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C O N T E N T

INTORDUCTION

In 2022, the covid-19 virus continues to spread around the world with serious environmental, social and economic consequences. The fatality of the SARAS-CoV-2 virus has decreased, compared to the deaths in 2020 worldwide, but the world is still facing a global crisis in all spheres of life. The pandemic has brought new shocks to agri-food systems that are already facing with terrible global impacts such as climate change, biodiversity loss and food production insecurity. During the pandemic, farmers from all over the world have been challenged in unprecedented ways to adapt their production, marketing, sales, food safety and labor practices to follow the covid-19 restrictions on health system and on society in general. Under all these difficult conditions, building adaptive capacities in the agriculture system get a new urgency with important learned lessons to share among nations for planning to safer global food system after the pandemic.

In the past three years, science was also not safe from the consequences of the pandemic. Especially in the agricultural production sector, all activities were aimed for adapting to the new conditions. Researchers certainly have not stopped their research activities, which is confirmed with the publication of this issue of JAPS.

The Editorial Board of JAPS, in the conditions of a covid-19 pandemic, continuously had published all journal issues in order to share with the scientific and professional community the new research results in the field of agricultural production and plant sciences. We are honoured and pleased to share with you four peer-reviewed scientific papers in JAPS issue No. 20., Vol. 1 and also to invite and encourage our colleagues from the Republic of North Macedonia, the region and wider to publish their research results in JAPS.

Editorial Board, **Editor in chief,**

June, 2022 **Prof. Liljana Koleva Gudeva, PhD**

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Original scientific paper

DETERMINATION OF RADIONUCLIDE CONCENTRATION IN MILK SAMPLES CONSUMED IN REPUBLIC OF NORTH MACEDONIA AND POPULATION DOSE RATE ESTIMATES

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Abstract

Milk is one of the most important food products in the human diet and contains all the macronutrients, that are, proteins, carbohydrates, fat, vitamins (A, D and B groups) and trace elements, especially calcium, phosphorus, magnesium, zinc and selenium. Milk contamination is largely due to the grazing of animals on contaminated grass and drinking water. Grass is a direct source or route of radionuclides to animals and humans through the consumption of meat and milk. One of the important tasks of the veterinary activity is veterinary-sanitary supervision of the production and sale of milk and dairy products, whose main goal is the provision of biologically good milk and dairy products from healthy animals. The purpose of this study was to determine the activity concentrations of ^{226}Ra, ^{232}Th ^{40}K and ^{137}Cs in milk samples most commonly used in daily consumption in the Republic of North Macedonia and based on the results, the risk of radiation to the population can be estimated. An instrument - gamma spectrometer (Canberra Packard) with a high purity germanium detector and GENIE 2000 programme was used for measurement of the samples. On the basis of the performed tests, the main activity concentrations of ^{226}Ra, ^{232}Th, and ^{40}K were 1.76 ± 0.23; 1.05 ± 1.00; 31.9 ± 5.07 (Bq·kg^{-1}), respectively. ^{40}K has the highest value compared to other radionuclides due to the process of transfer from soil to grass and from grass and water to milk. The activity of ^{137}Cs is below the detection limit for all tested milk types. This shows that there is no risk of radiation to the population, i.e., the safety limits are not exceeded, which points out the insignificant threat of radiation arising from radionuclides that are naturally or artificially present in the tested milk, and that reach humans through the food chain.

Key words: *radioactivity; milk; gamma spectrometry; radiation risk*

INTRODUCTION

Measurements of radioactivity in the environment and food products are extremely important for controlling the levels of radiation that humans are directly or indirectly exposed to. Milk is one of the basic food products for the human diet and it contains most of the macronutrients, that is, proteins, carbohydrates, fats, vitamins (A, B and D groups) and trace elements such as calcium, phosphorus, magnesium, zinc and selenium (Kanai et al., 2013; Vreman et al.,1989). Milk contamination is largely due to the grazing of animals on contaminated grass and the drinking water they consume. Grass is a direct source or route of radionuclides to animals and humans through the consumption of meat and milk. One of the key roles of veterinary medicine is the veterinary-sanitary supervision of the production and sale of milk and dairy products, whose main goal is to provide biologically good milk and dairy products from healthy animals. If milk samples contain high levels of radioactivity when they reach the person who uses them in the daily diet, they can accumulate in certain parts of the body (radium-226 is accumulated in the lungs and kidneys, thorium-232 is accumulated in the human liver, skeleton, tissue, and lungs, while potassium-40 is usually accumulated in the muscles). Due to the presence of these radionuclides in all vital organs of the human body, health problems may emerge that can cause various forms of diseases and weakening of the immune system and contribute to the increase in the mortality rate. The radionuclides ^{134}Cs, ^{137}Cs, ^{131}I, ^{89}Sr, ^{90}Sr are of most interest when it comes to milk, which is a food product in the daily diet (IAEA 295, 1989) and considering the fact that they have different decay rates, the doses of these elements will be different. Therefore, monitoring the concentrations of radionuclide activity will provide important information that can contribute to knowledge about the exposure of the population and the establishment of the original baseline. This study copes with the research, i.e., determination of the activity concentrations of ^{232}Th, ^{226}Ra, ^{40}K and ^{137}Cs in milk samples that are available in our markets, i.e., in the Republic of North Macedonia. The results of the measurement of the activity concentration will be used to calculate several parameters that are important for radiological risk assessment, and the data can be used to determine the baseline for natural and artificial radioactivity in milk.

MATERIAL AND METHODS

Sampling

In order to collect samples of milk that is part of the dairy products consumed in the Republic of North Macedonia, a survey was conducted during 2021, which includes analysis from supermarkets, fast food restaurants and large milk distributors throughout the country. The research showed that there are about 8 leading brands of liquid milk that were consumed by a large part of the population in the Republic of North Macedonia. Two specimens were taken from each sample within the envisaged months for taking samples, in fact, different UHT milk types from a total of 8 producers were included. The samples were placed in 0.5 L Marinelli containers which were fully filled, sealed and stored in order to establish a balance between ^{226}Ra and ^{222}Rn before the measurements were made.

Instrument

The research is focused on determining the level of radioactivity in raw milk by using a high-resolution HPGe detector. The gamma ray spectrometry technique was used for radioactivity determination of the tested samples. The spectrometer consisted of an HPGe detector, model 3020 (Canberra Packard, Meriden, CT, USA), with active volume of 180 cm^2, relative efficiency of 30 %, operating voltage 3000 V, and resolution of 2 keV at 1332.5 keV. The detector was enclosed in massive 12 cm thick lead shielding and internal lining of 2 mm high purity cooper. Data acquisition and analysis were performed with 8192 channel digital analyzer; duration of acquisition interval for each sample was 65 ks. The activity of ^{226}Ra was determined from the gamma lines associated with low half-live time daughters of 214Bi (609.31, 1120.29, and 1794.49 keV) and ^{214}Pb (351.93 keV). The ^{232}Th activity was determined by 338.4, 911.2 and 969.1 keV gamma lines form 228Ac and its decay products. The gamma line at 1460.8 keV was used to determine the activity of 40K.Efficiency calibration was performed with mixed calibration standard sources MBSS2, supplied from the Czech Metrological Institute, Inspectorate for Ionizing Radiation. In order to determine the background distribution in the detector environment, empty sealed Marinelli beaker with the same geometry was measured at equal counts as the soil samples. The analysis procedure included the subtraction of the background spectrum.

-Activity calculation

The specific activity (A) is determined according the equation

$$A = \frac{\frac{N}{t} - \frac{N_0}{t_0}}{\varepsilon \cdot \gamma \cdot m} \quad (\mathrm{Bq \cdot kg^{-1}})$$

Where, N is clean surface of peak accumulated from a specific radionuclide in analysis of a specific sample (number of readings), N_0 is clean surface of peak accumulated from the spot of a specific radionuclide without an analysis of sample (number of readings), t is live time of accumulation of the sample spectrum (s), t_0 is live time of accumulation of the phone spectrum (s), ε is detector efficiency for a given energy (for a specific peak), γ is intensity of gamma transition in radioactive decay for a respective radionuclide (%), and m is sample mass (kg).

-Air absorbed dose rate (D)

A direct connection between radioactivity concentrations of natural radionuclides and their exposure is known as the absorbed dose rate in the air at 1 meter above the ground surface. The mean activity concentrations of ^{232}Th, ^{226}Ra and ^{40}K(Bq·kg^{-1}) in the samples are used to calculate the absorbed dose rate given by the following formula. (Belivermis et al., 2010) D (nGy / h) = 0,462 A_{Ra} + 0,604 A_{Th} +0,0417 A_k +0,030 A_{Cs}

-Radium equivalent activity (Raeq)

The model of the radium equivalent activity establishes the use of a single index to define the gamma output or compare the specific activities of materials containing ^{226}Ra, ^{232}Th, and ^{40}K by a single quantity, which takes into consideration the radiation risk associated with these NORMs (Roy et al., 2000). The calculation of the radium equivalent activity (Raeq) is a quantity for comparing the specific activities of the samples with different contents of ^{226}Ra, ^{232}Th and ^{40}K. The uniformity with respect to radiation exposure was defined in terms of the radium equivalent activity (Raeq) in Bq·kg^{-1} in order to compare the specific activity of the materials containing different amounts of ^{226}Ra, ^{232}Th and ^{40}K. It is assumed that 370 Bq·kg^{-1} of ^{226}Ra, 259 Bq·kg^{-1} of ^{232}Th and 4810 Bq·kg^{-1} of ^{40}K produce the same gamma-ray dose rate. It is calculated by using the following ratio Ra_{eq} (Bq/kg) = A_{Ra}+1.43A_{Th}+0.07 A_k (Beretka et al., 1985) A_{Ra}, $A_{Th,}$ A_k – specific activities (Bq·kg^{-1}) of ^{226}Ra, ^{232}Th and ^{40}K, respectively. The value of the radium equivalent activity of 370 Bq·kg^{-1} corresponds to the maximum allowed dose for a population of 1 mSv.

-External and internal hazard index

In order to assess the equivalent average of the annual effective dose imposed to the residents of each area, the external hazard index for the soil samples was calculated.

$$H_{eks} = A_{Ra}/370 + A_{Th}/259 + A_k/4810 \leq 1$$

A_{Ra}, $A_{Th,}$ A_{k-}specific activites (Bq·kg^{-1}) ,^{226}Ra^{232} Th and ^{40}K, respectively (Kurnaz et al., 2007).

