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CONTENT / SADRŽAJ

Nebojša NOVKOVIĆ, Nataša VUKELIĆ, Miljko JANOŠEVIĆ,
Srboljub NIKOLIĆ, Slaviša ARSIĆ
ANALYSIS AND FORECAST OF THE PRODUCTION PARAMETERS OF MAJOR CEREAL CROPS IN SERBIA /
ANALIZA I PREDVIĐANJE PROIZVODNIH OBELEŽJA VAŽNIJIH VRSTA ŽITA U SRBIJI 45

Dragana TEKIĆ, Beba MUTAVDŽIĆ, Tihomir NOVAKOVIĆ
USE OF STATISTICAL CONTROL CHARTS FOR MONITORING THE QUALITY OF FLOUR /
KORIŠĆENJE STATISTIČKIH KONTROLNIH KARATA U OCENI KVALITETA BRAŠNA 50

Ajibola Olajide OJEDOKUN, Olufemi Adedotun YESUFU, Victoria Ayomide AYORINDE
FACTORS INFLUENCING TOMATO MARKETING
IN ILE-IFE, OSUN STATE, NIGERIA / UTICAJNI FAKTORI
PRODAJE PARADAJZA U ILE-IFE REGIJI, DRŽAVA OSUN U NIGERIJI 53

Dragana MLADENOVIĆ, Aleksandra ĐUKIĆ-VUKOVIĆ, Jelena PEJIN, Sunčica KOCIĆ-TANACKOV,
Milica RADOSAVLJEVIĆ, Marija MILAŠINOVIĆ-ŠEREMEŠIĆ, Ljiljana MOJOVIĆ
EFFECT OF LACTIC ACID FERMENTATION ON THE QUALITY
OF BREWER'S SPENT GRAIN AS RUMINANT FEED / UTICAJ MLEČNO-KISELINSKE
FERMENTACIJE NA KVALITET PIVSKOG TROPA KAO HRANIVA ZA PREŽIVARE 57

Zorana RONČEVIĆ, Ida ZAHOVIĆ, Nikolina DANILOVIĆ,
Siniša DODIĆ, Jovana GRAHOVAC, Jelena DODIĆ
POTENTIAL OF DIFFERENT Xanthomonas campestris STRAINS FOR XANTHAN
BIOSYNTHESIS ON WASTE GLYCEROL FROM BIODIESEL PRODUCTION /
POTENCIJAL RAZLIČITIH Xanthomonas campestris SOJEVA ZA BIOSINTEZU
KSANTANA NA OTPADNOM GLICEROLU IZ PROIZVODNJE BIODIZELA 62

Ľubomír KUBÍK, Monika BOŽIKOVÁ, Peter HLAVÁČ, Viera KAŽIMÍROVÁ
FRACTAL ANALYSIS OF THE FLOUR / FRAKTALNA ANALIZA BRAŠNA 67

Ivana PAJČIN, Vanja VLAJKOV, Zorana RONČEVIĆ, Jelena DODIĆ,
Mila GRAHOVAC, Maja IGNJATOV, Jovana GRAHOVAC
IN VITRO POTENTIAL OF BACILLUS SPP. ANTAGONISTS FOR SUPPRESSION
OF XANTHOMONAS EUVESICATORIA PHYTOPATHOGENS /
IN VITRO POTENCIJAL ANTAGONISTA BACILLUS SPP. ZA
SUZBIJANJE FITOPATOGENA XANTHOMONAS EUVESICATORIA 72

Milica RADOSAVLJEVIĆ, Marija MILAŠINOVIĆ-ŠEREMEŠIĆ, Dušanka TERZIĆ,
Života JOVANOVIĆ, Jelena SRDIĆ, Valentina NIKOLIĆ
GRAIN CHEMICAL COMPOSITION OF DENTS, POPPING
MAIZE AND SWEET MAIZE GENOTYPES / HEMIJSKI SASTAV ZRNA
GENOTIPOVA KUKURUZA ZUBANA, KOKIČARA I ŠEĆERCA 77

Mirko BABIC, Ljiljana BABIC
ADVANTAGES AND DISADVANTAGES OF DIRECT AND INDIRECT
DRYING OF MERCANTILE GRAIN AND SEEDS / PREDNOSTI I NEDOSTACI
DIREKTNOG I INDIREKTNOG SUŠENJA MERKANTILNOG ZRNA I SEMENA 81

Miladin KOSTIĆ, Vojin ĐUKIĆ, Aleksandar ILIĆ, Danka DUJOVIĆ,
Velimir LONČAREVIĆ, Milivoj RADIN, Miloš ROGIĆ
SPECIFICS OF SOYBEAN SEED PRODUCTION AND PROCESSING IN 2019 /
SPECIFIČNOSTI PROIZVODNJE I DORADE SEMENA SOJE U 2019. GODINI 85

INFORMACIJE ..... D1 – D5

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ANALYSIS AND FORECAST OF THE PRODUCTION PARAMETERS OF MAJOR CEREAL CROPS IN SERBIA

ANALIZA I PREDVIĐANJE PROIZVODNIH OBELEŽJA VAŽNIJIH VRSTA ŽITA U SRBIJI

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ABSTRACT

This paper deals with the production parameters of major cereal crops in Serbia (namely the cultivation area, annual production and yields of the cereal crops considered) in the period 2005–2018. The objective of this study was to determine trends in and the volume and stability of the production parameters of the most important cereal crops in Serbia. Furthermore, a forecast of changes in the parameters observed was made for the five-year period 2019-2023. The following cereal crops were under consideration: corn, wheat, barley, oats, rye and triticale. The area devoted to corn in Serbia was found to be very stable, indicating a slight downward trend. However, the Serbian corn production lacked stability due to variations in yield, suggesting a very slight downward trend. Wheat was found to be far more stable with slightly growing trends in all the parameters considered. Moreover, positive trends in all the parameters considered were also noticed in the Serbian triticale production.

Key words: *cereals, areas, production, yields, Serbia.*

REZIME

Predmet istraživanja u ovom radu su proizvodna obeležja, odnosno površine, godišnja proizvodnja i prinosi najzastupljenijih vrsta žita u Srbiji u intervalu od 2005 do 2018. godine.

Cilj istraživanja je da se utrvdi obim, stabilnost i tendencije proizvodnih parametara proizvodno i ekonomski najvažnijih vrsta žita i da se na osnovu toga predvidi njihova promena u periodu od narednih pet godina (2019-23). Analizirani su: kukuruz, pšenica, ječam, ovas, raž i tritikale.

U radu je primenjena desktriptivna analiza proizvodnih karakteristika značajnijih vrsta žita. Na osnovu rezultata deskriptivne statistike su predviđene vrednosti proizvodnih obeležja za narednih pet godina (2019-2023). Predviđanje je urađeno na bazi prosečnih godišnjih stopa promene (r) koje su primenjene na prosečnu vrednost pojave u posmatranom vremenskom interval. Stopa nije primenjena na poslednju godinu, zato što postoje velika variranja pojava pa bi se dobili nerealni rezultati uzrokovani specifičnošću poslednje godine.

Na osnovu primenjene metodologije i dobijenih rezultata može se zaključiti da kukuruz ima veoma stabilne površine i tendenciju blagog smanjenja. Prozvodnja je manje stabilna, što je posledica variranja prinosa i ima slabo izraženu tendenciju pada. Pšenica je daleko stabilnija po svim posmatranim parametrima. Svi proizvodni parametri imaju tendencije blagog povećanja. Od ostalih žita jedino tritikale ima pozitivne tendencije kod sva tri obeležja, dok kod ostalih površine se smanjuju, a prinosi rastu, što za posledicu ima rast proizvodnje kod ječma i pad proizvodnje kod ovsa i raži.

Ključne reči: *žita, površine, proizvodnja, prinosi, Srbija.*

INTRODUCTION

Forecasts of agricultural production and prices are intended to be useful for farmers, governments, and agribusiness industries. As food production is a significant aspect of the national security, governments have become both principal suppliers and main users of agricultural forecasts. They need internal forecasts to execute policies that provide technical and market support for the agricultural sector (*Geoffrey, 1994*). Crop yield forecasting has a growing importance in the public and private sector as a method of anticipating crop production and market fluctuations, ensuring food security, and optimizing agro-management practices and resource use (*Meinke and Stone, 2005).*

This paper deals with the production parameters of major cereal crops in Serbia: corn, wheat, barley, oats, rye and triticale. The production parameters examined include the cultivation area, annual production and yields of the cereal crops considered in the period 2005–2018. On the basis of the parameter values recorded in the period under consideration, a forecast of changes in the parameters considered was made for the period 2019-2023.

A number of studies have dealt with the forecasting of the production and economic parameters of agricultural products. *Novković et al. (2013, 2013a, 2013b)* focused on the forecasting and development of the production parameters of potato. *Michel and Makowski (2013)* have compared statistical models for analyzing wheat yield time series. *Miljanović et al. (2014)* forecast the production parameters of tomato in Serbia. *Mutavdžić et al. (2013)* dealt with the forecasting of major vegetable crops in Republika Srpska. *Mutavdžić et al. (2016)* made analyses and forecasts of wheat and corn price parity in Serbia. *Mutavdžić et al. (2017)* analyzed quarterly movements of wheat and maize sales prices in Serbia and Republika Srpska for the period 2010-2015. *Mutavdžić et al. (2017a)* analyzed monthly variations in the cereal prices in Serbia. *Ivanisevic et al. (2015)* analyzed the movement of tomato prices in Serbia, using the method of descriptive statistics, and made a forecast of the

Serbian tomato prices for the upcoming period based on time series analysis. *Novković and Mutavdžić (2016)* used descriptive statistics to analyze the bean prices in Serbia and forecast the movement of Serbian bean prices in the upcoming period using the analysis results and the adequate ARIMA model. *Mutavdžić et al. (2018)* analyzed the production parameters of cucumber in Serbia and Republika Srpska. *Novkovic et al. (2018)* performed a comparative analysis of the production parameters of potato in Serbia, Macedonia and Republika Srpska. *Lecerf et al. (2019)* used the MARS-Crop Yield Forecasting System (M-CYFS) to forecast the yields of all major crops in the European Union using the gridded runs of the WOFOST crop model. After 28 years of observation (from 1988 to 2015), the overall variability in crop yields was reported for all 28 EU member states. Using descriptive statistics, *Choudhury et al. (2017)* analyzed trends in the area, irrigated area, production and productivity of rice, wheat and maize. The Box–Jenkins ARIMA modeling technique was used for analyzing the data recorded in the period 1951-2015, as well as for modeling and forecasting purposes.

MATERIAL AND METHOD

In the present paper, descriptive analysis was used as a method of researching the production parameters of major cereal crops in Serbia in the period 2005-2018. The cultivated area, production and yields of corn, wheat, barley, oats, rye and triticale were the parameters considered for analysis. On the basis of the descriptive analysis results, the production parameter values were forecast for the forthcoming five-year period (2019-2023). The forecast was made according to the average annual rates of change (r) applied to the average values of the parameters considered in the period under consideration. The average annual rate of change was calculated according to the following formula:

$$G = \left(\frac{Y_n}{Y_1} \right)^{\frac{1}{n-1}}$$

and the average rate of change:

$$r = (G - 1)$$

where:
r is the average annual rate of change
G is the average annual index of change
Y_1 is the absolute value of the first member of the time series
Y_n is the value of the last number of the time series
n is the length of the series (number of years)

The data on the production parameters of the cereal crops considered were obtained from the website of the Statistical Office of the Republic of Serbia.

RESULTS AND DISCUSION

The results obtained include descriptive statistics for the cultivation area, production and yields of all the cereal crops examined in the period under consideration, as well as a forecast of these parameter values for the following five years.

Table 1. Descriptive statistics for the production parameters of corn (2005-18)

Corn	Average value	Variation interval		Variation coefficient (%)	Rate of change (%)
		Minimum	Maximum		
Area (ha)	999.547	901.753	1,057.877	3.89	-0.83
Production (t)	6,029.387	3,532.602	7,951.583	22.72	-0.28
Yield (t/ha)	6.03	3.60	7.70	22.57	0.63

Source: Authors' calculations

Corn is the predominant cereal crop in Serbia (Table 1), accounting for approximately 56 % of the total cultivated area devoted to cereals. The cultivated area devoted to corn was found to be stable, with moderate variations both in yield and annual production. A slight downward trend was noticed in all the production parameters of corn in the period under consideration.

Wheat is the second most prevalent cereal crop in Serbia. It accounts for about 35 % of the total cultivated area devoted to cereals. Therefore, corn and wheat jointly claimed a share exceeding 90 % of the total area devoted to cereals in Serbia in the period under consideration. Moreover, wheat is the predominant small grain cereal in Serbia. As in the case of corn, the cultivated area devoted to wheat in Serbia in the period under consideration was found to be stable, with relatively small variations in yield and annual production (even far smaller than those recorded in corn). The wheat area in Serbia increased very slightly (it was almost stagnant), whereas wheat yield and production increased at an annual rate of change exceeding 1 % (Table 2).

Table 2: Descriptive statistics for the production parameters of wheat (2005-18)

Wheat	Average value	Variation interval		Variation coefficient (%)	Rate of change (%)
		Minimum	Maximum		
Area (ha)	612.493	556.115	643.083	3.69	0.16
Production (t)	2,511.740	2,085.529	2,941.601	9.30	1.18
Yield (t/ha)	4.10	3.40	4.80	8.50	1.08

Source: Authors' calculations

According to the cultivated area, barley is the second most prevalent small grain and bread cereal in Serbia (Table 3). It is far less prevalent than wheat, accounting for approximately 5 % of the total area devoted to cereals in Serbia. Moderate coefficients of variation were computed for all the barley production parameters considered. The cultivated area devoted to barley in Serbia in the period under consideration indicated a slight downward trend, whereas a slight increase was recorded in the annual production and yields of barley.

Table 3. Descriptive statistics for the production parameters of barley (2005-18)

Barley	Average value	Variation interval		Variation coefficient (%)	Rate of change (%)
		Minimum	Maximum		
Area (ha)	94.020	80.803	110.551	9.23	-0.34
Production (t)	352.687	260.998	410.138	14.29	1.74
Yield (t/ha)	3.46	2.80	4.30	13.36	2.04

Source: Authors' calculations

Table 4 presents the descriptive statistics for the production parameters of oats. Oats are not a commonly grown small grain cereal in Serbia. They account for about 2 % of the total area devoted to cereals in Serbia. Moderate coefficients of variation were computed for all the oat production parameters considered. The annual production of oats in Serbia in the period under consideration indicated a slight downward trend, whereas an increasing trend was noticed in the oat yields.

Rye is the least common cereal grown in Serbia of all the crops considered in this paper. It accounts for less than half a percent of the area devoted to cereals in the country (Table 5). The descriptive analysis parameters are similar to those of oats. Moderate coefficients of variation were computed for all the rye production parameters considered. A moderate downward trend was recorded in the area devoted to rye. Moreover, a decrease in rye production was also recorded, whereas the rye yields increased. Triticale is a cereal crop which has been recently introduced into Serbia. It is used predominantly as livestock feed to compensate for decreases in oat and rye production (Table 6). The average area devoted to triticale in Serbia is now three times larger than that devoted to rye and almost equal to one-half of the area devoted to oats. High coefficients of variation were computed for the cultivated area and production of triticale, whereas moderate coefficients of variation were computed for the triticale yields. A growing trend was noticed in the Serbian triticale production in the period under consideration due to a significant increase in the cultivated area devoted to triticale and a moderately growing trend in the triticale yields.

Table 4. Descriptive statistics for the production parameters of oats (2005-18)

Oats	Average value	Variation interval		Variation coefficient (%)	Rate of change (%)
		Minimum	Maximum		
Area (ha)	37.462	26.111	52.583	22.57	-5.24
Production (t)	85.881	69.538	110.620	13.58	-2.51
Yield (t/ha)	2.35	1.90	3.00	14.81	2.90

Source: Authors' calculations

Table 5. Descriptive statistics for the production parameters of rye (2005-18)

Rye	Average value	Variation interval		Variation coefficient (%)	Rate of change (%)
		Minimum	Maximum		
Area (ha)	5.423	4.375	7.332	15.94	-3.31
Production (t)	13.001	10.640	16.137	13.49	-1.41
Yield (t/ha)	2.42	2.00	2.90	11.47	1.87

Source: Authors' calculations

Table 6. Descriptive statistics for the production parameters of triticale (2005-18)

Triticale	Average value	Variation interval		Variation coefficient (%)	Rate of change (%)
		Minimum	Maximum		
Area (ha)	17.603	9.775	27.233	30.94	8.20
Production (t)	66.788	32.853	113.439	38.88	10.00
Yield (t/ha)	3.71	3.10	4.30	11.85	1.64

Source: Authors' calculations

Table 7. Forecast of the cultivated areas devoted to the cereal crops considered (2019-23) in ha

Cereal	Average 2005-18	Forecast				
		2019	2020	2021	2022	2023
Corn	999.547	991.125	983.023	974.864	966.773	958.749
Wheat	612.493	613.473	614.455	615.438	616.422	617.409
Barley	94.020	93.794	93.569	93.344	93.121	92.897
Oats	37.462	35.499	33.639	31.876	30.206	28.623
Rye	5.423	5.243	5.070	4.902	4.740	4.583
Triticale	17.603	19.046	20.608	22.298	24.127	26.105
Total	1,766.548	-	-	-	-	1,728.366

Source: Authors' calculations

Table 8. Production forecast of the cereal crops considered (2019-23) in tons

Cereal	Average 2005-18	Forecast				
		2019	2020	2021	2022	2023
Corn	6,029.387	6,012.505	5,995.670	5,978.882	5,962.141	5,945.447
Wheat	2,511.740	2,541.378	2,571.367	2,601.709	2,632.409	2,663.471
Barley	352.687	358.824	365.067	371.419	377.882	384.456
Oats	85.881	83.725	81.624	79.575	77.578	75.631
Rye	13.001	12.818	12.637	12.332	12.035	11.745
Triticale	66.788	73.467	80.813	88.894	97.784	107.564

Source: Authors' calculations

The forecast distribution of areas devoted to the cereal crops considered for the period 2019–2023 are shown in Table 7. The area devoted to cereals in Serbia is expected to decrease by approximately 38,000 hectares in 2023 compared to the average cereal area in Serbia in the period 2005–2018. In the forecast period, the areas devoted to corn, barley, oats and rye will decrease by 40,000 ha, 1,100 ha, 9,000 ha and900 ha, respectively. However, the areas devoted to wheat and triticale are expected to increase by 5,000 ha and 8,500 ha, respectively (Table 7). The largest relative decrease in the cultivated area was forecast for oats (23%), whereas the largest relative increase is expected in the area devoted to triticale (48 %). The total area devoted to cereals in Serbia will be reduced by 2 %.

The corn production in Serbia is expected to decrease by approximately 84,000 tons in 2023, or by about 1.4 %, compared to the average Serbian corn production in the period 2005-2018 (Table 8). The wheat production in Serbia will increase by about 150.000 tons in 2013, or by about 6 %, compared to the average Serbian wheat production in the period 2005-2018. The barley production in Serbia is expected to increase by approximately 32,000 tons in 2023 compared to the average Serbian barley production in the period 2005-2018. The largest relative increase of 61 %, or over 40,000 tons, was forecast for triticale. In addition to corn, the production of oats and rye in Serbia is expected to decrease by 12 % and 9.6 % in 2023, respectively.

The yields of all the cereal crops considered is expected to increase in 2023 compared to the average yields recorded in the period 2005–2018 (Table 9). The largest relative increase in yield of 14.9 % was

forecast for oats, followed by barley (10.7 %), wheat (9.8 %), rye (9.5 %) and triticale (8.4 %). The smallest yield increase of 3.15% was forecast for corn

Table 9. Yield forecast of the cereal crops considered (2019-23) in tons per ha

Cereal	Average 2005-18	Forecast				
		2019	2020	2021	2022	2023
Corn	6.03	6.07	6.11	6.14	6.18	6.22
Wheat	4.10	4.18	4.25	4.33	4.42	4.50
Barley	3.46	3.53	6.60	3.68	3.75	3.83
Oats	2.35	2.42	2.49	2.56	2.63	2.70
Rye	2.42	2.47	2.51	2.56	2.61	2.65
Triticale	3.71	3.77	3.83	3.90	3.96	4.02

Source: Authors' calculations

CONCLUSION

On the basis of the results obtained in the present paper, the following conclusions can be drawn:

- Slight downward trends were observed in the area and total production of corn in Serbia in the period under consideration, whereas the corn yields were found to be slightly increasing. At the end of the forecast period (2023), the area devoted to corn in Serbia is expected to decrease by approximately 4 %, whereas the Serbian corn production will decrease by 1.4 % compared to the average corn production in the period 2005–2018.
- A slightly growing trend was noticed in the area devoted to wheat in Serbia in the period under consideration (which was almost stagnant). The slight increase in the Serbian wheat production recorded in this period can be attributed to the increase in wheat yields. The wheat production in Serbia is expected to increase by 150.000 t in 2023 compared to the average Serbian wheat production in the period 2005–2018, representing a decrease of 9.5% from the maximum production in Serbia in 2018.
- A slight downward trend was observed in the area devoted to barley in Serbia in the period under consideration, whereas the Serbian barley production increased due to the increase in the barley yields. The area devoted to barley in Serbia is expected to decrease by 1.2 % in 2023, whereas the Serbian barley production will increase by 9 % in 2023 compared to the average Serbian barley production in the period 2005-2018.
- Similar downward trends were noticed in the areas and annual production of oats and rye in Serbia in the period under consideration, whereas the oats and rye yields were found to be increasing.
- The area and annual production of triticale in Serbia indicated strong growing trends in the period under consideration, as well as a slight increase in yield. At the end of the forecast period, the area devoted to triticale in Serbia is expected to increase by 48 %, whereas the triticale production in Serbia will increase by 61 % compared to the average Serbian triticale production in the period 2005–2018.
- The total area devoted to cereals in Serbia is expected to decrease by 38,000 ha in 2023, or by 2 %, compared to the average Serbian cereal production in the period under consideration.
- The yields of all the cereal crops considered will increase in 2023 compared to the average yields recorded in the period under consideration, ranging from 3.15 % in maize to 14.9 % in oats.
- The area devoted to corn in Serbia will amount to 960 thousand hectares in 2023, whereas the Serbian corn production is expected to reach 5.95 million tons. Moreover, the area devoted to wheat in Serbia will amount to 618 thousand hectares in 2023, with a production of 2.66 million tons.

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USE OF STATISTICAL CONTROL CHARTS FOR MONITORING THE QUALITY OF FLOUR

KORIŠĆENJE STATISTIČKIH KONTROLNIH KARATA U OCENI KVALITETA BRAŠNA

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ABSTRACT

The use of statistical quality control in the production and storage processes significantly enhances the overall quality of products. Although various techniques are used for quality monitoring purposes, control charts are considered of paramount importance. In this paper, the ash and moisture contents of the wheat flour type 500 were analyzed using descriptive statistics. Consequently, the control charts for the variations in and the mean values of the parameters considered were created. The control charts obtained for the mean parameter values show that the flour production process was in control relative to both parameters considered, exhibiting only random variations. However, the control charts obtained for the variability in the parameter values considered show special variations, the causes of which require further research in order to maintain the satisfactory quality of flour production.

Key words: *quality, quality control, control charts, flour.*

REZIME

Primena statističke kontrole kvaliteta proizvodnog procesa i procesa skladištenja u velikoj meri doprinosi povećanju kvaliteta samog proizvoda. U svrhu praćenja kvaliteta koriste se razne tehnike, od kojih se kao najznačajnija izdvajaju kontrolne karte. Osnovna svrha primene statističkih kontrolnih karata jeste otkrivanje specijalnih uzroka varijacije. Ukoliko se utvrdi postojanje specijalnih uzroka varijacije može se konstatovati da je proces proizvodnje ili skladištenja van kontrole. U radu su analizirani podaci koji se odnose na sadržaj vlage i pepela u brašnu. Analiza podataka o sadržaju pepela i vlage u brašnu izvedena je na osnovu pokazatelja deskriptivne statistike. Osnovni preduslovi za primenu kontrolnih karata jeste da su podaci raspoređeni po zakonima normalne distribucije, nekolinernost opservacija, homogenost varijansi i aritmetičkih sredina, s toga su u radu najpre testirane ove pretpostavke, a zatim su na bazi izračunatih aritmetičkih sredina i intervala varijacije konstruisane kontrolne karte za srednju vrednost i varijabilitet posmatranih obeležja, tj. sadržaja pepela i vlage u brašnu tipa 500. Na bazi formiranih kontrolnih karata za srednje vrednosti uočeno je da je proces proizvodnje brašna pod kontrolom za oba posmatrana parametra. Utvrđeno je postojnje samo slučajnih uzroka varijacije, a analizom kontrolnih karata za varijabilitet posmatranih obeležja, uočeno je postojanje specijalnih uzroka varijacija koje treba dodatno ispitati kako bi kvalitet proizvodnje ostao na zadovoljavajućem nivou.

Ključne reči: *kvalitet, kontrola kvaliteta, kontrolne karte, brašno.*

INTRODUCTION

The finalization of agricultural production requires adequate quality control, implemented to the greatest extent possible *(Đevic and Dimitrijevic, 2005).* Quality represents the totality of characteristics that meet the users' needs and/or demands, whereas functioning security is a unified feature of maintaining the quality within the set limits of time, working mode and conditions *(Filipović and Đurić, 2009)*.

The application of statistical methods, with precisely and clearly defined parameters, provides the basis for consistent implementation of the quality policy. Statistical methods are considered suitable for defining the standards of production and services, which ensure high levels of quality and better business results *(Drenovac A., et al, 2013).* Statistical quality control is a set of methods and procedures for collecting, processing, analyzing, interpreting and presenting data. It is used to ensure the quality of production processes and final products. The proper implementation of statistical quality control can reduce production costs *(Horvat et al, 2006).* A number of different types of diagrams serve as the basic tools for statistical quality control: control charts, the Pareto diagrams, histograms, scatter diagrams and the Ishikawa diagrams *(Hadživuković, 1989)*.

Control charts are the basic instrument for controlling the quality of production processes and final products. The control chart is a statistical technique used to differentiate between common and special variations in a given process. It represents a graphical analysis of the process stability and instability over time, which is performed in order to ensure and maintain the stability of the process considered *(Okwonu and Ogini, 2017).*

There are a number of different versions of control charts that can be used to detect process irregularities. However, the Shewhart control chart ($\bar{X}$ chart) is considered the most common and easily interpretable of them all. It was based on the assumption that the variations which occur in every process can be understood and statistically monitored *(Savić, 2006).* The control chart consists of a center line, which represents the mean value of the in-control process, and two horizontal lines (namely the upper control limit (UCL) and the lower control limit (LCL)) *(Muhammad R and Muhammad, 2012).* The center line represents the mean value of the observed feature, whereas the upper and lower limits represent the range in which almost all feature values should be found if the process is in control. Control limits indicate the qualitative movement of a process, i.e. the stability and capability of a process. Moreover, they represent the possibilities of a process and normal process variations that can be expected *(Drenovac et al., 2013).*

Wheat is one of the most important cereals in the world, and wheat bread is one of the global staple foods *(Tarjan et al., 2009).* The properties of flour reflect its condition and quality. Various changes occur in flour during processing, which greatly affect its technological quality. Therefore, the production and storage of flour must be strictly controlled. The purpose of this

paper is to examine the use of control charts for monitoring the quality of wheat flour (namely the wheat flour type 500) according to the contents of ash and moisture of the flour type considered.

MATERIAL AND METHOD

In order to be properly created, control charts must contain the following entries: uncorrelated measurements, the normality of data, the homogeneity of variance and means. The Kolmogorov-Smirnov and Shapiro-Wilks normality tests, autocorrelation coefficients, the Levene's homogeneity tests and the analysis of variance (ANOVA) were used to test all the control chart considerations stated above.

The control chart used for monitoring changes in the average parameter values was based on the arithmetic mean and standard deviation calculations for the parameters considered. The estimate of the parameter μ equals the overall average of the m sample means. It was assumed that each sample contains the n observations. The average of the sample arithmetic means was computed using the following equation:

$$\mu = \frac{\sum \bar{X}_j}{m} \quad (1)$$

The estimate of σ equals $\bar{S}$, which is computed using the following equation:

$$\bar{S} = \bar{I}/dn \quad (2)$$

The range I represents the difference between the maximum and minimum values of the sample, and d_n denotes the coefficient by which $\bar{I}$ is transformed into $\bar{S}$.

The lower and upper control limits are calculated using the following equation:

$$\mu \pm 3\frac{\bar{I}/d_n}{\sqrt{n}} \quad (3)$$

The $\bar{X}$ charts are usually accompanied by the process variability R charts. In the R chart, the center line is the average range of the m samples observed, whereas the lower and upper control limits are expressed using the transformation coefficient (f_n).

