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CONTENT

INTORDUCTION

In the past twelve years the educational, research and applicative activities of the Faculty of Agriculture – Stip, Goce Delcev University – Stip, contributed to the development of agriculture sector in the country and broader region.

The Faculty of Agriculture organized the 2nd International Meeting Agriscience & Practice (ASP 2019), giving an opportunity to the participants for presentation and discussion of original scientific and practical results in different fields of agriculture.

The 2nd International Meeting Agriscience & Practice (ASP 2019), heled on 12 April 2019 at the Faculty of Agriculture - Stip, was organized with intention to bring together all agricultural stakeholders for sharing their knowledge, experience and obstacles. One of the main aims was to link research and field work in agricultural sector in the country and broader, giving it an international dimension. All oral presentations as well as poster presentations at ASP 2019 were organized in several scientific sessions:

- Agricultural economics,
- Plant biotechnology,
- Plant production,
- Plant protection,
- Quality control and food safety,
- Soil science and hydrology,
- Viticulture, enology and fruit production.

The main goal of the Meeting was linking and promoting scientific achievements and practical knowledge, presented in different thematic areas, which were achieved in the Republic of Macedonia and wider in the region.

Journal of Agriculture and Plant Sciences Vol. 17, No. 1 mostly contains the presented papers from the 2nd International Meeting Agriscience & Practice (ASP 2019).

Editorial Board, **Editor in chief,**

June, 2019 **Prof. d-r Liljana Koleva Gudeva, PhD**

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EVALUATION OF IRRIGATION SCHEDULING TECHNIQUES: A CASE STUDY OF WHEAT CROP SOWN OVER PERMANENT BEDS UNDER SEMI-ARID CONDITIONS

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Abstract

The present study was carried out at Water Management Research Centre (WMRC), University of Agriculture Faisalabad, Pakistan to optimize the water usage for wheat crop in water scare regions of the country. Two main irrigation scheduling approaches i.e soil-moisture-based and climatic based were adopted and compared. There were overall six treatments; three treatments were for testing of climatic approach of irrigation scheduling i.e. application of irrigations at 20 mm (TC1), 30 mm (TC2) and 40 mm (TC3) Cumulative Pan Evaporations (CPE), and three treatments were for soil-moisture-based approach of irrigation scheduling i.e. 30% (TS1), 45% (TS2) and 60% (TS3) of Management Allowable Depletion (MAD) levels, and one farmer's practice (Fp). Another aspect of the study was to check the ability of an in-season Normalized Difference Vegetation Index (NDVI) measurements to predict wheat yield potential. Results showed that the soil-moisture-based treatments significantly increased wheat grain yields over climate-based treatments (TC2 and TC3) and non-significantly over TC1. Results of NDVI measurements showed that it can be used for accurate in-season wheat grain yield estimation with R2, RMSE, Cv, ME, RE, Bias and NSE estimated 0.768, 17, 4.4, 0.07, 0.019, 0.073 and 0.84 respectively and can be used as a valuable crop management tool.

Key words: *soil moisture, cumulative pan evaporation, management allowable depletion, normalized difference vegetation index, grain yield*

INTRODUCTION

In Pakistan, wheat (Triticum aestivum L.) is one of the major crops and it is grown on a large area. It contributes 9.1% of the total value added of the agricultural sector and 1.7% of the gross domestic product of Pakistan (GoP, 2018). It has a dominant status in the formulation of agriculture sector policies in the country (Mangrio et al., 2016). Wheat production in the country for the economic year 2017-18 was 25.492 million tons from an area of 8734 thousands hectares (GoP, 2018). Punjab is the largest wheat producing province of Pakistan with a contribution of around 76% of the wheat production from 75% wheat area of the country (GoP, 2015). Irrigation water is one of the important inputs controlling the productivity of wheat crop.

The water availability in Pakistan has approached about 1000 m3/capita, categorizing the country as a water deficit country (Bakhsh et al., 2015 and Ansari et al., 2017). Moreover, projections show that the current pace of increasing population may lower water availability to 915 m3/capita in 2020 (GoP, 2014). Under these circumstances, there is a need to apply irrigation water efficiently to increase water productivity, which has been reported to be as low as 0.2 kg/m3 of water (Ceema et al., 2016). The traditional practice used for increasing productivity is extensive utilization of resources like fertilizer and water. Eventually, it increases the production cost and overuse of the inputs which in turn not only suppress overall yield

but cause problems like weed growth, plant diseases, soil degradation, and ineffectiveness of pesticide. Hence, an appropriate management of irrigation water is considered necessary for sustainable production and high-water use efficiency.

Crop water requirement varies from crop to crop and it fluctuates daily with the climate conditions. Pan evaporation (PE) integrates all the climatic parameters (Solar radiation, temperature, wind speed, relative humidity etc.) for determining crop water demand (Joshi et al., 2017). In this study the crop water requirement was assessed from pan evaporation method. However, PE does not be similar to the actual use of water by crops but it is driven by similar atmospheric variables and correlates relatively well with crop water use or potential ET (Penman, 1948). The crop evapotranspiration models and equations, for example Penman or the later Penman-Monteith equation requires a large number of climatological datasets (Solar radiation, maximum and minimum temperature, wind speed, relative humidity, etc) and it is also a complex method. Allen et al., (1998) found that the PE method provide a good estimate of reference evapotranspiration (ETo) when suitable pan coefficients of that area are applied. Lee et al., (2004) compared eight methods of estimation of evapotranspiration with daily data of 30 years and found no significant difference among PE method, Penman-Monteith method and Blaney-Criddle method at 0.05 probability.

Management Allowable Depletion (MAD) in soil moisture was used to schedule irrigation for wheat crop. MAD is the level of moisture depletion in available soil moisture to which a crop withstands without any adverse effect on it (Markewitz et al., 2010). Panda et al., (2003) observed maximum field water use efficiency at 45 % MAD even for the non-critical growth stages of wheat crop. Laghari (2009) found that maximum yield and crop water use efficiency was obtained when irrigation was scheduled at 55% MAD of Available Soil Moisture (ASM) for wheat crop and at 65% MAD of ASM for cotton crop.

Crop yield predictions play supporting role in decision-making and policy formulations on both regional and global scale. At the field level, the crop yield prediction information, particularly related to crop management decisions as crop performance, growth, and development are the desegregating factors that evaluate the efficiency of the adopted agricultural management practices within the boundaries of the agro-ecological environment (Choudhary et al., 2012). Particularly, much of the grain yield production capacity of wheat crop is related to growth during a few weeks before anthesis (Marti et al., 2007). Thus, the in-season yield prediction is used as a tool for deciding on management options for the rest of the crop stages. The use of spectral reflectance indices, such as the NDVI, has been proposed as a fast, nondestructive way to monitor crop condition and estimate crop growth capacity (Mutiibwa and Irmak 2013; Waqas et al. 2019). NDVI is an index which is a measure of vegetation density and condition. The NDVI can be calculated from reflectance measurements in the red and near infrared (NIR) portion of the spectrum. Its values range from −1 (usually water deficit) to +1 (strongest vegetative growth). The optical properties of the leaf tissues, their cellular structure and the air-cell wall protoplasm-chloroplast interfaces determined the reflectance in the NIR range (λ = 700–1300 nm) and in the Red range (λ = 550–700 nm) (Kumar and Silva, 1973).

The present study was an attempt to assess the effect of different irrigation schedules on the crop yield parameters. The whole study was carried out with the two main objectives; i) To study the effects of climate based and soil-moisture-based irrigation schedules on wheat yield parameters and ii) To determine the ability of NDVI to predict an in-season estimation of wheat yield potential.

MATERIAL AND METHODS

Experimental Site

The study was carried out at the experimental plots of WMRC, Faisalabad, Pakistan, on wheat crop for Rabi 2014-15. The local climate of the area is semi-arid with an average annual rainfall of 350mm, concentrated mostly over the months of June to August. A total of 133 mm rainfall was received in the whole growing season (from November to April), especially during March to April. Monthly

means that maximum temperature observed was 33.2 °C in the month of April and monthly mean minimum temperature observed was 5.9 °C in the month of December. The weather data of the experimental site for the whole growing season are given in Table 1. Three bulk soil samples were made from nine sub samples taken from three depths (0-15 cm, 16-30 cm, and 31-45 cm) at three different locations within the field. The soil texture in the study area was tested and found as sandy loam. All physical and chemical properties show a little variation throughout the study area and at different depths. The soi'sl physical and chemical properties at the experimental crop field are given in Table 2.

Table 1. Weather data of the experimental site throughout the growing season.

Month	Monthly Mean Max. Temperature (°C)	Monthly Mean Min. Temperature (°C)	Monthly Mean Relative Humidity (%)	Rainfall (mm)	Monthly Mean Sunshine Hours (Hrs)	Monthly Avg. Wind Speed (Km/hr)
November	26.3	11.5	61.7	0	7.6	3.1
December	18.5	05.9	75.0	0	4.7	2.0
January	16.6	06.9	75.3	11.12	5.0	3.6
February	22.0	11.1	66.0	20.5	5.6	5.3
March	24.5	13.6	64.0	67.9	4.9	5.6
April	33.2	20.7	43.9	32.8	9.1	6.2

Table 2. Soil physical and chemical properties of the experimental site.

Soil Depth Layers (cm)	Physical characteristics							Chemical characteristics				
	Sand (%)	Silt (%)	Clay (%)	Soil Type	Bulk Density (g/cc)	Field Capacity (%)	Wilting Point (%)	pH	EC (dS/m)	OM (%)	P (ppm)	K (ppm)
0-15	63	23	14	Sandy loam	1.54	21.7	8.42	8.40	1.32	0.45	1.8	100
16-30	67	19	14	Sandy loam	1.56	21.3	8.00	8.27	1.30	0.43	1.7	87
31-45	66	18	16	Sandy loam	1.55	21.8	8.45	8.25	1.33	0.46	1.5	73

P: Phosphorus, K: Potassium, OM: Organic Matter

Field Layout and Experimental Details

The experiment was comprised of three main plots; two plots for testing irrigation scheduling techniques (Climate based and soil moisture based) with three levels on each plot and replicated three times arranged in Complete Randomized Design (CRD), one plot for farmer practice treatment (Fp). Climate-based irrigation scheduling was accomplished using predefined cumulative pan evaporation (CPE) and Soil-moisture-based irrigation scheduling was done using the temporal soil moisture measurements. The three levels of each irrigation scheduling technique is given below:

1) Climate based
   TC_1= 20 mm CPE
   TC_2= 30 mm CPE
   TC_3= 40 mm CPE

2) Soil-moisture-based
   TS_1= 30% MAD of ASM
   TS_2= 45% MAD of ASM
   TS_3= 60% MAD of ASM

Sowing

The wheat crop was sown at mid-November using bed-furrow wheat planter. The seeding was done at the rate 125 kg/hectare at 0.0254 meter depth. The selected plot contained permanently raised beds. The beds were made two years earlier. Before sowing of wheat crop, cotton was sown over the same beds. Traditional soaking irrigation (Rouni irrigation) was not applied as enough moisture was present in soil. The total area was 1170 square meters. There were total 18 beds. Each bed was 67 meters long and 0.61 meters in width with 0.3048-meter space for furrow. Nine beds were reserved for trials for irrigation schedules based on climatic indicator (i.e. CPE) and remaining nine beds were reserved for the trials of irrigation schedules based on soil moisture depletions. Farmer practice was conducted on the adjacent field. The sowing method, plant protection and fertilizer application were same as other treatments.

Plant protection and fertilizer application

Three types of fertilizers were applied; i) Urea as a source of nitrogen at the rate of 48 Kg-N/ha, ii) Di-Ammonium Phosphate (DAP) as a source of phosphorus at the rate of 52 Kg-P/ha and iii) Muriate of Potash (MOP) as a source of potassium at the rate of 64 Kg-K/ha. Plant protection measures including herbicides and all other cultural practices were also carried out during its growth period whenever required for all the treatments. Buctril Super (Bromoxynil) and Topik (clodinafop-propargyl) were used for broadleaf and grass weeds at the rate of 740 mL/ha and 296 g/ha, respectively.

Irrigation Scheduling

Climate-based irrigation scheduling was accomplished using predefined CPE. Class A type evaporation pan was used for the estimation of pan evaporations. The average value of pan coefficient (K_p) was considered as 0.7 (Ken University lectures: Accessed online) and the crop coefficient (K_c) values for different growth stages were taken from the working paper 24 of International Water Management Institute (IWMI) (Ullah et al., 2001). Amount of water applied was calculated by using following formula:

$$I = E_{pan} \times K_{pan} \times K_c \quad (2)$$

Where, I is irrigation water depth (in mm), E_{pan} is pan evaporation (in mm), K_{pan} is pan coefficient (0.7), K_c is the crop coefficient

Soil-moisture-based irrigation scheduling was done by using the temporal soil moisture measurements using Time Domain Reflectometer (TDR). Similarly, irrigation was applied at 18%, 16% and 14% soil moisture content for 30% MAD, 45% MAD and 60% MAD respectively to fill up the root zone up to field capacity.

The percent depletion of ASM in the effective root zone was estimated using following Eq. 3 (Martin et al., 1990),

$$MAD(\%) = 100 \times \frac{1}{n} \sum_{1}^{n} \frac{FC_i - \theta_i}{FC_i - WP} \quad (3)$$

Where, n is the number of sub-divisions of the effective rooting depth, θ_i is the soil moisture in i^{th} layer (in %), FC_i is the soil moisture at field capacity for i^{th} layer (in %), WP is the soil moisture at permanent wilting point (in %)

The volume of irrigation was calculated by the Eq. 4.

$$V_d = \frac{MAD(\%) \times (FC - WP) \times R_z \times A}{100} \quad (4)$$

Where, V_d is the volume of irrigation water (in m^3), R_z is the effective rooting depth (in m), A is the surface area of plot (in m^2)

In Fp treatment, water was applied according to farmer's practice (irrigation water was applied at different growth stages without considering the soil moisture, evapotranspiration or crop stress) and implemented in adjacent field of the research area.

Crop Yield Prediction

Active remote sensing based NDVI measurements were taken using a spectro-radiometer (handheld Greenseeker optical sensor) above the canopy at 50 cm height at different growth stages during the season. The linear relationship between NDVI and grain yield was determined using correlation coefficient (r) for measuring the strength of association between NDVI and grain yield at all growth stages. A simple linear regression model was developed between NDVI and actual grain

yield at maximum correlated stage. Twelve plots (67% of the total plots) were randomly selected for this purpose. The remaining 33% (6 plots) were used for validation purpose. The mean error (ME), the relative error (RE), the root mean square error (RMSE), coefficient of variation (Cv), R^2 and Nash-Sutcliffe model efficiency (NSE) were used for model evaluation (Saeed et al. 2017).

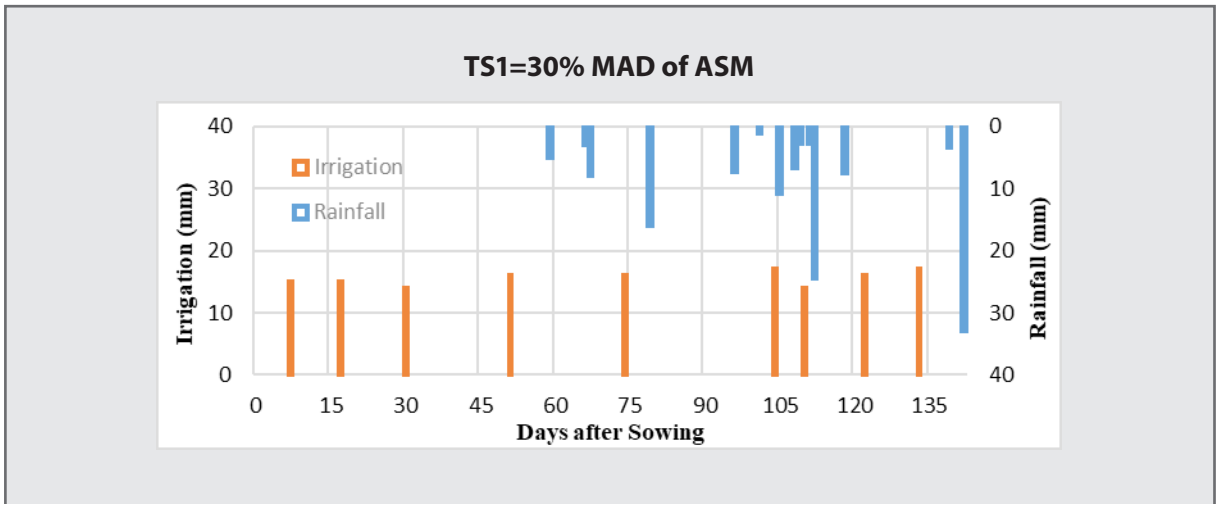
Harvesting

Crop was harvested in the last week of April. An area (0.6 m^2) at head, mid and tail section from each plot was harvested and sub samples of 10 plants were selected randomly for the determination of different yield components. All samples were threshed manually for the estimation of grain yield and biological yield. The grain yield data and other crop parameters (spike length, no. of grains per spike, plant height, and biological yield) were statistically analyzed using analysis of variance (ANOVA) with comparison of means using Least Square Difference (LSD) test at $P \leq 0.05$.

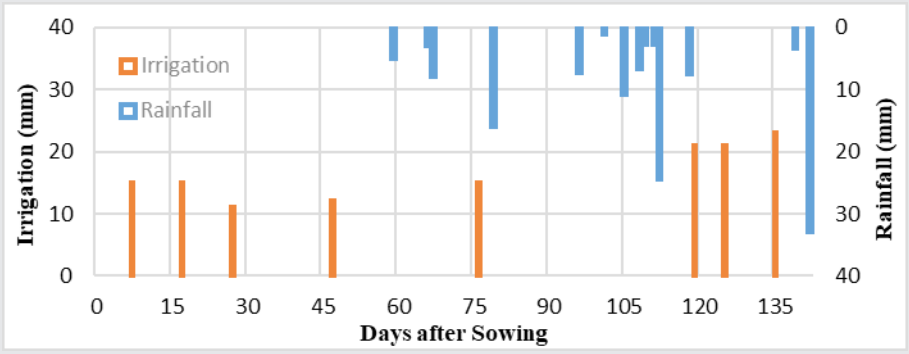
RESULTS AND DISCUSSION

Irrigation Scheduling

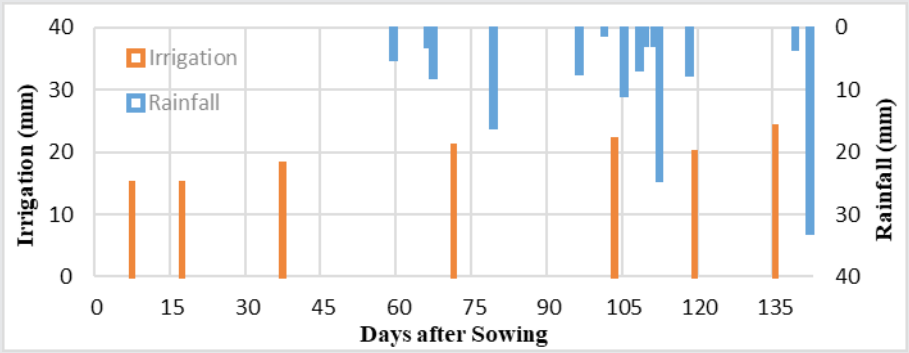
The frequency and quantity of irrigation water are critical in wheat crop production. The water stress timing can cause significant variation in grain yield (Akram, 2011; Mirbahar et al., 2009; Patel et al., 2008; Behra and Panda, 2009). The crop water needs were fulfilled by rainfall and irrigation water. The irrigations were applied through drip irrigation system except first watering (seven days after sowing) which applied through flooding method as proportionate amount of irrigation water was applied (30 mm total) for uniform germination of wheat. The rainfall used by plants was taken 70 percent of total rainfall and termed as effective rainfall. The whole growing season (from November 15 to April 21) received 92 mm effective rainfall. In first half of the growing season as no rainfall event occurred, and the crop water requirements were fulfilled by irrigation water only. In the later part of the growing season, temperature conditions turned into warmer, and total fourteen rainfall events were recorded which partially fulfilled the crop water requirements. The last rainfall of the crop season was lesser utilized by the crop due to maturity stage of the crop. In soil-moisture-based schedules, soil water depletion rates were used to determine the time of irrigation. In case of 30% MAD, Irrigation water was applied when soil moisture contents reached at 18% to fill up the root zone up to field capacity. Similarly, irrigation was applied at 16% and 14% soil moisture content for 45% MAD and 60% MAD respectively. The number of irrigations for TS1was greater than TS2 and TS3, as TS1 was low volume and high frequency treatment (least stressed) whereas TS3 was high volume and low frequency treatment (most stressed).



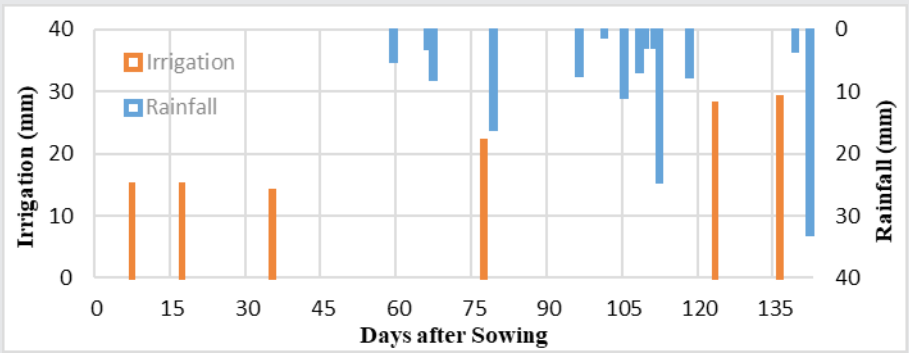
TC1=20 mm CPE



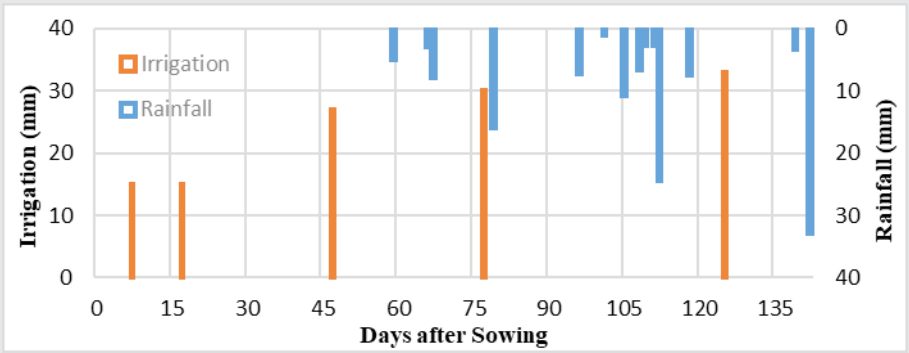
2=45% MAD of ASM



TC2=30 mm CPE



TS3=60% MAD of ASM



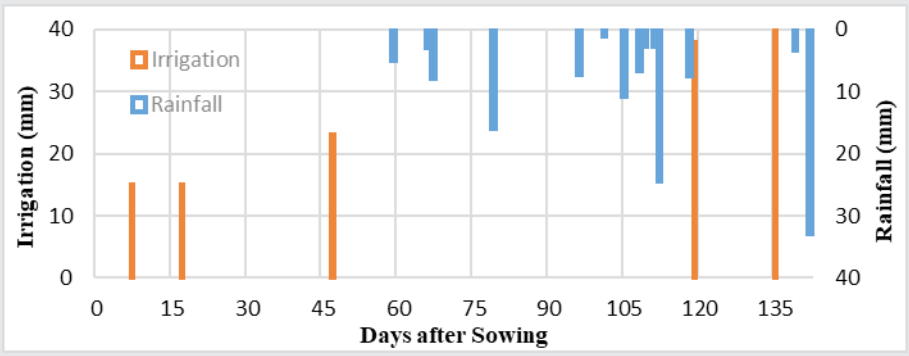


Figure 1. Irrigations scheduled and rainfall events recorded for all treatments.

The predefined levels of MAD were achieved throughout the growing season by applying total nine, seven and five irrigations in TS1, TS2 and TS3 treatments, respectively. In climate-based schedules, evaporation rates were used to determine the time of irrigation. For TC1 treatment, irrigation was applied, when cumulative pan evaporation reached at 20 mm. In the same way, water applied at 30 mm and 40 mm CPE for TC2 and TC3 treatments. The TC1 is the low volume high frequency treatment (least stressed) while TC3 is the high volume and low frequency treatment (most stressed). In the whole growing season, total eight, six and five irrigations were applied in TC1, TC2 and TC3 treatments, respectively. The water application in terms of rainfall and irrigation for all treatments are shown in Figure 1. The total water application from both sources irrigation and rainfall varied from 212 mm to 232 mm for all treatments except farmer practice, in which water was applied at a rate of 350 mm per season. Overall, the quantity of water applied based on soil moisture approach were greater than that of applied based on climatic approach.

Effect of water stress timing on grain yield

The effects of different irrigation schedules, based on climate and soil moisture data, on the development of grain yield of wheat crop was investigated during the study. The growth parameters considered in the current study were plant height at harvest, spike length at harvest, average number of grains per spike, biological yield, grain yield are shown in Table 3 with statistical analysis. Grain Yield, biological yield, and irrigation water productivity followed the same trend (i.e. TC1>TC2>TC3 and TS1>TS2>TS3) within each set of treatments of climate and soil-moisture-based approach. However, average plant height, average spike length and average number of grains per spike showed irregular trends. Regarding about grain yield, TS1 gave maximum yield (4.35 t/ha) while minimum yield was obtained with TC3 treatment (3.32t/ha). In case of biological yield, maximum (12.50 t/ha) and minimum (11.55 t/ha) yield were recorded with TS1 and TC3 treatments respectively. The significant difference was observed for all parameters except crop water use, plant height and spike length at harvest. Results of LSD pair-wise comparison test for three climate-based treatments showed that the irrigation schedule at 20 mm CPE resulted in significantly higher grain yield over 40 mm CPE and non-significantly over 30 mm CPE. Irrigation schedule at 30 mm and 40 mm CPE generated non-significant results. The results of three soil based treatments showed non-significant difference for grain yield. Overall, soil moisture-based treatments (30%, 45% and 60% MAD) significantly increased wheat grain yields over climate-based treatments (30 mm and 40 mm CPE) and non-significantly with 20 mm CPE climatic treatment.

Table 3. Effect of different irrigation schedules on wheat yield and its component.

Treatments	Crop water use (mm)	Plant height at harvest (cm)	spike length at harvest (cm)	No. of grains per spike	Biological yield (t/ha)	Grain Yield (t/ha)
TC1	225	99	12.67	65a	12.12cd	4.03bc
TC2	215	98	12.00	57b	11.81e	3.67d
TC3	225	97	12.00	55b	11.55f	3.32e
TS1	232	95	12.00	61ab	12.50a	4.35a
TS2	227	98	12.33	60b	12.28b	4.24ab
TS3	212	99	11.67	57b	12.05d	3.94c
LSD 5%				5.42	0.15	0.27
Significance	ns	ns	ns	*	*	*

*=Significant ns =non-significant

The maximum yield obtained among soil-moisture-based treatments (i.e. in TS1) was 7.94% higher than that of obtained among climate-based treatments (i.e. TC1), and gave 31% and 28 % more grin yield than TC3 and Fp treatment. The yield difference among all treatments were attributed to the first half of the season. The yield reduced for high volume and low frequency treatments as crop is its initial stage and it's become difficult for root system to extract sufficient soil water from a greater depth, thus limiting its water uptake (Ahmed et al., 2012), which consequently effect the plant growth and yield. Shiva et al., (2017) reported higher root water uptake for least stressed schedule (70% of FC) and decreased with less frequent amounts of irrigation (50% and 60% of FC). These results are in concord with the findings of Blonquist Jr et al., (2006) and Khalilian et al., (2007) while opposite results were found in Taber (2007) study. The irrigation water productivity was used to check the efficient utilization of water and presented in Figure 2. It is the ratio of grain yield produced per volume of water applied. Regarding the irrigation water productivity, its indicates the how efficiently water applied and used. The highest value was obtained for TS1 treatment (1.875 kg/m^3) and lowest for farmer practice (0.971 kg/m^3). Although lowest grain yield was recorded for TC3 treatment but it's still better schedule than farmer practice, as irrigation water productivity was higher for TC3 (1.475 kg/m^3) than Fp treatment (0.971 kg/m^3). Comparable results were reported by Shiva et al., (2017) who found the highest water productivity(2.08 kg/m^3) in drip irrigation method scheduled at 60% of field capacity and was the lowest in surface flooding method scheduled at 70% of field capacity (1.86 kg/m^3).

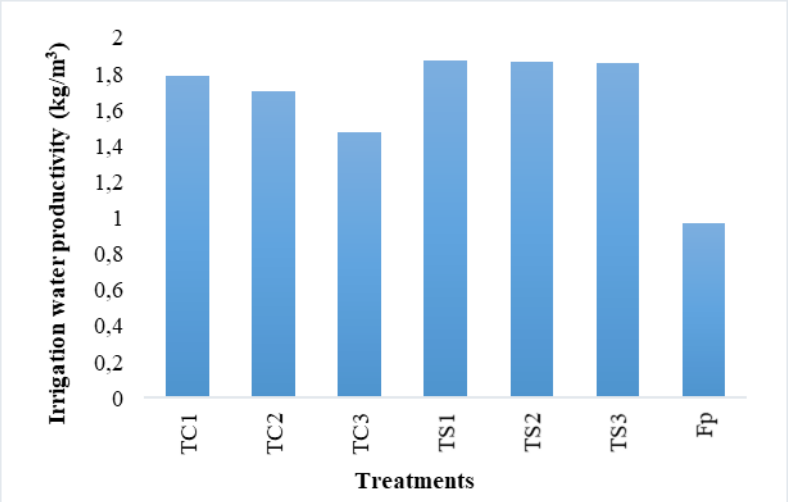


Figure 2. Graphical representation of grain yield and crop water use.

NDVI and yield prediction

NDVI based crop growth dynamics curves were developed for the same experimental data and shown in Figure 3. As expected, the NDVI values were low in early season and gradually increased with crop growth and reached a plateau in mid-season at the time when the ground surface was completely covered by the crop canopy. The plateau in NDVI curves seems to correspond to the saturation of the sensor and eventually loss of its sensitivity to measure changes in vegetation when leaf area index (LAI) is higher than 3 (Aparicio et al., 2000; Carlson and Ripley, 1997; Duchemin et al., 2006; Naser, 2012). The decrease in NDVI values at the end of the season is due to the physiological crop maturity, change in crop color and senescence leaf, which increases red band reflectance and decreases NIR band reflectance.

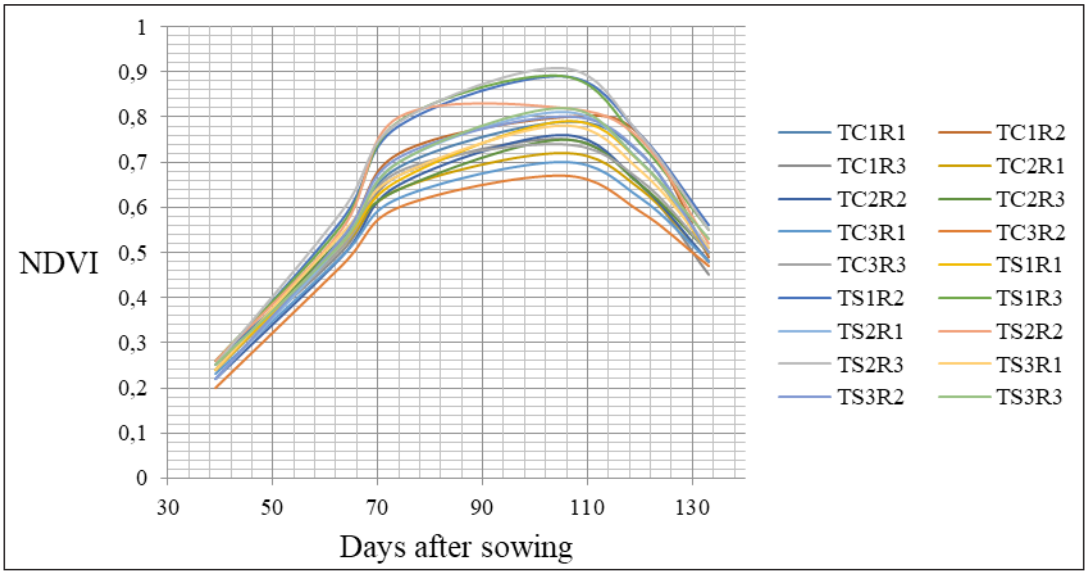


Figure 3. NDVI based crop growth dynamics curves.

Time of measurement is an important factor in determining the feasibility of integrating predicted yield under different water management practices. The correlation coefficient (R^2) between grain yield and NDVI was determined for all treatments at all growth stages. At the time of tillering, shooting, booting, anthesis, and milk maturity NDVI values were highly positively correlated with grain yield. Results of correlation coefficient suggested that NDVI measurements taken at heading stage could assist with more accuracy in predicting grain yield as compared to other developmental crop growth stages. Teal et al., (2006) studied that the NDVI readings after heading stage were unable to discriminate variations, whereas at early growth stages yield potential had not yet completely developed. A simple linear regression model was developed between actual grain yield and NDVI at heading stage for randomly selected 12 plots (67% of the total), shown in Figure 4. It showed a good agreement (R^2=0.873). According to Walsh et al., (2013) there is a good relationship between NDVI and actual wheat grain yield. at the Feekes 5 growth stage (end of tillering stage) and explained 82%, 69% and 88 % of variation in grain yield at Lahoma in 2008, at Perkins in 2008 and at Lahoma in 2009, respectively. The regression model developed between NDVI and grain yield by Naser (2012) showed a strong relationship at mid-grain filling stage with coefficient of variation 0.83. Identical results were found in the studies of Sultana et al., (2014) which were performed in similar environmental conditions. At country level, Ren et al., (2008) found good agreement between NDVI and grain yield with R^2=0.8, whereas Liaqat et al., (2017) reported this relationship with R^2=0.50.

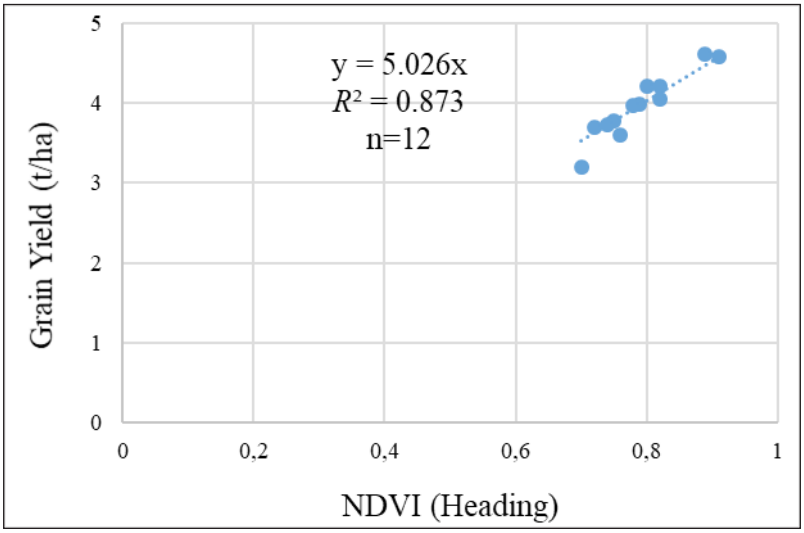


Figure 4. Relationship between Actual grain yield and NDVI at heading stage.

In validation process, a cross check analysis was performed. The accuracy of the developed regression model was evaluated. Validation analysis was done on the remaining 33% of the total plots (six plots). The comparison between actual and estimated values of grain yield were made as a percentage deviation and presented in Table 4. The validation showed that there exists a good agreement between predicted and actual wheat grain yield. Different evaluation parameters for the developed regression model are also given in Table 4. Ren et al., (2008) found similar results in Shandong, China with relative error varied from 4.62% to 5.40% and that regression coefficient (R^2) reached to 0.87.

Table 4. Measured vs. simulated results and statistical assessment.

Actual Yield (t/ha)	Estimated yield (t/ha)	
4.1	3.97	
3.8	3.77	
3.05	3.37	
4.44	4.47	
4.05	4.07	
3.79	4.02	
Evaluation Parameters		
R2		0.768
RMSE (%)		17
CV (%)		4.4
Bias		0.073
ME		0.07
RE		0.019
NSE		0.84

CONCLUDING REMARKS

In conclusion, the soil moisture information is more reliable for irrigation scheduling than climatological information (cumulative pan evaporation). The soil-moisture-based treatment TS1 (30% of MAD), yielded 7.94% more crop yield than irrigation scheduling at even lower level such as TC1 (20 mm CPE) of climate-based treatments for wheat crop grown in sandy loam soil.

Results of NDVI measurements showed that NDVI can be used for accurate (R^2=0.768) in-season wheat grain yield estimation at heading

stage (105 days after sowing), compared with other development stages and can be used for crop management. Additional plant spectral indices, less prone to saturation at high biomass situations, may be used to help prediction of crop yield in higher crop growth stages.

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ЕВАЛУАЦИЈА НА ТЕХНИКИТЕ НА РАСПОРЕДУВАЊЕ НА НАВОДНУВАЊЕТО: СТУДИЈА НА СЛУЧАЈ НА ПЧЕНИЦА ЗАСЕАНА ВО ПОСТОЈАНИ ЛЕИ ВО ПОЛУСУШНИ УСЛОВИ

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Резиме

Оваа студија беше спроведена во Истражувачкиот центар за управување со вода (WMRC) на Универзитетот за земјоделство Фаислабад, Пакистан, за да се оптимизира потрошувачката на вода за културата пченица во региони на земјата сиромашни со вода. Беа усвоени и споредени два главни пристапи за планирање на наводнување, т.е. базирано на почвена влага и на климатски фактори. Имаше вкупно шест третмани: три третмани беа за тестирање на климатскиот пристап за планирање на наводнувањето т.е. примена на наводнување на 20 mm (TC1), 30 mm (TC2) и 40 mm (TC3) кумулативно испарување мерено во сад за испарување (CPE), и три третмани се однесуваа на пристап базиран на почвена влага за планирање на наводнување т.е. 30% (TS1), (MAD) 45% (TS2) и 60% (TS3) од нивоата на максималната количина на вода достапна за растенијата (MAD) и една практика на фармерите (Fp). Друг аспект на истражувањето беше да се провери способноста на сезонско мерење на сушата (NDVI) за да се предвиди потенцијалниот принос на пченица. Резултатите покажаа дека третманите базирани на почвена влага значително го зголемиле приносот на пченица во зрно во однос на третманите базирани на клима (TC2 и TC3) и незначително во однос TC1. Резултатите од NDVI мерења покажаа дека тоа може да се користи за точна проценка на сезонскиот принос на зрно пченица со R2, RMSE, C_v, ME, RE, Bias и NSE проценка 0,768; 17; 4,4; 0,07; 0,019; 0,073 и 0,84, соодветно, и може да да се користи како важна алатка за управување со култури.

Клучни зборови: *почвена влажност, кумулативно испарување мерено во сад за испарување, максималната количина на вода достапна за растенијата, нормализиран индекс за разлика на вегетацијата, принос на зрно.*

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THE CONTENT OF INORGANIC NITROGEN IN THE WATER FROM THE FIFTH CHANNEL NEAR THE CITY OF BITOLA

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Abstract

Water plays an important role in everyday life. Therefore, the subject of the analysis was the water along the upstream of the fifth channel MM1, MM2, MM3, which is located in the city of Bitola and the Crna River - MM4 where the channel inflows into the river. The survey was conducted in May and November. The following chemical parameters were made: the content of ammonia and nitrites in the water was determined spectrophotometric, pH value with a pH-meter, determination of nitrates with UV pastel, the consumption of potassium permanganate was determined by boiling in acidic environment and titration according to Kubel-Tiemann. From the obtained results, it can be concluded that the largest ammonia load (2.2 mg/L) is in the measuring point MM3, while the same values where the channel inflows in Crna Reka in MM4 are reduced. One of the recommendations is the implementation of treatment for the wastewater treatment before being discharged into the rivers.

Key words: *wastewater, communal water, pollution, environment*

INTRODUCTION

According to Kannel et al., (2007) with increasing of the human activity increases the quantity of wastewater. The fifth channel that is the subject of this research is located in the City of Bitola and is filled with water from the river Siva Voda which is relatively pure mountain water with low water level and it drains the wastewater from the industrial sector located in the city of Bitola and its surroundings. It drains the wastewater from the factory for yeast and alcohol, the sugar factory, the beer factory, the factory for production of paper packaging and cardboard, printing house " Kiro Dandaro " as well as faecal or wastewater from the village of Kravari and part of the City of Bitola. The water from the fifth channel not untreated is discharged into Crna Reka. The main purpose of this research is to determine the content of inorganic nitrogen, the quality of the water in the fifth channel, and provide guidelines for proper channel management.

MATERIAL AND METHODS

Material for analysis in this research is the water from the fifth channel and part of Crna Reka. The Measuring point 1 MM1 is located at the exit of the city of Bitola. In this part of the channel, wastewaters are mainly from: the sugar factory and the yeast and alcohol factory, from the beer factory, the factory for production of paper and cardboard packaging, the printing house "Kiro Dandaro", as well as part of the faecal waters from the city of Bitola. Measurement point 2 MM2 is the fifth channel in the village of Kravari. In this part of the channel flow waste and faecal waters from the village of Krivari. The measure point 3 MM3

is the fifth channel before it enters the Crna Reka. 4 MM4 is a measuring point where the fifth channel flows into Crna Reka. The survey was conducted in May and November. The following chemical parameters were made: the content of ammonia and nitrites in the water was determined spectrophotometric, pH value with a pH-meter, determination of nitrates with UV pastel, the consumption of potassium permanganate was determined by boiling in acidic environment and titration according to Kubel-Tiemann.

RESULTS AND DISCUSSION

One of the physico-chemical indicators of the state of water is the pH value.

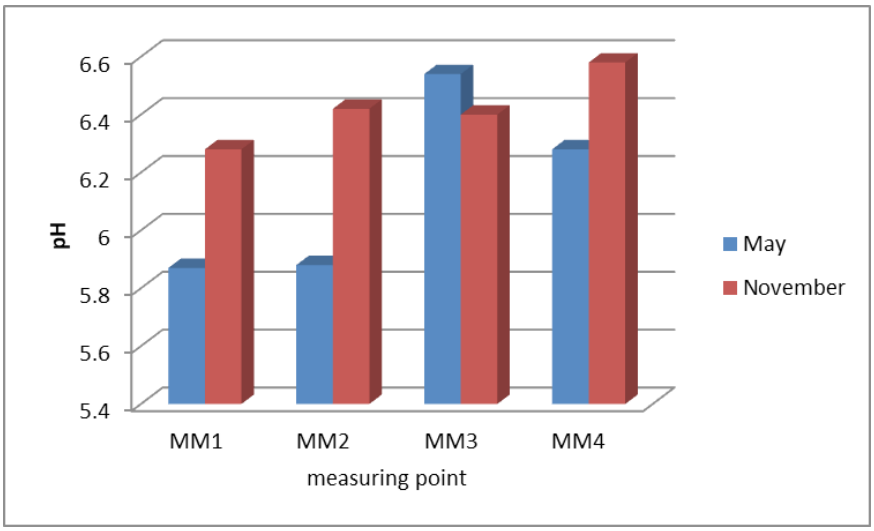

Figure 1. Water pH value in May and November.

From Figure 1 it can be concluded that the highest pH was measured in November in MM4 6.58 mg/L. In the same month, in all measuring points there are no large variations in pH due to the buffer capacity of the water. The lowest value is recorded in MM1 5.87 mg/L as a consequence of the influence of the waste waters from the industrial capacities.

Potassium permanganate consumption is an indicator of the content of organic matter present in the water.