-Annual effective dose equivalent (AEDE)

The annual effective dose equivalent received was computed from absorbed dose rate by applying a dose conversion factor of 0.7 Sv Gy−1 and the occupancy of 0.8 (19/24) recommended by UNSCEAR. Therefore, the annual effective dose equivalent (μS vy−1) was calculated using the formula (UNSCEAR., 2000)

AEDE(μSvy−1) = absorbed dose (nGyh−1)×8760h×0.7SvGy−1×0.8×10−3

- **Excess lifetime cancer risk**

Excess life cancer risk predicts the likelihood of developing cancer over a lifetime at a certain exposure rate. It is a value representing the number of extra cancers expected in a given number of people on exposure to a carcinogen at a given dose.

Excess lifetime cancer risk is given as (Taskin et al., 2009)

ELCR=AEDE×DL×RF

The parameters used are defined; thus, AEDE is the annual effective dose equivalent, DL is the average duration of life (estimated to be 70 years), and RF is the risk factor (S/v), i.e., fatal cancer risk per Sievert. ICRP uses a RF of 0.05 for the public for stochastic effects.

If a person consumes milk that contains elevated levels of radionuclides, this may increase the chances of cancer. If the radioactivity in milk is higher than the world average, this could be a source of radiation to the human body and some specific organs, whereby their ELCR would be higher than the world average of 0.29 mS vy-1 in such a body.

RESULTS AND DISCUSSION

The activity concentrations of ^{232}Th, ^{226}Ra, ^{40}K and ^{137}Cs in selected brands of fresh milk are presented in Table 1.

Table 1. ^{232}Th, ^{226}Ra ,^{40}K and ^{137}Cs activity concentrations of milk in January, 2021

	Specific activity* (Bq·kg⁻¹)			
Month	January, 2021			
Sample	^{226}Ra	^{232}Th	^{40}K	^{137}Cs
S1	1.80±0.20	0.75±0.15	34.9 ± 6.20	<MDA*
S2	1.99±0.21	1.18±0.15	32.7 ± 5.3	<MDA
S3	1.91±0.15	1.26±0.20	31.9± 5.5	<MDA
S4	1.65±0.16	0.63±0.14	32.0 ± 4.2	<MDA
S5	1.22±0.18	1.09±0.16	29.1 ± 3.5	<MDA
S6	2.52±0.15	1.64±0.15	36.2 ± 5.0	<MDA
S7	2.44±1.00	1.21 ± 0.17	36.4±5.2	<MDA
S8	1.11±0.50	0.72±0.19	21.0±5.5	<MDA
Average	1.83±0.31	1.06±0.16	31.0±5.05	<MDA

*MDA - minimum detectable activity, number of repetitions for each sample (n=3)

Table 2. ^{232}Th, ^{226}Ra ,^{40}K and ^{137}Cs activity concentrations of milk in April, 2021

	Specific activity* (Bq·kg⁻¹)			
Month	April, 2021			
Sample	^{226}Ra	^{232}Th	^{40}K	^{137}Cs
S1	1.81±0.24	0.85±0.15	35.6 ± 7.80	<MDA
S2	2.04±0.24	1.18±0.16	33.2 ± 5.21	<MDA
S3	1.86±0.17	1.21±0.24	34.2± 5.66	<MDA
S4	1.45±0.18	0.60±0.18	31.4 ± 4.6	<MDA
S5	1.15±0.18	0.99±0.16	29.7 ± 3.0	<MDA
S6	2.22±0.16	1.64±0.17	36.8 ± 4.1	<MDA
S7	2.14±0.20	1.21 ± 0.17	37.4±6.8	<MDA
S8	1.02±0.10	0.75±0.20	21.6±6.8	<MDA
Average	1.70±0.18	1.05±0.17	32.4±5.50	<MDA

*MDA - minimum detectable activity, number of repetitions for each sample (n=3)

Table 3. ^{232}Th, ^{226}Ra ,^{40}K and ^{137}Cs activity concentrations of milk in August, 2021

	Specific activity* (Bq·kg⁻¹)			
Month	August, 2021			
Sample	^{226}Ra	^{232}Th	^{40}K	^{137}Cs
S1	1.82±0.21	0.77±0.14	35.0 ± 5.00	<MDA
S2	2.24±0.24	1.26±0.14	33.4 ± 5.00	<MDA
S3	1.97±0.15	1.11±0.20	32.1± 5.00	<MDA
S4	1.52±0.11	0.68±0.11	31.7 ± 4.80	<MDA
S5	1.66±0.10	1.29±0.17	29.6 ± 3.50	<MDA
S6	2.44±0.19	1.52±0.15	36.9 ± 4.50	<MDA
S7	2.33±1.0	0.99 ± 0.17	35.4±6.50	<MDA
S8	1.17±0.55	0.75±0.18	20.9±6.80	<MDA
Average	1.83±0.31	1.04±0.15	31.87±4.53	<MDA

*MDA - minimum detectable activity, number of repetitions for each sample (n=3)

Table 4. ^{232}Th, ^{226}Ra ,^{40}K and ^{137}Cs and activity concentrations of milk in October, 2021

	Specific activity* (Bq·kg^{-1})			
Month	**October, 2021**			
Sample	^{226}Ra	^{232}Th	^{40}K	^{137}Cs
S1	1.81±0.18	0.88±0.10	36.1 ± 6.80	<MDA
S2	2.04±0.17	1.11±0.11	33.2 ± 5.20	<MDA
S3	1.86±0.17	1.22±0.23	31.2± 5.30	<MDA
S4	1.45±0.15	0.61±0.15	31.4 ± 4.50	<MDA
S5	1.15±0.15	0.99±0.16	28.7 ± 3.00	<MDA
S6	2.22±0.10	1.51±0.16	36.1 ± 4.00	<MDA
S7	2.14±0.15	1.06 ± 0.17	35.9±6.50	<MDA
S8	1.02±0.13	0.66±0.19	21.6±6.50	<MDA
Average	1.71±0.15	1.05±0.15	31.77±5.22	<MDA

*MDA - minimum detectable activity, number of repetitions for each sample (n=3)

Table 5. ^{232}Th, ^{226}Ra ,^{40}K and ^{137}Cs activity concentrations of milk samples, mean values from the four seasons

Radionuclides	**Bq·kg^{-1}**
226Ra	1.76±0.23
232Th	1.05±1.00
40K	31.9±5.07
137Cs	<MDA Bq·kg^{-1}

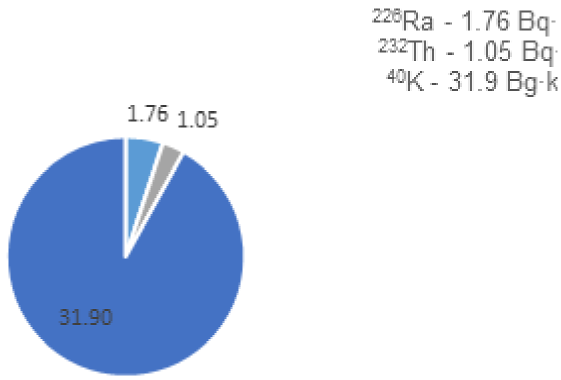

Figure1. Graphical representation of mean values of the three natural radionuclides measured in milk

^{40}K is the dominant radionuclide in the tested samples. More specifically, all samples, in all seasons, have the highest concentration of activity of ^{40}K with an average value of 31.9 ± 5.07 Bq·kg^{-1}. The higher concentration of activity of ^{40}K in milk samples can be justified since potassium is very mobile in the environment, it is one of the major radionuclides in the soil and naturally forms part of potassium, which is a major nutrient for plants, animals and humans (Hafsi et al., 2014; Bilgici Cengiz et al., 2019). These reasons lead to a high concentration of activity of ^{40}K compared to other radionuclides due to the process of transfer from soil to grass and from grass and water to milk. The average concentration of radioactivity of ^{226}Ra in liquid milk is 1.76 ± 0.23 Bq·kg^{-1}, while the average concentration of activity of ^{232}Th is 1.05 ± 1.0 Bq·kg^{-1}. Actually, the value of cesium 137 is below the detection limit. The values of the analysed samples are below the permitted limits UNSEAR 2000. If the results for all radionuclides are summarized, it can be seen that there are no statistically significant differences between the radioactivity concentrations between the samples. Furthermore, the activity of the concentrations of ^{232}Th, ^{226}Ra, ^{137}Cs and ^{40}K in milk that was found in this study is compared with reports from other authors in different countries where it can be seen that there are variations of the concentrations and the values in our study are below the obtained values for milk consumed in some countries such as Iran/France (Hosseni et al., 2006), Jordan (Zaid et al., 2010), Egypt (Harb et al., 2010), Turkey (Cengiz., 2020), Brazil (Melquiades et al., 2002) and other countries. Although the differences in the levels of radioactivity in different brands of milk are small, it is considered that the source of raw materials used for milk production is the contributing factor. Previous researches shown that the average annual value of the concentration of 137Cs activity in milk samples in Serbia in 1985 was 0.11 Bq / l. After the Chernobyl accident, its activity concentration values reached 72.6 Bq / l for cesium. Since 1987, this value has been declining exponentially, and in 2013, minimum values of 0.038 Bq / l for 137Cs were measured. (Bogojevic et al., 2016). From the obtained results shown in Tables 1,2,3 and 4, we can conclude that there is a non-significant difference in terms of specific activity for all examined radionuclides in different months of the year.

Based on the mean values of the specific activities for the radionuclides ^{232}Th, ^{226}Ra, ^{40}K and ^{137}Cs, the following values were calculated: the radium equivalent activity (Raeq), the absorbed dose rate D (nGy/h), the internal hazard indices (Hin), the received annual effective dose equivalent (AEDE) and the excess lifetime cancer risk (ELCR) for the different samples of dairy products, which are presented in Table 6.