The lower and upper control limits of the process variability chart are calculated using the following equation:

$$\bar{I} \pm 3f_n\bar{I}/d_n \quad (4)$$

In the present study, the moisture and ash contents of the wheat flour type 500 were observed relative to the monitoring standards as defined in the HACCP plan. A flour mill in Novi Sad, used for the experimental purposes in this study, was monitored during July and August in 2018. The capacity of the mill is 100 tons per 24 hours, whereas its silo capacity approximates to 35,000 tons. The moisture and ash contents of the flour considered were measured over a 50-day period. Based on the data obtained for 250 observation units, a total of 50 groups of five units each were formed. Mill products that meet the quality requirements have a maximum moisture content of 15 % and a maximum ash content of 0.6 % (*Sl. Glasnik RS 56/2018*). The IBM SPSS 21.0 software was used for statistical data processing.

RESULTS AND DISCUSSION

The descriptive statistics results for the moisture and ash contents of the wheat flour type 500 are presented in Table 1. The results obtained indicate that the average moisture content of the wheat flour type 500 was 14.03 %, with a minimum moisture content of 3.13 % and a maximum moisture content of 1.67 % (a coefficient of variation of 5.61 %). Furthermore, the average ash content of the wheat flour type 500 was 0.49 %, with a minimum ash content of 0.36 % and a maximum ash content of 0.57 % (a coefficient of variation of 6.84 %).

Table 1. Descriptive indicators of the quality properties of the wheat flour type 500

	Mean	Range		Coefficient of variation (%)
		Minimum	Maximum	
Moisture content (%)	14.03	10.90	15.70	5.61
Ash content (%)	0.49	0.36	0.57	6.84

The data normality was tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The tests performed indicate that the parameter values examined had a *normal* probability *distribution* (Sig. > 0.05). The autocorrelation coefficients computed suggest that no autocorrelation of the data was found. The Levene's test results show the homogeneity of variance for both parameters considered (Sig. > 0.05), whereas the ANOVA results indicate the homogeneity of their means (Sig. < 0.05) (Table 2). The tests performed confirmed that the basic assumption about the use of control charts in the process considered was fully met. Upon the basic assumption testing, the control charts for the variations in and the arithmetic means of the parameter values considered were created. Fig. 1 shows the control chart for the average moisture content of the wheat flour type 500. The value of the center line on this chart is 14.03 %, whereas the lower and upper limit values are 12.95 % and 15.12 %, respectively. All the control points are located within the control limits, indicating the stability of the process.

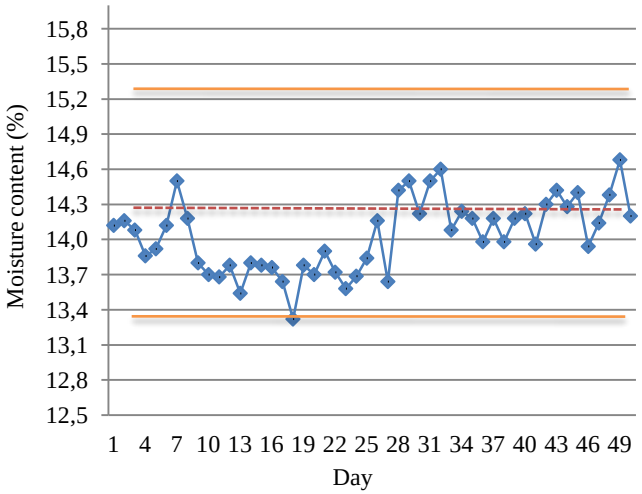

Fig. 1. Control chart for the average flour moisture content

Table 2. Basic assumption tests for the use of control charts

	Kolmogorov-Smirnov		Shapiro-Wilk		Levene's test		ANOVA	
	Statistic	Sig.	Statistic	Sig.	Statistic	Sig.	Statistic	Sig.
Moisture content (%)	0.096	0.200	0.980	0.543	0.453	0.532	24.627	0.003
Ash content (%)	0.108	0.200	0.963	0.121	0.875	0.467	11.729	0.001

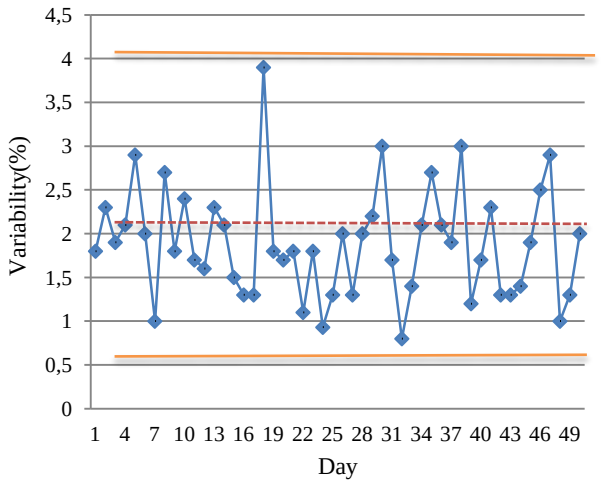

Fig. 2. Control chart for variations in the flour moisture content

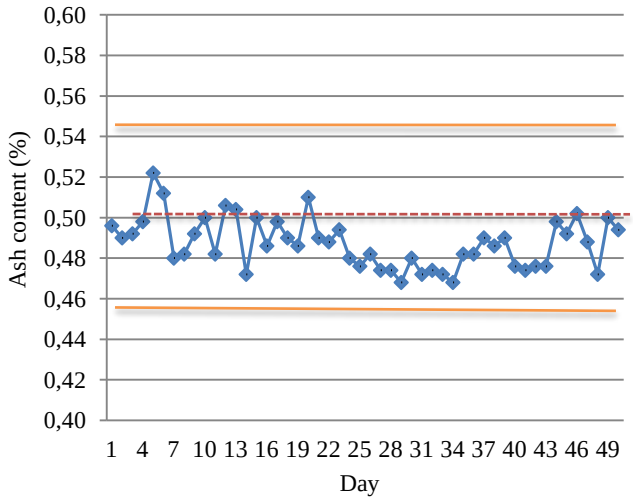

Fig. 3. Control chart for the average flour ash content

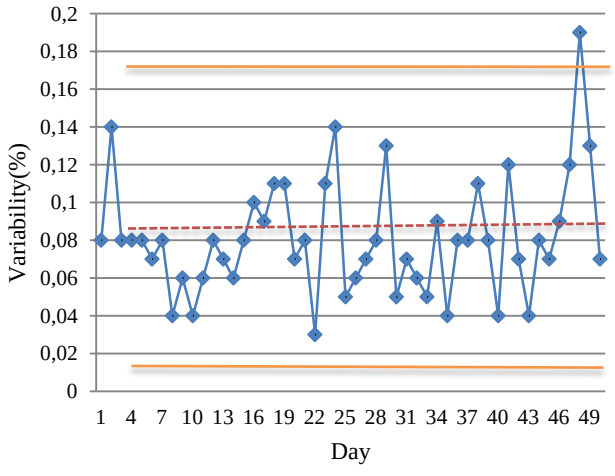

Fig. 4. Control chart for variations in the flour ash content

The control chart for variations in the flour moisture content (Fig. 2.) shows an average center line value of 1.88 % and control limit values of 0.00 % and 3.97 %, respectively. Although all the control points are within the control limits, a variability increase in this parameter value was recorded in the sample 18. Such occurrence should be further examined, as well as the quality of raw material used in the flour production considered. The control chart for the average ash content of the wheat flour type 500 (Fig. 3.) shows a center line value of 0.49 % and lower and upper limit values of 0.44 % and 0.53 %, respectively. The process was found to be stable with an acceptable content of flour ash. The control chart for variations in the flour ash content (Fig. 4) shows an average center line value of 0.08 %, and control limit values of 0.00 % and 0.17 %, respectively. A variability increase in this parameter value was recorded in the sample 48, indicating special variations and the need for further research. Special variations are most often caused by human errors or the use of poor quality raw material

CONCLUSION

In this study, the average moisture and ash contents of the wheat flour type 500 were 14.03 % and 0.49 %, respectively. On the basis of the calculated central line values and the lower and upper limit values, control charts for variations in and mean values of the parameters considered were created.

The control charts created for the mean parameter values indicate that the process of storing flour is in control, with acceptable contents of flour moisture and ash. However, the control charts for the variability in the parameter values considered show special variations, the causes of which require further research in order to eliminate them and thus render the flour storage process more stable in the future.

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FACTORS INFLUENCING TOMATO MARKETING IN ILE-IFE, OSUN STATE, NIGERIA

UTICAJNI FAKTORI PRODAJE PARADAJZA U ILE-IFE REGIJI, DRŽAVA OSUN U NIGERIJI

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ABSTRACT

This study was conducted to identify the factors influencing tomato marketing in Ile-Ife, Osun State, Nigeria. Primary data were collected from a total of 100 tomato marketers using a multistage sampling technique. The data were analysed using descriptive statistics, gross margin analysis and multiple regression analysis. The result obtained revealed that wholesalers had a higher gross margin than retailers. Gender, education level and transportation costs were found to influence the marketing margin of retailers, whereas household size and transportation costs influenced the marketing margin of wholesalers. The study therefore recommends that tomato marketers should be encouraged into wholesale marketing. This can be achieved by subsidization and the implementation of an efficient marketing system.

Key words: *tomato, retailer, wholesaler, gross margin, determinants, Ile-Ife region*

REZIME

Ova studija je sprovedena kako bi se identifikovali faktori koji utiču na prodaju paradajza u Ile-Ife, država Osun, Nigerija. Primarni podaci prikupljeni su od ukupno 100 prodavača paradajza koristeći tehniku višestrukog uzorkovanja. Podaci su analizirani koristeći opisnu statistiku, bruto maržu i višestruku regresionu analizu. Rezultat je pokazao da veletrgovci imaju veću bruto maržu od trgovaca na malo. Rezultat je takođe otkrio da su pol trgovca, nivo obrazovanja i troškovi prevoza uticali na maržu marketa na malo, dok su pol, veličina domaćinstva i troškovi prevoza uticali na maržu marketa na veliko. Studija je, dakle, preporučila da trgovce paradajzom treba motivisati da se bave prometom na veliko. To se može postići subvencionisanjem i uspostavljanjem efikasnog marketing sistema.

Ključne reči*: paradajz, maloprodaja, veletrgovac, bruto marža, determinante, Ile-Ife oblast.*

INTRODUCTION

Fruits and vegetables play a very important role in human nutrition and are considered a major source of essential vitamins and minerals. Vegetable production claims a substantial share (about 25 %) of the major food crops cultivated in the tropics, providing a source of livelihood to a considerable section of the population (*Shehu and Mohammed, 2017*). Among the different vegetables grown in Nigeria, the tomato (*Solanum lycopersicum*) is the most important vegetable in terms of scale of production and level of consumption (*Adejobi et al., 2011*). The tomato, which is grown either as a principal or secondary crop in Nigeria by most producers, can be consumed raw in salads or processed into paste or puree, which can be used for cooking savoury dishes (*Haruna et al., 2012; Shehu and Mohammed, 2017*).

Tomatoes and tomato-based foods provide a wide variety of nutrients and many health-related benefits to the body. The tomato contains a substantial amount of lycopene, which is a crucial antioxidant responsible for reducing the incidence of some chronic diseases such as cancer and many other cardiovascular disorders (*Arab and Steck, 2000; Basu and Imrhan, 2007; Freeman and Reimers, 2010*). In Nigeria, a total area of one million hectares is reportedly used for tomato cultivation, which accounts for about 18 percent of the average daily consumption of vegetables in Nigerian homes (*Chidi, 2012*). It is cultivated almost throughout Nigeria, thus generating employment both at urban and rural levels *(Adenegan and Adeoye, 2011).*

The tomato, like other agricultural products, has unique characteristics which pose certain problems to farmers, marketers (wholesalers and retailers) and final consumers alike. In addition to the lack of storage facilities, the seasonality of tomatoes exerts pressure on sales, with an attendant effect on the market price hindering large-quantity purchases by tomato sellers, thus discouraging farmers from large-scale production (*Obayelu et al., 2014*). Consequently, the tomato marketing system in Nigeria is poorly developed. Moreover, the Nigerian government has focused more on agricultural production with little emphasis on the follow-up marketing of vegetables such as tomatoes (*Idachaba, 2000*). This has resulted in reducing the profit margins of marketers who purchase vegetables mostly at the farm gate, where vegetable marketing practically begins (*Haruna et al., 2012*). Such costs incurred discourage vegetable marketers (namely wholesalers and retailers) from large-quantity vegetable purchases.

Tomato production and marketing involves a series of relationships and arrangements which are based on structure-conduct relationship paradigms at each marketing level, i.e. from the producers to the consumers. To ensure a stable and consistent supply of tomato throughout the year, tomato marketing needs to be considered across all the marketing levels and channels. According to *Haruna et al. (2012)*, this can best be achieved through a critical analysis of the factors influencing the profitability of tomato marketing, which would lead to maximum returns on vegetable and tomato sales for marketers.

The purpose of this study is to identify and analyze the factors influencing the marketing margin of tomato marketers (namely wholesalers and retailers) in Nigeria.

MATERIAL AND METHOD

Study Area

This study was conducted in Ile-Ife, Osun State, which is one of the major towns in the state where tomato marketing is predominantly carried out by marketers. A multistage sampling was used in selecting respondents. The first stage involved the sampling of all major markets in Ile-Ife, whereas the second stage involved a random selection of 100 respondents from all the major markets. Therefore, a total of 100 marketers were sampled. Primary data were collected using a pretested structured questionnaire. The data on respondents' socio-economic characteristics such as age, level of education, household size, marital status, selling price, purchase price, etc. were collected. A descriptive analysis of the data showed that there were more retailers (68.0 %) than wholesalers (32.0 %) (Table 1).

Table 1. Distribution of the respondents by type of seller

Type of seller	Frequency	Percentage
Wholesaler	32	32.0
Retailer	68	68.0
Total	100	100.0

Source: Field Survey, 2018

Method of Data Analysis

Various analytical tools and procedures were employed for this study. Descriptive statistics such as percentages, frequencies and means were employed to explain the socio-economic characteristics of the respondents. Budgetary analysis was used to determine the gross marketing margin of tomato marketers, whereas multiple regression analysis was used to determine the factors affecting the gross margin of tomato marketers.

Budgetary Technique

Budgetary technique was employed to determine the gross marketing margin of tomato marketing. Gross marketing margin measures the difference between the selling price and purchase price. The selling price was obtained by multiplying the unit price of each basket of tomato by the quantity sold, whereas the purchase price was obtained by multiplying the cost of each basket of tomato by the quantity purchased. The variable costs are those costs that vary with the total level of output and they include the cost of offloading/loading, association levy, agent fee and transportation. The addition of total variable costs and total fixed costs gives the tomato marketing costs incurred. Using the straight line method, the depreciation expenses were calculated on the fixed items, which were then used in the analysis.

The equations are:

$$SP=P_i \times Q_i \quad (1)$$

$$PP=C_i \times Q_i \quad (2)$$

$$TMC=TVC+TFC \quad (3)$$

$$GMM=SP-PP \quad (4)$$

where GMM is the gross marketing margin, TMC is the total marketing cost, TVC is the total variable cost, TFC is the total fixed cost, SP is the selling price, PP is the purchased price, C_i is the cost of tomato per unit of basket, P_i is the price per unit of basket of tomato, and Q_i is the price per unit quantity of tomato basket sold.

Regression Model

To identify the factors influencing the gross margin of tomato marketing, multiple regression analysis was used. The dependent variable was the computed gross margin for each marketer. A number of explanatory variables were identified and included in the model. The implicit function is given as follows:

$$Y = f(X_1, X_2, X_3, ---, X_6) \quad (7)$$

where
Y = Gross Marketing Margin;
X_1 = Age of tomato marketer (Years);
X_2 = level of education (1 = formal, 0 = otherwise);
X_3 = Household size (Number);
X_4 = Marketing experience (Years);
X_5 = Transportation cost (₦);
X_6 = Labour cost (₦);
e = Error term.

Different regression functions such as linear, exponential, semi-log and Cobb-Douglas were used. Of the regression functions used, the Cobb-Douglas regression function was adopted as the lead equation based on the highest coefficient of determination (adjusted R^2), with the highest F-statistics, the number of significant variables and the signs of the estimated coefficients.

RESULTS AND DISCUSSION

The mean age of retailers was 41.00 years, whereas that of wholesalers was 47.69 years. This finding indicates that respondents were mostly middle-aged and able-bodied. This is an indication of active youth involvement in tomato marketing activities. The gender distribution of the respondents presented in Table 2 show that all (100%) the marketers (retailers and wholesalers) considered were female. This implies that tomato marketing in the study area is predominantly a female-dominated enterprise. As tomato marketing is generally accepted to be gender-biased and largely associated with females, the results obtained herein are consistent with such claims made by *Adejobi et al. (2011)*. Previous research (*Adejobi et al., 2011; Achoja and Okoh, 2013; Camillus et al., 2014*) suggests that African women dominate small-scale agricultural marketing, but with less participation in the wholesaling of perishable items. The income they thus generate is mostly used to support the household and make some savings. The results obtained also indicate that the majority of the wholesalers and retailers interviewed were married ((93.8 % and 88.3 %, respectively). This suggests that they make use of cheap family labour, thus reducing overall labour costs**.** The majority of the tomato wholesalers (90.6%) and retailers (89.7%) were also found to have a formal education.

The mean household size of the tomato wholesalers and retailers was approximately 7 and 5 members, respectively. Although this could also suggest the employment of family labour in tomato marketing, *Ogundele and Okoruwa (2006)* report that a large family size does not necessarily translate into a higher use of family labour because some of the young able-bodied family members may prefer other jobs than marketing. The mean years of marketing experience of the wholesalers and retailers were 18 and 12 years, respectively. This indicates that the majority of the tomato marketers considered had been in the tomato marketing business for a long period of time.

Table 2. Socio-economic characteristics of the tomato marketers considered

Socio-economic characteristics	Wholesalers		Retailers	
	Frequency	Percentage	Frequency	Percentage
Age				
25 – 44	10		47	
45 – 64	22	31.3	21	69.1
Mean (Standard deviation)	47.69 (6.89)	68.7	41.00 (8.78)	30.9
Gender				
Male	0	0.0	0	0.0
Female	32	100.0	68	100.0
Marital status				
Single	2	6.2	8	11.7
Married	30	93.8	60	88.3
Level of education				
No formal education	3	9.4	7	10.3
Primary education	8	25.0	12	17.6
Secondary education	19	59.4	47	69.1
Tertiary education	2	6.3	2	2.9
Household size				
1 – 5	9	28.1	36	52.9
6 – 10	20	62.5	31	45.6
11 – 15	3	9.4	1	1.5
Mean (Standard deviation)	6.62 (2.27)		5.41 (1.57)	
Years of marketing experience				
1 – 20	24	75.0	61	89.7
21 – 40	8	25.0	6	8.8
41 – 60	0	0.0	1	1.5
Mean (Standard deviation)	18.0 (6.92)		12.0 (8.05)	

Source: Data Analysis, 2018

Analysis of the Marketing Margin of Tomato Retailers and Wholesalers

The marketing costs, estimated as the sum of variable costs and fixed costs, were ₦14,216.23 and ₦52,126.85 for the tomato retailers and wholesalers, respectively (1€≈420₦ - Nigerian naira). The selling prices were ₦169,529.40 and ₦984,625.00 for the retailers and wholesalers, respectively. However, the purchase prices were ₦140,835.30 and ₦805,937.50 for the retailers and wholesalers, respectively. The gross marketing margins were ₦28,694.10 and ₦178,687.50 for the retailers and wholesalers, respectively. However, the net marketing margins for the retailers and wholesalers were ₦14,447.84 and ₦126,560.65, respectively. The results obtained indicate that the wholesalers had a higher average marketing margin than the retailers. These results are consistent with those of *Obasi (2008) and Camillus et al. (2014)*, who reported that rice and tomato wholesalers had higher gross and net marketing margins than retailers.

Table 3. Analysis of the marketing margin of the tomato marketers considered

Item	Retailers	Wholesalers
	Average amount (₦)	Average amount (₦)
Variable Costs		
Cost of transportation	9,742.65	35,437.50
Cost of LG levy	479.12	993.91
Cost of loading/offloading	774.12	8,406.56
Cost of labour	302.94	5,587.50
Cost of nylon	2233.23	486.15
Association levy	92.06	170.37
Total Variable Costs	**13,624.12**	**51,081.99**
Fixed Costs		
Depreciation cost	146.03	233.66
Space rent	446.08	811.20
Total fixed costs	**592.11**	**1,044.86**
Total Marketing Costs	**14,216.23**	**52,126.85**
Purchase Price (PP)	140,835.30	805,937.50
Selling Price (SP)	169,529.40	984,625.00
Gross Marketing Margin (GMM)	**28,694.10**	**178,687.50**
Net Marketing Margin (NMM)	**14,447.87**	**126,560.65**
Percentage Marketing Margin	**16.93%**	**18.15%**

Source: Data Analysis, 2018

Factors Affecting the Marketing Margin of Tomato Marketers

The factors affecting the marketing margin of tomato retailers and wholesalers are shown in Table 4. The model employed had adjusted R^2 values of 0.9892 and 0.9854 for retailers and wholesalers respectively, indicating 98.92 % and 98.54 % of variation in the marketing margin of tomato retailers and wholesalers (as expressed by the explanatory variables included in the model). The models for the two different types of marketers were significant at the 1 % level, suggesting the models' goodness of fit. A total of six explanatory variables were included in the models, i.e. three per each type of tomato marketers considered: gender, level of education and transportation cost for the retailers, and gender, household size and transportation cost for the wholesalers.

The results obtained show that the gender coefficients for both tomato retailers and wholesalers were positive and statistically significant at the 1 % and 5 % levels, respectively. This implies that an increase in the number of female retailers and wholesalers will lead to an increase in the marketing margin accruable to tomato marketers. Furthermore, the coefficients of transportation costs for both types of tomato marketers considered were negative and statistically significant at the 10 % and 1 % levels, respectively. This implies that the marketing margin accruable to tomato retailers and wholesalers would reduce with increased transportation costs.

Although the education level coefficient for the tomato retailers was positively significant at the 5 % level, the coefficient of household size was negatively significant for the tomato wholesalers at the 1 % level. This indicates that the net

marketing margin accruable to the tomato retailers would increase with higher levels of their formal education, whereas a decrease in the number of wholesalers' household members would increase their marketing margin. The positive relationship between the retailers' level of education and their net marketing margin is consistent with the results of *Obasi (2008)*, who observed that higher levels of marketers' education improved their marketing strategy and compliance with marketing conditions.

Table 4. Factors influencing the marketing margin of the tomato marketers considered

Variable	Retailer		Wholesaler	
	Coefficient	t	Coefficient	t
Gender	8.726787	3.91***	8.410947	2.13**
Household size	.6684644	1.51	-1.165524	-2.94***
Level of education	.4620632	1.84**	.6428941	0.89
Years of experience	-.4279191	-1.55	.9240114	1.59
Transportation cost	-.2743932	-1.66*	-.1958655	-2.79***
Labour cost	.0243881	0.49	-.0169211	-0.18
R^2	0.9906		0.9891	
Adj R^2	0.9892		0.9854	
F-value	693.88		271.87	

Source: Data Analysis, 2018
****=Significant at 1 %;**=Significant at 5%; * = Significant at 10 %*

CONCLUSION

This study was conducted to identify the factors influencing tomato marketing in Ile-Ife, Osun State, Nigeria. The results obtained revealed that wholesalers had a higher gross margin than retailers. Gender, education level and transportation costs were found to influence the marketing margin of retailers, whereas household size and transportation costs influenced the marketing margin of wholesalers. The present study therefore recommends that tomato marketers should be encouraged into wholesale marketing. This can be achieved by subsidization and the implementation of an efficient marketing system. Tomato marketers should also be informed about the benefits of education and how it can be harnessed towards achieving maximum returns. Moreover, the government should assist tomato marketers by improving the transportation system in order to reduce transportation costs and transportation-related tomato quality losses.

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EFFECT OF LACTIC ACID FERMENTATION ON THE QUALITY OF BREWER'S SPENT GRAIN AS RUMINANT FEED

UTICAJ MLEČNO-KISELINSKE FERMENTACIJE NA KVALITET PIVSKOG TROPA KAO HRANIVA ZA PREŽIVARE

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ABSTRACT

Brewer's spent grain (BSG) was used in this study as a support for the immobilization of Lactobacillus paracasei NRRL B-4564, thus enabling the recirculation of immobilized biomass in repeated-batch fermentation. The chemical composition and the energy parameters of the fermented and non-fermented BSG were analyzed and compared. Moreover, the probiotic features of L. paracasei were analyzed to examine the possibility of using fermented BSG as a functional ingredient in ruminant diets.

The results obtained indicate that the fermented BSG had significantly higher protein and ash contents, as well as a significantly lower content of fiber fractions. Furthermore, the fermentation process increased the BSG energy content. The analysis of probiotic potential revealed a high tolerance of L. paracasei to pH 2.5 and bovine bile, autoaggregation ability and antimicrobial activity, suggesting that the fermented BSG with immobilized microbial biomass can be used as functional feed in ruminant diets.

Keywords: *brewer's spent grain, lactic acid, probiotics, ruminant feed*

REZIME

Globalna potražnja za hranom animalnog porekla raste kao posledica kontinuiranog rasta populacije, urbanizacije i porasta prihoda. Kako bi se zadovoljile potrebe tržišta, upotreba nekonvencionalnih hraniva i sporednih agro-industrijskih proizvoda u ishrani životinja postaje uobičajena praksa. U ovom radu je ispitivan uticaj mlečno-kiselinske fermentacije na kvalitet pivskog tropa kao hraniva za preživare. Pivski trop je korišćen kao nosač za imobilizaciju Lactobacillus paracasei NRRL B-4564, što je omogućilo recirkulaciju imobilisane mikrobne biomase u više uzastopnih šaržnih ciklusa. Po završetku poslednje fermentacione šarže, pivski trop sa imobilisanom biomasom je odvojen od fermentacionog medijuma i osušen, nakon čega su ispitivani hemijski sastav i energetski parametri relevantni za njegovu upotrebu u ishrani preživara. Dodatno, analizirana su probiotiska svojstva L. paracasei, kako bi se u potpunosti sagledala mogućnost primene fermentisanog pivskog tropa kao funkcionalnog hraniva.

Utvrđeno je da fermentisani pivski trop ima značajno veći sadržaj proteina i pepela, kao i znatno manji sadržaj svih frakcija vlakana u odnosu na nefermentisane uzorke. Takođe, fermentacija je dovela do povećanja sadržaja energije pivskog tropa. Analizom probiotskih karakteristika, utvrđeno je da L. paracasei ima visoku stopu preživljavanja pri pH 2.5 i u prisustvu goveđe žuči, sposobnost autoagregacije, kao i antimikrobnu aktivnost prema Gram-pozitivnim (Bacillus cereus) i Gram-negativnim (Escherichia coli) patogenim bakterijama.

Na osnovu fenotipskih karakteristika L. paracasei, kao i povoljnog uticaja mlečno-kiselinske fermentacije na kvalitet pivskog tropa, može se zaključiti da se fermentisani pivski trop sa imobilisanom mikrobnom biomasom može koristiti kao funkcionalno hranivo u obrocima namenjenim ishrani preživara.

Ključne reči: *pivski trop, mlečna kiselina, probiotici, hranivo za preživare.*

INTRODUCTION

The global demand for food of animal origin is increasing significantly due to the continued growth of the world population, urbanization, and economy. In the period 2005-2050, the global demand for meat and milk is expected to increase by 57 % and 48 %, respectively *(Mottet et al., 2017)*. Increased livestock production has become a great challenge, particularly for developing countries, imposing the use of different unconventional feed resources in animal nutrition. Using nutrient resources that are inedible for humans is a possible strategy for both reducing the competitiveness between food and feed and mitigating the environmental impacts of livestock production. Moreover, the costs of conventional feeds are very high and account for 60-70 % of the production costs in intensive dairy farming *(Salami et al., 2019)*. Therefore, supplanting common feed ingredients with some alternative feeds could largely contribute to the sustainability of the livestock industry.

A number of production residues and by-products are considered a great source of nutrients in livestock diets *(Čolović et al., 2018; Šćiban et al., 2013; Semenčenko et al., 2014)*. However, the vast majority of these side streams are still underutilized. The impediments for their broader application in animal farming entail the fluctuating nutrient composition and availability, high fiber contents and low digestibility, the presence of anti-nutritional compounds, a relatively short storage life, the need for further processing, and uncertain supplies. Different methods for tackling these obstacles have been

investigated. The fermentation with fungi or yeast *(Shrivastava et al., 2014)*, the supplementation with cellulases and hemicellulases *(Abdel-Aziz et al., 2015; Li et al., 2016)* and using inoculants containing selected strains of lactic acid bacteria (LAB) have shown promising results in improving nutrient content and digestibility of unconventional feeds. In addition to the favorable effects on the feed quality, many LAB are traditionally used as probiotic feed additives either in ruminant or monogastric diets, significantly contributing to host health through various mechanisms. The interest in using probiotics in the livestock industry has increased considerably, especially after the prohibition of antibiotics as growth promoters. A number of studies have shown that probiotics containing LAB prevent acidosis in ruminants fed with high-concentrate diets, increase weight gain, and reduce the incidence of diarrhea *(Abe et al., 2010; Lema et al., 2001; Stover et al., 2015)*.

