minutes and their composition was determined by high performance liquid chromatography on a Agilent LC 1220 unit equipped Zorbax Carbohydrate column (150 mm x 4.6 mm, 5 m Agilent and Zorbax Reliance Cartridge guard column (Agilent) and refractive index detector (RID 1260)(USA). The mobile phase was acetonitrile/water (80/20) at 1.0 mL/min [20]. All standards (individual pure mono- and disaccharides (more 98.0% purity) were purchased from Sigma Chemical Company (USA).

2.7 Statistics

2.7.1 Statistical analysis. All analyses were made in triplicate determinations. Statistical differences between samples were tested using ANOVA. Data were expressed as mean ± SD. The level of significance was set at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Generalcomposition of the seeds

The results of the analyses carried out on the seeds isolated from the four grape varieties are presented in Table 1.

Table 1

The comparative determination shows that the structural composition of the white and red wine varieties was found to be different but not in vary large limits. The levels of glyceride oil, proteins, total carbohydrates and ash varie in close values. The obtained experimental results are in accordance with data reported earlier by Canbay *et al*. [21] and Campos *et al*. [8]. Relatively high content of glyceride oil (16.5%) was detected in the seeds of the *Super ran bolgar* variety and saccharides - in seeds separated from *Mavroud* (6.3%)

The general composition of water soluble saccharides is given in Table 2.

Table 2

The main part of them, in all varieties,are disaccharides; they were discovered in the highest levels in the fraction of *Shiroka melnishka loza* (72.6%) and *Super ran bolgar* (70.2%). High values of monosaccharides (42.2%) were identified in the seeds of *Mavroud* at the expense of the lower content of trisaccharides (10.1%). Relatively lower content of monosaccharides was observed in the seeds of *Super ran bolgar* (13.5%).

Table 3

Glucose and galactose predominate in the monosaccharide fraction followed by fructose(Table 3). Xylose, rhamnose and arabinose were detected in negligible amounts. The were fact suggest that saccharide composition of fruits and seeds is identical. Iso-maltose was the main component in all discchaharide fractions (more 80%). Saccharose and maltose were detected too.This individual profil is in accordance with other investigations about saccharides, but extracted from grape pomace [13].

Table 4

Table 4 presents the variance of the general composition of the seeds during growing. The content of glyceride oil rised gradually, especially in the third stage when the accumulation was found to be from 3.4% to 16.5%. The level of carbohydrates was increased too. This increasing was at the expense of reduction of the quantities of all other constituents of the seeds- proteins, ash and moisture.

Table 5

The content of soluble saccharides during development (Table 5) increased gradually,as well as the level of trisaccharides was increasedat the expense of other components, especially of monosaccharides.

Table 6

The individual profil of the monosaccharides presented in Table 6 varies significantly during growing. The level of fructose increasedfrom 23.7% in first stage to 33.9% in the third at the expense of the sizable reduction of galactose (from 32.7% to 17.6%). A considerably higher content of iso-maltose (82.1%) in the third stage was established in the disaccharide fraction versus 55.8% in the first stage at the expense of the lower levels of other disaccharides whose amounts were found to be about four times lower than in the first stage.

4. FIGURES AND TABLES

Table 1

General composition of grape seeds*

Substances, content, % wt	***Bolgar***	***Super ran bolgar***	***Mavroud***	***Shirokamelnishkaloza***
Glyceride oil	11.6 ± 0.3	16.5 ± 0.7	15.7 ± 0.5	13.9 ± 0.6
Proteins	6.9 ± 0.3	6.3 ± 0.1	8.9 ± 0.4	8.9 ± 0.4
Ash	2.4 ± 0.1	2.5 ± 0.1	2.1 ± 0.1	2.8 ± 0.1
Carbohydrates	70.9 ± 1.4	68.2 ± 2.7	65.5 ± 3.3	68.2 ± 2.7
- Unsoluble	67.3 ± 2.7	63.1 ± 1.9	59.2 ± 1.2	64.0 ± 2.6
- Soluble	3.6 ± 0.1	5.1 ± 0.2	6.3 ± 0.3	4.2 ± 0.2
Moisture	8.2 ± 0.3	6.5 ± 0.3	7.8 ± 0.2	6.2 ± 0.2

*****mean of three separate determinations

Table 2

General composition of soluble saccharides*

Saccharides, content % wt	***Bolgar***	***Super ran bolgar***	***Mavroud***	***Shirokamelnishkaloza***
Monosaccharides	24.3 ± 1.0	13.5 ± 0.5	42.2 ± 1.7	21.1 ± 0.4
Disaccharides	56.5 ± 2.3	70.2 ± 2.8	47.7 ± 1.4	72.6 ± 2.9
Trisaccharides	19.2 ± 0.8	16.3 ± 0.7	10.1 ± 0.4	6.3 ± 0.3