Table 6. D, Raeq, Hex, AEDE, ELCR Risk of lifetime cancer values of dairy products

Sample (Month)	D (nGy/h)	Raeq (Bq·kg^{-1})	Hex	AEDE(μSv/year)	ELCR (μSv/year)
January	2.80	5.51	0.01	13.73	48
April	2.79	5.46	0.01	13.68	47.88
August	2.83	5.54	0.01	13.88	48.58
October	2.77	5.43	0.01	13.58	47.53
Average	2.80	5.48	0.01	13.71	47.99

The mean value of the radiation risk index Heks is 0.01 whose value is less than the maximum allowed value which for Heks is <1. The value of the radium equivalent activity Raeq is below the maximum recommended limit, i.e., 370 Bq kg^{-1} which is 5.48 Bq kg^{-1}. The values of the specific activity and the calculated Radiation Risk Index (Heks) and Radiation Equivalent (Raeq) obtained in this study also did not exceed the safety limits, emphasizing the insignificant radiation hazard which arises from naturally occurring terrestrial radionuclides. The values of the absorbed dose rate D (nGy/h) and the annual effective dose equivalent (AEDE) obtained in this study, also did not exceed safety limits, emphasizing the negligible radiation hazard arising from naturally present terrestrial radionuclides. The estimated ELCR obtained in all measured samples is lower than the international standard limit.

CONCLUDING REMARKS

The gamma-spectrometry assessment of natural radioactivity in milk consumed in the Republic of North Macedonia is presented in this study. It was found that the concentration of radionuclides in milk samples is determined by the source from which the milk was obtained. The mean values of the concentration of specific activity in the milk samples were lower than the permitted public dose limit worldwide. All calculated radiological risk parameters show that none of the milk samples exceeded the recommended permitted level. From the research it can be concluded that the milk consumed in the Republic of North Macedonia is radiologically safe and cannot cause immediate or significant threat to the health of the consumers of the examined milk brands. However, continuous control of the radiological safety of milk and all dairy products consumed by the population in the Republic of North Macedonia is recommended.

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ОПРЕДЕЛУВАЊЕ НА КОНЦЕНТРАЦИЈАТА НА РАДИОНУКЛИДИ ВО ПРИМЕРОЦИ НА МЛЕКО КОРИСТЕНИ ВО РЕПУБЛИКА СЕВЕРНА МАКЕДОНИЈА И ПРОЦЕНКИ НА РИЗИК ОД РАДИЈАЦИЈА НА НАСЕЛЕНИЕТО

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Резиме

Млекото е еден од најважните прехранбени производи во човековата исхрана и ги содржи сите макронутриенти, односно протеини, јаглени хидрати, масти, витамини (А, Д и Б групи) и елементи во траги, особено калциум, фосфор, магнезиум, цинк и селен. Контаминацијата на млекото во голема мера се должи на пасењето на животните на контаминирана трева и вода за пиење. Тревата е директен извор или пат на радионуклиди до животните и луѓето преку потрошувачката на месо и млеко. Една од важните задачи на ветеринарната дејност е ветеринарно-санитарниот надзор на производството и продажбата на млеко и млечни производи, чија основна цел е обезбедување на биолошки добро млеко и млечни производи од здрави животни. Целта на оваа студија беше да се утврдат концентрациите на активност на 226Ra, 232Th 40K и 137Cs во примероците на млеко кои најчесто се користат во секојдневната потрошувачка во Република Северна Македонија и врз основа на резултатите може да се процени ризикот од зрачење на населението. За мерење на примероците е користен инструмент - гама спектрометар (Canberra Packard) со детектор на германиум со висока чистота и програма GENIE 2000. Врз основа на извршените тестови, средните концентрации на активност на 226Ra, 232Th и 40K беа 1,723 ± 0; 1,05 ± 1,00; 31,9 ± 5,07 (Bq·kg-1), соодветно. 40K има најголема вредност во споредба со другите радионуклиди поради процесот на пренос од земја на трева и од трева и вода во млеко. Активноста на 137Cs е под границата за детекција за сите тестирани типови млеко. Ова покажува дека не постои ризик од зрачење за населението, односно не се надминуваат безбедносните граници, што укажува на незначителна закана од зрачење што произлегува од радионуклиди кои природно или вештачки се присутни во тестираното млеко, а кои стигнуваат до луѓето преку синџир на исхрана.

Клучни зборови: *радиоактивност, млеко, гама спектрометрија, ризик од зрачење*

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EXAMINATION OF RADIOACTIVITY AND PRESENCE OF ADDITIVES IN WINES FROM TIKVEŠ REGION

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Abstract

In Republic of North Macedonia, the production of grapes and wine has been known since ancient times. Many grape varieties are grown in this region, but one of the most important and largest regions for wine production is the Tikveš region. The aim of the research was to determine the presence of natural radionuclides, as well as total and free SO_2 that are added as additives in wine during its production. The analyses were performed by gamma spectrometry and the obtained spectra were analyzed by the GENIE 2000 programme. The sulfites were determined using OIV accredited methods after prior validation and verification. The results show that 40K is present in all wine brands with a larger size than the other radionuclides observed. 40K levels ranged from low 24.15 ± 2.30 to 38.22 ± 1.50 Bq/L for white wines and 16.28 ± 3.20 to 22.80 ± 3.50 Bq/L for red. As for sulfites, differences can be noticed in terms of the content of total and free SO_2 in all examined wines. Regarding red wines, the lowest value for the content of both total and free SO_2 is observed in Merlot wine (free SO_2 = 10.20 ± 0.54mg/L and total SO_2 = 51.79 ± 0.55mg/L) and in terms of white wines, the lowest content of total and free SO_2 is observed in Muscat Ottonel wine (free SO_2 = 6.84 ± 0.36mg/L and total SO_2 = 40.24 ± 0.43mg/L).

Key words: *wine, radionuclides, additives, sulfites, quality*

INTRODUCTION

Traceability of wines requires knowledge of their characteristics, which are associated with the geographical origin of grape, soil, water, climate (Fabani et al., 2010) In Republic of North Macedonia viticulture is the most important and most strategic industry in the field of crop production. (Economic chamber of Macedonia, 2005) In this region the process of winemaking is not something new, because it has been made since the ancient times. The wine was cultivated 4,000 years ago and this is confirmed by a number of artifacts found on ancient sites. According to Wine Export Comparative Analysis from 2010-2016, a total of 28 grape varieties are grown in Republic of North Macedonia, and there is a presence of white and black varieties equal to 50%. This country has all the prerequisites for the production of high-quality wines such as favourable climate, moderate amount of water, heat and proper soil with balanced content of all nutrients, which makes it region with great export potential. Tikveš is the most well-known wine region which represents around one third of all grapes grown in Republic of North Macedonia. Tikveš region, has been producing wine since the 4th century BC. Due to the perfect southern Mediterranean climate, full of long and warm summers, Tikveš' local grapes generally retain an ideal sugar concentration (17% to 26%). Over the years it was determined which varieties of grapes are most suitable and possess all the necessary characteristics for producing quality wine (pleasant taste, resistance to various diseases and pests, yielding high yields, etc.). (Angelovska et al., 2022) From the data obtained from the wine industry from 2015, the type of grapes used for production largely determines both the quality and the specific characteristics of the wine, such as the taste and colour of the wine, the presence of residual sugar, the content of alcohol, acidity and the presence of tannins. Sulphur dioxide (SO_2) and its salts have been added during winemaking since the 17th century. They still remain an essential winemaking additive as there is no one other additive that has the same dual properties of anti-oxidation and preservation. But, used in greater amounts can cause some toxic reaction for wine consumers such as, breathing difficulty, sneezing, hives, migraine and other problems and should be handled with care. Moreover, sulphites are also used as biocide agent in disinfection for sanitation of barrels. (WHO, 2012). If oxygen is present, it will be 'captured' by SO_2. A redox reaction to sulphite and further on to sulphate will take place. Due to this reaction the oxygen concentration reduces and aerobic microorganisms cannot increase anymore e.g., in wine. These properties are very important in the process of winemaking for two reasons: the anti-oxidant effect of SO_2 prevents the alteration of natural aromas of the grapes and wine due to the contact with oxygen and the preservative effect of SO_2 helps inhibiting the development of 'undesirable bacteria' in the wine. When SO_2 is incorporated into a must or a wine, a fraction of it will react with sugars, aldehydes or ketones. The remaining fraction, called free, is the one with the most important properties. So, the total amount of total SO_2 is sum of free and reacted SO_2. (OIV, 2021) More recently, the determination of radionuclide activity in wine has been increasingly used to control the authenticity of wine. Contamination can occur as a result of fruit contamination, which can result from direct deposition on fruit surfaces, absorption from the fruit peel and transport to the pulp, soil deposition, root absorption and fruit transfer. Some researchers have studied the transport of different radionuclides from vines to the wine, but these studies have only dealt with the addition of radioactive traces to the vines to confirm the behaviour and distribution of these radionuclides in the plant and the wine product. (Carini et al., 2005) The consumption of contaminated wines will increase the amount of radioactivity and chemical contamination inside a human being and therefore increases the health risks associated with radiation exposure. (Caridi et al., 2019)

So, the aim of our research was to determine the content of total and free SO_2 in wine samples from Tikveš region as well as radionuclide activity.

MATERIAL AND METHODS

In recent years, many studies have been conducted in which the content of radionuclides as indicators of the origin of the food industry products have been discussed. Wine analysis seems to draw much attention because of its importance in assessing the quality of a food product, possible verification of adulteration and analysis of its position in the production chain in the agriculture and the food industries. (Gajek et al., 2021)

Wine is produced by the fermentation process of wine juice under the action of enzymes such as *Saccharomyces cerevisiae*. SO_2 can be represented as endogenous (which occurs during the fermentation process) and exogenous (which is added during technological processes). Endogenous SO_2 occurs during the process of enzymatic transformation of sulphur-containing substances, such as thiamine acids (cysteine, cystine, methionine, glutathione, elemental sulphur, etc.). It is present in low concentrations from several mg/L up to 30 mg/L (Kubáň et al., 2018). Therefore, before adding exogenous SO_2 during technological processes, it is very important to determine the content of endogenous SO_2.

Wine samples: Total of 31 samples of red and white wine from different manufacturers from Tikveš region were subject of our research. The aim was determination of total and free SO_2 as well as radionuclide activity, by using standard accredited methods according to the Law on Wine and Wine Products of Republic of North Macedonia.

SO_2 determination: The presence of total SO_2 is the total amount of free SO_2 plus the one that is bound to sugars, pigment, aldehydes. It is very important the concentration (mg/L) of total and free SO_2 to be in balance due to the quality characteristics of the wine. The OIV-MA-AS323-04B official method was used to determine the content of total and free SO_2. For determination

of free SO_2, standard H_2SO_4 solution is used and for determination of the total SO_2 content, standard solutions of NaOH and H_2SO_4 are used and then the samples are titrated on Titrino plus titrators by using standard Iodine solution. Before the analysis, verification on each method was performed by determining accuracy, precision (standard deviation and relative standard deviation), repeatability and reproducibility by using standard reference material and proficiency testing.