Using brewer's spent grain (BSG) as a physical support for *Lactobacillus paracasei* NRRL B-4564, we examined the effects of lactic acid fermentation and associated biofilms on the quality of BSG as ruminant feed. The chemical composition and energy values of the fermented and non-fermented BSG were analyzed and compared. Moreover, the probiotic features of *L. paracasei* were assessed to determine the additional value provided by the microbial biomass attached to the BSG surface.

MATERIAL AND METHOD

Lactic acid fermentation with the recirculation of microbial biomass immobilized onto brewer's spent grain

Lactic acid fermentation was performed using a substrate composed of distillery stillage and sugar beet molasses. The distillery stillage and sugar beet molasses used were kindly provided by the Reahem ethanol plant (Reahem d.o.o., Srbobran, Serbia) and the Alpis-SLC ethanol plant (Swan lake d.o.o., Belgrade, Serbia), respectively. The distillery stillage was centrifuged (Sigma® model 2–16P, Osterode am Harz, Germany) and the resulting supernatant (thin stillage) was mixed with molasses in the ratio of 6:1. Dry BSG, provided by Carlsberg Serbia d.o.o., Čelarevo, Serbia, was used as a carrier for cell immobilization. The adsorption of *L. paracasei* NRRL B-4564 onto BSG was performed according to the previously described procedure *(Mladenović et al., 2017)*.

Lactic acid fermentation was conducted in the repeated-batch mode at an agitation speed of 150 rpm and a temperature of 41 °C (KS 4000i control, IKA®, Staufen, Germany), with the adjustment of pH to 6.5. After the sugar content in the media was reduced to less than 15 g/l, the BSG with immobilized biomass was separated by centrifugation and used as an inoculum for initiating the subsequent batch cycle. A total of five fermentation batches were performed. During the fermentation process, the concentration of total sugar and lactic acid and the number of immobilized cells were determined as described by *Mladenović et al., (2017)*.

Analysis of the fermented and non-fermented brewer's spent grain

After the last fermentation batch, the BSG with immobilized biomass was separated by centrifugation and dried in an oven to a constant mass. The total nitrogen content of the samples was estimated using the Kjeldahl method, and the factor of 6.25 was used to calculate the content of crude protein *(AOAC, 2005)*. The sample oil content was determined according to the Soxhlet method, and the sample ash content was estimated by the sample combustion in a muffle furnace at 650 °C *(AOAC, 2005)*. The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) of the samples were estimated using the Van Soest detergent method *(Goering and Van Soest, 1970)*. The digestible energy at production level of intake ($DE_{3\times}$), metabolizable energy at production level of intake ($ME_{3\times}$), and net energy for lactation at production level of intake ($NEL_{3\times}$) of the samples were calculated using the summative approach of the National Research Council *(NRC, 2001)*. The net energy for maintenance (NEm) and the net energy for growth (NEg) of the samples were calculated using the equations suggested by the *NRC, (1996)*. The non-fermented BSG was analyzed in the same fashion, serving as a control sample.

***In vitro* analysis of the probiotic features of *L. paracasei* NRRL B-4564**

Low pH and bile tolerance

The tolerance of *L. paracasei* NRRL B-4564 to low pH and bovine bile was studied according to the modified method of *Arasu et al., (2014)*. For this purpose, a total of three different MRS broths were prepared. For a low pH tolerance assay, the pH value of MRS broth was adjusted to pH 2.5 by addition of a 2 M HCl solution. For a bile tolerance test, the MRS broth was supplemented with 0.3 % (w/v) bovine bile (Torlak, Serbia), whereas the MRS with pH 6.5 was used as a control. The experiments were performed in 100 ml flasks containing 60 ml of sterile MRS broths, which were inoculated by the overnight culture of *L. paracasei* (5 % v/v). The samples were incubated under microaerophilic conditions at 37 °C for 4 h. The viable cell number at different points of incubation was determined using the pour plate method on MRS agar. The number of colonies was counted after incubation at 37 °C for 48 h. The survival of *L. paracasei* was expressed as a percentage of the control sample.

Antimicrobial activity

L. paracasei was propagated overnight in MRS broth at 37 °C under anaerobic static conditions. The culture was centrifuged (6000×*g*, 10 min), and the supernatant and neutralized supernatant were further subjected to the agar well diffusion assay. Two pathogenic bacteria (namely *Bacillus cereus* ATCC 11778 and *Escherichia coli* ATCC 25922) were used as indicator microorganisms. A ceramic well (5 mm diameter) was placed in a Petri dish, and the soft nutrient agar inoculated with 1 % (v/v) overnight culture of the indicator strain was poured and left to solidify. After removing the well, 100 µl of the cell-free supernatant was added in the hole. To confirm the production of antimicrobial compounds of proteinaceous nature, a small amount of crystalline proteolytic enzyme pronase E (4000 U mg^{-1}, Sigma-Aldrich, USA) was placed close to the edge of well. The Petri dishes were incubated at 37 °C for 12 h. A bright (halo) zone around the well indicated antimicrobial activity, whereas the absence of an inhibition zone around the pronase E confirmed the proteinaceous nature of the inhibitory compound.

Autoaggregation ability

The autoaggregation of *L. paracasei* was determined according to the procedure of *Collado et al., (2008)*. Briefly, the overnight culture of *L. paracasei* was centrifuged (6000×*g*, 10 min), the cells were washed in the sterile PBS buffer (pH 7.2) and resuspended in the same buffer to absorbance (A_{600nm})=0.25±0.25. Consequently, the concentration of cells in suspension was standardized to 10^7-10^8 CFU/ml. The cell suspensions (4 mL) were mixed briefly on a vortex mixer and incubated at 37 °C for 24 h. The autoaggregation process was monitored by measuring the absorbance at 600 nm. The percentage of autoaggregation was calculated as follows: $(1 - A_t/A_{0h}) \times 100$, where A_t is the absorbance at time t, whereas A_{0h} is the absorbance at t = 0h.

Statistical analysis

All the experiments and analytical methods were performed in triplicate. The results were presented as means ± standard deviation. The differences between the means were assessed using a one-way analysis of variance (ANOVA) followed by the Tukey's test. The *p*-values of less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The quality of brewer's spent grain as animal feed

This study was aimed to investigate the effect of lactic acid fermentation on the nutritional quality of BSG. The fermentation was performed using BSG as a physical support for *L. paracasei*, enabling the recirculation of immobilized biocatalyst in several successive batch cycles. The chemical composition and the energy parameters of the fermented BSG, alongside the immobilized biomass of *L. paracasei*, were assessed relative to their use in ruminant nutrition. The chemical analysis revealed that lactic acid fermentation considerably affected the composition of BSG (Fig. 1). The fermented BSG had significantly higher crude protein and ash contents compared to the non-fermented sample. Simultaneously, the fiber content of the fermented BSG sample was significantly lower after lactic acid fermentation. Among the cell wall fractions, the content of NDF was the most affected, followed by ADL, and ADF. The fermentation did not cause significant changes in the oil concentration of BSG.

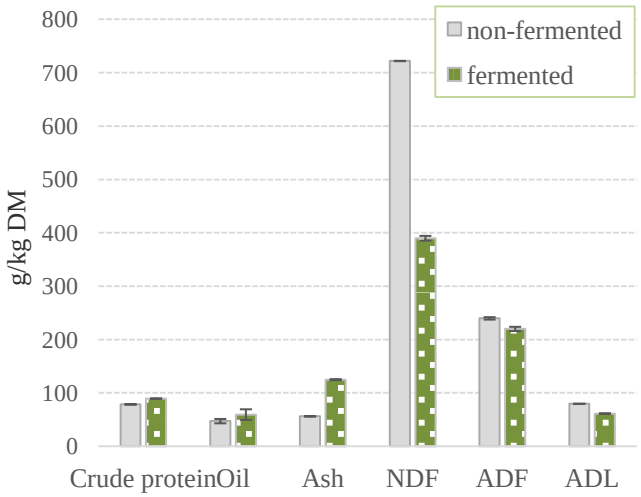

Fig. 1. Chemical composition of the fermented and non-fermented brewer's spent grain

Agro-industrial residues and by-products are often characterized by high fiber and low protein contents, suggesting their poor nutritional quality. Many of these residues are not suitable for monogastric animals and, in some cases, they are not even convenient for ruminant nutrition due to their low digestibility *(Villas-Bôas et al., 2002)*. Therefore, the main challenge of using agro-industrial residues and by-products in animal nutrition is enhancing their nutritional value, digestibility and dry matter intake. The protein content of the fermented BSG was 13.7 % higher than that of the non-fermented BSG sample. In addition, the content of fiber fractions of the fermented BSG sample was decreased by 8.26-46.09 %, indicating that lactic acid fermentation had a very favorable effect on the quality of BSG. *L. paracasei* NRRL B-4564 is not capable of producing cellulolytic enzymes, so the reduction of fiber fractions in BSG was not a result of enzymatic hydrolysis. On the other hand, *L. paracasei* NRRL B-4564 has been proven to be capable of producing lactic acid in high concentrations *(Mladenović et al., 2017)*, indicating that a decrease in fiber content could be a result of acid hydrolysis during repeated batch fermentation. Similar to the findings of this study, *Liu et al., (2015)* reported that treatments of rice straw using LAB improved its feed quality and digestibility, as evidenced by higher crude protein concentrations and lower contents of NDF and ADF.

The energy values of the non-fermented and fermented BSG samples were calculated following the NRC recommendations and nutrient requirements of dairy and beef cattle. It can be seen that the predicted energy parameters were significantly higher after lactic acid fermentation (Fig. 2). The energy values of the feed are related to its chemical characteristics, and in general, these values are lower in the feed having higher fiber contents. In the present study, the reuse of BSG with immobilized biomass in successive fermentation batches led to significant reductions in the fiber content of BSG, consequently improving its energy value.

The number of viable cells per gram of BSG at the end of the fifth fermentation batch was very high and amounted to 5×10^{10} CFU/g, indicating a satisfactory adsorption ability of *L. paracasei* and a stable cell attachment on the BSG surface. In addition to the improved chemical composition and energy content of BSG, the biomass of *L. paracasei* NRRL B-4564 attached to its surface could provide an additional advantage to animal diet. To fully consider the possibility of using fermented BSG as a functional feed ingredient, the probiotic characteristics of *L. paracasei* NRRL B-4564 strain were further assessed.

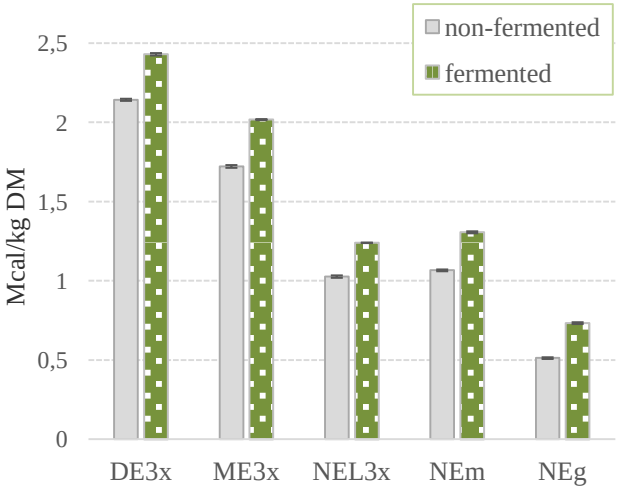

Fig. 2. Energy values of the fermented and non-fermented brewer's spent grain

Probiotic potential of lactic acid-producing strain

In order to provide health benefits when consumed, the probiotic microorganisms should be able to survive the passage through the gastrointestinal tract (GIT), i.e. the acidic environment of the stomach and the presence of bile salts in the small intestine. In an attempt to evaluate the ability of *L. paracasei* NRRL B-4564 to survive the unfavorable environment of the GIT, the strain was exposed to a pH value of 2.5 and a bovine bile concentration of 0.3 %. The results of acid and bile tolerance are presented in Fig. 3. *L. paracasei* exhibited high tolerance to low pH value and bovine bile during 4 h of exposure. After 4 h of incubation, the survival rates of *L. paracasei* at pH 2.5 and 0.3 % of bovine bile were 91 % and 96 % respectively, compared to a control sample. The ability of LAB to survive the conditions of the GIT was reported to be very variable, even within the same bacterial species, indicating

that these characteristics are strain-specific *(Mishra and Prasad, 2005; Ren et al., 2014)*. *Jacobsen et al., (1999)* reported a complete loss of viability for 15 out of the 44 tested strains after 4 h of the exposure to pH 2.5, whereas almost all the examined strains survived the bile salt concentration up to 0.3 %. The high survival rate of *L. paracasei* NRRL B-4564 under the testing conditions of the present study (Fig. 3) indicates that this strain can provide health benefits when consumed, thus confirming its potential as a probiotic additive.

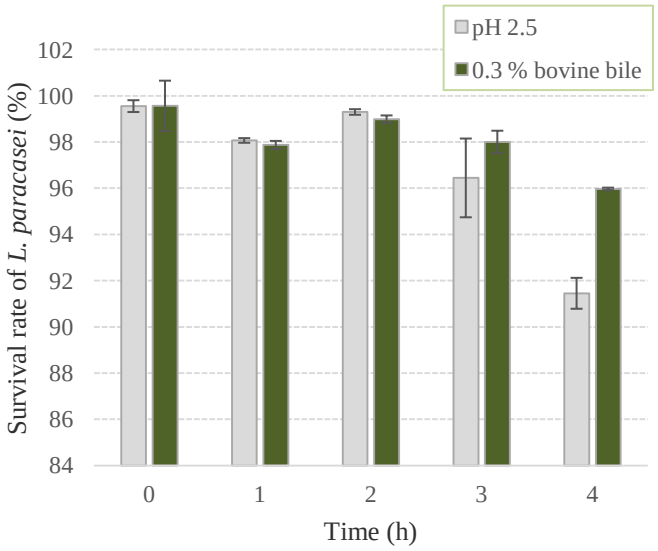

Fig. 3. Survival of L. paracasei NRRL B-4564 at low pH value and in the presence of bovine bile

The ability of bacterial cells to attach or adhere to the intestinal surface is another essential feature of probiotic strains. In many cases, this feature is related to the aggregation ability of a probiotic strain *(Del Re et al., 2000; Pérez et al., 1998)*. The ability of probiotic strains to aggregate is considered a desirable characteristic as such strains can potentially inhibit the binding of pathogenic microorganisms to intestinal surfaces. This can be achieved either by forming a barrier via autoaggregation, coaggregation with commensal microorganisms on the intestinal mucosa, or providing a direct coaggregation with intestinal pathogens, thereby facilitating their removal from GIT. The autoaggregation ability of *L. paracasei* NRRL B-4564 was estimated by measuring the optical density of cell suspensions for 24 h (Fig. 4). It can be observed that the percentage of autoaggregation increased during the incubation period. A very intensive formation of aggregates occurred during the first 3 h when an autoaggregation percentage of 20.7 % was achieved. The percentage of autoaggregation of *L. paracasei* after 24 h of incubation in the PBS buffer (44.1 %) obtained in the present study was close to the values previously reported for *L. casei* (40.4 %), *L. acidophilus* (33.5 %), *L. rhamnosus* (38.7 %) and *L. salivarius* (54.5 %) *(Collado et al., 2008)*. It is noteworthy that high autoaggregation values are not necessarily associated with the *in vivo* adhesion, which involves various host factors such as defense mechanisms, resident (permanent) microbiota and peristaltic movements. These factors can significantly affect and modify bacterial cell adhesion to the intestinal surface *(Verón et al., 2017)*. For the evaluation of the *L. paracasei* antimicrobial activity, the cell-free supernatants were tested against two indicator pathogenic microorganisms using the agar well diffusion method. The results obtained indicate that the cell-free supernatant at the acidic pH inhibited the growth of Gram-positive (*B. cereus*) and Gram-negative bacteria (*E. coli*). The supernatant of *L. paracasei* NRRL B-4564 was not sensitive to pronase E.

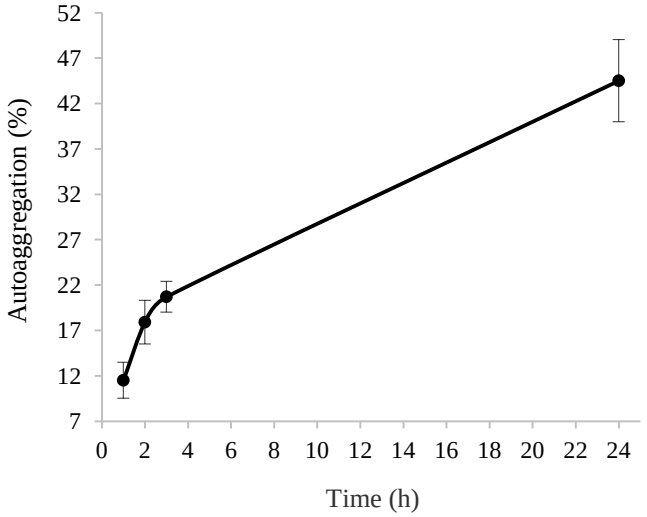

Fig. 4. Autoaggregation of L. paracasei NRRL B-4564 during the incubation in the PBS buffer

Moreover, the absence of an inhibition zone around the well containing the neutralized cell-free supernatant confirmed that the antimicrobial effect was caused by the produced lactic acid and not by the compounds of proteinaceous nature such as bacteriocins. Lactic acid is a major metabolite of many *Lactobacillus* and *Bifidobacterium* species, leading to a decrease in pH and the associated antimicrobial effect *(Tejero-Sariñena et al., 2012)* (which significantly contributes to the safety of fermented products and the stability of the gut microbiota *(Bendali et al., 2011; Cizeikiene et al., 2013))*. The mechanism of the inhibitory effect caused by lactic acid is based on the fact that the undissociated form of acid diffuses through the cell membrane and dissociates within the cell releasing of H^+ ions, which creates an acidic environment within the cell. In addition to the pH effect, undissociated acid disrupts the electrochemical proton gradient, causing bacteriostasis and, ultimately, the death of susceptible bacteria *(Magnusson and Schnurer, 2005)*.

According to the phenotypic characteristics of *L. paracasei* NRRL B-4564 confirmed in this study, the microbial biomass attached to the BSG surface could be of additional value to host health.

CONCLUSIONS

In the present study, the possibility of improving the quality of BSG as an animal feed ingredient was studied. The results obtained indicate that using BSG as a support for *L. paracasei* NRRL B-4564 in repeated-batch fermentation significantly improved its nutrient composition and energy value. The findings obtained on the probiotic features of *L. paracasei* NRRL B-4564 suggest that fermented BSG and the *L. paracasei* biomass attached to its surface can be used as a functional ingredient in ruminant diets.

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POTENTIAL OF DIFFERENT *Xanthomonas campestris* STRAINS FOR XANTHAN BIOSYNTHESIS ON WASTE GLYCEROL FROM BIODIESEL PRODUCTION

POTENCIJAL RAZLIČITIH *Xanthomonas campestris* SOJEVA ZA BIOSINTEZU KSANTANA NA OTPADNOM GLICEROLU IZ PROIZVODNJE BIODIZELA

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ABSTRACT

A rapid expansion of the biodiesel industry has created various ecological issues relative to crude glycerol disposal. Xanthan biosynthesis is considered one of the sustainable solutions for minimizing the adverse effects of waste crude glycerol on the environment. The initial phase of xanthan production on crude glycerol entails the screening of producing microorganism. Therefore, the purpose of this study is to examine the possibility of xanthan production on a crude glycerol-based medium using different Xanthomonas campestris strains. The bioprocesses performed were assessed according to the rheology of the media considered, amounts of xanthan produced and conversion degrees of the most important nutrients present. The pseudoplastic behavior of all the media considered, the amounts of xanthan produced (5.22-7.67 g/L) and the degrees of crude glycerol, total nitrogen and phosphorus conversion (34.44-57.61 %, 23.04-30.35 % and 18.20-22.28 %, respectively) suggest that crude glycerol, after additional bioprocess optimization, can be a suitable raw material for the industrial production of xanthan.

***Key words**: waste utilization, crude glycerol, xanthan production, Xanthomonas campestris*

REZIME

Ubrzana ekspanzija industrije biodizela tokom poslednjih nekoliko decenija rezultovala je različitim ekološkim problemima usled odlaganja velikih količina netretiranih efluenata, naročito sirovog glicerola. Kako bi se smanjio negativan uticaj na životnu sredinu, predložene su brojne strategije za upravljanje sirovim glicerolom koje su u skladu sa konceptom održivog razvoja. Među njima je biosinteza ksantana jedno od perspektivnih rešenja. Prvi korak u razvoju postupka proizvodnje ksantana na sirovom glicerolu je skrining proizvodnih mikroorganizama. Stoga je cilj ovih sitraživanja bio ispitivanje mogućnosti proizvodnje ksantana na medijumu čija je osnova sirovi glicerol primenom različitih Xanthomonas campestris sojeva. U okviru eksperimentalnog dela, izvedena je kultivacija četiri soja Xanthomonas campestris izolovana sa listova različitih biljaka iz porodice kupusnjača (CB, CF, 12-2 i Xp3-1) na medijumu sa sirovim glicerolom kao izvorom ugljenika. Biosinteza ksantana izvedena je u Woulff-ovim bocama zapremine 2,0 l u aerobnim uslovima, submerznom tehnikom kultivacije, pri optimalnim vrednostima procesnih parametara u toku 168 h. Reologija kultivacionog medijuma, količina proizvedenog ksantana i stepen konverzije važnih nutrijenata su određeni kako bi se ispitala uspešnost izvedenih bioprocesa. Pseudoplastično ponašanje svih medijuma, dobijene vrednosti za količinu ksantana (5,22-7,67 g/l) i stepeni konvezije sirovog glicerola, ukupnog azota i ukupnog fosfora (34,44-57,61%, 23,04-30,35% i 18,20-22,28%, respektivno) ukazuju na to da sirovi glicerol, nakon dodatne optimizacije bioprocesa, može biti pogodna sirovina za proizvodnju ksantana u uvećanim razmerama, što ukazuje da rezultati dobijeni u ovim istraživanjima predstavljaju osnovu za potencijalnu industrijalizaciju ispitivanog biotehnološkog postupka.

***Ključne reči:** iskorišćenje otpada, sirovi glicerol, proizvodnja ksantana, Xanthomonas campestris.*

INTRODUCTION

Increased global concerns about growing energy demands, limited reserves of fossil fuels and negative impacts of the conventional fossil fuels use on the environment (particularly greenhouse gas emissions) have placed a new emphasis on alternative energy sources. Liquid biofuels produced from renewable resources are considered important alternative energy sources (*Quispe et al., 2013*). Therefore, biodiesel has recently been receiving increased attention because it is renewable, sustainable, biodegradable, efficient, environmentally friendly, and directly usable in diesel engines without major engine modifications (*da Silva et al., 2009*). Accordingly, the biodiesel industry has become one of the most rapidly growing industries in the world, with a global biodiesel production of about 25.2 million tons in 2014 (indicating a constantly growing trend) (*He et al., 2017*).

The biodiesel production involves the utilization of different categories of catalysts such as alkalis, acids, and enzymes. Alkalis and acids are divided into homogeneous and heterogeneous catalysts. Due to their high activity and low costs, sodium hydroxide and potassium hydroxide are the most commonly used catalysts for the transesterification of oils in biodiesel production (*Chatzifragkou and Papanikolaou, 2012*). Homogeneous base catalysts can be corrosive, and thus their removal from the resulting biofuel is particularly problematic (often resulting in the formation of stable emulsions and soaps). Heterogeneous catalysis is more efficient as it ensures an easier separation of the catalyst from the final product. Moreover, the possibility of reusing this catalyst renders the process of

heterogeneous catalysis economically justified (*Sulaiman et al., 2020*).

Owing to the increasing global demand for biodiesel and the necessity for larger biodiesel production capacities, the appropriate utilization of generated effluents is of paramount importance to the competitiveness of biodiesel in the market and the economic and environmental sustainability of commercial biodiesel production (*Hejna et al., 2016*). Crude glycerol is the main by-product of the biodiesel industry. On balance, crude glycerol accounts for a 10-20% share of the total biodiesel production. Increased biodiesel production results in large surpluses of crude glycerol, which poses a serious environmental hazard and thus a disposal issue (*Quispe et al., 2013*).

Glycerol from biodiesel production is an impure compound requiring significant assets for purification, which impedes its use in the food and pharmaceutical industries (*Brandao et al., 2013*). Therefore, the use of this effluent as a carbon and energy source for microbial growth in industrial biotechnology is a more suitable application. However, the presence of different impurities (namely methanol, ethanol, inorganic salts, metals, long chain fatty acids and soaps) in crude glycerol may inhibit the metabolic activity of producing strains, which can result in the reduced biomass growth and bioconversion of glycerol into desired products (*Konstantinović et al., 2016*). Accordingly, the use of this effluent as a carbon and energy source for microbial growth is possible provided producing microorganisms are tolerant to impurities in crude glycerol or additional treatments are performed. Untreated glycerol may substitute conventional carbohydrates (namely glucose, sucrose and starch) in some bioprocesses, e.g. the production of bioethanol, butanol, biosurfactants, pigments and some acids such as lactic, citric, propionic and succinic acids (*da Silva et al., 2009*). In addition, crude glycerol is an interesting substrate often exploited in xanthan production (*de Jesus Assis et al., 2014*). The bioconversion of glycerol into xanthan and other valuable bioproducts reduces the production costs of desired metabolites and mitigates the ecological impacts caused by the accumulation of this industrial stream in the environment (*Johnson and Taconi, 2007; Hejna et al., 2016*).

Xanthan is the most commercially produced microbial polysaccharide obtained by the *Xanthomonas campestris* cultivation. It is widely used as a thickening or stabilizing agent in the food, pharmaceutical, and oil-recovery industry. This biopolymer is well-known for its unique rheological properties or pseudoplastic behavior, i.e. high viscosity at low shear, stability over a broad range of temperature and pH value, and high resistance to shear degradation in aqueous solutions (*García-Ochoa et al., 2000; Petri, 2015*). Xanthan is frequently employed in the food industry to obtain a higher viscosity of food products (*Gilani et al., 2011*).

On an industrial scale, xanthan is generally produced in a batch mode under aerobic conditions by the submerged cultivation of the pure bacterial culture *Xanthomonas campestris* in bioreactors with mechanical mixing on appropriately formulated media under optimal conditions (*Ozdal and Kurbanoglu, 2018*). The only effluent that remains after xanthan biosynthesis is stillage. The stillage from xanthan production is comparable to distillery stillage and is characterized by high values of chemical and biological oxygen consumption, low pH values and a high content of organic matter. There are numerous treatments for the degradation and utilization of organic matter present in the stillage, but the production of high-value products is one of the most promising alternatives (*Đuran et al., 2018*).

Xanthan production is usually performed using the reference strain *Xanthomonas campestris* ATCC 13951 on a medium with glucose or sucrose (*Kumara et al., 2012*). However, the increasing market price of and the demand for these sugars suggest that they may no longer be economically acceptable raw materials. In order to reduce the costs of medium preparation and overall xanthan production, the use of different waste streams as less expensive carbon sources are recommended (*Reis et al., 2010*). Their underutilization can be accounted for by the inability of the reference strain to successfully metabolize other carbon sources such as glucose or sucrose (*Roseiro et al., 1992*). In addition, it is important to note that xanthan can be produced by different bacteria of the genus *Xanthomonas* such as *Xanthomonas campestris*, *Xanthomonas phaseoli* and *Xanthomonas malvacearum.* However, *Xanthomonas campestris* is the most widely used for the industrial production of xanthan (*Leela and Sharma, 2000*). All the *Xanthomonas campestris* strains are phytopathogenic and infect various plants, some of which are important for agriculture such as cabbage, tomatoes, beans and peppers (*García-Ochoa et al., 2000*).

The purpose of this study is to examine the possibility of xanthan biosynthesis on a crude glycerol-based medium using different *Xanthomonas campestris* strains. The bioprocesses performed were assessed according to the rheology of the media considered, amounts of xanthan produced and conversion degrees of the most important nutrients present.

MATERIAL AND METHOD

Producing microorganism

A total of four *Xanthomonas campestris* strains isolated from different cruciferous plants (CB, CF, 12-2 and Xp3-1) were used as the producing microorganisms in the experiments conducted. The cultures were stored at 4 °C on an agar slant (YMA®, HiMedia, India) and subcultured at four-week intervals.

Cultivation media

A commercial medium (YMB®, HiMedia, India) was used for the inoculum preparation, whereas xanthan production was performed on a crude glycerol-based medium as the sole carbon source. A total of 20.0 g/L of crude glycerol was used, which was obtained from a domestic biodiesel factory (Belgrade, Serbia) that uses waste oil as raw material for biodiesel production. The following properties of the crude glycerol considered were determined: a glycerol content of 60.88 % (w/w), a phosphorus content of 0.015 % (w/w), a sulfur content of 1.58 mg/kg, a sodium content of 199.83 mg/kg, a potassium content of 3.73 % (w/w), a calcium content of 6.76 mg/kg, a magnesium content of 2.31 mg/kg, a moisture content of 4.84 % (w/w), a methanol content of 0.45 % (w/w), an organic matter content of 95.12 % (w/w), a density of 1.12 g/cm^3 and a kinematic viscosity of 74.97 mm^2/s. The cultivation medium also contained yeast extract (3.0 g/L), $(NH_4)_2SO_4$ (1.5 g/L), K_2HPO_4 (3.0 g/L) and $MgSO_4 \cdot 7H_2O$ (0.3 g/L). The pH value of the medium was adjusted to 7.0 ± 0.2 and then sterilized by autoclaving at 121 °C and 2.1 bar for 20 min.