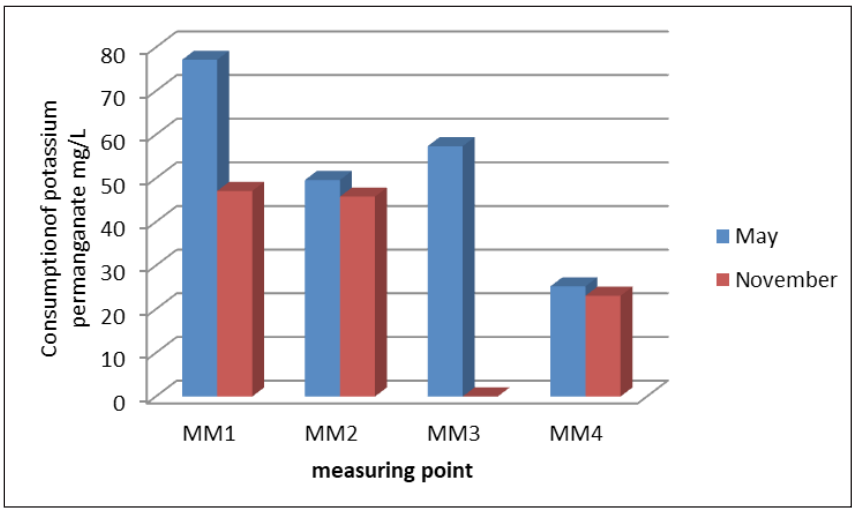

Figure 2. Consumption of $KMNO_4$ in the water from the fifth channel and Crna Reka for May and November.

The lowest value of $KMNO_4$ consumption was measured in MM4 on Crna Reka 23.07 mg/L in November (Fig. 2), due to the high water level of the river that affects the decomposition of organic matter. The highest value was found in MM1 and it was 77.16 mg/L due to the continuous input of industrial wastewater in this measuring point. Such results coincide with the research of Vasileska (2002), on Velgoshka River, where the high values are derived from the industrial waste water, communal water from households, and wastewater from agrarian areas. According to Dalmacija et al., (2011), ammonia is an indicator of pollution that occurred recently.

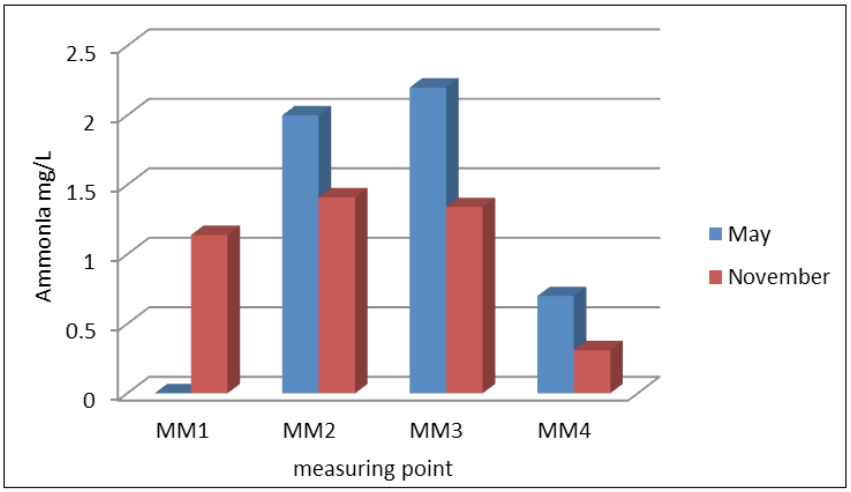

Figure 3. Ammonia content in water from the fifth channel and Crna Reka for May and November.

From Figure 3 it can be concluded that the highest values were found at the measuring point MM3 2.2 mg/L in May. The high content of ammonia in this measuring point is due to the continuous discharge of industrial wastewater, part of the faecal waters from the city of Bitola, faecal waters from the villages of Kravari and Egri. According to the Decree on Classification of Waters, the maximum permissible concentrations are exceeded. With the distances of the measuring points from the populated area and the value of the ammonia in the water it decreases to 0.34 mg/L in the MM4 of the River Crna. According to Blazevska (2016), the values of ammonia in the fifth channel are continuously high during the entire research period. With the distance from the settlements, the anthropogenic pressure decreases and consequently the ammonia content decreases.

The origin of nitrites in the water may be different: from degradable organic matter, from oxidation of ammonia to nitrites or as a result of reduction of nitrates (John, 2016).

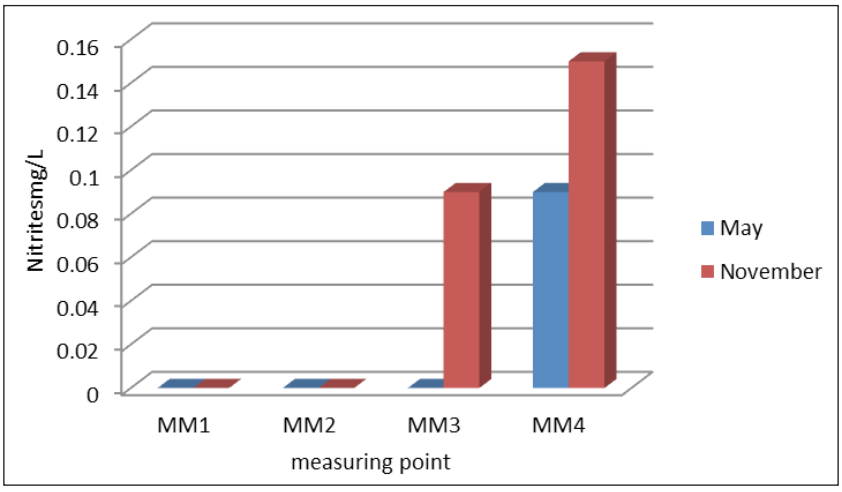

Figure 4. Content of nitrites in the water from the fifth channeland Crna Reka for May and November.

The high content of nitrites, which is most pronounced on MM4 (0.15 mg/L) in November (Fig. 4), is due to the intense decomposition of organic matter, as well as from the additional nitrites from the manure that comes to this measuring site with the rains. In MM1 and MM2 in the two months a value of 0.0 mg/L of nitrites was registered. And Milanovic (2007), examining the Vardar River at the Vardarobasi measuring point, explains the high values of 4.3 mg/L from the alochtonic input of nitrites originating from manure.

The following Figure gives the content of nitrates in the water from the fifth channel (Fig.5).

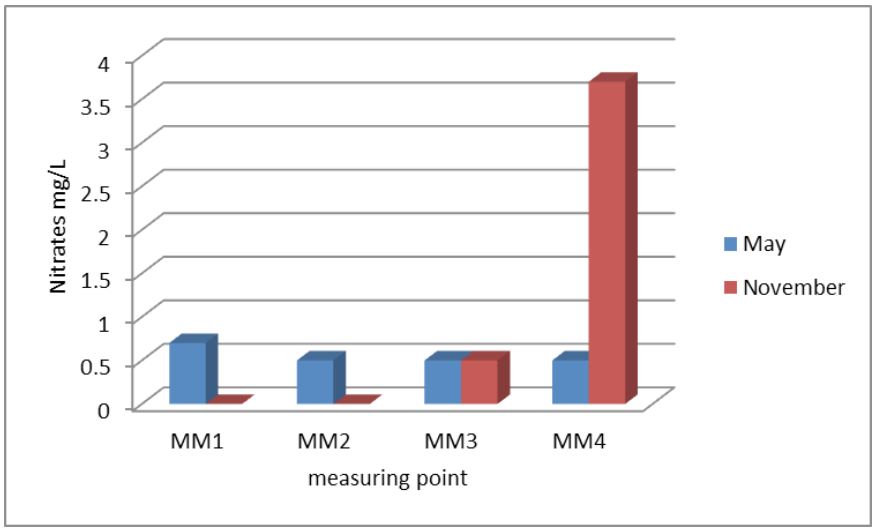

Figure 5. Content of nitrates in the water from the fifth channel and Crna Reka for May and November.

The high content of nitrates was found in MM4 3.7 mg L in November (Fig. 5), indicating that in this measuring point the degradation of organic matter is in the "final phase". According to Blazevska (2016), at this measuring point, the flow of waste industrial and communal water from the settlements and fertilizers used for fertilizing the agricultural crops, in the whole area of village of Kravari affect the increase in the nitrate concentration. In MM1 and MM2 where it was established value 0.0 mg/L for nitrites (Fig. 4), at the same measuring points the nitrate value (Fig.5) is the same (0.0 mg/L).

CONCLUDING REMARKS

From the conducted research of the water from the fifth channel and the Crna Reka, the following conclusions can be drawn: fifth canal, although it has low water capacity, yet the load that it makes with the waste organic matter on Crna Reka is large, close to the village of Kravari and Egri. The highest content of ammonia is recorded on MM3 whose value decreases with distances from settlements where the anthropogenic pressure is smallest. At the measuring point where the fifth channel flows into Crna Reka (MM4) the value of nitrates is the highest which indicates high degradation of organic matter.

Therefore, the results obtained from this research indicate that continuous cleaning of the channels and purification of industrial wastewater should be carried out before they are discharged into the rivers.

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СОДРЖИНАТА НА НЕОРГАНСКИ АЗОТ ВО ВОДАТА ОД ПЕТТИОТ КАНАЛ ВО БЛИЗИНА НА ГРАДОТ БИТОЛА

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Резиме

Водата има важна улога во секојдневниот живот.Токму за тоа материјал за анализа беше водата долж течението на петтиот канал ММ1, ММ2, ММ3 и реката Црна - ММ4 по влевањето на каналот. Истражувањето беше спроведено во мај и во ноември. Беа направени следниве хемиски параметри: pH вредност со пехаметар, содржината на амонијак и нитрити во водата се одредува спектрофотометриски со Spectroqvant UV / VIS Pharo 300. Определување на нитрати со УВ пастел - алатка за директно читање на вредностите, потрошувачката на калиум перманганат се одредуваше со варење во кисела средина и титрација според Kubel-Tiemann. Од добиените резултати може да се констатира дека најголемо оптоварување со амонијак (2,2 mg/L) и нитрити има во мерното место ММ3, додека истите овие врености по влевањето на каналот во Црна Река се намалуваат. Една од препораките е спроведување на третман на прочистување на отпадните води.

Клучни зборови: *отпадна вода, комунална вода, загадување, животна средина.*

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IMPACT OF AMPELOTECHNICAL MEASURES IN THE GRAPEVINE PROTECTION FROM OCCURRENCE OF GREY MOULD (Botrytis cinerea)

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Abstract

Grey mould (*Botrytis cinerea*) provokes severe damages and yield loses in grapevine (*Vitis vinifera*). This research determined ampelotechnical mesaures can be used to influence the development of the disease by reducing the number of chemical treatments. The black grapevine variety Vranec was continuously observed in the experimental field located at Kraiste, Kavadarci, Republic of Macedonia. The working hypothesis was to follow the development of the disease 14 days after the last treatment, when the impact of the fungicide decreases and by applying agrotechnical measures and green operations to control the development of the disease. Those variants where there was a reduction of irrigation and which they were applied ampelotechnical measures was significantly lower intensity of infection in relation of control.

Key words: *ampelotechnical measures, chemical treatments, agrotechnical measures, green operations, intensity of infection, control*

INTRODUCTION

Grey mould (*Botrytis cinerea*) is one of the most significant disease in grapevine (*Vitis vinifera*) causing not only serious economic losses but the development of the plant pathogen. Consequently, additional chemical treatments must be applied immediately before harvesting the grapes raising concerns regarding human health and environmental pollution. Globally, about 15 to 25 million dollars a year is spent on fungicides, to suppress *B.cinerea* (Elad et al., 2004). For these reasons research is directed towards applying certain mesaures during the cultivation of the grapevine that will lead to less use of fungicides.

The disease occurs in all wine regions except that it is more expensive the production of grapes on a unit area, with its enzyme effect worsens the quality of the wine. Considering that external microclimatic influences dictate biological development, appropriate agrotechnical and ampelotechnical measures could influence the health of the grapes. Previous analysis shows that when cultivating the grapevine, micro-location is also important, which could be a significant factor when it comes to profitable grape production, which means lower costs per unit area, (i.e. less of use of fungicides). Field observations found that the use of fungicides and the combined implementation of the ampelotechnical measures, such as the defoliation of the leaves around the bunches, increases the action of the fungicide and reduces the aggressiveness of the disease.

It is important to minimize irrigation at certain stages of disease development that will not create conditions around the bunches favourable to the development of spores of the disease. The current research was undertaken to determine whether from the time of the last fungicide treatment until the time of harvesting (i.e., the period of the fungicide

breakdown), is it possible to keep the disease under control without the use of chemicals. Instead of chemicals, simple agrotechnical and ampelotechnical measures were applied, to obtain an environmentally-justified product that would be less harmful to human health. The level of significance was set at 5%. This level in field conditions is acceptable to see the real effect of the treatments.

Infectious features

Grey mould or *Botryotinia fuckeliana* (de Bary) Whetzel (syn. *Sclerotinia fuckeliana* (de Bary) Fuckel) (anamorph **B. cinerea** Pers.), according to taxonomic characteristics, belongs to the genus Botryotinia (family *Sclerotiniaceae*). As a pleomorphic species, it is highly variable and produces conidiophores, conidia, phialoconidia, microconidia, sclerotia, apothecia, accuses and ascospores. Differences between isolates in terms of the speed of growth, the formation of conidia and sclerotia, production of enzymes and pathogenicity, have been noted by several authors (Di Lenna et al., 1981; Grindle, 1979; Leone, 1990; Movahedi & Heale, 1990), who have explored grey mould. In order to simplify the biological cycle of development to explain the infectious features of grey mould, the following phases are emphasised: attachment of conidia to the surface of the grape berry, germination of conidia, differentiation of infection structures on the host surface, penetration of the host surface and excretion of a spectrum of phytotoxic compounds (necrotrophic activity).

Attachment of conidia for the surface of the grape berry

Spores are dispersed in the wind. Once deposited on the surface of the plant, the spores adhere to the surface tissue of the grape berries. This phase represents the interaction of the spore with the cuticle surface. Initially, hydration of conidia occurs, which typically involves weak adhesive forces, resulting from hydrophobic interactions between the host and conidial surfaces (Doss et al., 1995). A few hours after inoculation, the conidia germination germ tubes are covered with a fibrillar-like extracellular matrix material (Doss et al., 1995). These adhesive structures are excreted by the spore and consist of carbohydrates and proteins (Doss, 1999). The spores are attached to the surface of the cuticle by the fibrillar extracellular matrix material, which also protects it from dehydration and the various defence mechanisms of the plant cells.

Germination of conidia

Several factors influence the germination of a conidium. Free surface water or high relative humidity (>93%) is essential to germinate and penetrate the host epidermis (Williamson et al., 1995). When dry conidia are inoculated on plant surfaces and incubated in the absence of free surface water, the emerging germ tube usually remains short before it penetrates the surface (Cole et al., 1996; Salinas & Verhoeff. 1995; Williamson et al., 1995). Inoculation with conidia in an aqueous suspension requires the addition of nutrients (Harper et al., 1981; Van Den Heuvel, 1981). These food nutrients can mimic the damaged epidermis of the plant, thereby allowing a realistic impression of the infectious characteristics of spores and their behaviour in such a situation. An example of this is the high germination efficiency of spores in an aqueous suspension rich in phosphates and sugars, to which prior primordial incubation was performed at a certain temperature and applied to the leaf tissue on a tomato plant test where it showed a high degree of infectivity. It means that any injury to the surface tissue of grape berry under certain conditions favourable for development produces a specified intensity of infection. Certain gaseous compounds, such as ethylene, can stimulate the germination of conidia. Previously, the germination of conidia on a hydrophobic surface was stimulated exogenously by ethylene, but the length of the germ tube under these conditions remained unchanged (Kepczynski & Kepczynska, 1977). In a hydrophilic environment, ethylene stimulated germ tube elongation without impacting on the percentage of germination of conidia . The production of ethylene in plants increases with the ageing of the tissue and ripening of the fruits, which is a signal for conidial germination and initiation of infection.

Differentiation of infection structures

After germination of the spore in a drop of water, the germ tube grows and extends. The energy necessary for the growth and elongation of the germ tube is supplied in part from the spore contents and partially by the environmental nutrients. When the tip of the germ tube touches the surface of the plant tissue, the wax of the cuticle degrades forming a recess or hole for appressorium formation. The appressoria are thick-walled swellings at the end of a germ tube, of non-standard form and heterogeneous appearance. Several authors (Akutsu et al., 1981; Cole et al., 1996) observed the swelling of hyphal tips of germ tubes and interpreted these as appressorium-like structures. Recent microscopic, histological–chemical researches and gene function analysis indicate that these structures act as functional appressoria, useful for attaching the pathogen to the host surface before penetration of the tissue, due to a fibrillar-like extracellular matrix material covering, which retains water while the polysaccharide component is extremely hygroscopic, allowing the pathogen to adapt to external factors.

Penetration of the host surface

The invasion of the plant tissue by B. cinerea can involve active penetration or penetration over natural openings and plant wounds of the tissue. The wounds of the tissue can be caused by an abiotic or biotic agent. Exclusively physical damage or brutal mechanical penetration through the cuticle by B. cinerea has not been seen (Cole et al., 1996; Williamson et al., 1995). Most often, penetration is followed by enzyme activity by the pathogen. Hence, the cutinolytic enzyme (18 kDa cutinase) activity was investigated by creating mutants that lacked genes encoding for cutinase activity. Studies carried out on the fruits of tomato and in gerbera flowers have shown that the wild isolates of B. cinerea and its mutants are capable of penetrating through the layer of cuticle, which leads to the conclusion that the enzyme 18 kDa cutinase is not essential for penetration. Another enzyme assumed to be meaningful for penetration is 60 kDa lipase (Comménil et al., 1995). There is an opinion (Comménil et al., 1999) that this enzyme is induced by grape berry cuticle components. The lipase possesses cutinolytic activity with clearly expressed kinetic properties, which differ from the typical cutinase, which was previously mentioned (Comménil et al., 1998). While the use of polyclonal antibodies against the conidia of B. cinerea prevent the germ tube from penetrating the cuticle, the antibodies did not affect the germination of the conidia (Comménil et al., 1998).

Excretion of a spectrum of phytotoxic compounds (necrotrophic activity)

Botrytis cinerea kills host cells before they are invaded by hyphae. The invasion of the plant tissue by *B. cinerea* triggers nuclear condensation and plant membrane damage, indicators for programmed cell death, in a ring of cells around the hyphae (Govrin & Levine, 2000). Culture filtrates of *B. cinerea*, grown on a nutritional basis, may induce toxic effects when applied to plant tissue. Phytotoxic compounds were identified as botcinolide, a highly substituted lactone (Cutler et al., 1993), and botrydial, a tricyclic sesquiterpene (Colmenares et al., 2002). The observation that both secondary metabolites were only secreted by *B. cinerea* in medium with high glucose levels, initially raised doubts about their physiological relevance in plants. Recent analytical chemistry studies have, however, demonstrated that botrydial accumulates in infected tissue under physiologically-relevant concentrations (Deighton et al., 2001; Muckenschnabel et al., 2003). The production of botrydial may be an important factor in the infection of (some) host plants but cannot be confirmed because the genes that participate in its synthesis have not yet been identified and, for this reason, appropriate mutants have not yet been created, which will experimentally confirm their role in the pathogenesis.

Resistance and susceptibility of the grape bunch and grape berries towards Botrytis cinerea

In grapevine, *B. cinerea* predominantly infects the inflorescences and grape berries. The development of the pathogen in grape clusters begins with the fruit ripening. Before the ripening of the bunches, the green grape berries possess a high amount of fungitoxic or fungistatic compounds known as phytoalexins, which prevent the development of grey mould. The level of resveratrol, a phytoalexin stilbenoid, is correlated with the resistance of grape berries towards grey mould. Besides phytoalexins, green grape berries also contain proteinase inhibitors that prevent cell wall-degrading enzymes. The concentration of polygalacturonase inhibitor proteins decreases during ripening (De Lorenzo et al., 2001). The skin of grape berry, especially the epicuticle and cuticle, play a major role in resistance towards grey mould. Particularly, epicuticle and cuticle waxes were described as very important features of skin grape berry in relation to susceptibility towards grey mould. In this context, warm temperatures, high air humidity and water on the berry surface are known as major reasons for the incidence of microscopic cracks in the cuticle membrane of berries (Becker & Knoche, 2012). The formation of these microscopic cracks largely determines the susceptibility or resistant of the grape berries to disease, because the entry of small quantities of water contribute to the additional cracking of the protective layers of grape berry. Cuticular forms on the surface of grape berry have an additional role as a regulator of molecular diffusion of food nutrients, which can stimulate the development of the conidia. Pores on the surface of grape berry contribute to the diffusion of nutrients, especially in conditions of hot weather and high relative air humidity when the exchange of matter has increased. The intra- and epicuticular waxes form a hydrophobic layer that repels water from the surface of the grape berry, contributing to the rapid drying of isolated water drops. The hydrophobic layer of the grape berry and the fast drying of excess water on the surface of grapes are the main factors in reducing susceptibility towards *B. cinerea*.

Protection measures against grey mould

Reliable methods for prognosis of the occurrence of disease in the vineyard do not currently exist (Leroux et al., 2002). Only with a good knowledge of all factors that directly or indirectly influence the development of the disease, is it possible to apply measures for preventing damage from the disease. By applying adequate agrotechnical and ampelotechnical measures, conditions can be created that endow a disabling attack,via creatingunfavourable conditions for the development of the pathogen or inability to establish the plant–pathogen interaction. Preventive protection and disease control rely mostly on preventing the establishment of contact between the pathogen and the host. In this context, ampelotechnical measures, such as defoliation around the grape bunches, preclude the establishment of micro-conditions that favour pathogen development. Defoliation is of great importance because of the physiology of guttation in grapevine. Since partial exposure of grape bunches and berries to solar insolation leads to rapid drying of the surface of fruits, the surface diffusion of nutrients between the berries and external environment is reduced. Shallow plowing of the soil in summer during the veraison phase (onset of ripening) of the fruit, as an agrotechnical measure,influences the development of the disease because of the rise in the relative humidity in the zone of the grape bunches. Irrigation and its reduction in the vineyard have also shown to have a key role in creating the conditions for the development of *B. cinerea*. From fungicides was used active substance boscalid with which the last treatment was carried out, and we started field monitoring of the disease.

MATERIAL AND METHODS

The research was completed in a vineyard located at Kraiste, near Kavadarci, Macedonia (41°27`14.3676" N, 22°0`38.5236" E) on black grape variety Vranec. A double Guyot pruning system was applied in the vineyard. The area of the vineyard was 0.6 ha, the plantation was 13 years old, and soil was degraded clay. The variants set in the experiment considered almost all possible situations encountered in practice during cultivation of grapevine. The goal was

to note how the individual variants, during cultivation of grapevine with the application of some agrotechnical and ampelotechnical measures, affect thedevelopment of the disease. Each variant was placed in an area of two rows, and the samples were taken from the middle of the variant, to prevent any external influence. Except for the control (no treatments against grey mould), which was represented by only one row, treatments against downy mildew and powdery mildew were regularly performed, but no active substances were used which could have a side effect against grey mould. The development of the grey mould was followed during the working hypothesis. From each variant, five plants were marked, and from each plant, six bunches were selected, which were marked on the rachis (handle) of the bunches with red tape. In the control, the disease was monitored in three plants. Monitoring for development of the disease was carried out every 4 days starting from 10 August 2018.

Variants and calculations

Six variants plus control were installed. In each of the variants, the bunches were placed under different microclimatic conditions encountered during the production of grapes, bearing in mind the agrotechnical and ampelotechnical measures undertaken. From each variant, a random sample of 30 bunches was taken. For each of the variants, the following were calculated: average number of grape berries in the bunch, average number of healthy grape berries in the bunch, average number of diseased grape berries in the bunch, infection index according to Mc-Kinney's formula (Pejchinovski & Mitrev, 2007), and efficiency of fungicide according to Abott's formula (Pejchinovski & Mitrev, 2007).

Table 1. Overview of variants and the calculated results.

VARIANTS		Average number of grape berry in the bunch	%	Average number of healthy grape berry in the bunch	%	Average number of disased grape berry in the bunch	%	Infection index according to formula of Mc-Kinney(%)	Efficiency of fungicide according to formula of Abott (%)
Without defoliaton around the bunches	Treatment against *B.cinera* under irrigation condition	110	100	90	81,8	20	18,2	28	65,4
	Treatment against *B.cinera* in dry condition	95	100	90	94,7	5	5,3	22,2	72,5
I. Pyrimethanil II. Boscalid III. Boscalid	Treatment against *B.cinera* in dry condition with summer plowing	90	100	78	86,6	12	13,4	27,7	65,8
With defoliation around the bunches	Treatment against *B.cinera* under irrigation condition	110	100	95	86,3	15	13,7	30,8	61,9
	Treatment against *B.cinera* in dry condition	95	100	93	97,8	2	2,2	16,6	79,5
I. Pyrimethanil II. Boscalid III. Boscalid	Treatment against *B.cinera* in dry condition with summer plowing	90	100	81	90	9	10	16,6	79,5
CONTROL		100	100	30	30	70	70	80,8	

Table 2. Formulas for determining the disease parameters.

Category of infection of the bunch	Calculating the index of infection according to Mc-Kynney`s formula	Calculating the efficiency of fungicide according to Abott`s formula
0 – without of infection 1 – rare infection 2 – significant infection 3 – strong infection 4 – whole infection	$I = \frac{\Sigma(n \times k)}{N \times K} \times 100$ I – index of infection n – no. of bunches by category N – simple size (30 bunches) K – no. of categories Σ – sum	$E = \frac{It}{Ik} \times 100$ E – efficiency of fungicide It – index of infection in treated plants Ik- index of infection in untreated plants (control)

To increase the accuracy of the assessment of the infection of the bunches, BRAT (Bunch Rot Assessment Trainer) software was used (http://bunchrot.co.nz/), which measures the percentage of the total area of the bunch that is affected by the disease. The results obtained using this software were compared with statistical analysis of diseased and healthy grape berries. The statistical difference was minimal and amounted to ±1%. The diseased and healthy bunches were categorised as described in Tab. 3.

Table 3. Categorisation of the diseased bunches.

Numerical category of infection	Terminological category of infection	Expressed in %
0	without of infection	/
1	rare infection	1 – 5
2	significant infection	6 – 30
3	strong infection	31 – 70
4	whole infection	71 – 100

The categorisation of the diseased bunches was based on the virulence of *B. cinerea* and the susceptibility of the grapevine variety. At the beginning of infection in the control (untreated plants) when infection was rare, the spread of the disease was checked using arithmetic progression while, in the further stages of the disease, the spread was monitored using geometric progression. This categorisation cannot be used as a general model in determining the diseased bunches,because of heterogeneous structural and mechanical differences in the bunches of different varieties and the natural resistance towards grey mould.

Working hypothesis

Three treatments were carried out against grey mould. The first treatment was performed with the active substance pyrimethanil while, in the other two treatments, boscalid was used as the active substance. The first treatment was executed on 17 June, the second treatment on 4 July, and the third treatment on 27 July (Tab. 4), respectively. On 7 September, when the technological maturity of the grapes was also established, the samples were taken, and their calculations were determined.The working hypothesis was to monitor the development of the disease from14 days after the last treatment against *B. cinerea* and with ampelotechnical measures to control the development of the disease in the period when the activity of the fungicide decreases. The level of significance was set at 5%. Under field conditions, this level is acceptable to see the real effect of the treatments.

Monitoring of the disease began on 10 August after the initial fungicidal effect of boscalid had passed and when the decomposition period occurs. Decomposition of boscalid requires 28 days (product Cantus WG). The analysis of the samples for each of the variants was consistently performed on the 28th day of the decomposition period, i.e., on 7 September. Boscalid is a mitochondrion respiration inhibitor with a broad spectrum of action that stops spore germination, germ tube extension and attachment of conidia to the surface of the grape berry. It also impacts on the stages of mycelial growth and is water-resistant. The characteristics of boscalid to act against *Erysiphe necator* was of essential importance to the working hypothesis,because its use on the last treatment on 27 July, allowed protecting the grapevine from powdery mildew besides the persistent effect of the decomposing active substance against grey mould. The veraison phase (onset of ripening or colour change of grape berries from green to black) in variety Vranec started on 17 August when the conditions for the development of B. cinerea occur. Defoliation, summer plowing and irrigation were carried out on 15 August.

Table 4. Dates of the chemical treatments.

Number of chemical treatments	Active substance	Date of treatments
I	Pyrimethanil	17 june
II	Boscalid	4 July
III	Boscalid	27 July

Table 5. Schematic presentation of treatments and working hypothesis.

June	July	August	September
		Working hypothesis	
17	4 and 27	10 and 7	

Monitoring of the disease

In order to understand a phenomenon, the tendency of its development should be followed. Observation of the development of the disease was carried out during the working hypothesis. Five vines of each variant were selected, which were marked by six clusters (bunches), to obtain a sample size of 30 clusters while three vines were chosen for the control because it was represented by one row. In the control vines, on average, there were 10 clusters, which again obtained a sample size of 30 clusters. Samples were collected every 4 days to note the change in the development of the disease. Since disease monitoring is a visual method, to avoid the subjectivity of the individual assessment in the categorization of diseased bunches, the BRAT software was used. Following the results of the BRAT software assessment, statistical processing of bunches at the sample level was performed, especially for each observation date. Then, a sample for each of the variants was categorized according to Tab. 3. Observation of the disease began on 10 August and ended on 3 September.

For the 28-day duration, the disease was observed seven times in each of the variants and control and every change in the marked vines (plants) and bunches was recorded. Ampelotechnical measures (defoliation of the leaves around the bunch), irrigation and summer plowing, depending on the variant, was performed on 15 August. The veraison phase (onset of ripening) began on 17 August. On 22 August, it rained up to 20 mm. In addition to the monitoring of the disease, grape sugar was regularly measured. Technological maturity in the variety Vranec was noticed on 1 September. On 7 September, a sample of 30 bunches was taken from each variant, and together with the control, a statistical calculation of the results was carried out.

RESULT AND DISCUSSION

This analysis aimed to create a model where the infectious characteristics of the grey mould would be elaborated. In developing this model, the infectious properties of grey rot depended on external factors. Base on the way in which the *B. cinerea* infection occurs, the working hypothesis can be roughly divided into two parts: (i) infections arising from conidia that first adhere to the surface of the grape berries, build a germ tube, form an appressorium and, finally, hyphae are produced that penetrate the tissue; (ii) formation of mycelium structures by which the infection spreads among the grape berries. The way of infection who make the conidia differ from the infection they make mycelium structures from grape berry to grape berry (Elmer & Michailides, 2007). The infection of grape berry by conidia that arrive by air and stick to the surface of the epicuticle is characterized by a lower speed relative to the infection that occurs with already formed mycelium structures on the bunches. The first mode of contamination that arises from conidia adhered to the surface of the grape

berry depends on the micro-conditions of the grapevine, and so certain ampelotechnical measures can be undertaken that influence the speed of development of the disease, i.e., slow down the infectious process. In contrast, in the second mode of contamination when mycelium structures have already formed, the infectious process is very difficult to control only with ampelotechnical measures and can reduce the production of grapes.

Table 6. Development of *Botrytis cinerea* disease during the working hypothesis.

VARIANTS 10 Aug.		Period of disease observation and assessment of diseased bunches with software BRAT (Bunch Rot Assessment Trainer) in %							Statistical calculation of results in %
		14 Aug.	18 Aug.	22 Aug.	26 Aug.	30 Aug.	03 Sept.	07 Sept.	
Without defoliaton around the bunches	Treatment against *B.cinera* under irrigation condition	0	0	1	2,1	4,9	8,7	13,5	18,2
	Treatment against *B.cinera* in dry condition	0	0	0,5	0,7	1,3	2,7	3,5	5,3
I . Pyrimethanil II.Boscalid III. Boscalid	Treatment against *B.cinera* in dry condition with summer plowing	0	0	1,3	2,2	5,7	6,3	9,1	13,4
With defoliation around the bunches	Treatment against *B.cinera* under irrigation condition	0	0	1	2,3	4	7,8	10,3	13,7
	Treatment against *B.cinera* in dry condition	0	0	0	0,5	1	1,2	1,7	2,2
I.Pyrimethanil II.Boscalid III.Boscalid	Treatment against *B.cinera* in dry condition with summer plowing	0	0	1	1,5	3	5,7	6,3	10
CONTROL		0	0	1,7	5,6	12,8	23,7	48,7	70

Control of *B. cinerea* is difficult because the pathogen creates a huge number of conidia, the grey mould attacks the grapevine at various stages of its development, and there is still no reliable method for prognosis of the disease. Consequently, often fungicidal treatments are applied, quite unjustifiably,which pollutes the environmentand places the health of the consumers at risk. It is very difficult for an organism to be placed in a certain frame, such as statistical formulas, and to obtain relevant indicatorssince many unknown factors are likely to act. At the beginning of the infectious process, the rate of infection varied with one arithmetic progression, as can be noted in Tab. 6 variant " Treatment against *Botrytis cinerea* in dry conditions" (0.0, 0.5, 1.0, 1.2, 1.7, 2.2). However, when mycelium structures of the pathogen had formed on the surface of the bunch, the rate of the infection moved according to a geometric progression, and this was most visible in the control (1.7, 5.6, 12.8, 23.7, 48.7, 70.0, Tab. 6). If the control is presented in the form of a graph, the curve displays exponential features (Fig. 1).

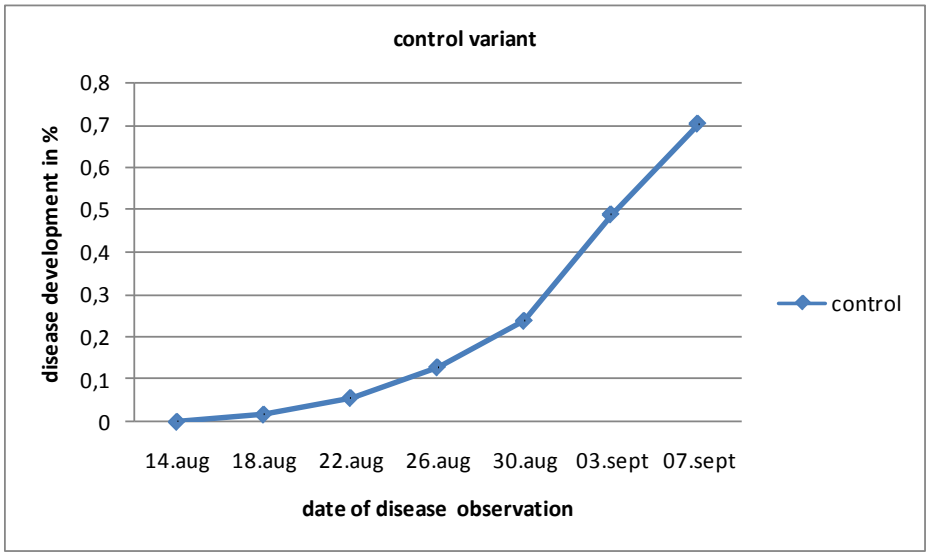

Figure 1. Graphic for disease observation and disease development in %.

When the variants with and without defoliation were compared (Tab. 6) where identical agrotechnical measures were assumed (i.e., treatment against *B. cinerea* in dry conditions, summer plowing in the veraison phase (onset of ripening) and irrigation), although there were no drastic differences in the percentage of numerical values of diseased bunches, the infectious process was somewhat faster in the variants without defoliation, possibly due to the higher epercentage of relative air humidity around the bunches. The assumption determined by the working hypothesis that it is possible in field conditions to control the disease using ampelotechnical measures proved accurate in variants with defoliation: treatment against *B. cinerea* in dry conditions (96.1%), and treating in dry conditions with summer plowing in the veraison phase (onset of ripening) (96.1%), at the 5% level of significance (Tab. 7).

Table 7. Sum of data between variables for determining the working hypothesis.

VARIANTS		Infection index according to formula of Mc-Kinney(%)	Efficiency of fungicide according to formula of Abott (%)	Allowed a level of significance of 5%
Without defoliaton around the bunches I . Pyrimethanil II.Boscalid III. Boscalid	Treatment against *B.cinerea* under irrigation condition	28	65,4	93,4
	Treatment against *B.cinerea* in dry condition	22,2	72,5	94,7
	Treatment against *B.cinerea* in dry condition with summer plowing	27,7	65,8	93,5
With defoliation around the bunches I.Pyrimethanil II.Boscalid III.Boscalid	Treatment against *B.cinerea* under irrigation condition	30,8	61,9	92,7
	Treatment against *B.cinerea* in dry condition	16,6	79,5	96,1
	Treatment against *B.cinerea* in dry condition with summer plowing	16,6	79,5	96,1

CONCLUDING REMARKS

The most spreaded and scientific observed plant disease, grey mould (*Botrytis cinerea*) provokes severe damages and yield loses in grapevine (*Vitis vinifera*). This is first research determined that ampelotechnical measures that can be used to influence the development of the disease by reducing the number of chemical treatments. The black grapevine variety Vranec was continuously observed in the experimental field located at Kraiste, Kavadarci, Republic of Macedonia. The working hypothesis follow the development of the disease 14 days after the last treatment, when the impact of the fungicide decreases and by applying agrotechnical measures and green operations to control the development of the disease. Those variants where there was a reduction of irrigation and which they were applied ampelotechnical measures was significantly lower intensity of infection in relation of control.

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ВЛИЈАНИЕ НА АМПЕЛОТЕХНИЧКИТЕ МЕРКИ ВО ЗАШТИТА НА ВИНОВАТА ЛОЗА ОД ПРИЧИНИТЕЛОТ НА СИВО ГНИЕЊЕ *(Botrytis cinerea)*

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Резиме

Сивото гниење кај виновата лоза (*Botrytis cinerea*) предизвикува сериозни оштетувања и големи економски загуби во производството на винова лоза (*Vitis vinifera*). Ова истражување стави посебен акцент на влијанието на ампелотехничките мерки кои може да се користатат да влијаат на спречување на развојот на болеста со намалување на бројот на хемиски третмани.

Црната винска сорта вранец беше предмет на постојано набљудување во експерименталното поле лоцирано во Краиште, Кавадарци, Република Северна Македонија. Поставената работна хипотеза имаше за цел да го следи развојот на болеста 14 дена по последниот третман, кога влијанието на фунгицидот се намалува и преку примена на агротехнички мерки и зелени операции, да се изврши контрола на развојот на болеста. Оние варијанти каде што имаше намалување на наводнувањето и за кои се применуваа ампелотехничките мерки значително го намалија интензитетот на инфекција во однос на контролата.

Клучни зборови: *ампелотехнички мерки, хемиски третмани, агротехнички мерки, зелени операции, интензитет на инфекција, контрола.*

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NON – DESTRUCTIVE STUDY OF ACID MILK COAGULATION

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Abstract

The acid coagulation is the main processing step in the yoghurt production and has a great impact on yogurt texture, microstructure and rheology, contributing to the overall sensory and visual product perception by the consumer. The objective of this paper was to study the different stages of acid gel formation by turbidimetric and conductometric methods. The non-destructive methods were used to allow the differentiation of three regions: latent phase (pH = 6.3 ÷ 6.0), exponential phase (pH = 5.9 ÷ 5.4) and phase of protein structure formation of demineralized casein micelles (pH = 5.3 ÷ 4.6). The mathematical model obtained by the three-parameter sigmoid function can be used in future studies to predict the change in conductivity during acid coagulation.

Key words: *turbidimetric method, conductometric method, acid coagulation*

INTRODUCTION

The acid coagulation is the main processing step in the yoghurt production. This process of acid gel formation has a considerable impact on yogurt texture, microstructure and rheology, contributing to the overall sensory and visual product perception by the consumer.

The gradual accumulation of lactic acid and respective decrease in pH, significant physical and chemical changes in micellar structure of casein are observed. The acidification of milk disturbs the internal structure of casein micelles, which results from the dissolution of colloidal calcium phosphate (Dalgleish & Law, 1982).

As reported by Lucey (2004), the physicochemical mechanism of acid gel formation can be divided at three pH range:1) 6.7 ÷ 6.0; 2) 5.8 ÷ 5,0 and 3) ≤ 4.8.

By lowering the pH from 6.7 to 6.0 the negative charge of casein micelles decreases, which leads to a weakening of electrostatic repulsion (Lucey, 2007; Phadungath, 2005). At this pH range no change in the size of the casein micelles is observed since only a small portion of the colloidal calcium phosphate is dissolved at pH> 6.0.

At pH values between 5.8 and 5.0 a significant amount of colloidal calcium phosphate dissolves, which causes the weakening of the intracellular interactions between the casein fractions and increasing the size and degree of hydration of the micelles.

Lowering the pH to 4.6 (the isoelectric point of casein) leads to a weakening of electrostatic repulsion between casein molecules and an increase in attraction forces between casein particles (Horne, 1998). As a result of the physical and chemical changes in micellar structure, it is formed a three-dimensional protein network of clusters and chains of demineralized casein micelles (Lucey, 2004).

The objective of this paper was to study the different stages of acid gel formation by turbidimetric and conductometric methods. The change in the optical properties of milk during acid coagulation as well as the change in electrical conductivity allows the use of these physical methods for the study the overall process of formation of the acid gel.

MATERIAL AND METHODS

Starter culture

Commercial freeze-dried yogurt cultures (Laktina, Bulgaria) which are a mixture of Streptococcus salivarius subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus were used (LAT BY 8).

The low fat ultra – high temperature (UHT) processed cow's milk was incubated at 45 °C which is the optimal growth temperature of the strains. The process was considered finished when pH value of 4.6 was reached.

Measurement of pH

The pH was measured using a pH meter equipped with a combined electrode (Schott instruments, GmbH, Deutschland, Germany). The pH meter was calibrated with standard buffer solutions of pH = 4.0 and 7.0 before use. The pH was monitored until the pH value of 4.6 was reached (isoelectic point of casein).

Turbidity monitoring

Turbidity experiments were carried out in a 2 L vessel (5) connected to a circulating water bath (6) at 45 °C. The changes in turbidity (Δτ) of milk were monitored by a portable turbidity meter (3) (McVanAnalite NEP 160 Series, Mulgrave, Australia).This device uses near-infrared (NIR) light (860 nm), allowing each particle in the fluid to reflect the falling beam at 180˚. The reflected beam at 180˚ is captured by a second fibre optic network and transmitted to turbidity sensor (7) that transforms the signal into Nephelometric Turbidity Units (NTU).

The recording of data was performed using a Data logger (Almemo 8990-8V5, Ahlborn, Holzkirchen, Germany), connected to a personal computer.

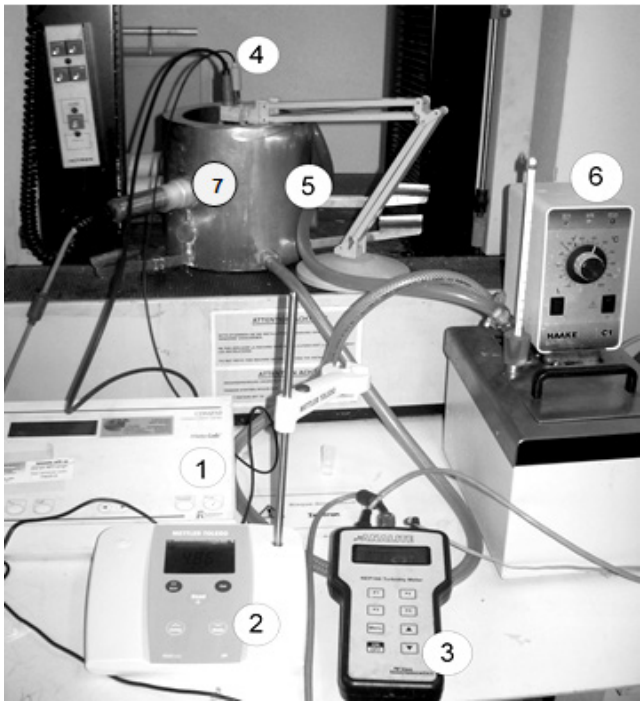

Figure 1. Experimental setup of acid milk coagulation by using aturbidity meter and a conductivity meter (1 – conductivity meter, 2 - pH meter, 3 – turbidity meter, 4 - pH electrode, 5 - thermostatic vessel, 6 - thermostat, 7 – turbidity sensor).