*****mean of three separate determinations

Table 3

Individual composition of monosaccharides and disaccharides*

Saccharides	***Bolgar***	***Super ran bolgar***	***Mavroud***	***Shirokamelnishkaloza***
Monosaccharides,% wt				
Fructose	16.2 ± 0.5	33.9 ± 1.0	18.7 ± 0.6	20.1 ± 0.6
Glucose	40.6 ± 1.6	43.5 ± 0.9	44.4 ± 1.8	38.2 ± 1.5
Galactose	39.2 ± 1.6	17.6 ± 0.7	30.9 ± 1.2	27.4 ± 1.4
Xylose	2.4 ± 0.1	1.8 ± 0.1	2.7 ± 0.1	3.5 ± 0.1
Rhamnose	1.2 ± 0.1	3.1 ± 0.1	2.8 ± 0.1	10.8 ± 0.3
Arabinose	0.4 ± 0.02	-	0.5 ± 0.02	-
Disaccharides,%wt				
Saccharose	8.4 ± 0.3	4.9 ± 0.2	5.4 ± 1.6	7.8 ± 0.4
Maltose	3.7 ± 0.1	3.0 ± 0.1	2.3 ± 0.1	3.6 ± 0.1

Iso-maltose	87.9 ± 3.5	82.1 ± 3.3	91.5 ± 2.7	85.6 ± 2.6
Other disaccharides	-	10.0 ± 0.2	0.8 ± 0.02	3.0 ± 0.2

***mean of three separate determinations**

Table 4

The change of general composition of grape seeds during development*

Substances, content, % wt	**I stage**	**II stage**	**III stage**
Glyceride oil	1.3 ± 0.04	3.4 ± 0.1	16.5 ± 0.7
Proteins	11.7 ± 0.5	8.5 ± 0.3	6.3 ± 0.3
Ash	4.8 ± 0.1	3.7 ± 0.1	2.5 ± 0.1
Carbohydrates	62.7 ± 2.5	69.7 ± 2.1	68.2 ± 2.7
- Unsoluble	55.8 ± 2.2	63.4 ± 1.9	63.1 ± 1.9
- Soluble	6.9 ± 0.3	6.3 ± 0.2	5.1 ± 0.2
Moisture	12.6 ± 0.5	8.4 ± 0.3	6.5 ± 0.2

***mean of three separate determinations**

Table 5

Change of the content of soluble saccharides during development*

Saccharides, content % wt	**I stage**	**II stage**	**III stage**
Monosaccharides,% wt	19.4 ± 0.6	15.3 ± 0.6	13.5 ± 0.5
Disaccharides.**%wt**	73.5 ± 2.9	73.8 ± 3.0	70.2 ± 2.8
Trisaccharides. %	7.1 ± 0.3	10.9 ± 0.3	16.3 ± 0.7
Total content in the seeds. % wt	6.9± 0.3	6.3± 0.3	5.1± 0.2

***mean of three separate determinations**

Table 6

Change of the composition of soluble saccharides during development*

Monosaccharides. content % wt	**I stage**	**II stage**	**III stage**
Fructose	23.7 ± 0.9	29.7 ± 1.2	33.9 ± 1.4
Glucose	41.0 ± 1.2	39.3 ± 1.6	43.5 ± 1.7
Galactose	32.7 ± 1.3	27.4 ± 1.1	17.6 ± 0.7
Xylose	0.9 ± 0.04	1.3 ± 0.1	1.8 ± 0.1
Rhamnose	1.7 ± 0.1	2.3 ± 0.1	3.1 ± 0.1
Disaccharides.%wt			
Saccharose	0.2 ± 0.01	2.2 ±0.1	4.9 ± 0.2
Maltose	-	1.7 ± 0.1	3.0 ± 0.1
Iso-maltose	55.9 ± 2.8	68.0 ± 2.7	82.1 ± 3.3
Other disaccharides	43.9 ± 1.3	28.1 ± 1.1	10.0 ± 0.4

***mean of three separate determinations**

5. CONCLUSION

The data obtained by the investigations show that the general composition concerning the quantities of glyceride oils, proteins, saccharides and ash varies depending mainly on variety and stage of growing. The quality and quantity general composition of water soluble carbohydrates is similar to this of grape fruits. During development, the individual saccharide profile changes the content of fructose and iso-maltose increase while the amounts of maltose and other disaccharides decrease.

The received information about grape seed composition can be useful for the evaluation of the possibilities to use of the seeds to produce some valuable food products.

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