The measurement of the control reference material (PT FAPAS 1389 - set 1 and 2, Quality indicators in wine) was performed in 10 repetitions for each method separately and for the calculation of the extended measurement uncertainty as a source of uncertainty were taken into account the repetition, bias, as well as errors arising from the equipment used. (Angelovska et al., 2022).

The results for the extended measurement uncertainty for each method are as follows: total SO_2 ± 1.07% and free SO_2 ± 5.33%. (Extended measurement uncertainty for k = 2, 95 % probability level).

Radioactivity: The analyses for the presence of radionuclides in wine were made without prior preparation of the wines. The samples of wine were measured with an instrument - gamma spectrometer (Canbera Packard) with a high purity germanium detector. The measurement was carried out in beakers that were hermetically sealed so that 222Rn produced by the decomposition of 226Ra would not result in gas leakage. After ensuring a time balance between the successors of the 238U and the 232Th series, these sealed samples were prepared for analysis. GENIE 2000 software was used for data acquisition and analysis. The specific activity of 226Ra is calculated for the energy line of 186.1 (keV) and 232Th through its decay descendant 228Ac (second in the decay chain), that is, through its three gamma decay energy lines which occur at 338.4; 911.07 and 968.9 (keV).

The activities of 40K were determined from its γ-line of 1460 keV. The time interval for calculation (counting) was 108000 seconds. (Angeleska et al., 2021).

RESULTS AND DISCUSSION

Table 1 contains results for free and total SO_2 determined in red and white wines from Tikveš wine region.

Table 1. Wines from Tikveš region, free and total SO_2 content (mg/L)

Red wine	**Free SO_2 (mg/L)**	**Total SO_2 (mg/L)**
Merlot 1	10.20 ± 0.54	51.79 ± 0.55
Merlot 2	39.88 ± 2.12	119.30 ± 1.27
Merlot 3	39.96 ± 2.12	125.90 ± 1.34
Aleksandar	36.53 ± 1.94	141.67 ± 1.51
Tempranillo	36.30 ± 1.93	151.30 ± 1.61
Vranec 1	30.18 ± 1.60	113.77 ± 1.21
Vranec 2	43.62 ± 2.32	119.09 ± 1.27
Vranec 3	20.32 ± 1.08	88.60 ± 0.95
Vranec 4	32.30 ± 1.72	123.33 ± 1.31
Cabernet Sauvignon 1	43.19 ± 2.30	162.76 ± 1.74
Cabernet Sauvignon 2	44.29 ± 2.36	106.11 ± 1.13
Cabernet sauvignon 3	38.04 ± 2.02	84.35 ± 0.90
Mekedonsko crveno trpezno vino	33.05 ± 1.76	69.32 ± 0.74
Red cuvee	38.88 ± 2.07	145.20 ± 1.55
mean value (MV)	**34.77 ± 1.85**	**114.46 ± 1.22**

White wine	**Free SO_2 (mg/L)**	**Total SO_2 (mg/L)**
Traminec	66.41 ± 3.53	179.81 ± 1.92
Temjanika 1	38.40 ± 2.04	124.19 ± 1.32
Temjanika 2	27.88 ± 1.48	108.85 ± 1.16
Muscat Temjanika	38.35 ± 2.04	131.00 ± 1.40
Chardonay 1	40.80 ± 2.17	214.42 ± 2.29
Chardonay 2	25.80 ± 1.37	118.16 ± 1.26
Chardonay Aristokrat	27.01 ± 1.43	114.15 ± 1.22
Riesling 1	29.98 ± 1.60	133.35 ± 1.42
Riesling 2	29.90 ± 1.59	128.23 ± 1.37
Pinot Grigio	21.89 ± 1.16	90.50 ± 0.96
Muscat Ottonel	6.84 ± 0.36	40.24 ± 0.43
Zilavka 1	23.48 ± 1.25	122.70 ± 1.31
Zilavka 2	21.91 ± 1.16	87.12 ± 0.93
Smederevka 1	22.73 ± 1.21	125.26 ± 1.34
Smederevka 2	16.56 ± 0.88	68.79 ± 0.74
Smederevka 3	32.23 ± 1.71	112.03 ± 1.19
Sauvignon blanc	40.16 ± 2.14	150.20 ± 1.60
mean value (MV)	**27.75 ± 1.48**	**120.53 ± 1.29**

Table 2. Wines from Tikves region, radionuclide activity

Red wine	**^{40}K(Bq/L)**	**^{226}Ra(Bq/L)**	**^{232}Th(Bq/L)**	**^{137}Cs(Bq/L)**
Merlot 1	31.20 ± 5.00	0.47 ± 0.20	0.36 ± 0.20	<0.10
Merlot 2	37.12 ± 5.20	0.92 ± 0.50	0.52 ± 0.45	<0.07
Merlot 3	34.00 ± 5.00	0.94 ± 0.50	0.46 ± 0.33	<0.10
Aleksandar	31.23 ± 2.50	1.71 ± 1.55	0.92 ± 0.90	<0.09
Tempranillo	31.20 ± 2.00	1.22 ± 1.00	0.97 ± 0.85	<0.07
Vranec 1	32.78 ± 1.50	1.81 ± 1.22	1.22 ± 1.00	<0.10
Vranec 2	33.12 ± 1.50	1.96 ± 1.22	1.35 ± 1.10	<0.08
Vranec 3	33.75 ± 1.05	1.95 ± 1.00	0.89 ± 0.75	<0.10
Vranec 4	32.50 ± 1.50	1.23 ± 1.20	1.07 ± 1.00	<0.10
Cabernet Sauvignon 1	28.33 ± 2.50	0.95 ± 0.55	0.47 ± 0.32	<0.08
Cabernet Sauvignon 2	24.15 ± 2.30	0.83 ± 0.70	0.33 ± 0.25	<0.08
Cabernet sauvignon 3	24.50 ± 2.00	0.91 ± 0.75	0.55 ± 1.52	<0.10
Mekedonsko crveno trpezno vino	31.25 ± 1.00	0.77 ± 0.75	0.26 ± 0.20	<0.10
Red cuvee	38.22 ± 1.50	0.75 ± 0.65	0.62 ± 0.64	<0.10
mean value (MV)	**31.66 ± 2.46**	**1.17 ± 0.84**	**0.64 ± 0.58**	
White wine	**^{40}K(Bq/L)**	**^{226}Ra(Bq/L)**	**^{232}Th(Bq/L)**	**^{137}Cs(Bq/L)**
Traminec	29.17 ± 3.50	0.96 ± 0.50	0.38 ± 0.22	<0.10
Temjanika 1	25.40 ± 4.00	0.40 ± 0.32	0.38 ±0.20	<0.07
Temjanika 2	21.18 ± 4.50	0.41 ± 1.22	0.40 ± 0.15	<0.09
Muscat Temjanika	27.22 ± 4.04	1.22 ± 1.40	0.92 ±0.55	<0.09
Chardonay 1	22.80 ± 3.50	0.58 ± 1.50	0.51 ±0.30	<0.10
Chardonay 2	20.44 ± 3.50	0.65 ± 1.20	0.55 ±0.20	<0.10
Chardonay Aristokrat	26.01 ± 3.90	0.80 ± 1.25	0.58 ±0.33	<0.10

Riesling 1	22.65 ± 4.05	0.88 ± 1.50	0.76 ±0.55	<0.10
Riesling 2	22.90 ± 4.50	1.20 ± 1.35	1.28 ±1.20	<0.10
Pinot Grigio	20.10 ± 3.00	0.90 ± 0.95	0.66 ±0.50	<0.09
Muscat Ottonel	19.14 ± 3.80	1.70 ± 1.50	1.55 ±1.00	<0.10
Zilavka 1	16.28 ± 3.20	0.96 ± 1.00	0.92 ±0.65	<0.10
Zilavka 2	19.20 ± 3.33	0.98 ± 1.00	0.95 ±0.70	<0.10
Smederevka 1	22.50 ± 2.50	1.70 ± 1.33	0.92 ±0.50	<0.09
Smederevka 2	21.97 ± 2.55	1.75 ± 1.70	0.92 ±0.50	<0.10
Smederevka 3	21.13 ± 3.10	1.22 ± 1.20	1.30 ±0.95	<0.10
Sauvignon blanc	27.34 ± 3.30	1.50 ± 1.60	1.28 ±1.00	<0.08
mean value (MV)	**22.66 ± 3.56**	**1.04 ± 1.25**	**0.83 ± 0.57**	

*The results are average value after 5 repetitions for each sample and each analyte ± MU (measurement uncertainty)

From the results obtained, differences can be noticed in terms of the content of total and free SO_2 in all examined wines. Regarding red wines, the lowest value for the content of both total and free SO_2 is observed in Merlot 1 wine (free SO_2 =10.20 ± 0.54mg/L and total SO_2=51.79 ± 0.55mg/L) and the highest in Cabernet Sauvignon 2, for free SO_2 (44.29 ± 2.36mg/L) and Tempranillo for total SO_2 (151.30 ± 1.61mg/L). In terms of white wines, the lowest content of total and free sulphur dioxide is observed in Muscat Ottone wine (free SO_2 =6.84 ± 0.36mg/L and total SO_2=40.24 ± 0.43mg/L). If a comparison is made between the same types of wine, but from different producers, we notice differences in the content of total and free SO_2. Thus, there is a significant difference between Merlot 1 and Merlot 2 and 3 both in terms of total SO_2 and free SO_2 content. Compared to Vranec and Cabernet Sauvignon, no significant differences were observed in the content of free SO_2 (from 20.32 ± 1.08mg/L to 44.29 ± 2.36mg/L), but higher differences were observed in the content of total SO_2 (from 88.60 ± 0.95mg/L to 162.76 ± 1.74mg/L). Regarding white wines, the most significant differences were observed in the content of total SO_2 in Chardonnay 1 and 2 wines from different producers, where the content is significantly lower in Chardonnay 2 (118.16 ± 1.26mg/L), compared to Chardonnay 1 (214.42 ± 2.29mg/L). Due to the quality standards prescribed in the Law of wine and wine products of Republic of North Macedonia and in accordance with the Rulebook published in Official Gazette of the Republic of Macedonia, No.16 of 02.02.2012, the amount of total SO_2 should not be higher than 200mg/L for white and rose wines and 150mg/L for red wines. The results show that some of the samples does not meet the requirements of the rulebook and exceed the value of the allowed presence of total SO_2, such as red wines Tempranillo (total SO_2=151.30 ± 1.61mg/L) and Cabernet Sauvignon 1 (total SO2=162.76 ± 1.74mg/L) and white wine Chardonnay 1 (total SO2=214.42 ± 2.29mg/L). All wines contain sufficient amount of free and total SO2 that protects wines from oxidation, the main role of this additive. According to previously published data on determination of total and free SO_2 in Macedonian wines, we can notice that in Vranec wine there are no significant differences in the concentration in terms of free SO_2 content, but there are differences in terms of total SO_2 content compared to our results. (Ivanova-Petropulos et al., 2014)40K is present in all wine brands with a size larger than the other radionuclides observed. 40K levels ranged from 24.15 ± 2.30 to 38.22 ± 1.50 Bq / L for white wines and 16.28 ± 3.20 to 22.80 ± 3.50 Bq / L for red. Potassium is a mineral element present in grapes and wine, it is also used as a fertilizer in vineyards. To a large extent, potassium in wine comes from the potassium in the soil where the vines are grown and at the same time the K content can be increased in the vineyard and from potassium sorbate which is often added to preserve certain types of wine. Thus, the vinification process will directly affect the amount of potassium stored in the alcoholic beverage. The radionuclide values of radium and thorium were significantly lower than those of K, which is consistent with other studies and the anthropogenic radioactive contamination of the examined samples.