Xanthan production

The xanthan production was carried out in a 2.0 L Woulff bottle with 1.5 L of the cultivation medium. The sterile medium was inoculated by adding 10 % (v/v) of inoculum prepared by a double passage procedure under aerobic conditions at 25 °C and 150 rpm (using the laboratory shaker KS 4,000i control, Ika® Werke, Germany) for 48 h. The biosynthesis was carried out in a batch mode under aerobic conditions (an air flow rate of 1 vvm in the first 48 h, and 2 vvm afterwards) for 168 h. In the first 48 h, the temperature was 25 °C and the agitation rate was 200 rpm, which thereafter increased to 30 °C and 300 rpm, respectively.

Xanthan separation

At the end of biosynthesis, the xanthan obtained was separated from the cultivation medium supernatant by precipitation with cold 96 % (v/v) ethanol in the presence of potassium-chloride as electrolyte. The supernatant was obtained using an ultracentrifuge (Hettich Rotina 380 R, Germany) at 10,000 rpm for 15 min. Ethanol was gradually added to the supernatant with constant stirring until the alcohol content in mixture was 60 % (v/v). Prior to this, a saturated solution of potassium-chloride was added into the cell-free supernatant to obtain a final content of 1 % (v/v). The obtained mixture was kept at 4 °C for 24 h in order to dehydrate the precipitated xanthan, and then centrifuged at 4,000 rpm for 15 min (Tehtnica LC-321, Slovenia). The precipitated biopolymer was dried at 60 °C to a constant weight in order to determine the quantity of xanthan produced. The ethanol used for xanthan precipitation was recycled by distillation.

Determination of the cultivation medium rheological properties

The rheological behavior of the cultivation medium samples taken at the end of the bioprocesses performed were evaluated using a rotational viscometer (REOTEST 2 VEB MLV Prufgerate-Verk, Mendingen, SitzFreitel) with the double gap coaxial cylinder sensor system spindle N. Relative to the measuring instrument deflection (α, Skt), the shear stress (τ, Pa) was calculated (at the specific values of shear rates (D, 1/s)) using the following equation:

$$\tau = 0.1 \cdot z \cdot \alpha \quad (1)$$

where z is a constant of 3.08 dyn/cm^2·Skt. The pseudoplastic behavior of the cultivation medium was confirmed by fitting the experimental data to the Ostwald-de-Waele model using the power regression. The values of the consistency factor (K, Pa·s^n), the flow behavior index (n) and the coefficient of determination (R^2) were determined using Excel 2013, which was employed for computing the medium apparent viscosity (η_a, mPa·s) using Eq. 2:

$$\eta_a = K \cdot D^{n-1} \quad (2)$$

where D is a shear rate of 100 1/s.

Determination of nutrients content

The samples of cell-free cultivation media taken after inoculation and 168 h of cultivation, obtained by centrifugation at 10,000 rpm for 15 min (Rotina 380 R, Hettich Lab Technology, Germany), were analyzed for crude glycerol, total nitrogen and total phosphorus contents.

The glycerol content was determined using the high pressure liquid chromatography (HPLC). Prior to analysis, the samples were filtered through a 0.45 μm nylon membrane (Agilent Technologies Inc, Germany). The HPLC instrument (Thermo Scientific DionexUltiMate 3000 series) was equipped with the HPG-3200SD/RS pump, the WPS-3000(T)SL autosampler (10 μL injection loop), the ZORBAX NH2 column (250 x 4.6 mm, 5 μm) (Agilent Technologies Inc, USA) and the Refracto Max 520 detector (ERC Inc, Japan). The acetonitrile solution (70 % v/v) was used as eluent at a flow rate of 10 mL/min and the elution time of 10 min at a column temperature of 30 °C.

The nitrogen content was determined using a method proposed by Kjeldahl (*Helrich, 1990*), whereas the phosphorus content was estimated using the spectrophotometric method (*Gales et al., 1966*).

The nutrient content results were used to calculate crude glycerol, total nitrogen and total phosphorus conversion (K, %) using Eq. 3:

$$K = \frac{(S_0 - S)}{S_0} \cdot 100 \quad (3)$$

where S_0 is the initial nutrient content (g/L), whereas S is the residual nutrient content (g/l).

Statistical analysis

All the experiments were carried out in triplicate and the results were averaged. The data obtained were processed using the analysis of variance (One-Way ANOVA). Significant differences between the means were determined by the Duncan's multiple range test at a significance level of α = 0.05 using the Statistica 13.2 software (Dell Inc., USA).

RESULTS AND DISCUSSION

The xanthan production under the experimental conditions was evaluated according to the rheological behavior of the crude glycerol-based media after the cultivation of the *Xanthomonas campestris* strains considered. The rheological properties were determined relative to the shear rate and shear stress values obtained (Figure 1). The flow curves represent a pseudoplastic flow type, which is a well-known property of xanthan solutions (*García-Ochoa et al., 2000*).

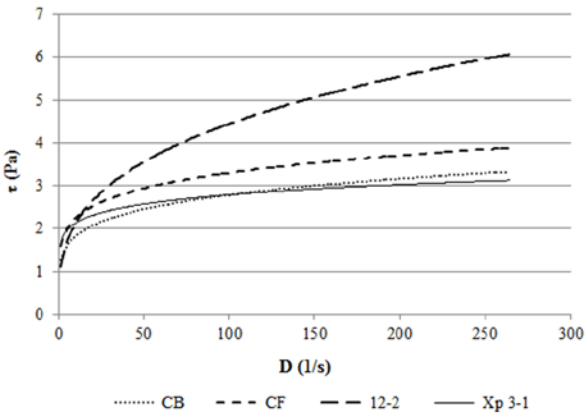

Figure 1. Effect of the shear rate on shear stress for the crude glycerol-based media considered after the cultivation of different Xanthomonas campestris strains

The pseudoplastic properties of the crude glycerol-based cultivation media were also confirmed by the flow behavior index values (n) shown in Table 1. The flow behavior index represents a level of deviation from the Newtonian flow behavior. The flow behavior index is equal to 1 for Newtonian fluids, greater than 1 for dilatants, and less than 1 for pseudoplastic fluids (*Björn et al., 2012*). The flow behavior index values obtained for the media samples analyzed in this research were in the range of 0.1178-0.3204.

As the consistency factor is proportional to the viscosity, the different values of this parameter shown in Table 1 indicate the difference in the quantity and quality of xanthan produced under the experimental conditions by the cultivation of different *Xanthomonas campestris* isolates on a crude glycerol-based medium. The Ostwald-de-Waele model results were in good agreement with the experimental data because the determination coefficients were higher than 0.90 in all the tests conducted (Table 1). Moreover, the values of the apparent viscosity in Table 1 indicate that the highest cultivation medium viscosity

(44.41 mPa·s) was obtained when using the strain 12-2, whereas the lowest apparent viscosity of the cultivation medium (27.87 mPa·s) was achieved by the CB strain cultivation.

Table 1. Rheological parameters of the crude glycerol-based media after the cultivation of different Xanthomonas campestris strains

Producing microorg.	Consistency factor, K [Pa·s^n]	Flow behavior index, n [1]	Coefficient of determination, R^2	Apparent viscosity, η_a [mPa·s]
CB	1.2005	0.1829	0.925	27.87
CF	1.3609	0.2311	0.930	39.45
12-2	1.0156	0.3204	0.924	44.41
Xp 3-1	1.6207	0.1178	0.916	27.88

The values of glycerol, total nitrogen and total phosphorus conversion in the crude glycerol-based media after the cultivation of different *Xanthomonas campestris* strains are shown in Table 2. In addition to xanthan production, the conversion of important nutrients is a very significant indicator of the bioprocess success. The results obtained indicate that the glycerol, total nitrogen and total phosphorus content of the media considered decreased during the xanthan biosynthesis. It can be seen that the glycerol conversion ranged from 34.44 % to 57.61 %, the nitrogen conversion ranged from 23.04 % to 30.35 %, and the phosphorus conversion ranged from 18.20 % to 22.28 %. The highest conversion values of crude glycerol (57.61 %), total nitrogen (30.35 %) and total phosphorus (22.28 %) were obtained when the Xp 3-1 strain was cultivated under the experimental conditions. This value of crude glycerol conversion is greater than that obtained in previous research when the reference strain *Xanthomonas campestris* ATCC 13951 was applied on a crude glycerol-based medium (*Zahović et al., 2019*). The lowest values of crude glycerol (34.44 %), total nitrogen (23.04 %) and total phosphorus (18.20 %) conversion were obtained using the CF, CB and 12-2 isolates, respectively. Although all the nutrient conversion values obtained are not high, the cultivation of the *Xanthomonas campestris* strain Xp3-1 on the crude glycerol-based medium considered results in reduced glycerol, nitrogen and phosphorus contents, which is important for minimizing the negative impact of such waste on the environment.

Table 2. Conversion degree of the most important nutrients present in the crude glycerol-based media considered after the cultivation of different Xanthomonas campestris strains

Producing microorganism	Crude glycerol conversion [%]*	Total nitrogen conversion [%]*	Total phosphorus conversion [%]*
CB	48.75 ± 0.67^b	23.04 ± 0.37^a	19.81 ± 0.54^c
CF	34.44 ± 0.43^a	24.64 ± 0.35^b	19.01 ± 0.42^b
12-2	35.31 ± 0.44^a	26.12 ± 0.62^c	18.20 ± 0.31^a
Xp 3-1	57.61 ± 0.54^c	30.35 ± 0.51^d	22.28 ± 0.41^d

**Values in the same column marked with the same letter are not significantly different at α = 0.05 according to the Duncan's multiple range test.*

According to the experimental plan, xanthan was precipitated and the conversion of sugar into product was calculated in order to determine the success of the performed bioprocesses. The results obtained are summarized in Table 3. The highest xanthan concentration in the crude glycerol-based medium (7.67 g/L) was obtained using the Xp 3-1 strain. The results values are higher than the previously published data which showed that the cultivation of the reference strain *Xanthomonas campestris* ATCC 13951 on the crude glycerol resulted in the accumulation of 6.68 g/L xanthan in the medium (*Zahović et al., 2019*).

Sugar conversion into product represents the amount of carbon sources converted into xanthan. According to the results shown in Table 3, the initial sugar conversion into the desired metabolite under the experimental conditions ranged from 26.11 % to 38.36 %. Conversely, the metabolized sugar conversion into biopolymers ranged from 64.10 % to 79.18 %, depending on the *Xanthomonas campestris* strains employed.

Table 3. Xanthan concentration in the crude glycerol-based media after the cultivation of different Xanthomonas campestris strains and the conversion of sugar into product

Producing microorganism	Xanthan concentration, P [g/L]***	Initial sugar conversion* [%]***	Metabolized sugar conversion** [%]***
CB	6.61 ± 0.39^b	33.07 ± 0.48^c	67.66 ± 0.44^b
CF	5.22 ± 0.21^a	26.11 ± 0.37^a	73.12 ± 0.59^c
12-2	5.85 ± 0.44^{ab}	29.27 ± 0.27^b	79.18 ± 0.52^d
Xp 3-1	7.67 ± 0.53^c	38.36 ± 0.42^d	64.10 ± 0.40^a

** Initial sugar conversion [%] = $P/S_0 \cdot 100$*

*** Metabolized sugar conversion [%] = $P/(S_0\text{-}S) \cdot 100$*

****Values in the same column marked with the same letter are not significantly different at α = 0.05 according to the Duncan's multiple range test.*

The results obtained indicate that all the strains considered can be used as xanthan producing microorganisms on a crude glycerol-based medium. High values of all the indicators of bioprocess success suggest that *Xanthomonas campestris* Xp 3-1 represents the most appropriate producing strain under the set experimental conditions.

CONCLUSION

In this study, the possibility of xanthan production on a crude glycerol-based medium as a sole carbon source using different *Xanthomonas campestris* strains was confirmed. The efficacy of such production was estimated according to the rheology of the media considered, amounts of xanthan produced and conversion degrees of the most important nutrients present. The results obtained for all the parameters considered suggest that the *Xanthomonas campestris* Xp 3-1 strain can be a suitable producing microorganism for the industrial production of xanthan on a crude glycerol-based medium. The findings in this study can provide a basis for the optimization of xanthan production on a crude glycerol-based medium, with an aim of increasing the xanthan yield and quality. In addition to producing a high-value product, this research particularly focused on reducing xanthan production costs and minimizing the negative impact of waste crude glycerol on the environment.

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FRACTAL ANALYSIS OF THE FLOUR
FRAKTALNA ANALIZA BRAŠNA

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ABSTRACT

The aim of the paper was the evaluation of the microscopic, powder samples of flour by utilizing the fractal analysis. The powder particles were compared and submitted to fractal analysis. Three types of flour were studied, smooth flour, semi-flour and thick flour. The five samples of each sort of flour were tested by fractal analysis. The samples were digitized by the digital microscope Motic DM 1802-A with software Motic Image Plus ver. 2.0. Each image was processed by the thresholding operation and the fractal analysis was realized by the software Harfa ver. 5.1.0 and the samples were compared by the correlation analysis. The obtained fractal dimensions described the segmentation and distribution of flour powder and the fractions of the flour. The fractal dimension of the smooth flour was D_{WBW} = 1.29266, of the semi-flour D_{WBW} = 1.70734 and of the thick flour D_{WBW} = 1.57978. The smooth flour was composed of microscopic powder particles of wheat. Small particles of about 10 μm were mainly found in the smooth flour. However, sporadic particles greater than 47.6 μm were also observed. The size of the smooth flour particles was from 2.38 μm to 47.6 μm. The semi-flour contained mainly particles the size of up to 71.2 μm. Practically half the particles obtained from semi-flour were the size of up to 71.2 μm. The thick flour was created mainly by particles to the size of 73.78 μm. Greater particles, the size from 130.9 μm to 314.2 μm, were obtained in a small number. On the base of the particle distribution, the semi-flour and the thick flour were very similar, but on the base of fractal analysis they were different and we can distinguish them.

Keywords: *powder, flour, fractal analyse*

REZIME

Cilj istraživanja bio je procenjivanje mikroskopskih, praškastih uzoraka brašna pomoću fraktalne analize. Čestice praha su upoređene i podvrgnute fraktalnoj analizi. Ispitivane su tri vrste brašna: meko brašno, polu poluoštro i oštro brašna. Pet uzoraka svake vrste brašna testirani su fraktalnom analizom. Uzorci su digitalizovani pomoću digitalnog mikroskopa Motic DM 1802-A sa softverom Motic Image Plus ver. 2.0. Svaka slika obrađena je operacijom praženja, a fraktalna analiza realizovana je softverom Harfa ver. 5.1.0, a uzorci su upoređeni korelacionom analizom. Dobijene fraktalne dimenzije opisale su segmentaciju i raspodelu praha brašna u prahu i frakcije brašna. Fraktalna dimenzija meko brašna iznosila je DVBV = 1,229266, poluoštrog brašna DVBV = 1,770734, a oštrog brašna DVBV = 1,57978. Glatko brašno je bilo sastavljeno od mikroskopskih čestica pšenice. Male čestice od oko 10 μm nalaze se uglavnom u glatkom brašnu. Međutim, primećene su i pojedinačne čestice veće od 47,6 μm. Veličina čestica glatkog brašna bila je od 2,38 μm do 47,6 μm. Poluoštro brašno je sadržavalo uglavnom čestice veličine do 71,2 μm. Praktično, polovina čestica dobijenih iz poluoštrog brašna bila je veličine do 71.2 μm. Oštra brašna bila su, uglavnom, od čestica veličine 73,78 μm. Veće čestice, veličine od 130,9 μm do 314,2 μm, dobijene su u malom broju. Na osnovu raspodele čestica, poluoštro brašno i oštra brašna bili su vrlo slični, ali na osnovu fraktalne analize bila su različita i možemo ih razlikovati.

Ključne reči: *prah, brašno, fraktalna anliza.*

INTRODUCTION

The quality of flour is influenced by the geometrical properties of the flour grains and their distribution. The quality of the flour depends on the compounds. Wheat flour is composed of proteins, starch, lipids, sugars and enzymes. The properties of flour are very important for the quality of cereals and bread. Wheat flour is generally used for bread making due to its gluten network structure that imparts excellent formation of cohesive, extensible, elastic dough and retention of gas during fermentation (*Cappelli, Oliva, & Cini, 2020)*. Previous research into fine cereal flour fractions derived by various milling techniques revealed that particle size can largely affect the physicochemical properties of flours, such as water absorption, damaged starch content, pasting properties and thereby has a significant impact on dough rheological behavior and bread quality *(Angelidis et al., 2016).*

Particles of flour have different shapes and segmentation. It can be applied to the detection of different flours by the segmentation and fragmentation parameter – fractal dimension. Detection of flours on the base of the optical and fractal methods could be useful for the evaluation of the flour quality by the nonstandard method. *Kim et al., (2004)* studied the particle sizes of soft and hard wheat (*Triticum aestivum L.*) flours at isothermal temperatures were determined by laser diffraction analysis. Flour samples were suspended in water at temperatures ranging from 30 to 80 °C, for 20–60 min. All flour particles exhibited trimodal size distributions, with the particles of the first mode <10 mμ, the second mode from 10 to 40 mμ, and the third mode from 41 to 300 mμ. Control experiments with isolated starch and gluten indicated that the first and second modes were mainly associated with starch granules, while the third mode was related to gluten and particle clusters. Soft wheat flours showed higher volume fractions in the first and second modes, indicating more dissociated starch granules. Since, particle size, particle shape and surface roughness greatly influence the bulk and shear properties of the powders, various researches have been conducted to establish a relation between surface roughness and other properties like particle shape and particle size for different types of powders in various industries. Particle size has a major influence on powder flowability. The reduction in flowability at smaller particle size is due to the increased surface area per unit mass of powder *(Shumaila et. al.,2017).*

Fractal dimension is a very important parameter for topographical measurement of various materials *(Chen, 2007).* It

is used in several applications, such as measurement of irregularities in an image, texture segmentation, surface roughness estimation and many other functions *(Biswas, Ghose, Guha, & Biswas, 1998*). The fragmentation of the powders can be evaluated by fractal analysis. Fractals are of a rough or fragmented geometric shape that can be subdivided into parts, each of which is (at least approximately) a reduced copy of the whole. They are crinkly objects that defy conventional measures, such as length and are most often characterised by their fractal dimension. They are mathematical sets with a high degree of geometrical complexity that can model many natural phenomena. Almost all natural objects can be observed as fractals (coastlines, trees, mountains, and clouds). Their fractal dimension strictly exceeds the topological dimension *(Zmeškal, 2001)*. Fractal dimension is the number, very often non-integer, often the only one measure of fractals. It measures the degree of fractal boundary fragmentation or irregularity over multiple scales. It determines how fractal differs from Euclidean objects (point, line, plane, circle, etc.)

Just a small group of fractals have one certain fractal dimension, which is scale-invariant. These fractals are monofractals. Most natural fractals have different fractal dimensions depending on the scale. They are composed of many fractals with different fractal dimensions. They are called „multifractals“. To characterise a set of multifractals (e.g. set of different coastlines) we do not have to establish all their fractal dimensions, it is enough to evaluate their fractal dimension at the same scale *(Mandelbrot, 1983;Theiler, 1990)*.

Dannenber (2002) found a much more sophisticated program call HarFA (harmonic fractal analysis) which creates some beautiful data, is very fast and allows a larger range of boxes to be analyzed. It calculates the fractal dimension spectrum as well, so you can see structure in the dimension *D* that corresponds to the particle size distribution and distribution of spacings between particle edges.

The objective of the manuscript was the evaluation of fractal properties of the microscopic, powder image samples of flour by utilizing the fractal analysis to distinguish the different flours by the fractal dimension. The application of quality control of the granularity of the flour can be realized.

MATERIAL AND METHOD

The Box-Counting method was used for evaluating the fractal properties of the flour particle texture. The method is often used to determine the fractal box dimensions of digitized images of fractal structures. *Nežádal et al., (2001)* and *Buchníček et al., (2000)* have implemented the Box-Counting procedure in software called HarFA ver. 5.1.0. The HarFA software analyses black&white images. The Box-Counting method utilizes the covering fractal pattern with a raster of boxes (squares) and then evaluating how many boxes are of the raster. Repeating this measurement with different sizes of boxes $\varepsilon = 1/r$ will result in the logarithmical function of box size ε and the number of boxes $N(\varepsilon)$ needed to completely cover the fractal. ε is the size of the side of squares, which are covering the texture. The number of boxes is

$$N(\varepsilon) \sim \varepsilon^{-D} \quad (1)$$

and the fractal dimension D

$$D = -\lim_{\varepsilon \to 0} \frac{\ln N(\varepsilon)}{\ln \varepsilon} \quad (2)$$

The slopes of the linear functions give D_{BW}, D_{BBW} and D_{WBW} fractal dimensions.

$$\ln N_{BW}(\varepsilon) = \ln(K_{BW}) + D_{BW} \ln(\varepsilon) \quad (3)$$

$$\ln N_{BBW}(\varepsilon) = \ln(K_{BBW}) + D_{BBW} \ln(\varepsilon) \quad (4)$$

$$\ln N_{WBW}(\varepsilon) = \ln(K_{WBW}) + D_{WBW} \ln(\varepsilon) \quad (5)$$

D_{BW} characterizes properties of the border of fractal patterns. D_{BBW} characterizes a fractal pattern on a white background and D_{WBW} characterizes a fractal pattern on a black background. N_{BW}, N_{BBW} and N_{WBW} are the numbers of squares covered black&white, black + black&white and white+ black&white squares. K_{BW}, K_{BBW} and K_{WBW} are fractal measures of the dividing line.

Three commercial flours in Slovakia were studied: smooth flour (Wheat smooth Special 0Extra flour), semi-flour (Wheat semi-flour superior) and thick flour (Gold Spike).

Five samples were realized for each sort of flour. The flour was applied on the microscopic glass of the digital microscope Motic DM 1802-A (Motic, China) by imprinting of the glass to the flour. The software Motic image plus 2.0 was used for digitizing of the flour image samples. The calibration of the microscope was realized by the calibration glass with a scale of 0.1 mm (Fig.1). One hundred magnification of the microscope was applied. Dimensions of the images were 1280x1024 pixels. The length of the scale division 0.1 mm at 100 times magnification was 42 pixels and then one pixel was 2.38 µm. The size of the flour particles was measured on the base of the scale.

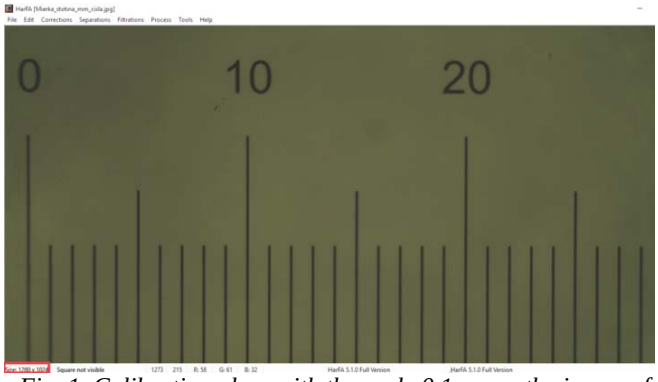

Fig. 1. Calibration glass with the scale 0.1mm as the image of size 1280x1024 pixels

The images of flour samples were evaluated in the software HarFA ver. 5.1.0. The original images were submitted to the thresholding intensity procedure. The thresholds of greyscale were determined for each sample image. Thresholding enabled the determination of the particles of the flour in the image sample. The inversion of the foreground and the background was realized, which was needed for the fractal analysis software. The fractal analysis of the image samples was realized on the base of the equations (3, 4 and 5). The image samples were covered by 28 square rasters with the square size from 1 pixel to 358 pixels and fractal dimensions were calculated from the slopes of the regression curves of the equations (3, 4 and 5).

RESULTS AND DISCUSSION

The original image of the smooth flour sample no. 1 is presented in Fig. 2. The dark particles represent the grains of the flour. The software Harfa required the intensity thresholding of the images for the next evaluation. The thresholding created negative images of the original images on the base of thresholding intensity. The level of intensity threshold was from level 0 (black color) to level 255 (white color). The image of the

sample no.1 of the smooth flour after thresholding is shown in Fig. 3 with the intensity threshold of 25 and 255. Determination of the fractal dimensions of the smooth flour for sample no. 1 by the software HarFA is presented in Fig. 4. The fractal dimension of white grains of smooth flour on a black background was evaluated.

Fig. 2. Image of the sample of the smooth flour sample no. 1 before thresholding in the software HarFA

It means the white and black&white squares of the rasters were used and dimension D_{WBW} = 1.3076 was determined from the slope of the straight line. The dimension D_{BW} represents the fraction of the border of the white flour grains and dimension D_{BBW} represents the fractionality of the black background. Flour was characterized only by the dimension D_{WBW}.

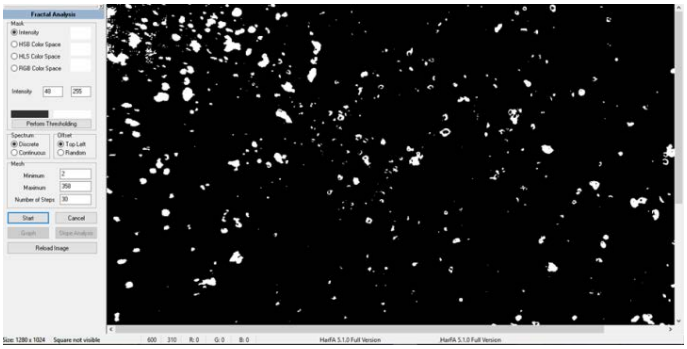

Fig. 3. Image of the smooth flour sample no. 1 after thresholding in the software HarFA. The threshold of intensity was 25 and 255.

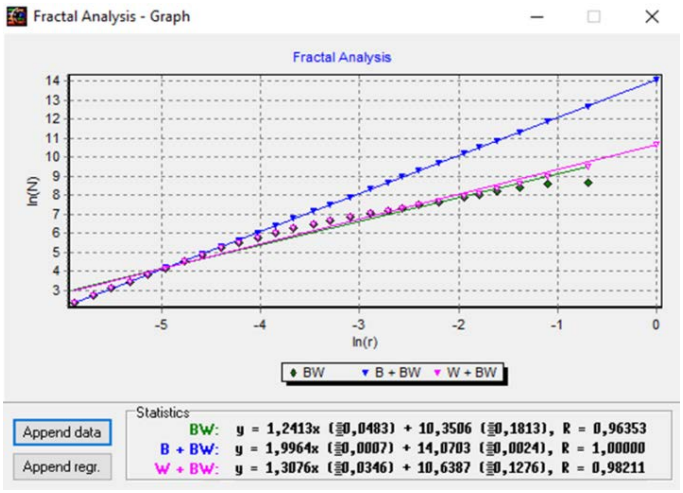

Fig. 4. Determination of fractal dimensions of the smooth flour of sample no. 1 from the slopes of the curves in the software HarFA

The results of the evaluation of the fractionality of the smooth flour are presented in the Tab. 1.

Table 1 Fractal dimensions of the smooth flour n – number of samples; D_{WBW} – fractal dimension of the flour particles; SD – standard deviation; K – fractal measure; SD_K – standard deviation; r – correlation coefficient r; CV – coefficient of variation.

n	D_{WBW}	SD	K	SD_K	r
1	1,3076	0,0346	10,6387	0,1276	0,98211
2	1,2392	0,0368	10,2879	0,1358	0,97754
3	1,3459	0,0372	10,8777	0,1371	0,98053
4	1,2561	0,0346	10,3557	0,1277	0,9806
5	1,3145	0,0354	10,6935	0,1307	0,98144
Average	1,29266	x	10,5707	x	x
SD	0,016103	x	0,089049	x	x
CV(%)	1,245747	x	0,842415	x	x

The smooth flour was composed of microscopic powder particles of wheat. Small particles of about 10 μm were mainly found in the smooth flour. However, sporadic particles greater than 47.6 μm were also observed. The size of the smooth flour particles was from 2.38 μm to 47.6 μm.

The original image of the semi-flour sample no. 1 is presented in Fig. 5. The image of sample no. 1 of the semi-flour after thresholding is shown in Fig. 6 with the intensity threshold of 47 and 255. Determination of the fractal dimensions of the semi-flour for sample no. 1 by the software HarFA is presented in Fig. 7. The fractal dimension of white grains of the semi-flour on a black background was evaluated. The dimension D_{WBW} = 1.7312 was determined from the slope of the straight line. The dimension D_{BW} represents the fraction of the border of the white flour grains and dimension D_{BBW} represents the fractionality of the black background.