The change in the turbidity (Δτ) was calculated as a difference between turbidity (τ) at time (t) and initial milk turbidity (τ_0) (OuldEleya et al.,1995).

$$\Delta\tau = \tau - \tau_0$$

where:

Δτ is the change of turbidity, NTU;

τ_0 - initial turbidity of the milk before inoculation, NTU;

τ - turbidity of milk at time t, NTU.

The first derivative (dτ/dT) of the turbidimetric profile was calculated as a function of time using 40 data points. The second derivative (d2τ/dT) was calculated using a similar procedure. All measurements were carried out at least in duplicate.

Conductometric monitoring

The electrical conductivity (G, $mS.cm^{-1}$) was measured using a conductivity meter (model CDM 210, Radiometer Analytical SAS, France). The conductivity meter was calibrated in the range of 0÷10 $mS.cm^{-1}$ by using a KCl solution.

Numerical differentiation of the pH and electrical conductivity data was performed to determine the rate of change of these parameters over time.

Statistical analysis

The statistical analysis, mathematical modelling and graphs were performed using Sigma Plot v.11.0. The resulting data was processed by dispersion analysis at significance level p = 0.05.

RESULTS AND DISCUSSION

Monitoring of acid coagulation by turbidimetric method

The change in milk turbidity during coagulation due to the increase in the size of casein micelles justifies the use of this optical method in the study of the process and its individual phases.

The numerical differentiation of the turbidity data (Δτ) obtained during the acid coagulation with LAT BY 1-8 is shown in Figure 2-B.

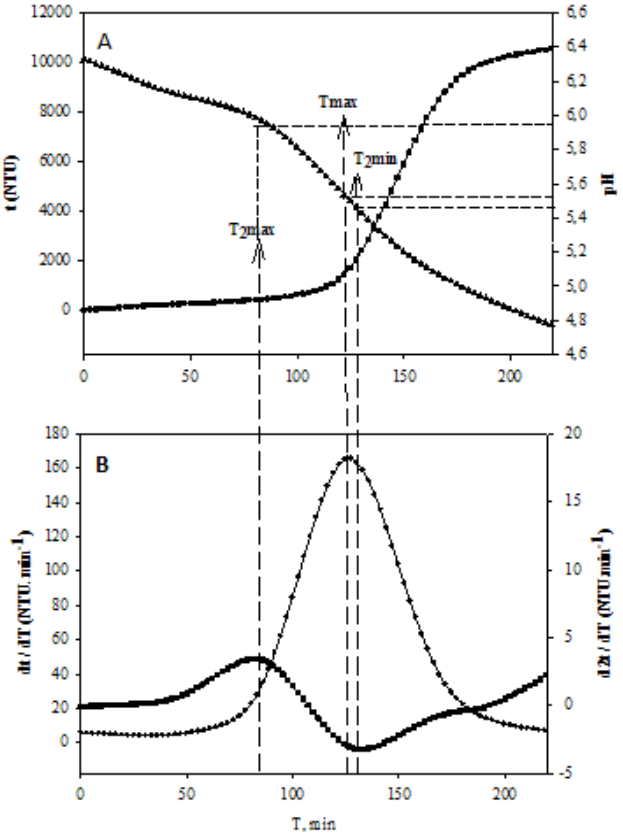

Figure 2. Change of Δτ (■) and pH (▲) during the acid coagulation of milk inoculated with a starter culture LAT BY 1-8 at 45 °C (A). First (dt / dT) (■) and second (d2t / dT) (●) derivative of Δτas a function of time (B).

Based on the identified inflection points (Tmax, T2max and Tmin) of the curve of turbidity (Δτ) can be differentiate three phases characterized by different dynamics in the velocity and acceleration of the ongoing process (Figure 2-A).

The first phase can be conventionally defined as a latent phase during which a slight change in Δτ was established. This change was in the order of 420 NTU (at baseline turbidity 1 NTU) for a time of 40 min. This phase was set at a pH values between 6.3 and 6.0.

In agreement with Lee and Lucey (2004), lowering the pH from 6.6 to 6.0 results in a reduction in electrostatic repulsion between casein micelles as a result of a decrease in their surface negative charge.

The second phase (pH = 5.5) of acid coagulation can be determine as an exponential phase and corresponds to a rapid increase of the values of the nephelometric units (NTU). This change can be explained by the increase in the size of the case in micellesin the indicated pH range. The significant increase in turbidity at the pH range of 5.4 ÷ 5.5 was also found by McMahon (2009) in a spectrophotometric study of acid coagulation at 40 °C.

After reaching the Tmax it was set up a delay in the rate of Δτ (T2min) at pH = 5.4. At this pH range the third phase is observed during which a protein structure of demineralised casein micelles is formed. At pH 5.2 ÷ 5.0 a significant amount of colloidal calcium phosphate dissolves (Dalgleish, 1989), which leads to weakening of the micellar interactions between casein fractions. In the pH range of 5.3 ÷ 4.6 the spatial structure characteristic of acid gels is finally formed, and thus the beginning of the last phase of the gel formation.

Similar changes in the turbidimetry profile of acid coagulation were also found by Bringe and Kinsella (1990) at 25 °C, Banon and Hardy (1991; 1992) at 30 and 42 °C and McMahon (2009) at 40 °C.

Monitoring of acid coagulation by conductivimetric method

The resulting curves for the change in active acidity and conductivity as well as their first derivatives (dpH / dt = f (t) and dG / dt = f (t)) are shown in Figure 3 and 4.

The pH curve (Figure 3) shows a decrease in its forming values, whereas that of the electrical conductivity (Figure 4) has an increasing profile. This is related to the amount of lactic acid produced, which leads to a decrease in pH and increase in the electrical conductivity (G) of milk due to the accumulation of H^+ and lactate ions during the lactic acid fermentation.

At pH 6.1 ÷ 6.2 for a period of 40 ÷ 45 min there was observed a slightly delay in the acidification rate ((dpH/dt)1) and up to 80 min had no effect on it. After this time interval, the second inflection point ((pH/dt)2) was established, indicating the attainment of the highest rate of reduction of the active acidity. According to the data obtained, the velocity was significant in the range of 110 ÷ 115 min, and the H^+ concentration achieved was 5.6 (Figure 3).

The highest rate of acid formation was reached after a period of 70 min into account the maximum in the rate of change of electrical conductivity ((dG/dt)1) (Figure 4) corresponding to the start of accumulation of lactic acid during of lactic acid fermentation.

After the intensive acidification process, a moderate acidification step, which starts in the range of 160 ÷ 170 min (pH = 5.3) follows the second conductivity minimum (Figure 4). From this moment to the end of the studied process, the process was monitored evenly in terms of lactic acid accumulation and conductivity.

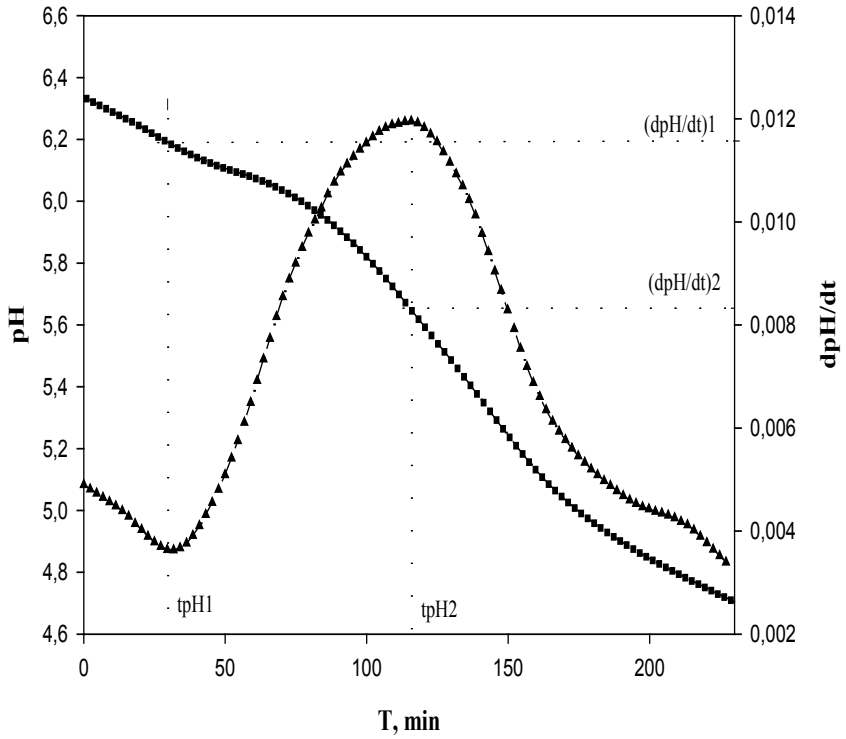

Figure 3. Change in pH of milk (■) inoculated with LAT BY 1-8 and the first derivative (dpH/dt) (▲) as a function of time.

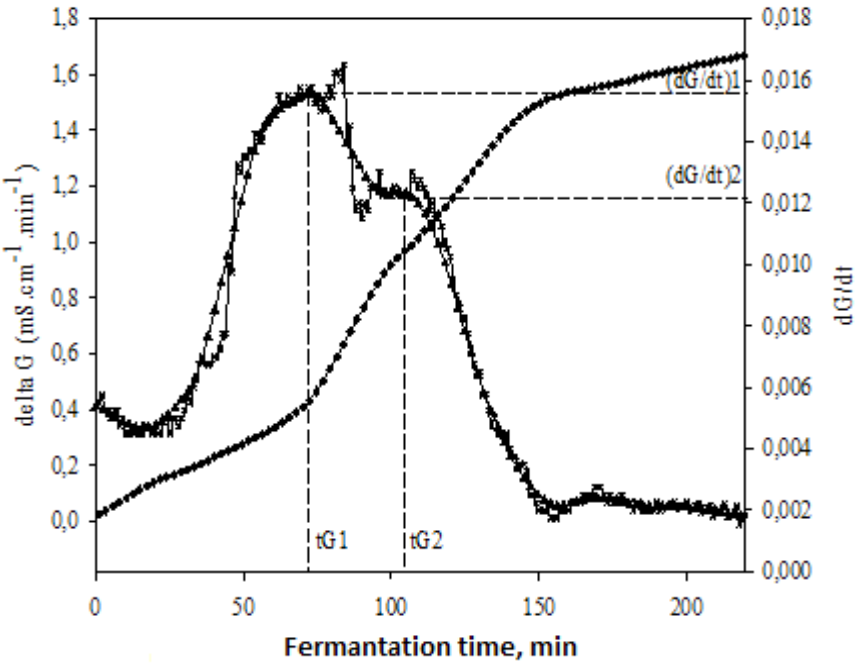

Figure 4. Changes in electrical conductivity (Δ G) (●) during acidic coagulation of milk inoculated and the first derivative (dG/dt) (▲) as a function of time.

The mathematical model of the data describing the change in conductivity during acid coagulation, was performed by non-linear regression (Figure 5). From the normality test it was concluded that the data are normally distributed (W = 0.9600) at a level of significance $p < 0.05$, which allows to continue statistical procedure.

The results of the statistical analysis, including regression coefficients (R) of 0.9993, are an indicator of the correct description of the experimental data from the selected function.

The function is a sigmoid with three parameters: a, b and x0.

$f = a/(1 + \exp(-(x-x0)/b))$

The pattern of change in conductivity during acid coagulation with LAT BY 1-8 has the following form:

$f = 1.5281/(1 + \exp(-(x-109.1578)/29.2098))$

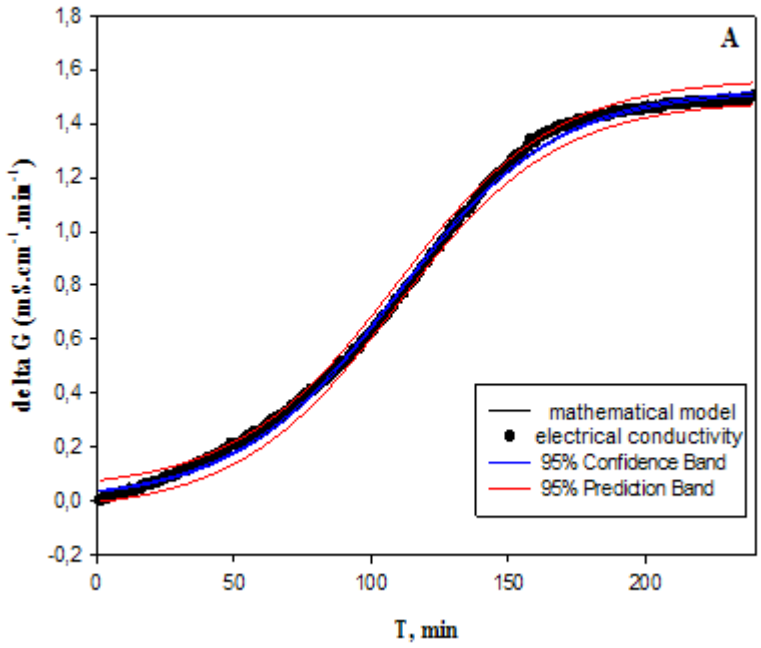

Figure 5. Regression model of the change of electrical conductivity during the acid coagulation.

The analysis of the results of the study of the change of conductivity in the course of the acid gel production showed a similar mechanism in the rate of lactic acid accumulation. Significant inflection points were found at close pH values, confirming the turbidimetry evaluation of acid coagulation.

CONCLUDING REMARKS

In consonance with the physicochemical changes established during acid gel formation, three pH ranges could be differentiated by the turbidimetric and conductometric methods.

A moderate acidification rate was demonstrated by an analysis of pH change and conductivity results in the range of pH = 6.0 ÷ 6.2 to 5.1 ÷ 5.2.

The analysis of the results of the study of the change of conductivity in the course of the acid gel production showed a similar mechanism in the rate of lactic acid accumulation. Significant inflection points were found at close pH values, confirming the turbidimetry evaluation of acid coagulation.

The resulting mathematical model by sigmoid function with three parameters can be used in future research to predict a change in electrical conductivity during acid coagulation.

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НЕДЕСТРУКТИВНА СТУДИЈА НА КОАГУЛАЦИЈА НА КИСЕЛО МЛЕКО

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Резиме

Коагулацијата на киселина е главниот чекор на производство на јогуртот и има големо влијание врз текстурата на јогурт, микроструктурата и реологијата, придонесувајќи за целокупната сензорна и визуелна перцепција на потрошувачот. Целта на овој труд беше да се проучат различните фази на формирање на киселински гел со турбидометриски и кондукционометриски методи. Недеструктивните методи кои се користат овозможуваат диференцијација на три региони: латентна фаза (rN = 6,3 ÷ 6,0), експоненцијална фаза (rN = 5,9 ÷ 5,4) и фаза на формирање на протеинска структура на деминерализирани казеински мицелии (pH = 5.3 ÷ 4.6). Математичкиот модел добиен со трипараметарската сигмоидна функција може да се користи во идните студии за да се предвиди промената на спроводливоста за време на коагулацијата на киселини.

Клучни зборови: *турбидометриски метод, кондукционометриски методи, коагулација на киселина.*

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STABILITY OF OIL FROM OIL SEED RAPE WITH GARLIC UNDER VARIOUS CONDITIONS

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Abstract

Rapeseed oil is obtained by cold pressing of rape seeds. It is performed at low temperature and therefore all valuable components are stored, which give it great biological value. At high temperature, light, oxygen and heavy metals, oils oxidize and harmful oxidation products, peroxides are created.

There were analysed rape seeds "Fila" in which the garlic was added to increase its stability. The peroxide number of this oil is determined under different conditions. Change the volume of the oil in the bottle, the light and the time of storage.

In the oil stored in the dark after 1 and 2 weeks, only the oil with a volume of 250mL after 2 weeks is not for use and has a peroxide number of 7.87. After 3 and 4 weeks, the oils of 250 and 500mL have a peroxide number greater than 7.5, or have peroxides more than the permitted. All is kept in the light have higher peroxide numbers of oils kept in the dark, under the same conditions. All analysed oils after 3 and 4 weeks in the light, have peroxides more than the permitted according to the Oils Rules and fats and oil quality

The light and time of storage are proportional, and the volume of the oil is inversely proportional to the peroxide number.

Key words: *cold pressed oil, peroxide number, analysis, oil volume*

INTRODUCTION

Rape seed contains a large number of unsaturated fatty acids, which play an important role in lowering LDL cholesterol levels, which is a major factor in cardiovascular disease (Schwingshackl et al., 2018; Gustafsson et al, 1994). The rapeseed oil has high polyphenols content and natural components with significant antioxidant activity (Kostadinovic-Velickovska & Mitrev, 2013; Szydłowska-Czerniak, 2013).

Cold pressing is used to obtain rapeseed oil, therefore all the valuable components that give a high biological value to the final product are stored (Gunstone, 2009; Dimic, 2005). The difference between cold pressed oils and refined oils is in the chemical properties (Pavlovska et al., 2016). Their biological value is higher, but they oxidize faster and their stability is lower.

Chemical changes in oils begin under the influence of air, light, temperature and heavy metals (Fe, Cu, Mn, Co, etc.). After longer standing, the oils might oxidize due to the influence of the oxygen in the air (Crapiste et al., 1999; Pavlovska et al., 2017). During the process of frying, increasing the temperature increases their oxidation. (Pavlovska et al., 2017, Berger, 1994; Sadoudi et al., 2014; Farhoosh et al., 2013). Peroxides are the main oxidation products in the oils and they are determined by the peroxide number. When the peroxides increase, their stability, decrease and they become harmful to use.

Oxidation products in fats and oils are very harmful to human health and are the cause of many diseases. Most of them are carcinogenic, and are thought to have contributed to the onset of Parkinson's disease (Barrera, 2012; Farooqui & Farooqui, 2011).

Oxidation products are very harmful to human health, therefore it is necessary to reduce their quantity as much as possible. For that purpose, antioxidants are added to the oils and foods with high percentage of oils. They

can be artificial and natural. Essential oils of rosemary, oregano, sage, ginger, cloves, mint, garlic, basil, fennel and others showed high antioxidant stability (Yanishlieva et al., 2006; Özcan, 2003; Piedrahita et al., 2015; Bravi, 2016; Al-Dalain, 2011).

The purpose of this paper is to determine the stability of oil from oilseed rape under different storage conditions. The influence of three factors on the stability of the oil is determined: the light, the amount of oxygen in the bottle and garlic as an antioxidant.

MATERIAL AND METHODS

For the analysis, cold-pressed oil from the seeds of oilseed rape from the producer "Fila" was taken. 8 bottles of oil with a volume of 1 litre were used. In two bottles, the volume of the oil remains 1L, and in 6 bottles, a portion of the oil is taken away, so the oil volume is reduced to 750mL, 500mL and 250mL. In all, 8 bottles of different volume, 20g of garlic is added. Four bottles with a volume of 1L, 750mL, 500mL and 250mL, containing garlic, are stored in a light, and four bottles with a volume of 1L, 750mL, 500mL and 250mL, containing garlic, are stored in the dark.

The peroxide number of these oils stored under different conditions is determined. The peroxide number is an indicator of the oxidation of the oil and it is determined according to the ISO 3960: 1998 method. Analyses were performed after one, two, three and four weeks.

RESULTS AND DISCUSSION

Figure 1 shows the peroxide number dependence of the oil volume and storage time when storing the oil in the dark.

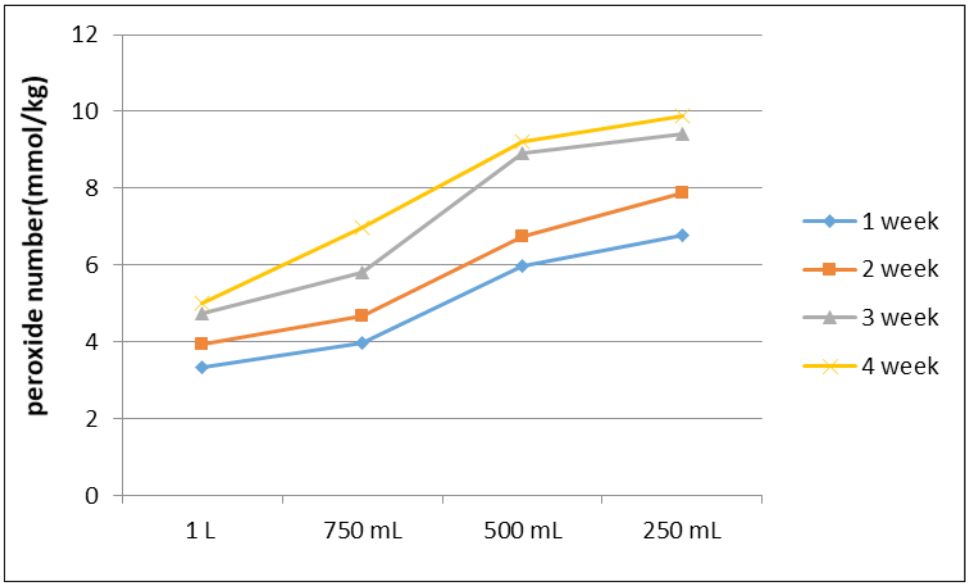

Figure 1. The dependence of the peroxide number on the storage time and volume of the oil from oilseed rape with garlic supplement, stored in the dark.

As seen in the picture, by reducing the volume of the oil in the bottle, the peroxide number increases. This is because when the volume of the oil reduces, the amount of oxygen in the bottle is increased. The smallest changes in the peroxide number are in the oil with a maximum volume of 1L. This oil, compared to the oils with smaller volume, has the smallest peroxide number after 1, 2, 3 and 4 weeks of storage. The peroxide number dependence on the storage time is proportional, or the longer the oils stored, the higher the peroxide numberis. The largest increase in the peroxide number after each week of storage is when the oil volume is reduced from 750mL to 500mL.

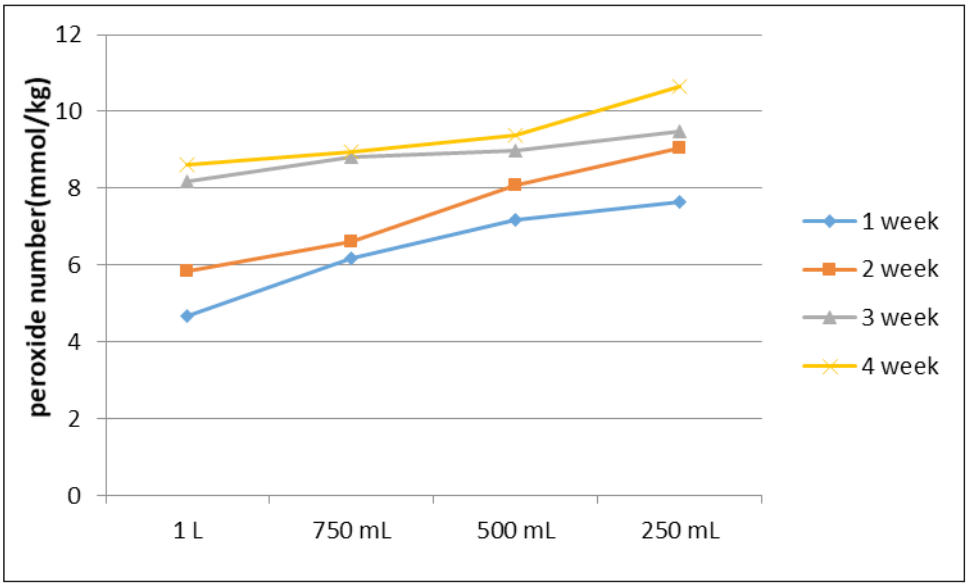

Figure 2. The dependence of the peroxide number on the storage time and volume of the oil from oilseed rape with garlic supplement, stored in the light.

Figure 2 shows the peroxide number dependence on the oil volume and storage time when the oil is stored in the light. For oils of 1L and 750mL, the biggest change in the peroxide number is between the second and third week. In the 750mL oil, the difference in the peroxide number between the third and fourth week is minimal. For all oils kept in the light, the peroxide number increases with increasing storage time and reducing the volume of the oil.

In figure 3, the values obtained for the peroxide number in the analysed oils stored in the dark are compared to the maximum allowed concentration (MAC) for the peroxide number in cold pressed oil given in the Rulebook for oils and the quality of fats and edible oils.

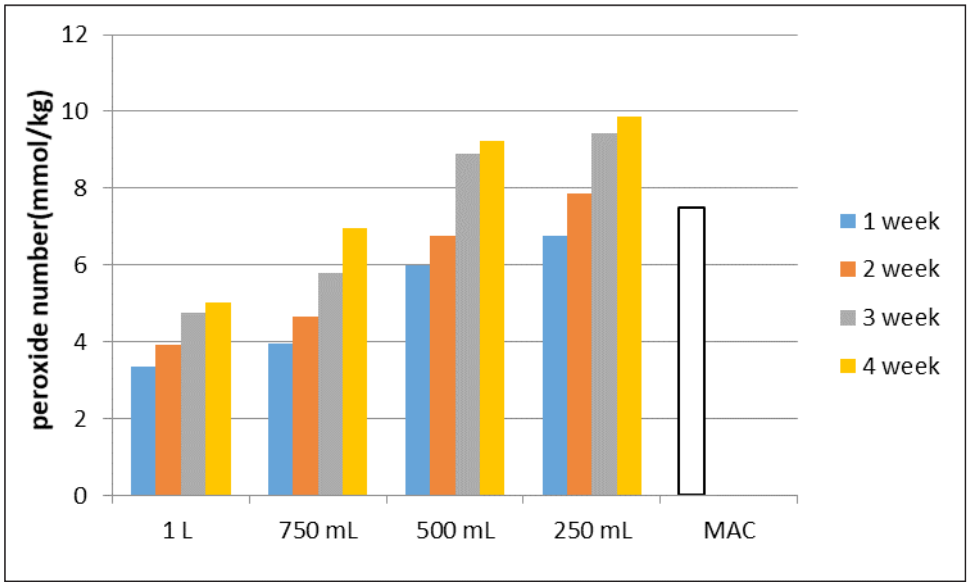

Figure 3. Peroxide number of cold pressed oils from oilseed rape with garlic supplement, stored in the dark.

Oils with a volume of 1L and 750mL, after 1, 2, 3 and 4 weeks of storage in the dark, have a lower peroxide number than the MAC. There is oxidation in them, which increases when the storage time increases as well, but it is very small and it is within the limits of the permitted values. The oil with volume of 500mL after 1 and 2 weeks of storage in the dark is good for nutrition, but after 3 and 4 weeks it should not be used because the peroxide number is greater than the MAC. The peroxide number in the oil with volume of 250mL, is lower than the MAC only after 1 week of storage in the dark, while after 2, 3 and 4 weeks the values are higher than the permitted values.

The obtained values for the peroxide number in the analysed oils, stored in the light, are compared to the MAC for the peroxide number in cold pressed oil given in the Rulebook for oils and the quality of fats and edible oils and are shown in Figure 4.

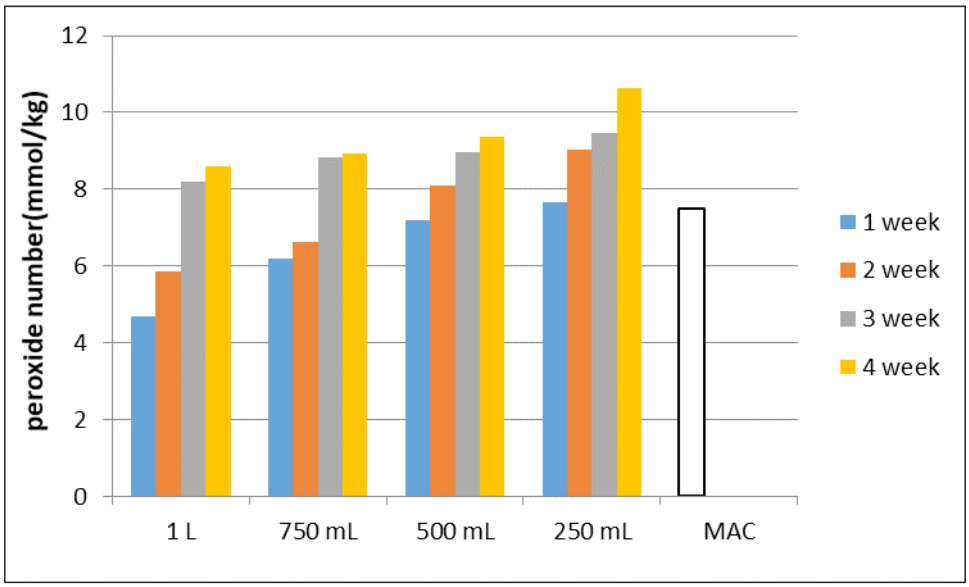

Figure 4. Peroxide number of cold pressed oils from oilseed rape with garlic supplement, stored in the light.

Oils with volume of 1L and 750mL, stored in the light, after 1 and 2 weeks of storage have a peroxide number smaller than the MAC. In these oils, after 3 and 4 weeks of storage, the peroxide number is higher than the MAC values and has occurred high oxidation, so they are harmful to use. For oil with a volume of 500mL, oxidation is within the permitted limits only after 1 week. The oil with volume of 250ml has a high oxidation rate after 1, 2, 3 and 4 weeks of storage and it is not recommended for use.

CONCLUDING REMARKS

We analysed cold pressed oils from oilseed rape seeds from producer "Fila". 8 bottles of oil were analysed, four of which with a volume of 1 L, 750mL, 500mL and 250mL, were stored in the light, and the other four with the same volumes were stored in the dark. Garlic was added in all bottles, as an antioxidant, to increase their stability. The peroxide number, which is a measure of the stability of the oils, is determined. From the obtained results we can conclude that the peroxide number increases when the storage time is increased and the volume of the oil is reduced. The longer the storage is, the lower the stability becomes. Light has also a major impact on the oil stability. Under the same conditions, the analysed oils stored in the light have a higher peroxide number than the analysed oils stored in the dark. Regarding the oils stored in the light, only the oils with a volume of 1L and 750mL, after storage of 1 and 2 weeks, and oil with volume of 500mL stored for 1 week, show a satisfactory stability. The remaining oils stored in the light have a peroxide number higher than the MAC. The peroxide number of oil stored in the dark is above the MAC in oils with a volume of 500mL, after 3 and 4 weeks of storage and in oils with a volume of 250mL, after 2, 3 and 4 weeks of storage.

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СТАБИЛНОСТ НА МАСЛО ОД МАСЛОДАЈНА РЕПКА СО ЛУК ПРИ РАЗНИ УСЛОВИ

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Резиме

Маслото од репка се добива со ладно пресување на семките од репка. Тоа се врши на ниска температура и затоа се зачувуваат сите вредни компоненти кои му даваат голема биолошка вредност. При висока температура, светлина, кислород и тешки метали, маслата оксидираат и се создаваат штетни оксидациони производи, пероксиди.

Анализирано е масло од семките од репка „Фила" во кое е додаден лук за зголемување на неговата стабилност. Определен е пероксидниот број на ова масло чувано при разни услови. Се менуваат волуменот на маслото во шишето, светлината и времето на чување.

Во маслото чувано на темно по 1 и 2 недели само маслото со волумен од 250 mL по две недели не е за употреба и има пероксиден број 7,87. По 3 и 4 недели маслата со волумен од 250 и 500 mL имаат пероксиден број поголем од 7,5, односно имаат пероксиди повеќе од дозволеното. Сите масла чувани на светло имаат повисоки пероксидни броеви од маслата чувани на темно при исти услови. Сите анализирани масла по 3 и 4 недели стоење на светло имаат пероксиди повеќе од дозволеното според Правилникот за масла и квалитет на масти и масла за јадење.

Светлината и времето на чување се правопропорционални, а волуменот на маслото е обратнопропорционален со пероксидниот број.

Клучни зборови: *ладно цедено масло, пероксиден број, анализа, волумен на масло.*

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DETERMINATION OF MINERAL COMPOSITION IN THE ALFALFA (*Medicago sativa* L.) COLLECTED FROM DIFFERENT REGIONS IN THE REPUBLIC OF NORTH MACEDONIA

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Abstract

The role of alfalfa (*Medicago sativa* L.), as the highest quality forage culture, in the development of agricultural production and the intensification of forage production is due to the ability to ensure high yield, has the ability to regenerate continuously and possess high nutritional value. The crops of alfalfa were analyzed from different locations in Tetovo, Skopje and Ovche Pole regions on the territory of the Republic of North Macedonia. For proper growth and development, it is necessary to have a sufficient amount of mineral matter in the soil. AAS (atomic absorption spectrophotometry) is used to determine the mineral composition. The experimental part in which the determination of the presence of mineral matter was made, according to the results of the measurements in the slopes and regions, only the manganese in the second slope was shown a significant difference between two groups: Tetovo region and the second group - Skopje and Ovche Pole region for $p < 0.05$. Also, the iron in the second slope was shown a significant difference for $p < 0.05$ between two groups of regions: the first group Tetovo region and the second group Skopje and Ovche Pole region.

From the examination it follows that the representation of macro and micro elements meets the basic criteria and that alfalfa can be recommended for growing in the examined regions, thus obtaining high yield and good quality.

Plant nutrition is the basis for obtaining high quality crop production.

Key words: *forage culture, mineral matter, macroelements, microelements*

INTRODUCTION

One of the most important processes in the growth and development of plants is the physiology of mineral nutrition. Mineral nutrients are essential for normal life of plants and animals. Plant nutrition through the root system is better known as plant mineral nutrition. The mineral nutrition of plants is the absorption of mineral elements from the external environment and their involvement in the physiological processes in the plant (Angeleska et al., 2011). Nutrients are all those chemical elements necessary for the normal growth and development of plants. The availability of minerals in the soil is important, as it affects the productivity of agric. crops. In our research the forage crop of alfalfa (*Medicago sativa* L.) is examined. It is one of the most widespread, most important and best quality perennial leguminous forage crops. Alfalfa is of very high nutritional quality as animal feed. It is characterized by the ability to ensure high yield and quality protein food, possesses high nutritional value and it has the ability to regenerate itself continuously (Julier et al., 2000). Alfalfa abounds with high content of raw proteins and is of excellent quality, thereby it surpasses almost all perennial forage plants (Dinic et al., 2005). Alfalfa is enriched with vitamins, carbohydrates, saponins, mineral elements and other active components of vital importance, essential for the growth and development of animals (Hao et al., 2008).

Alfalfa captures large amounts of nitrogen, one part of it is obtained through the soil, and the other part is accumulated by the symbiotic nitrogen fixation of the natural atmospheric nitrogen with the help of Rhisobium meliloti var. medicaginis (Ivanovski, 2000).

With the numerous scientific experiments, it is established that 17 chemical elements are essential for the life of plants. These are the elements that participate in the construction of plants that are often grouped, based on their presence in plants. That is why they are called necessary, essential or biogenic elements that are divided into macro and micro elements. Besides the essential elements, the plants can also absorb useful or beneficial elements and harmful (toxic) elements. The mineral elements, are part of many organic compounds, they participate in biochemical reactions and are important factors for maintaining the integrity of the cell and its parts (Spasenoski and Gadžovska-Simic, 2009).

For the proper growth and development of crops, a sufficient amount of macro and micro elements in the soil, available for the plants, is necessary. The meaning of the prefixes macro and micro only shows the needed quantity of a certain element without which the plants could not complete their life cycle if there is any deficiency in both of them, and in no way shows the general significance, because for the life of plants each of the previously mentioned 17 elements is necessary (Trajkova et al., 2017).

According to the way of their participation in the plant metabolism micro elements significantly differ from most macro elements. Namely, their effect is predominantly catalytic. They act on plants at very low concentrations, often strictly specific. However, their composition in the dry matter of plants is negligible compared to some constitutional macro elements (C, N and P) (Cvetanovska et al., 2015).

Plant nutrition is an agro-technical measure that replenishes nutrient reserves in the soil needed for growth, development and fruit-bearing of the plants Jekik (1983). Proper nutrition increases plant resistance to diseases and pests, as well as to high and low temperatures. It also has a positive impact on the quantity and quality of crop yields (Avramov 1999, Pemovski 1981).

MATERIAL AND METHOD

Plant material

The object of the examination was alfalfa (*Medicago sativa* L.), collected from three different regions, in three slopes on the territory of the Republic of North Macedonia:

- **Tetovo region:** Bogovinje, Vrutok, Dzepchishte, Galate, Zelino, Pechkovo and Jegunovce;
- **Skopje region:** Avtokomanda, Sopishte, Drachevo, Saraj, Radishani, Vlae and Glumovo and
- **Ovche Pole region:** Cheshinovo, Karbinci, Obleshevo, Lozovo and Mustafino.

The material was collected during the vegetative cycle of alfalfa. The experiments were carried out on prepared matrix solution using atomic absorption spectrophotometry (AAS). The experiment used a measured dry plant material of 1 g, which was then transferred to a combustion flask. Three repetitions have been made for AAS analysis.

Method for determination of mineral composition

In plants mineral nutrients are obtained by combustion of organic matter at high temperature. The method for determining the mineral elements in the plant material includes: combustion of organic matter, preparation of the matrix solution and quantitative determination of the mineral elements in the matrix solution.

The chemical analysis in the mineral part of the plant material is done after the combustion of the organic matter. The combustion of the organic matter can be both dry and wet. The method of dry combustion of the plant material is carried out in a high temperature furnace (450-550°C) for 60-90 minutes. During this process, organic substances combust and separate as a gas form CO_2, N_2, H_2O and the unburnt mineral part remains in a form of white ash. If the ash contains black spots that originate from the non-combustible organic matter, then several crystals of ammonium nitrate or several drops of alcohol are added. The wet combustion method includes treatment of the plant material with strong mineral acids. The dried and grinded plant material (1 g) is put in a

combustion flask. Then 10 ml of the combustion mixture are added to the flask and the flask is placed on a sand bath. When white steam starts to emerge from the flask, the combustion of the organic matter is completed. At the end of the combustion process, the mineral residue (white sediment) is repeatedly rinsed with distilled water and collected in 100 ml measuring flasks. The prepared solution is a matrix solution used to quantify the content of the mineral elements. When it comes to determining mineral composition, chemical methods increasingly give place to the various instrumental methods, such as AAS (atomic absorption spectrophotometry).

The AAS conditions for analysis of the metals are that the determination procedure itself is relatively simple, has high specificity, low detection limit for a large number of elements, which is very important when working with diluted solutions or with samples in which the elements to be determined are traced, possibility to determine a number of elements from one single solution, the possibility that a greater number of elements be determined by the same instrument, the duration of the analysis is short, the determination speed is large, which is undoubtedly one of the greatest advantages of AAS in relation to conventional chemical methods. This method allows direct results to be obtained.

The concentration area in which the AAS can be applied is very wide. Traces of metal by this method are very well determined. Also, the samples that contain up to 50% tested components are successfully analysed.

The concentrations determined in the samples must correspond to areas of the highest analytical accuracy. It is believed that this area is usually 20 to 200 times the value of the detection limit, although concentrations close to the detection limit can still be determined, but with less accuracy. Thus, when determining the trace elements, which are usually present in quantities close to the detection limits, the expected error is proportionately higher. The accuracy of the analysis undoubtedly depends on the extent to which the avoidance of chemical nature is avoided as well as the attention with which the sample is prepared for analysis, especially when determining the components present at higher concentrations than the optimum for this type of determination. With AAS today, a large number of elements can be determined.

Statistical analysis

For statistical processing of the research results, a software programme Statistical Package for Social Sciences (IBM SPSS Statistics Software v.23) with a one-way analysis of variance (ANOVA) was used in order to determine the significant differences ($p<0.05$) between the arithmetic features of the samples. Subsequent studies were made and the results were Post-Hoc analysed using the Duncan`s multiple range test. The test is used to determine the significance of the difference between the tested minerals and their diversity level ranging from 0.05% and 0.01%. A Karl Pearson`s coefficient of correlation between localities and regions was also used.

RESULTS AND DISCUSSION

Contents of mineral elements in the first slope

The results obtained from the determination of mineral elements are presented. We investigated the mineral elements: Na, K, Ca, Mg, Mn, Zn, Cu and Fe in alfalfa (*Medicago sativa* L.). Table 1 shows that in the first slope, can be seen, in which region was registered the highest and in which the lowest content of the examined elements. One of the tested useful elements was Na, which has the highest measured content in the Ovche Pole region and the lowest in the Skopje region. From the examined macro elements Ca, Mg and K, calcium has the highest measured content in Ovche Pole region, and magnesium has the lowest measured in Tetovo region. From the examined micro elements Fe, Mn, Zn and Cu, the highest level of iron was measured in Ovche Pole region, and the lowest level of copper was measured in the Tetovo region.

Table 1. Contents of mineral elements (mg/kg per dry matter) in alfalfa (*Medicago sativa* L.) from the examined regions in the territory of the Republic of North Macedonia in the first slope.

Regi-ons	Na		K		Ca		Mg		Mn		Zn		Cu		Fe	
	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD
Tetovo	241	±179	19138	±14408	45546	±64106	1070	±443	27	±17	17	±8	4	±1	641	±669
Skopje	201	±94	7936	±11032	52150	±88043	1235	±298	25	±14	23	±21	5	±1	386	±313
Ovche Pole	464	±389	14165	±14838	53060	±81883	1433	±427	29	±15	13	±2	4	±0	647	±470
All regions	285	±244	13702	±13534	49957	±73863	1226	±396	27	±15	18	±14	4	±1	548	±497

In the first slope in the Tetovo region, it was shown strong positive significant correlations the following pairs of elements: Mg-Mn (r =0.808, p=0.028 <0.05), Mg-Cu (r=0.773, p=0.042 <0.05) and Mn- Fe (r =0.785, p=0.036 <0.05).

In the first slope in the Skopje region, it was shown strong positive significant correlations the following pairs of elements: Mg-Mn (r=0.898, p=0.006 <0.05), Mg-Zn (r=0.882, p=0.009 <0.05) and Mg-Cu (r=0.910, p=0.004 <0.05), Mg-Fe (r =0.945, p=0.001 <0.05), Mn-Zn (r=0.945, p=0.001<0.05), Mn-Cu (r=0.859, p=0.013<0.05), Mn-Fe (r=0.926, p=0.003 <0.05), Zn-Cu (r =0.774, p=0.041<0.05), , Zn-Fe (r =0.898, p=0.006 <0.05), Cu-Fe (r =0.783, p=0.037 <0.05).

In the first slope in the Ovche Pole region, it was shown strong positive significant correlations the following pairs of elements: Na-K (r=0.928, p=0.023<0.05) and Mn-Fe (r=0.947, p=0.015 <0.05).

Table 2. Correlation between the elements in the first slope, in the three examined regions.