CONCLUDING REMARKS

Based on the results, we came to the conclusion that almost all types of white and red wine from Tikveš region satisfy the standards prescribed in the Law of wine and wine products of Republic of North Macedonia in terms of total and free SO_2 content. When it comes to the radiological activity, the values obtained in this study, regardless of the type of wine, did not exceed the safety limits, which highlights the insignificant danger of radiation arising from radionuclides that are naturally or artificially present in the examined wines most often used by the population in the Republic of North Macedonia. Prevention may be the continuous monitoring of the levels of natural and artificial radionuclides in wines in order to provide useful information on unwanted risks to human health. Many imported as well as domestic wines can be noticed in the markets across the country. In terms of quality, Macedonian wines belong to the group of high-quality wines among other wines with high quality characteristics and market price.

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ОПРЕДЕЛУВАЊЕ НА РАДИОАКТИВНОСТ И ПРИСУСТВО НА АДИТИВИ ВО ВИНАТА ОД ТИКВЕШКИОТ РЕГИОН

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Резиме

Во Република Северна Македонија производството на грозје и вино е познато уште од античко време. Во овој регион се одгледуваат поголем број сорти на грозје, но еден од најважните и најголемите региони за производство на вино е Тиквешкиот регион. Целта на истражувањето беше да се утврди присуството на природни радионуклиди, како и вкупен и слободен SO_2 кои се додаваат како адитиви во виното при неговото производство. Анализите се направени со гама спектрометрија и добиените спектри се анализирани со програмата GENIE 2000. Сулфитите беа утврдени со употреба на OIV акредитирани методи по претходна валидација и верификација. Од добиените резултати се забележува дека 40K е присутен во сите видови на вино во поголема концентрација од останатите радионуклиди. Нивоата на 40K се движеа од ниски 24,15 ± 2,30 до 38,22 ± 1,50 Bq/L за бели вина и 16,28 ± 3,20 до 22,80 ± 3,50 Bq/L за црвени вина. Што се однесува до сулфитите, може да се забележат разлики во однос на содржината на вкупниот и слободен SO_2 кај сите испитани вина. Во однос на црвените вина, најниска вредност за содржината и на вкупниот и на слободниот SO_2 е забележана кај виното Мерло (слободен SO_2 = 10.20 ± 0.54 mg/L и вкупен SO_2 = 51.79 ± 0.55 mg/L), а во однос на белите вина, најмала содржина на вкупен и слободен SO_2 е забележана во виното Muscat Ottonel (слободен SO_2 = 6.84 ± 0.36 mg/L и вкупен SO_2 = 40.24 ± 0.43 mg/L).

Клучни зборови: *вино, радионуклиди, адитиви, сулфити, квалитет*

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Original scientific paper

FORECASTING MODEL BASED ON CUMULATIVE DEGREE DAYS FOR INCUBATION PERIOD OF *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni

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Abstract

The overall development of *Plasmopara viticola* and its occurrence in time and space cause rapid disease increases. The incubation period is a part of the life cycle of *P. viticola,* between infection and the first appearance of symptoms. The forecasting based on cumulative degree days for determination of incubation allows the prediction of a small number of primary infections whose calculation is based on the temperature factor. This forecasting model, in essence, is a regression analysis that presents the relationship between average daily temperature and coefficient of incubation. The determination of the incubation allows precise management of the fungicides against *P. viticola* and gives the basic assumptions for the possible occurrence of the primary inoculum.

Keywords: *Plasmopara viticola, incubation period, average daily temperature, warning model, regression analysis, coefficient of incubation*

INTRODUCTION

The cause of grapevine downy mildew is the oomycete fungus *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni and belongs to the order *Peronosporales*. The asexual multiplication cycles appear throughout grapevine vegetative growth while sexual structures serve as an overwintering phase. The asexual spore multiplications lead to secondary infections during grapevine growing seasons, originating from specialized gametogenic structures that cause primary infections at the beginning of vegetation of grapevine. These secondary cycles of infection, under favourable weather conditions, can occur repeatedly throughout the grapevine growing season (Gessler et al.,2011). The aim of this research is to predict the incubation or latent period so we could determine precisely the chemical treatments against *P. viticola*. In the Republic of North Macedonia, fungicide management strategies are focused mainly on routine chemical applications against *P. viticola* without taking into account the actual need for spraying. The number of fungicide treatments against secondary infections during grapevine growing season is usually 5 to 7. A properly timed spray programme is essential for managing downy mildew in the vineyard. (Caffi et al., 2009). In plant pathogens latent period is the time between host infection and the onset of pathogen sporulation from that infection (Pariaud et al., 2009). The importance of the latent period for the understanding and prediction of pathogen development has long been recognized in plant disease epidemiology (Vanderplank, 1963). It is important to note that *P.viticola* often has a short time of latency following infection leading to rapid disease increases over time. Since the disease spreads very fast during the secondary infection cycles, successful control depends on controlling the primary infections (Schwinn, 1981). There seems to be very little information about the variability of the latent periods of fungal pathogens on foliar that can be, used as warning models. Designing a forecasting model for the determination of incubation of *P.viticola* based on cumulative degree days mostly depends on the climatic conditions of the areas, which represents only one side of the disease triangle. A forecasting model for the determination of incubation of the *P.viticola* is a simplified approach to the incubation process, but in no way is it a replica of reality itself. Hence, a model serves as an assessment to help research and organize knowledge, enhance understanding of phenomena, and eventually become a tool for decision-making. The main aim of the forecasting model for the determination of incubation of the *P.viticola* is to provide a prediction for the end of the latency period and omit sprays when weather conditions are unfavourable for downy mildew. Primary inoculum plays a key role in epidemics not only at the beginning of the growing season but also in overlapping secondary infection cycles in late spring and summer (Gessler et al., 2003).

MATERIAL AND METHODS

The research was conducted in a vineyard located at Smilica, near Kavadarci, Republic of North Macedonia (41°42`71.4" N, 22°0`10.75" E). The area of the vineyard was 9 ha. A double Guyot pruning system was applied in the vineyard. The aim was to note the incubation of the *P. viticola*, according to the widely known 3/10 empiric role. This rule is based on the simultaneous occurrence of the following conditions: (i) air temperature equal to or greater than 10°C; (ii) vine shoots at least 10 cm in length; (iii) a minimum of 10 mm of rainfall in 24- 48 h (Baldacci, 1947). When the criteria of empirical rules 3/10 by realized in the field, an incubation period begins. Different grapevine varieties (Vranec, Smederevka, Zilavka and Merlot) are present in the vineyard where monitoring was performed during incubation. The incubation of the disease occurred in the following phenological stages of the development of grapevines, according to the BBCH-scale (Hack et al., 1992): BBCH 15/53 - Five leaves separated; Shoots about 10 cm long; Inflorescence visible, BBCH 17/55 - Seven leaves separated; Inflorescence closely pressed together, and BBCH 19/57 - Nine or more leaves separated; Inflorescences fully developed (Figure 1). The extended BBCH-scale is a system for a uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species (Hack et al., 1992). The abbreviation BBCH derives from Biologische Bundesanstalt, Bundessortenamt and CHemical industry (Hack et al., 1992). BBCH- describes the phenological development of vines. The cumulative degree days-based model can be understood as an empirical model based upon statistical operation to calculate the incubation period. When the three elementary conditions of the above-mentioned empirical rule 3/10 for the occurrences of the primary infection from sexual structures (oospores), meet, the incubation period begins, which is the goal of observation and calculation (Table 1). The incubation was calculated as cumulative degree days, calculated as the sum of average daily temperatures. In other words, degree days can be understood as a period between infection and symptom detection, calculated based on temperature (°C). The incubation period was monitored every day from May 02 until May 12, 2022, when the first oil spot symptoms appeared on the upper side of the leaves.

Table 1. Observation of the incubation period at *P.viticola*

Varieties	ha	Observation of growth stage	Monitoring period	Incubation days	Σ of °C for cumulative degree days
Vranec	6,5	BBCH15/53-BBCH19/57	02.5.2022 -12.5.2022	10	181,1
Smederevka	1,5				
Zilavka	0,5				
Merlot	0,5				

Figure 1. Overview of phenological stages of the grapevines during the incubation period of *P. viticola:* A - BBCH 15/53 - Five leaves separated; shoots about 10 cm long; B - BBCH 17/55 - Seven leaves separated; Inflorescences closely pressed together; C - BBCH 19/57 - Nine or more leaves separated; Inflorescences fully developed. Smilica locality (authors photos)

MODEL DESCRIPTION

In explaining the model for predicting the length of incubation, we will start with the fact that the primary infection has already occurred. After that the incubation (latency) period is calculated according to the following parameters: average daily temperature, the effective temperature of incubation, and coefficient of incubation (table 2). Müller & Sleumer (1934) suggest that the minimum temperature of practical significance for the occurrence of an outbreak is 12 to 13°C. So, when calculating the incubation period, one should be based on average daily temperatures higher than 11 °C (Table 2). In order to estimate the duration of the incubation period of *P.viticola* the following formulas was used:

$$Ef = ADT - 11,$$

where: *Ef* - Effective temperature of incubation, *ADT* - Average daily temperature;

$$Coeff.In = Ef \div ADT,$$

where Coeff. In - Coefficient of incubation.