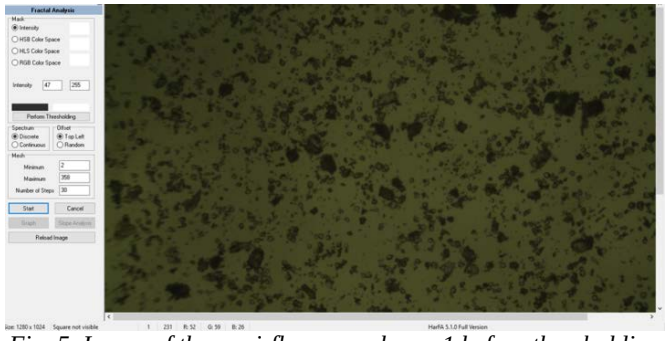

Fig. 5. Image of the semi-flour sample no. 1 before thresholding in the software HarFA

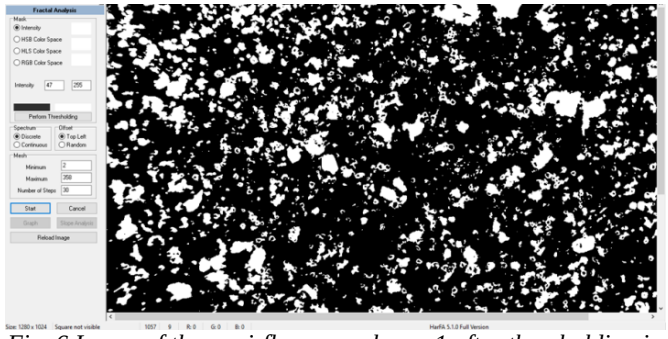

Fig. 6 Image of the semi-flour sample no. 1 after thresholding in the software HarFA. The threshold of intensity was 47 and 255.

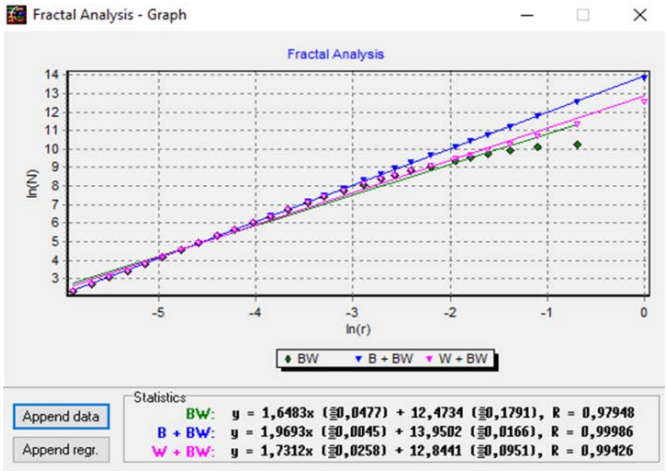

Fig. 7. Determination of fractal dimensions of the semi-flour of the sample no. 1 from the slopes of the curves in the software HarFA

The results of the evaluation of the fractionality of the semi-flour are presented in the Tab. 2.

Table 2 Fractal dimensions of the semi-flour n – number of samples; D_{WBW} – fractal dimension of the flour particles; SD – standard deviation; K – fractal measure; SD_K – standard deviation; r – correlation coefficient r; CV – coefficient of variation.

n	D_{WBW}	SD	K	SD_K	r
1	1,7312	0,0258	12,8441	0,0951	0,99426
2	1,7309	0,0266	12,8461	0,0979	0,99392
3	1,7085	0,0276	12,7366	0,1016	0,99328
4	1,6815	0,0283	12,6011	0,1043	0,9927
5	1,6846	0,0287	12,6206	0,106	0,99249
Average	1,70734	x	12,7297	x	x
SD	0,00869	x	0,042521	x	x
CV (%)	0,508994	x	0,33403	x	x

Semi-flour contained mainly the particles of the size up to 71.2 μm. Practically half the particles obtained from semi-flour were the size of up to 71.2 μm

The original image of the thick flour sample no. 1 is presented in Fig. 8. The image of the sample no.1 of the thick flour after thresholding is shown in Fig. 9 with the intensity threshold of 47 and 255. Determination of the fractal dimensions of the thick flour for sample no. 1 by the software HarFA is presented in Fig. 10. The fractal dimension of white grains of the smooth flour on a black background was evaluated. The dimension D_{WBW} = 1.5490 was determined from the slope of the straight line.

Fig. 8. Image of the sample of the thick flour sample no.1 before thresholding in the software HarFA

The dimension D_{BW} represents the fraction of the border of the white flour grains and dimension D_{BBW} represents the fractionality of the black background.

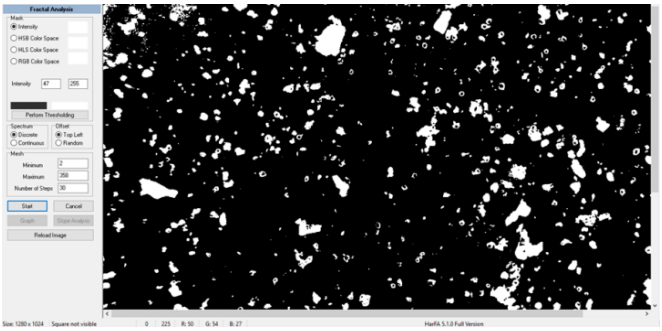

Fig. 9. Image of the sample of the thick flour sample no. 1 after thresholding in the software HarFA. The threshold of intensity was 47 and 255.

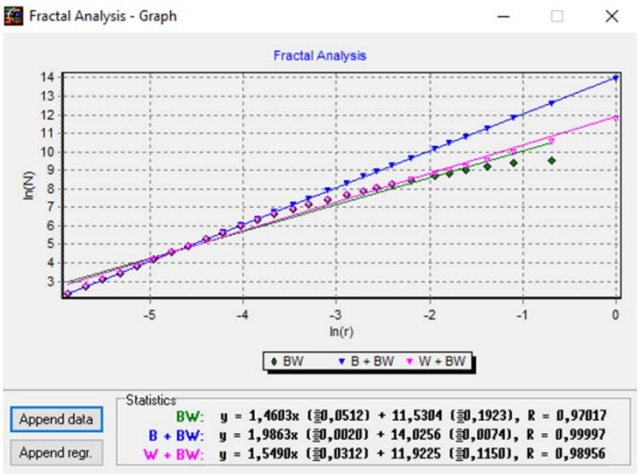

Fig. 10. Determination of fractal dimensions of the thick flour of the sample no. 1 from the slopes of the curves in the software HarFA

The results of the evaluation of the fractionality of the thick flour are presented in Table 3.

Table 3 Fractal dimensions of the thick flour n – number of samples; D_{WBW} – fractal dimension of the flour particles; SD – standard deviation; K – fractal measure; SD_K – standard deviation; r – correlation coefficient r; CV – coefficient of variation.

n	D_{WBW}	SD	K	SD_K	r
1	1,549	0,0312	11,9225	0,115	0,98956
2	1,6552	0,0282	12,4634	0,1039	0,99251
3	1,6976	0,0267	12,6749	0,0986	0,99359
4	1,6921	0,0258	12,6397	0,0951	0,994
5	1,305	0,0324	10,5755	0,1193	0,98425
Average	1,57978	x	12,0552	x	x
SD	0,05466	x	0,288435	x	x
CV (%)	3,45999	x	2,392618	x	x

The thick flour was created mainly by the particles to the size of 73.78 μm**.** Greater particles, of the size from 130.9 μm to 314.2 μm, were obtained in a small number.

CONCLUSION

The obtained fractal dimensions described the segmentation and distribution of flour powder and the fractions of the flour. The fractal dimensions of the smooth flour were D_{WBW} = 1.29266, of the semi-flour D_{WBW} = 1.70734 and of the thick flour D_{WBW} = 1.57978. The smooth flour was composed of microscopic powder particles of wheat. Small particles of about 10 μm were mainly found in the smooth flour. However, sporadic particles greater than 47.6 μm were also observed. The size of the smooth flour particles was from 2.38 μm to 47.6 μm. Semi-flour contained mainly particles the size of up to 71.2 μm. Practically half the particles obtained from semi-flour were the size of up to 71.2 μm. The thick flour was created mainly by particles to the size of 73.78 μm**.** Greater particles, the size from 130.9 μm to 314.2 μm, were obtained in a small number. On the base of the particle distribution, the semi-flour and the thick flour were very similar, but on the base of fractal analysis they were different and we can distinguish them.

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Original Scientific Paper
Originalni naučni rad

IN VITRO POTENTIAL OF BACILLUS SPP. ANTAGONISTS FOR SUPPRESSION OF XANTHOMONAS EUVESICATORIA PHYTOPATHOGENS

IN VITRO POTENCIJAL ANTAGONISTA BACILLUS SPP. ZA SUZBIJANJE FITOPATOGENA XANTHOMONAS EUVESICATORIA

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ABSTRACT

Bacterial pathogen Xanthomonas euvesicatoria, principal causer of bacterial spot, represents a significant problem in agricultural practice due to high yield losses in the production of pepper and tomato. The development of resistance to copper pesticides has shifted research, in the field of its suppression, towards biopesticides. In this study, several Bacillus strains were tested against Xanthomonas euvesicatoria strains, isolated from pepper leaves with symptoms of bacterial spot, to select a sufficiently effective antagonist. When it comes to the testing of cultivation broth, containing biomass of tested antagonists, the best results were achieved using isolate Bacillus sp3. On the other hand, when biomass-free supernatants, containing produced antimicrobial compounds, were tested, Bacillus sp1 and Bacillus sp2 have shown the highest antimicrobial activity. The results of this study represent a basis for further development of bioprocess solutions for the production of biopesticides based on Bacillus spp. biomass or antimicrobial compounds, showing high efficiency in suppression of pepper bacterial spot.

***Keywords**: antimicrobial activity, pepper, bacterial spot, biomass, antimicrobial compounds, sensitivity.*

REZIME

Bakterijski patogen Xanthomonas euvesicatoria predstavlja glavnog uzročnika bakteriozne pegavosti, stoga predstavlja značajan problem u poljoprivrednoj praksi usled izazivanja velikih gubitaka prinosa u proizvodnji paprike i paradajza. Uobičajena sredstva za suzbijanje i kontrolu ovog fitopatogena predstavljaju preparati na bazi bakra. Međutim, razvoj rezistentnosti prema pesticidima na bazi bakra doveo je do zaokreta u polju suzbijanja ovih patogena ka primeni biopesticida, odnosno bioloških kontrolnih agensa. Bakterije roda Bacillus i njihovi metaboliti sa izraženom antimikrobnom aktivnošću protiv ciljanih fitopatogena predstavljaju najperspektivnije aktivne komponente biokontrolnih preparata za zaštitu bilja. U ovom istraživanju nekoliko sojeva roda Bacillus ispitano je sa ciljem odabira antagonista dovoljno efikasnih u suzbijanju sojeva Xanthomonas euvesicatoria, koji su izolovani sa listova paprike sa simptomima bakteriozne pegavosti. Prilikom testiranja antimikrobne aktivnosti uzoraka kultivacionih tečnosti, koji sadrže i biomasu testiranih antagonista, najbolji rezultati u suzbijanju testiranih fitopatogena su postignuti primenom izolata Bacillus sp3. Sa druge strane, prilikom testiranja antimikrobne aktivnosti supernatanata oslobođenih biomase antagonista, koji sadrže samo produkovana antimikrobna jedinjenja, izolati Bacillus sp1 i Bacillus sp2 su pokazali najveću antimikrobnu aktivnost protiv fitopatogena Xanthomonas euvesicatoria. Rezultati ovog istraživanja predstavljaju osnovu za dalji razvoj bioprocesnih rešenja za proizvodnju biopesticida na bazi biomase ili antimikrobnih jedinjenja koja proizvode antagonisti roda Bacillus, a koji pokazuju visoku efikasnost u suzbijanju bakteriozne pegavosti paprike.

***Ključne reči**: antimikrobna aktivnost, paprika, bakteriozna pegavost, biomasa, antimikrobna jedinjenja, osetljivost.*

INTRODUCTION

Plant diseases caused by fungal and bacterial pathogens are still the most important source of yield losses worldwide (*Savary et al., 2012*). Bacterial plant diseases represent a more serious problem in terms of disease prevention, suppression and management since there is only a limited range of products allowed and efficient enough to suppress bacterial pathogens (*Sundin et al., 2016*). Some of the products that are usually used for the management of bacterial plant diseases include copper-based chemical pesticides and antibiotics, whose application in agriculture isn't allowed in the majority of countries (*Sundin and Wang, 2018*). The main problem when it comes to controlling bacterial pathogens is their ability to develop or acquire resistance to different chemical compounds used for their suppression in a short time period (*Sundin et al., 2016*). Therefore, the suggested alternative for the management of bacterial pathogens is the application of microbial biopesticides, which usually evince more than one mechanism of action (*Köhl et al., 2019*), making it harder for bacterial phytopathogens to develop resistance to these biocontrol agents.

Plant pathogens from the genus *Xanthomonas* represent one of the major pathogen groups responsible for massive yield losses in the wide range of host plants (*Mansfield et al., 2012*). Strains of the species *Xanthomonas euvesicatoria* are the main causative agents of pepper and tomato bacterial spot (*Potnis et al., 2015*). The symptoms of this bacterial disease in pepper plants could be observed as brownish and necrotic irregular-shaped spots in leaves, and as scab-like whitish lesions in fruits (*EPPO, 2013*), contributing to their lower market value. Furthermore, another problem is the long persistence of *Xanthomonas euvesicatoria* pathogens in the infected fields, even for 10 years (*Bashan et al., 1982*). Usual practices, when it comes to the management of pepper bacterial spot, rely on high-

quality and pathogen-free seeds and seedlings (*Šević et al., 2019*), as well as on the application of copper bactericides in combination with plant resistance activators (*Buonaurio et al., 2002*). In recent years, the application of bacterial biocontrol agents (*Sević et al., 2016*), as well as bacteriophages (*Gašić et al., 2018*), has gained significant attention in the field of pepper bacterial spot management.

The development of biocontrol products is based on an efficient and cost-effective production strategy, which implies biotechnological multiplication or production of a certain biocontrol agent, usually a highly-efficient microbial strain or some of its metabolites. The first step in this process is a search for a suitable biocatalyst, which expresses a high potential for suppression of target plant pathogens and simultaneously doesn't represent a threat to environmental biodiversity (*Mota et al., 2017*). Determination of a suitable biocontrol agent or antagonist for suppression of certain plant diseases caused by microbial phytopathogens usually relies on *in vitro* testing of its antimicrobial activity against target pathogens. Literature data could be a significant source of information when it comes to the selection of an appropriate antagonist for plant protection. Therefore, in this study several *Bacillus* strains were tested as potential antagonists for suppression of *Xanthomonas euvesicatoria* pathogens, causing pepper bacterial spots. *Bacillus* strains were selected due to several beneficial traits for application in biological control: high resistance to unfavorable environmental conditions (*Fira et al., 2018*), stability during the formulation or downstream steps in the production process (*Stamenković Stojanović et al., 2019*), as well as a genetic basis for the production of wide spectra of metabolites evincing antimicrobial activity against a wide range of plant pathogens (*Stein, 2005; Shafi et al., 2017; Fira et al., 2018*). Hence, the aim of this study was to select appropriate antagonist(s) in terms of their potential to suppress *Xanthomonas euvesicatoria* phytopathogenic strains using cultivation broth of antagonists and biomass-free supernatants as potential biocontrol agents.

MATERIAL AND METHOD

Microorganisms

Antagonistic microorganisms tested in this study were five *Bacillus* strains: two referent strains – *Bacillus subtilis* ATCC 6633 (I1) and *Bacillus cereus* ATCC 10876 (I2), and three isolates from fresh cheese – *Bacillus* sp1 (I3), *Bacillus* sp2 (I4) and *Bacillus* sp3 (I5). Isolation of these three strains was performed in the following way: successive dilutions of the fresh cheese sample (10^{-1}, 10^{-2} and 10^{-3}) were prepared and 1 mL of the last dilution was spread on the nutrient agar plate. After incubation at 28 °C for three days, colonies were picked according to the morphological traits of *Bacillus* species and subcultured to fresh nutrient agar plates. The procedure was repeated until obtaining a visually pure culture of three isolated strains. Afterwards, these strains were identified as members of the genus *Bacillus* according to their morphological and biochemical traits (*De Vos et al., 2009*). All antagonists were kept on a nutrient agar slant at 4 °C. Phytopathogenic *Xanthomonas* strains (P1, P2 and P3) were isolated using standard phytopathological techniques from pepper leaves with symptoms of bacterial spot, collected during 2015 in the cadaster municipality Pivnice, Serbia. PCR identification of phytopathogenic strains was carried out using the method described by Morreti et al. (2009). All phytopathogenic strains were identified as members of *Xanthomonas euvesicatoria* species. Phytopathogens were kept on YMA (yeast maltose agar) medium (*Pajčin et al., 2018)* at 4 °C. All microorganisms were subcultured on the media used for their preservation and incubated at 26 °C (phytopathogens) and 28 °C (antagonists) prior to further utilization.

Inoculum preparation and cultivation of antagonistic *Bacillus* spp.

After incubation of antagonistic strains at 28 °C on nutrient agar, *Bacillus* spp. were transferred to liquid media (nutrient broth) using an inoculation loop. *Bacillus* spp. inocula were incubated at 28 °C on a laboratory shaker (150 rpm, spontaneous aeration) during 48 h. After that, the inoculation of cultivation media (nutrient broth) was performed using 10% (v/v) of inocula compared to cultivation media volume (100 mL). Cultivation of *Bacillus* spp. was carried out under the same conditions as inoculum preparation, except cultivation time was 96 h.

Antimicrobial activity testing

After incubation of phytopathogenic *Xanthomonas euvesicatoria* strains at 26 °C on YMA slant, three suspensions of phytopathogenic *Xanthomonas euvesicatoria* strains were prepared using sterile saline to achieve 10^8 CFU/mL. Media containing phytopathogens were prepared by transferring 1 mL of suspension into melted and tempered (50±1 °C) YMA medium (15 mL). After pouring the medium into the Petri dish and its solidification, three paper discs (HiMedia, India) were placed on the medium surface to carry out testing of antimicrobial activity in triplicate tests for each sample (3x10 µL) against each of the phytopathogenic isolates. Samples used for antimicrobial activity assay were cultivation broths of five *Bacillus* strains (obtained after 96 hours of cultivation) and their supernatants obtained after centrifugation at 10000 rpm (13000 g) for 10 min (Rotina 380R, Hettich, Germany). Spectrophotometric measurement (600 nm, UV 1800, Shimadzu, Japan) of the optical density of cultivation broth samples was applied to determine the final concentration of *Bacillus* spp. in the samples used for antimicrobial activity assay, which was at the level of 10^8 CFU/mL. The incubation of media for antimicrobial activity assaying was carried out at 26 °C for 72 h, which was followed by the measurement of inhibition zone diameters. Furthermore, commercial streptomycin discs (Torlak, Serbia) containing 30 µg of streptomycin were also used as a positive control against each phytopathogenic *Xanthomonas euvesicatoria* strain, while sterile distilled water was used as a negative control.

Statistical data analysis

The obtained inhibition zone diameters were presented as average values (sum of the obtained values of inhibition zone diameters from triplicate tests divided by three due to the number of repetitions) with standard deviations, calculated using Microsoft® Excel 2010 software (Microsoft Corporation, USA). Statistical analysis of the experimental data was performed using Statistica 13.5 software (Tibco Software Inc., USA). Levene's test was applied to test the hypothesis of variance homogeneity, followed by ANOVA and post hoc testing using Duncan's multiple range test. All statistical analyses were performed at the significance level of 0.05.

RESULTS AND DISCUSSION

After the cultivation of five antagonists, samples of cultivation broth, as well as samples of biomass-free supernatants, were tested against three *Xanthomonas euvesicatoria* strains, isolated from diseased pepper leaves with symptoms of bacterial spot, using the diffusion-disc method. Since the testing of antimicrobial activity was performed in

triplicate tests, the results were statistically processed using Levene's test, ANOVA and post hoc Duncan's multiple range tests.

When it comes to the results of the inhibition zone diameters obtained as a result of antimicrobial activity testing using *Bacillus* spp. cultivation broth samples against *Xanthomonas euvesicatoria* strains, Levene's test with a *p*-value of 0.1377 has confirmed that there were no significant differences between repetitions for antimicrobial activity testing for each cultivation broth sample. ANOVA results, presented in Table 1, have also confirmed a significant effect of different antagonists on the obtained inhibition zone diameters against *Xanthomonas euvesicatoria* strains, with *p*-values less than 0.05.

Table 1. One-way ANOVA of inhibition zone diameters for cultivation broths of different antagonists used for suppression of Xanthomonas euvesicatoria

Effect	SS	MS	DF	F-value	*p*-value
Intercept	24875.57	24875.57	1	15318.96	**<0.0001**
Antagonist	5102.48	1020.50	5	628.45	**<0.0001**
Error	77.94	48.00	48		
SS – the sum of squares, MS – mean squares, DF –degree of freedom					

Mean values and standard deviations of the inhibition zone diameters obtained by testing of cultivation broth samples against *Xanthomonas euvesicatoria* strains are given in Table 2. These results were also processed using post hoc Duncan's multiple range test, to determine homogenous groups of independent variables at the same level of statistical significance. From the presented results it could be concluded that cultivation broth samples of the isolate *Bacillus* sp3 (I5) have shown the highest inhibitory activity against phytopathogenic *Xanthomonas euvesicatoria* strains. On the other hand, the lowest suppression of the phytopathogenic strains was achieved using the cultivation broth samples of the referent strain *Bacillus subtilis* ATCC 6633 (I1) and isolates *Bacillus* sp1 (I3) and *Bacillus* sp2 (I4), which are at the same level of statistical significance when it comes to antimicrobial activity against tested *Xanthomonas euvesicatoria* phytopathogens.

Table 2. Mean values and significance levels of inhibition zone diameters obtained using cultivation broth samples of different antagonists for suppression of Xanthomonas euvesicatoria

Antagonist	Inhibition zone diameter (mm)
I1	13.94 ± 0.81^{a}
I4	14.22 ± 0.83^{a}
I3	14.33 ± 0.41^{a}
I2	16.61 ± 1.54^{b}
I5	31.22 ± 1.56^{c}
S	38.44 ± 1.26^{d}

I1 - Bacillus subtilis ATCC 6633, I2 - Bacillus cereus ATCC 10876,
I3 - Bacillus sp1, I4 - Bacillus sp2, I5 - Bacillus sp3, S – streptomycin

The inhibition zone diameters obtained by testing *Bacillus* sp3 cultivation broth (A), streptomycin as a positive control (B) and sterile distilled water as a negative control (C) against *Xanthomonas euvesicatoria* are given in Figure 1. The second experimental stage has investigated the antimicrobial effect of supernatants (after the removal of *Bacillus* spp. biomass by centrifugation) against phytopathogenic *Xanthomonas euvesicatoria* strains. Once again, Levene's test has confirmed the homogeneity of variances with a *p*-value of 0.0876. ANOVA results are given in Table 3. These results have also confirmed a significant effect of the tested antagonist for suppression of *Xanthomonas euvesicatoria* phytopathogens using samples of cultivation broth supernatants (*p*-values less than 0.0001).

Table 3. One-way ANOVA of inhibition zone diameters for supernatants of different antagonists used for suppression of Xanthomonas euvesicatoria

Effect	SS	MS	DF	F-value	*p*-value
Intercept	8562.96	8562.96	1	9236.45	**<0.0001**
Antagonist	9091.04	1818.21	5	1961.21	**<0.0001**
Error	44.50	0.93	48		
SS – the sum of squares, MS – mean squares, DF –degree of freedom					

Afterwards, Duncan's multiple range test was applied in order to determine homogenous groups of the same statistical significance, which are given in Table 4, together with mean values and standard deviations of inhibition zone diameters resulting from testing of antimicrobial activity of supernatants against *Xanthomonas euvesicatoria*. As it could be seen in Table 4, supernatants of cultivation broths of referent strains *Bacillus subtilis* ATCC 6633 (I1) and *Bacillus cereus* ATCC 10876 (I2) didn't show any antimicrobial activity against tested phytopathogens. On the other hand, antimicrobial compounds produced by the isolates from fresh cheese have suppressed the growth of *Xanthomonas euvesicatoria* strains. The best results regarding antimicrobial activity were obtained using supernatants of *Bacillus* sp1 and *Bacillus* sp2 cultivation broths. At the same time, it can be concluded that these two isolates are at the same level of statistical significance. Therefore, any of these two isolates could be successfully applied as a biocontrol agent for the production of antimicrobial compounds effective against bacterial pepper spot causers. These results have confirmed the previously established thesis that wild strains usually express higher antimicrobial activity compared to referent strains, making the environment the richest source of microbial strains with a wide range of different biological activities (*Earl et al., 2008*). In this case, it has been also shown that wild *Bacillus* strains show better ability and genetic basis for the production of antimicrobial metabolites effective against *Xanthomonas* pathogens, in comparison to tested referent strains. Bacteria of the genus *Bacillus* are well known for their ability to produce vast antimicrobial compounds, including antibiotics (*Stein, 2005*), lipopeptides (*Ongena and Jacques, 2008*) and volatile organic compounds (*Gao et al., 2018*).

Table 4. Mean values and significance levels of inhibition zone diameters obtained using supernatant samples of different antagonists for suppression of Xanthomonas euvesicatoria

Antagonist	Inhibition zone diameter (mm)
I2	0.00 ± 0.00^{a}
I1	0.00 ± 0.00^{a}
I5	8.33 ± 1.32^{b}
I4	14.22 ± 0.97^{c}
I3	14.56 ± 1.13^{c}
S	38.44 ± 1.26^{d}

Furthermore, the sensitivity of the tested *Xanthomonas euvesicatoria* isolates towards the applied biocontrol agents has also been investigated, separately for the cultivation broth samples and the supernatant samples. ANOVA results are given in Table 5. According to the ANOVA results, the effect of the pathogen (or tested phytopathogenic *Xanthomonas euvesicatoria*

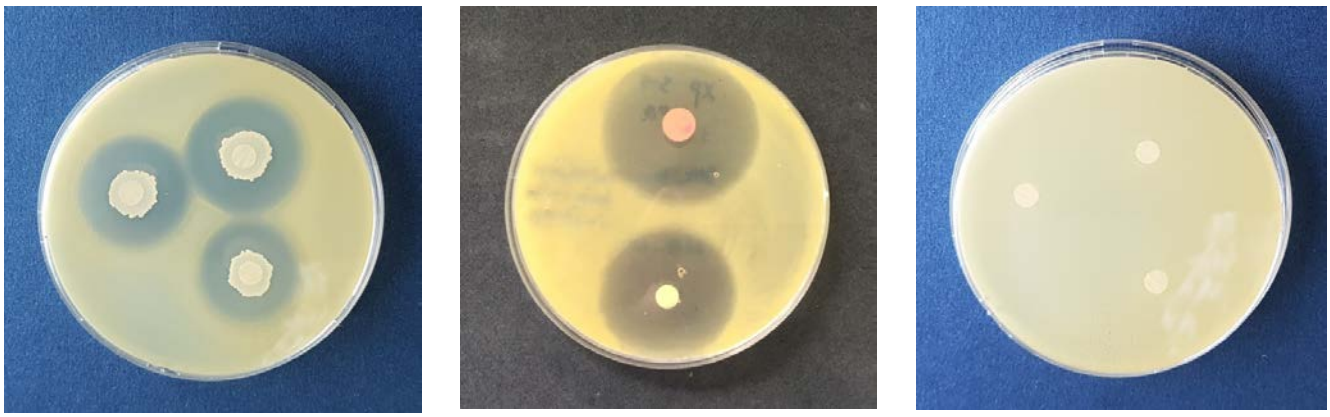

Fig. 1. Results of the antimicrobial activity assay against Xanthomonas euvesicatoria: A - Bacillus sp3 cultivation broth, B - streptomycin (positive control), C - sterile distilled water (negative control)

strain) couldn't be observed as significant when it comes to inhibition zone diameters obtained by testing both cultivation broth samples and supernatant samples as potential biocontrol agents.

ANOVA results were also confirmed by Duncan's multiple range test (Table 6), which has shown that all pathogenic isolates are at the same level of statistical significance when it comes to mean values of the obtained inhibition zone diameters. In other words, there weren't any significant differences when it comes to the sensitivity of *Xanthomonas euvesicatoria* strains to the tested *Bacillus*-based biocontrol agents. This means that the investigated biocontrol agents (cultivation broths and supernatants of the examined antagonists) could be successfully applied against all three tested phytopathogenic *Xanthomonas euvesicatoria* isolates, as the main causers of pepper bacterial spot.