		Na	K	Ca	Mg	Mn	Zn	Cu	Fe
Na	Pearson Correlation	1	0.262	0.162	-0.098	0.161	-0.183	0.054	0.211
	Sig. (2-tailed)		0.278	0.508	0.689	0.511	0.452	0.825	0.386
K	Pearson Correlation	0.262	1	-0.055	-0.228	-0.185	-0.203	-0.016	-0.088
	Sig. (2-tailed)	0.278		0.824	0.347	0.449	0.404	0.948	0.721
Ca	Pearson Correlation	0.162	-0.055	1	-0.240	-0.146	0.030	-0.108	-0.153
	Sig. (2-tailed)	0.508	0.824		0.322	0.551	0.904	0.660	0.533
Mg	Pearson Correlation	-0.098	-0.228	-0.240	1	**0.725****	0.241	**0.492***	**0.513***
	Sig. (2-tailed)	0.689	0.347	0.322		0.000	0.321	0.032	0.025
Mn	Pearson Correlation	0.161	-0.185	-0.146	0.725**	1	0.404	0.359	**0.821****
	Sig. (2-tailed)	0.511	0.449	0.551	0.000		0.086	0.131	0.000
Zn	Pearson Correlation	-0.183	-0.203	0.030	0.241	0.404	1	0.435	0.281
	Sig. (2-tailed)	0.452	0.404	0.904	0.321	0.086		0.063	0.243
Cu	Pearson Correlation	0.054	-0.016	-0.108	**0.492***	0.359	0.435	1	0.297
	Sig. (2-tailed)	0.825	0.948	0.660	0.032	0.131	0.063		0.216
Fe	Pearson Correlation	0.211	-0.088	-0.153	**0.513***	**0.821****	0.281	0.297	1
	Sig. (2-tailed)	0.386	0.721	0.533	0.025	0.000	0.243	0.216	
**. Correlation is significant at the 0.01 level									
*. Correlation is significant at the 0.05 level									

In the first slope, all regions together, in Table 2, it was shown strong positive significant correlations the following pairs of elements: Mg-Mn (r=0.725, p=0.000<0.05), Mg-Cu (r=0.492, p=0.032 <0.05), Mg-Fe (r=0.513, p=0.025<0.05) and Mn-Fe (r=0.821, p=0.000<0.05).

Contents of mineral elements in the second slope

Based on the results in Table 3, in the second slope, the content on one of the examined element from the group of useful elements was natrium. The highest measured value was in Tetovo region, and the lowest in Skopje region. From the examined macro elements (K, Ca and Mg), the highest measured content at the potassium in the Ovche Pole region, and the lowest of the magnesium in the Skopje region. From the examined micro elements (Mn, Zn, Cu and Fe), the highest determined content was that of iron , and the lowest of copper both in Skopje region.

Table 3. Contents of mineral elements (mg/kg per dry matter) in alfalfa *(Medicago sativa* L.) from the examined regions in the territory of the Republic of North Macedonia in the second slope.

Regions	Na		K		Ca		Mg		Mn		Zn		Cu		Fe	
	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD
Tetovo	704	761	8662	4967	13096	5062	1455	223	27	8	26	33	6	1	694	678
Skopje	116	81	8923	7159	16500	4312	1268	442	15	7	18	9	4	1	131	37
Ovche Pole	433	432	14972	10996	10565	3034	1709	770	16	6	15	5	5	1	165	96
All regions	416	551	10418	7747	13684	4765	1453	495	19	9	20	21	5	1	348	479

In the second slope in the Tetovo region, it was shown strong positive significant correlations the following pairs of elements: Mn-Zn (r =0.793, p=0.033<0.05), Mn-Fe (r =0.858, p=0.014 <0.05), Zn-Cu (r =0.772, p=0.042<0.05) and Zn-Fe (r =0.966, p=0.000<0.05).

In the second slope in the Skopje region, the elements did not show a significant correlations, but in the Ovche Pole region, it was shown strong positive significance correlation only the pairs of elements: K-Mg (r = 0.899, p = 0, 038 <0.05).

Table 4. Correlation between the elements in the second slope, in the three examined regions.

		Na	K	Ca	Mg	Mn	Zn	Cu	Fe
Na	Pearson Correlation	1	0.000	-0.265	-0.069	0.002	-0.105	0.104	-0.014
	Sig. (2-tailed)		0.998	0.272	0.778	0.994	0.669	0.673	0.954
K	Pearson Correlation	0.000	1	**-0.458***	0.378	-0.165	-0.048	-0.329	-0.091
	Sig. (2-tailed)	0.998		0.048	0.111	0.499	0.844	0.169	0.710
Ca	Pearson Correlation	-0.265	**-0.458***	1	-0.255	-0.121	-0.331	-0.344	-0.353
	Sig. (2-tailed)	0.272	0.048		0.292	0.623	0.166	0.149	0.138
Mg	Pearson Correlation	-0.069	0.378	-0.255	1	0.439	-0.060	-0.170	0.040
	Sig. (2-tailed)	0.778	0.111	0.292		0.060	0.808	0.487	0.869
Mn	Pearson Correlation	0.002	-0.165	-0.121	0.439	1	**0.488***	0.357	**0.760****
	Sig. (2-tailed)	0.994	0.499	0.623	0.060		0.034	0.134	0.000
Zn	Pearson Correlation	-0.105	-0.048	-0.331	-0.060	0.488*	1	**0.511***	**0.858****
	Sig. (2-tailed)	0.669	0.844	0.166	0.808	0.034		0.025	0.000
Cu	Pearson Correlation	0.104	-0.329	-0.344	-0.170	0.357	**0.511***	1	**0.626****
	Sig. (2-tailed)	0.673	0.169	0.149	0.487	0.134	0.025		0.004
Fe	Pearson Correlation	-0.014	-0.091	-0.353	0.040	**0.760****	**0.858****	**0.626****	1
	Sig. (2-tailed)	0.954	0.710	0.138	0.869	0.000	0.000	0.004	
*. Correlation is significant at the 0.05 level									
**. Correlation is significant at the 0.01 level									

In the second slope, all regions together, in Table 4, it was shown positive significant correlation in the following pairs of elements: Mn-Fe (r=0.760, p=0.000<0.05) strong correlation, Zn-Cu (r=0.511, p=0.025<0.05) medium strong correlation, Zn-Fe (r=0.858, p=0.000<0.05) strong correlation and Cu-Fe (r=0.626, p=0.004<0.05) medium strong correlation. The K-Ca couple (r=0.458, p=0.048<0.05) was shown a significantly week negative correlation.

Content of mineral elements in the third slope

The content of the examined minerals is shown in Table 5, where the highest and the lowest levels of presence of macro and micro elements can be seen, as well as that of one of the useful examined elements in the analysed regions. The highest level of sodium content was measured in the Skopje region, and the lowest in Ovce Pole region. From the macro elements the highest specific content was that of calcium in Tetovo region, and the lowest magnesium content was measured in the Ovche Pole region. From the micro elements, the highest content of iron was measured in Ovche Pole region, and the lowest content of copper in Skopje region.

Table 5. Contents of mineral elements (mg/kg per dry matter) in alfalfa (*Medicago sativa* L.) from the examined regions in the territory of the Republic of North Macedonia in the third slope.

Regions	Na		K		Ca		Mg		Mn		Zn		Cu		Fe	
	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD
Tetovo	467	±328	11850	±7373	17989	±5797	1265	±140	23	±4	20	±8	5	±1	137	±46
Skopje	477	±562	9605	±5288	17089	±4261	1396	±483	19	±10	19	±11	4	±1	123	±67
Ovche Pole	212	±52	12642	±5729	13030	±7308	1258	±705	22	±5	19	±6	5	±1	148	±106

In the third slope in the Tetovo region, it was shown strong significant negative correlations in the following pairs of elements: Na-K (r=0.803, p=0.030<0.05) and Ca-Fe (r=0.853, p=0.015 <0.05).

In the third slope in the Skopje region, it was shown strong positive correlations pairs of elements: Mg-Zn (r=0.939, p=0.002 <0.05), Mg-Cu (r=0.814, p=0.026 <0.05) and Zn-Cu (r=0.783, p=0.037 <0.05). A strong significant negative correlation was shown the pairs of elements: Zn-Fe (r=0.773, p=0.042 <0.05).

In the third slope in the Ovche Pole region, it was shown strong positive correlations the following pairs of elements: K-Mg (r=0.906, p=0.034 <0.05) and K-Fe (r=0.916, p=0.029 <0.05). A strong significant negative correlation was shown the pairs of elements: Mn-Zn (r=0.895, p=0.040 <0.05).

Table 6. Correlation between the elements in the third slope, in the three examined regions.

		Na	K	Ca	Mg	Mn	Zn	Cu	Fe
Na	Pearson Correlation	1	-0.251	0.191	0.285	0.170	0.152	0.228	-0.101
	Sig. (2-tailed)		0.300	0.432	0.236	0.487	0.534	0.347	0.680
K	Pearson Correlation	-0.251	1	-0.213	0.063	0.082	0.056	0.240	**0.641****
	Sig. (2-tailed)	0.300		0.380	0.797	0.738	0.820	0.323	0.003
Ca	Pearson Correlation	0.191	-0.213	1	0.056	-0.002	-0.327	-0.361	-0.273
	Sig. (2-tailed)	0.432	0.380		0.820	0.992	0.171	0.129	0.259
Mg	Pearson Correlation	0.285	0.063	0.056	1	-0.003	**0.498***	0.371	0.065
	Sig. (2-tailed)	0.236	0.797	0.820		0.990	0.030	0.118	0.793
Mn	Pearson Correlation	0.170	0.082	-0.002	-0.003	1	0.010	0.093	0.112
	Sig. (2-tailed)	0.487	0.738	0.992	0.990		0.968	0.705	0.647
Zn	Pearson Correlation	0.152	0.056	-0.327	**0.498***	0.010	1	**0.488***	-0.210
	Sig. (2-tailed)	0.534	0.820	0.171	0.030	0.968		0.034	0.388
Cu	Pearson Correlation	0.228	0.240	-0.361	0.371	0.093	**0.488***	1	0.001
	Sig. (2-tailed)	0.347	0.323	0.129	0.118	0.705	0.034		0.998
Fe	Pearson Correlation	-0.101	**0.641****	-0.273	0.065	0.112	-0.210	0.001	1
	Sig. (2-tailed)	0.680	0.003	0.259	0.793	0.647	0.388	0.998	
**. Correlation is significant at the 0.01 level									
*. Correlation is significant at the 0.05 level									

In the third slope, all regions together, in Table 6, it was shown medium strong significant positive correlations of the following pairs of elements: K-Fe (r =0.641, p=0.003 <0.05), Mg-Zn (r =0.498, p=0.030 <0.05) and Zn- Cu (r =0.488, p=0.034 <0.05).

The content of the mineral elements in all the slopes together

From the results shown in Table 7, it can be seen that the highest measured sodium content was in Tetovo region, and the lowest in Skopje region. From the macro elements the highest calcium content was measured in Skopje region, and the lowest magnesium content in Tetovo region. From the micro elements the highest iron content was measured in Tetovo region, and the lowest copper content in Skopje region.

Table 7. Contents of mineral elements (mg/kg per dry matter) in alfalfa (*Medicago sativa* L.) from the examined regions in the territory of the Republic of North Macedonia for all slopes together.

Regi-ons	Na		K		Ca		Mg		Mn		Zn		Cu		Fe	
	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD	Mean value	SD
Tetovo	471	±503	13217	±10304	25544	±38274	1263	±325	26	±11	21	±20	5	±1	491	±582
Skopje	265	±352	8821	±7795	28580	±51266	1300	±400	20	±11	20	±14	4	±1	213	±216
Ovche Pole	370	±333	13926	±10384	25552	±48374	1467	±633	23	±11	16	±5	5	±1	320	±355
All regions together	368	±413	11784	±9585	26664	±45266	1330	±450	23	±11	19	±15	5	±1	344	±429

Table 8. Contents of micro and macro elements (mg/kg per dry matter) which are significant different at the three examined regions in all done sloping of alfalfa.

Slopes	Regions	Na	K	Ca	Mg	Mn	Zn	Cu	Fe
		Mean value	Mean value	Mean value	Mean value	Mean value	Mean value	Mean value	Mean value
1	Tetovo	241[a]	19138[a]	45546[a]	1070[a]	27	17[a]	4[a]	641[a]
	Skopje	201[a]	7936[a]	52150[a]	1235[a]	25	23[a]	5[a]	386[a]
	Ovche Pole	464[a]	14165[a]	53060[a]	1433[a]	29	13[a]	4[a]	647[a]
2	Tetovo	704[a]	8662[a]	13096[a]	1455[a]	27[b]	26[a]	6[a]	694[b]
	Skopje	116[a]	8923[a]	16500[a]	1268[a]	15[a]	18[a]	4[a]	131[a]
	Ovche Pole	433[a]	14972[a]	10565[a]	1709[a]	16[a]	15[a]	5[a]	165[a]
3	Tetovo	467[a]	11850[a]	17989[a]	1265[a]	23[a]	20[a]	5[a]	137[a]
	Skopje	477[a]	9605[a]	17089[a]	1396[a]	19[a]	19[a]	4[a]	123[a]
	Ovche Pole	212[a]	12645[a]	13030[a]	1258[a]	22[a]	19[a]	5[a]	148[a]
All slopes	Tetovo	471[a]	13217[a]	25544[a]	1263[a]	26	21[a]	5[a]	491[a]
	Skopje	265[a]	8821[a]	28580[a]	1300[a]	20[a]	20[a]	5[a]	213[a]
	Ovche Pole	370[a]	13926[a]	25552[a]	1467[a]	23[a]	16[a]	5[a]	320[a]

Means within each column having different letters are significantly different according to Duncan's test at $p<0.05$

Means within each column having different numbers are significantly different according to Duncan's test at $p<0.01$

According to the results of measurements by slopes and regions, (Table 8), only Mn in the second slope was shown a significant difference between two groups: Tetovo and the second group - Skopje and Ovche Pole region for $p<0.05$. Also, the iron in the second slope was shown a significant difference for $p<0.05$ between two groups of regions: one Tetovo region and the second group Skopje and Ovche Pole region.

CONCLUDING REMERKS

Modern agricultural production, strives to obtain higher yields of good quality, as well as products that are health-safe and environmentally sound, by the proper use of mineral nutrition. Based on the theoretical framework of the topic, as well as the experimental part in which the determination of the presence of mineral elements is made, the following conclusions can be drawn:

- In the first slope, all regions together, it was shown strong positive significant correlations the following pairs of elements: Mg-Mn ($r=0.725$, $p=0.000<0.05$), Mg-Cu ($r=0.492$, $p=0.032<0,05$), Mg-Fe ($r=0.513$, $p=0.025<0.05$) and Mn-Fe ($r=0.821$, $p=0.000<0.05$).
- In the second slope, all regions together,it was shown positive significant correlation

in the following pairs of elements: Mn-Fe (r=0.760, p=0.000 <0.05) strong correlation, Zn-Cu (r= 0.511, p=0.025 <0.05) medium strong correlation, Zn-Fe (r=0.858, p=0.000 <0.05) strong correlation and Cu-Fe (r=0.626, p=0.004 <0.05) medium strong correlation. The K-Ca (r=0.458, p=0.048 <0.05) was shown a significantly week negative correlation.

- In the third slope, all regions together, it was shown significant, medium strong positive correlations in the following pairs of elements: K-Fe (r=0.641, p=0.003 <0.05), Mg-Zn (r= 0.498, p=0.030 <0.05) and Zn-Cu (r=0.488, p=0.034 <0.05).

According to the results of the measurement made in slopes and regions, only Mn in the second slope shows a significant difference between two groups: Tetovo region and the second group - Skopje and Ovce Pole region for p <0.05. Also, Fe in the second slope was shown a significant difference for p <0.05 between two groups of regions: the first group Tetovo region and the second group Skopje and Ovche Pole region.

All of the above shows that the mineral composition is satisfactory and that alfalfa can be recommended for growing in similar agro-ecological conditions, since in terms of the chemical composition and the presence of the examined macro and micro elements as well as the presence of the useful elements meets the appropriate standards, for high yield and good quality. Plant nutrition is the basis for obtaining high quality crop production.

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ОДРЕДУВАЊЕ НА МИНЕРАЛНИОТ СОСТАВ КАЈ ЛУЦЕРКА (*Medicago sativa* L.) КОЛЕКЦИОНИРАНА ОД РАЗЛИЧНИ РЕГИОНИ ВО РЕПУБЛИКА СЕВЕРНА МАКЕДОНИЈА

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Резиме

Улогата на луцерката (*Medicago sativa* L.), како најквалитетна фуражна култура, во развитокот на земјоделското производство и интензивирање на фуражното производство се должи на способноста да осигурува висок принос, има способност непрекинато да се регенерира и поседува висока хранлива вредност. Анализирани се култури на луцерка од различни локалитети во Тетовскиот, Скопскиот и Овчеполскиот регион на територијата на Република Северна Македонија. За правилен раст и развој е неопходно присуство на доволна количина на минерални материи во почвата. За одредување на минералниот состав се користи ААС (атомска апсорпциона спектрофотометрија). Во експерименталниот дел во кој е извршено одредувањето на застапеноста на минералните материи, според резултатите на мерењата по откоси и региони, единствено манганот во вториот откос покажа сигнификантна разлика меѓу две групи: Тетовскиот и втората група – Скопскиот и Овчеполскиот регион за $p<0,05$. Исто така, железото во вториот откос покажа сигнификантна разлика за $p<0,05$ меѓу две групи на региони: едната група Тетовскиот регион и втората група Скопскиот и Овчеполскиот регион. Од испитувањето произлегува дека застапеноста на макроелементите и микроелементите ги задоволува основните критериуми и луцерката може да се препорача за одгледување во испитуваните региони, а со тоа добивање на висок принос и добар квалитет.

Исхраната на растенијата е основа за добивање на висококвалитетно растително производство.

Клучни зборови: *фуражна култура, минерални материи, макроелементи, микроелементи.*

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EFFECT OF EXTENDED AGING UPON TEXTURAL ASPECTS OF TRADITIONAL BULGARIAN DRY-CURED HAM

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Abstract

The objective of this study was to determine the effect of prolong ripening and drying on quality aspects of traditional Bulgarian dry cured ham. After 8, 18 and 36 months of aging comparative textural profiling of dry cured ham were established. Ham sensory profile as well as physicochemical, morphological characteristics, proteolysis index and Warner-Bratzler shear force (WBSF) were investigated. Excessive aging up to 36 months of dry-cured ham lead to decreased hardness, but lower sensory values, characterizing overall textural perception tenderness of the samples. Hams of higher ripening and drying had statistically significant increased proteolysis index and lower values of WBSF. Morphological analysis revealed significant qualitative and quantitative differences between sample groups. Thus, miofibrilar fragments increased remarkably in samples with higher proteolysis index scores. In addition, longest aging hams showed increased degradation for a total myofibrillar structure. Warner-Bratzler shear force (WBS) had a significant relationship with the sensory tenderness variables, such as softness, chewiness, and rate of breakdown in mouth.

Key words: *dry-cured ham, excessive ripening, texture, morphological changes, Warner-Bratzler Shear Force, proteolytic index*

INTRODUCTION

Traditional dry-cured meat products from whole muscle meat are highly fascinating foods, both in social and market aspects (Talon et al., 2007; Laranjo et al., 2017; Rantsiou & Cocolin, 2008). They have a clear function in the global gastronomic heritage (Iaccarino et al., 2006) and are often associated with a certain organoleptic superiority (Panagou et al., 2013). These products can be categorized on the basis of different factors such as the specificity of the used raw materials and/or the applied technology (fermentation, salting, smoking), country of origin, degree of drying, maturation, etc. (Zdolec, 2017; Lücke, 1994; Lücke, 2000). Their production technology is closely tied to the local identity of the country or ethnicity concerned. Historically, the reasons for the wide variety of dry-cured meat products are related to the climatic and resource conditions of local production (Zdolec, 2017) and to the influence and nutritional practices of the various civilizations that have settled in the area Stearns, 2010; Gagaoua & Boudechicha, 2018). The sensory, physicochemical, microbiological and textural characteristics of the different European-produced dry ham vary greatly depending on the specifics of the technological process adopted by the different producers (Leroy et al., 2013; Toldrá, 2014, Zeng, et al., 2016; Rather et al., 2016). In their traditional production technology, the following general technological stages have been adopted: salting, post salting or process of achieving equilibrium salt concentration, drying and maturing. The continuance of drying and ripening varies from 2-3 months to 2-3 years in the highest quality dry ham, such as 48 months in some Spanish species such as Seraño ham (Petrova et al., 2016). Increasing the aging time provides for a more significant degree of enzyme digestion,

which contributes to the production of a higher quality product (Toldra, 2002; 2004).

Numerous studies have been conducted on the physicochemical and sensory properties of a number of Italian and Spanish ham and variations of these properties depending on the technology used (Andrés et al., 2004a,b, Flores et al., 2006; Gou et al. 2008; Huang, & Huang, 2010; Ruiz-Ramirez, et al., 2006; Serra et al., 2005). However, in existing literature sources, no attempt has been made to combine two important aspects of the production of this group of dry-cured meat products: the biochemical and morphological changes in the protein fraction as a result of the long maturation and drying period over 1 year and their effect on the texture of meat products. Also, almost no data is available about Balkan traditional dry-cured meat products from whole muscle meat, despite the fact that traditional technologies exist for these products (Gagaoua & Boudechicha, 2018; Gök, Obuz, & Akkaya, 2012, Rajkovic, 2012), though not as popular and exported worldwide as Italian and Spanish ham.

During socialism, with the establishment of the amalgamated Economic Union in the Republic of Bulgaria, called "Rodopa", industrial and mass production occurred in meat processing, which gradually oppressed traditional meat crafts and replaced them with unified so called now "classical" meat technologies. Nevertheless, even today, in a variety of regions of Bulgaria there are preserved and produced even a few traditional meat products. Therefore, the aim of the present work is focused on physicochemical, morphological and textural changes occurring in the traditional Bulgarian dry-cured meat product "Elena ham" during a long period of aging of this product in natural drying chamber.

MATERIAL AND METHODS

Materials

The research was carried out with dry-cured traditional meat product "Elena ham" for the production of which fresh chilled pork green ham of cross-breed pigs up to 12 months old and live weight over 90 kg were bought by Biltrans Ltd, Elena town, and the preparation and production of studied hams was carried out in the same company, producing this traditional meat product. The shaping of the hams is done by separating them from the carcass by cutting between the last lumbar and the first cross vertebrae and cutting through the hock joint. The pelvic bone was gently released without cutting the muscles, leaving only a small part of the pelvic wing (2-3 cm), the inner surface of the thigh was formed by removing the fat, as the outer surface had a perfectly preserved skin on the surface and without damage. The skin was cut out of the outside and the fat, giving the ovoid shape to the buttocks, and the shaped hams were salted with table salt in special chambers with temperature from 3 to 5 °C and approx. humidity 85-90%. Salting was done manually by scrubbing and massaging the entire surface. The time for salting in these conditions lasted about 45 days and depended on the weight of the individual hams (approximately 5-6 days per kilogram), during this time hams were salted 3-4 times by sprinkling and massaging. After salting the hams were cleaned from the surface adhering salt mixture, washed with clean drinking water, drained and hung for ripening and drying in special natural drying rooms – at the specific mountain climate of town Elena, Bulgaria. This have been done in the months of February, March and early April when the air is cool and dry and the temperature varies between 2 - 5 ° C to plus 10-12 ° C. After about 20 days of initial drying, the hams are covered with a thin, but dense layer of specially prepared leaf fat and rice flour, especially to the areas without skin and around the joints and bones. After this manipulation, the hams were aged under the above conditions until they reached the organoleptic characteristics of the final product, but not shorter time than 6 months.

The research was carried out with samples of the traditional Bulgarian dry-cured "Elena ham", aged for 8, 18 and 36 months and in some analysis with raw meat. The samples taken included the following muscle groups: m.Semimembranosus, m. Semitendinosus and m. Biceps femoris. Samples were analysed to monitor changes during the ripening process in water content, water activity, pH, degree of hydrolysis of the protein fraction, morphological and texture profile of muscle tissue.

Methods

To determine moisture, drying of a homogenous mixture of the samples at 104 ± 1°C was performed using an electronic moisture analyser model KERN MLS-A (Kern & Sohn GmbH, Germany).

Water activity (aw) was measured using a LabSwift-aw system (Novasina AG, Switzerland) at 25°C. Three independent measurements were made for each sample.

The degree of hydrolysis of the protein fraction was determined as the ratio of the amount of free alpha-amine nitrogen to the total amount of nitrogen in the sample determined by the Lowry method. Extraction of the muscle proteins was performed with phosphate buffer with pH 7.3 and ion strength 0.55 M. From an average sample, 2.5 g were weighted, transferred to a 50 cm3 measuring flask and made up to the mark with phosphate buffer. The extraction was carried out with periodic stirring at 4 ± 2 ° C for 24 hours. After extraction, the homogenate was centrifuged at 10,000 g for 20 min, after which the above clear layer was separated and used to determine the content of protein by the Lowry method (1951) and the amount of α-amine nitrogen by the ninhydrin method described by Moore & Stein (1954).

For Warner–Bratzler (W–B) analyses, samples were cut into 30 × 30 × 30 mm chops (thickness × length × width) and cooled overnight at 4°C. After cooling, three slices (15 mm thick) without fat or connective tissue, parallel to the longitudinal orientation of the muscle fibres, were taken from each sample chop. Warner–Bratzler Shear Force (WBSF) was determined using a texture analyser TA-XT.Plus (Stable Micro Systems, Surrey, Great Britain) with a Warner-Bratzler stainless cutter blade. Samples were sheared perpendicular to the long axis of the core at a speed of test – 2 $mm.s^{-1}$, and WBSF was taken to be the peak force of the curve (Honikel, 1998; Iseya et al., 1996). Prior to analysis samples chop were left for 30 min at room temperature. Determinations were repeated six times per sample and were averaged.

Sensory analysis was focused on hardness, firmness and overall texture perception (Larmond, 1976) for the samples of "Elena" ham with different aging period. The overall consistency assessment involved the evaluation of three aspects: the easiness of initial dental penetration in the meat cut; the easiness with which the meat was broken into fragments, and the amount of residue left after chewing (Choe et al., 2016; Lawrie and Ledward, 2006;). The analysis was conducted according to the method described by Iseya et al., (1996) with some modifications. Hardness and firmness was evaluated by chewing a testing sample with molars and by biting off with front teeth the sample. Sensory analysis was performed by 7 trained assessors. The panel evaluated each characteristics according to a 5-point category scale (1=least, 5 = highly). Before analysis, the panel members had attended a preliminary training session where they were examined samples that varied in the evaluated attributes and the meaning of hardness, firmness and overall textural perception had been also discussed. The preliminary session was concluded when individual scores did not vary more than 1 unit for the mean scores, and all members understood and could use the scoring system.

One-way ANOVA was applied to the assessment of the effect of aging time (Factor I) on the water content, proteolytic index, WBSF and sensory evaluations of the examined hams. Duncan's test was applied for multiple comparisons between all mean value pairs. All calculations were made at confidence level α = 0.05. The experiments were carried out with three replications of three samples of one aged ham or green ham. The data in tables and figures are shown as average ± standard deviation (SD). The statistical procedures were performed using the Microsoft Excel 5.0 software and the Statgraphics 16 programme.

RESULTS AND DISCUSSION

Physicochemical and biochemical characteristics

To evaluate the influence of the time of ripening in natural drying chamber of the traditional dry cured meat product "Elena ham" changes in their physicochemical parameters listed in Table 1 are tracked.

Table 1. Change in moisture content, aw – value and proteolytic index of testing sample during extended aging.

Sample	Indicator			
	moisture content, $g.kg^{-1}$	a_w	proteolytic Index	pH
8 months	467,72±18,00c	0,895±0,003c	24,81±8,88a	6,10±0,02c
18 months	422,6±7,69b	0,845±0,005b	35,04±13,57a,b	6,04±0,04b
36 months	226,96±27,46a	0,813±0,006a	53,27±16,51b	5,85±0,06a

Means within each column having different letters are significantly different according to Duncan's test at $p<0,05$.

For all three samples, statistically significant differences were found for the results for pH, water content and water activity ($p<0.05$) (Tab. 1). The reason for significant differences in water content of the samples at 36 months compared to the other two samples of ham can be explained not only with the longer drying and ripening stage (36 months) in the natural climate chamber, but also with the lower average pH values measured for these samples (5.85 ± 0.06) (Tab. 1). At a lower pH, the resulting coagulation changes of meat proteins speed up drying and favour the loss of moisture from the product (Ockerman & Basu, 2008). The established decrease in pH with increasing maturation is in some contradiction with the increase in pH as a result of the accumulation of biogenic amines or ammonia due to deeper muscle proteolysis during the maturation process observed by other authors (Toldra, 2002). Cause for these results may also be related to the more intense hydrolysis reactions of the meat lipids (data not shown) and the subsequent chemical transformations of the protein and lipid degradation products accompanying the long maturation period in the ham aged 36 months.

For the values obtained for the water activity of the samples, statistically significant differences were recorded, as in the samples with higher water content, higher values for a_w were also measured. Comparing the a_w values measured for "Elena" ham samples with those reported from other authors that investigating more popular European dry cured ham, makes it clear that our results are significantly lower, with the exception of a_w values received for 8-month samples. For example, the reported water activity at the end of the production of Italian hams, aging for 12 ÷ 16 months, varied between 0.94 ÷ 0.87, for Spanish hams with a production cycle duration of 12 ÷ 48 months - 0.899 ÷ 0.860 (Martínez- Martínez-Onandi et al., 2016; Petrova et al., 2016), and for the French hams with production of about 9 months or over - 0.89 (Petrova et al., 2016; Parafita et al., 2015). Enzymatic degradation of proteins plays an important role in the formation of the aroma and taste of dry-cured hams. An important result associated also with proteolysis is the formation of ham consistency, which is mainly due to the breakdown of myofibrillar structures (Chandek-Potokar & Škrlep, 2012; Mora, Sentandreu, & Toldra, 2011; López-Pedrouso et al., 2019). The constant increase in the proteolytic index found in the studies is a direct indication for the extent of proteolytic changes in Elena ham, as maturation and drying progresses until the end of the investigated period (Tab. 1). These results are in agreement with data reported by other authors (Garcıa-Garrido et al., 1999; Pugliese et al., 2015, Zhao et al., 2008). In Spanish dry-cured ham, proteolysis index (PI) reflecting good quality could be considered between 33 and 36, while in Italian dry-cured ham between 22 and 30 (Careri et al., 1993; Pérez-Santaescolástica et al., 2018). In the present work we observed values between 24.81 and 53.27 in dry-cured hams, which can explain the fact that the 18-month aging sample was evaluated as most perceptional and wanted in sensory aspect. An increase in proteolysis during dry-cured ham processing has been associated with negative effects on the taste and aroma of the dry-cured ham (Pérez-Santaescolástica et al., 2018) which, as a result, may lead to consumer rejection of the product. However, the proteolysis index well explains the established relationship between water content and WBSF values of samples of dry-cured meat products and can serve as a marker for their consistency at different extent of drying and ripening (Ruiz- Ramirez et al., 2006; Pérez-Santaescolástic et al., 2018).

Textural analysis

The Warner-Bratzler device is known as a tool for tenderness measurement of livestock meat and in some cases for fish (Iseya et al., 1996; Kemp et al., 1968). Shear force values were similar in samples aged 18 and 36 months, but generally favoured the hams in trials 36 months (Tab. 2). These mechanical parameter were attenuated as the time of ripening and drying of ham increased, as a marked change in this textural parameter occurred in samples aged over 8 month ($p < 0.05$) (Fig. 1).

Table 2. Changes in Warner-Bratzler Shear force of testing sample during extended aging.

Sample	cutting force, N/mm	coefficient of variation
raw meat	7,53±0,63^{c}	8,380%
"Elena ham"- 8 months	2,26±0,456^{b}	20,202%
"Elena ham"-18 months	1, 57±0,640^{a}	40,983 %
"Elena ham"- 36 months	1,414±0,410^{a}	28,955%

Means within each column having different letters are significantly different at $p<0,05$

Coefficient of variations were 8.38% for the shear force of raw meat and ranged between 20,20 – 40,98 % for hams. Other studies have shown similar high coefficient of variation for WBS testing (Caine et al., 2003). There were significant differences in the cutting force between 8 month and 18 month as well as between 8 and 36 month ($p<0.05$). The texture profile is related to water activity and water content by observing a negative non-linear relationship between ham hardness and measured water content and water activity (Ruiz-Ramírez et al., 2005, Andrés et al., 2005). However, the increasing rate of shear force in the Warner-Bratzler shear test for samples, that have been aged 18 and 36 months and have lower moisture content, was higher than that received for the ham sample aged for 8 months and having higher moisture content.

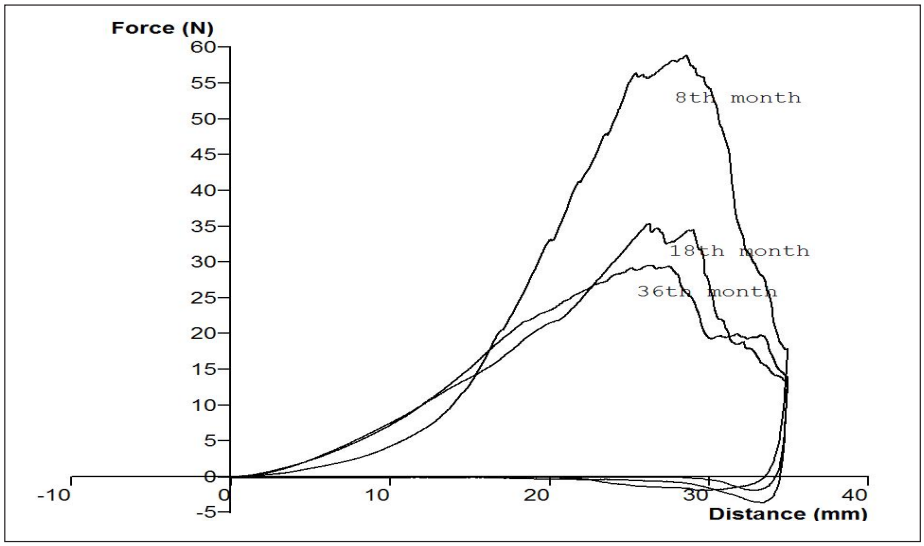

Figure 1. WBSF of "Elena" hams aged for 8, 18 and 36 months.

Morphological analysis

Group of cytoskeletal proteins consisting mainly myofibrillar proteins, and proteins of the stroma are of great importance in respect of the meat texture (Damodaran et al., 2008, Petrova, 2016). At the microstructural level, it has been shown that myofibrillar proteins are mainly proteins that undergo substantial proteolytic changes during the production of raw-dry-cured meat products (Larrea et al., 2007).

The morphological images (Fig.2) representing changes in transverse and longitudinal sections of muscle tissue, used for the preparations of the tested samples, shown that, as the drying and maturing progresses,

deeper structural changes were observed, most pronounced at the sample of 36 months. These changes were mainly related to a decrease in the diameter of the muscle fibres as well as to the appearance of more enlightened areas in them due to the ongoing hydrolysis of muscle proteins (Fig.2).

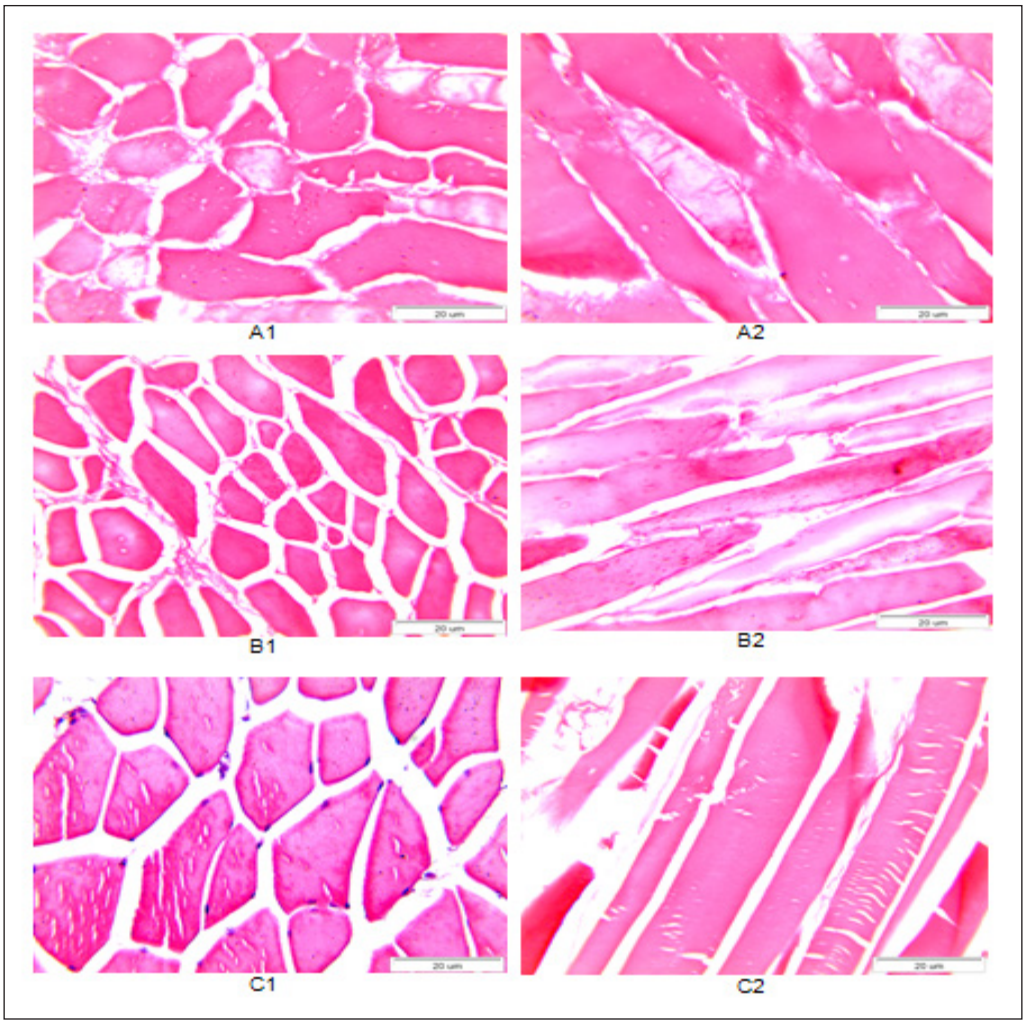

Figure 2. Light microscopic morphological images of samples A- Elena Ham at 8 months; B – "Elena ham" at 18 months; C – "Elena ham" at 36 months; haematoxylin stained; magnification 400×.

Morphological changes in the studied ham pointed the occurrence of proteolysis that caused destruction of the endomysial envelope and partial lysis of the structure of muscle fibres (Fig. 2). The presence of multiple light sections in both the muscle fibres and the intracellular space with a formed network of finely granulated protein mass and muscle nuclei were the consequence of the myolemma disruption. This significant changes in muscle fibre structure due to the ongoing hydrolysis of protein substances over the entire volume of muscle fragments was associated with a decrease in water retention capacity and correlates well with the water content of the tested samples. The greater degree of muscle fibres destruction leads to more significant degradation of myofibrillar proteins, as evidenced by the higher values for the proteolytic index and the sensory scores characterizing the consistency of the samples at 18 and 36 months received by the sensory analysis performed (Tab. 3).

Sensory Evaluation of Texture

Most sensory analyses presented in the literature point at consistency and flavour complex as the most important characteristics influencing the overall organoleptic quality of dry-cured ham (Laureati et al., 2014). Changes in the texture profile of the tested samples during aging were also evaluated by the sensory analysis and the results are presented in Table 3.

Table 3. Sensory changes in the texture of the testing sample during aging.

Sample	Hardness	Firmness	Overall texture perception
8 months	4,60±0,50[b]	4,65±0,35[b]	4,10±0,65[a]
18 months	4,50±0,40[b]	4,25±0,20[b]	4,75±0,45[b]
36 months	3,50±0,50[a]	3,90±0,10[a]	4,40±0,50[a,b]

Means within each column having different letters are significantly different at $p<0,05$

Sensory evaluated hardness and firmness decreased significantly after the 18th month of the extended aging in the natural drying chamber. There were no significant differences between scores obtained by each panel member ($p < 0.01$). As a result of multiple range test, there were significant differences in the sensory scores between tested samples. The significant increases in sensory evaluated tenderness were observed at 36 months of aging as assessors easily detected this textural change. Sensory assessments for the samples at 36 months, compared to the 18-month samples (Table 3), were expressed as a more undesirable solid consistency. The reason for this can be found in the established lower water content in sample of 36 months and respectively reduced water activity. This have a significant influence on the required strength to chew the product, despite deeper proteolytic changes in the muscle tissue of this ham (Andrés et al., 2005; Benedini, Parolari, Toscani, & Virgili, 2012; Ruiz- Ramirez et al., 2006). For example, Ruiz et al. (2002) and Laureati et al (2014) attribute this negative influence to the drier and fibrous structure obtained from both insoluble collagen and myofibrillar proteins aggregation as a result of dehydration during drying and maturation (Córdoba et al. 1994; Chizzolini et al., 1996). However, overall maturation of over 8 months leads to better texture perception as a result of improved sensory tenderness variables, such as softness, chewiness, and rate of breakdown in the mouth.

CONCLUDING REMARKS

As the aging process progressed, there were significant changes not only in the water content and the proteolytic index but also in the morphological and texture profile of the studied traditional dry-cured meat product. These changes were also compared with the data obtained by the textural sensory analysis and the period of ripening and drying of the samples at 18 months was most appreciated by the panellist. The dry-cured traditional meat product "Elena ham" differed from the most popular and offered on the European market raw-dried pork hams. The study on changes during the initial stages of the production of "Elena ham" until the 8th month will allow more detailed and clearer understanding of the ongoing transformations in this traditional dry meat product as well as the overall assessment of its qualitative characteristics as a function of drying and ripening time. Examination of microbiological safety in the course of its technology and antioxidant capacity will allow for the production of better quality and safer traditional meat products and will meet the increasing demands of modern food safety.

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ЕФЕКТ НА ЗГОЛЕМЕНО СТАРЕЕЊЕ НА ТЕКСТУРАЛНИТЕ АСПЕКТИ НА ТРАДИЦИОНАЛНАТА СУШЕНА БУГАРСКА САЛАМА

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Резиме

Целта на ова истражување е да се одреди ефектот од продолженото зреење и сушење на бугарската салама. По 8, 18, и 36 месеци стареење беа дадени параметрите на сушената салама. Профилот на саламата како и физичките, хемиските и морфолошките карактеристики со протеолитичкиот индекс на Ворнер-Брацлер беше истражуван. Продолженото стареење до 36 месеци доведе до зголемена тврдост но со мали сензорни вредности кои карактеризираа преголема мекост. Саламите со подолго зреење и сушење имаат статистички зголемен протеолитички индекс. Морфолошката анализа открива значајна квалитативна и квантитативна разлика помеѓу примероците. Притоа, се зголемија и миофибриларните фрагменти. Тоа доведе до зголемена деградација на целосната миофибрична структура. Силата на волната на Ворнер-Брацлер имала значајна врска со сензорната мекост како ровкост, џвакање и ниво на топење во устата.

Клучни зборови: *сушена салама, зголемено зреење, текстура, морфолошки промени, сила на волна, протеолитички индекс.*

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ASSESSMENT OF AGRO-MORPHOLOGICAL VARIABILITY IN RICE USING MULTIVARIATE ANALYSIS

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Abstract

The research was carried out to assessment the agro-morphological variability in fourteen rice varieties using principal component analysis, linear correlation and cluster analysis. All rice varieties have Italian origin and were grown in 2014 and 2015 under agroecological conditions in Kocani, the Republic of North Macedonia. Principal component analysis was utilized to examine the variation and to estimate the contribution of traits for total variability. Three components in the PCA analysis with Eigen value > 1 contributed 75.59% variability existing in the rice varieties for yield contributing traits. PC1 accounted 30.81% of the total variability, contributed by traits like 1 000 grain weight, panicle length, weight of grains per panicle and plant height. PC2 had the contribution from the traits as number of plants per m^2, plant height and panicle length which accounted for 25.08% of the total variation. Grain yield and panicle length had contributed 19.71% of the total variation in PC3. Only Ulisse and San Andrea showed positive values among all three main components. Grain yield showed positive correlation with number of plants per m^2 (r= 0.185). On the other side, number of plants per m^2 was in negative correlation with weight of grains per panicle (r= -0.593). Also, negative correlation was established between number of grains per panicle and 1 000 grain weight (r= -0.752). Using cluster analysis, two main cluster groups with subgroups were extracted. The results revealed existence of variability in the studied varieties which can help breeders to achieve higher yield in rice.

Key words: *Oryza sativa* L., *principal component analysis, cluster analysis, linear correlation*

INTRODUCTION

The existence of genetic diversity in the initial material is one of the basic prerequisites for the success of each breeding programme and the achievement of the basic goals for plant improvement, such as increasing the yield potential, adaptability, quality and resistance to biotic and abiotic stress. For this aim, breeders use the natural populations, wild relatives of cultivated species, populations created by hybridization, induced mutations and other biotechnological methods. Introduction is also one of the methods used to increase genetic variability. Using introduction as a method in breeding programme, it is necessary to have the correct characterization and evaluation of the introduced material, especially from geographically more remote regions. Characterization of rice germplasm increases its value in any breeding programme. Agro-morphological characterization of germplasm variety is fundamental in order to provide information for plant breeding programmes (Lin, 1991; Nascimento et al., 2011). The use of agro-morphological traits is the most common approach utilized to estimate relationships between genotypes (Bajracharya et al., 2006).

Genetic diversity has an important and significant role in plant production. It is considered as a means of survival and adaptation to changing environments (Rao and Hodgkin, 2002; Gao, 2003). Evaluation of genetic resources for various agronomic traits and the assessment of the amount of genetic variation within them are useful to allow more efficient genetic improvement (Haussmann et al., 2004). Assessment of genetic and agro-morphological variability is very important in rice breeding from the standpoint of selection, conservation of different land races variety of rice and proper utilization (Jayasudha and Sharma, 2010).

Statistical analyses are necessary for proper examination and analysis of agro-morphological properties. The application of statistical methods gives an insight into the legality of the relationship between the tested properties, genotypes and factors that influence the development of the culture itself. The large number of agro-morphological properties and their interconnectedness make the statistical analysis extremely complex, especially if a large number of genotypes are examined.

Multivariate analysis is the most commonly used approach for genetic variability, estimation to illuminate the patterns of variation in germplasm collections. Principal component analysis (PCA), cluster analysis (CA) and correlation are the most important multivariate analysis methods (Oyelola, 2014). Among multivariate techniques, PCA and cluster analysis are preferred tools for morphological characterization of genotypes and their grouping on similarity basis (Peeters and Martinelli, 1989; Mohammadi and Prasanna, 2003). Principal component analysis, significantly contribute to reducing the data and highlighting the properties that most influence the sample's variability. Combination of these two approaches gives comprehensive information of characters which are critically contributing for genetic variability in crops (Rachovska et al., 2002).

Many researchers had successfully used agro-morphological characters to classify and estimate diversity in a variety of rice using multivariate analyses (Nachimuthu et al., 2014; Ravikumar et al., 2015; Pachauri et al., 2017).

The objective of this study was to characterize and classify 14 new introduced rice varieties from Italy and also to identify morphological traits responsible for selection of important characters and study the interrelationship among the traits.

MATERIAL AND METHODS

Plant material and experimental design

Fourteen rice varieties were used as an experimental material for this research. All rice varieties have Italian origin. San Andrea variety for a long period is used as a main cultivar in commercial rice production in the Republic of North Macedonia, while the other Italian varieties (Arsenal, Nembo, Ronaldo, Galileo, Sprint, Ulisse, Krystalino, Mirko, Sfera, Gloria, Pato, Creso and Vasco) were newly introduced. The trials were set up during the period 2014 and 2015, on alluvial soil type in the region of Kocani. The experimental material was placed by using randomized block design in three replications. The standard agronomic packages of practices were followed throughout the season for regular growth of crop.

Determination of agro-morphological traits

Ten randomly selected plants from each repetition have been analysed for some agro-morphological traits such as plant height (cm), panicle length (cm), number of grains per panicle and weight of grains per panicle (g). The number of plants per m^2 was determined by counting the plants from m^2 of each repetition. 1 000 grain weight, has been determined to measure 1 000 grain of each repetition. Grain yield obtained from the 5 m^2 was calculated in $t \cdot ha^{-1}$. When calculating the 1000 grain weight and grain yield, the moisture content was reduced in 14%.

Statistical analyses

All tested traits were statistical analysed by SPSS (2010) system and JMP 5.0.1 (2002) software. Principal component analysis and cluster analysis were done using the methodology given by Gomez and Gomez (1984). The correlation between yield and yield components was calculated according Singh and Chaudhary (1985).

RESULTS AND DISCUSSION

Principal component analysis (PCA) is one of the important multivariate tool of diversity analysis. This statistical technique analyses a data table in which observations are described by several inter-correlated quantitative dependent variables and can be helpful for identification of plant character that categorize the distinctiveness among promising varieties. The objective of principal component analysis is reduction of dimensionality of a data set with a large number of correlated variable or traits (Jolliffe, 2002).

The results of PCA from our research are depicted in Table 1. PCA was carried out by using 14 rice varieties and seven agro-morphological traits. Three main components in the PCA analysis with Eigen value > 1 contributed 75.59% variability existing in the rice varieties for yield contributing traits. In this study, PC1 accounted 30.81% of the total variability, PC2 showed 25.08% and PC3 accounted 19.71% of variations among characters. PC1 accounted for the maximum variability and highly loaded with characters such as 1 000 grain weight (0.65), panicle length (0.54), weight of grains per panicle (0.36) and plant height (0.32) contributed in positive direction. The second principal component accounted 25.08% of the total variation and was in positive correlation with number of plants per m^2 (0.56), plant height (0.32) and panicle length (0.30). Grain yield (0.74) and panicle length (0.62) were important traits contributing to the third PC, which accounted 19.71% of the total variation (Tab. 1).

Through PCA we could identify the number of plant characters, which are responsible for the observed genotypic variation within a group. Four principal components with Eigen value greater than >1 and explained 72.48% of the total variance were recorded by Pachauri et al. (2017).

PCA has been used by various researchers like Gana et al. (2013), Asfaq et al. (2014), Worede et al. (2014), Kumar et al. (2015), Ravikumar et al. (2015), Ojha et al. (2017), Pachauri et al. (2017) and Yugandhar et al. (2018) for characterization different rice germplasm lines.

Table 1. Eigen value, contribution of variability and factor loadings for the principal component.

	Principal Components		
	PC1	PC2	PC3
Eigen value	2.16	1.76	1.38
Percentage of variability (%)	30.81	25.08	19.71
Cumulative percentage (%)	30.81	55.88	75.59
Traits	**Factor loadings**		
Plant height	0.32	0.32	-0.10
Panicle length	0.54	0.30	0.62
Number of grains per panicle	-0.47	-0.43	-0.01
Weight of grains per panicle	0.36	-0.54	0.20
1 000 grain weight	0.65	0.03	0.13
Number of plants per m^2	-0.30	0.56	0.07
Grain yield	-0.19	-0.13	0.74

In Table 2 are given the factor loadings by main components of the analysed rice varieties. Only two varieties, Ulisse and San Andrea, showed positive values by all main components. Those varieties had a higher value for grain yield and favourable values by other yield-related components.

Table 2. Factor loadings by main components of the analysed rice varieties.

Rice varieties	PC1	PC2	PC3
Arsenal	-0.94	0.2	-1.02
Nembo	0.34	-1.94	-1.93
Ronaldo	-0.73	-1.02	1.71
Galileo	1.77	-1.98	2.26
Sprint	-1.22	1.52	-0.30
Ulisse	1.41	0.75	0.80
Krystalino	-0.98	-2.10	-1.17
Mirko	-2.47	0.64	0.57
Sfera	-1.97	0.72	0.54
Gloria	1.76	0.90	-1.09
Pato	1.73	0.23	-1.02
Creso	-0.25	-0.59	0.01
Vasko	-0.11	0.35	0.33
San Andrea	1.66	2.10	0.31

Correlations help the breeder to understand the mutual component characters on which selection can be based for genetic improvement (Chakravorty et al., 2013). In order to assess trait association, phenotypic correlation analysis was done and the result is depicted in Table 3. 1 000 grain weight was highly and significantly negative correlated with number of grains per panicle (r = -0.752). Positive significant correlation was established between the number of plants per m^2 and grain yield (r= 0.185). On the other hand, the number of plants per m^2 was negatively correlated with weight of grains per panicle (r= -0.593).

Significant phenotypic correlations of yield and yield-related traits were reported by other workers in inbred lines, hybrids, varieties and landrace rice (Sürek and Beşer 2003; Bastian et al., 2008; Chakravorty et al., 2013; Janwan et al., 2013; Seesang et al., 2013).

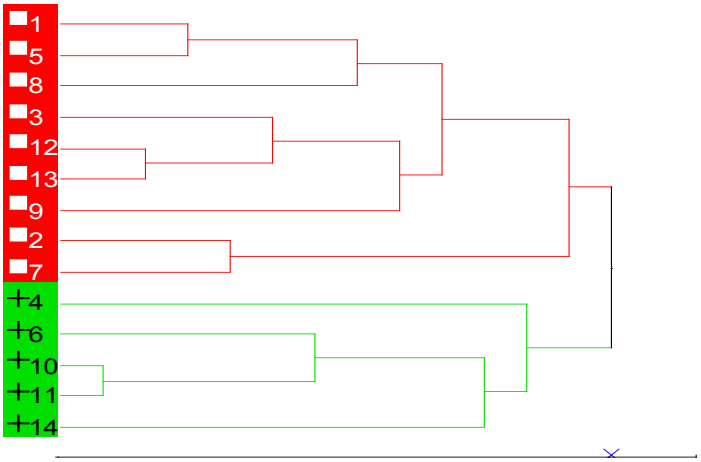
Table 3. Phenotypic linear correlation between grain yield and yield components.

Traits	Plant height	Panicle length	Number of grains per panicle	Weight of grains per panicle	1 000 grain weight	Number of plants per m^2	Grain yield
Plant height	1	0.128	-0.246	0.167	0.317	0.248	-0.292
Panicle length		1	-0.208	-0.125	0.113	0.126	0.337
Number of grains per panicle			1	0.180	-0.752**	-0.056	0.224
Weight of grains per panicle				1	0.485	-0.593*	0.207
1 000 grain weight					1	-0.326	-0.098
Number of plants per m^2						1	0.185*
Grain yield							1

** Correlation is significant at the P<0.01

* Correlation is significant at the P<0.05

Cluster analysis was carried out based on available data for grain yield and yield related components (Fig. 1). Two main cluster groups were extracted. The first one contains 9 varieties: Arsenal, Sprint, Mirko, Ronaldo, Creso, Vasko, Sfera, Nembo and Krystalino. The varieties Creso and Vasko were genetically the closest with the least remote units, forming subgroup in the first cluster. The second cluster group contains the remaining varieties Galileo, Ulisse, Gloria, Pato and San Andrea (Fig. 1).



	Legend
1	Arsenal
2	Nembo
3	Ronaldo
4	Galileo
5	Sprint
6	Ulisse
7	Krystalino
8	Mirko
9	Sfera
10	Gloria
11	Pato
12	Creso
13	Vasko
14	San Andrea

Figure 1. Cluster analysis of rice varieties based on grain yield and yield related components.

CONCLUDING REMARKS

The performed research showed variability between analysed agro-morphological traits. Principal component analysis was utilized to estimate the relative contribution of different traits for total variability. This tool has identified some characters that plays prominent role in classifying the variation existing in the germplasm. The results of the PCA revealed that the 75.59% of the total variability was explained by the first three main principal components. The analysis identified that traits such as 1 000 grain weight, panicle lenght, number of plants per m^2 and grain yield in different principal components are the most important for classifying the variation. Only Ulisse and San Andrea had positive values by all main components. Positive correlation between number of plants per m^2 and grain yield was established, but also the significant high factor loading values for panicle length and 1 000 grain weight were determinate which indicate on the importance of those traits in breeding programs for yield improvement.

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ПРОЦЕНКА НА АГРО-МОРФОЛОШКАТА ВАРИЈАБИЛНОСТ КАЈ ОРИЗОТ СО МУЛТИФАКТОРИЈАЛНА АНАЛИЗА

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Резиме

Целта на овој труд е да се изврши проценка на агроморфолошката варијабилност на четиринаесет сорти ориз, користејќи ги компонентната векторска анализа, линеарната корелација и кластер анализата. Сите анализирани сорти имаат италијанско потекло и се одгледувани во 2014 и 2015 година во агроеколошки услови во Кочани, Република Северна Македонија. Со цел да се утврди варијабилноста на својствата и да се процени уделот на анализираните својства во однос на вкупната варијабилност беше применета компонентна векторска анализа. Со оваа анализа се добиени три главни компоненти со гранична вредност на оптоварување поголема од 1, а кумулативниот процент на овие три главни компоненти изнесува 75.59% од вкупното варирање. Првата главна компонента учествува со 30.81% и е позитивно поврзана со следниве својства: маса на 1000 зрна, должина на метличка, маса на зрна од метличка и висина на растение. Втората главна компонента учествува со 25.08% од вкупното варирање и е во корелација со позитивните вредности на својствата: број на растенија на m^2, висина на растение и должина на метличка. Третата главна компонента учествува со 19.71% од вкупното варирање и е позитивно поврзана со приносот на зрно и должина на метличката. Само сортите Ulisse и San Andrea покажаа позитивни вредности кај сите три главни компоненти. Приносот на зрно покажа позитивна корелација со бројот на растенија на m^2 ($r = 0.185$). Од друга страна, бројот на растенија на m^2 беше во негативна корелација со масата на зрна од метличка ($r = -0.593$). Исто така, беше утврдена негативна корелација помеѓу бројот на зрна од метличка и масата на 1000 зрна ($r = -0.752$). Со кластер анализа се издвоени две главни кластер групи со подгрупи. Резултатите укажуваат на постоење на варијабилност во проучуваните сорти која може да им помогне на селекционерите за постигнување на повисок принос кај оризот.

Клучни зборови: *Oryza sativa L., компонентна векторска анализа, кластер анализа, линеарна корелација.*

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STARTER CULTURES EFFECT ON pH AND SH DYNAMICS OF INOCULUM DURING FERMENTATION PERIOD OF PROBIOTIC YOGURT

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ABSTRACT

Fermented dairy products are produced by fermentation process of lactose by using microorganisms especially lactic acid bacteria. Probiotics are living microorganisms, which -ingested in sufficient amounts, beneficially influence the health of the host by improving the composition of intestinal microflora. Nowadays, the popularity of these products is growing, not only because of its organoleptic properties, but also because of its nutritional value and health benefits. Probiotic yogurt is a dairy product obtained by milk fermentation process, by adding a probiotic starter culture. The goal of this survey was to follow the pH and SH values of inoculum during fermentation period of probiotic yogurt manufactured with three different starter cultures, which are with the following commercial names: ABT-6, ABT-750 and ABT-10 consisting of *Streptococcus thermophilus, Lactobacillus acidophilus* and *Bifidobacterium bifidus.*

Key words: *probiotic yogurt, inoculum, active acidity, titrable acidity, fermentation period*

INTRODUCTION

Fermented dairy products are products that can be produced by lactose fermentation by microorganisms especially by lactic acid bacteria. Fermented dairy products are usually produced by using lactic acid bacteria and yeasts (Ozer and Kirmaci, 2010). Probiotics are defined as living microorganisms, which when ingested in sufficient amounts, beneficially influence the health of the host by improving the composition of intestinal microflora, (Ejtahed et al., 2011). Probiotic microorganisms that are known to be beneficial to human health can be ingested through fermented dairy products, enrichment of various foods with these bacteria and consumption of pharmaceutical products that are obtained by using viable cells, (Kanmani et al., 2013). Probiotics also are defined as viable microorganisms which can be consumed separately or with different foods, which assist dietary and microbial balance by regulating the mucosal and systemic immunity and beneficially affect the consumer's health, (Gibson and Fuller, 2000).

The main milk function in the diet of young mammals is providing nutrients that are essential for their normal growth, development and also providing immune protection. Milk is a major source of protein, essential fatty acids, vitamins and minerals that are needed for normal functioning of the human metabolism. To improve the functional, sensory, nutritional, immunologic therapeutic and even technological properties of milk and dairy products substances are added in small amounts. This group of substances includes probiotics that always go together with substances called prebiotics When combined with probiotics, the two become known as a symbiotic, which is a characteristic of foods that have both good bacteria and the strength to keep them going, (Trajchevski, 2012).

Yogurt is a dairy product with excellent nutritional value, and is a favorite food of all generations (Rašić and Kurmann, 1978). This

product can be consumed by people who are lactose intolerant. Yogurt is characterized by slightly sour taste and pleasant aroma. Specific taste comes from the created lactic acid, which comes from the work of bacteria during the fermentation process of the lactose, while the yogurt flavor comes from the presence of multiple chemical substances produced during fermentation of the lactose in milk.

If we want to produce probiotic yogurt with specific properties, we need to use different probiotic cultures. Recently for yogurt production "ready-set" cultures (dried, deep-frozen or freeze-dried) are used. The effect of this functional food increases by inoculation of probiotic bacteria and prebiotic that operate in symbiosis. Probiotic product must maintain the vitality of probiotic culture during the production process, and also have to be sustainable and stable during storage time and shelf life of the product.

Probiotics are defined as a single or mixed culture of live microorganisms that have positive effect on human metabolism and also improve the properties of autochthonous microflora.

Titratable and active acidity are very important parameters, which mostly affect the shelf life and acceptability of fermented dairy products (Mahmoudi et al., 2012).

MATERIAL AND METHODS

Milk composition

As a material for this research were used the following: standardized cow milk, (Table 1), 0.1% skimmed milk powder in an amount of 0.7%, three different types of frozen probiotic starter cultures in an amount of 0.2% (Chr Hansen, Copenhagen, Denmark) and addition oligofructose as prebiotic, in an amount of 1.5%.

Table 1: Chemical composition of standardized cow milk

Milk components (%)	
Protein	3.46±0.05
Milk fat	1.0 ±0.03
Lactose	4.53±0.01
Dry fat matter	9.0±0.06

Probiotic starter cultures

The probiotic cultures that were used in this research paper (ABT-6, ABT-750 and ABT-10) were composed with the same types of bacteria, but in different proportion: Streptococcus thermophilus St-M5; Lactobacillus acidophilus, LA–5 and Bifidobacterium bifidus, BB-12; All starter cultures that were used during production process were prepared as operating instructions of the probiotic cultures manufacturer.

Probiotic yogurt manufacture

Pasteurized cow milk (1.0 % standardized milk fat) was heated at 37oC. Three variants of probiotic yogurt were produced (A, B and C). Variant A with probiotic cultre ABT-6, Variant B – with probiotic starter culture ABT-750 and Variant C with probiotic starter culture ABT-10. All samples were inoculated with the activated starter culture (0.02% v/v) and prebiotic-oligofructose (1.5%) The fermentation was finished at pH 4.6 and the samples stored at 4±1°C for 21 days. Fisrt 24 hours the active and titrable acidity of inoculm was measured.

Measurement of active acidity

The pH (active acidity) was determined by using digital pH MP120 Meter, Mettler Toledo, Switzerland. After each usage the pH-meter was calibrated with buffer solution pH=7 and pH=4. The concentration of hydrogen ions present in the inoculum of all variants probiotic yogurt was measured during fermentation (in a period time of six hours).

Measurement of Titratable Acidy

The measurement of tirtable acidity (oSH) was according the method described by Caric et al. (2000). In erelenmaer with transfer pipette 20 ml inoculum and 1 ml of 2% w/v solution of phenolphthalein. Content is titrated with 0.1 M NaOH solution till appearance of faint pink color that will not change in a period of 2 minutes. Acidification of inoculum was calculated by the formula: K= V•2, where: V-volume of NaOH spent during titration. The titrable acidity of inoculum of three variants probiotic yogurt was measured during fermentation (in a period time of six hours).

RESULTS AND DISCUSSION

Dynamic of inoculum titrable acidity during fermentation

In Table 2 are presented the results of inoculum titrable acidity of three different variants probiotic yogurt, during fermentation process.

Table 2: Dynamic of inoculum titrable acidity during fermentation

Starter culture	Titrable acidity of inoculum (n=5)							
	Period of fermentation (hours)							
	0	1	2	3	4	4.5	5	6
ABT-6	6.13±0.01	8.12±0.02	10.80±0.2	15.90±0.35	24.70±0.3	27.20±0.7	/	/
ABT-750	6.13±0.03	8.15±0.01	10.90±0.05	16.85±0.55	23.53±0.6	25.65±0.9	26.40±0.9	27.50±0.9
ABT-10	6.13±0.03	8.17±0.01	10.80±0.29	16.30±0.4	25.10±0.55	26.85±0.95	27.40±0.9	/

According to the results obtained in this research, constantly increasing of inoculum titrable acidity can be noticed. At the beginning of fermentation process the inoculum titrable acidity was almost equal in all three variants of probiotic yogurt (6.13 oSH). Minimal difference of titrable acidity between inoculums of three different probiotic yogurts was noticed, during the fermentation period. The time for achieving the required inoculum titrable acidity was different at all three examined variants probiotic yogurt. The probiotic yogurt produced with starter culture ABT-6, has achieved the required titrable acidity of inoculum (27oSH) in shortest time, for four hours and thirty minutes, the probiotic yogurt produced with starter culture ABT-10, have achieved the required titrable acidity of inoculum in five hours and probiotic yogurt produced with starter culture ABT-750 have achieved the required titrable acidity of inoculum in six hours (Figure 1).

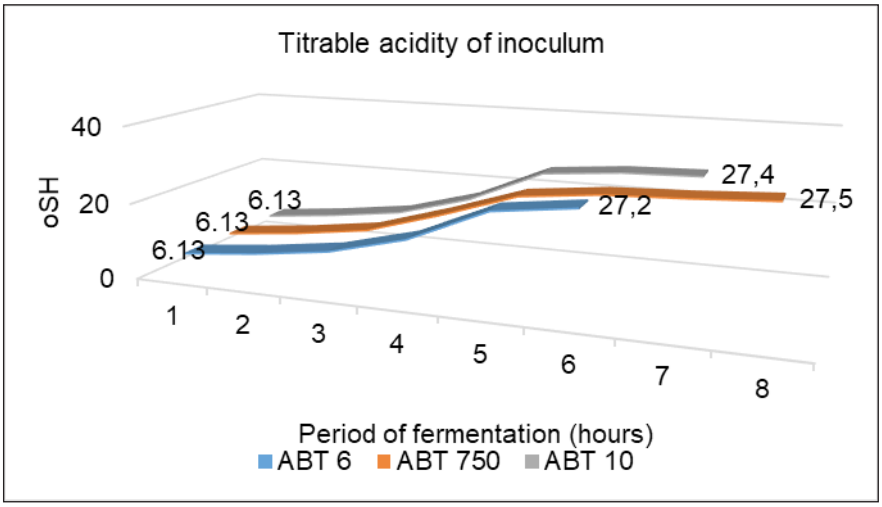

Figure 1. Dynamics of inoculum titrable acidity of probiotic yogurt variants.

Dynamic of inoculum active acidity during fermentation

In Table 3 are presented the results of inoculum active acidity during fermentation process.

Table 3. The results of inoculum active acidity during fermentation process

Starter culture	Active acidity of inoculum (n=5)							
	Period of fermentation (hours)							
	0	1	2	3	4	4.5	5	6
ABT-6	6.50±0.02	6.26±0.01	5.81±0.07	5.12±0.08	4.69±0.02	4.55±0.02	/	/
ABT-750	6.51±0.06	6.20±0.05	5.85±0.05	5.21±0.05	4.95±0.01	4.80±0.03	4.70±0.03	4.55±0.03
ABT-10	6.52±0.02	6.31±0.03	5.75±0.03	5.32±0.07	4.82±0.03	4.70±0.02	4.53±0.03	/

According to the results obtained in this research, constantly decreasing of inoculum active acidity can be noticed. At the beginning of fermentation process the inoculum active acidity was almost equal in all three variants of probiotic yogurt (pH=6.50). Minimal difference of active acidity between inoculums of three different probiotic yogurts was noticed, during the fermentation period. The time for achieving the required inoculum active acidity was different at all three examined variants probiotic yogurt. The probiotic yogurt produced with starter culture ABT-6, has achieved the required active acidity of inoculum (pH=4.55) in shortest time, for four hours and thirty minutes, the probiotic yogurt produced with starter culture ABT-10, have achieved the required active acidity of inoculum in five hours and probiotic yogurt produced with starter culture ABT-750 have achieved the required active acidity of inoculum in six hours (Figure 2).

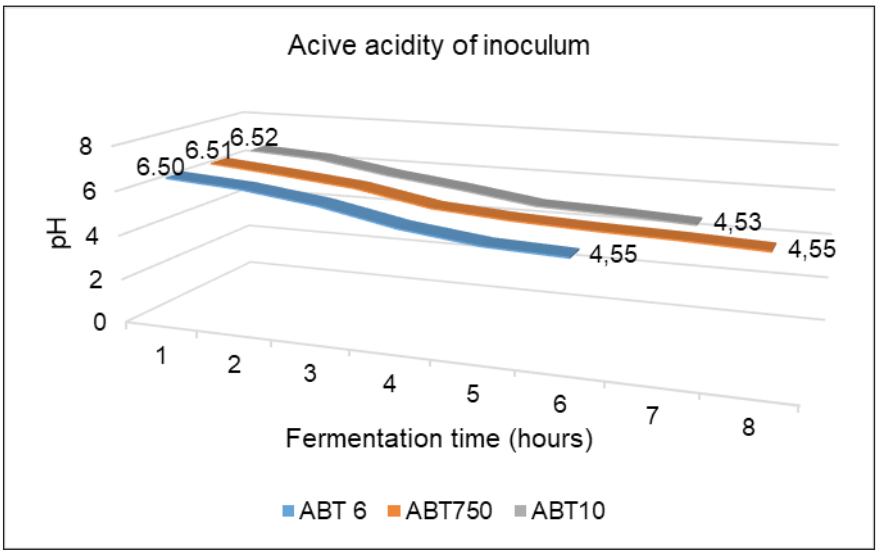

Figure 2. Dynamics of inoculum active acidity of probiotic yogurt variants.

CONCLUSION

The probiotic starter cultures that were used in the production process of three variants probiotic yogurt have a different time for achieving the required titrable and active acidity of yogurt inoculum. The probiotic starter culture ABT-6 has reached the required values for titrable acidity (27oSH) and active acidity (pH=4.55) of yogurt inoculum in shortest time, 4 hours and 30 minutes. The probiotic starter culture ABT-10 has reached the required values for titrable acidity and active acidity in 5 hours and probiotic starter culture ABT-750 has reached the required values for titrable acidity and active acidity of yogurt inoculum in 6 hours. The probiotic starter cultures have significant impact on the time that is needed for achieving the necessary titrabale and active acidity value for yogurt inoculum during fermentation process.

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ВЛИЈАНИЕ НА СТАРТЕР КУЛТУРИТЕ ВРЗ ДИНАМИКАТА НА pH И SH НА ИНОКУЛУМОТ ЗА ВРЕМЕ НА ФЕРМЕНТАЦИЈА НА ПРОБИОТИЧКИ ЈОГУРТ

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Резиме

Ферментираните млечни производи се произведуваат преку процесот на ферментација на лактозата, со помош на микроорганизми, особено млечно-киселинските бактерии. Пробиотиците се живи микроорганизми, кои доколку се внесени во доволна количина во организмот позитивно влијаат на здравјето на домаќинот, преку подобрувањето на составот на интестиналната микрофлора. Денес популарно стана овие производи сè повеќе и повеќе да се зголемуваат, не само поради органолептичките карактеристики, туку и заради нивната нутритивна вредност и здравствени бенифити. Пробиотичкиот јогурт е течен млечен производ добиен преку процес на ферментација на млекото, со додавање на пробиотички стартер култури. Целта на истражувањето е да се следи активната (pH) и титрационата (SH) киселост на инокулумот за време на ферментациониот период кај три различни варијанти на пробиотички јогурт, кои се произведени со следните стартер култури: ABT-6, ABT-750 и ABT-10, кои во својот состав се состојат од *Streptococcus thermophilus, Lactobacillus acidophilus* и *Bifidobacterium bifidus*.

Клучни зборови: *пробиотички јогурт, инокулум, активна киселост, титрациона киселост, ферментационен период.*

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SENSORY EVALUATION OF PROBIOTIC YOGURT INOCULATED WITH DIFFERENT STARTER CULTURES

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ABSTRACT

Today's consumer choice is mostly based on products that provide health benefits, such as probiotic yogurt. The consumer does not tolerate consistency defects, poor firmness or gel viscosity, and also its acid taste, so our goal was to make comparison between different probiotic yogurts (different starter cultures) and to choose the one that is most suitable for consumer use, product that have the best consumer acceptance. Starter cultures that were used in production process were with the following commercial names: ABT-2, ABT-6 and ABT-10 consisting of S. thermophilus, L. acidophilus and B. bifidus. The sensory evaluations showed that the sample C was most preferred by the panelists.

Keywords: *probiotic yogurt, sensory evaluation, starter cultures*

INTRODUCTION

Yogurt is fermented dairy product which is worldwide known for its acceptance in terms of nutritional and health benefits, Özer and Kirmaci (2010). This product is a mixture of milk fermented by lactic acid that is produced by lactic acid bacteria (mostly used for yogurt production: Lactobacillus bulgaricus and Streptococcus thermophilus), (Yadav et al. 2015).

Probiotic yogurt is a functional dairy product that contains probiotic bacteria and has a lot of positive effect on people's health, (Trajchevski, 2015). By using this products, people can get lot of health benefits. It can be improved their intestinal microbial balance, the stimulation of the digestive system, the lactose metabolism, the reduction of blood cholesterol level, the prevention against urinary infections, cardiovascular diseases, osteoporosis, diarrhea, anti-mutagenic and anti-carcinogenic properties. The probiotics are defined as microbial cell supplement that have positive impact on the health of the host when ingested alive in sufficient amount, (Marinaki, 2016).

The development of this novel functional foods that contain probiotic is a highly growing area of food industry and also attracts a special interest from the field of nutrition, due to their beneficial properties to human health. If we want these products to deliver their health benefits, probiotics must be present in it, at level higher then 6 log cfu g^{-1}, at the time of consumption, in order to survive the passage through the gastrointestinal tract, (Saxelin, 2008).

The objective of this work was to determine the effects of the starter cultures on sensorial properties of three types probiotic yogurt produced by using commercial starter cultures (ABT-2, ABT-6 and ABT-10), which contain S. thermophilus, L. acidophilus and B. Bifidus in different portions. The probiotic yogurt samples were evaluated during storage of 21 days at refrigerated temperature (4±1°C).

MATERIALS AND METHODS

The probiotic cultures that were used in this research paper (ABT-2, ABT-6 and ABT-10) were composed with the same types of bacteria, but in different proportion: *Streptococcus thermophilus* St-M5; *Lactobacillus acidophilus*, LA–5 and *Bifidobacterium bifidus*, BB-12; All starter cultures that were used during production process were prepared as operating instructions of the probiotic cultures manufacturer.

Yogurt manufacture

Pasteurized cow milk (3.2 % standardized milk fat) was heated at 37°C. Three variants of probiotic yogurt were produced (A, B and C). Variant A with probiotic culture ABT-2, Variant B – with probiotic starter culture ABT-6 and Variant C with probiotic starter culture ABT-10. All samples were inoculated with the activated starter culture (0.3% v/v). The fermentation was finished at pH 4.65 and the samples stored at 4±1°C for 21 days.

Sensory evaluation

100 panelists familiar with the consumption of yogurts from the Faculty of Biotechnical sciences, were used to evaluate the produced probiotic yogurts for (external appearance, viscosity, color, smell, taste and overall acceptability, using a 50-point system method (Presilski, 2002). The tests were made in a room which met the standard ISO 6658:1985. The sensory evaluation was also made in order to determine the best variant of probiotic yogurt (A, B and C), that is chosen by the final users-consumers.

RESULTS AND DISCUSSION

The score from the sensory evaluation of three variants of probiotic yogurt are presented in Table 1. According to the results presented in Table 1 the probiotic yogurt produced with starter culture ABT 10 (Variant C) has had the best overall acceptability form the consumers.

For external appearance the probiotic yogurt produced with starter culture ABT-2 (Variant A) has got score of 7.65 points, probiotic culture produced with starter culture ABT-6 (Variant B) has got score of 10 points and probiotic yogurt produced with starter culture ABT-10 (Variant C) has got score of 10 points.

For viscosity the probiotic yogurt produced with starter culture ABT-2 (Variant A) has got score of 6 points, probiotic yogurt produced with starter culture ABT-6 (Variant B) has got a score of 10 points and probiotic yogurt produced with starter culture ABT-10 (Variant C) has got a score of 10 points.

For quality parameter color, each of examined probiotic yogurt variants (A, B and C) produced with starter culture ABT-2, ABT-6 and ABT-10 have got score of 10 points.

For quality parameter odor the probiotic yogurt produced with starter culture ABT-2 (Variant A) has got score of 10 points, probiotic yogurt produced with starter culture ABT-6 (Variant B) has got score of 8 points and probiotic yogurt produced with starter culture ABT-10 (Variant C) has got score of 9.5 points.

For quality parameter taste the probiotic yogurt produced with starter culture ABT-2 (Variant A) has got score of 7.5 points, probiotic yogurt produced with starter culture ABT-6 (Variant B) got score 8.0 and probiotic yogurt produced with starter culture ABT-10 (Variant C) has got score 9.5.

The probiotic yogurt (Variant A) produced with starter culture ABT-2 for all sensory quality parameters has got a score of 41.15 points, probiotic yogurt (Variant B) produced with starter culture ABT-6 for all sensory quality parameters has got a score of 44.5 points and probiotic yogurt (Variant C) produced with starter culture ABT-10 for all sensory quality parameters has got the best score of 49 points.

Table 1. Sensory evaluation of probiotic yogurt (Sensory evaluation test).

Product: Probiotic yogurt (Variant A, B and C)					
Sensory properties	**Description of sensory properties**	**Score**	**Average score**		
			Variant A (ABT-2)	Variant B (ABT-6)	Variant C (ABT-10)
External appearance	**Smooth structure without foam on the surface**		7.65	10	10
	Smooth structure without separate serum				
	Grain structure				
	Decomposition				
Viscosity	**Optimal density**		6	8.5	10
	Too high density				
	Low density				
Color	**White or yellow white**		10	10	10
	Untypical				
Odor	**Pleasant sour milk smell**		10	8	9.5
	Poorly expressed sour milk smell				
	Strange odor, the smell of yeast				
Taste	**Pleasant sour taste**		7.5	8	9.5
	Poorly expressed sour milk taste				
	Acid taste of acetic fermentation				
	Bitter taste				
	External taste				
	Unnatural taste				
Total score			**41.15**	**44.5**	**49.0**

CONCLUSIONS

According to the presented data it can be concluded that starter cultures have significant impact on the sensory properties of probiotic yogurt. Probiotic yogurt produced with starter culture ABT 10 (St- M5, LA – 5, BB – 12) is the most acceptable for the consumers with a total score of 49 points. Starter cultures have significant effect on sensory quality of probiotic yogurt.

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СЕНЗОРНО ОЦЕНУВАЊЕ НА ПРОБИОТИЧКИ ЈОГУРТ ИНОКУЛИРАН СО РАЗЛИЧНИ ЧИСТИ КУЛТУРИ

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Резиме

Денешниот избор на потрошувачите е насочен кон производите кои придонесуваат за здравствени бенефити. Еден од тие производи е и пробиотичкиот јогурт. Потрошувачите на овие производи не толерираат неправилности во конзистенцијата, недоволна цврстина, несоодветен вкус, па нашата цел беше да направиме споредба помеѓу различни варијанти на пробиотички јогурт произведени со различни стартер култури и да се избере една која ќе биде најсоодветна и најприфатлива за консуматорите. Стартер културите кои беа користени во процесот на производството се со следниве комерцијални имиња: ABT-2, ABT-6 и ABT-10, и се состојат од *S. thermophilus, L. acidophilus* и *B. bifidus.* Сензорната анализа покажа дека примерокот С беше најдобро прифатен од панелистите кои беа вклучени во оценувањето.

Клучни зборови: *пробиотички јогурт, сензорно оценување, стартер култура.*

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HYGIENIC SANITATION IN THE DAIRY INDUSTRY IN THE PRODUCTION OF COW YELLOW CHEESE AS A FACTOR FOR OBTAINING HYGIENICALLY PROPER PRODUCTS IN ACCORDANCE WITH THE EU STANDARDIZATION AND CERTIFICATION STANDARDS

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Abstract

Hygienic sanitation processes in the dairy industry are a major factor in obtaining hygienically healthy dairy products. The subject of the analysis is the production equipment, human resources and the produced finished product – cow yellow cheese, in which different procedures for sanitation are used due to the application of various technologies at work.

Studies have shown that hygienic sanitation gives satisfactory results. The responsibility and positive influence of the employees has been confirmed. The results of the examined bacteriological correctness of the equipment showed a greater and better effect when performing the machine compared to the manual cleaning. From the analysis of the key parameters in the CIP (Cleaning-In-Place) system it was found that the shorter duration of cleaning is correlated with a faster flow and a higher temperature and concentration of the agents. From the recirculating agents, the base and the acid, more clouding occurs in the base material. Finally, from the examined bacteriological correctness of the finished product – yellow cheese, it has been confirmed that the product is completely hygienically proper and healthy for consumption.

Hygienic sanitation in the dairy industry is the most critical operation in the milk processing processes and it is of great importance for obtaining a quality, healthy and hygienically straightforward finished product.

Key words: *CIP system, human resources, production equipment, finished product*

INTRODUCTION

In their composition milk and dairy products are food rich with protein, fat and milk sugar as their specific products, and then with mineral substances and vitamins, necessary for the growth and development of organisms. Hence the fact that milk in its foodstuffs is a product in which people find relatively inexpensive, full-time, easily digestible and suitable food, children are the best replacement for breast milk, and the sick and elderly foods that favourably affect their health.

As a result of certain economic changes and technical achievements, the rapid pace of the development of dairy in the real sense of the word is closely related to the rapid industrial development in the first half of the 19th century, a period characterized by large industrial rise caused by a number of significant inventions of the field the technique, and especially the machine area.

A key challenge facing all segments of the food processing industry is how to apply reproducible and consistent cleaning methods with proper process control, monitoring, as well as a series of documents that are needed to meet the requirements for validity. Contemporary dairy industries, as well as many other industries, are characterized by

revolutionary changes, as well as accelerated transformation and continuous upgrading of their technologies. All these changes during the functioning of a whole necessitate the constant monitoring of a number of parameters, their constant control and improvement. In order to extract the maximum in the working process, in the framework of the modern milk production, constantly improves the production process. The improvement of the same is done both in terms of quality production of individual products and utilization of the capacities, where all this should be done in accordance with the world requirements for good manufacturing and good hygiene practice, the implementation of the ISO standard and the HACCP system. This involves the continued engagement and performance of the staff, the use of appropriate equipment and appliances that enable a high degree of effectiveness and record ability of all individual phases in the course of production.

In the framework of such a modern dairy processing facility, besides the basic starting point for the production of a different product range, the equipment should meet the envisaged conditions for use in a specific production process. The purpose of such requests is to obtain finished product that is completely upright, not only organoleptic, but also from the food and microbiological aspect. In this way, there is a need to use a new modern technology that stands out as one of the most important components in the production chain. In line with what has been said so far, the link which stands out as an element of special importance is the microbiological hygienic correctness during the whole production process which is in direct dependence on the maintenance of the hygiene of all the equipment and apparatus involved in the transformation of the starting raw material to the finished product. This implies continuous control of the working conditions and the parameters envisaged for the respective operations, as a first step during the whole day and as the last step after the completion of the production, which would ensure the production of safe food and satisfy the needs and expectations of the consumers.

There are four main cleaning processes that are used within production processes:

- CIP (Cleaning-in Place)
- COP (Cleaning-out-of-place)
- Manual cleaning
- Cleaning with immersion

All these cleaning techniques have their own characteristics, and in order to meet all the necessary requirements, standards and regulations, they should constantly be perfected and complement each other. Starting from this fact, it is necessary to pay great attention to each separate segment that participates in the process, especially the sanitation which is one of the most critical moments of production and all this in order to maintain consumer confidence and to avoid negative publicity due to failures related to food safety, as well as to prevent economic losses in the operations arising as a result of inadequate execution of the procedures and procedures for maintaining the technological and microbiological quality of the food products.

The quality parameters of food products are made according to standard and prescribed methods according to the applicable national Rulebooks and EU Directives.

MATERIAL AND METHODS

The role of human resources in the process of hygienic sanitation was examined and determined that is a significant factor in the purification and production processes.

The three basic parameters of the CIP system - time, flow and concentration used in the dairy were examined, because of their significance in machine cleaning of the equipment.

The extent of changes in the recycling solvents-base and acid, which was used to perform the machine cleaning of the equipment, was investigated. The success of the cleaning of production equipment, both on the one that has been cleaned manually and on the equipment that is cleaned by machine, has been fully investigated.

The bacteriological correctness of the cleaned surfaces was also examined. In addition, five points were taken for taking swabs from the equipment that was cleaned manually and the same as the equipment cleaned by machine.

Finally, the bacteriological correctness of the finished products – cow yellow cheese - was examined.

Test Material

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Finally, the bacteriological correctness of the finished product – cow yellow cheese was examined.

All experimental samples were correctly and timely taken for analysis. The sampling time was accurately observed, and the control points for the effectiveness and success of the hygienic sanitation regime applied were laid down in the rulebooks used during the work. The results obtained are comparable with the results of other researchers

RESULTS AND DISCUSSION

Table 1 shows the habit of hand washing at the workplace among employees, according to 4 subcategories which are described in more detail in the table itself.

Table 1. Habit of hand washing, expressed in (%).

I wash my hands in the workplace	Reply %
-upon arrival	36.4
-before and after leaving	15.2
-after brushing nose, sneezing, coughing	27.3
-after touching the hair / skin	21.3

Table 2. Frequency for changing work clothing at the workplace, expressed in (%).

I change my work clothes	Reply %
-every day	25
-more times a day	16,7
-2-3 times a week	58,3
-once a week	0

From the result of the Table 2 it was confirm that each of the employees in production and the employees in charge of sanitary hygienic sanitation change their work clothes depending on the nature of the work tasks.

According to the results of comparison of the duration of each phase of cleansing with the CIP system in the US and Macedonia, shown in Table 3, it has been established that cleansing with CIP in the United States lasts (75 min) and in the Republic of North Macedonia (60 min) that in North Macedonia, it saves time, and the necessary cleaning effect is achieved.

Table 3. Comparison of the duration of each phase of cleansing with the CIP system in the Republic of North Macedonia and the US.

Comparison of duration							
Phases of the CIP system	I Phase		II Phase	III Phase	IV Phase	V Phase	Total duration (min)
	I sub-phase - cold tap water (min)	II sub-phase - hot water (min)	Base Wash (min)	Interphase rinse (min)	Wash with acid (min)	Final rinse (min)	
R. N. Macedonia	5	5	12.5	10	12.5	15	60
US	5	5	30	5	20	7.5	75

Table 4 shows that the flow of CIP cleaning in the Republic of N. Macedonia is (3m/s), and in the United States (1.5m/s), where it is determined that the flow velocity and duration of each phase in these two countries is inversely proportional, and the necessary effect with different parameters is achieved.

Table 4. Comparison of flow through pipes in each phase of cleansing with the CIP system in the United States, and Republic of North Macedonia.

Comparison of duration							
Phases of the CIP system	I Phase		II Phase	III Phase	IV Phase	V Phase	Total duration (min)
	I sub-phase - cold tap water (min)	II sub-phase - hot water (min)	Base Wash (min)	Interphase rinse (min)	Wash with acid (min)	Final rinse (min)	
R. N. Macedonia	3	3	3	3	3	3	3
US	1.5	1.5	1.5	1.5	1,5	1.5	1.5

From the comparison of the solubility of the solutions shown in Figure 1, it can be concluded that the blurring of the solution is greater with the base cleaning agent, which is probably due to the daily use immediately after the end of the production processes due to the milk deposits that have not been removed during rinsing with water, which is performed as a first step. While the acidification of the acid cleaning agent is much smaller than that of the base solution, since the acid as the agent is used after having been previously treated with a base agent, and furthermore it is characterized by a smaller number of recycled ones, once a week or about four times for thirty days.

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From the results shown in Figures 2 and 3, after the analysis, it can be determined that the colour of the litmus paper used as an indicator fully corresponds to the anticipated tint for a successful cleaning process, from which it can be concluded that the requirements for manual and machine cleaning of the production equipment.

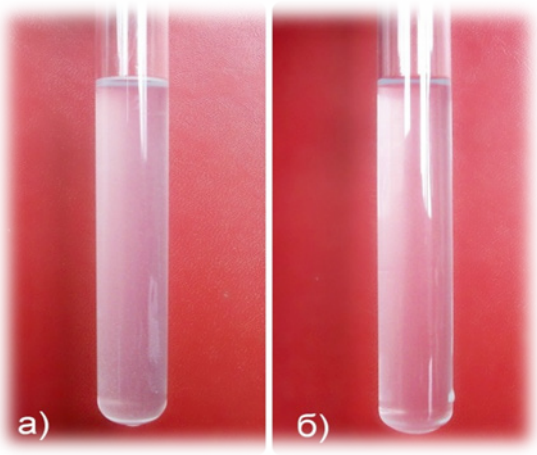

Figure1. Comparison of recycled solutions: (a) base solution and (b) acid solution.

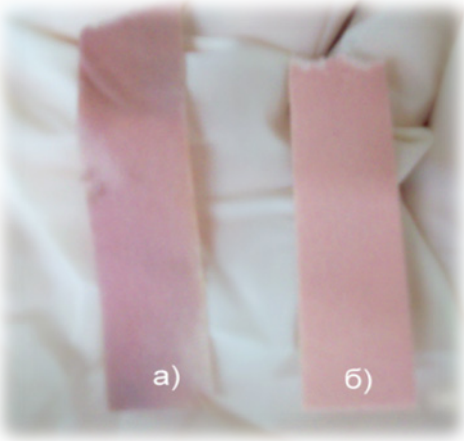

Figure 2. The litmus paper used for verification of manual cleaning, (a) after and (b) before the test.

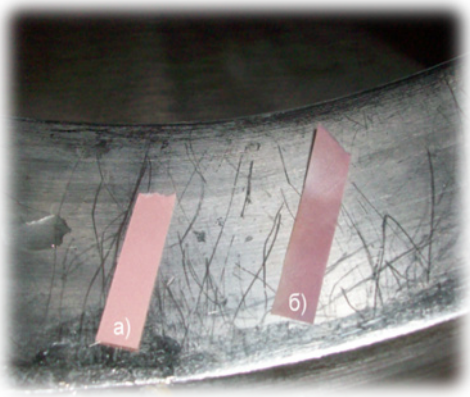

Figure 3. The litmus paper used to verify machine cleaning, (a) before (b) after the test.

Table 5. Presence of total number of bacteria and Enterobacteriaceae on areas subjected to manual cleaning (CFU / cm^2).

Working surfaces-cleaned manually	Type of bacteria	N	(CFU/cm2)	Sd	Min (CFU/cm2)	Max (CFU/cm2)	Cv (%)
Work desk	Total number of bacteria		5.7	7.4	0	14	130.1
	Enterobacteria	3	0	0	0	0	0
Molds	Total number of bacteria		1.3	1.2	0	2	86.6
	Enterobacteria	3	0	0	0	0	0
Prominent wheelbarrows	Total number of bacteria	3	2.7	2.9	1	6	108.3
	Enterobacteria		0	0	0	0	0
Stalk	Total number of bacteria	3	1.7	2.1	0	4	124.9
	Enterobacteria	3	0	0	0	0	0

From the results of the Table 5, it can be concluded that in none of the examined surfaces - work desk, moulds, prominent wheelbarrows and stalks treated with manual cleaning, contamination with Enterobacteriaceae has not been established. Regarding the total number of bacteria found on the investigated surfaces, it is noted that there is a certain number of occurrences, but within the permitted limits according to the milk policy.

Table 6: Presence of total number of bacteria and Enterobacteriaceae on surfaces subjected to machine cleaning (CFU / cm^2).

Working surfaces - cleaned by machine	Type of bacteria	N	(CFU/cm2)	Sd	Min (CFU/ cm2)	Max (CFU/ cm2)	Cv (%)
Machine / unit	Total number of bacteria		1.7	2.9	0	5	173.2
	Enterobacteria	3	0	0	0	0	0
Pasterizator	Total number of bacteria		0	0	0	0	0
	Enterobacteria	3	0	0	0	0	0
Filter	Total number of bacteria		0.3	0.6	0	1	173.2
	Enterobacteria	3	0	0	0	0	0
Vehicle tank	Total number of bacteria	3	0.7	0.6	0	1	86.6
	Enterobacteria		0	0	0	0	0
Raw milk storage tank	Total number of bacteria	3	0.7	1.2	0	2	173.2
	Enterobacteria		0	0	0	0	0

From the results of the Table 6, it can be concluded from none of the tested surfaces - machine / aggregate, pasteurizer, filter, vehicle tank, crude milk storage tank, which are treated with machine cleaning, as well as in the manual cleaning procedure, contamination with Enterobacteriaceae has not been established. Regarding the total number of bacteria found on the investigated areas, it is noted that there is an insignificant number within the permitted limits according to the milk policy.

From the obtained results of the microbiological analysis of the yellow cheese in the form of a finished product, it can be concluded that none of the analysed yellow cheese has not been established the presence of bacteria and therefore the quality of the product fully corresponds to the Rulebook of specific requirements for food safety in terms of microbiological criteria, Official Gazette of RM No. 78 of 2008.

The legal matter regulating the behaviours, the manner and the methods of production is regulated and certified as follows:

- Determination of fat content in milk (ISO 2446: 1976, ISO 488: 1983)
- 32008R1020, REGULATION OF THE COMMISSION (EC) No 1020/2008 of 17 October 2008 amending Annexes II and III to Regulation (EC) 2076/2005 of the European Parliament for the placing on the market of milk products
- Rulebook on the special requirements for safety and hygiene and the manner and procedure for performing official controls of milk and milk products-Official Gazette of RM 151/07 and Official Gazette of RM 93 from 12.07.2010
- Determination of acidity of milk (Rulebook on methods of physical and chemical analysis of milk and milk products Official Gazette of the SFRJ, No. 32/83)
- Determination of dry matter of milk (Rulebook on Methods of Physical and Chemical Analysis of Milk and Milk Products Official Gazette of the SFRJ, No. 32/83)

CONCLUDING REMARKS

The production of dairy products as a sector that our country participates with a good percentage in the gross domestic product, as well as the developed production capacities and products thereof, are subject of certification, standardization and regulation in accordance with the European positively applicable norms and directives. From the above stated it was necessary our rules, regulations and laws to become compatible with the European ones.

Hygienic sanitation in the dairy industry is the most critical operation in the milk processing processes and is extremely important for the complex production of hygienic products. Quality hygienic sanitation is conducted in accordance with the HACCP system and ISO standards, and for the best performing operations manual cleaning is increasingly being replaced by machine. In addition, with the CIP system, cleaning is carried out faster, with less labour and with reduced chemical risk for human health. In manual cleaning, visual inspection of the performed operation is provided, but it is necessary to increase the share of human labour. Finally, the overall sanitation of the plant, and hence the quality of the products implies continued engagement.

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ХИГИЕНСКАТА САНИТАЦИЈА ВО МЛЕКАРСКАТА ИНДУСТРИЈА ПРИ ПРОИЗВОДСТВО НА КРАВЈИ КАШКАВАЛ КАКО ФАКТОР ЗА ДОБИВАЊЕ НА ХИГИЕНСКИ ИСПРАВНИ ПРОИЗВОДИ ВО СОГЛАСНОСТ СО СТАНДАРДИТЕ НА ЕУ ЗА СТАНДАРДИЗАЦИЈА И СЕРТИФИКАЦИЈА

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Резиме

Процесите на хигиенска санитација во млекарската индустрија се носечки фактор за добивање на хигиенски исправни млечни производи.

Предмет на анализа се производната опрема, човечките ресурси и произведениот готов производ – кравји кашкавал, при чие производство поради примената на различните технологии на работа се користат различни постапки за санитација.

Испитувањата покажаа дека хигиенската санитација дава задоволителни резултати. Потврдени се одговорноста и позитивното влијание на вработените. Резултатите од испитуваната бактериолошка исправност на опремата покажаа поголем и подобар ефект при спроведување на машинското во споредба со рачното чистење. Од анализата на клучните параметри во CIP (Cleaning-In-Place) системот е утврдено дека пократкото времетраење на чистењето е во корелација со побрз проток и повисока температура и концентрација на средствата. Од рециркулирачките средства, база и киселина поголемо заматување се јавува кај базното средство. На крај, од испитуваната бактериолошка исправност на готовиот производ - кашкавал е потврдено дека производот е потполно хигиенски исправен и здрав за консумација.

Хигиенската санитација во млекарската индустрија е најкритична операција при млекопреработувачките процеси и има големо значење за добивање на квалитетен, здрав и хигиенски исправен готов производ.

Клучни зборови: *CIP-систем, човечки ресурси, производна опрема, готов производ.*

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EVALUATION OF THE QUALITY OF RAW MILK FOR YOGURT PRODUCTION

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Abstract

Yogurt is one of the most popular fermented products. From a nutritious aspect yogurt is very similar to the milk from which it was made, partly changed, with certain therapeutic effects linked with the starter cultures added in the production process.

The research includes the examination and presentation of results for the physical-chemical composition, the inhibitory substances and the hygienic quality of the raw milk for yogurt production, the technology, the physical-chemical composition and the microbiological quality of the yogurt.

According to the results obtained from the research, the milk fat content amounts to 3.78%, proteins 3.23%, lactose 4.68%, and dry matter 12.76%. The average number of somatic cells in the milk is 248.400/ml, and the total number of bacteria 199.000/ml. No antibiotics had been detected in the milk, and the content of aflatoxins is 0.010μg/kg. The average value of the acidity and pH of the milk are 6.68 and 6.79 ^{0}SH respectively. The microbiological quality of the produced yogurt is in accordance with the requirements of the Regulations for the special requirements for the microbiological criteria of food.

Key words: *quality, raw milk, yogurt*

INTRODUCTION

Fermented milk products have been consumed in Europe for perhaps 4000 years and yogurt is one of the oldest and most popular of these products worldwide (Buttriss, 1997). Fermented milk products contain all important food ingredients in such relation that a human body can optimally use them and because of that they belong to a group of very important food in human nutrition. They, as well as milk, contain all the basic ingredients needed for growth of a human body, for development, reproduction, maintenance and satisfying energy needs. These ingredients entail lactose, lactic acid, protein, milk fat, minerals and vitamins. During fermentation there is a change of some constituents of milk and with creating of new constituents, fermented products get new features compared to milk (Carić, 2000).

The fermented dairy products are traditional products that are present in milk processing facilities, markets and our table (Tamime and Robinson, 2004). One of the most consumed sour-milk products in our country is the yogurt. Yogurts are produced by a lactic acid fermentation process using thermophilic strains of bacteria. From a nutritious aspect yogurt is very similar to the milk from which it was made, partly changed, with certain therapeutic effects linked with the starter cultures added in the production process. For the production of yogurt, a good quality milk is necessary (Srbinovska, 2007). The physico-chemical quality of the milk for yogurt production is defined based on the following parameters: proteins, fats, lactose, dry matter without fat and casein, the degree of acidity of the milk (Antunac et.al., 1997). The variations in the composition of the milk affect its total quality, as well as certain technological operations in the processing of the milk, and manifest themselves on the

composition, properties and quality. Indicators of the hygienic correctness of the milk are the total number of bacteria and the number of somatic cells, which is correlated with changes in the physico-chemical composition of the milk and is used to evaluate its quality (Kalit et. al. 1998). As a determining factor in establishing the suitability of the milk for yogurt, processing is the ascertainment of the upper limit of the number of somatic cells (Katić & Stojanović, 2002). An increased number of somatic cells and enzymatic activity adversely affect the milk's processing suitability and its technological properties. The milk for yogurt must not have the presence of antibiotics and other residues that negatively affect its technological suitability and human health. Antibiotics inhibit the development of lactic acid bacteria, and can cause allergic reactions in humans or reduce the sensitivity of harmful bacteria in the human body to some antibiotics used in the treatment of certain diseases. (Presilski, 2004). Milk can also contain aflatoxins M1, which are the most toxic collection of mycotoxins that are secondary fungal metabolites. The aflatoxin present in milk over an extended period of time even in extremely small quantities poses a health hazard to humans. (Sassahara et. al., 2005).

MATERIAL AND METHODS

The samples used for this research were taken from the aggregate milk for production of yogurt, following a standard technological process in the Dairy "Bistra" Kicevo. The technology of yogurt includes the following steps:

Raw milk
↓
Filtration
↓
Pasteurization (92^{0}C/10 min)
↓
Cooling to 43^{0}C
↓
Inoculation with starter cultures YF- L812
↓
Fermentation to pH 4.6
↓
Cooling and mixing to 15^{0}C
↓
Packaging of the yogurt
↓
Storage of the yogurt at 4^{0}C

Physico-chemical composition of the milk

The analysis of the physico-chemical composition and antibiotics in the milk was done in the internal laboratory of the dairy, while the other analyses were done in the Institute of Food" at the Faculty of Veterinary Medicine in Skopje: Analysis of the physico-chemical composition (proteins, milk fat, dry matter, lactose and minerals) was performed with Lacto Scope. The active acidity of the milk was determined with the digital pH meter TESTO 720.

Microbiological analysis of the milk

Total number of bacteria (Bactocount - ISO 21187: 2004) and number of somatic cells (Fossomatic-ISO 13366). Antibiotics from the group of tetracyclines and β-lactams in the milk were determined by rapid tests with the Twin Sensor incubator. The analysis of aflatoxins in the milk was performed with (ELISA R-biopharm 1113).

Physico-chemical composition of the yogurt

Physico-chemical composition of yogurt was determined by accredited methods: fats (ISO2446:1976), dry matter (ADAC/2005/990.20), acidity of yogurt (SH^0) with titration. The pH value was determined by a pH meter.

Microbiological analysis of the yogurt

Listeria monocytogenes (ISO 11290-1), Enterobacteriaceae - (ISO 21528-2), Escherichia coli (ISO 16649-2).

RESULTS AND DISCUSSION

The parameters for the quality of raw milk (physical chemical composition and hygienic quality) used for the production of yogurt are shown in Table 1.

Table 1. Physico-chemical analysis and hygienic quality of milk for yogurt.

Parameters	$\bar{x}$	S_d	Cv (%)	min	max
Proteins	3.23	0.04	1.59	3.19	3.31
Milk fat	3.78	0.06	1.68	3.68	3.89
Lactose	4.68	0.05	1.18	4.61	4.79
Dry matter	12.76	0.16	1.32	12.64	13.16
^{0}SH	6.79	0.07	1.11	6.67	6.88
pH	6.68	0.06	1.04	6.58	6.78
*TCSC/ml	248.400	18.260	7.35	220.000	277.200
*TCB/ml	199.000	17.336	8.71	176.000	228.000

*TCSC/ml (Total count of somatic cells)

*TCB/ml (Total count of bacteria)

The average content of fat in the milk is 3.78 %, proteins 3.23%, dry matter 12.76%, and lactose 4.68%. The average value of the active acidity of the milk is 6.68 and 6.79 ^{0}SH respectively. The average values of all the parameters of the physico-chemical composition are within the value limit prescribed by the Macedonian regulations on requirements for the quality of raw milk and are in accordance with the results of other authors (Srbinovska, 2007), (Dimitrovska et al., 2016). According to (Heeschen, 1987), the quality of milk, in addition to the composition, is also determined by the parameters for hygienic correctness. The number of somatic cells in the milk is in accordance with the regulations, while the total number of bacteria in the milk notes certain deviations from the permissible limit according to the regulations. A correctly performed pasteurization is expected to destroy the microorganisms present in the milk. Similar results were obtained in the research of (Dimitrovska et al., 2016), while deviating results were determined by (Srbinovska, 2007). An increased number of somatic cells in milk indicates poor hygienic quality and is a basic indicator of hygiene conditions for milk production (Srbinovska, 2007), (Kirin, 2001).

Table 2. Residues and contaminants in the milk.

Cow's milk	**Results**
Aflatoxin	<0,010 mg/kg
Antibiotics	not found

The presence of antibiotics was not established in the milk samples. The content of aflatoxins in the examined milk samples for the production of yogurt is within the limits of the permitted quantities according to the food safety regulations in relation to the max levels of certain contaminants.

Physico - chemical characteristics of the yogurt after the production are shown in table 3.

Table 3. Physico-chemical analysis of yogurt.

Parameters	(%)
dry matter	9.65
fats	3.30
^{0}SH	38
pH	4.45

From Table 3 it can be concluded that the average content of dry matter in yogurt is 9.65% and fat is 3.30%. Similar results about the physico chemical composition of yogurt were obtained in the research of (Dozet et al., 1979). The pH value of yogurt after the completed fermentation is 4.45, while the titration acidity is 38^0SH. These results are in accordance with previous researches (Milanović et al., 2013).

The results of the microbiological analysis of the yogurt are presented in Table 4.

Table 4. Microbiological analysis of the yogurt.

Parameters	CFU/ml
L. monocytogenes	not found in 25ml
Enterobacteriaceae	0
E. coli	0

From the presented results of the microbiological analysis of the yogurt, it can be concluded that the presence of L. Monocytogenes, Enterobacteriaceae and E. Coli cfu/ml, was not established. From the results of the microbiological analysis it can be noted that it complies with the regulations on special demands for microbiological criteria for food.

CONCLUDING REMARKS

Based on the performed tests and the results obtained for the physico-chemical composition, the hygienic quality and the presence of residues and contaminants in the milk, it can be concluded that the milk for the production of yogurt is of good quality and is technologically suitable for the production of fermented products, which is confirmed by the quality of yogurt produced.

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ОЦЕНУВАЊЕ НА КВАЛИТЕТОТ НА СУРОВО МЛЕКО ЗА ПРОИЗВОДСТВО НА ЈОГУРТ

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Резиме

Еден од најпопуларните ферментирани производи е јогуртот. Од нутритивен аспект Еден од најпопуларните ферментирани производи е јогуртот. Од нутритивен аспект јогуртот е многу сличен на млекото од кое е произведен, делумно променет и со одредени терапевтски ефекти поврзани со стартер културите додадени во неговото производство.

Истражувањето вклучува испитување и презентирање на резултати за физичко-хемискиот состав, хигиенскиот квалитет на суровото млеко за производство на јогурт, технологијата, физичко-хемискиот состав и микробиолошкиот квалитет на јогуртот.

Според добиените резултати од истражувањето, содржината на масти изнесува 3,78%, протеини 3,23%, лактоза 4,68% и сува материја 12,76%. Просечниот број на соматски клетки во млекото изнесува 248,400/ml, а вкупниот број на бактерии 199.000/ml. Во млекото не се утврдени антибиотици, а содржината на афлатоксини изнесува 0,010 μg/kg. Просечната вредност на киселост и pH на млекото изнесуваат 6,68 и 6,79^{0}SH соодветно. Микробиолошкиот квалитет на произведениот јогурт одговара на условите од Правилникот за посебните барања на микробиолошките критериуми на храната.

Клучни зборови: *квалитет, сурово млеко, јогурт.*

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COMPARISON OF THE RHEOLOGICAL CHARACTERISTICS OF BIO-FORTIFIED FLOUR OBTAINED FROM SOFT WHEAT VARIETIES TRESKA AND RADIKA

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Abstract

The dough rheology is very important for the prediction of the final bakery product quality such as mixing behavior, sheeting and baking performance, and based on starch gelatinization data the overall cooking behavior and product properties can be determined. The aim of this research was to examine the impact of agronomic bio-fortification on the rheological properties of dough. In this research were included 7 samples obtained by adding high quality chelate fertilizers at different stages of wheat growth: Fe soil (1), Fe soil + foliar (2), Fe foliar (3), Control (4), Zn soil (5), Zn soil + foliar (6) and Zn foliar (7). From farinograph data for water absorption it was concluded that all variants for variety Treska and Radika had approximate values with minimal differences compared with variant 4. According to the data obtained for the level of softness, it was concluded that all variants for both varieties of dough are with medium quality. According to the qualitative number, all variants of both varieties belong to quality group B_2, with the exception of variant 2 for the variety Treska and variant 5 for the variety Radika which belonged to the quality group C_1. From the farinographc analysis it can be concluded that bio-fortification did not have a significant effect on the technological quality of the flour. Extensographic analysis showed that for variety Radika from variants 1, 2 and 7 were obtained flour with higher extensibility, resistance and energy in comparison with the variety Treska.

Keywords: *dough, farinograph and extensigraf*

INTRODUCTION

Biofortification is the process of increasing the content and/or bioavailability of essential nutrients in crops during plant growth through genetic and agronomic pathways (Bouis et al., 2011). Genetic biofortification involves either genetic engineering or classical breeding (Saltzman et al., 2013). Agronomic biofortification is achieved through micronutrient fertilizer application to the soil and/or foliar application directly to the leaves of the crop. Biofortification is mainly focused on starchy staple crops (rice, wheat, maize, sorghum, millet, sweet potato and legumes), because they dominate diets worldwide – especially among groups vulnerable to micronutrient deficiencies - and provide a feasible means of reaching malnourished populations with limited access to diverse diets, supplements, and commercially fortified foods (Saltzman et al., 2013). There is evidence that agronomic biofortification can increase yields and the nutritional quality of staple crops, but there is a lack of direct evidence about changes in rheological properties as a result of the application of micronutrient fertilizer.

Rheological properties of dough are very important indices for product development in terms of product quality and process efficiency (Mondal and Datta 2008). Rheology concerns the flow and deformation of a material (Vergnes 2003) and is particularly important technique in revealing the influence of flour constituents and additives on dough behaviour during breadmaking (Dapčević et al., 2014). Among the cereal technologists, rheology is widely recognized as a valuable tool in quality assessment of flour. However, acceptance of biofortificated crops from the producers in the

milling and bakery industry is another issue that needs to be paid special attention. This study was aimed to evaluate the rheological changes that take place in the dough as a result of addition of zinc and iron chelating fertilizers during the cultivation of wheat from the variety Treska and Radka.

MATERIAL AND METHODS

Material

Plant material

The variant of the type Radika soft wheat, and Treska soft wheat (Triticum aestivum L.), which belongs to the group of high quality bread varieties was used as a plant material in this research.

Location and setting experiment

On the property belonging to the Agricultural Institute in Skopje (Republic of North Macedonia), in the testing economy "Dolno Lisiche", a test was placed according to the method of randomized block system, with 7 variants, in three replications.

The following variants were included in the test of this research:

- Fe application in soil (variant 1)
- Fe application in soil and foliar (variant 2)
- Fe foliar application (variant 3)
- Control – without fertilizing (variant 4)
- Zn application in soil (variant 5)
- Zn application in soil and foliar (variant 6)
- Zn foliar application (variant 7)

Basic characteristics of the fertilizers

Yara Vera™ Amidas is a highly qualitative granular fertilizer which contains nitrogen and sulfur. Nutrichem folifer-Fe EDTA chelate product, which is used for a foliar nutrition of the plants.Yara Vita Rexolin is a product for prevention of a shortage of zinc formulated in the form of EDTA chelate. The nutrition of the wheat is conducted in the different stages of development (Menkinoska et.al., 2018)

Methods

Determination of the rheological properties of the biofortificated dough was carried out with the pharynograph and extensograph from the company BRABENDER.

- Pharographic analysis with the method AACC 54-21 (American Association for Cereal Chemistry - AACC 1995)
- Extensographic analysis with the method AACC 54-10 (American Association for of Cereal Chemistry - AACC 1995)

Grinding and testing of the flour was carried out in the farinological laboratory of "Zito Luks AD" - Skopje. (Menkinoska et.al., 2018)

RESULTS AND DISCUSSION

There are many test methods available to measure rheological properties, which are commonly divided into empirical (descriptive, imitative) and fundamental (basic) (Weipert, 1990). According to the empirical rheological parameters it is possible to determine the optimal flour quality for a particular purpose. The empirical techniques used for dough quality control are generally recognized as standard methods by ICC, AACC, ISO and different national standards. In comparison to rheological methods generally applicable in food quality control, dough rheological tests are probably the most diverse (Dapčević et al., 2014).

The most popular and accepted device for measuring dough physical properties is Brabender Farinograph. It measures and records the mechanical resistance of the dough during mixing and kneading. In this research the rheological properties of two soft wheat cultivars Treska and Radika were analyzed and evaluated. Rheological testing included empirical rheological methods with farinograph.

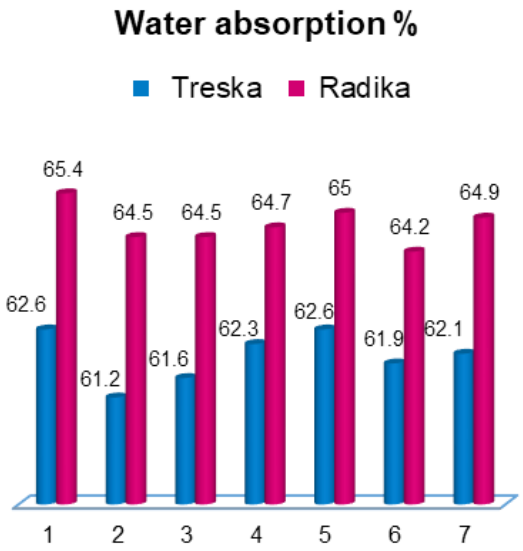

Figure 1. Comparison of water absorption values flour samples.

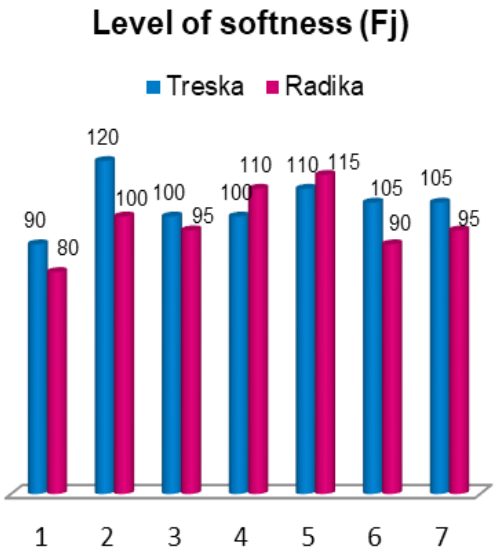

Figure 2. Comparison of level of softness values of flour samples.

The Farinogram parameter with the greatest practical value is the water absorption. Water absorption is directly related to the yield of final bakery product and it is one of the most important parameters in assessing the "flour strength" and in product price calculations. Farinograph water absorption is mainly influenced by the properties of flour main components: gluten and starch. In order to be properly interpreted, it must be compared to the other Farinograph parameters

The results presented in the Figure 1, showed that all variants have approximate values with minimal differences in relation to variant 4. However, the variety Radika showed higher values of water absorption compared to the variety Treska.

Another important technological feature for the baking industry is the degree of softening the dough, expressed in Farinoghraphic units (in Fj). If the degree of softening is higher, the flour, i.e. the dough is harder to tolerate fermentation and vice versa. It is considered that the 75 Fj softening grade flour is of good quality, from 75 to 125 Fj with medium and over 125 Fj of poor quality. The lower the value, dough has better

quality. Thus, high water absorption, combined with low degree of softening indicates good quality flour, whereas a high water absorption combined with a high degree of softening indicates poor quality flour.

According the results presented on Figure 2, dough for all varieties was within the limits of 80 to 120 Fj and belongs to the dough group with medium quality. The comparison of the results of Figure 1 and 2 show that the highest value of water absorption and the lowest degree of softening were noted for the variant 1 variety Radka, which indicates good quality flour. Farinograph also enables monitoring the influence of additives, and thus allows optimization of flour processing in terms of standardization of flour quality produced from raw materials of variable quality. Flour quality is defined and classified differently in European countries depending on its end-use purpose (Dapčević et al., 2014).

The technological quality of the flour based on farinograph is evaluated in three quality groups. In quality group A belong strong flours with optimal baking ability; in quality group B belong medium flours with good baking characteristics, while in quality group C belong weak flours, i.e. with poorer quality and lower absorption power (Albrecht, 2010).

From the presented results in Table 1 it is noted that all variants belong to the quality group B_2, with the exception variant 2 variety Treska and variant 5 variety Radika, which belongs to the quality group C_1.

Table 1. Comparison of qualitative number and quality group values of flour samples.

	Qualitative number		Quality group	
Variant	**Treska**	**Radika**	**Treska**	**Radika**
1	53.7	54.4	B_2	B_2
2	43.5	48.6	C_1	B_2
3	50.2	49.6	B_2	B_2
4	52	48.8	B_2	B_2
5	49.2	43.4	B_2	C_1
6	50.6	53.3	B_2	B_2
7	48.4	52	B_2	B_2

The Brabender Extensograph is an internationally accepted standard method that is in compliance with ISO 5530-2, ICC 114/1, AACC 54-10. It is applicable for measurement of physical properties of dough subjected to mechanical handling and resting. Precisely, an extensograph provides information about dough resistance to stretching and extensibility by measuring the force to pull a hook through a cylindrically shaped piece of dough (Dapčević et al., 2014). During the measurement of resistance of dough to stretching and the distance the dough stretches before breaking is recorded in the form of diagram extensogram. The shape of extensogram curve gives an indication of results that could be expected for baking performance (Freund and Kim, 2006).

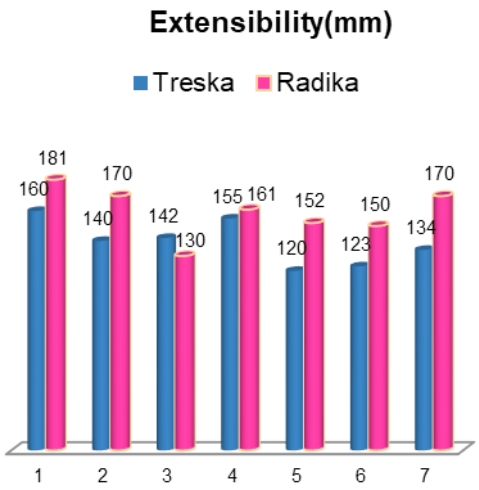

Figure 3. Comparison of extensibility (mm) values of flour samples.

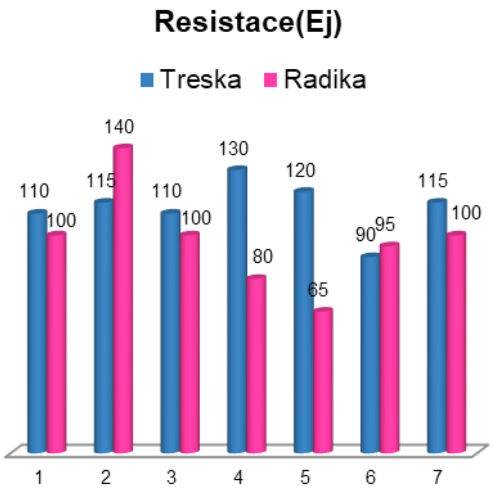

Figure 4. Comparison of resistance (Ej) values of flour samples.

As shown in Figure 3, for the variety Treska variant 1 had a higher value of dough extensibility as compared to control variant 4, while variant 2 and 3 had lower values. Significantly less extensibility showed the variants 5, 6 and 7 as compared to variant 4. Also, for variety Radika it was noted that the varieties 1, 2 and 7 have higher dough extensibility as compared to variant 4, while variant 5, 6 and 3 have lower values. Generally, the variety Radika showed higher extensibility compared to the variety Treska for all variants, with an exception of variant 3.

The resistance was expressed in extensiographic units and for good quality it is desirable a higher value of this parameter. Curves characterized by low resistance to extension indicates a small baking volume and vice versa.

The energy, the same the resistance, is desirable to be a higher value, because it is an indicator of the volume, i.e. the strength of the dough.

According the results presented in Figure 4 and Figure 5, the greatest resistance and energy for the variety Treska were measured in control variant 4, and the lowest value in variant 6. The variants 1, 2, 3, 5 and 7 showed lower values in relation to variant 4. Regarding the variety Radika, the highest resistance and energy were measured in variant 2, and the lowest value for variant 5. In all other variants, higher values were found in relation to the variant 4, with the exception of the variant 3.

The fact that wheat breeders have developed varieties with lower but more intensive protein content we would like to point out that the energy values of the extensogram cannot be interpreted as in previous decades in the evaluation of technological wheat quality (Anderssen et al., 2004; Torbica et al., 2011). On one hand, it stresses the need to increase the understanding of the parameters obtained in extensibility tests and its relevance to baking performance, and on the other hand, to relate the gliadin content and ratio of gliadin and gluten in to extensional properties of wheat dough.

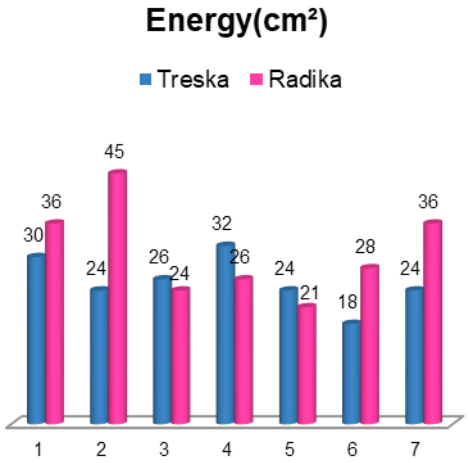

Figure 5. Comparison of Energy (cm^2) values of flour samples.

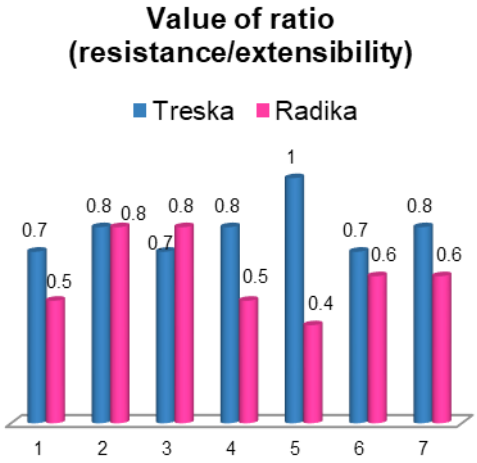

Figure 6. Comparison of resistance / extensibility values of flour samples.

The value of ratio (resistance/extensibility) represents the relationship between the resistance and the extensibility. It gives information on the behaviour of the dough during processing and the best indicates of quality the dough.

As it is shown in Figure 6, the highest value of the ratio resistance/extensibility for variety Treska was measured in variant 5. In all other variants 1, 2, 3, 6 and 7, approximately the same values were found with the control variant 4. In contrast, the variety Radika showed the highest values in variant 2 and 3, and the lowest in the variant 5. For the variants 6 and 7, higher values have been found as compared to the variant 4.

CONCLUDING REMARKS

The effect of using different types of Zn and Fe fertilizers on rheological characteristics may depend on several factors such as climatic conditions and wheat genotypes. However, although both varieties were grown under the same conditions and same agrotechnical measures were applied equally, analyses from pharynograph and extensograph generally showed that there are differences between the varieties resulting from their genetic characteristics.

From the farinograph analysis it can be concluded that:

- bio-fortification did not have a significant effect on the technological quality of the flour for variety Treska and
- bio-fortification with Fe application in soil have an effect on the technological quality of the flour for variety Radika.

From the extensographic analysis it can be concluded that:

- bio-fortification did not have a significant effect on the technological quality of the flour for variety Treska and
- bio-fortification with Fe application in soil, Fe soil + foliar and Zn foliar for variety Radika were obtained flour with higher extensibility, and energy in comparison with the variety Treska and control variant.

Based on the obtained results of the research for determining the effects of agronomic bio-fortification, it can be concluded that variety Radika can considered as a raw material of a suitable quality for baking production.

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СПОРЕДБА НА РЕОЛОШКИТЕ КАРАКТЕРИСТИКИ НА БИОФОРТИФИЦИРАНО БРАШНО ДОБИЕНО ОД МЕКА ПЧЕНИЦА СОРТА ТРЕСКА И РАДИКА

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Резиме

Реологијата на тестото е многу важна за предвидување на крајниот квалитет на пекарските производи како што се однесување при мешање, перформанси на печење и желатинизација на скроб. Врз основа на овие податоци може да се одредат својствата на производот и неговото целокупно однесување при готвење.

Целта на ова истражување беше да се испита влијанието на агрономската биофортификација врз реолошките својства на тестото. Во ова истражување се вклучени 7 варијанти добиени со додавање на висококвалитетни хелатни ѓубрива во различни фази од растот на пченицата: Fe почвено (1), Fe почвено + фолијарно (2), Fe фолијарно (3), Контрола (4), Zn почвено(5), Zn почвено + фолијарно (6) и Zn фолијарно (7). Од анализите добиени од фариноrрафот за апсорпција на вода може да заклучиме дека сите варијанти за сортите треска и радика имаат приближни вредности со минимални разлики во споредба со варијанта 4. Според добиените податоци за степенот на омекнување, се констатира дека сите варијанти за двете сорти на пченица се со среден квалитет. Според квалитативниот број, сите варијанти од двете сорти припаѓаат на квалитетна група $Б_2$, со исклучок на варијанта 2 за сортата треска и варијанта 5 за сортата радика која припаѓа на квалитетна група $С_1$.

Од фариноrрафските анализи може да се заклучи дека биофортификацијата нема значително влијание врз технолошкиот квалитет на брашното. Од резултатите добиени од екстензоrрамот се констатира дека за сортата радика од варијанти 1, 2 и 7 се добива брашно со поголема растегливост, отпорност и енергија во споредба со сортата треска.

Клучни зборови: *тесто, фариноrраф, екстензиоrраф.*

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PLACEMENT OF INFORMATION SYSTEM IN AGRIBUSINESS

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Abstract

Today, the need for timely information is essential, especially for business entities working in the field of agribusiness, which are on a constant race on the world market to prove their own quality and services they offer.

Therefore, the organizational ownership of agribusiness is very important, because with good ownership and teamwork, it is possible to obtain quality use of collected information and data for the benefit of the company.

What is missing in terms of mode of acceptance, direction, and classification of information in agribusiness is the use and presentation of information.

In today's modern concept of agribusiness development, what is lacking in every company is to have a presenter who will be tasked with collecting all agribusiness information and data to classify the information and, as a priority, to make such information online on a daily basis the portal from the company.

The quality presentation with timely received information may have led to the successful operation of any business entity in the area of agribusiness economic market.

That is the essence of this paper, to show the general functional structure and exchange of information in agribusiness, and, as such make a more competitive economic market

Key words: *information, agribusiness, development, business entities, management*

INTRODUCTION

Management in the agribusiness in order to anticipate with a plan in the future development of the company needs to make the required amount of information in the decision-making process.

The information itself and the decision made about management has the significance of a strategic resource, especially in the domain of making strategic decisions for the enterprise.

In fact, the information received that corresponds to the basic functions of agribusiness such as: production, finance, management, human resources, create complete information about a particular area in the management of a business entity within the functional system of agribusiness. However, the strategic role of management in an agribusiness is perceived precisely by the way of providing information and making decisions that will further support the creation of goals and the realization of the benefits in terms of competitive advantage and increase of the profit on the services market.

Making decisions by the management team in a business entity is defined as the investment of personal effort to achieve the business goals implemented through the planning, organization, management and control functions.

From this it follows that planning in the decision-making process refers to determining the business goals and instruments that will be realized, the organization includes those managerial activities in the decision-making process that carries out the schedule of tasks to perform the assigned tasks.

However, what needs to be taken into account is that the procedure for obtaining information and making decisions about it is that information has its own cost, which means that the value of the information covers the costs incurred for its generation, therefore that investing in information in itself brings results that are hardly measurable in contrast to other resources.

Regarding the management function, managers in agribusinesses have the opportunity, on the basis of the received information and made decisions to use various motivating means for the employees in order to achieve the highest results in the management of the business entities,

As for the office as a segment in the information retrieval and decision-making process, it also plays an important role especially in the monitoring of management in order to discover and resolve the gaps faster and faster in carrying out the given tasks from the activity of the business entity in agribusiness.

In fact, the topic of this paper is to give a general conceptual understanding of the placement and exchange of information of business entities in agribusiness as a condition for their successful development.

Concept of decision making and exchange of information in agribusiness

Management, in order to anticipate with the plan for the future development of the business entity in the agribusiness, should make the necessary amount of information in the decision making process. The information itself and the decision made about the management have the significance of a strategic resource, especially in the domain of strategic decision making for the development of agribusiness.

The received information that corresponds with the basic functions of the enterprise as they are; marketing, production, finance, management, human resources, generate complete information about a particular area in the management of a business entity.

Otherwise, if the reception of information is generated only from one specific area of the business entity then that information will only affect the decision-making process of the manager only in that area. On that basis, decision making is a special dimension of management, implemented in all stages of decision making carried out by management at all levels of decision making. So the decision process itself is shown in Figure 1, and it is characterized by several stages as it is;

- determining the time for decision making by the management to resolve a particular problem,
- finding alternative solutions by management to solve a particular problem,
- choice of the most adequate solution and its reception,
- evaluation of the results of the decision.

Figure 1. decision making process in the business entities of agribusiness.

However, the strategic role of management is perceived precisely by the way of providing information and making decisions which will further support the creation of goals and realize the benefits in terms of competitive advantage and increase the profit in the services market. (Dr. M. Sekulovska, "Management Information Systems", Ohrid 2003. p.45).

On the contrary, according to traditional, classical theories of decision making by the management team in a business entity, it is defined as the investment of personal labour to achieve business goals implemented through the functions of planning, organizing, managing and controlling.

From this it follows that planning in the decision-making process refers to the determination of the business goals and instruments that will be realized, the organization covers those managerial activities in the decision-making process, which are used for scheduling task execution tasks.

Regarding the management function, managers have the opportunity based on the received information and made decisions to use various motivating means for the employees in order to achieve the highest results in the management of the business entities. As for the office as a segment in the process of obtaining information and decision-making, it also plays an important role as all the previously listed phases, especially in the monitoring of management in order to be quickly and better detected and resolved the blanks that would gloss over the performance of the given tasks.

However, what needs to be taken into account is that the procedure for obtaining information and making decisions about it is that information has its own cost, which means that the value of the information covers the costs incurred for its generation, therefore that investing in information in itself brings results that are hardly measurable in contrast to other resources.

Trends in management of information in management

Regarding the trends in information management, it can be said that in today's conditions the business environment has changed as a result of the present world trends such as; the globalization of the economy, information economies, and the transformation of business businesses. The changed business environment of the business entities imposes a number of challenges in terms of managing or managing the information in the decision-making process that relates to the application of information technology and the implementation of information systems in the function of managers.

On this basis, information systems provide basic organizational solutions to the challenges and problems imposed by the modern business environment. From here, it emerges that information and telecommunication technology overnight develops and with their performance causes an explosion of information, in which inevitably any modern enterprise should be involved in order to provide information for it. (Blagoja Georgievski, PhD "Information Management", Skopje2003). As for the competition on the world markets, it is becoming sharper with the emergence of the global economy.

Therefore, in order for business entities to bear this phenomenon, they need to be more efficient and more flexible as market participants, and thus to implement more powerful information and communication systems, as soon as possible they will come to the timing and the necessary information. Otherwise, in the process of obtaining information, the moment of speed that needs to be processed and to get the information itself, which is of primary importance, to carry accurate and timely and optimal decisions in a very short period of time. (Blagoja Georgievski, PhD "Information Management", Skopje, 2003). However, what means a trend in decision-making and decision-making is that in the modern world there is a shortening of the importance of data which means that in dynamic conditions of management, managers should respond with the same dynamic and skilful management, how to get the most out of your own company.

It can therefore be said that the management that follows the management trends and is guided by the application of scientific methods, models and techniques that are in the scope of business communication becomes successful and strives to maintain that level and achieve even greater success .