Table 2. Temperature parameters on a model for cumulative degree days

Dates	Average daily temperature (*ADT*)	Effective temperature of incubation (-11°C) (*Ef*)	Coefficient of incubation (*Coeff. In*)
02.5.2022	13,6	2,6	0,191176471
03.5.2022	12,9	1,9	0,147286822
04.5.2022	14,6	3,6	0,246575342
05.5.2022	17,8	6,8	0,382022472
06.5.2022	16,6	5,6	0,337349398
07.5.2022	16,6	5,6	0,337349398
08.5.2022	15,8	4,8	0,303797468
09.5.2022	16,7	5,7	0,341317365
10.5.2011	17,4	6,4	0,367816092
11.5.2022	18,3	7,3	0,398907104
12.5.2022	20,8	9,8	0,471153846
	∑=181,1	∑=60,1	

The results obtained from Table 2 were used for further calculation, using regression analysis [$y = \beta o + \beta 1(incubation\ days)$] for investigating and modeling the relationship between variables. Where: βo -*intercept*, $\beta 1$ - *x variable* and $y = (incubation\ days)$ - *number of observations*

RESULT AND DISCUSSION

The development of disease happens in time and space, and the incubation period is a part of the life cycle of *P.viticola*. The grapevine downy mildew is an obligate parasite, which means when zoospores swim through stomata and enter leaves, consequently the incubation process begins. Figure 1 gives an overview of the phenological stages of the development of grapevines (A - BBCH 15/53, B - BBCH 17/55, C - BBCH 19/57) during the incubation period of *P. viticola* in the Smilica locality. That means that in the intensive vegetative growing stage from-BBCH 15/53 till BBCH 19/57th growth stage, vines are in a sensitive development period towards downy mildew. When the leaf is 1/3 its full size, it exports more food than it uses and begins to contribute to vine growth happening in the already mentioned phenological stages: BBCH 15/53, BBCH 17/55, and BBCH 19/57. Due to favourable conditions at the beginning of the growing season, the open stomata in the above-mentioned phenological stages allow for disease development. Further disease development depends on the impact of temperature because the zoospores already in the leaf tissue are encysted (zoospore which has shed its flagellum) then germinate and produce an appressorium while penetrating through the stoma. In this context linear regression is used to find the relationship between average daily temperature and coefficient of incubation (Table 2), which allows determining the incubation period, i.e., Its length expressed in a period of 24 hours.

Table 3. Statistical analysis of cumulative degree days-model

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0,98498
R Square	0,970186
Adjusted R Square	0,966459
Standard Error	0,016147
Observations	10

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95,0%*	*Upper 95,0%*
Intercept	-0,35047	0,042689	-8,20982	3,62E-05	-0,44891	-0,25203	-0,44891	-0,25203
X variable	0,040825	0,00253	16,13467	2,19E-07	0,034991	0,04666	0,034991	0,04666

The essential benefit of regression analysis is determining how changes in the independent variable x are associated with shifts in the dependent variable y.That means that the values of the independent variable x allow us to explain the variations of the dependent variable y. When setting up the forecasting model, the *x* variable represents the average daily temperatures on which the incubation coefficients represented by the *y* variable depends. The interdependence of variable quantities expressed by Multiple R shows how strong the linear relationship is. The Multiple R = 0,98498 is significant in this case and shows that *y* dependent variable is in interdependent connection with *x* independent variable. Frequently R-squared values range from 0 to 1 and are commonly stated as percentages from 0% to 100%. Further, the coefficient of determination (R^2) or R Square is an indication of how much changes in independent variable cause changes in the dependent variable and the convection is expressed in percentage, respectively R^2=0,970186 x 100= 97%. This means that the other 3% belong to the category of unknown factors. Adjusted R Square typically always lower than the R Square (R^2), respectively Adjusted R Square=0,966459. The adjusted R-squared can tell you how useful a model is. In essence, Adjusted R-squared is a modified version of R-squared and decreases when a predictor (dependent variable) improves the model by less than expected. The parameters Standard Error = 0,016147 (SE) and R Square = 0,970186 (R^2) are related because the lower coefficient for SE causes a more predictable R^2, which gives the model validity.

y= βo+β1(incubation days);
$y = -0{,}35047 + 0{,}040825 \times (10);\ \ y = 0{,}057$

The parameter (0,057) of calculations shows the end of the incubation process (Table 4). This result of the *y* variable (0,057) got on May 12, 2022, was confirmed in the field when the first oil spot symptoms on the upper side of leaves at grapevines were noticed (Figure 2). Until May 12, the vineyard was not fungicide sprayed meaning there was no obstacle to the development of incubation.

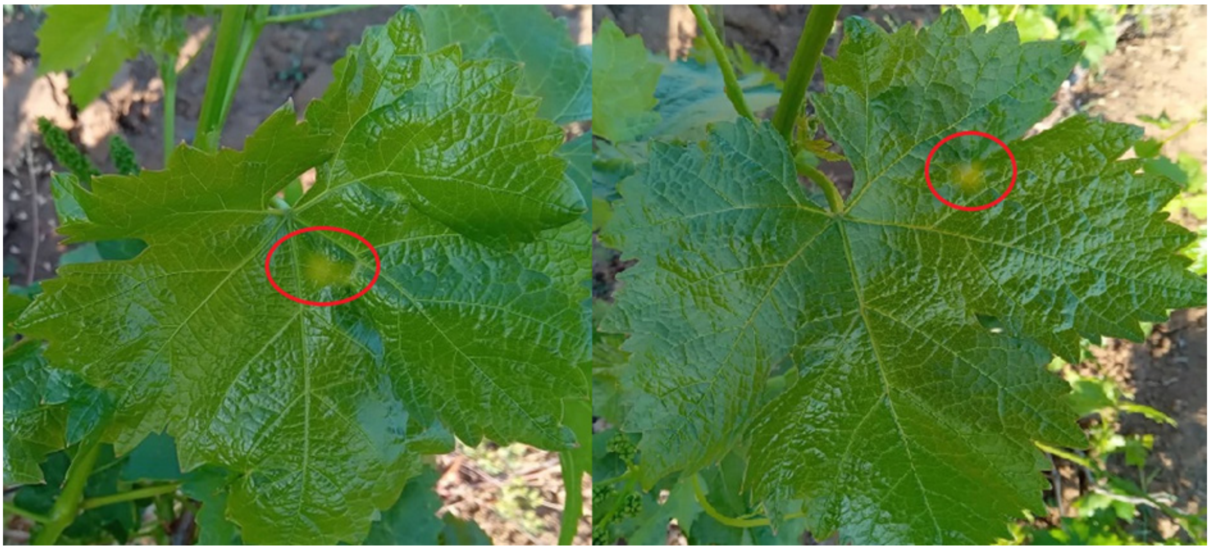

Figure 2. The occurrence of oil spot symptoms on the upper side of leaves indicates the end of incubation period of downy mildew at Smederevka variety. (photo of the author, 12.05.2022)

Any increase or decrease of disease intensity is in range of: $0<y\leq1$. The regression analysis result of the *y* variable in the days before May 12, 2022, have a negative sign and are not taken into account. The first day when the value of the *y* variable will have a positive coefficient marks the end of the incubation period and meets the expectations for predictions of the model (Table 4).

Table 4. Overview of values of the *y* prediction variable

Dates	Values of the *y* variable duration of incubation period $y = \beta o + \beta 1(incubation\ days)$
03.5.2022	-0,78694
04.5.2022	-0,42772
05.5.2022	-0,26144
06.5.2022	-0,21983
07.5.2022	-0,21983
08.5.2022	-0,17362
09.5.2022	-0,12661
10.5.2022	-0,07604
11.5.2022	-0,02118
12.5.2022	0,057-the end of incubation and occurrence of first symptoms

CONCLUSIONS

P.viticola the causal agent of downy mildew is very important fungal disease in grapevine production in the Republic of North Macedonia. The overall development of *P. viticola* and its occurrence in time and space cause rapid disease increases. The incubation period is a part of the life cycle of *P. viticola* between infection and the first appearance of symptoms. This simple model for determining the incubation period provides a quick prediction of the disease. According to Gobbin et al (2005), primary infections play a significant role in the downy mildew epidemic. This model serves to determine the end of the incubation period of the primary infection. Since the zoospores it's installed in plant tissue, which represents the invasive structure of *P.viticola*, obtain nutrients from the leaves, and the temperature remains the main driving factor for disease development, probably humidity of the environment has an indirect impact at this stage of the development of the disease Moreover, the inoculum is less present due to the delayed incubation period that depends on temperature conditions. At lower temperatures, the incubation is delayed and thus the appearance of the reproductive organs. The goal was to trace the incubation period through the temperature and discover when it ended. In practice, spraying is not applied during the first primary and the first secondary infection because the symptoms are visually less noticeable. Refraining from chemical treatments at the beginning of the vegetation is due to the lower temperatures that slow down the development of the disease in contrast to the period when the temperatures are higher and when we have an accelerated cycle of disease development that causes significant economic damages. The significance of determining the primary incubation lies in detecting the timing for the start of the first chemical treatments against the *P.viticola*. When we discover the end of incubation, if we have the appearance of favourable microclimatic conditions for the development of the disease, we must carry out spraying. If the weather is rainy, the chemical treatments can be carried out much earlier, i.e., for the duration of incubation. Due to the influence of lower temperatures at the beginning of the vegetation period, it often happens that the vine has a reduced intensity of growth, which does not exclude the possibility of the parasite carrying out infection and starting the incubation period. It is important to note that the prediction of the *P.viticola* should be based on temperature factors for disease development than on the grapevine's phenology.

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ПРОГНОЗЕН МОДЕЛ ЗАСНОВАН НА АКУМУЛИРАНИ ДЕНОВИ СО ТЕМПЕРАТУРНА СУМА ЗА ИНКУБАЦИСКИОТ ПЕРИОД НА *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni

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Резиме

Појавата на *Plasmopara viticola* со нејзиниот комплетен развоен циклус во времето и просторот овозможува брзо интензивирање на болеста. Периодот на инкубацијата ја претставува фазата на развој од биолошкиот циклус на *P. viticola* помеѓу инфекцијата и првото појавување на симптомите на болеста. Прогнозата базирана на акумулирани денови со температурна сума за определување на инкубацијата овозможува предвидување на мал број примарни инфекции чиешто пресметување се врши врз основа на температурниот фактор. Овој модел за прогноза, во суштина, е регресивна анализа која ја прикажува врската помеѓу просечната дневна температура и коефициентот на инкубација. Одредувањето на инкубацијата овозможува правовремено користење на фунгицидите против *P. viticola* и ги дава основните претпоставки за можна појава на примарниот инокулум.

Клучни зборови: *Plasmopara viticola, инкубациски период, просечна дневна температура, модел за предупредување, регресиона анализа, коефициент на инкубација*

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Original scientific paper

COMPARATIVE STUDIES OF THE CONTENT OF ANTIOXIDANTS IN FRUITS OF SOME AUTOCHTHONOUS CHERRY VARIETIES

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Abstract

The content of vitamin C, total phenols, anthocyanins, flavan-3-ols and the fruit antioxidant activity of 6 cherry varieties (Ohridska brza, Ohridska rana, Ohridska crna, Dolga shishka, Dalbazlija and Ohridska bela) was examined.

The fruits of autochthonous cherry varieties (*Prunus avium* L.) averagely contained 12.83 mg% vitamin C, 1386.25 mg/kg FW total phenols, 394.30 mg/kg FW anthocyanins, 69.150 mg/kg FW flavan-3-ols and had antioxidant activity 43.36% inhibition.

Some of the autochthonous cherry varieties compared to the proposed standard variety showed higher values in the examined parameters. The highest content of vitamin C and the fruit antioxidant activity were recorded in Dalbazlija and Dolga shishka varieties. The highest content of total phenols had the standard variety Burlat. High content of total phenols was also found in Ohridska rana, Dalbazlija, Ohridska brza and Ohridska crna varieties. Ohridska rana and Ohridska crna varieties had higher content of anthocyanins compared to the standard variety and other autochthonous varieties.

A moderate positive correlation was found between the content of anthocyanins and vitamin C. There was a moderate negative correlation between the content of flavan-3-ols and phenols. A very high positive correlation was found between the content of vitamin C and the antioxidant activity, while other chemical compounds had little or no effect on the antioxidant activity of cherry fruits.

Keywords: *Prunus avium* L., *vitamin C, total phenols, anthocyanins, flavan-3-ols, antioxidant activity*

INTRODUCTION

Fruits are rich source of antioxidants that have a preventive and therapeutic effect on human health, destroying free radicals that cause cancer and degenerative diseases. The content of antioxidants according to Bassi et al. (2017) depends on the genotype, environmental conditions, cultivation type, fruit storage method and processing. Unlike fresh fruits, industrially processed agricultural products contain fewer natural antioxidants.

Vitamins are necessary for maintaining human life and health, ensuring growth and development of the body. Insufficient intake of certain vitamins in the body leads to various diseases called hypovitaminosis and avitaminosis. One of the strongest antioxidants is vitamin C. It is contained in smaller or bigger quantities in all fruits. It is mostly found in rose hips and actinidia (634.1-1008.3 mg/100g), hawthorn (500 mg/100g), black currant, pomegranate (300 mg/100g), wild strawberry and blueberry (80 mg/100g) and citrus fruits (50 mg/100g). In smaller quantities it is found in raspberry, chestnut (40 mg/100g), apricot, cherry, peach (20 mg/100g), plum, walnut (10 mg/100g) and pear (4 mg/100g) (Latocha et al., 2010; Selamovska and Miskoska-Milevska, 2021; Rahman et al., 2008).

Polyphenols are very important compounds in plants that have antioxidant, anti-inflammatory, anti-cancer effects (Hertog et al., 1993; Goldner et al., 2015), anti-mutagenic, antiallergenic, antimicrobial action, they provide protection against infections, reduce the risk of chronic diseases, cardiovascular and neurodegenerative diseases (Vauzour et al., 2010). More than 40 phenolic compounds have been identified. **Flavonoids** are a class of plant secondary metabolites, polyphenolic antioxidants, which belong to the group of soluble coloured pigments. They are classified into 12 subclasses in terms of chemical structure, such as: flavonols, flavons, catechins, isoflavonoids etc. (Panche et al., 2016). They are most common in fruits (especially berry fruits) and grapes. Flavonoids are necessary for the production of vitamin C (Cook and Sammon, 1996). **Flavonols** belong to flavonoids, they are building blocks of proanthocyanidins. They are found in apples, strawberry fruits and grapes. Campferol, quercentin, myricetin, rutin and ficetin are especially present. **Flavanols** or catechins are the 3 hydroxy- derivatives of flavanones. These include flavan-3-ols, which are a large family of phenolic compounds, mainly responsible for the astringency, bitterness, and nutrient structure (Ivanova and Dimovska, 2010). They are mostly present in apple, blueberry, strawberry, and grape.

Anthocyanins are a group of over 500 different compounds that contribute to the red, purple, and blue colour of many plants, especially fruits and vegetables. Anthocyanin aglycones are known as anthocyanidins. The most important representatives of the group of anthocyanidins found in fruits and grapes are: pelargonidine, cyanidin, peonidine, delphinidine, petunidine and malvidin. They are most common in berry fruit species (black currant, blueberry, strawberry, raspberry), grapes and some tropical species, and to a lesser extent are found in medlar, mulberry, plum, apricot, pomegranate (Paz and Fredes, 2015; Panche et al., 2016; Khoo et al., 2017). Cevallos-Casals et al., (2002), Werner et al. (1989) in Prunus fruits established presence of several anthocyanins: cyanidin-3-glycoside, cyanidin-3-rutinoside, peonidin-3-glycoside and peonidin-3-rutinoside, while Tešović (1985, 1987) found the presence of cyanidin-3-ramnoglycoside, cyanidin-3-gentiobioside, cyanizide-3-monoglycoside and cyanidin-3-diglycoside.

Republic of N. Macedonia is plentiful in autochthonous varieties of fruit species which represent a rich starting material in further selection processes. The aim of this study is to detect and determine the content of specific groups of active substances (antioxidants) in the fruits of autochthonous cherry varieties.

MATERIAL AND METHODS

The study was part of the scientific project ***Antioxidant activity of fruits of autochthonous varieties and populations of fruits, vegetables and grapes,*** financed by the Ministry of Education and Science in 2021. The laboratory tests were performed in the oenological laboratory at the Institute of Agriculture in Skopje.

Six autochthonous varieties of cherry, *Prunus avium* L. (Ohridska brza, Ohridska rana, Ohridska crna, Dolga shishka, Dalbazlija and Ohridska bela) were taken as material for analysis from the region of Ohrid. Comparative studies were performed in relation to the standard variety Burlat.

The content **of vitamin C** (mg%) was examined by volumetric method, i.e. by titration of the filtrate with 2.6 dichlorophenol indophenol, (Shrestha et al., 2016) The end point of the titration was to obtain a faint pink colour. Samples with a higher degree of red staining in the final stage, before titration, were treated with 50 mg of activated carbon, until complete decolourization.

The content **of total phenols, anthocyanins and flavan-3-ols** was determined by spectrophotometric method, expressed in mg/kg FW. Their content was determined with Agilent 8453 UV-VIS spectrophotometer. Before proceeding to their determination, samples were prepared. Approximately 5 g of material was taken from the homogenized sample and transferred to laboratory flask, where 20 ml of pre-prepared solution (methanol: water: hydrochloric acid in a ratio of 70:30:0.1) was added. It was placed in an ultrasonic bath for 15 minutes and then for 30 minutes on a magnetic stirrer. The obtained clarified solution was transferred to a 25 ml laboratory flask and filled up to the mark with the same extraction solution.

Determining of total phenols: Total phenols in the samples were determined by the Folin-Ciocalteu method (Slinkar and Singelton, 1977). A blank sample was prepared, where distilled water was used instead of the tested sample, while the other reagents remained the same.

Determining of total anthocyanins: Determining the content of total anthocyanins was performed according to the Acid ethanol method (Somers and Evans, 1977). Ethanol chloride solution was used as a blank test. The content of anthocyanins was measured on a spectrophotometer at a wavelength of 550 nm.

Determining of total flavan-3-ols: P-dimethylaminocinnamaldehyde (p-DMACA) was used to quantify total flavan-3-ols in the tested samples (Di Stefano et al., 1989). Methanol was used as a control sample. The absorbance was measured at a wavelength of 640 nm.

These methods are significantly suitable for routine analysis, they are fast and can be used to monitor changes in polyphenols during fruit ripening.

Determining of antioxidant activity: This method was performed as an anti-radical activity against stable product DPPH (2,2-diphenyl-1-picrylhydrazil). Ascorbic acid was used as standard for preparing a series of standard solutions. The determining was performed spectrophotometrically, at a wavelength of 517 nm. (Shetty et al., 1995) (Shori & Baba, 2014).

According to XLStat test 2014 5.03, a correlation analysis was made between the examined parameters. The coefficient of determination R^2 and certain general standard parameters, such as maximum and minimum values, average value and standard deviation for each of the parameters were calculated.

RESULTS AND DISCUSSION

In Table 1, results from the chemical analysis performed on the fruits of the autochthonous cherry varieties were given.

Table 1. Chemical analysis on fruits of some cherry varieties

Variety	Vitamin C mg% FW	Total phenols mg/kg FW	Anthocyanins (mg/kg FW)	Flavan-3-ols mg/kg FW	Antioxidant activity (% inhibition)
Ohridska brza	11.0	1582.59	33.67	19.214	41.8
Ohridska rana	12.0	1604.91	1007.30	47.288	43.0
Ohridska crna	13.0	1488.41	742.36	100.564	40.2
Dolga shishka	19.0	1027.31	150.12	70.057	46.7
Dalbazlija	15.0	1589.00	61.34	37.717	50.7
Ohridska bela	7.0	785.32	67.18	104.966	36.9
Burlat	15.0	1626.24	698.42	104.282	44.2
Average	12.83	1386.25	394.30	69.150	43.36

The content of vitamin C in the fruits of the autochthonous cherry varieties had an average of 12.83 mg%. The varieties Dolga shishka (19.0 mg%) and Dalbazlija (15.0 mg%) had highest content of vitamin C, more than the standard variety and the other autochthonous varieties. The obtained results for the content of vitamin C were about 30% of the total antioxidant activity. In Dolga shishka variety, the content of vitamin C was about 40.7% of the total antioxidant activity, and in Dalbazlija variety was 29.6% of the total antioxidant activity. A moderate positive correlation was found between the content of vitamin C and anthocyanins. There was very high and statistically significant correlation between vitamin C and the antioxidant activity.