Table 5. One-way ANOVA of inhibition zone diameters for different Xanthomonas euvesicatoria pathogenic strains

Effect	SS	MS	DF	F-value	*p*-value
Intercept	14688.20^{CB} 2479.02^{S}	14688.20^{CB} 2479.02^{S}	1^{CB} 1^{S}	302.63^{CB} 54.70^{S}	**<0.0001^{CB}** **<0.0001^{S}**
Pathogen	14.80^{CB} 1.64^{S}	7.40^{CB} 0.82^{S}	2^{CB} 2^{S}	0.15^{CB} 0.02^{S}	0.8591^{CB} 0.9820^{S}
Error	2038.50^{CB} 1903.33^{S}	48.54^{CB} 45.32^{S}	42^{CB} 42^{S}		

SS – the sum of squares, MS – mean squares, DF –degree of freedom
^{CB} – samples of cultivation broths, ^{S} – samples of supernatants

Table 6. Mean values of inhibition zone diameters obtained against different Xanthomonas euvesicatoria pathogenic strains

Antagonist	Inhibition zone diameter – samples of cultivation broths (mm)	Inhibition zone diameter – samples of supernatants (mm)
P2	17.33±6.28^{a}	7.20±6.66^{a}
P1	18.13±7.59^{a}	7.40±6.40^{a}
P3	18.73±6.97^{a}	7.67±7.12^{a}

CONCLUSION

The results of this study have confirmed the significant potential of different *Bacillus* strains to be used as biocontrol agents for suppression of *Xanthomonas euvesicatoria*, as principal pathogens responsible for the occurrence of pepper bacterial spot. Natural isolate *Bacillus* sp3 has shown the highest antimicrobial activity when it comes to the application of cultivation broth, while the other two natural isolates *Bacillus* sp1 and *Bacillus* sp2 have shown the highest inhibitory activity due to the production of antimicrobial metabolites, which was drawn as a conclusion from testing of antimicrobial activity of supernatants. This study has also confirmed the thesis that natural isolates usually express a higher potential for application in biological control and plant protection compared to referent isolates. Also, statistical analysis has revealed a similar sensitivity of three tested phytopathogenic isolates towards the applied biocontrol agents. Further steps will include molecular identification of the selected antagonists and their genetic basis to produce different antimicrobial metabolites characteristic for *Bacillus* species. The selection of potential antagonists done in this study represents a promising basis for further development of bioprocesses for the production of cultivation broth and antimicrobial compounds as biocontrol agents effective in suppression of pepper bacterial spot.

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GRAIN CHEMICAL COMPOSITION OF DENTS, POPPING MAIZE AND SWEET MAIZE GENOTYPES

HEMIJSKI SASTAV ZRNA GENOTIPOVA KUKURUZA ZUBANA, KOKIČARA I ŠEĆERCA

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ABSTRACT

Maize is one of the most important field crops both in the world and in our country. All commercially grown maize hybrids can be classified into one of five elementary types: dent, flint, floury, popping and sweet maize. The objectives of this study were to characterize the grain chemical compositions of yellow-seeded and white-seeded maize dents, popping maize and sweet maize genotypes. The results show that grains contents of starch, protein, oil, crude fibre, and ash of four selected maize genotypes ranged in the intervals: 53.54-68.13%; 9.19-13.00%; 4.35-5.39%; 2.13-3.93% and 1.28-2.85%, respectively. The amylose to amylopectin ratio varied from 21:79 to 28:72, which is a principal property of normal maize starch. The content of lignocellulosic fibres: NDF, ADF, ADL, hemicellulose and cellulose ranged from 11.31-15.27%; 2.51-3.54%, 0.24-0.52%, 8.10-12.68% and 2.14-3.02%, respectively. The solubility index of albumin, globulin, zein and glutelin ranged from 9.46-29.42%, 5.64-13.13%, 21.11-28.10% and 18.81-23.69%, respectively.

***Keywords**: maize, grain, chemical composition*

REZIME

Kukuruz je jedna od najznačajnijih ratarskih biljka u našoj zemlji i u svetu. Na osnovu strukture zrna i sastava endosperma svi komercijalno gajeni hibridi kukuruza mogu se svrstati u jedan od pet osnovnih tipova: zubani, tvrdunci, brašnasti, kokičari i šećerci. Hemijski sastav kukuruznog zrna predstavlja njegovo najbitnije svojstvo. U ovom radu su prikazani rezultati ispitivanja hemijskog sastava zrna različitih genotipova kukuruza (zuban žutog i belog zrna, kokičari i šećerci). Hemijski sastav zrna odabranih genotipova kukuruza ispitivan je određivanjem sardžaja skroba, amiloze i amilopektina, lignoceluloznih vlakana (NDF - vlakna nerastvorna u neutralnom deterdžentu, ADF - vlakna nerastvorna u kiselom deterdžentu, ADL - lignin nerastvorljiv u 72% rastvoru sumporne kiseline, celuloza i hemiceluloza), proteina i proteinskih frakcija (% rastvorljivih proteina i indeks rastvorljivosti albumina, globulina, zeina, glutelina), ulja, sirove celuloze i pepela. Određivan je i sadržaj nestrukturalnih ugljenih hidrata (NFC–non fiber carbohydrate) i bezazotnih ekstraktivnih materija (BEM).

Rezultati ispitivanja hemijskog sastava zrna odabranih genotipova kukuruza zubana, kokičara i šećerca su pokazali da su se sadržaji skroba, proteina, ulja, sirove celuloze i pepela kretali u sledećim intervalima: 53,54-68,13%; 9,19-13,00%; 4,35-5,39%; 2,13-3,93% i 1,28-2,85%. Odnos amiloze i amilopektina skroba ispitivanih genotipova bio je u rasponu od 21:79 do 28:72, što je glavno svojstvo normalnog kukuruznog skroba. Sadržaj lignoceluloznih vlakana: NDF, ADF, ADL, hemiceluloze i celuloze bio je u rasponima od 11,31-15,27%; 2,51-3,54%, 0,24-0,52%, 8,10-12,68% i 2,14-3,02%. Sadržaj NFC kretao se od 67,16-73,97% i BEM od 74,83-83,05%. Indeks rastvorljivosti albumina je bio od 9,46-29,42%, globulina 5,64-13,13%, zeina 21,11-28,10% i glutelina 18,81-23,60%.

***Ključne reči**: kukuruz, zrno, hemijski sastav*

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important field crops both in our country and in the world. Maize grain represents a well-organized entity consisting of the three essential parts: pericarp or a coat (5.3%), endosperm (82.8%) and the germ/embryo (11.9%) (*Bekrić, 1997; Watson, 2003*). Based on the grain structure and the endosperm composition, all commercially grown maize hybrids can be classified into one of five basic types: dent, flint, floury, popping and sweet maize hybrids. The chemical composition of maize grain is its most important trait both in the selection and breeding of new maize hybrids and in the improvement of its practical application in industrial processing and in human and animal nutrition (*Watson, 2003; Radosavljević et al., 2015; Milašinović-Šeremešić et al., 2018*). The largest percentage of the produced maize in our country, as well as in the world, is traditionally used in the nutrition of domestic animals. Furthermore, maize is a very important cereal used in the food industry in the process of wet (starch processing) and dry milling (mill processing). The following products are made in the process of maize wet milling: starch, gluten, bran, germ and corn steep liquor (CSL). The primary products of dry maize milling are semolina and flour. The main products of wet and dry maize milling are used to produce a whole range of different food products, while their by-products are used in animal nutrition (*Nuss and Tanumihardjo, 2010*). Maize grain, as well as the grain of other cereals, contains the following most important chemical components: starch (61-78%), non-starch polysaccharides (about 10%), proteins (6-12%) and fats (3-6%) (*Sinha et al., 2011*). Due to the large and wide

application of maize grain in various food products, the grain is an important source of these macronutrients. In the past few decades, many studies were carried out with the aim to improve the nutritional value of maize for food and feed (*Ai and Jane, 2016*). Therefore, the objective of the present study was to observe the chemical composition of different genotypes: yellow-seeded and white-seeded maize dents, popping maize and sweet maize.

MATERIAL AND METHOD

Four different maize genotypes (yellow-seeded and white-seeded maize dents, popping maize and sweet maize) were analyzed in the study. The sweet maize was harvested at the milk stage of maturity which is optimal for the technological quality of the specialty genotype. Other maize samples were harvested at the stage of physiological maturity which is the final stage in the maize growth process (maximum kernel dry weight has accumulated). The grain chemical composition of selected maize genotypes was observed by the determination of contents of starch, amylase and amylopectin, lignocellulose fibres (NDF - neutral detergent fibres, ADF - acid detergent fibres, ADL - acid detergent lignin, cellulose and hemicellulose), proteins and protein fractions (% of soluble proteins and the solubility index of albumin, globulin, zein and glutelin), oil, crude fibre and ash. Furthermore, the content of non-fibre carbohydrates (NFC) and nitrogen-free extracts (NFE) was determined. All methods applied in this study are described in detail in previously published papers (*Semenčenko 2013; Radosavljević et al., 2015*).

All results of chemical quality parameters presented in this paper are the average of a two-year research (2016, 2017).

RESULTS AND DISCUSSION

Table 1 shows the chemical composition of different maize genotypes (yellow-seeded and white-seeded maize dents, popping maize and sweet maize).

Obtained results show that contents of starch, protein, oil, crude fibres and ash in grain of observed maize genotypes ranged from 53.54 (sweet maize) to 68.13% (yellow dent); 9.19 (white dent) to 13.00% (sweet maize); 4.35 (white dent) to 5.39% (sweet maize); 2.13(white dent) to 3.93% (sweet maize) and from 1.28 (white dent) to 2.85% (sweet maize), respectively. The contents of NFC and NFE varied from 67.16 (sweet maize) to 73.97% (white dent) and from 74.83 (sweet maize) to 83.05% (white dent), respectively (Table 1). The highest content of grain starch was in the genotypes of yellow and white dent maize (68.13 and 68.11%), while the lowest starch content in grain was in the genotype of sweet maize (53.54%), which also had the highest content of protein (13.00%), oil (5.39%), crude cellulose (3.93%) and ash (2.85%). Besides the genotype of sweet maize had the lowest content of NFE (74.83%) and NFC (67.16%) in the grain. The white dent maize genotype had the highest content of NFE (83.05%) and NFC (73.76%) and the lowest content of protein (9.19%), oil (4.35%), crude cellulose (2.13%) and ash (1.28%) in the grain. Results presented in Table 1 are following previously published results (*Watson,* 2003; *Ai and Jane, 2016*; *Milašinović-Šeremešić et al., 2018 and 2019).*

Figure 1 shows the content of amylose and amylopectin in grain starch of different maize genotypes (yellow-seeded and white-seeded dents, popping maize and sweet maize).

The content of amylose and amylopectin, i.e. their ratio in grain starch of observed genotypes varied from 21:79 (sweet maize) to 28:72 (popping maize). The popping maize had the highest content of amylose (28%) which can be attributed to the kernel hardness (a high proportion of hard endosperm fraction) as well as specific genetics. Based on such obtained amylose to amylopectin ratios, starches of observed maize genotypes can be classified as normal maize starches (*Jane, 2009; Milašinović-Šeremešić et al., 2012*).

Table 1. Chemical composition of different maize genotypes

Genotype	Content (%)						
	NFE	NFC	Starch	Proteins	Oil	Crude fibres	Ash
Yellow-seeded dent	81.73	69.07	68.13	9.84	4.46	2.61	1.36
White-seeded dent	83.05	73.79	68.11	9.19	4.35	2.13	1.28
Popping maize	79.72	70.73	65.77	11.43	5.13	2.32	1.40
Sweet maize	74.83	67.16	53.54	13.00	5.39	3.93	2.85
Average	**79.83**	**70.19**	**63.89**	**10.87**	**4.83**	**2.75**	**1.72**
SD	**3.61**	**2.81**	**6.99**	**1.71**	**0.51**	**0.81**	**0.75**

NFE – nitrogen-free extracts; NFC - non-fibre carbohydrate

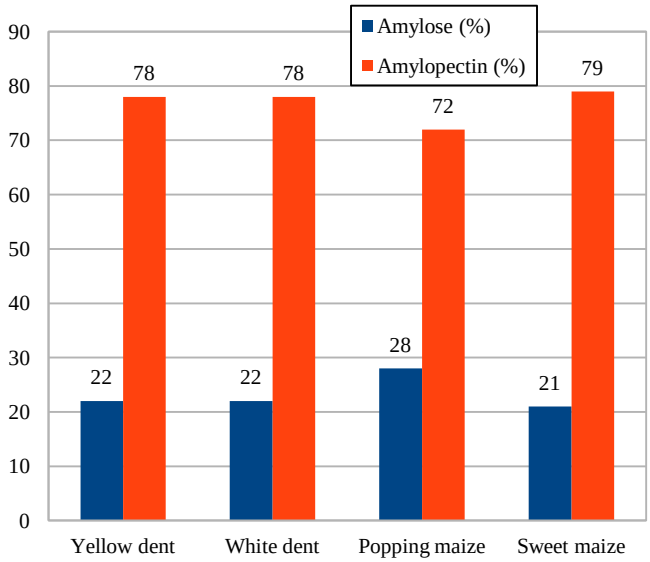

Fig. 1. Content of amylose and amylopectin in grain starch of different maize genotypes

In addition to the analyses of basic chemical composition, determinations of nutritional quality parameters such as the content of lignocellulosic fibres (NDF, ADF, ADL, hemicellulose and cellulose) and the protein fractions content (solubility and the solubility index of albumins, globulins, zein and glutelins) of selected maize genotypes also were done in this study. Lignocellulosic fibres are very valuable nutritional components of maize grain that affect grain nutritional and technological quality. Cellulose and hemicellulose are principal non-starch polysaccharides present in maize grain, especially in maize bran (*Watson, 2003).* The contents of NDF, ADF, ADL, hemicellulose and cellulose of grain of various maize genotypes (yellow- and white-seeded dents, popping maize and sweet maize) are presented in Table 2.

Table 2. Content of lignocellulosic fibres of grains of different maize genotypes

Genotype	Content (%)				
	NDF	ADF	ADL	Hemicellulose	Cellulose
Yellow-seeded dent	15.27	2.59	0.24	12.68	2.35
White-seeded dent	11.36	2.51	0.37	8.88	2.14
Popping maize	11.31	2.62	0.47	8.69	2.15
Sweet maize	11.61	3.54	0.52	8.10	3.02
Average	**12.39**	**2.82**	**0.40**	**9.59**	**2.42**
SD	**1.93**	**0.49**	**0.12**	**2.09**	**0.41**

NDF - neutral detergent fibres; ADF - acid detergent fibres; ADL - acid detergent lignin

The content of NDF, ADF, ADL, hemicellulose and cellulose of grain of observed maize genotypes ranged from 11.31 (popping maize) to 15.27% (yellow dent), 2.51(white dent) to 3.54% (sweet maize), 0.24 (yellow dent) to 0.52% (sweet maize), 8.10 (sweet maize) to 12.68% (yellow dent) and from 2.14 (white dent) to 3.02% (sweet maize), respectively (Table 2). The highest content of NDF (15.27%) and hemicellulose (12.68%) was detected in the grain of the yellow-seeded dent genotype. On the other hand, the highest contents of ADF (3.54%), ADL (0.52%) and cellulose (3.02%) was determined in the grain of the sweet maize genotype. At the same time, the results showed that the lowest content of grain lignocellulosic fibres NDF (11.31%), ADF (2.51%), ADL (0.24%), hemicellulose (8.10%) and cellulose (2.14%) was present in the genotypes of popping, white dent, yellow dent, sweet and white dent maize, respectively. Similar results have been obtained in previous studies (*Radosavljević et al., 2012; Milašinović-Šeremešić et al., 2017).*

Maize grain contains from 6% to 12% of proteins that are mainly located in the endosperm (70-79% of total grain proteins) and the germ (18-28% of total grain proteins) (*Watson, 2003).* Although maize is an important raw material for human and animal nutrition, maize grain proteins are deficient in the content of lysine, which is one of the essential and limiting amino acids in protein synthesis. The results of analyzing the protein content in grain of different maize genotypes (yellow-seeded and white-seeded dents, popping maize and sweet maize) are shown in Table 3.

The protein content of grain of four different maize genotypes is presented by the following parameters: solubility and the solubility index of proteins. Solubility of albumins, globulins, zein and glutelins ranged from 1.12 (popping maize) to 3.37% (sweet maize), 0.65 (sweet maize) to 1.27% (yellow and white dent), 2.43 (sweet maize) to 3.04% (yellow dent) and from 2.04 (yellow dent) to 2.80% (popping maize), respectively. Furthermore, the solubility index varied from 9.46 (popping maize) to 29.42% (sweet maize), 5.64 (sweet maize) to 13.13% (white dent), 21.11 (sweet maize) to 28.10% (yellow dent) and from 18.81 (yellow dent) to 23,60% (popping maize) in albumins, globulins, zein and glutelins, respectively. The results showed that the highest indexes of solubility of albumin, globulin, zein and glutelin were found in the genotypes of sweet (29.42%), white dent (13.13%), yellow dent (28.10%) and popping (23.60%) maize. However, the lowest solubility indices of albumin, globulin, zein, and glutelin were found in popping (9.46%), sweet (5.64%), sweet (21.11%) and yellow dent (18.81%) maize genotypes, respectively.

Table 3. Protein content in grain of different maize genotypes

Genotype	Albumins (%)		Globulins (%)		Zein (%)		Glutelins (%)	
	SP	SI	SP	SI	SP	SI	SP	SI
Yellow-seeded dent	1.42	13.13	1.27	11.74	3.04	28.10	2.04	18.81
White-seeded dent	1.51	15.62	1.27	13.13	2.45	25.34	2.08	21.51
Popping maize	1.12	9.46	0.85	7.15	2.66	22.46	2.80	23.60
Sweet maize	3.37	29.42	0.65	5.64	2.43	21.11	2.26	19.77
Average	**1.86**	**16.91**	**1.01**	**9.42**	**2.65**	**24.25**	**2.30**	**20.92**
SD	**1.02**	**8.72**	**0.31**	**3.59**	**0.28**	**3.11**	**0.35**	**2.11**

SP – soluble proteins; SI – solubility index

Due to its specific genetics and the harvesting stage, the kernel of sweet maize genotype had significantly different chemical composition (low starch content and high protein, fiber and ash contents) and very different nutritional quality parameters (high contents of ADF, ADL and cellulose and low content of hemicellulose, a high index of solubility of albumin and low solubility of globulin and zein) compared to other maize genotypes.

The results presented in this study could be useful for the improvement of maize utilization and the development of new maize-based products. In addition to this, they could be guidelines for maize breeders in the further research and development of new maize hybrids with desired properties for specific purposes.

CONCLUSION

The chemical composition of the four selected maize genotypes varied as shown by their proximate analyses.

Results obtained on grain basic chemical composition of the selected yellow-seeded and white-seeded maize dents, popping maize and sweet maize show that the contents of starch, protein, oil, crude fibres, and ash varied among tested genotypes. The highest content of grain starch was in the genotypes of yellow and white dent maize (68.13 and 68.11%), while the lowest starch content in grain was in the genotype of sweet maize (53.54%), which also had the highest content of protein (13.00%), oil (5.39%), crude cellulose (3.93%) and ash (2.85%). The ratio of amylose to amylopectin of all tested genotypes varied from 21:79 (sweet maize) to 28:78 (popping maize), which is a principal property of normal maize starch. Similarly, the nutritional quality parameters such as the content of lignocellulosic fibres (NDF, ADF, ADL, hemicellulose and cellulose) and the solubility index of albumin, globulin, zein and glutelin of observed maize genotypes were in a broad range as well.Thus, based on gained results, maize genotypes developed at the Maize Research Institute, Zemun Polje, can be classified as hybrids of high grain quality and as such are highly valuable naturally renewable raw materials for production and energy.

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Informative article – comment
Inforativni rad - komentar

ADVANTAGES AND DISADVANTAGES OF DIRECT AND INDIRECT DRYING OF MERCANTILE GRAIN AND SEEDS

PREDNOSTI I NEDOSTACI DIREKTNOG I INDIREKTNOG SUŠENJA MERKANTILNOG ZRNA I SEMENA

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ABSTRACT

The question again arises as to how to dry certain types of agricultural grain products and seeds. Should it be indirect or direct? The reason for this is the occasional fires that occur in dryers. It is often and unprofessionally stated that the cause of the fires is the use of a direct dryer. However, the causes of fires are multiple, and it is seldom due to the type of dryer. Two factors determine the differences between direct and indirect drying, and these are the nutritional and safety aspects. The ambition of this study is to clarify all the important differences between direct and indirect grain drying and to eliminate the established misconceptions on this issue. Drying of sunflower, oilseed rape and sorghum is especially delicate.

Keywords: *grain dryer, fire, direct and indirect grain dryers, sunflower.*

REZIME

Po ko zna koji put ponovo se postavlja pitanje kako sušiti pojedine vrste poljoprivrednih zrnastih proizvoda i semena. Da li da to bude indirektno ili direktno? Razlog ovome su povremeni požari koji se javljaju na sušarama, a tada se često i nestručno konstatuje da je razlog požara korišćenje direktne sušare. Međutim, razlozi požara su mnogostruki, a vrlo retko je to zbog tipa sušare. Dva faktora determinišu razlike između direktnog i indirektnog sušenja, a to su nutricionistički i bezbednosni aspekti. Presudni faktor pojava požara je nepažnja ili nestručna upotreba sušare. Suncokret se može sušiti i na indirektnim sušarama, ali se mora strogo voditi računa o adekvtanim merama bezbednosti i primerenoj tehnologiji. Požari se javljaju i na direktnim i na indirektnim sušarama. U radu se objašnjava mehanizam požara na indirektnim sušarama. Ambicija ove analize je da razjasni sve bitne razlike između direktnog i indirektnog sušenja zrna i da otkloni ustaljene zablude o ovom pitanju.

Ključne reči: *sušara za zrno, požar, direktne i indirektne sušare za zrno, suncokret.*

INTRODUCTION

Definition and description

Direct dryers are those dryers in which convective drying is performed with a mixture of air and combustion products (*Babic and Babic, 2012*). Thus, the products of combustion of fuel, used as an energy source, are present in the drying fluid (drying agent). Indirect dryers are characterized by convective drying in which the drying fluid is heated air without the presence of combustion products. Therefore, clean heated air.

Dilemmas and problems

Drying is the oldest preservation process of agricultural products. Even in the original community, the application of this technology began, so that meat and fish were dried in smoke by the fire. This was direct drying. Sun-drying was also present, which can be attributed to indirect drying because there was no contact with the combustion products. From then until today, both types of drying are used. In modern times, analyzes of the quality of food have begun, in the production of which the drying of raw materials has been applied. The presence of unpleasant odors was the first reason to eliminate the contact of smoke (combustion products) with the material being dried. However, it was later determined that heavier hydrocarbons and other organic compounds that appear in the products of incomplete combustion, if present in the dried product, can be the cause of cancer. The mass removal of direct drying as a type of drying began then due to the fear of disease. Such an approach has partially reached grain agricultural products. The professional public was divided on this issue in the 1960s when it came to grain. Namely, in then West Germany, the prevailing view was that grain agricultural products must be dried exclusively in indirect dryers. In Germany at that time, there was no corn and soybeans in production, they predominantly dried wheat, barley and oats. In France, as the leading producer of corn in Europe, and in the USA, as the leading producer of corn in the world, direct grain dryers were used. Interestingly, the division into supporters of direct and indirect drying was also present in SFR Yugoslavia. Serbia used direct grain dryers en masse, and Croatia exclusively indirect ones.

As for sunflower, there was an opinion earlier that only indirect dryers should be used for its drying. In this case, the reason for such an attitude was the fire hazards, which were more pronounced in the case of sunflowers. At the faculties of technology, teachers educated technologists that indirect drying must be used for drying sunflowers as a safer drying method. This position, in the general case, is not correct and we will make a somewhat more detailed analysis and clarification of the matter later because there are different ways of indirect drying.

The dominant drying fuel was liquid fuel, extra light fuel oil (EL) and in a number of cases heavy oil.

Poor quality burners were the reason that with direct dryers there was a problem of incomplete combustion, i.e. the appearance of residues of heavy hydrocarbons (tar) on the dried grain.

Natural gas was available to a small number of grain dryers. The large increase in the price of liquid fuel has caused the need to look for cheaper solutions for the energy source. The

expansion of the natural gas distribution network was the answer to this problem. At that time (from the eighties of the last century onwards), this fuel was significantly cheaper than liquid fuel. There have been valuable and creative attempts to use renewable energy sources and coal.

Biomass as a fuel has not been domesticated in large silo dryers due to a number of technical, safety and organizational problems of use. It was concluded that biomass as a fuel can be used relatively easily and rationally on smaller energy units, except in special cases, such as seed processing centers. There have also been attempts to use geothermal water in grain dryers and to use straw bales for vertical gravity grain dryers (Hungary).

MATERIAL AND DISCUSSION

The nutritional aspects

Combustion of natural gas is relatively simple and is realized, mainly, as complete combustion. Combustion products contain carbon dioxide and water vapor as a result of combustion. None of these components (substances) are toxic or contaminate the grain. Of course, it is known that the produced carbon dioxide is harmful due to the greenhouse effect, but it is not harmful to the grain. The expansion of the natural gas distribution network to silos has led to a gradual transformation of attitudes towards the use of direct dryers. There are a large number of newer direct-type dryers in Germany. The reason for this is the tendency to reduce drying costs. Namely, indirect dryers require more heat energy than direct ones by about 10 to 25%. This loss is directly related to heat energy losses with combustion products released into the environment through the chimney. Direct dryers are significantly cheaper to invest in (up to 30%). In addition to reducing fuel consumption and lowering investment costs, there is also the argument of reduced carbon dioxide emissions into the atmosphere. The responsible professional public accepted these arguments and enabled the mass construction of direct dryers on natural gas. Furthermore, driers for liquefied petroleum gas (LPG), compressed natural gas (CNG) and biogas should be added to this category.

The question is what to do about liquid and solid fuel. Older burners on older dryers have become technically obsolete because new generations of modern burners are better. They are better because they provide quality fuel spraying in the form of equal fog droplets. In addition, turbulent mixing with air enables very high quality and complete combustion. This is very important. If we have very high quality (complete) combustion of liquid fuel, then there are no heavy hydrocarbons (herbal tars) in the combustion products that later condense on the grain. So, in this case, too, there is no fear of harmful effects on the material. It should be noted that the ratio of combustion products (according to stoichiometric balance) and added air in order to obtain a drying fluid (agent) of a certain temperature is very small. There is 20 to 70 times more air than combustion products in that mixture of fluids that dry the grain (*Babić and Babić, 2012*). This ratio depends on the type of gaseous fuel and the set temperature of the drying agent.

As for heavy oil, the issue is quite specific. Combustible sulfur is present in heavy oil to a greater or lesser extent. It can be about 2% or more. Sulfur combustion produces gaseous sulfur oxides. They will subsequently, during the flow through the grain, cool and condense on the grain itself, but also on the equipment. The presence of SO2, i.e. sulfuric acid, can be ascertained on the grain. Although this is not forbidden, it should be taken into account when trading. There is much more damage to the equipment, which will corrode quickly. Therefore, fuel oil requires very good combustion, i.e. a very high-quality burner, but also, uses heavy oil with very low sulfur content (less than 1%). It should be known that the sulfur content in fuel oil depends on the composition of crude oil used in rectification, i.e. the production of derivatives (and fuel oil) in refineries. Sulfur mainly remains in heavy oil and bitumen.

Solid fuel cannot burn with high quality on the heat air generators of the dryer. In this case, there are no dilemmas, the dryers must be indirect. When it comes to seed production, things are a little simpler. The seeds are not eaten or consumed for animal nutrition, so any residual tar on the grain (seed) is not so important. Here it is above all important to preserve germination. Germination is also important in beer barley. The temperature of seeds and beer barley must not exceed 42^{o}C. Everything previously analyzed concerned the nutritional aspects of direct grain drying.

The security aspects

The safety aspects of direct and indirect dryers will be considered here, i.e. the impact of the type of dryer on fires that occur in grain dryers will be analyzed. There are a large number of different technical solutions for indirect dryers. There are also differences in the type of heating medium in the technical solutions of the heat exchanger. Heating fluids can be warm and very hot. Warm heating media are all those with which we heat the air for drying, and whose temperature is lower than 200^{o}C. Examples are warm or hot water, dry saturated water vapor and thermal oil.

As for indirect grain dryers that use a warm heating medium, they are usually also factories that have a central power plant with the production of warm water, water vapor, or thermal oil. Typical places are oil factories, which produce water vapor for technological needs, and part of that co-saturated water vapor (usually 3-6 bar) is fed to tubular finned heat exchangers in which air is heated for drying. The ambient air, which is heated to obtain a drying agent, should not have dust or leaves in it, but even if it does, there is no great danger of ignition because the temperatures are lower than the ignition temperature (exothermic reaction). When the building of dryers in oil factories began, it was concluded that dryers should be indirect and that was the correct position, but only if the energy for heating the air for drying is realized with a warm heating medium. However, it should be noted that there are known cases of fires in such dryers (*Radmilo, 2005*). However, if the temperature of the heating medium is higher, usually over 500 ^{o}C, then it is a matter of very hot heating media.