In addition to the stated challenges that are determining for building and introducing new data management technologies, other important factors are present in today's business systems as a consequence of the already mentioned trends in the global systems for delivering new frequencies and services, especially in the area of agribusiness. Thus, effectively organized management provides a greater quantum of information in unit time, that is, the same amount of information with lower costs.

This way of managing information contributes to making optimal decisions and better control over the implementation phase. In addition to such trends in the management and use of information, today the business entities use the computer, the Internet and telecommunications, to a large extent as a technical tool, because only by their complete application is fast transmission and access to data and information, fast updating and processing data and information and quick exchange of information, regardless of the geographical position of the business entities.

Classification and ways of managing information in business entities from agribusiness

Basic methods through which a business entity is successfully implemented is the correct management of the information system and their proper targeting. In order for a business entity to function properly the information it has collected for a certain issue in the operation of certain segments of the organizational set-up of the company, leads to the formation and classification of certain information systems, which, depending on the problem that is the subject of resolution, will provide specific data , information or results for their further resolution or analysis, as this would further contribute to the successful positioning of the business entity on the market.

Since for any business function an appropriate information system can be created, the logistic information system is considered as basic and essential information systems for the functioning of a business entity.

Logistics information systems in agribusiness

What is responsible for the question of the need for a separate logistics system to classify the information in parts of this type of entities, it can be said that it is closely related to the need for the existence of a shadow whole, for the logistics of the business entity in the organizational sense. However, while the existence of the need for logistics is not at all in question, depending on the size of the firm, its activity, type of production, etc., logistical activities will be treated separately from the aspect of the organizational setting and aspect of the information setup.

Because logistical information is essentially very closely related to marketing and production, logistics management will largely rely on the structure and data created for the purpose of the business entity. Otherwise, the creation of special databases for this kind of information would lead to data reduction, their inexhaustibility which would lead to high costs of the cost of this type of information.

On the basis of this the best solution, in any case, with careful analysis, information about the information necessary for the logical activities, which are not present in these subsystems, is obtained, and the already existing database structure is rewarded with new fields that would satisfy these needs. Namely, this statement is also true for the reports needed for the modification of data on logistic information.

So this solution is good anyway because marketing management and logistics management are interested in information that is based on the same or similar data, but from a completely different aspect. However, the logistic information systems corresponding to one business entity, especially if it deals with the production activity, from a functional and organizational structure are composed of the following four basic information subsystems:

- ordering information systems,
- information systems for materials,
- information systems for ready-made products,
- Information transport systems.

Characteristic in this case is that these subsystems do not function individually, they intensively communicate with one another, and, of course, this also applies to other management managements.

Thus, the main connections of these subsystems of information with the business entities in the agribusiness are presented on Figure 2 (Blagoja Georgievski, PhD, "Information Management", Skopje2003)

Figure 2. Structure of logistics information systems - interconnections, flow of information and data among business entities in agribusiness.

This way of managing the information enables easy implementation of the connection of the database and information, as well as their availability on the Internet global network, provided with an adequate level of protection of the information itself and data.

Therefore, for the management of a company that is developing within the scope of agribusiness, such ways of information management are of great importance, because such methods of placing information are more competitive than others on the market.

CONCLUDING REMARKS

When making a general overview of the placement of information systems in agribusiness it can be said that it is a concept for the exchange of information between the business entities in the segment of agribusiness and the information that emerges as a need for market demand.

The very fact that such an attitude indicates that it is necessary to pay great attention to the concept of information exchange in business entities, since the main goal of such a system, especially in the agrarian sector, is to collect information from the demand and consumption market and as such process and place them until their final conversion realization.

In today's modern concept of development of agribusiness, what is lacking in every company as mentioned above is standardized working methods, which requires a managerial person to timely place the information and data in the field of agribusiness on the Internet portal from the company.

Lack of information about a management team in one business entity leads to business failure, therefore quality information exchange and their timely acquisition can lead to success in the business of the business in the area of agribusiness.

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ПОСТАВЕНОСТА НА ИНФОРМАЦИСКИТЕ СИСТЕМИ ВО АГРОБИЗНИСОТ

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Резиме

Во денешно време потребата од навремени информации е од битно значење, особено за деловните субјекти кои функционираат во областа на агробизнисот, а кои се во постојана трка на светскиот пазар за докажување на сопствениот квалитет и услуги што ги нудат.

Затоа мошне е важна организациската поставеност на еден агробизнис, бидејќи само со добра поставеност и тимско работење може да се дојде до квалитетно употребување на собраните информации и податоци во корист на компанијата.

Она што недостасува во однос на начинот на прифаќање, насочување и класифицирање на информациите во агробизнисот е тоа што недостасуваат начините за употреба и презентација на информациите.

Во денешниот современ концепт на развој на агробизнисот она што отсуствува во секоја компанија е тоа што во денешното време на стандардизирани начини на работење е потребно да има лице-презентер кое би имало задача да ги собира сите информации и податоци од областа на агробизнисот да ги класифицира информациите и според приоритет да ги поставува секојдневно таквите информации на интернет порталот од компанијата. Затоа што квалитетната презентација со навремено добиените информации може да доведе до успешно работење на секој деловен субјект во сферата на агробизнисот на економскиот пазар. Токму и суштината на овој труд е да ја прикаже општата функционална поставеност и размена на информации во агробизнисот и како таков да го направи поконкурентен на економскиот пазар.

Клучни зборови: *информации, агробизнис, развој, деловни субјекти ,менаџмент.*

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INFLUENCE OF THE STERILIZATION PROCESS ON THE PHYSICOCHEMICAL AND NUTRITIONAL PROPERTIES OF MEAT VEGETABLE PATE

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Abstract

The aim of this research was to investigate the possibility of meat-vegetable sterilized pate production with reduced fat content and functional ingredient supplementation, as well as to evaluate the thermal effect on physicochemical and lipid content of the final products. In the study, ten experimental groups of poultry pate were produced with different level and type of fat substitution with inulin gel and lentils flour. The residual quantity of fructans was examined to evaluate the thermal effect on functional ingredients. Analysis upon changes in lipid fraction was performed in order to determine the appropriated thermal effect in different pate formula. The pate from sample 4 and 6 in which the amount of recipe fat was reduced and partially substituted with inulin gel or lentils flour had the most acceptable nutritional characteristics.

Keywords: *canned meat, inulin, lentils, fructans, thermal effect*

INTRODUCTION

The meat and meat products are highly evaluated and very preferred foods (Baltic & Boskovic, 2015), as well as concerning their sensory properties, and linking their nutritive and biological value (Pereira & Vicente, 2013; McNeill, 2014). By the other side the high level of unsaturated fatty acids and cholesterol, the high level of sodium chloride (NaCl) and nitrates contents in meat products are associated with a number of health problems as obesity, cardiovascular diseases, diabetes, cancer and etc. (Richi et al., 2015). Although these benefits and risks associated with processed meat, the consumption of meat products should not be necessarily questionable if they are consumed in moderate amounts manner as a part of balanced human daily diets (De Smet & Vossen, 2016).

Even more, the enrichment of meat products with natural functional ingredients contemporarily has widespread usage in the modern meat processing technologies with the aim to improve the health status of foods (Bhat & Bhat, 2011). The meat pâtés are widespread and they are consumed by many people. In fact, the meat pates have a fat contain about and over 30%, which has a negative effect on their health perception (Lorenzo et al., 2014; Latoch et al., 2016).

The inulin is a very promising functional additive (Roberfroid, 2004; Shoaib, 2016), which is involved in different food e.g. in meat salami as fat replacer, as a technological additive for improving water-holding and emulsifying ability, as an agent reducing product's energy value, or aiming to improve structural

properties and viscosity of the product. By the other side the beans vegetables, and more especially the lentil, is accepted as good sources of proteins, slow-release carbohydrates, dietary fibres, mineral compound and vitamins (Iqbal et al., 2006). The lentil is successfully applied as a binding agent in the preparation of beef meatballs with low-fat content (Serdaroglu et al., 2005), but we have not enough information for its application in meat pate. At more long and severe thermal process as is sterilization, meat loses its colour, taste, its structure becomes too much crumbly, a formation of jelly increases that is not wanted. The use of binders and hydrocolloids is prospective to improve product consistency and to increase the jelly capacity in canned meat as well as the increasing density of a product and decreased diffusion of nutritive substances (Pasichinyi et al., 2017).

The inulin and lentil flour are interesting polyfunctional additives for the production of meat products in accordance with their structural and nutritive potential. However, there are no studies on their use as potential fat substitutes in sterilized canned foods. Therefore, the aim of the present study is to evaluate the changes in physicochemical, lipid and levels of oligofructoses in finished products, depending on the obtained sterilization effects in different samples of sterilized meat pâtés with reduced fat content and addition of inulin gel and flour of lentils.

MATERIAL AND METHODS

Materials

For the production of meat-vegetable pate it have been applied the following recipe for pate from poultry meat: turkey meat – 300 g/kg -1 as filling mass; poultry liver– 100 g kg-1; egg mélange – 150 g/kg-1; pork fat -250 g/kg-1; corn flour 20 g/kg -1; salt – 15 g/kg-1; sodium nitrite – 0,05 ; polyphosphate – 2 g/kg-1; black pepper -3 g/kg-1; nutmeg - 0,5 g/kg-1 ; coriander -1,5 g/kg-1 and drinking-water - 150 g/kg-1. Ten (10) samples with a different concentration of fat, inulin and lentil flour are developed according to Table 1.The added inulin Orafti®HPX was provided by the company „Artemis" Ltd - Sofia, representative of Beneo-Orafti Ltd., Belgium. The inulin was added on as gel, obtained by hydration in a ratio 1:4 (w/v) (Latochet al., 2016). Thus obtained suspension was heated to the 85°C until complete dissolution and then cooled to 50°C. Both functional additives were added to the filler mass during cutting.

Table 1. Samples of poultry meat pate with inulin and lentil flour.

Sample	Pork fat, g·kg $^{-1}$	Inulin gel, g·kg $^{-1}$	Lentil flour, g·kg $^{-1}$
1	0,25	0	0
2	0	0,25	0
3	0	0	0,25
4	0,125	0,125	0
5	0,125	0	0,125
6	0	0,125	0,125
7	0,0835	0,0835	0,0835
8	0,167	0,042	0,042
9	0,042	0,167	0,042
10	0,042	0,042	0,167

The samples were prepared in the meat cutter machine (model Fimar CL/5), adding the water during the cutting in quantity until 15% concerning the weight of meat mass.

The prepared filling mass was manually filled in the cans of 200 g and then sterilized in the following regime: $\frac{10-45-20}{121°C}, 2x10^5\,Pa$ at the laboratory autoclave (Hydroplast, Khaskovo). To determine the sterilization regimes of the cans, the F-value was determined by using the company's Ellab (Denmark) report device. The thermograms of the regime were set and the actual sterilization effects ($F^{10}_{121°C}$) were determined.

Methods

Prepared meat-vegetable pates were analysed for the following physicochemical parameters: pH value, TBA, fatty acid composition and residual fructan (inulin) [%].

The pH value of the pate mass was recorded immediately after preparation, as well as after sterilization of the meat-vegetable pates. The determination was carried out using a pH meter of MS 2004 (Microsyst, Bulgaria) on a pre-prepared aqueous extract of the sample at a sample/water ratio of 1: 9 (w/v). Prior to the measurement, the extract was filtered through filter paper. The remaining analyses were carried out after sterilization of the preserves, on the 4-th day of their storage. For the determination of TBA, the method described by Sörensen and Jörgensen (1996) was used. The fatty acid composition of the extracted lipids from the sample pates (Bligh & Dyer, 1959) was obtained by gas chromatography after pre-esterification of the higher fatty acids with methanol in order to obtain more readily volatizing methyl esters of fatty acids (ISO 5508). For determination of residual fructans (inulin) in the samples, a spectrophotometric method based on the qualitative reaction of Selivanov to prove ketoses was used (Petkova et al., 2014).

The Statistical Data Analysis was performed by the software program Statgraphics 16. The trials were performed in triplicate, the data in the tables and graphs being arithmetic mean ± standard deviation (SD). Statistically significant differences between the mean values were found with a probability of less than 0.05.

RESULTS AND DISCUSSION

Determination of the actual sterilization effects of meat-vegetable pates

Table 2 sets out the determined actual sterilization effects (F_{121}^{10}) of the different samples in the selected sterilization regime of the meat-vegetable pate and the sterilization effect required for the test microorganism Cl. sporogenes - 25, according to the pH value and volume of the package.

The obtained results show that in all meat-vegetable pate samples, achieved sterilization value is higher than the required sterilization effect, which ensures the production of practically sterile and safe for human health cans. The actual sterilization effects obtained (Tab. 2) are higher in samples with a higher addition of inulin, which is a direct result of the consistency of the pate mass. In these samples, the heating process under the same sterilization regime was much faster than that observed at lentils samples.

Table 2. Actual sterilization effects of meat-vegetable pates.

Sample	3	4	5	6	8	9	10
(F_{121}^{10})	24,83min	51,43min	31,88min	26,91min	31,07min	26,46min	33,35min
Necessary lethality for sterilization of cans with the volume of the package 200 cm^3 and pH 6,1 – 6,4						F_0 = 17,4 min	
Necessary lethality for sterilization of cans with the volume of the package 200 cm^3 and pH above 6,4						F_0 = 20,3 min	

In Figure 1 are shown curves of heat treatment (thermograms) during the sterilization of seven selected meat-vegetable pate samples at the selected sterilization mode. The intensity of heat treatment has not only a decisive impact on the inactivation of bacteria, but also on physicochemical properties and subsequently on organoleptic quality of the final product. In some of the samples has been seen a considerable deteriorate in colour and consistency after heat treatment (data not shown). However proper prevention measurement must be taken to avoid undesirable protein denaturation or fat oxidation and fat and jelly release due to emulsion destabilization from excessive heat application.

Changes in physicochemical characteristics of meat-vegetable cans

One of the most important factors on which the process of sterilization depends is the active acidity of the food products (Gavin et al., 1995). By comparing the samples, it was found that the used inulin and lentil flour had an effect on the hydrogen ion concentration of the raw pate mass and on the sterilized meat-vegetable pate (Tab. 3). There was a trend for increase of pH of the sample's raw meat masses in which the degree of fat replacement with inulin had been increased. The established difference between meat-vegetable pate samples can be explained by the different amount of inulin addition and its pH. According to the technical specification, the inulin has a pH value of 6.40, which leads to an increase in the pH value of the inulin added samples. An increase in pH value due to the addition of inulin was also found by Méndez-Zamora et al. (2015) in the production of frankfurter sausages. Contrary to what has been stated for inulin, higher concentrations of the lentils flour result in a decrease in the pH of the pate masses.

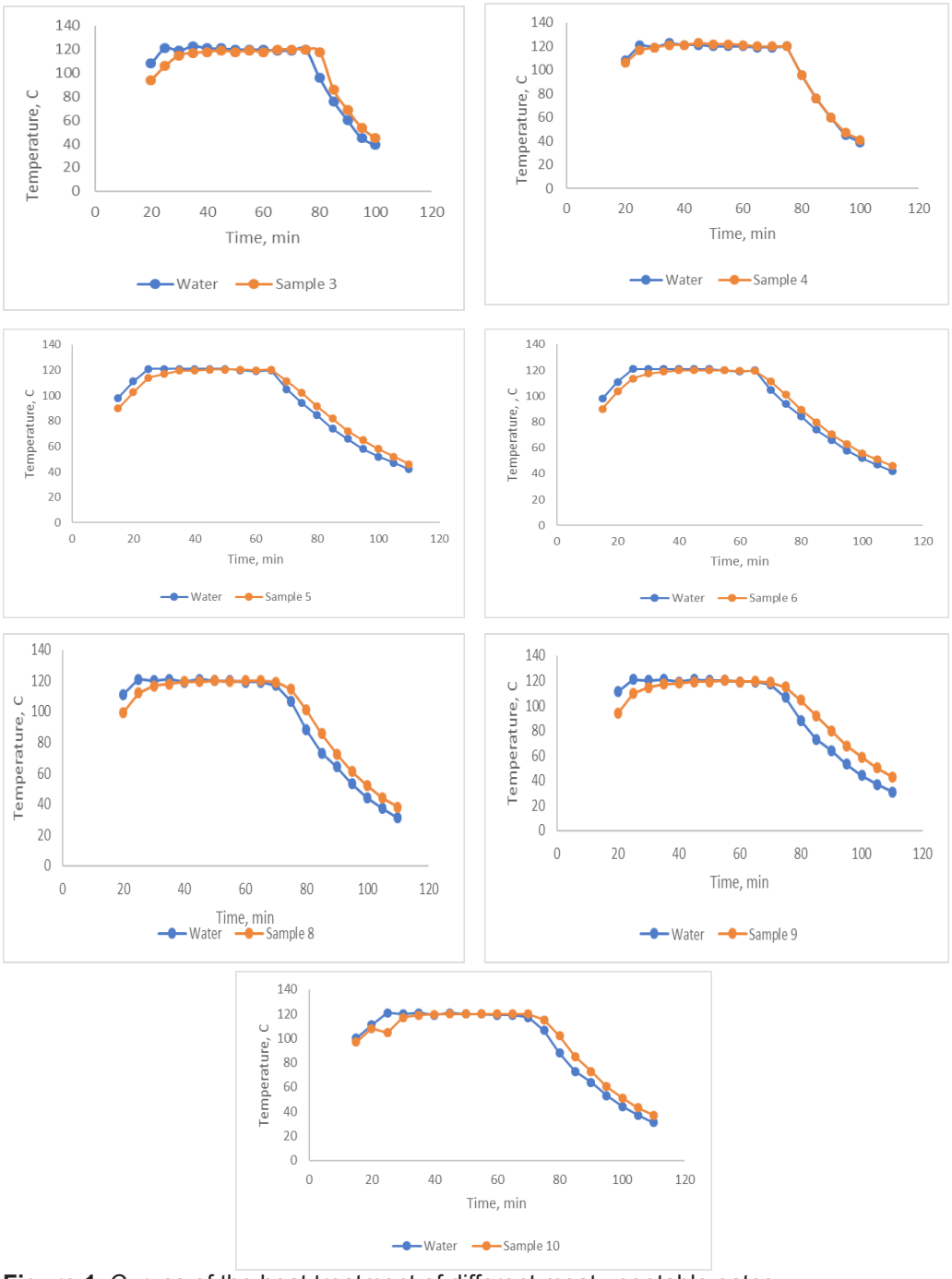

Figure 1. Curves of the heat treatment of different meat-vegetable pates.

After sterilization, the pH of the meat-vegetable pates was reduced. Again at the inulin added samples were measured significantly higher pH values than the other samples (P <0.05). Lower values of hydrogen ion concentration after sterilization can be explained by changes in total charge (Rao et al., 1996), as a result of both denaturation changes in protein molecules as well as thermal induced carbohydrate degradation processes at temperatures above 100°C. In the heat treatment, there are conditions in which the hydrogen bonds are mainly disrupted, which can be explained mainly by the greater difference in pH values of the raw pate masses and of the preservatives after sterilization.

Table 3. Physicochemical characteristics and fructan content (inulin) in the meat-vegetable pate.

Sample	Indicator			
	pH of the meat filling mass	pH of the can	Fructans* g/100gproduct	TBA, mg MDA.kg^{-1}
1	6,83±0,01^{g}	6,39±0,025b^{c}	0±0^{a}	0,94±0,11^{ab}
2	6,81±0,01^{g}	6,48±0,03^{c}	1,54±0,07^{c}	0,83±0,01^{a}
3	6,56±0,03^{c}	6,31±0,02^{ab}	0,21±0,02^{a}	1,11±0,02^{bc}
4	6,76±0,005^{f}	6,47±0,05^{c}	2,98±0,51^{d}	0,96±0,05^{ab}
5	6,61±0,01^{d}	6,46±0,09^{c}	0,17±0,01^{a}	1,25±0,08^{c}
6	6,44±0,04^{a}	6,29±0,02^{a}	1,36±0,01^{c}	1,51±0,14^{d}
7	6,55±0,02^{c}	6,33±0,02^{ab}	0,81±0,15^{b}	1,6±0,08^{d}
8	6,72±0,01^{e}	6,36±0,05^{ab}	0,62±0,05^{b}	1,89±0,05^{e}
9	6,71±0,01^{e}	6,3±0,12^{a}	1,59±0,13^{c}	2,94±0,22^{g}
10	6,52±0,05^{b}	6,27±0,04^{a}	0,67±0,03^{b}	2,17±0,2^{f}

* Fructans, including inulin, fructooligosaccharides, sucrose and fructose

Note: The values given are the arithmetic mean of the relevant sample of three measurements for the given indicator.

a-g - values on columns with the same alphabetic characters are statistically indistinguishable (P> 0.05).

Determination of fructans (inulin)

Comparing the fructan content in the meat-vegetable pates after the sterilization regime, large statistically significant differences (P <0.05) were found between the individual samples (Tab. 3). These differences cannot be explained alone by the different amounts of added fructo-oligosaccharides (inulin), due to the initial formulation of the samples. According to Bohm et al. (2005) and Wang et al. (2009), the content of fructans (inulin) decreases with increasing temperature. We also experienced loss of inulin versus input quantity after heat treatment. Obviously, there was also an additional factor influencing the changes in this indicator. Most likely, this was the different fat content as well as the different consistency of the tested samples and hence the different heating curves under the same sterilization regime and the achieved F-value effects in them.

This is confirmed by almost statistically indistinguishable results (P>0.05) for the number of fructans in samples 6 and 9, which is a consequence of both the amount of inulin used in these two samples and their close actual sterilizing effects. For the same reasons, between samples 8 and 10 there are also no significant differences in the final amount of fructans. In samples with the addition of a significant amount of lentils flour with or without less inulin gel (samples 3 and 5), minimal amounts of fructans were reported. At sample 4, the largest statistically distinct amount of residual fructans in the finished meat-vegetable pates (P<0.05) was observed. In this sample, although the highest actual sterilization effect was recorded, the amount of added pork fat can prevent the thermal destruction of the incorporated fructooligosaccharide in the form of an inulin gel.

Determination of the lipolytic and oxidative changes of the lipid fraction of the samples

It has been found that the amount of the lipid oxidation byproducts determined by TBA and expressed as mg MDA kg-1 of pate ranges from 2.94 mg MDA kg-1 in sample 9 to 0.83 mg MDA kg-1 for sample 2, where there were full substitution of fat with inulin (Tab. 3). High TBA values for samples 6, 7, 8, 9 and 10 are most likely due to inappropriate ratios in these samples between lentils flour, inulin gel and fat, necessary to form a stable meat emulsion. This is also confirmed by data on the emulsion stability of the samples of pates (data not shown here).

Table 4. Fatty acids content of meat-vegetable pates.

Fatty acids	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
C4:0 Buteric /Buanoic acid	ND	ND	0.08±0.01^{a}	0.09±0.01^{b}	0.05±0.01^{ab}	0.07±0.01^{a}	0.04±0.01^{a}	0.08±0.01^{a}	0.06±0.01^{a}	0.08±0.01^{a}
C6:0 (Caproic acid)	ND	0.07±0.01^{a}	0.05±0.01^{a}	0.14±0.01^{d}	0.11±0.01^{c}	0.15±0.01^{ab}	0.11±0.01^{a}	0.09±0.01^{a}	0.14±0.01^{a}	0.15±0.01^{ab}
C8:0 (Caprylic acid)	ND	0.05±0.01^{a}	0.04±0.01^{a}	0.06±0.01^{a}	0.04±0.01^{ab}	0.2±0.01^{b}	0.05±0.01^{a}	0.04±0.01^{ab}	0.07±0.01^{a}	0.08±0.01^{a}
C10:0 (Capric acid)	ND	0.07±0.01^{a}	0.07±0.01^{a}	0.15±0.01^{d}	0.03±0.01^{a}	0.08±0.01^{a}	0.03±0.01^{a}	0.14±0.01^{abc}	0.04±0.01^{a}	0.06±0.01^{a}
C11:0 Undecylic acid)	ND	ND	ND		0.09±0.01^{bc}	ND	ND	ND	ND	ND
C12:0 (Lauric acid)	0.91±0.01^{e}	1.03±0.01^{e}	0.95±0.01^{e}	1.09±0.01^{h}	0.96±0.01^{f}	0.98±0.01^{e}	0.94±0.01^{e}	0.16±0.01^{abc}	3.9±5.28^{b}	0.82±0.01^{d}
C13:0 (Tridecylic acid)	ND	0.03±0.01^{a}	ND	ND	0.02±0.01^{a}	ND	ND	ND	ND	ND
C14:0 (Mieistic acid)	ND	0.06±0.01^{a}	ND	0.07±0.01^{a}	0.03±0.01^{a}	ND	ND	0.09±0.01^{a}	ND	ND
C14:1 (Myristoleanic)	ND	ND	ND	0.11±0.01^{c}	0.06±0.01^{ab}c	ND	ND	ND	ND	ND
C16:0 (Palmitic acid)	25.06±0.01^{k}	25.7±0.1^{l}	25.56±0.01^{i}	25.73±0.1^{l}	26.04±0.01^{k}	25.28±0.01^{j}	25.43±0.1^{k}	26.2±0.01^{h}	24.4±0.1^{e}	24.46±0.01^{i}
C16:1 (Palmetoelic acid)	1.97±0.01^{h}	1.87±0.01^{j}	2.1±0.1^{f}	1.98±0.01^{i}	2.02±0.01^{h}	1.84±0.01^{g}	1.85±0.1^{h}	2.02±0.01^{h}	ND	2.02±0.01^{h}
C 17:0 (Heptadecanoic acid) /Margaric/	0.07±0.01^{a}	0.03±0.01^{a}	0.1±0.1^{ab}	ND	ND	ND	ND	ND	ND	ND
C17:1 (Margarinoleic acid)	0.34±0.01^{b}	0.41±0.01^{c}		0.48±0.01^{e}	0.32±0.01^{d}	0.41±0.01^{a}	0.43±0.1^{d}	0.4±0.01^{abc}	0.47±0.01^{a}	0.43±0.01^{bc}
C18:0 (Stearic acid)	7.96±0.01^{i}	9.95±0.01^{h}	9.69±0.01^{g}	10.96±0.01^{j}	10.6±0.1^{i}	11.02±0.1^{h}	9.4±0.01^{i}	10.6±0.1^{i}	9.2±0.1^{c}	9.4±0.1^{g}
C18:1 (Oleic)	44.07±0.01^{l}	40.84±0.01^{k}	43.37±0.01^{j}	39.99±0.01^{m}	41.78±0.01^{l}	40.54±0.01^{k}	41.54±0.01^{j}	43±0.01^{i}	39.5±0.1^{f}	41.54±0.01^{j}
C18:2 (Linolic) /Lnoleic	15.65±0.01^{j}	16.81±0.01^{i}	15.75±0.01^{h}	16.96±0.01^{k}	15.77±0.01^{j}	18.8±0.01^{i}	17.65±0.01^{j}	15.77±0.01^{j}	18.5±0.1^{d}	17.65±0.01^{h}
C18:3 (α-Linolenic)	1.06±0.01^{f}	1.28±0.1^{f}	1.02±0.01^{e}	1.01±0.01^{g}	1.11±0.01^{d}	1.6±0.01^{f}	1.29±0.01^{e}	0.88±0.01^{d}	1.32±0.01^{a}	1.29±0.01^{e}
C20:0 (Arachidic acid)	0.05±0.01^{a}	ND	ND	ND	ND	ND	ND	ND	ND	ND
C20:4 (Arachidonic)	1.42±0.01^{g}	0.31±0.1^{b}	0.16±0.01^{b}	0.09±0.01^{b}	0.05±0.01a^{b}	0.6±0.01^{d}	0.67±0.01^{e}	0.05±0.01^{ab}	0.58±0.01^{a}	0.67±0.01^{cd}
C22:0 (Behenic acid)	0.7±0.01^{c}	0.75±0.1^{d}	0.47±0.01^{c}	0.51±0.01^{f}	0.6±0.1^{e}	ND	0.38±0.01^{c}	0.49±0.1^{bcd}	0.4±0.1^{a}	0.83±0.49^{d}
C24:1 (Lignoceric acid)	0.75±0.01^{d}	0.73±0.1^{d}	0.6±0.1^{d}	0.51±0.01^{f}	0.65±0.01^{e}	ND	ND	ND	ND	ND
Saturated FA (SFA)	36.87	39.19	37.77	39.99	38.35	40.03	38.28	39.82	38.28	37.5
Unsaturated FA (UFA	62.8	60.8	62.24	59.94	58.66	62.78	62.33	61.67	59.33	62.5
Mono-unsaturatedFA (MUFA)	45.52	45.55	44.44	41.01	43.03	40.54	41.92	43.54	39.9	52.37
PolyunsaturatedA(PUFA)	18.13	18.4	16.93	18.06	16.93	21	19.61	16.7	20.4	19.61
PUFA/UFA	0.29	0.5	0.45	0.45	0.44	0.52	0.51	0.42	0.53	0.52
MUFA/PUFA	2.51	2.48	2.62	2.27	2.54	1.93	2.14	2.61	1.96	2.67

Note: The values given are the arithmetic mean of the relevant sample of three measurements for the given indicator.
a-m - values in rows with the same alphabetic characters are statistically indistinguishable (P>0,05)

According to some authors (Joo et al., 1999, Sasaki et al., 2001, Estevez et al., 2003, Lorenzo & Pateiro, 2013), the higher fat content results in a higher amount of the oxidation reaction products. However, in our samples, more significant oxidation processes were observed in samples with a fuller reduction of the fat, containing a lentil flour in combination with more inulin gel. In these samples, the more easily oxidized muscle phospholipids remain more vulnerable to the oxidation processes occurring during the heat treatment.

The analysis of the fatty acid composition of the pate samples revealed significant differences between the individual samples (Tab. 4). The different patterns of the three-component mixture used for the individual samples differ from one another in the presence, respectively, of inulin, animal fat, and lentils, as well as their concentration. The differences in the fatty acid composition of the individual pate samples are mainly due to the type and quantity of these ingredients and to a lesser extent to the sterilization effect achieved. The difference in the percentage of unsaturated fatty acids (PUFA) was obviously. The highest content of PUFA was marked at the samples 3, 6, 7, 10 and control sample 1, and the lowest percentage - at sample 5. A widely used indicator characterizing the healthy profile of lipid fraction in food is the PUFA/SFA ratio (Decker&Park, 2010; Hathwar et al., 2012; Wood et al., 2003). PUFA/SFA ratio values ≥ 0.40 are considered useful for human health (COMA, 1994). The highest value - 0.53, was recorded in sample 9, followed by 0.52 in samples 10 and 6 and 0.51 in sample 7. By comparing the fatty acid composition of all samples tested for essential fatty acids such as linoleic and linolenic, it should be noted that these two fatty acids were at the highest levels in sample 6, followed by samples 7,10 > 2 > 4. For each of these samples, the established amount of these two polyunsaturated fatty acids was higher than that obtained for the control sample 1. This can be considered as an additional nutritional advantage of these fortified with fibres and reduced fat content canned foods.

CONCLUDING REMARKS

By selected sterilization regime, a sterilization value was achieved in all samples of meat-vegetable pates which was higher than the sterilization effect required to ensure industrial sterility. In the all tested samples of pates, the fructan (inulin) content decreases as the highest amount of fructans (inulin) are recorded in sample 4, followed by sample 2 and 6. These amounts are acceptable in terms of the desired potential functional prebiotic effect and reported by Bodner and Sieg (2009) limiting amount of fructan (inulin) in meat products - 4 g / 100g. The results allow to conclude that the mode of sterilization, even if there were changes, preserves the positive effect of the incorporated functional ingredients on the nutritional, technological and functional characteristics of the tested meat-vegetable cans.

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ВЛИЈАНИЕ НА ПРОЦЕСОТ НА СТЕРИЛИЗАЦИЈА НА ФИЗИЧКО-ХЕМИСКИТЕ И НУТРИТИВНИТЕ КАРАКТЕРИСТИКИ НА ПАШТЕТА ОД МЕСО И ЗЕЛЕНЧУК

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Резиме

Целта на ова истражување беше да се испита можноста за производство на стерилизирана паштета од месо и зеленчук со намалена содржина на маснотии и дополнување на функционалните состојки, како и да се оцени термичкиот ефект на физичко-хемиските и липидните содржини на крајните производи. Во студијата беа произведени десет експериментални групи на паштети од живинско месо со различно ниво и тип на супституција на масти, додаток на инулин и леќа во прав. Преостанатата количина на фруктани се испитуваше за да се оцени термичкиот ефект врз функционалните состојки. Анализата по промените во липидната фракција беше изведена со цел да се одреди присвоениот термички ефект кај различни содржини на паштета. Паштетата од примероците 4 и 6, во која количината на маснотијата е намалена и делумно супституирана со инулин или леќа, имала најприфатливи нутритивни карактеристики.

Клучни зборови: *конзервирано месо, паштета, инулин, леќа, ефект на стерилизација.*

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POTENTIAL AND POSSIBILITIES OF REBO – NEW GRAPEVINE VARIETY (*Vitis vinifera* L.) IN GROWING CONDITIONS IN REPUBLIC OF NORTH MACEDONIA

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Abstract

Rebo (Rigotti 107-3) is a vine variety that originates from Trentino, North Italy. It was selected by the researcher Rebo Rigotti by crossing the varieties Merlot x Terlodego at the agriculture and forest experimental station in S. Michele all' Adige. According to the literature the idea of the breeder was to produce new variety that would be similar to Merlot but more resistant to cold climate conditions. Our idea to plant Rebo in Macedonia was to grow this variety on sites with higher altitude locations that are difficult for growing Merlot. The vineyard for this study is located in the north part of Macedonia on the mountain German near the town of Kriva Palanka, area typical for growing frost resistant varieties. In this research paper we analyzed the mechanical structure of the grape cluster and berries, the quantity of sugar and titratable acids in the grapes. In the wine we analyzed the quantity of total phenols, total anthocyanins, wine colour (CI;H), total flavan-3-ols and standard chemical analysis of the wine.

Key words: *Rebo, Rigotti 107-3, cold resistance*

INTRODUCTION

Republic of North Macedonia has centuries of tradition of growing vines and producing high quality wines. Located in the south part of Balkan peninsula, it's a country with very good viticultural conditions, dry and warm summers and not so cold winters, which are ideal conditions for the production of high quality red wines. As a result of the clime conditions the produced wines in general are characterized as high colored, full body, structured, well balanced wines with aromas of ripened and black fruit. The most planted is the local variety Vranec, but from the red varieties, Merlot, Cabernet Sauvignon, Cabernet franc, Syrah and Pinot noir are also grown.

The quality of a wine can be expressed as a balanced set of multiple components found in it. The phenolics substances are just one group that participated in the quality of the wine. The greater the quantity of this component is the more complex wine can be produced. There are numerous factors that influence the production of this components in the grape berry: grape variety, climatic and soil condition, agricultural practice (Stockham, K. et all., 2013; Maria G. et all., 2015).

In some parts of the country there are high altitude terrains for viticulture with not ideal winter temperatures. Because Merlot is one of the varieties that are demanded on the wine market the idea was to search for a variety that is similar to Merlot but is more frost tolerant. Rebo is grape variety created by the researcher Rebo Rigotti by crossing the varieties Merlot x Terlodego in S. Michele all' Adige. This grape variety is cultivated in Trentino northern Italy mainly in San Michele all'Adige, Volano, Calavino, Cavedine and Padergnone. In itali in 2000 there were 39ha according to agricultural census. (Ian D'Agata 2014; Jancis R. et all 2012). The aim of the research was to investigate the quality of the grapes and wine from Rebo, Italian variety that is related to Merlot and is more resistant to low winter temperatures grown in clime conditions

of N. Macedonia. By analytical methods and degustation tasting to determine if this variety can provide grapes with good yield and quality grapes with expected parameters for the production of high quality wine. To investigate the potential and possibilities of Rebo we analysed the mechanical composition of the harvested grapes and chemical composition of the produced wine (alcohol, extract, titratable acids, pH, free and total SO_2, total polyphenols, total anthocyanidins, total flavan-3-ol, Hue and wine intensity and colour).

MATERIALS AND METHODS

Field research

The vineyard is located in the north part of the country near the town Kriva Palanka in the mountain German. It is a young vineyard, the vines are four years old and it was its second harvest. The distance between the plants in the vineyard is 3.2,m x 1,2m. The pruning system is double guyot, leaving two spurs with 2 buds and two cane with 6-8 buds, which is 16 to 20 buds per grape vine in total. Standard agricultural practise was performed in the vineyard.

Mechanical composition of the grapes

The grape was harvested in September and has relatively high amount of sugar 24,5 Brix. The yield and the mechanical analysis of the grape clusters and the grape berries were performed in the experimental laboratory in the Institute of Agriculture, using standard methods (Avramov, 1991).

Figure 1. Left- grape cluster of Rebo; Right – young vine of Rebo with visible grape clusters.

Vinification

Processing of grape was performed in the microvinification cellar, according to the standard procedure for red wines production. The grapes were hand-picked in plastic cases from 13 kg at technological maturity, at ripeness of 24.5 Brix. The grapes were immediately destemmed and crushed on a small electric crusher and 30mg/l SO2 was added in order to prevent oxidation and to obtain microbiological protection. Lallemand EXV enzyme for maceration was added during the processing of the grapes and yeast culture Lalvin D254 with dosage of 25g/hl was used for the alcoholic fermentation. The grape mash was fermented in 225liters capacity stainless steel tanks. The temperature regime during the AF was 24±3°C. Yeast nutrient, Fermaid E (recommended dosage of 25 g/hL) was added during fermentation.

Spectrophotometric analyses of grape berries and wine

The analysed wine samples were performed by direct measurements or using appropriate dilution of wine in distilled water if it's necessary.

Total phenols content were assessed by the reduction method with Folin-Ciocalteu reagent. The results obtained are expressed as mg/L gallic acid equivalent (GAE/L).

Total flavan-3-ols were determined using DMCA (p-dimethylaminocinnamaldehyde) method. Quantity of 0.1 mL of the wine sample was transfered in a 10 mL flask, than few drops of glycerol and 5 mL DMCA solution were added and made up to the mark with methanol. The absorbance was measured at 640 nm against methanol as blank. The obtained results are expressed as mg/l catechine hidrate.

Determination of total anthocyanins in wine. Wine dilution (1:100) was added in 10 ml flask and made up to volume with a solution of ethanol: water: hydrochloric acid (70:30:1). The absorbance measured at 536 to 540 nm against ethanol chloride as blank was used for calculation the concentration of total anthocyanins (TA) in wines using the equation:

TA_{540nm} (mg/L) = A_{540nm}16.7d,
where "A_{540nm}" is the absorbance at 540 nm and "d" is the dilution factor
Wine intensity, Hue and colour were analysed according to Ivanova V. (2013).
Degustation rating of the wine was performed by UC Davis 20 point system.

RESULTS AND DISCUSSION

The results for the quantity of the harvested grape per vine are given in Table 1. From it we can see that that the yield of this variety is similar compared to Merlot. The average yield produced of Rebo is 2700 g/vine and the average cluster weight is 194.3g. From the measurements of cluster weight and weight of 100 berries in table 1 we can establish that Rebo has small grape clusters and small berry size, similar to Merlot. One of the most important parameters for the quality of the grapes and wine are the content of sugar and titratable acids and their mutual ratio. From the analysis of the grapes we measured high content of total soluble solids of 24,5° Brix and high content of titratable acids 7.5g/L. The level of Malic acid was also high 1,8 g/L.

Table 1. Mechanical analysis of Rebo grapes.

Parameter	Rebo
Yield (g/vine)	2700
Cluster weight (g)	194,3
Grape stem weight (g)	5
Weight of 100 berries (g)	86
Seed number/100 berries	202
Seed weight/100 seeds (g)	3,14
pH	3,23
Titratable acids (g/L)	7,5
Malic acid (g/L)	1,8
Total souluble solids (°Brix)	24,5

The obtained results in Table 2 for the general analysis of the wines (pH, alcohol content, total acidity, volatile acidity) demonstrated that the samples are in the expected range for red wines of this type of wine. Additionally, they showed that the wine from Rebo have high alcohol level which shows that this variety is capable to produce high level of sugar and still have a good level of total acidity in the grapes and in the wine. The ratio between these two parameters makes this variety very good for production of young fresh wines and wines that can have long shelf life which can be also confirmed from the obtained high level of

total extract (33.20 g/L). The high value for this parameter also indicates that from this variety structural, full body wines can be produced. The level of volatile acidity is 0.51g/L and the free and total SO2 have good ratio which indicates that there was no problem during the alcoholic fermentation. According to the degustation rating the wine had 18 points that showed that the produced wine from Rebo have very good potential for production high quality wines. These results are similar with the results from the research performed by Sartor et al. (2017).

Table 2. General analysis of the analysed wines.

Parameter	Rebo
Sp. Gravity, 20/20	0,99
Alchool, vol%	14,32
Total extract, g/l	33,20
Titratable Acid, g/l	6,8
Volatile Acid, g/l	0,51
Free SO2, mg/l	32,00
Total SO2, mg/l	62,72
Degustation rating	18,00

The phenolic components are one of the most important components in the wine. Their production is as a response of stress conditions and their accumulation is influenced by agricultural practise, clime condition and the grape variety (Fernandez-Mar, M et all. 2012). During the wine making process they are transferred from the solid parts of the grapes to the must (Monagas et al. 2003). They are the components responsible for the organoleptic properties of the wine, astringency, bitterness, wine colour and the healthy effect. The atnhocyanine through co-pigmentation with flavonols stabilised and increased the wine colour. In our study the level of total anthocyanine was 588.24 mg/L for Rebo which indicates that the obtained wine has a very nice dark colour and that is supported with the obtained value for color intensity, 7.25. During winemaking (crushing, maceration, and fermentation), flavonoids, anthocyanins,flavonols, flavan-3-ols, and procyanidins are transferred from the solid parts of the grape (the skin, seed, and stem) to the must (Monagas et al., 2003). The experimental wines have high level of total polyphenols (2920.95 mg/L) components and total flavan-3-ols (614.66 mg/L). The high level of these components suggests that the wine produced from this grape variety is suitable for ageing.

Table 3. Analysis of phenolic components Hue CI in wine.

Parameter	Rebo
Total polyphenols (mg/L)	2920,59
Total flavan-3-ols (mg/L)	614,66
Total Anhocyanins (mg/L)	588,24
Hue	0,2397
Colour Intensity	7,25
Yellow, %	9,70
Red, %	40,45
Blue, %	49,86

CONCLUDING REMARKS

The grape variety Rebo is perspective wine variety for Republic of North Macedonia. Its higher frost resistance compared to Merlot makes it more suitable for vine growing terrains with higher altitude. From the mechanical analysis it can be seen that this variety has small grape clusters and medium level yield per vine. The variety can produce high amount of sugar and titratable acidity which makes it good variety for producing quality wines. The

wines produced from this variety are complex, with deeply ruby red color and high content of anthocyanins. The taste of the wine is berry fruited, with light herbaceous and spicy notes, full body. The young wine has slightly higher level of tannins which makes it suitable for ageing.

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ПОТЕНЦИЈАЛ И МОЖНОСТИ НА РЕБО - НОВА ВИНСКА СОРТА (*Vitis vinifera* L.) ОДГЛЕДУВАНА ВО УСЛОВИ НА РЕПУБЛИКА СЕВЕРНА МАКЕДОНИЈА

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Резиме

Ребо (риготи 107-3) е сорта на винова лоза која потекнува од Трентино, северна Италија. Селекционирана е од страна на истражувачот Ребо Риготи со вкрстување на сортите Merlot x Terlodego на експерименталната станица за земјоделство и шумарство во С. Мишел Алто Адиџе. Според литературните податоци идејата на селекционерот била да произведе нова сорта која би била слична со мерло, но поотпорна на ладни климатски услови. Нашата идеја да засадиме ребо во Македонија беше со цел оваа сорта да се одгледува на локации со повисоки надморска височина кои не се погодни за одгледување на мерло. Лозовиот насад за оваа студија е лоциран

во северниот дел на Македонија на планината Герман, во близина на градот Крива Паланка, област која е типична за одгледување на сорти отпорни на измрзнување. Во овој истражувачки труд беа анализирани механичката структура на гроздот и зрното, количеството на шеќер и вкупни киселини во грозјето. Во виното беше анализирано количеството на вкупни феноли, вкупните антоцијани, бојата на виното (CI; H), вкупни флана-3-оли и стандардна хемиска анализа на вино.

Клучни зборови: *ребо, риготи 107-3, отпорна на ладни климатски услови.*

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YIELD AND MINERAL COMPOSITION OF GRAPEVINE AS AFFECTED BY MAGNESIUM AND IRON FOLIAR NUTRITION

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Abstract

The aim of this study was to evaluate foliar fertilizers (Magni mag helat and Magni fer helat) effects on yield, quality parameters and nutrient contents of the leaves of vine cultivar Cardinal in comparison to control variant (without foliar fertilizer). Field trials has been organised according the method of random block system with three variants (including control variant I) in three repetitions. Foliar fertilizers were applied in concentration of 0.5 %, four times during the vegetation period (before and after blooming, buckshot berries and version).

Results for 3 years indicated that Mg and Fe fertilizers application affected the yield of grape and its quality. Fertilized Variant III had higher average yield with 14.87 t/ha. Foliar fertilizing with Fe has significant effect on increasing of: total cluster weight, berries weight per cluster and percentage of berries. Treated variants had a lower weight of skin and seeds, compared with the control. A substantial influence of foliar fertilizers on analysed element contents in leaves has been identified, also. The 3-years average content of macro and micro elements showed higher content of P (0.35 %), Ca (3.03 %), N (2.25 %), Mn (133.24 mg/kg) and Cu (18.18 mg/kg), at Variant II. Results of leaf tissue confirmed some antagonistic relations between analysed elements, like: Fe/P, Mg/K and Fe/Mn.

Key words: *Cardinal, berries, element, fertilization, quality*

INTRODUCTION

All plants needs an adequate supply of macro and micro-elements in order to match their normal physiological and biochemical functions. Beside basic mineral nutrients (N, P and K), some other elements (Mg, Fe, Zn, B and etc.) are considered to be essential for plant metabolic processes because they areco-factors and/or activators of many metabolic enzymes (Marschner, 1995).

Nutrient availability can be improved by soil or foliar application of a needed element. Soil fertilization is the most ancient normal fertilization practice, but foliar fertilization, which has been developed in the last 60 years, may improve nutrient uptake when compared with soil application, particularly for nutrients that can be sorbed on the soil minerals (Kannan, 2010; Tejada and Gonzales, 2004). With this type of fertilization, in a short period of time, plants are provided with a adequate amounts of nutrients. The short period of response, allows its applications periods of highest necessity (Duletić & Mijović, 2014), with a major impact on grape yield increase (Shaaban et al., 2007). According Alshaal and El-Ramady (2017), this foliar fertilizing is utilized in agriculture by spraying the foliage with dilute solutions of the desired nutrients. Therefore, foliar fertilization is generally recommended for supplying additional N, magnesium (Mg) and micronutrients as well as P, K and sulphur (S).

Magnesium (Mg) is an important macronutrient with a number of physiological functions in the plant. The importance of

magnesium in the plant is in many ways connected with photosynthesis. It is the central atom of chlorophyll and it activates enzymatic processes. Magnesium also favourably influences assimilation (Mengel and Kirkby, 2001).Visually it is seen as chlorosis of leaves, especially older ones and causes premature abscission. Chlorosis is caused either by Mg deficiency, high content of soil Ca (calcareous soils) or a combination of these factors (Gluhić et al., 2009).Foliar spraying with fertilizers containing Mgis a common practice to correct nutrient imbalances in grape, but Mg doses beyond those required for maximum yield rarely induce further improvement of product quality (Gerendás & Führs, 2013).

While Fe is the most common nutrient in soils, 90 to 99.98% of soil Fe is unavailable for plants (Barber, 1995). Iron (Fe) is an important element in crop physiology, because it is essential for the activity of many important enzymes, including cytochrome that is involved in electron transport chain, synthese of chlorophyll, maintaining the chloroplast structure and enzyme activity (Mamatha, 2007). Iron in the soil is the fourth abundant element on earth, but its amount was low or not available for the plants and microorganisms needs, due to low solubility of minerals containing iron in many places the world, especially in arid region with alkaline soils (Eskandari, 2011). Iron (Fe) deficiency induces chlorosis is a major nutritional disorder in in calcareous soils (Álvarez- Fernández et al., 2006).Iron deficiency (iron chlorosis) in fruit trees is results from impaired acquisition and use of the metal by plants, rather than from a low level of Fe in soils. Therefore, Fe fertilizers, either incorporated through soil or applied to the foliage, are used every year to control Fe deficiency (Abadía et al., 2011).

Nutrients management in vineyards requires regular assessment of leaf, petiole and/or soil nutrient content and the application of fertilizers accordingly (Martin, 2012).Thus, the aim of this experiment was to study the effects of foliar applications of fertilizers containing Mgand Fe on the yield, cluster structure and concentration of macro and micro elements in the blades of grapevines variety 'Cardinal' (Vitis viniferaL.), planted on alkaline and high carbonate soil.

MATERIAL AND METHODS

Experimental site description

Three years (2012-2014) of field examinations has been conducted in area of "Tikvesh" vine region, Republic of North Macedonia, which has agro-ecologically favourable conditions for growing table vine varieties with different period of ripening. "Tikvesh" vine region is situated in the central part of the country, characterized with warm and dry climate. The predominant soil type is rendzinic soils (Table 1), formed on recent Pliocene sediments. The vineyard where the experimental site was situated was established 25 years ago, planted with cv. Cardinal, "2-cordon" system, with row spacing of 2,80 x 1,10 m (3247 vine/ha).

Table. 1. Soil properties at experimental site.

Depth (cm)	pH/H_2O	Total %	Active lime %	Total %	Available mg/100g soil		Available mg/kg	
		$CaCO_3$	CaO	N	P_2O_5	K_2O	Mg	Fe
0-30	8.06	17.33	5.50	0,13	22.10	22.52	422,46	4,58
	moderately alkaline	high	low	medium	very high	optimal	optimal	medium
30-50	8.09	17.28	2.0	0,11	19.22	21.46	422,73	3,95
	moderately alkaline	high	low	medium	high	optimal	optimal	medium
50-80	8.10	18.53	3.50	0.10	16.05	19.65	448,66	3,27
	moderately alkaline	high	low	medium	optimal	medium	optimal	medium

Treatments and experimental design

The experiment has been conducted according the method of random block system with three variants and three repetitions. During the period of investigation, all standard agro-technical measures were conducted: cultivation (deep and shallow tillage), soil fertilization (350kg/ha NPK 8-16-24 in autumn and 100kg/ha ammonium nitrate 33 % in spring), irrigation (July- August) and plant protection (up to eight times, due to the bad weather conditions in 2014). The monitored variants were:

1. Control NPK(without foliar fertilizer) in text Variant I;
2. NPK + Magni mag helat Mg EDTA (1.5 % Mg; 24.14 % MgK_2 EDTA; 9.89 % NH_4NO_3) in text Variant II;
3. NPK + Magni fer helat Fe EDTA (3.2 % Fe; 22 % FeK EDTA; 9.18 %. NH_4Cl) in text Variant III.

The fertilizers were foliary applied in concentration of 0.5 %, four times during the growing period, at the stages of: before and after blooming, buckshot berries and version. Applications were performed on mostly dry and cloudy weather, with no precipitation, in early morning hours.

Soil analysis

pH – electrometric, carbonate content, active lime by Drouineau-Galet; total N by Kjeldahl, available forms of P_2O_5 and K_2O by AL method; mobile fraction of Mg after extraction by ammonium acetate solution and mobile fraction of Fe after extraction by DTPA.

Plant analysis- leaf samples consisting of 10 leaves per replicate were taken opposite the basal cluster, every year after harvest. Leaves were gently washed, dried at room temperature and fine grinded. Analysed elements (N, P, K, Ca, Zn, Mn and Cu) in plant tissue were determined with ISP-AES technique after its digestion (Heating Digester DK 20)with concentrated HNO_3 + H_2O_2 (Cvetković, 2002). All tests were performed each year in three replications.

Yield and grape quality

The total yield was calculated by counting of clusters number and its weight per vine. Representative random samples of 24 clusters per variant were analysed to determine: cluster weight, berries and peduncle weight per cluster and percentage of berry's per cluster. Random samples of 100 berries from each replication were analysed to determine the weight of: berries, skin, seeds and pulp. The cluster and berries properties were determined according to the CODE system issued by the International Organization of Vine and Wine (O.I.V, 2011).

Statistical data analysis was carried out with SPSS 20.0 package program.

RESULTS AND DISCUSSION

Effects of Mg and Fe fertilizers on grape yield and cluster structure

There are many factors that influence on grape quantity and quality, as many ampelo technical measures, the growth regulators and proper plant nutrition (Prabhu and Singaram, 2001).

Out of the data presented in Table 2, it can be concluded that the different types of applied fertilizers showed a positive influence even in the first year of research, compared to the control variant. The results showed that all tested variants of fertilizing had a higher yield than the variant without foliar fertilization (control), where the average yield is lowest, with 4,18 kg/vine (13.59 t/ha). The variant III had the highest average yield of 4.58 kg/per vine or 14.87 t/ha. According Bozinović (2010), fruit yields can vary from year-to-year and vineyard to vineyard depending on environmental conditions and management practices, which was confirmed with our results as well (Tab. 2). Grape yield was significantly different in the 3rd year of exemination, when the yield was significantly lower compared to the previous years. The negative influence of high precipitations should be emphasized, especially rainfalls in April (135.8 mm) in a phase of buds opening and shoots growth, and in September (124.0 mm) in the period of intensive harvest. The unfavourable weather conditions during May2014, with appearance of hail in the beginning of the growing season and outburst of Plasmopara viticola, were the main reasons for low grape yield in this growing season, which was significantly lower compared to the

previous two growing seasons (2012/2013). Despite the bad weather conditions in 2014, foliar application in variant II and III had a positive effects on grape yield in these two variants. The differences among varieties may be related to the Mg and Fe roles in chlorophyll molecules, directly and photosynthesis indirectly, which increased their ability to recover from hail damages and resistance to infections.

Table 2. Grape yield (2012-2014).

Year	2012		2013		2014		Average	
Variant	kg/vine	t/ha	kg/vine	t/ha	kg/vine	t/ha	kg/vine	t/ha
Control	4.90	15.89[a]	4.94	16.03[a]	2.72	8.83[a]	4.18	13.59[a]
NPK+ MgEDTA	5.50	17.87[a]	5.04	16.35[a]	3.03	9.84[a]	4.52	14.68[a]
NPK+ Fe EDTA	5.40	17.54[a]	5.43	17.63[a]	2.91	9.44[a]	4.58	14.87[a]

Different letters (a, b) indicate significant differences among treatments.

Our results are in correlation with those obtained by Duletić and Mijović (2014) and Zlámalová et al. (2015), who observed that foliar treatment improved grape yield and its parameters.

During Duletić and Mijović (2014) researched, authors noted that foliar fertilization has positive influence on grape yield and cluster weight (cv. Cardinal). All treated variants in his research, had a higher yield (3.38-4.5 kg/vine) and higher cluster weight (363 g) compared with the not treated variant (2.9 kg/vine and 223 g). Zatloukalová et al. (2011) reported a 3.1–6.7% increase in yields of cv. Riesling italico after 5 times repeated 5% foliar nutrition to vine with the fertilizer Epso Top (9.65% Mg, 13% S) and Epso combitop (7.8% Mg, 13% S, 4% Mn and 1% Zn).

During the research period, significant differences were noted in yield components at tasted variants (Tab. 3). Foliar fertilizers have effect on increasing the total cluster weight (CW), berries weight per cluster (BWC) and percentage of berries (PB). Cluster weight and berries weight per cluster were significantly higher in treated variants ($p \leq 0.05$). The average weights have a significantly highest values in Variants III (362.33 g) compared to the control (variant I) with 330.92 g. Additionally, no significant differences were detected in pedical weight and percent of berries per cluster, weight of 100 berries, their skin, seeds and pulp weight. Out of data presented, it can be noted that Variant II has the highest weight of 100 berries (526.77 g) and 506.21 g pulp/100 berries. Treated variants had a lower weight of skin and seeds, compared with the control, where the average weights are higher, with 14.83 g skin/100 berries and 7.17 g seeds/100 berries.

The three main berry chemistry parameters were analysed to evaluate harvest juice quality: total sugar (TSS),pH and total acid (TA). Content of total sugar was significantly higher at Variant III with 165 g/l, compared with other variants. The reason for increase is due to iron serves as activator for enzymes in growth process and assist in soluble solids synthesis in grape vine (Christenson et al. 1982).

Yogeesha (2005) noted that Fe foliar spraying has significant effect on grape yield (quantity and quality). Namely, author obtained: yield of 7.91 kg/vine or 26.32 t/ha, cluster weight of 225.76 g, cluster width of 5.05cm, cluster length of 12.60 cm, number of berry 162 and etc. According the author accumulation of total sugar, reducing sugar and non-reducing sugar increased with increasing the soil iron level with foliar sprays. The highest total (20.08%), reducing (17.90%) and non-reducing sugars (1.63%) were recorded in treatments receiving: 50 kg $FeSO_4$/ha with foliar sprays, 0.2 % $FeSO_4$ at 20 and 40 DAP, which is confirmed with our results, as well. Increasing vine growth, grape yield, mass and volume of the cluster and berry, juice and total soluble solids percentage, during the foliar fertilization, have been noted by many authors, such as Yogeesha (2005) and Nikkhah et al. (2013).

Yield components showed positive and significant correlation with yield such as cluster weight and TSS (0.946**, 0.700**) and cluster weight and TSS (0.562**, respectively).

Effects of Mg and Fe fertilizers on contents of macro and micro elements in grapevine leaves

Leaf analysis (blades or petioles) is widely recognized as the most reliable laboratory method to determine the nutritional status in grapevines. The macro and micronutrient content in leaf blade is directly influenced by the type and dose of the fertilizer and by conditions of nutrition and vegetation. The average values of leaf blades samples from the examined grapevine are given in Table 4.

Nitrogen content was in range between 2.10 and 2.39% depending on the season, but with no statistical difference at 0.05 level. Magnesium fertilizer significantly increased P content. Phosphorus is present in leaf blade in quantities ranging from the lowest 0.32 % P in the control variant and 0.35 % P in the Variant II. Content of this element, at variant III, decreased during the research, which is probably resulting of the antagonistic relations between P and Fe. This phenomenon was also noted between Mg and K content. During the whole research period, Mg spray significantly decreased the potassium content in leaf blade, with significantly lower amount of 0.71%. Potassium has a major importance for grapevine nutrition. To obtain 1 ton of grapes, it's needed 3-3.5 kg K. Therefore, the knowledge of the antagonism between the potassium and the other elements is of great importance for grape fertilizing. It acts as a biocatalyst and is in antagonistic relations with Ca, Mg and Na, as the presence of these ions in large quantities hinders the absorption of potassium (Sala and Blidariu, 2012).

Spraying grapevine plants four times per season with 0.5% of Mg increased content of Ca in leaves. During the research, variant II has the significantly higher concentration of this element, from 2.28 to 3.29 % Ca. These results are opposite of many previous researches, which are based on antagonism between these elements. In the analyses conducted by the authors, a significant reduction was shown for the uptake of calcium cations by leaves, with an increase in the intensity of plant nutrition with magnesium. This might be result to the higher concentration of $CaCO_3$ (in carbonate soils more than 80% is Ca, while 4% is Mg) and Ca in soil, optimal level of Ca in analysed vine leafs, even in control variant and process of Ca reutilization in plant (once translocate Ca into the leaves, is practically immobile). These findings corresponds to the notes reported by Wyszkowski (2001) and Herak et al. (2008). The effect of magnesium in the accumulation of other nutrients is dependent on the species, plant organ and only to a slight degree on the manner of its application (Wyszkowski, 2001). According to the Herak et al. (2008), the amount of calcium in vine leaf varies depending on the amount of physiological active lime in the soil. Due to the unfavourable physical and chemical properties of carbonatesoils and the important role of magnesium in the chlorophyll, foliar application with magnesium has a great advantage over the root (soil) application (Takacs et al. 2007).

Presented results showed that foliar treatments had no influence on vine Zn status, because during the whole period of investigations, control variant has the higher content of this microelement, with average content of 35.85 mg/kg. During the research period, Mn concentration was higher at variant II with average content of 133.24 mg/kg. Our research data, confirmed antagonism between iron and manganese. Application of Fe chelate, during 2013 and 2014, decreased the content of Mn, but without statistical difference at 0.05 level. Results in Table 4, indicates that leaf content of Cu was not affected in vines treated with foliar fertilizers. The differences between treated variants and control are minimal and not significantly. Content of copper was statistically higher in 2014, from 33.28 to 35.07 mg/kg, as a result of copper fungicides application, due to bad weather conditions in that year.

Our results for macro and micro elements content are consistent with the ranges which are suggested by Mills and Jones (1996) and Reuter and Robinson (1997). Mills and Jones (1996) suggested critical leaf values for optimum grapevine growing as follows: 1.6-2.8 % for N, 0.2-0.6 % for P, 1.5-5.0 % for K, 0.4-2.5 % for Ca, 10-100 mg/kg for Zn, 40-600 mg/kg for Mn and 4-20 mg/kg Cu. As a long term of research, the authors Reuter and Robinson (1997) gives range values for the content of macro and micro elements in the grape leaf. According to the authors, grapevine has optimal levels by: 0,8-1,1 % N, 0,25-0,5 % P, 1,8-3,0 % K, 1,2-2,5 % Ca, >0,4

% Mg, 30-60 mg/kgMn, >26 mg/kgZn, 6-11 mg/kgCu, >30 mg/kgFe, 35-70 mg/kgB.

The multiple correlation analysis of the content elements determined in grapevine leaves led to the identification of some significant correlations: Ca/Cu (r=+.519**), Ca/Zn (r=-.591**), Mn/Cu (r=-.755**). Other elements are weakly correlated or not at all.

Table 3. *Cluster structure and content of harvest juice (2012-2014).*

Variant	Parameter										
	Cluster weight (g)	Pedical weight/cluster (g)	Berries weight/cluster (g)	Percent of berries/cluster	Weight of 100 berries (g)	Skin weight/100 berries (g)	Seeds weight/100 berries (g)	Pulp weight/100 berries (g)	Total sugar (g/l)	Total acid (g/l)	pH
I	344^{b}	13.08^{a}	330.92^{b}	96.19^{a}	516.67^{a}	14.83^{a}	7.17^{a}	494.66^{a}	155.55^{b}	5.15^{a}	3.39^{a}
II	371^{a}	12.78^{a}	358.22^{a}	96.55^{a}	526.77^{a}	14.18^{a}	6.38^{a}	506.21^{a}	158.55^{b}	4.95^{a}	3.25^{a}
III	376^{a}	13.67^{a}	362.33^{a}	96.36^{a}	502.31^{a}	13.93^{a}	6.38^{a}	481.96^{a}	165.00^{a}	5.08^{a}	3.36^{a}

Different letters (a, b) indicate significant differences among treatments.

Table 4. *Nutrient composition in leaves.*

Year	2012			2013			2014			average		
Variant	I	II	III	I	II	III	I	II	III	I	II	III
Element	%											
N	2.39^{a}	2.38^{a}	2.31^{a}	2.17^{a}	2.22^{a}	2.10^{a}	2.10^{a}	2.15^{a}	2.14^{a}	2.23^{a}	2.25^{a}	2.21^{a}
P	0.32^{a}	0.35^{a}	0.36^{a}	0.31^{a}	0.31^{a}	0.31^{a}	0.30^{b}	0.38^{a}	0.31^{b}	0.32^{b}	0.35^{a}	0.33^{a}
K	0.84^{a}	0.71^{b}	0.92^{a}	0.82^{a}	0.71^{a}	0.81^{a}	0.82a	0.70^{b}	0.83^{a}	0.82^{a}	0.71^{b}	0.85^{a}
Ca	1.85^{b}	2.28^{a}	1.86^{b}	2.42^{b}	3.20^{a}	3.06^{a}	2.40^{b}	3.61^{a}	3.29^{a}	2.23^{b}	3.03^{a}	2.74^{b}
	mg/kg											
Zn	43.57^{a}	36.02^{a}	34.56^{b}	32.08^{a}	31.95^{a}	32.54^{a}	31.91^{a}	30.29^{a}	31.75^{a}	35.85^{a}	32.75^{a}	32.95^{a}
Mn	134.55^{a}	146.17^{a}	142.79^{a}	147.19^{a}	149.98^{a}	145.41^{a}	101.76^{a}	103.57^{a}	100.62^{a}	127.84^{a}	133.24^{a}	129.61^{a}
Cu	10.94^{a}	10.52^{a}	10.15^{a}	9.37^{a}	9.37^{a}	9.10^{a}	33.28^{a}	34.65^{a}	35.07^{a}	17.86^{a}	18.18^{a}	18.11^{a}

Different letters (a, b) indicate significant differences among treatments.

CONCLUSION:

The experimental and biometric results of this three-year study indicate not only increasing grape yield production, but also some of the quality parameters were positively affected resulting in increased cluster and berries weights, TSS content and concentration of some macro and micro elements in grape leaves, also.

1. The highest average yield was noted at variant III, with 4.58 kg/vine or 14.87 t/ha;
2. Foliar fertilizing with Fe has significant effect on increasing of: total cluster weight, berries weight per cluster and percentage of berries. Treated variants had a lower weight of skin and seeds, compared with the control;
3. The average values from leaf blades samples showed that foliar fertilizing with Mg increased N, P, Ca and Mn, but decreased K and Zn content;
4. Results of leaf tissue confirmed some antagonistic relations between analysed elements, like: Fe/P, Mg/K and Fe/Mn.

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ПРИНОС И МИНЕРАЛЕН СОСТАВ НА ВИНОВА ЛОЗА ПОД ВЛИЈАНИЕ НА ФОЛИЈАРНО ЃУБРЕЊЕ СО МАГНЕЗИУМ И ЖЕЛЕЗО

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Резиме

Цел на ова истражување е да се утврди влијанието на фолијарните ѓубрива (Magni mag helat и Magni fer helat) врз висината на приносот, квалитетот и хемискиот состав на листовите кај сортата кардинал во споредба со контролната варијанта (без фолијарно ѓубрење).

Опитот е поставен по методот на случаен блок систем, со 3 варијанти, во 3 повторувања. Фолијарните ѓубрива со различен хемиски состав се аплицирани во концентрација од 0.5%, четири пати во текот на вегетацијата, во фазите: пред цветање, по цветање, пораст на зрно и прошарок.

Резултатите од тригодишното истражување покажаа дека ѓубривата врз база на магнезиум и железо имаат влијание врз висината на приносот и квалитетот кај виновата лоза. Кај варијантата 3 е утврден највисок просечен принос од 14.87 t/ha. Фолијарното ѓубрење со железо покажа сигнификантно влијание врз зголемувањето на: масата на гроздот, масата на зрна во грозд и процентот за зрна. Третираните варијанти имаат помала маса на покожица и семки, споредено со контролата. Сигнификантно влијание фолијарните ѓубрива имаат и врз содржината на анализираните елементи во листот од лозата. Највисока просечна содржина на P (0.35 %), Ca (3.03 %), N (2.25 %), Mn (133.24 mg/kg) и Cu (18.18 mg/kg) е утврдена кај варијантата 2. Резултатите од лисната анализа истовремено потврдија и антагонизам помеѓу некои од анализираните елементи, како: Fe/P, Mg/K и Fe/Mn.

Клучни зборови: *кардинал, зрна, елемент, ѓубрење, квалитет.*

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STUDY ON THE INFLUENCE OF PANAMIN LEAF FERTILIZER ON PLANT DEVELOPMENT, RESISTANCE TO ABIOTIC STRESS, PRODUCTIVITY AND GRAIN QUALITY OF WHEAT AND BARLEY

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Abstract

The second treatment influence of leaf fertilizer Panamin Agro has been studied on plant growth, resistance to biotic and abiotic stress, productivity and quality of the grain in wheat and barley. The aim of this study was to investigate the effect of Panamin Agro leaf fertilizer on plant development, resistance to abiotic stress, productivity and grain quality of wheat and barley. The study was conducted between 2017-2018 year at the Institute of Agriculture - Karnobat, Bulgaria. It was found that at 100% of the nitrogen fertilization dose and 2 treatments with Panamin Agro and at 50% of the nitrogen fertilization dose and 3 treatments with Panamin Agro wheat yields increased by 23.3% and 22.2% respectively and barley by 23.4% and 25.2%, respectively.

Key words: *bio-immunostimulant, leaf feeding, yields, cereal*

INTRODUCTION

To obtain greater yield and better quality of cereal crops, an effective alternative for an ecological and environmentally friendly approach is the organic product Panamin Agro. It is an innovative, globally unique blend of rock flours harvested from highest quality Austrian volcanic rocks, minerals and limestone. Due to its innovative grinding technology, the particle size is reduced to nanoparticles, which contributes to the maximum absorption of the product by the plant. It is applicable for plant and soil remineralization in all crops by means of foliar application. Panamin Agro is a bio-immunostimulant for leaf-feeding. It is also rich in most of the necessary micro- and macro-nutrients to feed the plants.

The aim of this study was to investigate the effect of Panamin Agro foliar fertilizer on plant development, resistance to abiotic stress, productivity and grain quality of wheat and barley.

MATERIAL AND METHODS

The study was conducted in a two-year period from 2017 to 2018, in the experimental field at the Institute of Agriculture in Karnobat, Bulgaria. Panamin Agro was tested in multifactorial experiments on wheat and barley. We used wheat varieties Mirjana and six-row barley Zemela created in the Institute of Agriculture - Karnobat, Bulgaria. Wheat variety Miryana is a high-yielding variety widely distributed in Bulgaria. Zemela variety is a new Bulgarian variety, high yield with very good grain quality.

The experience is set in six (I-VI) feeding variants:

Variant I – control. Varieties of wheat and barley were grown in technology adopted for the conditions of Bulgaria and recommendations of Hristova and Cheresharov (2002) and Gramatikov et al. (2004). Sowing of wheat and barley was carried out in optimal terms for the region on October 20. In the spring fertilization was carried out with ammonium nitrate at a dose for wheat 50 kg/1000 m^2 and for barley 30 kg/1000 m^2.

Variant II – three treatments with Panamin Agro were performed:

First treatment – It was administered in autumn, during the 3-6 leaf crop stage, in a dose of 150 g/1000 m^2.

Second treatment – It was administered in spring, at the beginning of active vegetation, in a dose of 150 g/1000 m^2.

Third treatment – It was administered 10-15 days after the second treatment. In this variant, the herbicide treatment was administered in a dose reduced by 50%, no nitrogen feeding was used and there was no fungicide treatment.

Variant III - three treatments with Panamin Agro were performed:

First treatment – It was administered in autumn, during the 3-6 leaf crop stage, with 150 g/1000 m^2.

Second treatment – It was administered in spring, at the beginning of active vegetation, in a dose of 150 g/1000 m^2.

Third treatment – It was performed 10-15 days after the second treatment. In this variant, the herbicide treatment was administered in a dose reduced by 50%, 50% of nitrogen fertilization was used and the plots were treated with a 50% dose of fungicide.

Variant IV – Pre-sowing seed treatment with Panamin Agro in dose 5 g/kg seed. Three treatments with Panamin Agro were performed:

First treatment – It was administered in autumn, during the 3-6 leaf crop stage, in a dose 150 g/1000 m^2.

Second treatment – It was administered in spring, at the beginning of active vegetation, in a dose of 150 g/1000 m^2.

Third treatment - It was administered 10-15 days after the second treatment. In this variant, the herbicide treatment was administered in a dose reduced by 50%, 50% of nitrogen fertilization was used and the plots were treated with a 50% dose of fungicide.

Variant V – Two spring treatments were administered:

First treatment – It was administered in spring, at the beginning of active vegetation, in a dose of 150 g/1000 m^2.

Second treatment - It was administered 10-15 days after the first treatmentIn this variant, the herbicide treatment was administered in a dose reduced by 50%, 100% of nitrogen fertilization was used and the plots were treated with a 50% dose of fungicide.

Variant VI - Two spring treatments were administered:

First treatment – It was administered in spring, at the beginning of active vegetation, in a dose of 150 g/1000 m^2.

Second treatment - It was administered 10-15 days after the first treatment. In this variant, the herbicide treatment was administered in a dose reduced by 50%, 60% of nitrogen fertilization was used and the plots were treated with a 50% dose of fungicide.

The size of the plot was 10 m^2. Each variant was set in 4 replications. During the testing period, yield and its structural elements were studied. The grain quality of wheat was determined by the indicators: protein content, test weight, 1000 grain weight, gluten, relaxation, and bread-making strength index (BMSI); and barley grain quality by the indicators: protein content, test weight, starch content and 1000 grain weight.

All the results were statistically processed by performing a Fit analysis using JMP 5.0.1 software (JMP, 2002). LSD values and VC% were calculated.

Figures 1 and 2 show the average monthly air temperatures and the monthly precipitation by months and years of experiment as well as for a multiannual period. The two experimental years of testing were very different in meteorological aspect. The crop vegetation in year 2016/2017 occurred under severe drought. The amount of precipitation in all the months was less than the multiannual period. The winter of 2017 was very cold, with average monthly temperatures significantly lower than the typical average for this region. No frost damage was reported, despite the low negative temperatures in January, due to significant snow cover. The second year, which was characterized by abundant precipitation, was less favourable for the development of barley. The vegetation in October began with heavy rainfall and flooding the crops. Along with the heavy rainfall during vegetation, another deviation from the typical weather for the region was the higher average monthly temperatures from November until the end of vegetation.

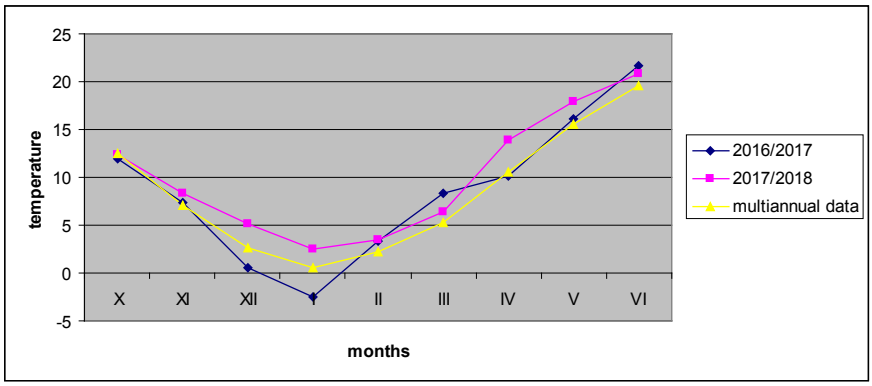

Figure 1. Average monthly air temperatures by months.

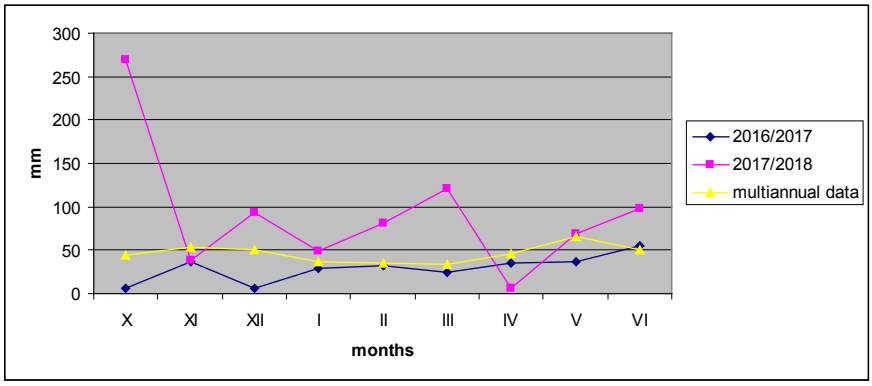

Figure 2. Sum of precipitation (mm) by months.

The monthly deviation ranged from +1.1^{0}C in March to +3.4^{0}C in April. The lack of negative temperatures, and having only low positive temperatures close to zero, made it possible for the vegetation to continue during the winter months. It enabled both barley and wheat tillering and at the beginning of March were reported 4-5 tillers per plant. The weather was colder and with snow cover in March (on 1.03.2018 and 21.03.2018), and instead of spring, winter came. April was characterized with the highest deviation in the average monthly temperatures with lack of precipitation. The draught and warm weather in April boosted the plant development and shortened the developmental stages. As a result, the plants remained short with small spikes and underfed grains. On 17.05.2018 the experiments were hit by hailstorm, which caused severe damages to the crops in the region. The conducted observations and measurements to account for the losses after the hail in the experiments with Panamin Agro showed losses of about 30%.

RESULTS AND DISCUSSION

Table 1 presents data on productivity elements by wheat variants. The best results were obtained in the variants with different % of nitrogen feeding + 2 to 3 treatments with Panamin Agro (Variants III, IV and V). The data on the number of productive tillers per m^2, as well as the other indicators convincingly proved that without nitrogen feeding the effect of Panamin Agro with 3 treatments was weaker than the untreated control. The higher tillering caused the lower values in Variant II. Very dense crops needed more nutrients. Their lack led to lower values than the other productivity elements. The highest numbers of productive tillers per m^2 were formed by Variants II, III and IV. The percentage of unproductive tillers, however, was also significant. The tree treatments apparently stimulated the feeding of tillers, but a high number of them also remained with no spikes. After the untreated control, Variant V formed the longest spikes – 4.72 cm.

Table 1. Productivity elements of wheat variety Miryana by variants.

Variants	Total number of tillers per m^2	Number of productive tillers per m^2	% unproductive tillers per m^2	Spike length (cm)	Number of grains per spike	Grain weight per spike (g)	1000-grain weight (g)
Variant I – control	910 c	792	12.97 ab	7.18 ab	35.72 a	1.83 a	48.70 a
Variant II	998 a	650 d	34.87 cd	5.30 c	26.76 b	1.15 b	42.55 b
Variant III	940 b	830 a	11.70 a	7.76 a	40.76 a	1.96 a	46.84 ab
Variant IV	966 b	844 a	12.63 ab	7.74 a	40.76 a	1.91 a	51.29 a
Variant V	950 b	840 a	11.58 a	7.62 a	35.96 a	1.93 a	48.35 a
Variant VI	900 c	790 b	12.22 ab	6.60 b	29.88 b	1.40 b	46.07 ab
Average	950	758	20.21	6.98	34.89	1.70	47.30
LSD	55.70	45.27	15.16	0.61	5.39	0.30	5.58
VC%	10.23	16.23	22.13	15.76	27.54	30.59	21.06

The highest number of grain per spike was formed in Variant III, but unfortunately, there was the highest number of sterile spikelets. In Variant V, during ear emergence was administered the second treatment +100% of the nitrogen dose, which led to a more favourable situation of flowering, fertilization, and feeding of germs. The spikes in Variant V were proven heavier, followed by the ones in Variants II and III. The greatest 1000-grain weight was reported for Variant III, followed by Variants II and V. Higher Grain weight per spike and 1000-grain weight of wheat reported Kenanov (2018; 2018a). Better results after treatment with Panamir Agro can be explained by the findings of other authors. Lucipidis and Bozhinova (2010) say in their research that the Miryana variety prefers balanced fertilization. Higher nitrogen levels, according to them, lead to an increase in the elements of productivity and yield (Nankova, 2009).

The results in Table 2 show that barley in Variant II also reported the highest total number of tillers per m^2. Unlike wheat, the very dense crops of barley more easily endured the lack of sufficient nutrients. Except for the number of sterile spikelets, all other indicators for Variant II showed values in group a and they were proven high. Although there was no good differentiation by number of grains per spike by variants, the highest number of grains was observed in Variants II, V and VI. The greatest grain weight per spike was in Variants I and V.

Table 2. Productivity elements of barley variety Zemela by variants.

Variants	Total number of tillers per m^2	Number of productive tillers per m^2	% unproductive tillers per m^2	Spike length (cm)	Number of grains per spike	Number of sterile spikelets	Grain weight per spike (g)	1000 grain weight (g)
Variant I – control	860	810 ab	5.81 a	4.84 a	46.80 a	8.40 a	1.60 a	41.13 a
Variant II	1046 a	845 a	19.22 c	4.66 abc	49.32 a	13.28 bc	1.42 ab	39.17 ab
Variant III	999 a	830 a	16.92 ab	4.28 bc	44.68 a	9.96 bc	1.44 ab	41.60 a
Variant IV	1020 a	844 a	17.25 ab	4.18 c	44.88 a	9.24 b	1.23 b	35.12 c
Variant V	905	840 ab	20.50 c	4.72 ab	47.28 a	6.72 a	1.57 a	38.90 ab
Variant VI	890	790 b	11.24 a	4.68 abc	47.76 a	14.16 c	1.23 b	36.26 bc
Average	937	803	15.16 ab	4.56	46.79	10.29	1.42	38.70
LSD	52.13	42.27	10.08	0.71	5.41	3.41	0.32	3.76
VC%	32.00	26.23	22.14	19.96	20.65	58.89	40.85	17.36

The yield results are presented in Table 3. The wheat data shows that high yield was obtained in Variants III, IV, V and VI. There was no good differentiation between them and the yields from the four variants were in group a, exceeding the untreated control by 22.2% to 26.6%. The highest barley yield was obtained in Variants V and VI, exceeding the control by 23.4% and 25.2%.

Table 3. Yield results for wheat variety Miryana and barley variety Zemela by variants.

Variants / Yield	Wheat variety Miryana		Barley variety Zemela	
	kg/da	% compared to control	kg/da	% compared to control
Variant I– control	433 c	100.0	461 c	100.0
Variant II	457 b	105.5	474 c	102.8
Variant III	530 a	124.4	543 b	117.8
Variant IV	548 a	126.6	536 b	116.3
Variant V	534 a	123.3	569 a	123.4
Variant VI	529 a	122.2	577 a	125.2
Average	505.06		526.83	
LSD	21.10		22.62	
VC%	5.84		5.26	

The results of the yield obtained in wheat and barley by variants confirm the findings of Kenanov (2017). In his studies the author claims that three sprays may increase the yield of cereal to 60%. Effects on the yield and the biomass from the increased fertilization norms are reported by Ivanova and Tzenov (2014).

Grain quality of winter wheat wheat Miryana is good for intensive and moderate mineral fertilization (Koteva and Marcheva, 2012). Table 4 presents results for the grain quality of cultivar Miryana.

Table 4. Grain quality of wheat variety Miryana by variants.

Variants	Protein content (%)	Test weight (kg/hl)	1000-grain weight (g)	Gluten (%)	Relaxation	BMSI
Variant I – control	16.10 ab	68.35 c	33.63 ab	29.33 ab	17.93 ab	42.50 b
Variant II	14.98 c	72.18 a	35.45 a	26.48 c	13.70 d	45.25 a
Variant III	15.95 b	70.58 b	32.75 b	28.98 ab	16.08 c	44.50 a
Variant IV	15.78 b	70.48 b	32.75 b	28.63 b	16.95 bc	43.25 b
Variant V	16.50 a	69.63 bc	32.00 b	30.08 a	18.55 a	42.25 b
Variant VI	16.00 ab	70.28 b	33.38 ab	28.88 b	17.53 b	43.00 b
Average	15.88	70.25	33.33	28.73	16.79	43.46
LSD	0.53	1.51	2.21	1.16	0.99	1.11
VC%	2.27	1.45	4.47	2.71	3.93	1.06

Table 5. Grain quality of barley variety Zemela by variants.

Variants	Protein content (%)	Hectoliter weight (kg/hl)	Starch content (%)	1000-grain weight (g)
Variant I – control	14.00 bcd	61.30 b	52.91 b	35.63 a
Variant II	13.85 cd	64.55 a	57.53 a	35.50 ab
Variant III	14.43 ab	62.88 ab	53.57 b	35.00 ab
Variant IV	14.38 abc	62.18 b	62.18 b	34.50 ab
Variant V	14.58 a	62.13 b	62.13 b	34.00 b
Variant VI	13.80 d	61.83 b	61.83 b	34. 00 ab
Average	14.17	62.48	54.02	34.92
LSD	0.55	1.79	2.06	1.49
VC%	2.61	2.57	2.57	2.89

The data shows that the highest values for grain protein were in Variants V and VI. The test weight had very high values in Variant II. This shows that Panamin Agro with 3 treatments, even without nitrogen feeding, can ensure grain of high hectoliter mass.

The 1000-grain weight was the highest in Variants II and VI, whereas gluten was convincingly the highest in Variant V. The best results in three of all indicators were shown by Variants II and V. Good Bread Wheat Quality is achieved with optimal fertilization (Yanchev et al., 2014). Researchers report that it is very important to achieve higher levels of protein and gluten.

Table 5 shows data on grain quality of barley by variants. The highest values of grain protein content were reported for Variant V, where 100% of the nitrogen dose +2 treatments resulted in the highest protein value. The test weight showed its highest values in Variants II and III, and starch – in Variant II. The biggest grains were in Variants II, III, IV and VI. For a better quality of the grain of barley with balanced fertilization, report Ivanova et al. (2014).

CONCLUDING REMARKS

As a result of this study can be drawn the following conclusions:

Regarding wheat: the highest statistically proven yield was formed by Variants III, IV, V and VI, where Variant IV had the highest values – 548 kg/da or 26.6% more than the untreated control. The effect on yield caused by 50% nitrogen feeding +3 treatments with Panamin Agro was equal to 60% of nitrogen feeding +2 treatments with Panamin Agro. Close yield values were observed for Variants IV and V, i.e. high yield can be obtained by 50% nitrogen feeding +3 treatments with Panamin Agro, or by 100% nitrogen feeding with 2 treatments with Panamin Agro.The grain quality was better in 3 of the indicators for Variants II and V.

Regarding barley: the highest statistically proven yield was formed by Variants V and VI – 569 kg/da and 577 kg/da, respectively, or 23.4% to 25.2% more than the untreated control. Close yield values to Variants V and VI were observed for Variants III and IV, i.e. high yield can be obtained by 50% nitrogen feeding +3 treatments with Panamin Agro or by 100% nitrogen feeding with 2 treatments with Panamin Agro. The highest values in three of the grain quality indicators were obtained in Variant II.

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ИСПИТУВАЊЕ НА ВЛИЈАНИЕТО НА ФОЛИЈАРНОТО ЃУБРИВО ПАНАМИН ВРЗ РАСТОТ НА РАСТЕНИЈАТА, ОТПОРНОСТА НА АБИОТСКИОТ СТРЕС, ПРОДУКТИВНОСТА И КВАЛИТЕТОТ НА ЗРНОТО КАЈ ПЧЕНИЦА И ЈАЧМЕН

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Резиме

Влијанието на фолијарното ѓубриво Панамин агро е испитувано врз растот на растенијата, отпорност на биотски и абиотски стрес, продуктивноста и квалитетот на зрното кај пченицата и јачменот. Целта на оваа студија беше да се испита ефектот на ѓубривото на Панамин агро лист врз развојот на растенијата, отпорноста на абиотски стрес, продуктивноста и квалитетот на зрното на пченицата и јачменот. Истражувањата беа спроведени помеѓу 2017 и 2018 година во Институтот за земјоделство - Карнобат, Република Бугарија. Утврдено е дека 100% од дозата на азотно ѓубриво со 2 третмани од Панамин агро и 50% од дозата на азотно ѓубриво со 3 третмани од Панамин агро ги зголемиле приносите кај пченица за 23.3% и 22.2% соодветно, а кај јачменот приносите се зголемиле за 23.4% и 25.2 % соодветно.

Клучни зборови: *биоимуностимулатор, лисна прехрана, приноси, житни култури.*

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