The content of total phenols in the fruits was 1386.25 mg/kg FW (fresh weight). The standard cherry variety Burlat had higher content of total phenols (1626.24 mg/kg FW), than all the autochthonous cherry varieties. High content of total phenols was also observed in Ohridska rana (1604.91 mg/kg FW), Dalbazlija (1589.00 mg/kg FW), Ohridska brza (1582.59 mg/kg FW) and Ohridska crna (1488.41 mg/kg FW) varieties. A positive correlation was found between the content of total phenols and the fruit antioxidant activity.

According to Mikulic-Petkovsek et al. (2016), bird cherry had higher content of total phenols (11053 mg GAE/kg) than wild cherry (2373 mg GAE/kg). Kaur et Kapoor (2005) found a positive correlation between the content of phenols and anthocyanins in some fruit species, while Murillo et al. (2012) found a positive correlation between polyphenol content and the antioxidant activity.

The average content of anthocyanins in the fruits of the autochthonous cherry varieties was 394.3 mg/kg FW. The varieties Ohridska rana (1007.30 mg/kg FW) and Ohridska crna (742.36 mg/kg FW) had a higher content of anthocyanins compared to the standard variety and other autochthonous varieties. Due to the high content of anthocyanins in the varieties Ohridska crna and Ohridska rana, it was necessary to add a larger amount of activated carbon for decolourization.

The accumulation of anthocyanins is primarily influenced by genetic and external factors (light, temperature, etc.). Chokeberry (300-2000mg/100g), blueberry (300-698 mg/100g), blackberry (82.5-325.9 mg/100g), pomegranate (15-252 mg/100g) were rich in anthocyanins (Cevallos-Casals et al., 2002; Ćujić et al., 2013). According to Tešović et al. (2012), different fruit species contained the same anthocyanin components: apple and dogwood fruits contained cyanidin-3-arabinoside, plum and juniper fruits contained cyanidin-3-glycoside and peonidin-3-rutinoside. In the fruits of wild and cultivated cherry varieties, the authors determined 4 anthocyanin components. Mikulic-Petkovsek et al. (2016) found a very high content of total flavonols in *Prunus mahaleb* and *Prunus avium*, represented by 19 quercetin derivatives, 10 campherol derivatives and 2 isoramenethine derivatives. Among the anthocyanin components in the *Prunus* species, according to the authors, the most common were cyanidin-3-glycoside and cyanidin-3-rutinoside.

The average content of flavan-3-ols in the fruits was 69.15 mg/kg FW. The highest contents were measured in Ohridska bela (104.966 mg/kg FW), Burlat (104.282 mg/kg FW) and Ohridska crna (100.564 mg/kg FW). There was a moderate negative correlation between the flavan-3-ols and total phenols.

According to the results of the study, cherry fruits had 43.36% inhibition antioxidant activity. The highest antioxidant activity was recorded in Dalbazlija (50.7% inhibition) and Dolga shishka (46.7% inhibition) varieties. A positive correlation was found between the content of total phenols and the fruit antioxidant activity.

The antioxidant activity of the fruits depended on the biotype, type and age of the plant material. The greatest antioxidant activity was found in strawberries, blackberry and red raspberry. In some cherry varieties of the species *Elaeagnus umbellate*, the antioxidant activity was 4.32-9.49 mM TE/100g (Lachowicz et al., 2019). The antioxidant activity in some cherry varieties of the species *Elaeagnus umbellate* according to Mikulic-Petkovsek et al., (2016) was 7.26-31.54 mM/kg. According to Wang and Lin (2000), fruits and leaves of fruit species had great antioxidant activity. With aging, the content of total phenols in leaves reduced and their antioxidant ability decreased.

In Tables 2, 3, 4 and 5 statistical data (maximum and minimum values, average value, standard deviation) and the correlation values of the examined parameters in the autochthonous varieties of cherries were given.

Table 2. Maximum and minimum values, average value and standard deviation with the tested parameters

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
Vitamin C	7	0	7	7,0000	19,0000	13,1429	3,7607
Phenols	7	0	7	785,3200	1626,2400	1386,2543	337,9980
Anthocyans	7	0	7	33,6700	1007,3000	394,3414	407,6255
Flavan-3-ols	7	0	7	19214,0000	104966,0000	69155,4286	35271,0688
Antiox.activity	7	0	7	36,9000	50,7000	43,3571	4,4725

A moderate positive correlation was found between the content of anthocyanins and vitamin C. There was a moderate negative correlation between the flavan-3-ols and phenols, i.e. with increase of the value of flavan-3-ols, while the value of phenols decreased.

There was very high and statistically significant correlation between the content of vitamin C and the antioxidant activity, while other chemical compounds had little or no effect on the antioxidant activity in cherry fruits (Tab. 3).

Table 3. Correlations with tested parameters

Variables	Vitamin C	Phenols	Anthocyans	Flavan 3-ols	Antiox. Activity
Vitamin C	**1**	0,2263	0,0596	-0,1009	**0,7793**
Phenols	0,2263	**1**	0,4700	-0,4357	0,4041
Anthocyans	0,0596	0,4700	**1**	0,2870	-0,1507
Flavan-3-ols	-0,1009	-0,4357	0,2870	**1**	-0,4681
Antiox.activity	**0,7793**	0,4041	-0,1507	-0,4681	**1**

Values in bold are different from 0 with a significance level alpha=0.05

The values of p were lower than the given alpha = 0.05 when comparing the values of vitamin C and antioxidant activity (p = 0.0389), which indicated a statistically significant correlation (Tab. 4).

Table 4. p values with tested parameters

Variables	Vitamin C	Phenols	Anthocyans	Flavan 3-ols	Antiox. activity
Vitamin C	**0**	0,6255	0,8990	0,8295	**0,0389**
Phenols	0,6255	**0**	0,2873	0,3285	0,3686
Anthocyans	0,8990	0,2873	**0**	0,5327	0,7470
Flavan-3-ols	0,8295	0,3285	0,5327	**0**	0,2895
Antiox. Activity	**0,0389**	0,3686	0,7470	0,2895	**0**

Values in bold are different from 0 with a significance level alpha=0.05

The highest coefficient of determination was found between vitamin C and the antioxidant activity, i.e. about 60.7% of the variations in the value of the antioxidant activity were due to variations in the value of vitamin C (Tab. 5).

Table 5. Coefficient of determination R^2 with tested parameters

Variables	Vitamin C	Phenols	Anthocyans	Flavan 3-ols	Antiox. activity
Vitamin C	**1**	0,0512	0,0036	0,0102	0,6073
Phenols	0,0512	**1**	0,2209	0,1898	0,1633
Anthocyans	0,0036	0,2209	**1**	0,0823	0,0227
Flavan-3-ols	0,0102	0,1898	0,0823	**1**	0,2191
Antiox. Activity	0,6073	0,1633	0,0227	0,2191	**1**

CONCLUDING REMARKS

Some of the autochthonous cherry varieties compared to the proposed standard variety showed higher values in the examined parameters. The varieties Dolga shishka and Dalbazlija had the highest content of vitamin C, more than the standard variety and the other autochthonous cherry varieties. The obtained results for the content of vitamin C were about 30% of the total antioxidant activity.

The highest content of total phenols had the standard variety Burlat. High content of total phenols was also found in Ohridska rana, Dalbazlija, Ohridska brza and Ohridska crna varieties.

The varieties Ohridska rana and Ohridska crna had higher content of anthocyanins compared to the standard variety and other autochthonous varieties.

The highest content of flavan-3-ols in the fruits were measured in Ohridska bela, Burlat and Ohridska crna varieties.

The varieties Dalbazlija and Dolga shishka had the highest antioxidant activity, more than the standard variety and the other autochthonous varieties.

A moderate positive correlation was found between the content of anthocyanins and vitamin C. There was a moderate negative correlation between the content of flavan-3-ols and total phenols. A very high positive correlation was found between vitamin C and the antioxidant activity, while other chemical compounds had little or no effect on the antioxidant activity of cherry fruits.

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СПОРЕДБЕНИ ПРОУЧУВАЊА НА СОДРЖИНАТА НА НЕКОИ АНТИОКСИДАНТИ ВО ПЛОДОВИТЕ ОД НЕКОИ АВТОХТОНИ СОРТИ ЦРЕШИ

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Резиме

Во трудот се испитувани содржината на витамин С, вкупни феноли, антоцијани, флаван-3-оли и антиоксидативната активност на плодовите од 6 сорти цреша (*охридска брза, охридска рана, охридска црна, долга шишка, далбазлија* и *охридска бела*).

Плодовите од автохтоните сорти цреша просечно содржеле 12.83 mg% витамин С, 1386.25 mg/kg FW вкупни феноли, 394.30 mg/kg FW антоцијани, 69.150 mg/kg FW флаван-3-оли и имале антиоксидативна активност на плодовите од 43.36% инхибиција.

Некои од автохтоните сорти цреша споредени со стандардната сорта покажале повисоки вредности во испитуваните параметри. Највисока содржина на витамин С и најголема антиоксидативна активност на плодовите биле регистрирани кај сортите *далбазлија* и *долга шишка*. Највисока содржина на вкупни феноли имала стандардната сорта *бурлат*. Висока содржина на вкупни феноли, исто така, била утврдена и кај *охридска рана, далбазлија, охридска брза* и *охридска црна*. *Охридска рана* и *охридска црна* имале повисока содржина на антоцијани, споредено со стандардната сорта и другите автохтони сорти.

Кај црешите е утврдена умерена позитивна корелација меѓу содржината на антоцијани и витаминот С. Утврдена е средна негативна корелација меѓу содржината на флаван-3-оли и вкупни феноли. Многу висока позитивна корелација е најдена меѓу содржината на витаминот С и антиоксидативната активност, додека другите хемиски компоненти имале мал или никаков ефект на антиоксидативната активност на плодовите цреши.

Клучни зборови: *цреша, автохтони сорти, витамин С, вкупни феноли, антоцијани, флаван-3-оли, антиоксидативна активност*

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