Heating media in these cases are the products of combustion. What is the situation in such cases from the aspect of safety, i.e. possible occurrence of fire? Applied exchangers in which the air is heated by means of a heating medium are a drum, pipe, or combined system (drum exchanger + pipe exchanger). In all these cases, there is a zone in which the exchange surface is of a very high temperature. The inner sheath of the multi-pass drum exchanger (Fig. 1) is usually red-hot. The situation is the same with a simple drum firebox with a mantle for heating the air. The material of these mantles is either fire-resistant stainless steel or less often boiler sheet steel. It is important to notice that a flammable particle, leaf, husk, etc., which comes from outside, can touch the red-hot sheet metal and that will be the cause of ignition and the formation of sparks that formed in clean air. That is important to understand. The question is often asked why a fire broke out in an indirect dryer if it is safe. Similarly, a fire occurs in the case of other types of exchangers, because there are always parts of the exchanger that are heated to a very high temperature where the husk or a piece of a leaf with the surrounding air can reach. An indirect dryer with very hot

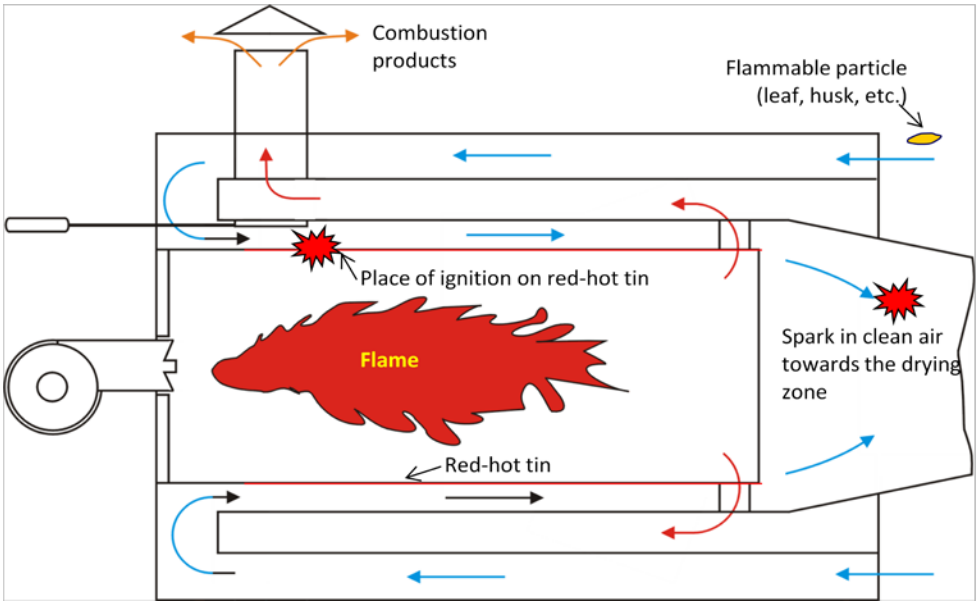

Fig 1. Demonstration of spark formation in a drum-type exchanger on an indirect dryer

heating media is almost as dangerous for fires as a direct dryer (*Poljak, 2000*).

Fire can also occur in the case of pipe exchangers, which are rare in the case of hot heating media because they are expensive and occur in combined heat exchange (example of the indirect dryer of former Serbian factory "Cer" based on combustion products).

Contemporary European dryers are almost all with partial recirculation of drying fluid (Fig. 2). In addition to them, there are two-stage dryers with air heating after the first pass by an open flame. Their safety is based on the elimination of husks in the recirculated fluid based on the closure of the airflow during the period of movement (discharge) of the grain into the bunker under the dryer (*Babić et al., 2007*). At that point, the presence of organic dust (and chaff) was reduced to less than 25 mg / m3. It is relatively safe, but in the case of sunflowers, the recirculation of the drying fluid is excluded and the dryer works as a single-pass. In this way, the possible occurrence of inflammation of the sunflower shell, which can be found in the recirculated air, is prevented. The dryer will be less energy efficient but will be safer to operate.

In addition to general safety measures, in the case of sunflowers, additional technical, technological and organizational measures are required, which are also important

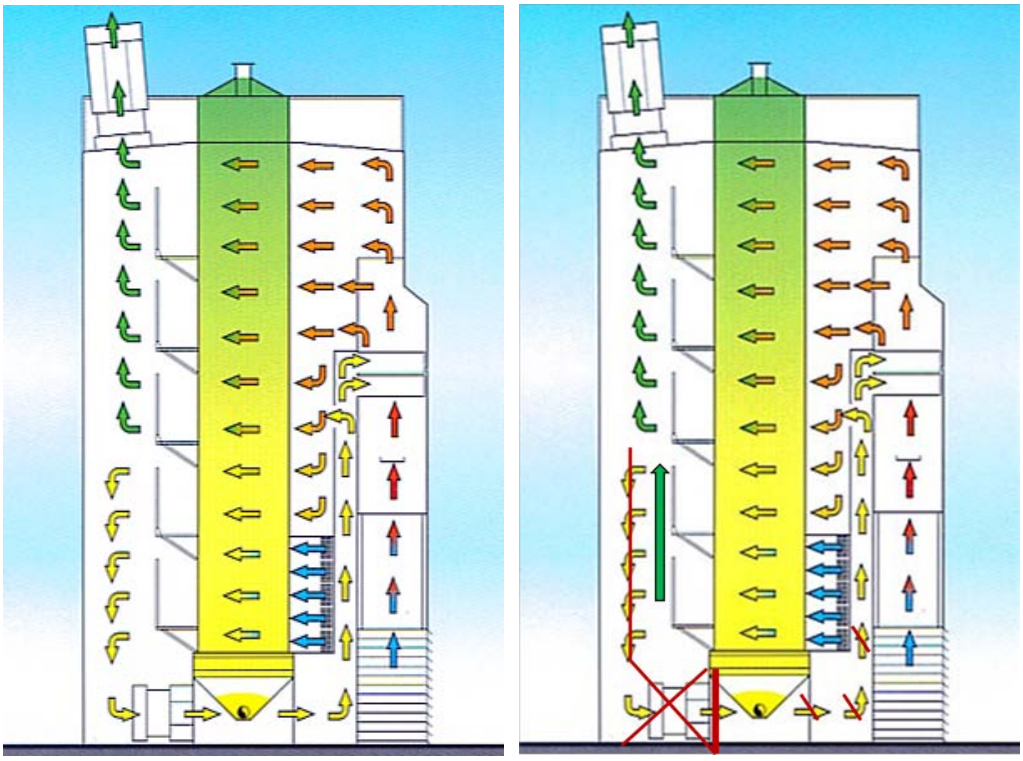

Fig 2. Direct dryer with partial recirculation of drying fluid (left - partial recirculation reduces fuel consumption and is used for all crops except sunflower; right - in case of sunflower drying partial recirculation is blocked to increase the safety of the dryer)

for the safe operation of the dryer (*Babić and Babić 2018*):

1) Use reduced airflow (grain must not be carried out into the dryer duct);

2) Turn off the recirculation fans in case of sunflower drying;

3) It is necessary to use especially low air (medium) temperatures for drying: max 65 °C. (best up to 60 °C)

4) Never fill the dryer with a sunflower that has started to ferment; the fermentation process develops a highly flammable gas. Such sunflowers should be actively ventilated beforehand;

5) Before starting the burner, the fan should run at least 45 minutes;

7) Increase the cooling time of the dryer to at least 60 minutes after drying;

8) Avoid downtime with a full sunflower dryer. If this happens, every 3 hours turn on the ventilation to work for at least 30 min. If the dryer will not work for more than 12 hours, then by recirculating the grain, dry all the sunflower present and send it to storage dried in this way;

9) Raw sunflower must be well cleaned before drying on a good grain cleaner with sieves and air separation;

10) Stop the dryer every 3-4 days (or maximum one time per week) and clean it (this is done in several hours or one shift);

11) The air entering the dryer must be clean, and for this purpose, there should be wire nets that stop leaves, husks and other larger impurities. Pay attention to the constant cleaning of these nets because they can get dirty quickly ("choke") and thus reduce the airflow, i.e. the capacity of the dryer;

12) The environment of the dryer must be clean;

13) Drying instrumentation, i.e. sensors, hardware and software must be constantly correct and checked before and during the drying season;

14) Workers must be well trained by a combination of theoretical instruction and practical work.

This applies to both direct and indirect dryers! Also, these tips should be applied to a greater extent in the case of drying other granular agricultural products.

The development of modern sensors and intelligent control reduces fire hazards. Timely registration of all important operating parameters on the dryer, but also the speed of parameter changes can shorten the time from the occurrence to the moment of noticing the accident situation (Dai et al., 2018; Dai et al., 2019). design of equipment and fire-fighting activities in case of fire (Xie et al., 2020)

CONCLUSION

There are major misconceptions about the role of direct and indirect grain drying in fire formation. Unfortunately, any fire that occurs in practice is abused by unfair competition by attributing the fire to the type and manufacturer of the grain dryer.

The analysis showed that the main causes of the fire were the technical condition of the equipment and the training of the dryer operator.

The type of dryer, i.e. whether the dryer is direct or indirect, can also participate in the danger of fire to some extent. Fires, however, occur in both dryers.

The installation of good and adequate sensors, which is an integral part of dryer automation, reduces the risk of fire. Such control systems must be fully operational at all times to ensure a high level of fire safety**.**

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Original Scientific Paper
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SPECIFICS OF SOYBEAN SEED PRODUCTION AND PROCESSING IN 2019

SPECIFIČNOSTI PROIZVODNJE I DORADE SEMENA SOJE U 2019. GODINI

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ABSTRACT

The soybean harvest in 2019 began at the end of August. The quality of the natural seed was very high. The moisture content of the harvested seeds ranged from 7.1 % to 14.1 %. Thanks to this humidity, there was no need to dry the seeds, which is very important given the fact that drying soybeans can reduce the quality. Seed germination ranged between 82 % and 96 %. It should be noted that 97 % of seeds were harvested with germination greater than 85 % while about 65 % of seeds had germination above 90 %. The weight of 1000 grains averaged about 160 g. Soybean seed processing started on time and without major problems. The purity of the processed seed was on average 99.4 %. Of the total amount of processed seeds, about 72 % of seeds had germination greater than 85 %. The achieved seed quality was better compared to the one in 2018.

Keywords: *soybean, production, harvest, seed reception, processing.*

REZIME

Iako su proizvodnju soje u 2019. godini pratili brojni problemi, na kraju možemo da kažemo da je bila uspešna kako po zasejanim površinama tako i po ostvarenom prinosu. Žetva soje započela je krajem avgusta. Prijem semena u doradnim centrima obavljen je bez većih problema zahvaljujući dobro pripremljenoj opremi i dobroj organizaciji prijema semena. Kvalitet naturalnog semena bio je veoma dobar sa zanemarljivo malim prisustvom zelenih i nedozrelih zrna. Vlažnost požnjevenog semena bila je u rasponu od 7,1% do 14,1% dok je oko 65% semena zaprimljeno sa vlažnošću iznad 10%. Zahvaljujući ovakvoj vlažnosti, nije bilo potrebe za sušenjem semena što je jako važno s obzirom na činjenicu da se sušenjem soje kvalitet može smanjiti. Klijavost semena, kao najvažniji parametar kvaliteta, bila je u rasponu između 82% i 96%. Treba istaći da je 97% semena požnjeveno sa klijavošću većom od 85% dok je oko 65% semena imalo klijavost iznad 90%. Masa 1000 zrna u proseku je iznosila oko 160 g. Na osnovu zaprimljenih količina semena u doradnim centrima može se reći da će količina semena nakon dorade biti dovoljna za setvu soje u 2020. godini. Dorada semena soje počela je na vreme i bez većih problema. Ostvarena čistoća dorađenog semena bila je u proseku 99,4%. Od ukupne količine dorađenog semena oko 72% semena imalo je klijavost veću od 85%, dok je oko 50% semena imalo klijavost iznad 90%. Ostvareni kvalitet semena bio je bolji u odnosu na 2018. godinu.

Ključne reči: *soja, proizvodnja, žetva, prijem, dorada.*

INTRODUCTION

According to the announcement of the Republic Bureau of Statistics of Serbia, the sown areas under soybeans in 2019 increased by 16.7 % compared to 2018 and amount to 229,372 ha *(www.stat.gov.rs).* According to the estimates of the Business Community for Industrial Plants, soybeans were sown on an area of about 220,000 hectares in 2019, with an average yield at the state level of 2,900 to 3,000 kg/ha *(Đukić et al., 2020),* which is by about 500 kg more when compared to the long-term average soybean yield in the Republic of Serbia. If we take into account the fact that the areas sown under soybeans in 2019 were also increased, then it can be said that soybean production in the previous year was very successful. Based on the presented results (Figure 1), a constant trend of increasing the area sown under soybeans in the Republic of Serbia in the last few years can be observed. Although soybean production is strongly influenced by climatic and other production conditions, thanks to a good assortment and increased investments in production, it can be said that the average yield, and thus the total soybean production, is growing from year to year.

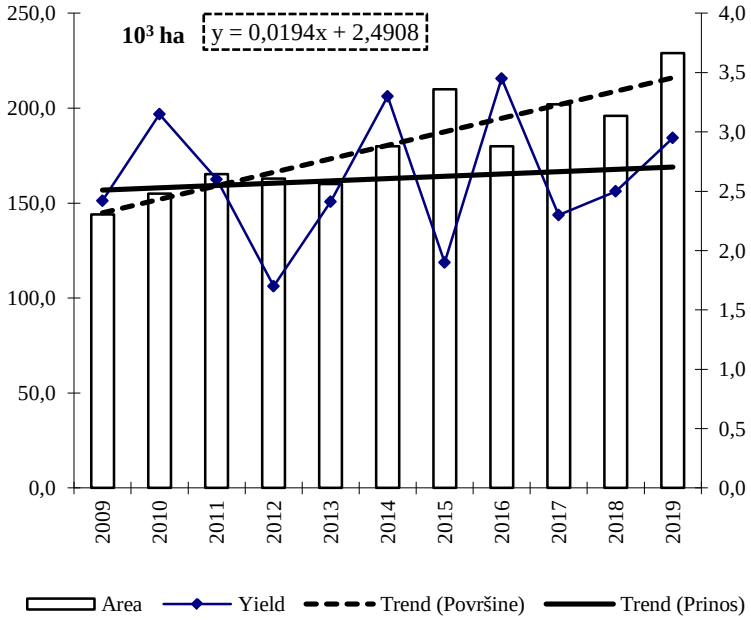

Fig. 1: Areas and yields of soybeans in the Republic of Serbia in the period 2009-2019

Production of soybean in 2019

In most production regions during 2019, the amount of precipitation, as well as the average monthly temperatures increased compared to the multi-year average. However, due to the very cold and rainy spring as well as the wet soil, sowing was delayed, while the period of germination and germination in the sown areas was significantly extended. In the first and second decade of May, the average daily temperatures were significantly lower compared to the multi-year average in all observed regions (by about 3°C), while the amount of precipitation was higher *(Đukić et al., 2020).* Such unfavourable conditions led to poor seed germination and thus to thinned assemblies, which is why producers had to sift a certain number of plots. After this cold period, much more favourable conditions occurred, which encouraged intensive growth and development of soybean crops. However, in July, a pronounced lack of precipitation prevailed in most production regions, especially in the second and third decades (lack compared to multi-year values by more than 30 mm). In the last decade of July and during August, the measured temperature values were 2-3°C above the multi-year average. As a consequence of stressful conditions during the seed watering period, the top pods on soybean plants were insufficiently watered, which led to a reduction in yield. The soybean harvest began in late August.

MATERIAL

Harvested soybean seeds were brought by truck and tractor conveyors to the processing centres, where they were measured on a weighbridge. After the measurement, it was sampled using appropriate probes according to the established seed sampling procedure for each vehicle. Then, the formation of samples was started, which were submitted to the authorized laboratory for analysis after marking. An analysis of the submitted samples was performed in the laboratory according to the rulebook on seed testing *(Official Gazette of the SFRY No. 47/87)*. Seed moisture, 1000 grain weight, germination energy, seed germination, the content of atypical germinate, inert substances, as well as the content of broken grain, were examined. Processed soybean seeds were sampled and tested by varieties and batches by the laboratory for testing the quality of seeds at the Institute of Field and Vegetable Crops from Novi Sad.

RESULTS AND DISCUSSION

Receiving, storage and processing of soybean seeds

Seed reception in processing centres was performed without major problems thanks to well-prepared equipment by the processor together with well-organized seed reception. The available storage capacities in the processing centres for receiving soybean seeds were sufficient to accept and store the planned amount of produced seeds without major problems. The quality of natural seeds was very good with an insignificantly small presence of "green" and immature grains. However, depending on the producer, smaller quantities of natural seeds had a certain presence of immature grains at the beginning of the harvest, which is why it was necessary to process the seeds on a rough purifier before storing them in silo cells. This measure is very important because storing soybean seeds (along with impurities) without prior purification would be very risky. The rough purifier has the role of extracting large impurities from the seeds, such as parts of harvest residues, unfinished pods (usually with physiologically immature grain), lumps of earth, as well as very small impurities and dust. The moisture content of harvested soybean seeds ranged from 7.1 % to 14.1 %. Most of the seeds of about 55 % were received with a moisture content above 10 %, which is very important considering that the moisture content in the grain is one of the important internal factors that affect the mechanical properties of the seeds. Furthermore, thanks to this humidity, there was no need to dry the seeds, which is very important given the fact that in addition to the increased cost of drying, the quality of the seeds would be significantly reduced. Numerous papers can be found in the literature that indicate that soybean seeds that have a low moisture content are exposed to mechanical damage. *Hoeft et al., (2000)* state that the soybean harvest should be completed before the seed moisture falls below 12 % due to cracking and damage to the seedling. It should be noted that about 45 % of soybean seeds in processing centres were received with humidity levels lower than 10 %. Therefore, it was necessary to receive and process soybeans carefully in order to minimize the risk of mechanical damage to the seeds. Germination of harvested seeds, as the most important quality parameter, ranged between 82 % and 96 %. It should be noted that as much as 97 % of seeds were harvested with germination greater than 85 % while about 61 % of seeds had germination above 90 %, which represents a very high quality of natural seeds. The weight of 1000 grains averaged about 160 g. It should be noted that this year the soybean grain was smaller compared to the previous year, so larger amounts of waste can be expected during seed processing. The content of inert substances in natural seeds averaged about 1.8 % and ranged from 0.1 % to as much as 9.3 %. The average content of broken grain in natural seeds was about 1.9 % and ranged from 0.1 % to extremely high 16.3 %. Based on the quantities of seeds received in the processing centres, it can be said that the number of seeds after processing will be sufficient for sowing soybeans in 2020. If we compare the average quality of natural soybean seeds produced in 2019. with the natural quality of seeds produced in the previous year, we can see that seed moisture, germination energy, seed germination and the content of atypical germinate have approximately the same values (Table 1). Only the values of the mass of 1000 grains, the content of broken grain and to some extent the content of inert substances are different.

Table 1. The average quality of natural soybean seeds in 2018 and 2019

Year	Humid. (%)	Inert substan. (%)	Broken seed (%)	Mass of 1000 grains (%)	Germination energy (%)	Seed germination (%)	Atypical germination (%)
2018	10.00	2.07	3.79	175.83	84	89	5
2019	10.13	1.77	1.87	159.87	85	90	5

In other words, it could be said that the quality of seeds in the observed two years was very uniform. However, unlike 2019, in which as many as 97 % of seeds had germination higher than 85 %, in 2018 this share of seeds was lower and amounted to 85 % (Table 2). Based on the results from Table 2, it can be

Table 2. The share of natural soybean seeds whose humidity is lower than 10 % and the share of seeds with germination above 85 % in 2018 and 2019

Year	Humidity < 10 %	Seed germination < 75 %	Seed germination > 85 %
2018	49 %	4.5 %	85 %
2019	45 %	0 %	97 %

said that the quality of natural seeds produced in 2019 was better.

Most of the processors this year started processing soybean seeds on time so the seeds were not stored in silo cells for a long time, which is very important. Namely, storing soybean seeds in warehouses for a longer period of time is not recommended because it can lead to reduced germination. Therefore, it was necessary to organize the processing of seeds as soon as possible so that the storage period of soybean seeds would be as short as possible. In the beginning, the most important thing was to provide the documentation necessary for the issuance of labels. It should be noted that the collection of the Certificate on the recognition of soybean seed crops this year was not delayed. In a short period of time, the agricultural expert services and the Ministry of Agriculture of the Republic of Serbia prepared the mentioned documentation so that the organization and processing of soybean seeds could be started in a timely manner. Since the quality of natural soybean seeds was very good, there were no major problems during seed processing, which is why this work was completed on time. Thanks to the favourable humidity of natural seeds, mechanical damage during manipulation and processing of seeds was minimal in most processors. The moisture content in the grain affects the physical properties of the seed, so the grain with a moisture content of 8 to 10 % is significantly more sensitive to shocks than a grain whose moisture content is 11 to 15 % *(Crnobarac et al., 2008).* Regardless of the favourable moisture content in the grain, it was necessary to equip the seed processing line with a sufficient number of seed drop dampers, since when handling soybean seeds from the hopper to the packaging device, there is a different degree of damage to the grain. However, some processors had certain organizational problems, which delayed the start of seed processing. Furthermore, they did not prepare their equipment to a sufficient extent, which all together influenced the fact that soybean seeds did not have the required quality after processing. The stated soybean seed which has poor quality will not be delivered to the market as seed material. The achieved purity of processed seeds was on average 99.4 %, which is a very good result considering that the legal minimum for the purity of soybean seeds is 96 % *(Official Gazette of SFRY No. 47/87. Rule on the quality of agricultural plant seeds. 1987)*, all finishing centres ranged from 96.8 % to 99.9 %. Such a wide range in seed purity was created as a consequence of different equipment of processing centres, applied seed processing technology, quality of natural seed as well as genotype. It has already been said that the production conditions did not affect the sowing and maturation of soybean seeds, which affected the weight of a thousand seeds to be lower than the usual values. However, in addition to the production conditions themselves, this parameter of seed quality largely depends on the genotype *(Kostić, 2016)*. It should be noted that the amount of waste generated during seed processing this year was pronounced, because natural soybean seeds were quite small and insufficiently watered, which is unfavourable since seed size can affect seed germination and initial plant growth *(Kostić, 2013)*. Yields for some varieties were very low, about 80 % and even lower, which makes the production in 2019 stand out from previous years. Of the total amount of processed seeds, about 72 % of seeds had germination greater than 85 %, while about 50 % of seeds had germination above 90 %. If we take into account that the legal minimum for soybean germination is 75 % *(Official Gazette of SFRY No. 47/87. Rule on the quality of agricultural plant seeds. 1987)*, then we can say that the achieved quality of soybean seeds in 2019 is very good, which confirms the fact that favourable conditions for seed production in the field and applied geotechnics together with quality processing and storage of seeds greatly contribute to the good quality of soybean seeds *(Tatić, 2007)*. However, there were processors in which the seeds of individual batches had a germination rate of less than 75 %, which is a consequence of mechanical damage to the seeds during manipulation and processing. These batches of seeds were denatured and as such, with all the accompanying documentation, were delivered to the processors as mercantile grain. If we compare the average quality of processed soybean seeds produced in 2019 with the quality of processed soybeans from 2018, we can see that the measured values of moisture and seed purity are approximately the same (Table 3.) while germination energy, seed germination and atypical germinate content in 2019 were significantly better.

Unlike in 2018, when as much as 25 % of processed seeds did not have the required quality, in 2019 there were only 6 % of such seeds. It is reliably known that the soybean grain is very sensitive to impact because the embryo is located just below the thin seed coat and can be easily damaged by mechanical action. Therefore, it is necessary for the processors to finish the soybean seeds very carefully and prepare the equipment for this important job, as already mentioned. The increase in soybean seed breakage during handling and transport depends on the impact force acting on each individual grain as well as on the seed moisture. The amount of crushed and broken seeds increases with each operation of manipulation and transport. It is very important to adjust the dynamics of processing so that the newly arrived quantities of seeds in the hopper are poured on the previously poured batch of seeds. In this way, the impact of the grain on the bottom and walls of the hopper is avoided, and thus the mechanical damage to the seed is reduced. *Shreekant et al., (2002)* found that soybean seed germination decreases by an average of 10 % to 31 % if the seed falls from a height of 1 to 2 meters on a concrete floor, while seed germination decreases by 7.5 % to 22 % if the seed falls from the same height on the galvanized metal floor. They also confirmed that seeds with a moisture content of 12 % suffer less damage compared to seeds whose humidity is lower than 10 %. In addition to the moisture content in the seed, *Kostić et al., (2012)* point out that the genotype also has an impact on the resistance of the seed to mechanical damage to the seed. *Hurburgh, (1995)* states that seeds with a lower humidity of 10% become very brittle and easily break in half during harvesting and manipulation, and even the processing of such seed soybeans reduces the germination of seeds. This indicates that during the harvest, reception, storage and processing of soybean seeds, it is necessary to respect and apply the correct technology, which requires great expertise, discipline and special care when handling seeds.

Table 3. The average quality of processed soybean seeds in 2018 and 2019

Year	Humidity (%)	Seed purity (%)	Mass of 1000 grains (%)	Energy germination (%)	Seed germination (%)	Atypical germinate (%)	Seed germination rate < 75 % (%)
2018	10.0	99.0	168.4	74	79	9	25
2019	10.2	99.4	162.6	80	87	6	6

CONCLUSION

The production of soybean seeds in 2019 was accompanied by favourable weather conditions. Thanks to that, quality assortment and adequate investments by producers, above-average grain yields were achieved in both mercantile and seed production of this important plant species. The quality of natural seed in seed production was high, with minimal presence of immature grains. Seed reception was done in a timely manner and without major problems, thanks to well-prepared equipment and organization of seed reception. Most of the seeds of about 65 % were received with a moisture content above 10 %, which is very important considering that the moisture content in the grain is one of the important internal factors that affect the mechanical damage to the seeds. Therefore, it is very important to complete the soybean harvest before the seed moisture drops below 12 %.

Moreover, it is necessary to manipulate the seed in the processing centre as little as possible, in order to reduce the risk of mechanical damage to the grain. Seed germination ranged between 82 % and 96 %, while 97 % of seeds were harvested with germination greater than 85 %. Furthermore, about 65 % of seeds had germination above 90 %, which is a very good quality of natural seeds. The weight of 1000 grains averaged about 160 g. It should be noted that agroecological conditions during seed production in the field have a great influence on seed quality. In addition to agroecological conditions, seed quality also depends on the elasticity of soybeans, which can be a varietal characteristic. Seed processing was done mostly on time, but some processors completed this work with a certain delay. The achieved purity of processed seeds was on average 99.4 %. Of the total amount of processed seed, about 72 % of seeds had germination greater than 85 %, while about 50 % of seeds had germination above 90 %. Therefore, it can be said that in 2020, producers will have very good quality seeds at their disposal. However, 6 % of the processed seeds did not have the required minimum germination of 75 %, which is a great damage that occurred as a result of mechanical damage to the seeds during manipulation and processing of seeds. In the end, it can be concluded that the quality of both natural and processed seeds in 2019 was better than the achieved quality of seeds produced in 2018.

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INFORMACIJE

FORUM O AKTULNIM PROBLEMIMA

Nacionalno društvo za procesnu tehniku i energetiku u poljoprivredi već više od 30 godina (uključujući i aktivnost Sekcije PTEP, kao prethodnice Društva PTEP) prati i raspravlja o aktuelnim problemima i pitanjima posleubirajućih tehnologija. Često su ove rasprave bile veoma korisne za praksu. Praksa PTEP-a je (kao nasleđe nekadašnjeg VDPT) negovala tradiciju da se zabeleži rasprava, odnosno da se publikuju tekstovi koji se tiču nekih stručnih dilema. Pamti se samo ono što je zapisano. Priče odnese vetar. Pozivam sve zainteresovane da se uključe u ovaj Forum i da svojim tekstom doprinesu raspravi o stručnim temama posleubirajućih tehnologija, ali i primarnih prehrambenih tehnologija. Pod primarnim prehrambenim tehnologijama podrazumevamo tehnološke operacije nad poljoprivrednim proizvodima iz domena mehaničkih, toplotnih, difuzionih i jednostavnih mikrobioloških postupaka (biogas, sušenje fermentisanih proizvoda…). Naše Društvo PTEP će biti moderator ovog foruma sa maksimalnim demokratskim mogućnostima, vodeći računa o opšte prihaćenim stručnim i naučnim znanjima. Pošaljite nam kraći ili duži tekst. Možete i komentare na objavljene tekstove na ovom forumu. Evo, za početak funkcinisanja ove tribine, ja sam napisao autorski tekst o nutricionističkim i bezbednosnim aspektima korišćenja direktnih i indirektnih sušara za zrno. Napišite argumentovani stručni komentar ili kao opovrgavanje stavova ili napišite dopunu, ako smatrate da treba.

Prof. dr Mirko Babi, generalni sekretar društva PTEP

DIREKTNO ILI INDIREKTNO SUŠENJE MERKANTILNOG ZRNA I SEMENA?

Po ko zna koji put ponovo se postavlja pitanje kako sušiti pojedine vrste poljoprivrednih zrnastih proizvoda i semena. Da li da to bude indirektno ili direktno? Razlog ovome su povremeni požari koji se javljaju na sušarama i tada se često i nestručno konstatuje da je razlog požara direktna sušara. Međutim, razlozi požara su mnogostruki, a vrlo retko je to zbog tipa sušare (direktna ili indirektna). Ambicija ovog teksta je da razjasni sve bitne razlike između direktnog i indirektnog sušenja zrna i da otkloni ustaljene zablude o ovom pitanju.

DEFINICIJA I OPIS

Direktne sušare su one sušare kod kojih se konvektivno sušenje obavlja mešavinom vazduha i produkata sagorevanja. Dakle, u fluidu za sušenje (agens sušenja) su prisutni produkti sagorevanja goriva koje se koristi kao energent. Indirektne sušare se karakterišu konvektivnim sušenjem u kome je fluid za sušenje zagrejan vazduh bez prisustva produkata sagorevanja. Dakle čist zagrejan vazduh.

DILEME I PROBLEMI

Sušenje je najstariji proces konzervisanja poljoprivrednih proizvoda. Još u prvobitnoj zajednici počela je primena ove tehnologije i to tako da su meso i riba sušeni na dimu, pored vatre. Ovo je bilo direktno sušenje. Bilo je prisutno i sušenje na suncu, što se može pripisati indirektnom sušenju, jer nije bilo kontakta sa produktima sagorevanja. Od tada pa do danas koriste se oba tipa sušenja. U savremeno doba počele su analize kvaliteta hrane pri čijoj je proizvodnji primenjeno sušenje sirovina. Prisustvo neprijatnih mirisa je bilo prvi razlog eliminisanja kontakta dima (produkata sagorevanja) i materijala koji se suši. Međutim, kasnije je utvrđeno da teži ugljovodnoci i druga organska jedinjenja koja se pojavljuju u produktima nepotpunog sagorevanja, ako su prisutni u sušenom proizvodu, mogu biti uzročnik obolenja od kancera. Tada je počelo masovno eliminisanje direktnog sušenja kao tipa sušenja zbog bojazni od obolevanja. Takav pristup je delimično stigao i do zrnastih poljoprivrednih proizvoda.

Stručna javnost se 60-tih godina prošlog veka podelila oko ovog pitanja kada je zrno u pitanju. Naime, u tadašnjoj Zapadnoj Nemačkoj preovladao je stav da se zrnasti poljoprivredni proizvodi moraju sušiti isključivo na indirektnim sušarama. U Nemačkoj tada nije bilo kukuruza i soje u proizvodnji, oni su dominantno sušili pšenicu, ječam i ovas. U Francuskoj, kao vodećem proizvođaču kukuruza u Evropi, i SAD-u, kao vodećem proizvođaču kukuruza u svetu, korišćene su direktne sušare za zrno. Interesantno je da je podela na pristalice direktnog i indirektnog sušenja bila prisutna i u SFR Jugoslaviji. Srbija je masovno koristila direktne sušare za zrno, a Hrvatska isključivo indirektne.

Što se tiče suncokreta ranije je bilo prisutno mišljenje da samo indirektne sušare treba koristiti za njegovo sušenje. U ovom slučaju razlog takvom stavu bile su požarne opasnosti koje su u slučaju suncokreta bile izraženije. Na tehnološkim fakultetima su nastavnici edukovali tehnologe da se za sušenje suncokreta mora koristiti indirektno sušenje kao bezbedniji metod sušenja Ovaj stav, u opštem slučaju nije tačan, o čemo ćemo kasnije napraviti nešto detaljniju analizu i razjašnjenja, jer postoje različiti načini indirektnog sušenja.

Dominantno gorivo za sušenje bilo je tečno gorivo i to ekstra lako ulje za loženje (EL) i u određenom broju slučajeva mazut. Nekvalitetni gorionici su bili razlog da se kod direktnih sušara pojavljivao problem nepotpunog sagorevanja, odnosno pojava rezidua teških ugljovodnika (katrana) na osušenom zrnu. Prirodni gas je bio dostupan manjem broju sušara za zrno. Veliki rast cena tečnog goriva uzrokovao je potrebu da se traže jeftinija rešenja za energetski izvor. Širenje distributivne mreže prirodnog gasa bio je odgovor na ovaj problem. Ovo gorivo je u to vreme (od osamdesetih godina prošlog veka pa nadalje) bilo znatno jeftinije od tečnog. Postojali su vredni i kreativni pokušaji korišćenja obnovljivih izvora energije i uglja. To se tada nazivalo alternativni izvori energije. Biomasa, kao gorivo, nije se odomaćila kod velikih sušara za zrno na silosima zbog niza tehničkih, bezbednosnih i organizacionih problema korišćenja. Zaključeno je da biomasa kao gorivo može relativno jednstavno i racionalno da se koristi na manjim energetskim jedinicama, izuzev posebnih slučajeva, kao što su semenski centri. Postojali su i pokušaji korišćenja geotermalne vode na sušarama za zrno i korišćenja rol bala slame za vertikalne gravitacione sušare za zrno (Mađarska).

STANJE I REŠENJA

Nutricionistički aspekti

Sagorevanje prirodnog gasa je relativno jednostavno i realizuje se, uglavnom, kao potpuno sagorevanje. U produktima sagorevanja, kao rezultat sagorevanja, nalaze se ugljen-dioksid i vodena para. Nijedna od ovih komponenti (supstanci) nije

otrovna niti zagađuje zrno. Naravno, znamo da je produkovani ugljen-dioksid štetan zbog efekta staklene bašte, ali on nije štetan za zrno. Širenje distributivne mreže prirodnog gasa do silosa dovelo je do postupne transformacije stava o upotrebi direktnih sušara. U Nemačkoj je veliki broj novijih sušara direktnog tipa. Razlog ovome je težnja ka smanjenju troškova sušenja. Naime, indirektne sušare zahtevaju više toplotne enrgije od direktnih za oko 10 do 25%. Ovaj gubitak je direktno povezan sa gubicima toplotne energije sa produktima sagorevanja koji se ispuštaju u okolinu kroz dimnjak. Direktne sušare su znatno investiciono jeftinije (do 30%). Uz smanjenje potrošnje goriva, niže investicione troškove tu je i argument o smanjenoj emisiji ugljen dioksida u atmosferu. Odgovorna stručna javnost je prihvatila ove argumente i omogućila masovnu gradnju direktnih sušara na prirodni gas. Ovoj kategoriji treba pridodati i sušare na tečni naftni gas TNG (LPG), na komprimovani prirodni gas KPG (CNG) i biogas. Postavlja se pitanje šta je sa tečnim i čvrstim gorivom. Stariji gorionici na našim starijim sušarama su u tehničkom smislu postali zastareli, jer su nove generacije savremenih gorionika bolje. Bolje su zato što se na njima ostvaruje kvalitetno raspršivanje goriva u vidu jednakih kapljica magle. Pored toga, turbuletno mešanje sa vazduhom omogućava veoma kvalitetno i potpuno sagorevanje. Ovo je veoma važno. Ako imamo vrlo kvalitetno (potpuno) sagorevanje tečnog goriva, tada u produktima sagorevanja nema teških ugljovodnika (biljni katrani) koji se kasnije kondezuju na zrnu. Dakle, i u ovom slučaju nema bojazni za štetno dejstvo na materijal. Treba znati da je odnos produkata sagorevanja (po stehiometrijskom bilansu) i pridodatog vazduha radi dobijanja fluida (agensa) sušenja određene temperature veoma mali. Vazduha ima više od 20 do 70 puta više od produkata sagorevanja u toj mešavini fluida kojom se suši zrno. Ovaj odnos zavisi od vrste gasovitog goriva i zadate temperature agensa za sušenje. Što se tiče mazuta stvar je dosta specifična. U mazutu je u manjoj ili većoj meri prisutan sagorivi sumpor. To može biti i oko 2% pa i više. Sagorevanjem sumpora dobijaju se gasoviti sumporni oksidi. Oni će sa naknadno, tokom prolaska kroz zrno, rashlađivati i kondezovati na samom zrnu, ali i na opremi. Na zrnu će moći da se konstatuje prisustvo $S0_2$, odnosno sumporne kiseline. Mada to nije zabranjeno, o tome treba voditi računa prilikom trgovine. Mnogo je veća šteta na opremi, koja će brzo korodirati. Zbog toga je kod mazuta potrebno veoma dobro sagorevanje, odnosno veoma kvalitetan gorionik, ali isto tako, koristiti mazute sa veoma malim sadržajem sumpora (manje 1%). Treba da se zna da sadržaj sumpora u mazutu zavisi od sastava sirove nafte koja se koristi pri rektifikaciji, odnosno proizvodnji derivata (i mazuta) u rafinerijama. Sumpor, uglavnom, ostane u mazutu i bitumenu. Čvrsto gorivo ne možemo visoko kvalitetno sagorevati na toplotnim agregatima sušara. U ovom slučaju nema dilema, sušare moraju biti indirektne. Kada je semenska proizvonja u pitanju stvar je malo jednostavnija. Seme se ne jede niti se troši za ishranu životinja, pa eventuane rezidue katrana na zrnu (semenu) nisu toliko bitne. Ovde je prevashodno važno da se sačuva klijavost. Klijavost je važna i kod pivskog ječma. Temperatura semena i pivskog ječma ne sme da premaši 42^{o}C

Štetne rezidue na pivskom ječnu će ostati i na sladu, a to znači i u pivu. Znači, i u ovom slučaju, pored klijavosti, brinemo o zdravstvenoj bezbednosti zrna. Sve što je prethodno prodiskutovano se ticalo nutricionističkih aspekata direktnog sušenja zrna.

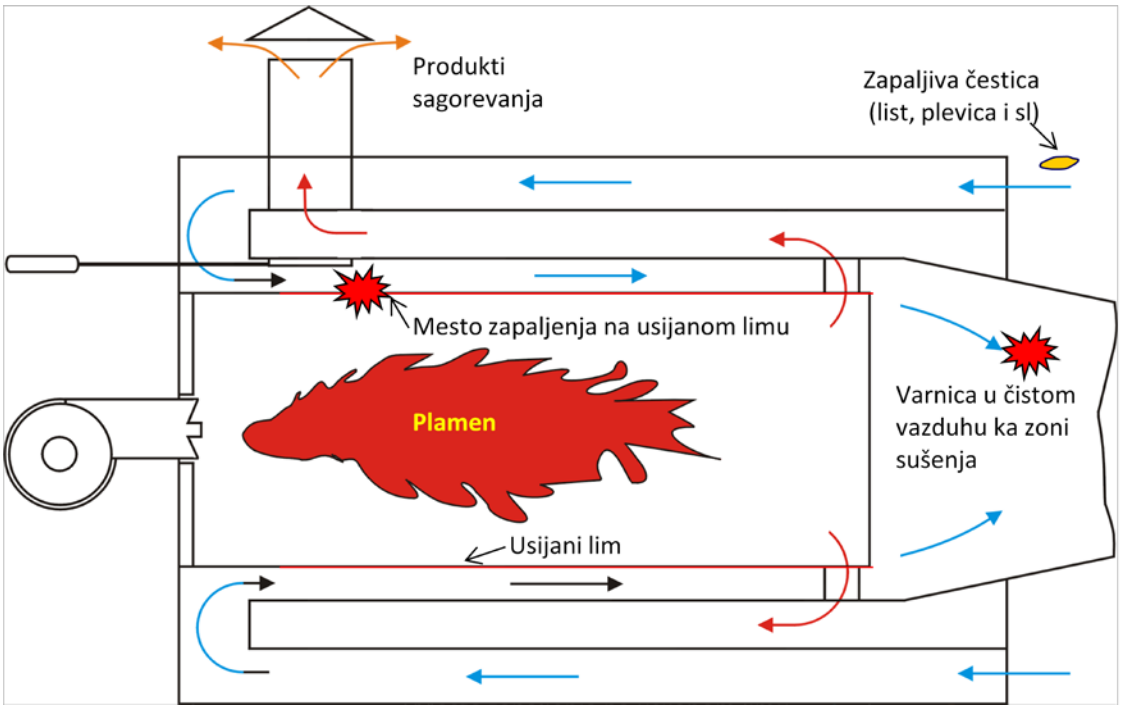
Bezbednosni aspekti

Sada će se razmotriti bezbednosni aspekti direktnih i indirektnih sušara, odnosno analiziraće se uticaj tipa sušare na požare koji se javljaju na sušarama za zrno. Postoji veliki broj različitih tehničkih rešenja indirektnih sušara. Razlike su u vrsti grejnog medijuma i u tehničkim rešenjima razmenjivača toplote. Grejni fluidi mogu biti topli i vreli. **Topli grejni medijumi** su svi oni kojima grejemo vazduh za sušenje, a čija je **temperatura niža od 200^{o}C**. Primer za to su topla ili vrela voda, suvozasićena vodena para i termalno ulje. Što se tiče indirektnih sušara za zrno na kojima se koristi topli grejni medijum one su obično i fabrikama koje imaju centralnu energanu sa produkcijom vrele vode, vodene pare ili termalnog ulja. Tipično mesto su uljare, koje proizvode vodenu paru za tehnološke potrebe, a deo te suvozasićene vodene pare (najčešće 3-6 bar) se dovodi do cevnih orebrenih razmenjivača toplote u kojima se greje vazduha za sušenje. Okolni vazduh, koji se greje da bi se dobio fluid (agens) za sušenje, ne treba da ima prašinu ni lišće u sebi, ali i ako ga ima, nema velikih opasnosti za zapaljenje jer su temperature niže od temperatura zapaljenja (egzotermna reakcija). Ipak, i u ovim slučajevima postoje manji rizici za pojavu požara. Kada su počele gradnje sušara u uljarama zaključeno je da sušare treba da su indirektne i to je bilo ispravno stanovište, ali samo u slučaju ako se energija za zagrevanje vazduha za sušenje ostvari toplim grejnim medijumom (topla voda, vodena para ili termo ulje). Međutim, ako je **temperatura grejnog medijuma** viša, najčešće **preko 500^{o}C,** tada je reč o **vrelim grejnim medijumima.** Grejni medijumi u ovim slučajevima su produkti sagorevanja. Kakva je situacija u ovakvim slučajevima sa aspekta bezbednosti, odnosno moguće pojave požara? Primenjeni razmenjivači u kojima se pomoću grejnog medijuma zagreva vazduh su bubnjasti, cevni ili kombinovani sistem (bubnjasti razmenjivač + cevni razmenjivač). U svim ovim slučajevima postoji zona u kojoj je razmenjivačka površina vrlo visoke temperature. Unutrašnji plašt višeprolaznog bubnjastog razmenjivača (sl. 1) je obično u crvenom usijanju. Ista je siutacija i sa jednostavnim bubnjastim ložištem sa plaštom za zagrevanje vazduha. Materijal ovih plaštova ili vatrootporni čelik ili ređe kotlovski lim. Bitno je da se uoči da zapaljiva čestica, **list, plevicai sl,** koja spolja dođe može da **dodirne usijani lim** i to će biti **uzrok zapaljenja i nastanka varnice** koja se pojavila u čistom vazduhu. To je važno da se razume. Često se pitamo zašto je nastao požar na indirektnoj sušari ako je ona bezbedna. Na sličan način požar nastaje i u slučaju drugih tipova razmenjivača, jer uvek postoje delovi razmenjivača koji su ugrejani na veoma visoku temperaturu gde može dospeti plevica ili delić lista sa okolnim vazduhom.

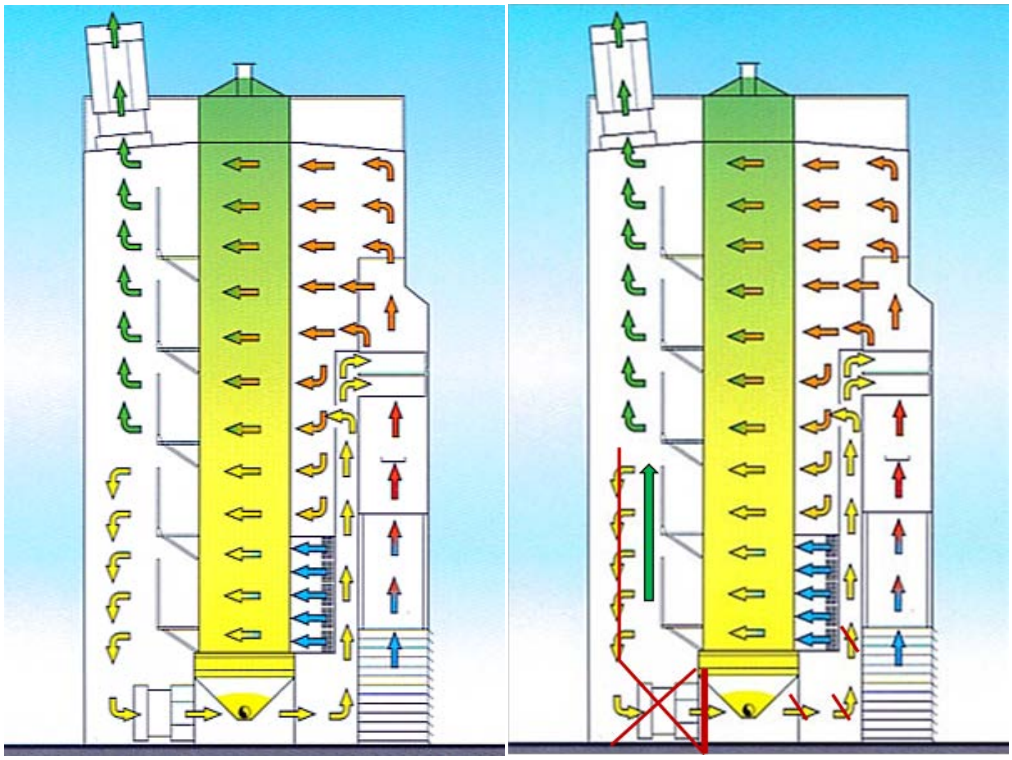
Indirektna sušara sa vrelim grejnim medijumima je skoro jednako opasna za nastanak požara kao i direktna sušara!

Požar može nastati i u slučaju cevnih razmnjivača koji su retki u slučaju vrelih grejnih medijuma, jer su skupi, a javlljaju se u kombinovanoj razmeni toplote (primer indirektne sušare "Cer" na bazi produkata sagorevanja).

Savremene evropske sušare su skoro sve sa delimičnom recirkulacijom fluida za sušenje (sl. 2). Pored njih su prisutne i dvostepene sušare sa dogrevanjem vazduha nakon jednog prolaza otvorenim plamenom. Njihova bezbednost je zasnovana na eliminaciji plevica u recirkulisanom fluidu na bazi zatvaranja protoka vazduha u periodu kretanja (ispuštanja) zrna u bunker ispod sušare. U tom momentu prisutvo organske prašine (i plevica) je redukovana na manje od 25 mg/m^3. To je relativno bezbedno, ali se u slučaju **suncokreta** isključuje recirkulacija fluida za sušenje i sušara radi kao jednoprolazna. Na ovaj način se sprečava eventualna pojava upaljenja ljuske suncokreta koja se može naći u recirkulisanom vaduhu. Sušara će biti manje energetski racionalna, ali će biti bezbednija za rad.



Sl. Prikaz nastanka varnice kod razmenjivača bubnjastog tipa na indirektnoj sušari



Sl. 2. Direktna sušara sa delimičnom recirkulacijom fluida za sušenje (levo – delimična recirkulacija smanjuje potrošnju goriva i koristi se za sve kulture osim suncokreta; desno – u slučaju sušenja suncokreta delimična recirkulacija je blokirana da bi se povećala bezbednost sušare)

Pored opštih mera bezbednost, u slučaju suncokreta, zahtevaju se dodatne tehničke, tehnološke i organizacione mere u koje su, takođe, važne za bezbedan rad sušare:

1) Koristiti smanjeni protok vazduha (ne sme dolaziti do iznošenja zrna u kanal sušare);
2) Isključitti ventilatore za recirkulaciju u slučaju sušenja suncokreta;
3) Koristite posebno niske temperature vazduha (medijuma) za sušenja: max 65°C. (najbolje do 60°C)
4) Nikad ne puniti sušaru suncokretom koji je počeo da fermentiše; proces fermentacije razvija visoko zapaljivi gas. Takav suncokret treba prethodno aktivno ventilisati;
5) Pre početka rada gorionika, rad ventilatora treba da traje najmanje 45 minuta;

7) Povećati vreme hlađenja sušare najmanje na 60 minuta nakon sušenja;
8) Izbegavati zastoje u radu sa punom sušarom suncokreta. Ako se to desi svaka 3 sata uključiti ventilaciju da radi bar 30 min. Ako sušara neće raditi duže od 12 h, tada recirkulacijom zrna osušti sav prisutni suncokret i tako osušen poslati ga na skladištenje;
9) Sirovi suncokret mora biti dobro očišćen pre sušenja na dobrom prečistaču sa sitima i vazdušnom separacijom;
10) Sušaru zaustavljati svaka 3-4 dana (ili u najgorem slučaju jedanput nedeljno) i očistiti je (to se obavi za nekoliko sati u zavisnosti od tipa sušare);
11) Vazduh koji ulazi u sušaru mora biti čist, a za tu svrhu treba da postoje žičana pletiva/mreže koja zaustavljaju lišće, plevicu i ostale krupnije nečistoće. Obratiti pažnju na stalno čišćenje ovih pletiva jer mogu brzo da se zaprljaju ("zaguše") pa tako da smanje protok vazduha, odnosno kapacitet sušare;
12) Okolina sušare mora biti čista;
13) Instrumentacija sušare, odnosno senzorika, hardveri i softveri moraju stalno biti ispravni i provereni pre sezone sušenja;
14) Radnici moraju biti dobro obučeni kombinacijom teorijske nastave i praktičnog rada.

Ovo važi i za direktne i za indirektne sušare! Takođe, ovo treba primenjivati u većoj meri i u slučaju sušenja drugih zrnastih poljoprirednih proizvoda, a naročito uljane repice, zrna sirka i sličnih osetljivijih zrnastih materijala.

Razvoj modernih senzora i inteligentne kontrole smanjuje opasnost od požara. Pravovremeno detektovanje svih važnih radnih parametara na sušari, ali i brzina promene parametara može skratiti vreme od pojave do trenutka uočavanja situacije aksidenta. Projektovanje opreme i vatrogasne aktivnosti u slučaju požara su, takođe, deo istraživačkih aktivnosti koje pomažu efikasnijoj odbrani.

ZAKLJUČAK

Postoje velike zablude oko uloge direktnog i direktnog sušenja zrna u nastajanju požara. Nažalost, svaki požar koji se desi u praksi, nelojana konkurencija zlouptrebljava pripisujući požar tipu i proizvođaču sušare za zrno.

Analiza je pokazala, da su glavni uzroci požara tehničko stanje opreme i obučenost rukovaoca sušara.

Na opasnosti od požara donekle može uticati i tip sušare, odnosno da li je sušara direktna ili indirektna. Požari se, ipak javljaju i u jednim i u drugim sušarama.

Na smanjenje rizika od požara utiče ugradnja dobre i adekvatne senzorike, koja je sastavni deo automatike sušare. Ovakvi upravljački sistemi u potpunosti moraju biti ispravni u svakom momentu, kako bi obezbedili viskoki nivo bezbednosti od požara.

Prof. dr Mirko Babi, dipl.inž.maš.
Nacionalno društvo PTEP

IN MEMORIAM
Prof. emeritus dr Dušan Petrovački

Krajem februara 2020. preminuo je prof. emeritus dr Dušan Petrovački. Profesor Petrovački ostavio je dubok trag u akademskoj zajednici Univerziteta u Novom Sadu, AP Vojvodini i Republici Srbiji. Obavljao je odgovorne funkcije, ali je sve vreme bio neposredno povezan sa razvojem automatike, računarstva i IT tehnologija.

Profesor Dušan Petrovački je rođen 09.09. 1943. u Pivnicama, pored Bačke Palanke, odakle ubrzo prelazi u Novi Sad. Osnovnu školu „Đorđe Natošević" i gimnaziju „Jovan Jovanović Zmaj" završio je u Novom Sadu. Diplomirao je 1968, magistrirao 1975, a doktorirao 1979. Po povratku iz vojske 1969. radio je u projektnom birou „Agroindustrija", Novi Sad. Školske 1971/1972 započinje svoju akademsku karijeru na Mašinskom fakultetu u Novom Sadu kao asistent. Docent postaje 1979, vanredni profesor 1983, a redovni profesor 1988. godine. Za profesora emeritusa izabran je 2011. godine.

Prvo je bio asistent na predmetima Automatika 1 i 2 kod prof. dr Dušana Jakšića. Bio je odmeren, ozbiljan i videlo se da pored teorije dobro poznaje i praksu, a to je bilo logično jer je došao na fakultet iz „Agroindustrije" gde se bavio projektovanjem termo-energetskih sistema. Veliki broj njegovih projekata je i izveden. Bio je član Katedre za elektrotehniku i automatiku. Kada je 1974. godine Mašinski fakultet prerastao u FTN, deo Katedre iz područja automatike prelazi na elektrotehnički odsek, kasnije Departman za računarstvo i automatiku i tu je proveo ceo svoj radni vek. U tom radnom veku se baš naradio. Bio je vrstan profesor, plodan istraživač i pouzdan partner privredi Vojvodine i Srbije.

Prof. dr Dušan Petrovački je držao nastavu na trideset predmeta na redovnim i poslediplomskim studijama. Unapredio je postojeće i uveo nove oblasti u obrazovni proces, na redovnim i poslediplomskim studijama i formirao je više od dvadeset novih savremenih predmeta. Bio je mentor na 23 doktorska i magistarska rada. Na Međunarodnim poslediplomskim studijama, na engleskom jeziku, održavanih u Internacionalnom UNESKO centru za upravljanje velikim sistemima, držao je internacionalnih kurseva i seminara iz oblasti upravljanja, simulacije i optimizacije.

Dugo godina je profesor Petrovački bio nastavnik studentima poljoprivredne tehnike na Poljoprivrednom fakultetu u Novom Sadu. Prvo kao asistent, a potom kao nastavnik na predmetu Automaika. On je bio učesnik PTEP-a u ranom periodu ovih savetovanja, a potom su njegovi saradnici nastavili da podržavaju i učestvovuju na našim konferncijama sve do danas.

Publikovao je preko 200 radova u časopisima ili ih saopštio na konferencijama. Bio je nosilac i učesnik značajnog broja naučnih tema, podprojekata i projekata koje su finansirale naučne institucije na saveznom, republičkom i pokrajinskom nivou, kao i 14 međunarodnih projekata.

Posebna vrednost i karakteristika njegovog rada se ogledala u činjenici da je rukovodio i učestvovao u projektovanju i realizaciji u praksi velikog broja postrojenja baziranih na visokim tehnologijama, značajnih za tehnološki razvoj Vojvodine i Srbije.

Profesor Petrovački je dao neosporan doprinos razvoju Univerziteta i svog fakulteta, obavljajući razne rukovodeće funkcije (dekan FTN-a, direktor instituta za računarstvo, automatiku i merenje, šef Odseka za automatiku i upravljanje sistemima i Katedre za signale, sisteme i upravljanje, predsednik Fonda za nadarene studente UNS-a, član Senata UNS-a i predsednik Stručnog veća za polje tehničko-tehnoloških nauka UNS-a, Pokrajinski sekretar za nauku i tehnološki razvoj AP Vojvodine, Izvršni sekretar stručne asocijacije Srbije – ETRAN, član stručnih tela kao predstavnik Srbije – EUPOS-a, itd).

Jedan od najvažnijih rezultata prof. Petrovačkog bio je sigurno razvoj i obezbeđenje visoko kvalitetnog i brojnog nastavno-naučnog podmlatka za koji se zna i izvan naše zemlje. Za svoj stvaralački rad i doprinos razvoju UNS-a i FTN-a dobio je više nagrada i priznanja, među kojima se ističe Oktobarska nagrada grada Novog Sada 1986. godine.

Posebno se ističe doprinos profesora Dušana Petrovačkog razvoju fakulteta i malih preduzeća u oblasti IT sektora. Bio je dekan fakulteta u tri mandatna perioda od 1991. do 1998. godine. To je bio težak period za Srbiju. Period raspada Zemlje, velike ekonomske krize, sankcija, inflacije, ratova na teritoriji Jugoslavije. Profesor Petrovački je uspešno i mudro vodio fakultet u tom periodu. Pokreno je studije Arhitekture sa profesorom Rankom Radovićem i sagradio novi prostor za njih. Sa profesorom Vladimirom Kovačevićem i saradnicima u rezultatu naučno-istraživačkog rada razvijali su nove proizvode i osnivali prva mala preduzeća na fakultetu koja su vodili naši profesori.

Za vreme mandata profesora Petrovačkog osnovano je 7 takvih preduzeća. Bio je pionir ovog poduhvata, mada to tada nije bilo dozvoljeno po zakonu. Tek je 2002. godine donet zakon koji je dozvolio da fakulteti mogu da učestvuju u osnivanju privatnih preduzeća. To je tada bilo hrabro i opasno. To je bio vizionarski poduhvat i zato sa pravom ističe da je profesor Petrovački bio jedan od pionira razvoja IT sektora u Novom Sadu, Vojvodini i Srbiji. Nema sumnje da je profesor dr Dušan Petrovački dao nemerljiv doprinos razvoju FTN-a i Univerziteta u Novom Sadu.

Otišao je čovek širokog srca i velikog znanja.

(veći deo ovog teksta preuzet je iz komemoracionog govora prof. emeritusa dr Ilije Ćosića)

NOTICE: