UDC: 63 ISSN 1450-8109

JOURNAL OF AGRICULTURAL SCIENCES BELGRADE

Vol. 67, No. 4, 2022

Published by University of Belgrade
Faculty of Agriculture
Republic of Serbia

UDC: 63 ISSN 1450-8109

JOURNAL OF AGRICULTURAL SCIENCES BELGRADE

Vol. 67, No. 4, 2022

Published by University of Belgrade
Faculty of Agriculture
Republic of Serbia

Journal of Agricultural Sciences (Belgrade) www.joas.agrif.bg.ac.rs
J. Agric. Sci (Bg).

PUBLISHED BY

University of Belgrade-Faculty of Agriculture
11081 Belgrade-Zemun, Nemanjina 6, PO Box 14, Serbia
Tel: + 381 11 4413-555/467; Fax: + 381 11 2193-659; E-mail: redakcija@agrif.bg.ac.rs
URL: http://www.agrif.bg.ac.rs/

DTP Service: Snežana Spirić

Printed by the Faculty of Agriculture, Belgrade-Zemun
Circulation: 100

Publishing is supported by the Ministry of Education, Science and Technological Development
of the Republic of Serbia, Belgrade

According to the opinion of the Ministry of Science of the Republic of Serbia
No. 413-00-1928/2001-01 dated November 6, 2001 this
Journal is exempt from general tax liability

Frequency: Four times per year

Abstracting and Indexing
CAB Abstracts, AGRICOLA, SCIndeks, EBSCO, Scopus, DOAJ

Number of institutions the Journal is exchanged with: 80

JOURNAL OF AGRICULTURAL SCIENCES BELGRADE
Vol. 67, No. 4, 2022

C O N T E N T S

JOURNAL OF AGRICULTURAL SCIENCES BELGRADE
Vol. 67, No. 4, 2022

S A D R Ž A J

Journal of Agricultural Sciences (Belgrade) https://doi.org/10.2298/JAS2204335K
Vol. 67, No. 4, 2022 UDC: 635.648.07:575.22
Pages 335-354 Original scientific paper

YIELD PERFORMANCE AND STABILITY ANALYSIS OF OKRA (*ABELMOSCHUS ESCULENTUS* L. MOENCH) ACCESSIONS USING AMMI AND GGE BIPLOTS

Ronke J. Komolafe[1*], Omolayo J. Ariyo[2] and Olusanya C. Alake[2]

[1]Department of Plant Science and Biotechnology,
Federal University Oye-Ekiti, Ekiti State, Nigeria
[2]Department of Plant Breeding and Seed Technology,
Federal University of Agriculture Abeokuta, Ogun State, Nigeria

Abstract: The identification of adaptable, stable and high yielding genotypes under varying environmental conditions prior to release poses a lot of challenge to plant breeders in selecting the best genotypes of okra. The genotype × environment interaction is a major challenge to plant breeders because a large interaction can reduce selection gain and make the identification of superior cultivars difficult. The objectives of this study were to evaluate the performance of okra accessions in different environments and identify a high yielding and stable accession so as to select a parent for further breeding work. Seventeen accessions of okra were evaluated at Akure during the rainy season of 2018, at Akure and Oye during the rainy season of 2019; and at Akure during the rainy season of 2020, making a total of four environments. The additive main effects and multiplicative interaction and GGE-biplots were employed for the evaluation of the G×E interaction and stability studies in the four environments. The AMMI analysis identified NGB00378a as the most stable accession and high yielder. Also, GGE biplot identified NGB00378a as highly stable and the high yielder while NGB00355 was the highest yielder, but fairly stable. However, NGB00378a combines good performance with stability. Therefore, NGB00378a is an ideal accession that should be recommended for further breeding work.

Key words: environment, genotype, interaction, performance, stability.

Introduction

Okra (*Abelmoschus esculentus* L. Moench) is one of the most important vegetable crops in the world, mainly grown for its tender leaves and pods (Chattopadhyay et al*.,* 2011). It is a nutritious vegetable which provides dietary fiber (Kumar et al., 2010), protein, fats and carbohydrates (Saifullah and Rabbani,

*Corresponding author: e-mail: ronke.komolafe@fuoye.edu.ng

2009), vitamins, minerals and medically important compounds (Kumar et al., 2010). Okra is highly nutritious and valuable, and as a result, its performances and production across locations and environments all over the continents should be highly encouraged. Okra is widely grown in Nigeria for its edible fresh pod for cooking soups. Several researchers have worked on the production, cultivation, diversity, correlation and heritability studies of okra in Nigeria (Aminu et al., 2016; Komolafe et al., 2021a; AdeOluwa and Kehinde, 2011; Bello et al., 2015). Komolafe et al. (2021b) carried out correlation studies on forty genotypes of okra in Akure, Nigeria and concluded that pod yield per plant had highly significant and positive genotypic and phenotypic correlations with plant height, number of leaves, petiole length, internode length, plant height at flowering, peduncle length, fruit length, fruit diameter, number of seeds and number of pods. Oyetunde and Ariyo (2015) investigated gene action controlling yield and related traits in okra and concluded that both additive and dominant gene actions controlled the expression of characters in okra. Alake and Ariyo (2012) evaluated twenty-five West African okra genotypes from diverse geographical backgrounds in five different environments in Nigeria for the stability of performance using AMMI and GGE biplot analyses and concluded that the GGE biplot explains higher proportions of the sum of squares of the G × E interaction and it is more informative with regards to environments and cultivar performance than the AMMI analysis. Twenty-nine okra accessions were evaluated for stability and yield performance in four environments in Nigeria and both AMMI and GGE-biplot models identified LD88/1-8-5-2, 47-4 and NH88/1-8-16-2 as the best accessions for cultivation across seasons (Nwangburuka et al., 2011).

Many genotypes or varieties of okra that perform very well as regards yield and component in a particular environment tend to perform poorly or differently in another environment. This results from the interaction between genotype and the environment, which is referred to as genotype × environment interactions. Kang (2004) defined genotype × environment interactions as the disparity in the performances of genotypes or cultivars across different environments. This has been a major problem in plant breeding because it leads to inconsistency in the yield performances of genotypes or accessions over different locations and environments, which makes the selection of the best genotype or consistent performing lines difficult (Ariyo and Ayo-Vaughan, 2000). Genotype × environment interactions retard progress in the crop improvement programme by complicating the breeding processes as a result of the unstable performance of accessions or genotypes across a wide range of locations or environments. As a result, the genotype × environment interaction study is imperative to plant breeders in order to determine stable and high yielding genotypes across different environments (Olayiwola and Ariyo, 2013), to test cultivar adaptation (Dias and Krzanowski, 2003), to evaluate newly enhanced genotypes across test environments before a specific genotype is released for cultivation (Akter et al*.,* 2015).

Many researchers have demonstrated the effectiveness of the AMMI model in understanding the G×E interaction in yield, estimating yields more accurately and selecting superior genotypes more reliably (Alake, 2018; Nassir and Ariyo, 2005). The AMMI analysis combines additive components in a single model for the main effects of genotypes and environments as well as multiplicative components for the interaction effect (Edugbo et al*.,* 2015). The AMMI model displays main effects of genotypes and environments and their interactions and also contributes to the improved genotype evaluation, recommendation and selection of test environments (Abay and Bjornstad*,* 2009). The GGE biplot model provides breeders with a more complete and visual evaluation of all aspects of the data by creating a biplot that simultaneously represents mean performance and stability, as well as identifying mega-environments (Yan, 2001; Yan and Kang, 2003). The GGE biplot analysis is another method which integrates the genotype and genotype by environment effects in the evaluation of cultivars. The GGE that uses graphic axes identifies superior cultivars in the mega environments (Akcura et al., 2011). Mega environments comprise groups of environments which consistently share the same test genotypes (Abay and Bjornstad, 2009). Miranda et al. (2009) reported that the GGE biplot informatively explains a higher proportion of the sum of squares of the G × E interaction with regards to environments and cultivar performance and makes visualization more logical and biological for practice than AMMI. The objectives of this study were to (i) evaluate the performance of seventeen (17) okra accessions in different environments using AMMI and GGE biplots and (ii) identify high yielding and stable accessions across environments so as to select a parent for further breeding work.

Material and Methods

Seventeen accessions of okra used for this research were sourced from five agro ecological zones of Nigeria, namely: Sudan savannah, Derived savannah, Southern Guinea savannah, Northern Guinea savannah and humid forest, through the gene bank of the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Oyo State, Nigeria (Table 1). The experiment was carried out in Akure, Ondo State and Oye, Ekiti State, over a three-year period (2018–2020). Seventeen accessions of okra were evaluated at Akure during the rainy season of 2018 (FUTA2018), Akure and Oye during the rainy season of 2019 (FUTA2019 and FUOYE2019); and in Akure during the rainy season of 2020 (FUTA2020), making a total of four environments (Figure 1). The experiments were carried out in a randomized complete block design (RCBD) with three replications. One-row experimental plots of 6-m length were used. The rows were 1 m apart while the plant-to-plant distance in each row was 0.6 m. Three seeds were sown per hole and later thinned to two plants per stand. Weeds were controlled manually at three-week intervals and insect pests were controlled using

Cypermethrin at the rate of 50ml/10 liters of water. Agro-meteorological data at FUTA and FUOYE experimental sites from January to December 2018–2020 (Table 2) revealed that there were differences in environmental conditions of the different locations from year to year.

Table 1. Okra accessions collected in Nigeria – sources, places of collection, agro-ecological locations and their qualitative traits.

S/N	Accession	Source	Place of collection	Agro-ecological location	Stem pubescence	Pod pubescence	Stem colour	Petal colour	Pod colour	Position of the pod on the main stem
1	NGB00297	NACGRAB	Kebbi State	Sudan savannah	Slightly conspicuous	Prickly	Green	Yellow	Green	Pendulous
2	NGB00298	NACGRAB	Nasarawa State	Derived savannah	Slightly conspicuous	Slightly rough	Green	Yellow	Green	Erect
3	NGB00299	NACGRAB	Oyo State	Derived savannah	Conspicuous	Prickly	Green	Yellow	Green	Erect
4	NGB00302	NACGRAB	Kano State	Sudan savannah	Conspicuous	Prickly	Green	Yellow	Green	Erect
5	NGB00303	NACGRAB	Unknown	Unknown	Glabrous	Downy	Purple-green	Yellow	Purple-green	Erect
6	NGB00304	NACGRAB	Niger State	Southern Guinea savanna	Conspicuous	Prickly	Purple-green	Yellow	Purple-green	Erect
7	NGB00331	NACGRAB	Oyo State	Derived savannah	Slightly conspicuous	Slightly rough	Green	Yellow	Purple-green	Erect
8	NGB00346	NACGRAB	Kebbi State	Sudan savannah	Conspicuous	Prickly	Green	Yellow	Green	Erect
9	NGB00347	NACGRAB	Unknown	Unknown	Slightly conspicuous	Slightly rough	Purple	Yellow	Purple-green	Erect
10	NGB00350	NACGRAB	Unknown	Unknown	Conspicuous	Prickly	Green	Yellow	Green	Erect
11	NGB00355	NACGRAB	Ondo State	Humid forest	Conspicuous	Prickly	Red-green	Yellow	Green	Erect
12	NGB00356	NACGRAB	Edo State	Humid forest	Slightly conspicuous	Downy	Green	Yellow	Green	Erect
13	NGB00369	NACGRAB	Niger State	Southern Guinea savanna	Conspicuous	Prickly	Green	Yellow	Green	Erect
14	NGB00371	NACGRAB	Niger State	Southern Guinea savanna	Slightly conspicuous	Slightly rough	Red-green	Yellow	Green	Erect
15	NGB00378a	NACGRAB	Delta State	Humid forest	Glabrous	Downy	Green	Yellow	Green	Erect
16	NGB00378b	NACGRAB	Delta State	Humid forest	Glabrous	Slightly rough	Green	Yellow	Green	Erect
17	NGB00430	NACGRAB	Unknown	Unknown	Glabrous	Slightly rough	Green	Yellow	Green	Erect

Source: NACGRAB = National Centre for Genetic Resources and Biotechnology.

Data collection

Data were collected from six plants in each row on the following traits:

(i) Plant height at 50% flowering (cm): This was determined by measuring the plant from the soil level to the tip of the main stem when half of the plants in a plot flowered;

(ii) Plant height (cm): This was measured from the soil level to the tip of the plant at maturity;

(iii) Number of seeds per pod: This was determined at maturity by counting the number of seeds in five randomly selected pods and averaging the pods;

(iv) Pod yield per plant (g/plant): This was determined by averaging and summing the weight of green pods harvested from five sampled plants at all picking times.

Data analysis

Data on the above traits were subjected to a combined analysis of variance to determine the effects of the environment (E), the genotype (G) and the genotype × environment interaction.

The data were subjected to the additive main effects and multiplicative interaction (AMMI) analysis in order to determine the stability of the accessions. The AMMI model first fits the additive effects for the genotypes and the environments and the multiplicative term for interactions of genotypes with the environments. According to Gauch and Zobel (1996), the linear model for AMMI is presented as:

$$Y_{ij} = \mu + B_i + C_j + \sum \lambda_x . \alpha_{ix} . \delta_{jx} + R_{ij} + E \quad (1)$$

where Yij is the value of the i^{th} genotype in the j^{th} environment; μ is the grand mean; Bi is the deviation of the i^{th} genotype from the grand mean; Cj is the deviation of the j^{th} environment from the grand mean; λx is the singular value for PC axis x; αix and δjx are the PC scores for axis x of the i^{th} genotype and j^{th} environment, respectively; Rij is the residual and E is the error (Gauch, 1992).

To measure the value of genotypes and rank based on yield stability, the AMMI stability value (ASV) proposed by Purchase et al. (2000) was calculated.

AMMI stability value (ASV) was calculated using the formula proposed by Purchase et al. (2000) as follows:

$$\text{ASV} = \sqrt{\left[\frac{IPCA\ 1SS}{IPCA\ 2SS}(IPCA\ 1_{Scores})\right]^2 + (IPCA\ 2_{Scores})^2} \quad (2)$$

where IPCA1 SS / IPCA2 SS is the weight given to the IPCA1-value.

The GGE biplot model was used according to Yan (2001) as:

$$Y_{ij} - \mu - \beta_j = \lambda_1 £_{i1}\, \eta_{j1} + \lambda_2 £_{i2} \eta_{j2} + \varepsilon_{ij} \quad (3)$$

where Y_{ij} is the mean of genotype i in environment j, μ is the grand mean, βj is the main effect of environment j, λ1 and λ2 are the singular values for the first and second principal component (PC1 and PC2), respectively, £$_{i1}$ and £$_{i2}$ are eigenvectors of genotype i for PC1 and PC2, respectively, η_{j1} and η_{j2} are eigenvectors of environment j for PCl and PC2, respectively, ε_{ij} is the residual associated with genotype i in environment j.

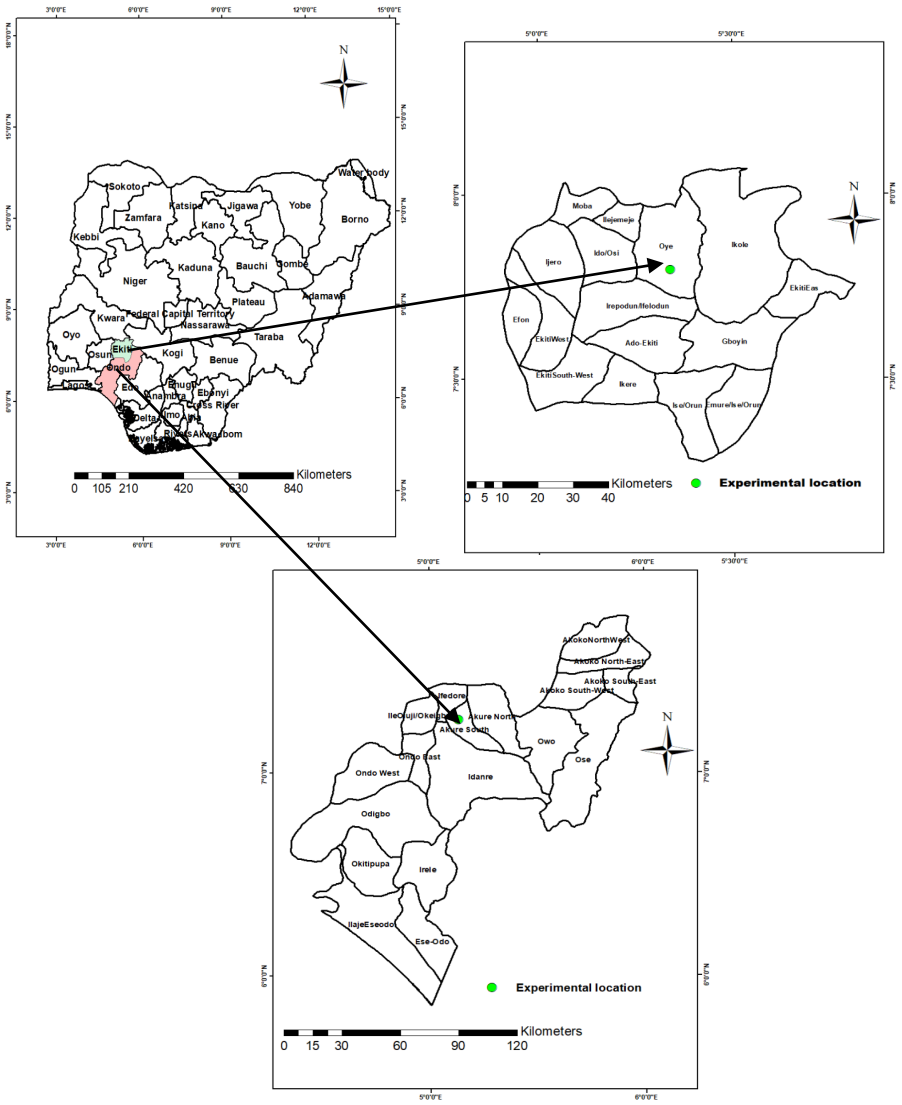

Figure 1. Experimental sites of the study location in Nigeria.

Genotype stability index

A stability index recommended by Farshadfar (2008) was calculated for each accession by summing the overall mean performances and ASV for each trait as given by:

$$GSIi = RASVi + RYi, \quad (4)$$

where: GSIi = the genotype stability index for the i^{th} genotype across the environment for each trait; RASVj = the rank of the i^{th} genotype across the environment based on ASV;

RYi = the rank of the i^{th} genotype based on mean performance across the environment. The genotype with the lowest GSI was considered the best for a particular trait across the environment.

Results and Discussion

Agro-metrological data at FUTA and FUOYE experimental sites from January to December 2018–2020 (Table 2) revealed that there were differences in environmental conditions of the different locations from year to year.

One of the major goals of every plant breeding programme is the selection and development of high yielding genotypes with wide stability across various agro-ecological environments. Genotypes do not perform exactly the same way in different environments as a result of genotype by environment interaction (GEI), making it difficult for breeders to select the appropriate genotype during cultivar development. Ariyo and Ayo-Vaughan (2000) have reported that in any multi-site evaluation programme where interactions are of practical importance, selection for stability is of prime consideration.

The combined analysis of the variance of the main effects of pod yield per plant and three agronomic traits of 17 accessions of okra grown in four environments are presented in Table 3. The results revealed that accessions, environments and their interactions were highly significant for final plant height, plant height at 50% flowering, number of seeds per pod and pod yield. Results of the combined analysis of variance show that the response of accessions was influenced by the environments. This is in agreement with the reports by Ariyo and Ayo-Vaughan (2000), Javia (2014), Alake (2018), Alake and Ariyo (2012) who reported the high influence of environment on the assessed traits, which makes it difficult to identify stable genotypes among the available genotypes.

The result of the AMMI analysis of variance for pod yield of seventeen accessions of okra grown in four environments is presented in Table 4. This showed that the accession and environment were highly significant as well as the accession x environment interaction. The total sum of squares shows that the environment had a greater proportion of 57.54% of the total variation; interaction had 24.96% while 17.5% was attributed to the accession, indicating that there were

substantial differences among studied environments which advocated the adequacy of running stability analysis. This is in accordance with the findings of several authors like Irfan (2018), Gebremedhin et al. (2014), Munaro et al. (2014). The large variance due to the effect of environment obtained in this present study also agrees with the findings of Rad et al. (2013), who obtained 51.9% of the total variation as the effect due to environment but contrary to the findings of Akter et al. (2015), Alake and Ariyo (2012), who had only 12.49% and 4.6%, respectively, of the total sum of squares attributable to the environmental effect. The magnitude of the G x E interaction sum of squares higher than that for accessions indicated that there were substantial differences in genotypic response across environments. This is in agreement with previous reports (Alam et al., 2015; Vaezi et al., 2017).

Table 2. Average monthly rainfall, maximum and minimum temperatures, relative humidity, and sunshine duration during growing periods of multi-environment trials of okra at the Federal University of Technology, Akure and Federal University Oye Ekiti, Nigeria

Year/Location	Month	Temperature		Relative humidity		Rainfall (mm)	Sunshine (hr)
		Max (◦C)	Min (◦C)	Max (%)	Min (%)		
2018/FUTA	April	29.8	23.9	95.6	71.2	130.6	8
	May	28.7	23.2	96.3	70.1	187.0	8
	June	26.9	23.2	96.1	82.0	218.6	7
	July	26.3	22.6	95.8	84.2	203.3	7
	August	26.0	22.5	96.5	84.2	227.7	6
	September	26.5	22.0	98.5	83.3	360.8	7
	October	27.6	22.8	98.4	79.2	197.5	8
	November	28.7	23.3	99.1	74.0	30.6	7
	December	29.8	19.8	93.1	50.0	7.2	9
2019/FUTA	April	29.4	23.8	94.4	72.0	103.4	8
	May	27.8	23.5	95.7	76.2	175.0	7
	June	27.1	23.4	96.6	84.3	256.6	7
	July	26.0	22.3	95.7	80.9	225.7	6
	August	25.8	22.2	96.5	83.9	180.1	6
	September	26.4	22.3	97.5	83.2	326.2	6
2019/FUOYE	April	30.8	24.0	91.19	62.69	76.9	3.5
	May	28.1	23.4	93.25	73.05	164.0	3.1
	June	27.5	23.4	92.81	78.65	253.1	2.8
	July	26.3	22.3	94.28	78.66	203.1	2.8
	August	26.0	22.1	95.24	80.78	171.4	2.8
	September	26.6	22.1	96.45	82.40	324.8	2.8
2020/FUTA	March	30.7	24.1	93.6	58.8	47.0	8
	April	29.4	24.0	97.1	68.9	125.3	8
	May	27.7	23.6	96.9	80.8	177.7	7
	June	26.8	22.8	97.0	83.3	205.9	7

The GEI was partitioned into the first two IPCA axes and the residual. The first two interaction principal component axes (IPCA) were highly significant and cumulatively contributed 78% of the total GEI. IPCA 1 and IPCA 2 explained 41.33% and 36.79%, respectively, of the total GEI variance. The mean yield of 17 accessions of okra grown in four environments and the values of the first IPCA scores from the AMMI analysis are presented in Table 5. Pod yield/plant ranged from 120.70g to 299.40g plant^{-1} for NGB00304 and NGB00355, respectively. The environmental means ranged from 114.50 for FUTA 2019 to 312.90 for FUTA 2020. NGB00304 had the largest IPCA score of 7.58 while NGB00355 had the smallest IPCA score of -8.57. Similarly, FUTA 2018 had the largest IPCA score of 9.72 while FUTA 2020 had the lowest IPCA score of -13.43. Thus, when an accession and environment have the same sign on their respective first IPCA axes, their interaction is positive; if different, their interaction is negative.

Table 3. The analysis of variance for the pod yield (g/plant) and three agronomic traits of 17 okra accessions in four environments.

Source	df	Final plant height	PHAF	NSPP	PYPP
REP	2	20.7	181.7	37.0	11374*
ENV	3	48038.5**	26006.7**	28955.9**	442814**
ACC	16	4796.6**	2291.0**	1818.0**	25261**
ENV*ACC	48	1354.1**	1005.7**	577.3**	12004**
Error	134	323.0	168.3	50.7	1769
Total	203				

*, ** = significant at P < 0.05, < 0.01, respectively. df = degree of freedom; REP = replication, ENV = environment, ACC = accession; PHAF = plant height at flowering, NSPP = number of seeds per pod, PYPP = pod yield per plant.

Table 4. The AMMI analysis of variance for the pod yield of 17 okra accessions tested over four environments.

Source of variation	DF	SS	MS	% Total SS	% Treatment	% interaction (G×E)
Treatments	67	2308810	34460**	89.89		
Accession	16	404175	25261**		17.5	
Environments	3	1328443	442814**		57.54	
Block	2	37803	4725			
Interactions	48	576192	12004**		24.96	
IPCA 1	18	238125	13229**			41.33
IPCA 2	16	211970	13248**			36.79
Residuals	14	126097	9007**			
Error	136	222005	1734	10.11		
Total	203	2568618	12653			

** = significant at P < 0.01.

Table 5. Means and the first PCA scores of the AMMI analysis of pod yield of 17 okra accessions evaluated in four environments.

Accession	FUTA18	FUTA19	FUOYE2019	FUTA2020	Mean (g)	IPCA1
NGB00297	102.60	93.30	122.60	268.10	146.60	1.18
NGB00298	116.20	101.10	50.50	223.80	122.90	3.27
NGB00299	155.40	122.00	286.30	320.60	221.10	1.88
NGB00302	124.80	95.50	150.10	245.70	154.00	3.24
NGB00303	111.50	90.70	246.20	308.60	189.20	0.46
NGB00304	135.00	71.50	121.60	154.60	120.70	7.58
NGB00331	164.90	113.60	333.00	306.80	229.60	3.16
NGB00346	131.60	107.30	106.00	238.20	145.80	3.57
NGB00347	150.40	137.70	101.30	272.40	165.50	2.73
NGB00350	100.70	89.30	210.30	307.30	176.90	-0.12
NGB00355	138.40	193.70	323.70	542.00	299.40	-8.57
NGB00356	83.60	142.60	87.90	403.10	179.30	-5.88
NGB00369	107.70	110.30	78.50	276.20	143.20	0.75
NGB00371	62.80	87.20	68.80	301.10	130.00	-2.20
NGB00378a	133.50	139.30	172.50	344.50	197.40	-0.76
NGB00378b	81.20	134.90	116.30	404.10	184.10	-5.84
NGB00430	94.30	117.10	242.20	401.70	213.80	-4.45
Mean (g)	117.30	114.50	165.80	312.90	177.63	
IPCA1	9.72	1.62	2.09	-13.43		

AMMI IPCA 1 and IPCA 2 scores of okra fruit yield, the AMMI stability value (ASV) and the genotype stability index (GSI) for 17 accessions of okra are presented in Table 6. The genotype ranking based on GSI, which combines both ASV and mean pod yield performance rankings revealed that NGB-00378a, NGB-00350, NGB-00299 and NGB-00297 were the most desirable accessions as they combine high yield with stability. Considering the ASV ranking values alone, NGB00378a and NGB00297 with the lowest values would be the most stable accessions even with low mean pod yield while NGB-00355, NGB-00331, NGB-00304, NGB-00356 and NGB-00378b would be unstable accessions. Unfortunately, NGB00331 and NGB00355 ranked the first and second best, respectively, in yield but the most unstable accessions according to ASV ranking. However, they were found to be fairly stable according to GSI ranking scores. Accession NGB-00378a was found to be the most stable accession according to ASV and GSI rankings and as a result, the best accession to be selected.

The AMMI I biplot for okra pod yield grown in four environments is presented in Figure 2. The Y-axis represents the first IPCA while the X-axis is the main effect, the yield of okra pods. The accessions NGB-00356, NGB-00378b, NGB-00378a, NGB-00350, NGB-00430, NGB-00303, NGB-00299, NGB-00355 and NGB-00331 yielded above average while FUTA 2020 was the most favourable

environment since AMMI placed them on the right-hand side of the midpoint while NGB-00298, NGB-00347, NGB-00369, NGB-00371, NGB-00346, NGB-00297, NGB-00304 and NGB-00302 were low yielding because they were placed on the left side of the midpoint of the biplot. Results showed that NGB-00350, NGB-00303, NGB-00378a, NGB-00302 and NGB-00297 were very close to the equator of the IPCA1 and so the most stable accessions. On the other hand, NGB-00355, NGB-00331, NGB-00304, NGB-00356 and NGB-00378b though high yielding were the most unstable accessions because they were located far from the IPCA1.

Table 6. The ranking of 17 accessions of okra by mean performance, AMMI stability value (ASV) and genotype selection index (GSI) for pod yield evaluated in four environments.

Accession	Mean yield (g)	Yield rank (YR)	ASV	ASV rank (RASV)	GSI	GSI rank
NGB00297	146.60	12	1.65	2	14	4
NGB00298	122.90	16	6.01	12	28	16
NGB00299	221.10	3	5.69	10	13	3
NGB00302	154.00	11	3.44	4	15	6
NGB00303	189.20	6	4.96	8	14	4
NGB00304	120.70	17	8.04	15	32	17
NGB00331	229.60	1	8.60	16	17	8
NGB00346	145.80	13	4.57	7	20	11
NGB00347	165.50	10	5.12	9	19	9
NGB00350	176.90	9	3.31	3	12	2
NGB00355	299.40	2	9.90	17	19	9
NGB00356	179.30	8	7.93	14	22	15
NGB00369	143.20	14	4.04	6	20	11
NGB00371	130.00	15	4.00	5	20	11
NGB00378a	197.40	5	1.16	1	6	1
NGB00378b	184.10	7	6.96	13	20	11
NGB00430	213.80	4	5.94	11	15	6

The finding that the AMMI plot accounted for a substantial part of the total sum of squares suggested that the model was more appropriate in explaining the G×E interaction. The variability in the amount of rainfall in different locations was probably responsible for the differences in the performance of the accessions. This is in agreement with the report of Ariyo and Ayo-Vaughan (2000), who also reported significant interaction.

NGB00298 and NGB00304 had similar low yields but a different large positive interaction. NGB00355, NGB00356, NGB00378a, NGB00378b and NGB00430 had high mean yield and a different high negative interaction. Based on the magnitude of the interaction score, only NGB00303 and NGB00350 could be

considered fairly stable. This is in agreement with Ariyo and Ayo-Vaughan (2000), who reported only G13 and G9 to be fairly stable. FUTA 2018 had the largest interaction, while FUTA 2020 had the least. NGB00303 was favoured by FUTA 2020 and FUOYE 2019, but it was not favoured by FUTA 2019.

NGB00350 and NGB00302 were located close to the origin and proved highly stable, but they had low mean yields. This is similar to the findings of Irfan (2018), Singh et al. (2019), Mohammadi and Amri (2013) and Oral et al. (2018), who also reported the stability of genotypes with low IPCA 1 values.

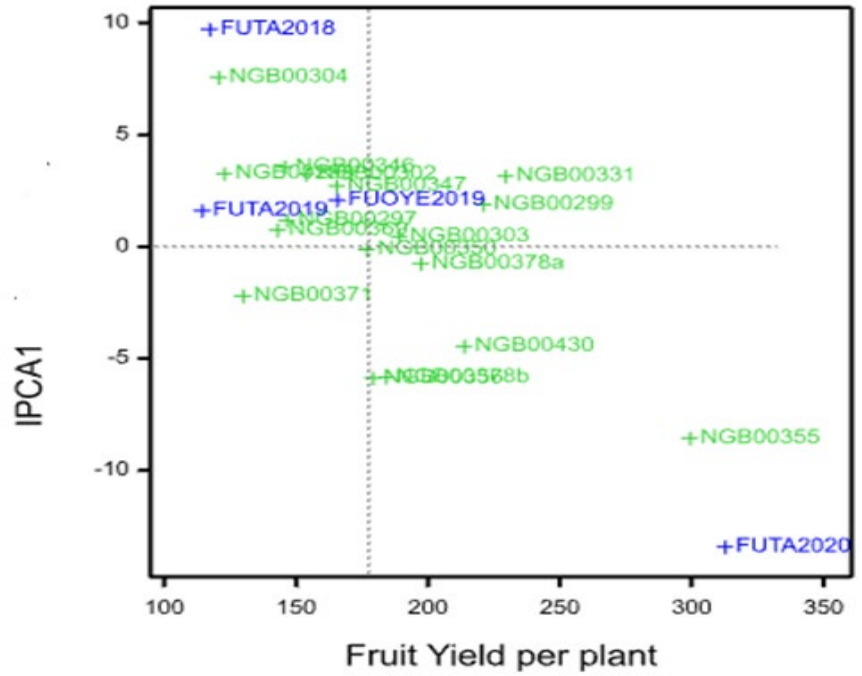

Figure 2. The biplot analysis of GEI based on the AMMI 1 model for the PCA1 scores and fruit yield/plant.
FUTA2018=environment 1, FUTA2019=environment 2, FUOYE2019=environment 3, FUTA2020=environment 4.

The polygon view of the GGE biplot which shows the which-won-where pattern of the seventeen accessions of okra evaluated in four environments with respect to pod yield per plant is presented in Figure 3. The quadrilateral is divided into six sectors by six projecting lines from the origin and the four environments fell into only two of the sectors. Akure 2018 (E1) and Akure 2019 (E2) fell into sector one with NGB00331 (G7) being the vertex accession while Oye 2019 (E3)

and Akure 2020 (E4) fell into the second sector with NGB-00355 (G11) as the vertex accession. NGB-00331 (G7) and NGB-00355 (G11) accessions were, therefore, the highest yielding accessions. No environment was associated with sectors three, four, five and six where NGB-00356 (G12), NGB-00371 (G14), NGB-00298 (G2) and NGB-00304 (G6) were located. NGB-00331 (G7) won in two environments – FUTA 2018 (E1) and FUTA 2019 (E2), while NGB-00355 (G11) won in FUOYE 2019 (E3) and FUTA 2020 (E4). According to Ariyo and Ayo-Vaughan (2000), who have stated that the high influence of the environment on the performance of traits makes it difficult to identify stable ones among the studied genotypes.

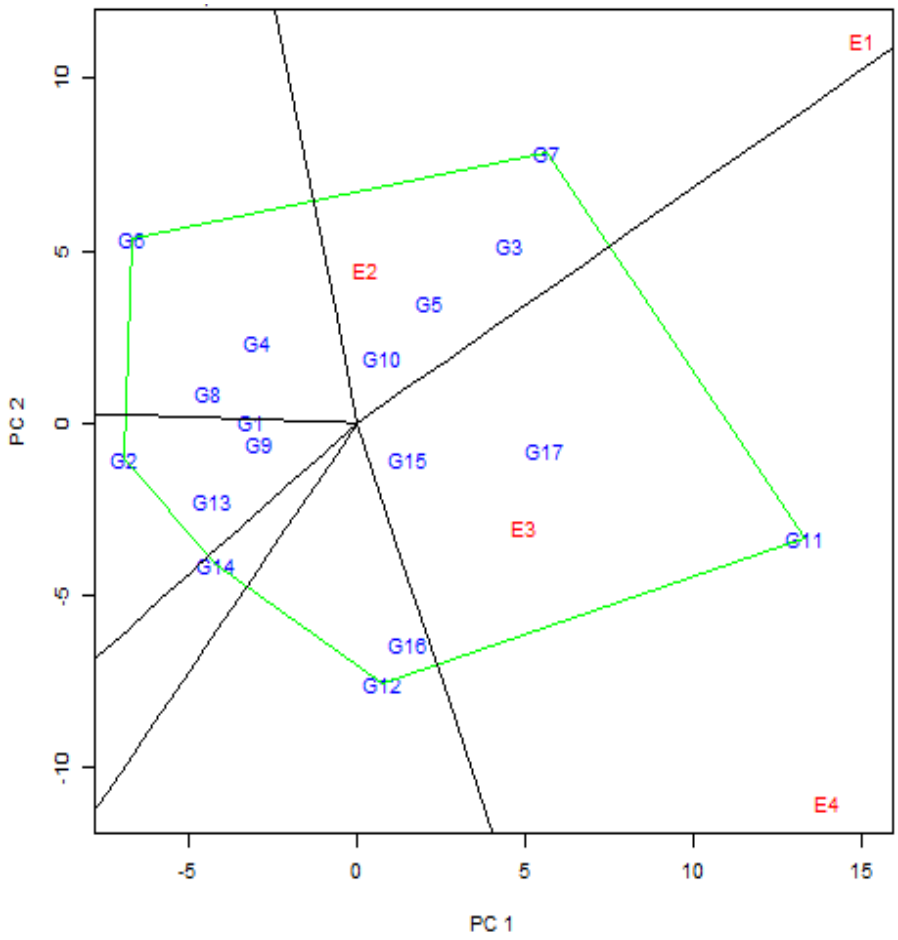

Figure 3. The polygon view of the GGE biplot for pod yield data of 17 okra accessions evaluated across 4 environments between 2018 and 2020.

This means that there is an interaction between genotype and environment. According to GSI ranking, genotypes NGB00378a, NGB00350, NGB00299 and NGB00297 were identified as the stable accessions with high mean yield across the test environment, which makes them the best accessions for selection. According to Yan and Tinker (2006), the genotypes found at the vertex of the sectors had the highest yield than those that are not found at the vertex in all environments (Yan, 2001). NGB00331 (G7) was the best performing accession in Akure 2018 and Akure 2019 while NGB00355 (G11) was the high yielding accession in Oye 2019 and Akure 2020.

The average environment coordinate line (AEC) was used to estimate the yield and stability of accessions in the GGE biplot (Figure 4). The line with a double arrow passing through the biplot origin is referred to as the average environment coordinate (AEC). Proximity to concentric circles indicates a high mean yield while remoteness to it shows a low mean yield. Projections from the biplot origin in either direction on the axis indicate greater instability. Also, the accessions with yield performance greater than the mean yield are those on the right side of the AEC line, while those with a yield lower than the mean yield are on the left side of the line. NGB-00355 (G11) was the highest yielding accession, followed by NGB-00331 (G7), NGB-00430 (G17) and NGB-00299 (G3). Those with mean yield below average include NGB-00304 (G6), NGB-00298 (G2), NGB-00371 (G14), NGB-00369 (G13), NGB-00346 (G8), NGB-00297 (G1), NGB-00302 (G4) and NGB-00347 (G9). The projection from the origin to the AEC indicates the stability of the genotypes. A greater projection into the AEC line regardless of the direction indicates greater instability. Therefore, accessions NGB00331 (G7), NGB00304 (G6), NGB00356 (G12) and NGB00378b were highly unstable while NGB00297 (G1), NGB00347 (G9), NGB00298 (G2), NGB00378a (G15) and NGB00430 (G17) with shorter projections were relatively stable over environments. The accession that combines good performance with stability is NGB00378a (G15) and, therefore, the most desirable accession. The estimation of the yield and stability of genotypes showed that NGB00304 and NGB00298 were the least stable; while NGB00378a (G15) proved to be the most stable and high yielder across the environments. For selection, the desirable accessions are those with both high mean yield and stability. In this study, the accession that combines good performance with high stability is NGB00378a (G15). Therefore, it should be selected. This result is in agreement with the findings of Akter et al. (2015) and Nahief and Mohammad (2013).

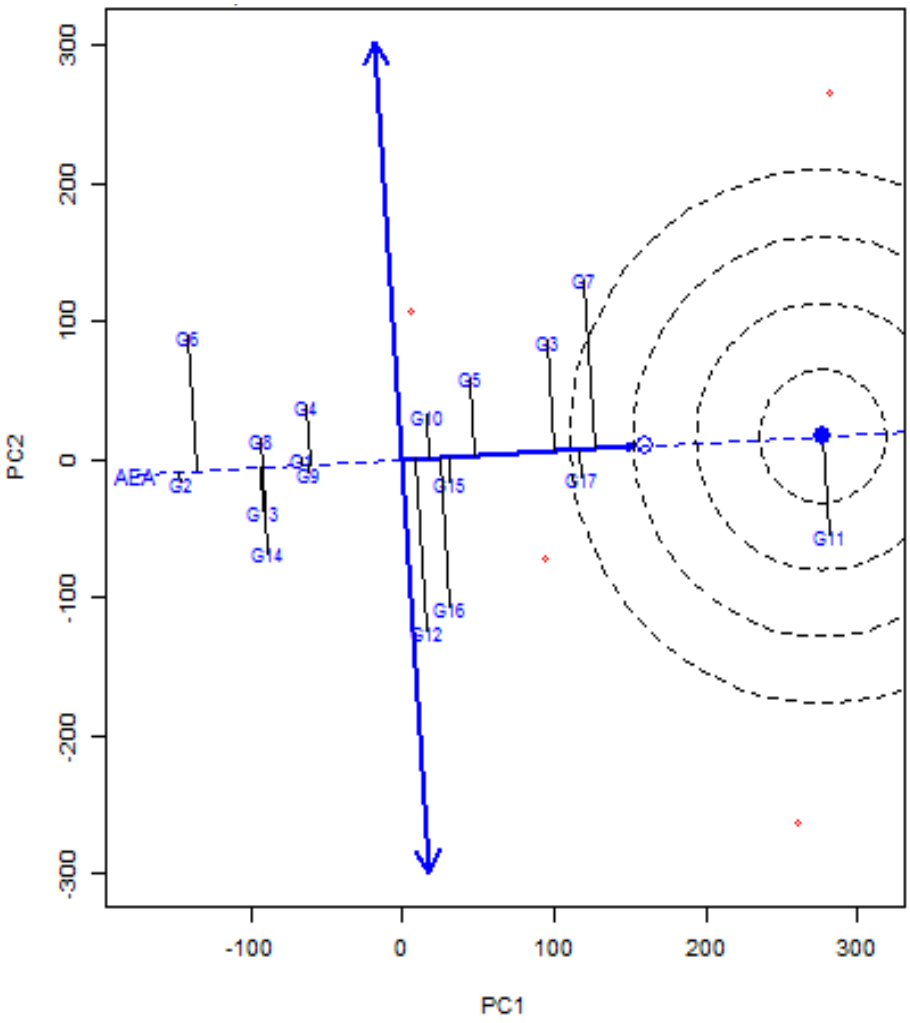

Figure 4. The GGE biplot showing the ranking of accessions for both pod yield and stability performance of 17 accessions of okra evaluated across 4 environments between 2018 and 2020.

The discriminatory power and representativeness of the test environments among the four environments are displayed in Figure 5. A small circle with an arrow pointing to it depicts an ideal environment. From the vector view of the biplot, the length of the environment vectors is a measure of their discriminating ability. In this study, Akure 2020 was the most discriminatory environment. The second most important aspect of test environment evaluation is its representativeness of the mega environments. The smaller the angle, the more

representative the test environment would be. The important properties of an ideal environment are its discriminatory ability and representativeness (Yan, 2001). An ideal environment should be highly discriminating for the tested genotype as well as a representative of the target environments (Yan and Kang, 2003). Hence, FUOYE 2019 (E3) was the most representative of other varieties in this study.

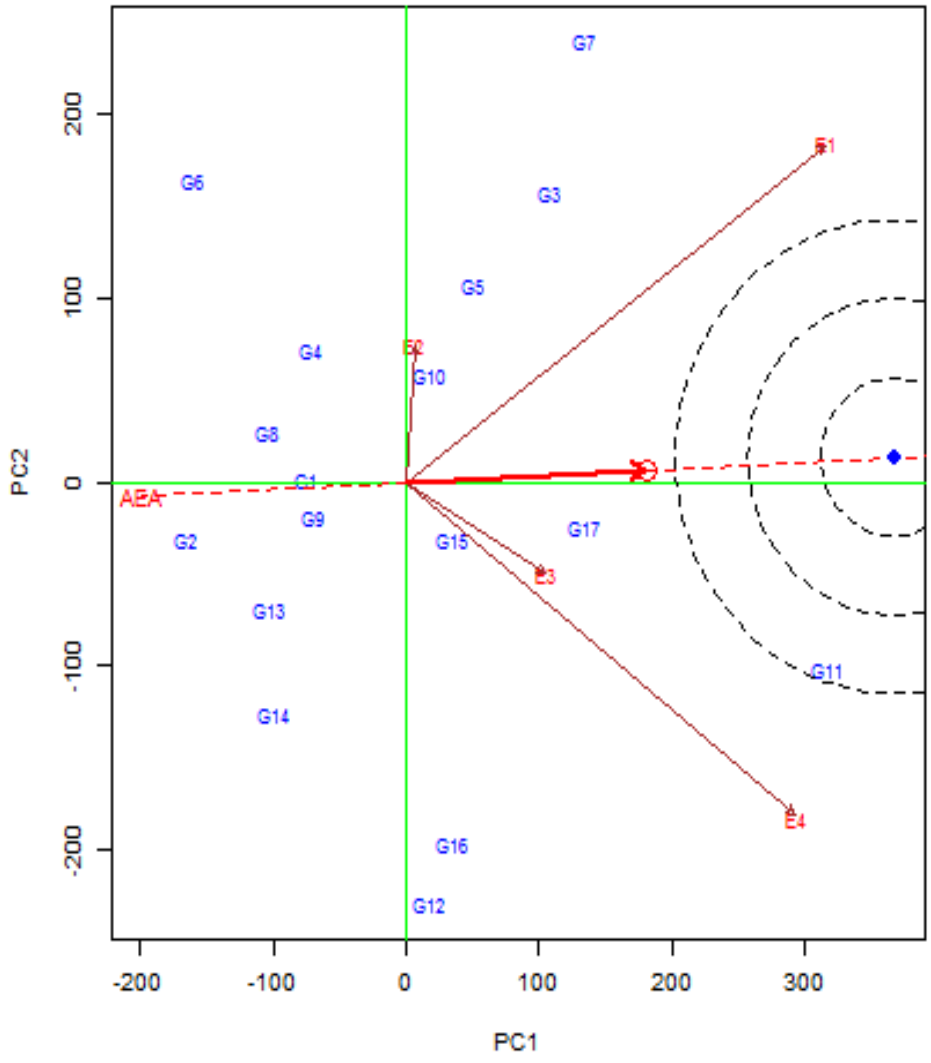

Figure 5. The biplot showing discriminativeness and representativeness based on genotype by environment yield data of 17 accessions of okra evaluated across 4 environments between 2018 and 2020.

The ranking of other environments based on the ideal nature of environments showed that Akure 2020 (E4) was the most discriminatory. Environment E3 has a large PC1 score and a low PC2 score, so this environment is more stable and suitable for all genotypes. This is in accordance with the result of Akter et al. (2015), who reported that environment E3 had a large PC1 score and a small PC2 score, and, as a result, the environment is more stable and suitable for all accessions and that E4 was a discriminating environment due to its large PC2 score.

Conclusion

The AMMI analysis identified NGB00378a to be the most stable accession and high yielder, whereas NGB00331 and NGB00355 also identified as high yielders were highly unstable. Similarly, the GGE biplot identified NGB00378a as a highly stable and high yielder, whereas NGB00355 was the highest yielder, but fairly stable. On the other hand, NGB00378a combines good performance with stability. Therefore, NGB00378a is the ideal accession and should be recommended. The GGE biplot method gave a superior and more thorough description of the stability of the seventeen accessions in identifying the ideal accession and the environment, and reduced the four environments into two mega environments.

References

Abay, F., & Bjornstad, A. (2009). Specific adaptation of barley varieties in different locations in Ethiopia. *Euphytica*, *167,* 181-195.

AdeOluwa, O.O. & Kehinde, O.B. 2011. Genetic variability in West African okra (*Abelmoschus caillei*). *Agricultural Biology Journal North America 2* (10), 1326-1335.

Akcura, M., Taner, S., & Kaya, Y. (2011). Evaluation of bread wheat genotypes under irrigated multi-environment conditions using GGE biplot analyses. *Agriculture, 98* (1), 35-40.

Akter, A., Hasan, M.J., Kulsum, U., Rahman, M.H., Khatun, M., & Islam, M.R. (2015). GGE biplot analysis for yield stability in multi-environment trials of promising hybrid rice (*Oryza sativa* L.) *Bangladesh Rice Journal, 19*, 1-8.

Alake, C.O. (2018). Genotype × environment interaction effects on pod yield and pod number in West African Okra. *Archives of Agronomy and Soil Science, 64*, 1503-1517.

Alake, C.O., & Ariyo, O.J. (2012). Comparative Analysis of Genotype x Environment Interaction Techniques in West Africa Okra (*Abelmoschus caillei, A. ChevStevel). Journal of Agricultural Science (Canada) 4*, 135-149.

Alam, M.A., Sarker, Z.I., Farhad, M, Hakim, M.A., Barma, N.C.D., Hossain, M.I., Rahman, M.M., & Islam, R. (2015). Yield stability of newly released wheat varieties in multi-environments of Bangladesh. *International Journal of Plant & Soil Science, 6,* 150-161.

Aminu, D., Bello O.B., Gambo, B.A., Azeez, A.H., Agbolade, J.O., Abdulhamid, U.A., & Iliyasu, A. (2016). Varietal performance and correlation of okra pod yield and yield components. *Bangladesh Journal of Plant Breeding and Genetics, 29* (1), 11-20

Ariyo, O.J., & Ayo-Vaughan, M.A. (2000). Analysis of genotype x environment interaction of okra. (*Abelmoschus esculentus (L.) Moench). Journal of Genetics & Breeding, 54*, 35-40.

Bello, O.B., Aminu, D., Gambo, B.A., Azeez, A.H., Lawal, M., Agbolade, J.O., Iliyasu, A., & Abdulhamid, U.A. (2015). Genetic diversity, heritability and genetic advance in okra [*Abelmoschus esculentus* (L.) Moench]. *Bangladesh Journal of Plant Breeding and Genetics*, *28* (2), 25-38.

Chattopadhyay, A., Dutta, S., & Chattearjee, S. (2011). Seed yield and quality of okra as influenced by sowing dates. *African Journal of Biotechnology*, *10* (28), 5461-5467.

Dias, C.T., dos, S., & Krzanowski, W.J. (2003). Model selection and cross validation in additive main effect and multiplicative interaction models. *Crop Science, 43*, 865-873.

Edugbo, R.E., Nwofia, G.E., & Fayeun, L.S. (2015). An Assessment of Soybean (*Glycine max,* L. Merrill) Grain Yield in Different Environments Using AMMI and GGE Biplot Models in Humidorest Fringes of Southeast Nigeria. *Agricultura Tropica et Subtropica*, *48* (3-4), 82-90.

Farshadfar, E. (2008). Incorporation of AMMI stability value and grain yield in a single non parametric index (GSI) in bread wheat. *Pakistan Journal of Biological Science, 11*, 1791-1796.

Gauch, H.G. (1992). *Statistical analysis of regional yield trials: AMMI analysis of factorial designs.* Elsevier, Amsterdam.

Gauch, H.G., & Zobel, R.W. (1996). AMMI analyses of yield trials. In: Kang MS, Gauch HG (Eds.) *Genotype by Environment Interaction*. (pp 85-122). CRC Boca Raton, Florida.

Gebremedhin, W., Firew, M., & Tesfye, B. (2014). Stability analysis of food barley genotypes in northern Ethiopia. *African Crop Science Journal 22*, 145-153.

Irfan, E. (2018). Investigation of genotype × environment interaction in chickpea genotypes using AMMI and GGE biplot analysis. *Turkish Journal of field crops*, *23* (1), 20-26.

Javia, R.M. (2014). Stability analysis for fruit yield and its attributing characters in Okra. *International Journal of Plant Science, 9* (1), 35-39.

Kang, M.S. (2004). Breeding: Genotype-by-environment interaction. In: R.M. Goodman (Ed.) *Encyclopedia of Plant and Crop Science.* (pp. 218-221). Marcel-Dekker, New York.

Komolafe, R.J., Ariyo, O.J., & Alake, C.O. (2021a). Diversity in Phenotypic Traits and Mineral Elements of Okra (Abelmoschus esculentus L. Moench) Genotypes. *International Journal of Agronomy*, https://doi.org/10.1155/2021/5528703.

Komolafe, R.J., Ariyo, O.J., & Alake, C.O. (2021b). Correlation and Path Coefficient Analysis of Fruit Yield Attributes in Forty Genotypes of Okra (*Abelmoschus esculentus*). *Journal of Agricultural Researc*h. https://doi.org/10.1007/s40003-021-00549-9.

Kumar, S., Dagnoko, S., Haougui, A., Ratnadass, A., Pasternak, D., & Kouame, C. (2010). Okra (*Abelmoschus* spp L.) in West and Central Africa: Potential and Progress on its Improvement. A special review. *African Journal of Agricultural Research, 5* (25), 3590-3598.

Miranda, G.V., Souza, L.V., Guimarães, L.J.M., Namorato, H., Oliveira, L.R., & Soares, M.O. (2009). Multivariate analyses of genotype x environment interaction of popcorn. *Pesquisa Agropecuaria Brasileira Brasília, 44* (1), 45-50.

Mohammadi, R., & Amri, A. (2013). Genotype x environment interaction and genetic improvement for yield and yield stability of rainfed durum wheat in Iran. *Euphytica 192*, 227-249.

Munaro, L.B., Benin, G., Marchioro, V.S., Franco, F.A., Silva, R.R., Lemes da Silva, C., & Beche, E. (2014). Brazilian spring wheat homogeneous adaptation regions can be dissected in major mega environments. *Crop Science, 54*, 1-10.

Naheif, E., & Mohammad, M. (2013). Genotype by environment interactions for grain yield in bread wheat *(Triticum aestivum* L.). *Global Science Research Journal 1* (1), 045-052.

Nassir, A.L., & Ariyo, O.J. (2005). Genotype X Environment stability analysis of grain yield of rice (*Oryza sativa) (L.), Journal of Tropical Agriculture (Trinidad), 82*, 269-276.

Nwangburuka, C.C., Kehinde, O.B., Ojo, D.K., Denton, O.A., & Popoola, A.R. (2011). Morphological classification of genetic diversity in cultivated okra (*Abelmoschus esculentus* (L)

Moench) using principal component analysis (PCA) and single linkage cluster analysis (SLCA). *African Journal of Biotechnology*, *10* (54), 11165-11172.

Olayiwola, M.O., & Ariyo, O.J. (2013). Relative discriminatory ability of GGE biplot and YSi in the analysis of genotype x Environment interaction in okra (*Abelmoschus esculentus* (L.) Moench). *International Journal of Plant Breeding and Genetics, 7* (3), 146-158.

Oral, E., Kendal, E., & Dogan, Y. (2018). Selection of the best barley genotypes to multi and special environments by AMMI and GGE biplot models. *Fresenius Environmental Bulletin, 27*, 5179-5187.

Oyetunde, B.O., & Ariyo, O.J. (2015). Genetics of seed yield and related traits in biparental crosses of okra, *Abelmoschus esculentus* (L.) Moench. *Nigerian Journal of Genetics, 28*, 8-14.

Purchase, J.L., Hatting, H., & Van Deventer, C.S. (2000). Genotype x environment interaction of winter wheat (*T. aestivum*) in South Africa: Stability analysis of yield performance. *South African Journal of Plant Soil, 17* (3), 101-107

Rad, M.R., Kadir, M.A., Rafii, M.Y., Jaafar, Z.E., Naghavi, M.R., & Ahmadi, F. (2013). Genotype × environment interaction by AMMI and GGE biplot analysis in three consecutive generations of wheat (*Triticum aestivum*) under normal and drought stress conditions. *Australian Journal of Crop Science*, *7* (7), 956-961.

Saifullah, M., & Rabbani, M.G. (2009). Evaluation and Characterisation of okra *(Abelmoschus esculentus* L. Moench.) genotypes. *SAARC Journal of Agriculture, 7* (1), 92-99.

Singh, C., Gupta, A., Gupta, V., Kumar, P., Sendhil, R., Tyagi, B.S., Singh, G., Chatrath, R., & Singh, G.P. (2019). Genotype x environment interaction analysis of multi-environment wheat trials in India using AMMI and GGE biplot models. *Journal of Crop Breeding and Applied Biotechnology, 19* (3), 309-318.

Vaezi, B., Pour-Aboughadareh, A., Mohammadi, R., Armion, M., Mehraban, A., Hossein-Pour, T., & Dorii, M. (2017). GGE Biplot and AMMI Analysis of Barley Yield Performance in Iran. *Cereal Research Communications, 45,* 500-511.

Yan, W. (2001). GGE biplot a windows application for graphical analysis of multi-environment trial data and other types of two-way data. *Agronomy Journal*, *93*, 1111-1118.

Yan, W., & Kang, M.S. (2003). *GGE biplot analysis: a graphical tool for breeders, Geneticists and Agronomists.* 1st Edn. CRC Press LLC., Boca Raton, Florida.

Yan, W., & Tinker, N.A. (2006). Biplot analysis of multi-environment trial data: Principles and applications. *Canadian Journal of Plant Science*, *86*, 623-645

Received: April 6, 2022
Accepted: October 10, 2022

ANALIZA VISINE I STABILNOSTI PRINOSA GENOTIPOVA BAMIJE (*ABELMOSCHUS ESCULENTUS* L. MOENCH) KORIŠĆENJEM AMMI I GGE BIPLOTA

Ronke J. Komolafe[1*], Omolayo J. Ariyo[2] i Olusanya C. Alake[2]

[1]Odeljenje za nauku o biljkama i biotehnologiju,
Federalni univerzitet Oje Ekiti, Država Ekiti, Nigerija
[2]Odeljenje za oplemenjivanje biljaka i tehnologiju semena,
Federalni poljoprivredni univerztet Abeokuta, Država Ogun, Nigerija

R e z i m e

Identifikacija prilagodljivih, stabilnih i visokoprinosnih genotipova u različitim uslovima spoljne sredine pre puštanja u promet, predstavlja veliki izazov za oplemenjivače biljaka pri odabiru najboljih genotipova bamije. Interakcija genotip × sredina je veliki izazov za oplemenjivače biljaka, jer velika interakcija može smanjiti selekcionu dobit i otežati identifikaciju superiornih sorti. Ciljevi ovog istraživanja bili su da se proceni vrednost genotipova bamije u različitim spoljnim sredinama i da se identifikuju visokoprinosni i stabilni genotipovi, kako bi se izabrao roditelj za dalji selekcioni rad. Sedamnaest genotipova bamije je ocenjeno na lokalitetu Akure tokom kišne sezone 2018. godine, zatim na lokalitetima Akure i Oje tokom kišne sezone 2019. godine i na lokalitetu Akure tokom kišne sezone 2020. godine, što čini ukupno četiri spoljne sredine. AMMI i GGE biplotovi korišćeni su za procenu interakcije G × E i proučavanje stabilnosti u četiri spoljne sredine. AMMI analiza je identifikovala, NGB00378a kao najstabilniji genotip i genotip sa visokim prinosom. Takođe, GGE biplot je identifikovao, NGB00378a kao visoko stabilan genotip sa visokim prinosom, dok je NGB00355 bio identifikovan kao najprinosniji genotip, ali umerene stabilnosti. Ipak, u slučaju genotipa NGB00378a, dobar učinak se kombinuje sa stabilnošću. S tim u vezi, NGB00378a predstavlja idealan genotip, koji treba preporučiti za dalji oplemenjivački rad.

Ključne reči: spoljna sredina, genotip, interakcija, prinos, stabilnost.

Primljeno: 6. aprila 2022.
Odobreno: 10. oktobra 2022.

[*]Autor za kontakt: e-mail: ronke.komolafe@fuoye.edu.ng

Journal of Agricultural Sciences (Belgrade) https://doi.org/10.2298/JAS2204355L
Vol. 67, No. 4, 2022 UDC: 635.648.07:575.22
Pages 355-366 Original scientific paper

THE SEED VIGOUR OF SPELT PRODUCED AT THE MAIZE RESEARCH INSTITUTE "ZEMUN POLJE"

Tijana D. Lazarević[1], Tanja B. Petrović[2], Goran N. Todorović[2], Mile D. Sečanski[2], Jelena M. Golijan-Pantović[1*] and Slavoljub S. Lekić[1]

[1]University of Belgrade, Faculty of Agriculture, Belgrade, Serbia
[2]Maize Research Institute "Zemun Polje", Belgrade, Serbia

Abstract: Spelt (*Triticum spelta* L.) has been increasingly attracting producers due to the biological properties and chemical composition of its seeds. As high-quality seeds are necessary for successful production, the vigour of organically and conventionally produced spelt seeds has been studied and results are presented in this paper. The seeds of the variety Nirvana produced by both methods at the Maize Research Institute "Zemun Polje" in 2015 were observed. According to the results, the germination energy of conventionally and organically produced seeds amounted to 30% and 69%, respectively. The total germination of conventionally and organically produced spelt seeds amounted to 99% and 93%, respectively. The percentage of abnormal seedlings of spelt produced by both methods amounted to 1% on average. The participation of diseased and dead seeds was higher in organically produced seeds (6%) than in conventionally produced seeds (0%). After the seed accelerated ageing test, a higher germination was observed in conventionally produced seeds (75%) than in organically produced seeds (68%). The electric conductivity of conventionally produced seeds amounted to 189.4 μS/cm and 195.2 μS/cm in the first and the second replication, respectively, while the values of organically produced seeds amounted to 95.5 μS/cm and 98.6 μS/cm, in the first and the second replication, respectively. The results obtained by the electrical conductivity test indicated that the conventionally produced spelt seeds (32.33 μS/cm g) were classified into the category of low vigour seeds in comparison to organically produced spelt seeds (27.65 μS/cm g).

Key words: *Triticum spelta* L., germination, accelerated ageing, electrical conductivity.

Introduction

According to data of the FiBL survey reported by Willer et al. (2022), more than 60 percent of the arable land was located in Europe. Cereals, including rice

*Corresponding author: e-mail: golijan.j@agrif.bg.ac.rs

(5.1 million ha), green fodder (3.2 million ha) and oilseeds (1.8 million ha) were mostly grown on the arable cropland. In 2020, global organic production of cereals was performed on almost 5.1 million hectares (0.7%). Within the global organic production of cereals, wheat ranks first (30.88%), maize second (14.85%) and rice third (12.25%). On the other hand, in Serbia in 2020, organic crops were grown on the area of 20,970.75 ha in the following way: cereals on the area of 3,623.15 ha, while organic spelt was produced on only 256.14 ha (Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, 2022).

The interest in growing spelt (species: *Triticum spelta* L., family: *Poaceae*) has been growing, as alternative crops have been gaining in their importance (Lacko-Bartošová et al., 2010). Spelt, due to its biological traits and chemical composition of its grain, belongs to resistant cereal species that do not require special growing conditions (Ugrenović, 2003). Its needs in terms of soil quality, climate conditions and cropping practices are modest, and it also does not require chemical treatments, which makes spelt suitable for the organic production (Golijan et al., 2019). Organic agriculture is defined as a system that manages agriculture and preserves biological diversity, and uses processes and technologies based on biological principles without the use of artificial inputs, i.e., genetically modified organisms (FAO/WHO Codex Alimentarius Commission, 1999). Based on its modest requirements for growing conditions and expressed qualitative properties that are in line with contemporary nutritional requirements, spelt ranks high within the organic system of cultivation (Nikolić et al., 2015). Vigour is the most important seed property, which, in the broadest sense, includes several interconnected traits due to which seeds germinate and develop under various (favourable and unfavourable) conditions (Lekić, 2009). The aim of seed vigour testing is to provide data on the sowing value for the wide range of environmental conditions and/or properties of seeds stored in warehouses. Since practice and literature data show the differences between seed germination in the laboratory and the field, it is assumed that the observed spelt varieties will also express differences between field and laboratory germination.

The objective of this study was to observe and compare the vigour of organically and conventionally produced spelt seeds, as well as to establish possible differences between them.

Material and Methods

Seeds of the conventionally and organically grown spelt variety Nirvana, used in this study, were produced in the experimental field of the Maize Research Institute “Zemun Polje” in 2015. The spelt variety Nirvana has been developed at the Department of the Organic Production and Biodiversity of the Institute of Field and Vegetable Crops in Novi Sad. It is a late variety, very resistant to low temperatures

during winter. It forms covered kernels with a test weight of 75–78 kg, 1000-kernel weight of approximately 41 g and a protein content of about 15%. The variety contains all vitamins of the B group (except vitamin B12). Its content of Ca, Mg, P and Se is 7–8 times higher than the content in other cereals. It also contains a significant amount of Zn (nsseme.com).

The following tests were performed in the Seed Testing Laboratory of the Maize Research Institute "Zemun Polje" in May of 2016:

1) the standard seed germination test,
2) the seed accelerated ageing test, and
3) the electrical conductivity test.

1. The standard seed germination test. The standard seed germination test was performed in the standard germination cabinet. The filter paper was used as a germination medium. Maize and spelt seeds (4 x 100 seeds) were selected. Spelt seeds were cleaned of impurities. Counted samples were placed on the moist filter paper, covered with another filter paper, rolled and placed in the germination cabinet at the alternating temperature of 20⇔30°C (ISTA, 2016). The energy of germination was read on the fourth day from the beginning of the germination test. The standard seed germination test for maize and spelt is carried out for seven and eight days, respectively.

2. The seed accelerated ageing test. The accelerated ageing test of seeds was performed in the accelerated ageing chamber (ISTA, 2016). Two hundred seeds were placed in each container. Once samples were drawn, the seed moisture content and the seed initial weight were determined. The tray mesh screen was placed into each container. Seeds were placed on the screen under which there were 40 ml of distilled water, taking care that the seeds did not come into contact with water. The containers were then covered with lids and placed into the accelerated ageing chamber. The test period lasted for 72 hours from the moment when the chamber temperature reached 43°C and the maximum humidity was reached. After that, seeds were placed to germinate in the same way as when the standard seed germination test was performed.

Based on the initial moisture content, the initial seed weight and the final seed weight, the final seed moisture content was calculated using the following formula:

$$FM = 100 - IW \times \frac{100 - IM}{FW} \quad (1)$$

where:

FM is final moisture, IW is initial weight, IM is initial moisture and FW is final weight.

3. The electrical conductivity test of seeds. An electrical conductivity metre was used to measure the electrical conductivity of seeds (ISTA, 2016). A total of 2 x 50 spelt seeds were randomly counted from the pure seed fraction with moisture

ranging between 10% and 14% and their weight was determined. Erlenmeyer flasks were used to perform electrical conductivity. Flaks were first washed with distilled water and then filled with 250 ml of distilled water with a conductivity below 5μS/cm and finally they were covered. Erlenmeyer flasks prepared in this way were stored at the temperature of 20°C for 24 h. After this time, each seed sample was placed in the prepared Erlenmeyer flasks, which were gently stirred to immerse all seeds. The remaining flasks were filled with distilled water to serve as a control. Flasks were then covered and returned to the temperature of 20°C for 24 h. Conductivity was read after 24 h.

Conductivity per gram of seed weight for each replicate was calculated after the reading of the basic water conductivity and the average of two replicates was a testing result of a certain seed lot. Conductivity for each replicate was calculated using the following formula:

$$\text{conductivity}\left(\frac{\mu S}{cm \times g}\right) = \frac{\text{conductivity value}\left(\frac{\mu\text{S}}{\text{cm}}\right) - basic \text{ value}}{\text{replication weight (g)}} \quad (2)$$

The obtained data were processed and graphically displayed by using the Microsoft Excel 2010 program.

Results and Discussion

Viability is the most important biological property of seeds. Seed viability not only shows the percentage of viable seeds in a particular sample, but also the ability of seeds to successfully develop normal seedlings under unfavourable field conditions (ISTA, 2014). This concept is reflected by the rate of germination, emergence and rooting in the field, as well as the general effect of storage substances in the endosperm in relation to the seedling growth (Lekić, 2009). Seed viability is most simply evaluated via seed germination (Lekić, 2003).

Standard germination tests are indicators of seed quality that can be used to predict the emergence of plants in the field if the soil conditions are almost ideal (Durrant and Gummerson, 1990). However, the conditions under which seeds are tested are often more favourable than those in the field. The plant emergence in the field actually depends on seed vigour (Milošević et al., 2010).

Figure 1 shows the germination energy of conventionally and organically produced seeds that was read on the fourth day of the standard germination test. The germination energy of conventionally and organically produced seeds amounted to 30% and 69%, respectively. The total germination of spelt seeds was read on the eighth day of the standard germination test. The final results of the germination of conventionally and organically produced seeds are presented in Figure 2.

The total germination of conventionally and organically produced spelt seeds averaged 99% and 93%, respectively, which indicates that, in addition to large differences in the percentage of seed germination of these two types of seed production, the differences in total germination were not large. As already stated, higher germination (99%) was expressed by conventionally produced spelt seeds. Morphological traits of spelt seeds (chaffiness) can, to the greatest extent, affect seed germination. In 2002 and 2003, Acko (2004) performed the two-year germination test under laboratory conditions using chaffy and hulless spelt. The obtained results differed from one another. The average germination of chaffy and hulless spelt seeds amounted to 96.4% and of 80.5%, respectively. According to these results, it can be concluded that the higher germination percentage was determined in chaffy seeds, and, therefore, these seeds are the best choice for the crop establishment.

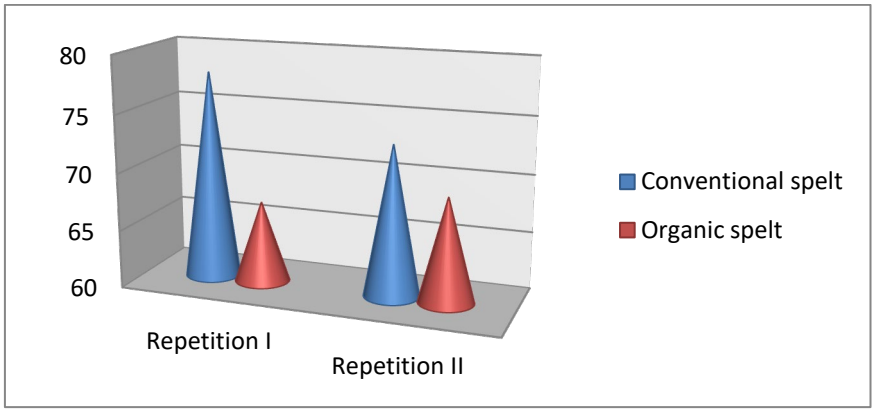

Figure 1. The germination energy of conventionally and organically produced spelt seeds.

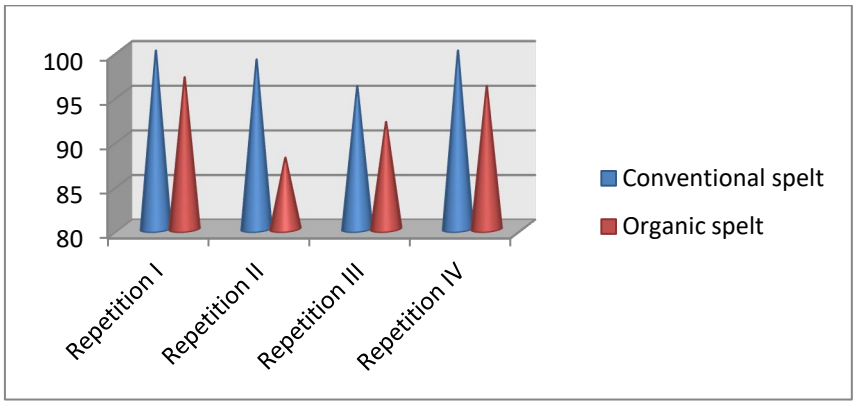

Figure 2. The seed germination of conventionally and organically grown spelt.

The accelerated ageing test is one of the most commonly used tests to determine seed vigour because it is closely related to the ability of seeds to germinate in the field (Lovato et al., 2001). In the course of the test, seeds absorb moisture from the humid environment, thus increasing their moisture content, which, together with the high temperature, results in accelerated seed ageing. High vigour seeds are more tolerant to stress conditions and will age slower than low vigour seeds. Results obtained on the effects of accelerated ageing on the germination of conventionally and organically produced spelt seeds are presented in this paper. Prior to the accelerated ageing test, the initial moisture content of seeds was measured. This content amounted to 10% and 8.3% in conventionally and organically produced spelt seeds, respectively. After this test, the seed weight was measured.

The final moisture content of conventionally produced spelt seeds amounted to 32% in the first replicate and to 33.9% in the second replicate, while the corresponding content of organically produced spelt seeds was slightly lower and equal in both replications and amounted to 31.3%. The final moisture content was compared to the values allowable by the ISTA. These values for spelt range from 28% to 30% (ISTA, 1995) and are somewhat lower than those obtained in the accelerated ageing test. This indicates that the spelt seeds that underwent accelerated ageing had poorer vigour than seeds that did not.

After accelerated ageing, seed germination was determined by the standard procedure. Seeds of conventionally and organically grown spelt were exposed to accelerated ageing and Figure 3 presents their germination energy (read on the fourth day from the day of placing seeds for germination testing).

The germination energy of conventionally and organically produced spelt seeds averaged 7% and 5%, respectively. The total germination energy of spelt seeds after accelerated ageing was read on the eighth day from the day of testing seeds and the final result is presented in Figure 4. After accelerated ageing, the seed germination of conventionally and organically grown spelt averaged 75% and 68%, respectively.

Obtained values of spelt seed germination after accelerated ageing were very low compared to the values gained by the standard germination test, and thus they are an indicator of lower seed vigour. These values also indicate the fact that the seeds, under environmental conditions, which are not ideal for germination, will probably express a low degree of germination. The percentage of abnormal seedlings of conventionally and organically grown spelt averaged 5% and 11%, respectively. The percentage of diseased and dead seeds in conventionally and organically grown spelt averaged 20% and 21%, respectively. A large number of papers describe various changes that occur in aged seeds, which are later manifested in seedlings, but some research has shown that there was no increase in the percentage of abnormal sunflower seedlings after accelerated ageing (Draganić,

2011). Many authors studied the effects of seed immersion (in aqueous or osmotic solutions) on different parameters of seed vigour. Thus, moistening of aged watermelon seeds (45°C, relative air humidity of 79% for 6 days) with vermiculate (25°C for 24h) resulted in the partial restoration of the initial germination of tested seeds (Chiu et al., 1995).

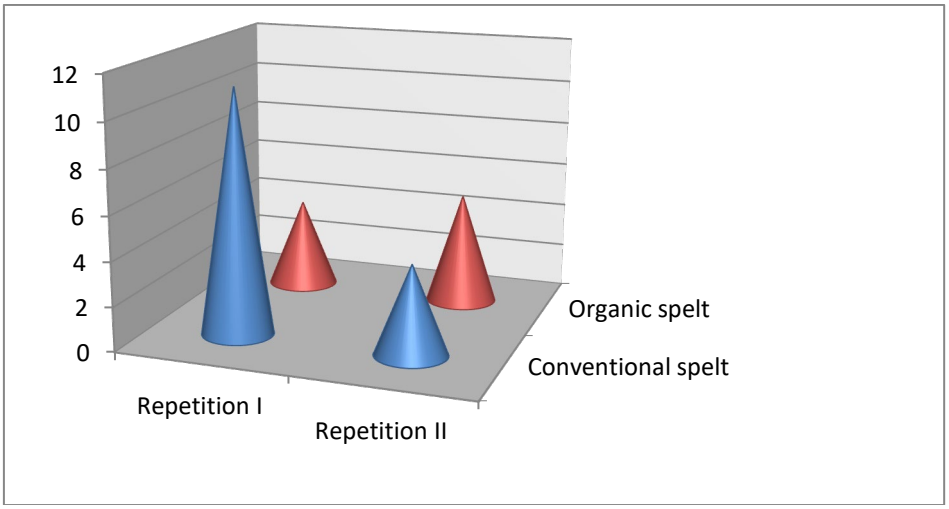

Figure 3. The germination energy of conventionally and organically produced spelt seeds after accelerated ageing.

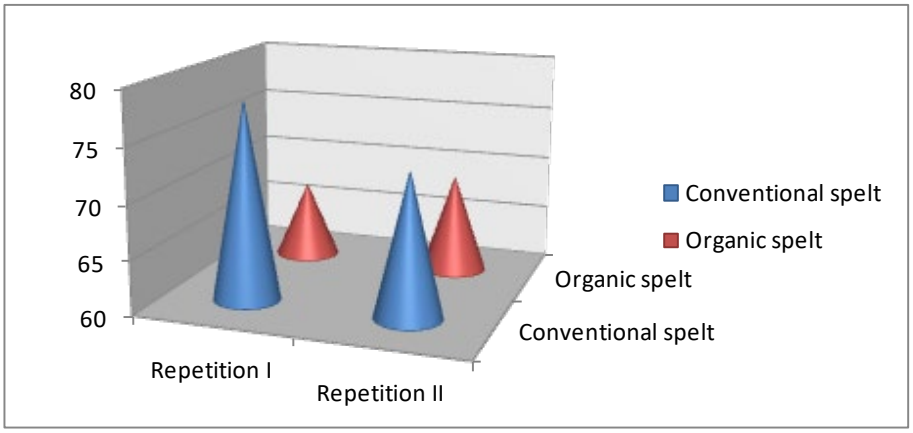

Figure 4. The germination of conventionally and organically produced spelt seeds after accelerated ageing.

The electrical conductivity measurement of seed extracts provides an assessment of the degree of loss by releasing electrolytes from plant tissues. Seed

vigour can be established by the conductivity measurement of the immersion water in which seed samples were soaked. If the release of electrolytes is strong, i.e., if the extract conductivity is high, the seed is considered to be of poor vigour, but if the release of electrolytes is poor (low conductivity) then the seed is of high vigour (ISTA, 1995).

According to the conductivity formula, the basic value was obtained by measuring the conductivity of water (control), which, in the case of spelt seeds, amounted to 3.92 and 3.56 or 3.74 on average.

The spelt seed weight was determined prior to seed soaking and reading of the conductivity, and the obtained values are presented in Table 1, while the read values of seed conductivity are shown in Table 2. The electric conductivity of conventionally produced seeds amounted to 189.4 μS/cm and 195.2 μS/cm in the first and the second replication, respectively. The corresponding values of organically produced seeds amounted to 95.5 μS/cm and 98.6 μS/cm, in the first and the second replication, respectively. The obtained values (conductivity, initial seed weight and basic values) are inserted into the conductivity formula and the conductivity of the extract per gram of the seed weight was calculated and presented in Figure 5. The conductivity per gram of commercially and organically produced spelt seeds amounted to 32.33 μS/cmg and 27.65 μS/cmg, respectively (Figure 4). The obtained values were compared with the tabular values of conductivity, i.e., with allowable values (Milošević et al., 2010).

Table 1. The spelt seed weight before the reading of conductivity (g).

Spelt seed production	Weight of 50 seeds (g)	
	1st repetition	2nd repetition
Conventional spelt	5.73	5.93
Organic spelt	3.40	3.35

Table 2. The values of seed electrical conductivity (μS/cm).

Spelt seed production	Conductivity (μS/cm)	
	1st repetition	2nd repetition
Conventional spelt	189.4	195.2
Organic spelt	95.5	98.6

Allowable values of conductivity amount to:

1. 43 μS/cmg – the low vigour seed that is not suitable for sowing;
2. 25–29 μS/cmg – the seed can be used for early sowing under unfavourable environmental conditions, but at risk;
3. 30–43 μS/cmg – the seed is not suitable for early sowing, especially not under unfavourable environmental conditions, and
4. >43 μS/cmg – the low vigour seed that is not suitable for sowing.

According to the literature data accessible, rapid seed vigour tests that give reliable information about seed physiological potential indicating their association with enzymatic and respiratory activities as well as cell membrane integrity are the tests such as the tetrazolium test and the electrical conductivity test (Szemruch et al., 2015). The latter test is based on the strength of resistance to the flow of electric current imposed on the steep water of seeds. Resistance is a function of the amount of electrolytes in the solution. The electrical resistance of pure water is strong, but electrolytic substances, such as ionic substances, enable the flowing of electric currents. Numerous cells are composed of bases, acids or their salts, i.e., electrolytes. The efflux of electrolytes from seeds during their imbibition most probably demonstrates the condition of the seed cell membrane. The weaker seeds are the poorer the membrane cell is due to which the electrolyte loss and conductivity measurements are higher (Pandey, 1992).

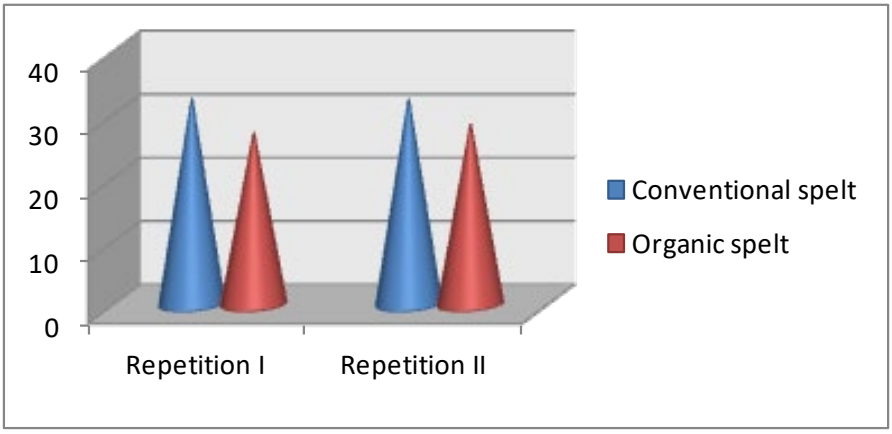

Figure 5. The conductivity of extract per gram of spelt seed weight.

Khan et al. (2010) performed a study related to seed quality tests and the field emergence of old and new wheat varieties during the 2003–2004 period. These authors used 32 samples of four wheat varieties and determined that the best estimation of seed vigour of the observed wheat varieties was achieved by the germination index, accelerated ageing and electrical conductivity, not only for ranking the quality of seed lots but also for predicting field emergence.

Results of the electrical conductivity test were feebly related to field emergence and the standard germination of seed lots of the four observed wheat varieties that varied in their vigour. Moreover, both tests, accelerated ageing and electrical conductivity, were very sensitive tests regarding seed lot quality ranking and showed a stronger correlation with field emergence than the standard

germination test for all four varieties in two years of investigation. According to the electrolyte outflow determined by the conductivity test of wheat seed lots that differed in vigour, the cell membrane integrity was the primary result of vigour degradation. Kaya (2014) showed that the conductivity test could be used in the determination of safflower seed vigour due to its negative correlation with the germination and accelerated ageing tests, while the control deterioration test was ineffective in the seed vigour evaluation.

Conclusion

The standard seed germination test showed that commercially produced spelt seeds had higher germination (99%) than organically produced spelt seeds (93%). The seed accelerated ageing test showed that the germination of commercially and organically produced spelt seeds amounted to 75% and 68%, respectively. Test results show that there was a great difference in electrical conductivity between organically (95.5 and 98.6 μS/cm) and conventionally produced spelt seeds (189.4 and 195.2 μS/cm). The electrical conductivity testing of conventionally produced spelt seeds (32.33 μS/cmg) points out that these seeds are low vigour seeds. On the other hand, the corresponding value of organically produced spelt seeds was 27.65 μS/cmg, which means that these seeds have a greater potential for storage and higher resistance to stressful conditions during germination.

References

Acko, K. (2004). Comparison between seed germination abilities: shelled and grind spelt wheat (*Triticum aestivum* L. var. *spelta*) and chaff spelt wheat seed germination. *Acta agriculturae Slovenica*, *38* (2), 331-339.

Chiu, K.Y., Wang, C.S., & Sung, J.M. (1995). Lipid peroxidation and peroxide-scavenging enzymes associated with accelerated aging and hydration of watermelon seeds differing in ploidy. *Physiologia Plantarum*, *94* (3), 441-446.

Draganić, I. (2011). Uticaj ubrzanog starenja na životnu sposobnost semena suncokreta. Magistarska teza. Poljoprivredni fakultet, Zemun.

Durrant, M.J., & Gummerson, R.J. (1990). Factors associated with germination of sugarbeet seed in the standard test establishment in the field. *Seed Science and Technology, 18,* 1-10.

FAO & WHO (1999). Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission. Report of the 30th Session of the Codex Committee on Pesticide Residues, 2025 April 1998. The Hague.

Golijan, J., Kolarić, LJ., Popović, A. & Živanović, LJ. (2019). Proizvodnja organskog krupnika (*Triticum spelta* L.) u Srbiji. *Selekcija i semenarstvo, 25* (1), 23-32

ISTA (1995). *Handbook of vigour test methods* (3rd edition). J.G. Hampton and D.M.TeKrony (eds). Zurich: International Seed Testing Association.

ISTA (2014). International Rules for Seed Testing. International Seed Testing Association, Switzerland.

ISTA (2016). International Rules for Seed Testing, Vol. 2016, Full Issue i–19-8 (284) http://doi.org/10.15258/istarules.2016.F

Kaya, M.D. (2014). Conformity of vigor tests to determine the seed quality of safflower (*Carthamus tinctorius* L.) cultivars. *Australian Journal of Crop Science*, *8* (3), 455-459.

Khan, A.Z., Shah, P., Mohd, F., & Zubair, M. (2010). Vigour tests used to rank seed lot quality and predict field emergence in Wheat. *Pakistan Journal of Botany*, 3147-3155. https://www.researchgate.net/publication/287739275pdf

Lacko-Bartošová, M., Korczyk-Szabó, J., & Ražný, R. (2010). Triticum spelta-a specialty grain for ecological farming systems. *Research Journal of Agricultural Science, 42* (1), 143-147.

Lekić, S. (2003). *Životna sposobnost semena.* Društvo selekcionera i semenara, Beograd.

Lekić, S. (2009). *Ispitivanje semena*. Izdanje autora, Beograd.

Lovato, A. E., Noli, A, Lovato, F.S., Beltrami, E., & Grassi, E. (2001). Comparison between three cold test low temperatures, accelerated ageing test and field emergence on maize seed. *Seed Symposium, ISTA Congres, Angers, France.*

Milošević, M., Bujaković, M., & Karagić, Đ. (2010). Vigour tests as indicators of seed viability. *Genetika, 42* (1), 103-118.

Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia (2022). Organic production. http://www.minpolj.gov.rs/organska/?script=lat Access: 27.7.2022.

Nikolić, O., Pavlović, M., Savurdić, A., & Jelić, M. (2015). Mogućnost gajenja spelte u organskoj poljoprivredi. *Zbornik radova XX savetovanja o biotehnologiji,* (pp. 117-122). Čačak.

Pandey, D.K. (1992). Conductivity testing of seeds. *Modern Methods of Plant Analyses. New Series, 14*, 273-299.

Szemruch, C., Del Longo, O., Ferrari, L., Renteria, S., Murcia, M., Cantamutto, M., & Rondanini, D. (2015). Ranges of vigor based on the electrical conductivity test in dehulled sunflower seeds. *Research Journal of Seed Science*, *8* (1), 12-21.

Ugrenović, V. (2003). *Uticaj vremena setve i gustine useva na ontogenezu, prinos i kvalitet zrna krupnika (Triticum spelta L.).* Doktorska disertacija. Poljoprivredni fakultet, Zemun.

Willer, H., Trávníček, J., Meier, C. & Schlatter, B. (Eds.) (2022). *The World of Organic Agriculture. Statistics and Emerging Trends 2022*. Research Institute of Organic Agriculture FiBL, Frick, and IFOAM – Organics International, Bonn. http://www.organic-world.net/yearbook/yearbook-2022.html

Received: March 9, 2022
Accepted: October 6, 2022

ŽIVOTNA SPOSOBNOST SEMENA KRUPNIKA PROIZVEDENOG U INSTITUTU ZA KUKURUZ „ZEMUN POLJE”

Tijana D. Lazarević[1], Tanja B. Petrović[2], Goran N. Todorović[2], Mile D. Sečanski[2], Jelena M. Golijan-Pantović[1*] i Slavoljub S. Lekić[1]

[1]Univerzitet u Beogradu, Poljoprivredni fakultet, Beograd, Srbija
[2]Institut za kukuruz „Zemun Polje”, Beograd, Srbija

R e z i m e

Krupnik (*Triticum spelta* L.) privlači sve veću pažnju proizvođača zbog svojih bioloških osobina i hemijskog sastava semena. Kako je za uspešnu proizvodnju neophodno kvalitetno seme, u ovom radu je ispitivana životna sposobnost semena krupnika proizvedenog konvencionalnim i organskim načinom proizvodnje. Ispitivano je seme krupnika sorte Nirvana, konvencionalno i organski proizvedeno 2015. godine u Institutu za kukuruz „Zemun polje”. Prema dobijenim rezultatima, energija klijanja organskog i konvencionalno proizvedenog semena krupnika iznosila je 30% odnosno 69%. Ukupna klijavost konvencionalno proizvedenog semena krupnika iznosila je 99%, dok je klijavost organski proizvedenog semena bila niža i iznosila je 93%. Broj nenormalnih klijanaca konvencionalno proizvedenog krupnika u proseku je dao 1% nenormalnih klijanaca, a takođe i organski krupnik. Organski proizvedeno seme krupnika beleži veći udeo bolesnog i mrtvog semena u odnosu na seme iz konvencionalnog useva (0%), te u proseku daje 6% bolesnih i mrtvih semena. Nakon testa ubrzanog starenja semena, zabeležena je viša ukupna klijavost konvencionalnog semena krupnika (75%), u poređenju sa semenom organskog krupnika (68%). Električna provodljivost konvencionalno proizvedenog semena iznosila je 189,4 μS/cm i 195,2 μS/cm u prvom i drugom ponavljanju, dok su vrednosti organskog semena iznosile 95,5 μS/cm i 98,6 μS/cm, u prvom i drugom ponavljanju. Rezultati ispitivanja elektroprovodljivosti ukazuju na to da ispitivano seme konvencionalnog krupnika (32,33 μS/cmg) spada u kategoriju semena niže životne sposobnosti u odnosu na organsko seme krupnika (27,65 μS/cmg).

Ključne reči: *Triticum spelta* L., klijavost, ubrzano starenje, elektroprovodljivost.

Primljeno: 9. marta 2022.
Odobreno: 6. oktobra 2022.

[*]Autor za kontakt: e-mail: golijan.j@agrif.bg.ac.rs

Journal of Agricultural Sciences (Belgrade) https://doi.org/10.2298/JAS2204367R
Vol. 67, No. 4, 2022 UDC: 633.11:631.427
Pages 367-380 Original scientific paper

SOIL BIOGENICITY IN THE RHIZOSPHERE OF DIFFERENT WHEAT GENOTYPES UNDER THE IMPACT OF FERTILIZATION TREATMENT

Svetlana M. Roljević Nikolić[1*], Željko K. Dolijanović[2], Dušan Đ. Kovačević[2], Snežana I. Oljača[2] and Helena J. Majstorović[1]

[1]Research and Development Institute Tamiš,
Novoseljanski put 33, Pančevo, Serbia
[2]University of Belgrade, Faculty of Agriculture,
Nemanjina 6, Belgrade, Serbia

Abstract: The rhizosphere is a dynamic environment in which many parameters may influence biogenicity. The important factors determining the microbial community in the rhizosphere are plant and soil nutrient supply. The aim of this paper was to determine the abundance of basic microbiological groups in the rhizosphere of four wheat subspecies, in three fertilization treatments in the organic farming system. A field experiment was conducted using a randomized complete block design with four replicates. It was carried out on the leached chernozem soil type. There was significant variability in the abundance of the studied physiological groups of microorganisms between the wheat subspecies, as well as between the fertilization treatments. The rhizosphere of common wheat had the greatest abundance of fungi (24.37 x 10^3 g^{-1}). The rhizosphere of compactum wheat had the largest abundance of oligonitrophilic bacteria (361.47 x 10^5 g^{-1}) and amonificators (119.27 x 10^5 g^{-1}). There were no significant differences in the abundance of actinomycetes between the cultivars of common, compactum and durum wheat, but their lowest number was found in the spelt wheat cultivar (11.25 x 10^3 g^{-1}). The combined application of biofertilizer and organic fertilizer resulted in a significantly greater abundance of amonificators (56.6%), fungi (28.2%) and oligonitrophiles (14.6%) than in the control treatment. The results show that the crop variety and application of appropriate fertilizer formulations can influence the abundance of the studied groups of microorganisms. This is particularly the case in organic farming, which relies completely on natural resources and processes.

Key words: wheat, organic farming, biohumus, biofertilizer, fungi, actinomycetes, amonificators, oligonitrophiles.

[*]Corresponding author: e-mail: roljevic@institut-tamis.rs

Introduction

Intensive farming causes soil degradation (Purwanto and Alam, 2020), excessive water consumption (Pfister et al., 2011), and greenhouse gas emissions (Shakoor et al., 2021), failing to provide the necessary sustainability of food production. The concern for natural resources, high costs and a decrease in oil and gas reserves used in the Haber-Bosch process that produces nitrogen fertilizers industrially has brought some essential changes in agricultural practices based on sustainable management (Singh et al., 2011). Consequently, over the last decades, studies in the field of agriculture have been directed at the development and application of alternative solutions that can potentially provide satisfactory yields without negative consequences on the environment (Ye et al., 2020). Therefore, it is very important to examine the rhizosphere microbial communities and their impact on crop productivity and ecological implications of their application in agriculture (Gqozo et al., 2020; Karličić et al., 2016). Previous researches have shown that the application of microorganisms in crop production increases the accessibility and efficiency of nutritive matter, which decreases the requirements for mineral fertilization by 50% while not lowering the plant yield (Hayat et al., 2010; da Costa et al., 2013).

The rhizosphere community consists of different types of microbes which have beneficial effects on the plant growth and development, including the ones which are capable of atmospheric nitrogen intake, fungi, protozoa and rhizomicrobes promoting plant growth and conducting biocontrol (Zhao et al., 2018). The presence of fungi in soil provides numerous services to crops including improved soil physical properties, mineralization of organic matter and synthesis of humus, efficient use of fertilizer and soil nutrients, protection against drought stress and diseases (Singh et al., 2012). On the other side, actinomycetes perform not only a decomposition of organic matter in the soil, but also perform nitrogen fixation (15% of total fixation) and phosphate solubilization (AbdElgawad et al., 2020). They produce various antibiotics (chloramphenicol, neomycin, streptomycin), biologically active matter such as B vitamins, auxines and others (Jarak et al., 2006). Studies on wheat and rice have shown that reduction of N fertilizer by 20% and replacement of 50% of N fertilizer by organic manure increased the number of actinomycetes by 11–153%, without significantly reducing crop yields (Guan et al., 2011).

The free-living diazotrophic bacteria are known to affect, directly or indirectly, plant growth. This occurs as a result of the synthesis and export of organic compounds such as phytohormones (e.g., indole-3-acetic acid) that enhance root growth and through a contribution of biological nitrogen fixation to nitrogen acquisition by the plant (Venieraki et al., 2011). Oligonitrophilic bacteria represent a specific group of microorganisms capable of reducing the molecular

form of nitrogen from the atmosphere and converting it into the organic ammonia form, using very small quantities of mineral nitrogen content from soil (Rasulić et al., 2021).

Specific characteristics of a microbial community are affected by physical and chemical soil properties, grown plant species, fertilizer use and other agrotechnical measures (Sivojiene et al., 2021). Organic matter is a vital component of soil quality and fertility. At the same time, it represents the main substrate and energy source for microorganisms (Xu et al., 2013), as well as the limiting factor for the abundance and diversity of the microbial population (Fließbach et al., 2007). It has been determined that the increase in the organic matter content leads to the increase in the weight and diversity of heterotrophic microorganisms (Ding et al., 2016), which consequently results in greater soil respiration (Araújo et al., 2009). Numerous examinations have shown that the application of organic fertilizers improves physical and chemical properties of the rhizosphere soil (Bibhuti and Dkhar, 2011), and consequently increases the biomass and activity of microorganisms (Chang et al., 2007; Mohammadi, 2011).

The plant genotype also has a significant impact on the microbial community in the rhizosphere (Compant et al., 2019). Namely, different plant species have a specific relationship and interaction with microorganisms promoting the plant growth and development, as well as their resistance to stress and diseases (Wei and Jousset, 2017). The examination of the bacterial rhizosphere of *Triticum monococcum* PI 167549, *T. aestivum* cv. CDC Teal and T. *aestivum* cv. Red Fife has shown that *Aureobacter* species differed significantly between cultivars (Germida and Siciliano, 2001). Similarly, significant differences between spelt cultivars were found in the total number of rhizosphere fungi (Korniłłowicz-Kowalska et al., 2022).

In the literature, scanty information is available on the influence of host genotype on the number of different physiological groups of microorganisms in the rhizosphere, so the aim of this research is to study the soil rhizosphere biogenicity of different wheat genotypes under the influence of fertilization treatments.

Material and Methods

The examination of soil biogenicity regarding the abundance of different physiological groups of microorganisms in the rhizosphere of four wheat subspecies, under the influence of fertilization in organic farming, was conducted at the experimental field of the Faculty of Agriculture, Radmilovac. The soil type was a luvic chernozem, with the characteristics: pH-(H_2O) 8.04, total N 0.13%, available K 19.10 mg (K_2O 100 g^{-1}) and P 22.18 mg (P_2O_5 100 g^{-1}), content of SOC 14.2%. According to the content of basic mechanical fractions (sand, silt, clay), the study site belongs to silty clay loam soil (Dolijanović, 2002). The field experiment

was conducted using a randomized complete block design with four replicates in the period 2009–2012. The elementary plot was 6 m^2.

Figure 1 shows the average monthly temperatures and the amount of precipitation in the long-term period, as well as during the experiment. The average amount of precipitation in the long-term period (1970–2008) amounted to 698.3 mm, while the average monthly air temperature was 12.3°C. However, during the three-year examination period (2009–2012), there were certain deviations from the long-term average values. The average annual amount of precipitation was slightly lower (by 14.9 mm), while the average annual air temperature was greater by 1.2°C in comparison to the long-term average values. The lower amount of precipitation was recorded in April (32.7 mm), August (29.1 mm) and September (33.5 mm), while abundant precipitation was registered in the winter months (December, January and February). Contrary to the tendency of the precipitation regime change, lower average air temperatures were recorded in January (1.0°C) and February (1.2°C), while higher air temperatures were recorded in August (24.8°C) and September (20.6°C), as well as in November (9.3°C) in comparison to the long-term average values.

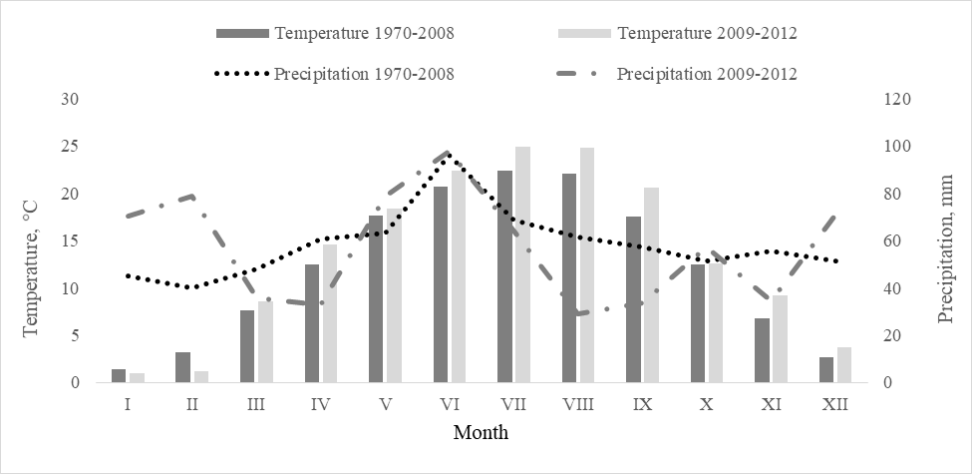

Figure 1. The average monthly mean air temperature (°C) and the precipitation sum (mm).
Source: RHMS of Serbia

In all three years, the previous crop to wheat was maize (*Zea mays* L.). After the maize harvest, conservation tillage of soil at the depth of 25 cm was performed and the organic fertilizer was plowed in. Presowing preparation was performed using a disc harrow and a spike-tooth harrow. Sowing was done manually at the end of the second decade of October. The original seed from the Institute of Field and Vegetable Crops in Novi Sad was used.

In order to study the abundance of basic microbiological groups in the rhizosphere of different wheat genotypes, three cultivars of alternative wheat subspecies were included in the research (*Triticum aestivum* ssp. *spelta* – cv. Nirvana, *Triticum durum* – cv. Dolap, *Triticim aestivum* ssp. *compactum* – cv. Bambi) and one cultivar of common soft wheat (*Triticum aestivum* ssp. *vulgare* – cv. NS 40S). The seed densities were: NS 40S – 550; Dolap – 600; Bambi – 600; Nirvana – 550 germinating seeds per m^2.

The fertilization treatment involved: T0 – control, without fertilization, T1 – microbiological fertilizer "Slavol"(5.0 l ha^{-1}), T2 – biohumus (3,000 kg ha^{-1}) + microbiological fertilizer "Slavol" (5.0 l ha^{-1}). "Slavol" ("Agrounik" Serbia) is a liquid foliar microbiological fertilizer containing: *Bacillus megaterium* 10^{-6} cm^3, *Bacillus licheniformis* 10^{-6} cm^3, *Bacillus subtilis* 10^{-6} cm^3, *Azotobacter chroococcum* 10^{-6} cm^3, *Azotobacter vinelandii* 10^{-6} cm^3, *Derxia* sp. 10^{-6} cm^3. It was applied during the BBCH 31-33 phenophase.

Biohumus ("Biohumus Royal offert", "Altamed" Serbia) is an organic fertilizer, certified for use in organic farming, and plowed in during the autumn within the primary tillage. The chemical properties of biohumus are as follows: pH 8.63, N 2.2%; P_2O_5 4.8% and K_2O 2.8%.

In all three years, the soil samples for microbiological analyses were taken aseptically at a 0–10 cm depth, in the zone of 0.5 cm from wheat root (Đurić and Jarak, 2006). Soil samples were stored in sterile polyethylene bags. The soil samples were taken at the end of the BBCH 33 phase of wheat, in four replications.

The number of microorganisms was calculated in 1 gram of absolutely dry soil. The number of fungi was determined on the Czapek-Dox medium, and actinomycetes on a synthetic medium by Krasilnikov (1965). The presence of oligonitrophyls was determined on a medium without nitrogen by Fjodorov (Sarić, 1989), and the number of amonificators on the meat peptone agar (Pochon and Tardieux, 1962). The sown media were incubated at the temperature of 28°C. The incubation depended on the microorganism group and lasted from 3 to 7 days.

The obtained results of the research on the abundance of different physiological groups of microorganisms were analyzed using the analysis of variance (ANOVA) procedure of the Statistical Analysis Software (SPSS software, 19.0). The comparisons among different treatments were made with the least significant difference (LSD) test, at the significance levels of $p<0.01$ and $p<0.05$.

Results and Discussion

Table 1 shows the research results regarding the abundance of physiological groups of microorganisms in the rhizosphere of different wheat subspecies under the influence of fertilization treatments. The analysis of variance shows that the

genotype, treatments, as well as the interaction of these two factors, had a significant impact on the abundance of fungi in the rhizosphere. The greatest number of fungi was recorded in the rhizosphere zone of common wheat (24.37 x 10^3 g^{-1}), which was significantly higher than in spelt (22.25x 10^3 g^{-1}), durum (20.08 x 10^3 g^{-1}) and compactum (20.60 x 10^3 g^{-1}) wheats. The variant with the combined application of organic fertilizer and biofertilizer (26.63 x 10^3 g^{-1}) had a significantly higher number of fungi than the variant with the application of biofertilizers (20.07 x 10^3 g^{-1}) and control (20.78 x 10^3 g^{-1}). The analysis of the interaction between the studied factors, i.e., the analysis of differences in the mean values between treatments, shows that the combined application of fertilizers provided the best results in the spelt wheat cultivar (51.10%).

The greatest difference in the abundance of actinomycetes was determined between durum (21.49 x 10^3g^{-1}) and spelt (11.25 x 10^3g^{-1}) wheats. Although the examined treatments did not have a significant influence on this group of microorganisms, there was an increase at the levels of 30.89% and 16.30% in T1 and T2 in comparison to the control. In addition, there were certain differences between the cultivars regarding their reaction to the applied fertilizers. The greatest differences between T1 and T2 treatments in comparison to the control were registered in the compactum wheat cultivar (90.1% and 58.6%, respectively), while the smallest differences were observed in the durum wheat cultivar (6.8% and 3.1%, respectively).

The number of microorganisms-amonificators was found in the rhizosphere of compactum wheat (119.27 x 10^5g^{-1}) and it was higher compared to the other wheat subspecies. The abundance of amonificators recorded in the treatment with the combined application of organic fertilizer and biofertilizer was higher by 56.6%, while their abundance in the variant with the independent application of biofertilizer was higher by 39.2% than in the control. In all studied wheat subspecies, there was a greater number ($p < 0.01$) of amonificators in the variants with the applied fertilizers than in the control. The obtained differences were the biggest in spelt wheat – 65.7% and 111.6% in T1 and T2 in comparison to the control.

Within the studied microbial community, oligonitrophiles represent the most abundant group with significant ($p<0.01$) differences between the studied cultivars. The abundance of this group of saprophytes ranged from 229.84 to 361.47 x 10^5g^{-1} in durum and compactum wheat, respectively. The effect of treatment was also significant. Treatment with a combined application of organic fertilizer and biofertilizer (321.21 x 10^5g^{-1}) resulted in an increase in the number of oligonitrophiles by 14.6% compared to the control. The analysis of the differences between the treatments indicates that the fertilizer application in the spelt wheat cultivar increased the number of oligonitrophiles by 22.6% in T1 and by 37.7% in T2, which is significantly more than in other cultivars.

Table 1. The average abundance of the physiological groups of microorganisms in the rhizosphere of different wheat subspecies in the three-year period (2009/2010–2011/2012).

Wheat subspecies/cultivars	T0	T1	T2	Average
Fungi 10^3 g^{-1}				
T. aestivum ssp. *vulgare* – NS 40S	20.40±10.9	23.65±18.7	29.07±21.3	24.37
Triticum durum – Dolap	20.48±11.3	19.27±10.5	20.50±10.9	20.08
T. aestivum ssp. *compactum* – Bambi	24.05±15.7	16.30±9.7	21.45±15.3	20.60
Triticum spelta – Nirvana	18.20±9.4	21.05±13.1	27.50±23.5	22.25
Average	20.78	20.07	26.63	
Actinomycetes 10^3 g^{-1}				
T. aestivum ssp. *vulgare* – NS 40S	15.80±3.2	18.10±4.5	19.75±4.7	17.88
Triticum durum – Dolap	20.80±5.85	22.22±9.73	21.45±9.33	21.49
T. aestivum ssp. *compactum* – Bambi	13.73±3.6	26.10±11.7	21.78±7.6	20.54
Triticum spelta – Nirvana	11.15±2.1	14.05±5.3	8.54±1.84	11.25
Average	15.37	20.12	17.88	
Amonificators 10^5 g^{-1}				
T. aestivum ssp. *vulgare* – NS 40S	92.90±37.3	125.70±53.9	129.60±67.5	116.07
Triticum durum – Dolap	75.40±25.1	91.40±36.7	92.30±38.9	86.37
T. aestivum ssp. *compactum* – Bambi	85.00±30.0	123.20±53.6	149.60±77.0	119.27
Triticum spelta – Nirvana	45.80±26.9	75.90±27.0	96.90±41.7	72.87
Average	74.78	104.05	117.10	
Oligonitrophilic bacteria 10^5 g^{-1}				
T. aestivum ssp. *vulgare* – NS 40S	310.50±81.6	230.82±63.5	230.22±60.6	257.18
Triticum durum – Dolap	219.60±53.7	183.87±49.3	286.05±75.7	229.84
T. aestivum ssp. *compactum* – Bambi	317.35±85.3	375.60±93.7	391.45±103.3	361.47
Triticum spelta – Nirvana	273.85±71.8	335.80±88.2	377.10±96.6	328.92
Average	280.33	281.52	321.21	

*All measurements are the means of three years of testing (± standard deviation) of four replicates.

Microorganisms represent the most numerous component of the soil biological phase, which has a direct impact on the agroecosystem stability (Le Guillou et al., 2019). The research results show that the wheat genotypes significantly affected the biogenicity of the rhizosphere. The similar results were recorded in other crops. The discriminant analysis clearly differentiated rhizosphere microbial communities in relation to the maize genotype (Aira et al., 2010), while in barley, it determined a small but significant host genotype effect on the diversity of root-associated bacterial communities (Bulgarelli et al., 2015). The greatest abundance of fungi was registered in the rhizosphere of the common wheat (NS 40S). Some studies dealt with the diversity of fungal communities in the rhizosphere of the roots in common wheat and spelt (Salamon et al., 2020), but studies of the abundance of fungi in the rhizosphere of different wheat subspecies are very limited. The analysis of the differences between the treatments showed that, in the treatment with the combined application of biohumus and biofertilizer (T2), the abundance of

fungi was greater by 18.5% than in the control. Similarly, Zhu et al. (2020) found that the soil treated with organic fertilizer had a larger number of fungi than the soil where no fertilizer was applied, which could be explained by the balanced content of nutritive matter, primarily organic carbon, nitrogen and potassium (Kumar et al., 2017; Zhu et al., 2020). The differences in the mean values between the treatments showed that the fertilizer application did not significantly increase the abundance of fungi in the rhizosphere of durum wheat and compactum wheat, while the obtained differences were significant for the cultivars of common wheat and spelt wheat. Therefore, understanding the dynamics of the abundance of the fungal population in the rhizosphere represents the first step towards the successful management of the microbial community with the aim of production and yield sustainability (Bever et al., 2012).

Table 2. The analysis of variance of the abundance of the examined physiological groups of microorganisms.

Microorganisms	2009/10 – 2011/12				
			G	T	G*T
Fungi	LSD	F	11.747	30.571	12.208
		Sig.	0.000	0.000	0.000
		0.05	1.386	1.201	2.401
		0.01	1.901	1.646	3.293
Actinomycetes	LSD	F	5.434	1.914	0.816
		Sig.	0.005	0.169	0.568
		0.05	5.098	4.415	8.830
		0.01	6.992	6.055	12.110
Amonificators	LSD	F	764.065	929.104	54.906
		Sig.	0.000	0.000	0.000
		0.05	2.111	1.828	3.656
		0.01	2.895	2.507	5.014
Oligonitrophiles	LSD	F	4502.743	867.018	918.446
		Sig.	0.000	0.000	0.000
		0.05	2.346	2.032	4.064
		0.01	3.218	2.787	5.574

G – genotype, T – treatment.

The genotype represented the key source of the variable abundance of actinomycetes. The greatest number of actinomycetes was found in the rhizosphere of durum wheat (21.49x $10^3 g^{-1}$), followed by compactum wheat (20.54x $10^3 g^{-1}$) and common wheat (17.88x $10^3 g^{-1}$), while their smallest number was recorded in spelt wheat (11.25x $10^3 g^{-1}$). However, the only significant difference in the number of actinomycetes was determined between durum wheat and spelt wheat. Studying the biodiversity of bacteria and their metabolic profile in the rhizosphere of four winter

wheat (*Triticum aestivum* L.) varieties, Wolińska et al. (2020) have established that the number of Actinobacteria was dependent on the wheat variety. Fertilization did not significantly affect the abundance of actinomycetes. Some authors (Liang et al., 2020; Wang et al., 2017) indicate that there is a correlation between the abundance and diversity of the bacterial community in the wheat rhizosphere and pH, nitrates, accessible phosphorus and available potassium. Since the analysis of macromineral accessibility was not conducted in this study, we speculate that one of the factors controlling the bacterial structure and actinomycete abundance is the accessibility of minerals rather than their total content and organic matter content *per se*.

Amonificators are the most represented group of microorganisms in the soil (Rasulić et al., 2021). A significantly greater number of amonificators was recorded in the rhizosphere of compactum wheat than in the rhizosphere of the cultivars of common, durum and spelt wheats. The results reported by Jezierska-Tis et al. (2012) showed that the highest number of proteolytic bacteria was found in the control soil under winter wheat compared to durum winter wheat and spelt winter wheat lines. While analyzing root-associated bacterial and fungal communities in modern wheat cultivars, *Triticum aestivum* ssp. *spelta* and ancestors of wheat (T. *turgidum* ssp. *dicoccum*, T. *monococcum* ssp. *monococcum* and T. *monococcum* ssp. *aegilopoides*), Kinnunen-Grubb et al. (2020) observed a clear separation of bulk soil microbial communities of all wheat accessions. In addition, significant differences between the treatments were registered in this trial. The abundance of amonificators recorded in the treatment with the combined application of biohumus and biofertilizer was greater by more than 50% than in the control, while the greatest differences between the treatments were found in spelt wheat. Some studies found significant differences in the abundance of amonificators between different variants of organic and mineral fertilizer applications (Raičević et al., 2005). The greatest abundance of amonificators in the control was registered in the common wheat rhizosphere, which might indicate its tendency towards forming associations with this bacterial group.

The greatest number of the members of oligonitrophilic bacteria was detected in the rhizosphere of compactum and spelt wheats, as well as in the variant with biofertilizer +biohumus. From the aspect of soil fertility, free-living nitrogen-fixers are extremely important, which underlines the significance of the selection of an appropriate variety within the soil and plant nutritional management.

The research results showed the differences in the abundance of the examined physiological groups of microorganisms in the rhizosphere of the studied species (wheat) which could be used in selecting the genotype adjusted to the specific characteristics of soil and the applied agricultural practices. The same has been indicated by other authors (Abhilash et al., 2012). Nevertheless, this approach to genotype selection has to be supported by crop management practices favoring beneficial microflora.

Conclusion

The obtained results show that the selection of a suitable crop variety, accompanied by the application of appropriate fertilizer formulations, can affect the specific characteristics of the microbial community that represent a significant factor of soil fertility. The greatest abundance of fungi was recorded in the common wheat cultivar (24.37 x 10^3 g^{-1}), while the rhizosphere of compactum wheat had the largest number of amonificators (119.27 x 10^5 g^{-1}) and oligonitrophilic bacteria (361.47 x 10^5 g^{-1}). There was no significant difference in the number of actinomycetes between common, compactum and durum wheats, but their number was the lowest in the spelt wheat cultivar (11.25 x 10^3 g^{-1}). The analysis of differences between the mean values of the examined treatments showed that the combined application of biohumus and biofertilizer had a significant influence on the abundance of fungi, amonificators and oligonitrophiles. The selection of the appropriate variety, along with the suitable manner of nutrient supply, can significantly affect soil biogenicity and, consequently, its fertility. Such an integral approach can represent a basis for the development of innovative technologies within the concept of sustainable development of agriculture, particularly in organic farming, which completely relies on natural resources and mechanisms.

Acknowledgements

This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 451-03-68/2022-14/200116.

References

AbdElgawad, H., Abuelsoud, W., Madany, M.M., Selim, S., Zinta, G., Mousa, A.S., & Hozzein, W.N. (2020). Actinomycetes enrich soil rhizosphere and improve seed quality as well as productivity of legumes by boosting nitrogen availability and metabolism. *Biomolecules*, *10* (12), 1675.

Abhilash, P.C., Powell, J.R., Singh, H.B., & Singh, B.K. (2012). Plant–microbe interactions: novel applications for exploitation in multipurpose remediation technologies. *Trends in biotechnology*, *30* (8), 416-420.

Araújo, A.S., Leite, L.F., Santos, V.B., & Carneiro, R.F. (2009). Soil microbial activity in conventional and organic agricultural systems. *Sustainability, 1* (2), 268-276.

Aira, M., Gómez-Brandón, M., Lazcano, C., Baath, E., & Domínguez, J. (2010). Plant genotype strongly modifies the structure and growth of maize rhizosphere microbial communities. *Soil Biology and Biochemistry*, *42* (12), 2276-2281.

Bever, J.D., Platt, T.G., & Morton, E.V. (2012). Microbial population and community dynamics on plant roots and their feedbacks on plant communities. *Annual Review of Microbiology*, *66*, 265-283.

Bibhuti, B.D., & Dkhar, M.S. (2011). Rhizosphere Microbial Populations and Physico Chemical Properties as Affected by Organic and Inorganic Farming Practices. *American-Eurasian Journal of Agricultural & Environmental Sciences, 10* (2), 140-150.

Bulgarelli, D., Garrido-Oter, R., Münch, P. C., Weiman, A., Dröge, J., Pan, Y., & Schulze-Lefert, P. (2015). Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell host & microbe*, *17* (3), 392-403.

Chang, E.H., Chung, R.S., & Tsai, Y.H. (2007). Effect of different application rates of organic fertilizer on soil enzyme activity and microbial Population. *Soil Science and Plant Nutrition*, *53,* 132-140.

Compant, S., Samad, A., Faist, H., & Sessitsch, A. (2019). A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research*, *19*, 29-37.

da Costa, P.B., Beneduzi, A., de Souza, R., Schoenfeld, R., Kayser Vargas, L., & Passaglia, L.M.P. (2013). The effects of different fertilization conditions on bacterial plant growth promoting traits: guidelines for directed bacterial prospection and testing. *Plant and Soil, 368,* 267-280.

Ding, J.L., Jiang, X., Ma, M.C., Zhou, B.K., Guan, D.W., Zhao, B.S., Zhou, J., Cao, F.M., Li, L., & Li, J. (2016). Effect of 35 years inorganic fertilizer and manure amendment on structure of bacterial and archaeal communities in black soil of northeast China. *Applied Soil Ecology*, *105*, 187-195.

Dolijanović, Ž. (2002). Uticaj aditivnog načina združivanja i prihranjivanja na produktivnost kukuruza i soje. Magistarska teza, Univerzitet u Beogradu, Poljoprivredni fakultet, Zemun.

Đurić, S., & Jarak, M. (2006). The effect of sulphonylurea herbicides on the microbial activity in soil under maize. *Annals of the Faculty of Engineering Hunedoara*, *4*, 93-96.

Fließbach, A., Oberholzer, H.R., Gunst, L., Mäder, P., & Fliessbach, A. (2007). Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. *Agriculture, Ecosystems and Environment*, *118*, 273-284.

Germida, J., & Siciliano, S. (2001). Taxonomic diversity of bacteria associated with the roots of modern, recent and ancient wheat cultivars. *Biology and Fertility of Soils*, *33* (5), 410-415.

Gqozo, M.P., Bill, M., Siyoum, N., Labuschagne, N., & Korsten, L. (2020). Fungal diversity and community composition of wheat rhizosphere and non-rhizosphere soils from three different agricultural production regions of South Africa. *Applied Soil Ecology*, *151*, 103543.

Guan, G., Tu, S.X., Yang, J.C., Zhang, J.F., & Li, Y.A.N.G. (2011). A field study on effects of nitrogen fertilization modes on nutrient uptake, crop yield and soil biological properties in rice-wheat rotation system. *Agricultural Sciences in China*, *10*, 1254-1261.

Hayat, R., Ali, S., Amara, U., Khalid, R., & Ahmed, I. (2010). Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of Microbiology,* 60, 579-598.

Jarak, M., Protić, R., Janković, S., & Čolo, J. (2006). Response of wheat to Azotobacter-actinomycetes inoculation and nitrogen fertilizers. *Romanian Agricultural Research*, *23*, 37-40.

Jezierska-Tys, S., Rachoń, L., Rutkowska, A., & Szumiło, G. (2012). Effect of new lines of winter wheat on microbiological activity in Luvisol. *International Agrophysics*, *26* (1), 33-38.

Karličić, V., Radić, D., Jovičić-Petrović, J., Lalević, B., Jovanović, L., Kiković, D., & Raičević, V. (2016). Isolation and characterization of bacteria and yeasts from contaminated soil. *Journal of Agricultural Sciences (Belgrade)*, *61* (3), 247-256.

Kinnunen-Grubb, M., Sapkota, R., Vignola, M., Nunes, I. M., & Nicolaisen, M. (2020). Wheat domestication has dramatically affected the root-associated microbiome. https://doi.org/10.21203/rs.3.rs-20588/v1

Korniłłowicz-Kowalska, T., Andruszczak, S., Bohacz, J., Kraska, P., Możejko, M., & Kwiecińska-Poppe, E. (2022). The effect of tillage and no-tillage system on culturable fungal communities in the rhizosphere and soil of two spelt cultivars. *Applied Soil Ecology*, *174*, 104413.

Krasilnikov, N.N. (1965). *Biology of some actinomycetes groups.* Science. Moskva.

Kumar, U., Shahid, M., Tripathi, R., Mohanty, S., Kumar, A., Bhattacharyya, P., & Nayak, A.K. (2017). Variation of functional diversity of soil microbial community in sub-humid tropical rice-rice cropping system under long-term organic and inorganic fertilization. *Ecological indicators*, *73,* 536-543.

Le Guillou, C., Chemidlin Prévost-Bouré, N., Karimi, B., Akkal-Corfini, N., Dequiedt, S., Nowak, V., & Ranjard, L. (2019). Tillage intensity and pasture in rotation effectively shape soil microbial communities at a landscape scale. *MicrobiologyOpen*, *8* (4), e00676.

Liang, R., Hou, R., Li, J., Lyu, Y., Hang, S., Gong, H., & Ouyang, Z. (2020). Effects of different fertilizers on rhizosphere bacterial communities of winter wheat in the North China Plain. *Agronomy*, *10* (1), 93.

Mohammadi, K. (2011). Soil Microbial Activity and Biomass as Influenced by Tillage and Fertilization in Wheat Production American-Eurasian. *Journal of Agriculture and Environmental Sciences*, *10* (3), 330-337.

Pfister, S., Bayer, P., Koehler, A., & Hellweg, S. (2011). Projected water consumption in future global agriculture: Scenarios and related impacts. *Science of the total environment, 409* (20), 4206-4216.

Pochon, J., & Tardieux, P. (1962). *Techniques d'analyse en microbiologie du sol.* Paris, France.

Purwanto, B.H., & Alam, S. (2020). Impact of intensive agricultural management on carbon and nitrogen dynamics in the humid tropics. *Soil Science and Plant Nutrition*, *66* (1), 50-59.

Raičević, V., Lalević, B., Ličina, V., Vasić, G., & Antić-Mladenović, S. (2005). Microbiological activity of barley rhizosphere grown on deposol. *Savremena poljoprivreda*, 54 (3-4), 487-491.

Rasulić, N., Delić, D., Stajković-Srbinović, O., Buntić, A., Kuzmanović, Đ., Knežević, M., & Sikirić, B. (2021). Microbiological and basic agrochemical properties of Eutric Cambisols in western and southwestern Serbia. *Zemljište i biljka*, *70* (2), 1-9.

Republic Hydrometeorological Service of Serbia, Meteorological Yearbooks, http://www.hidmet.gov.rs/latin/meteorologija/klimatologija_godisnjaci.php

Salamon, S., Mikołajczak, K., Błaszczyk, L., Ratajczak, K., & Sulewska, H. (2020). Changes in root-associated fungal communities in *Triticum aestivum* ssp. *spelta* L. and *Triticum aestivum* ssp. *vulgare* L. under drought stress and in various soil processing. *Plos one*, 15, e0240037.

Sarić, Z. (1989). *Praktikum iz mikrobiologije*. Naučna knjiga, Beograd.

Singh, A.K., Hamel, C., DePauw, R.M., & Knox, R.E. (2012). Genetic variability in arbuscular mycorrhizal fungi compatibility supports the selection of durum wheat genotypes for enhancing soil ecological services and cropping systems in Canada. *Canadian journal of microbiology*, *58,* 293-302.

Singh, J.S., Pandey, V.C., & Singh, D.P. (2011). Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. *Agriculture, Ecosystems & Environment*, *140* (3-4), 339-353.

Sivojiene, D., Kacergius, A., Baksiene, E., Maseviciene, A., & Zickiene, L. (2021). The Influence of Organic Fertilizers on the Abundance of Soil Microorganism Communities, Agrochemical Indicators, and Yield in East Lithuanian Light Soils. *Plants*, *10* (12), 2648.

Shakoor, A., Shakoor, S., Rehman, A., Ashraf, F., Abdullah, M., Shahzad, S. M., & Altaf, M.A. (2021). Effect of animal manure, crop type, climate zone, and soil attributes on greenhouse gas emissions from agricultural soils—A global meta-analysis. *Journal of Cleaner Production*, *278*, 124019.

Venieraki, A., Dimou, M., Pergalis, P., Kefalogianni, I., Chatzipavlidis, I., & Katinakis, P. (2011). The genetic diversity of culturable nitrogen-fixing bacteria in the rhizosphere of wheat. *Microbial Ecology*, *61*, 277-285.

Wang, J., Song, Y., Ma, T., Raza, W., Li, J., Howland, J.G., Huang, Q., & Shen, Q. (2017). Impacts of inorganic and organic fertilization treatments on bacterial and fungal communities in a paddy soil. *Applied Soil Ecology*, *112,* 42-50.

Wei, Z., & Jousset, A. (2017). Plant breeding goes microbial. *Trends in Plant Science*, *22* (7), 555-558.

Wolińska, A., Kuźniar, A., & Gałązka, A. (2020). Biodiversity in the rhizosphere of selected winter wheat (*Triticum aestivum* L.) cultivars-genetic and catabolic fingerprinting. *Agronomy*, *10* (7), 953.

Xu, X., Thornton, P.E, & Post, W.M. (2013). A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. *Global Ecology and Biogeography*, *22*, 737-749.

Ye, L., Zhao, X., Bao, E., Li, J., Zou, Z., & Cao, K. (2020). Bio-organic fertilizer with reduced rates of chemical fertilization improves soil fertility and enhances tomato yield and quality. *Scientific Reports*, *10* (1), 1-11.

Zhao, J., Liu, J., Liang, H., Huang, J., Chen, Z., Nie, Y., Wang, C., & Wang, Y. (2018). Manipulation of the rhizosphere microbial community through application of a new bio-organic fertilizer improves watermelon quality and health. *PLoS One. 13* (2), e0192967

Zhu, Z., Bai, Y., Lv, M., Tian, G., Zhang, X., Li, L., & Ge, S. (2020). Soil fertility, microbial biomass, and microbial functional diversity responses to four years fertilization in an apple orchard in North China. *Horticultural Plant Journal, 6* (4), 223-230.

Received: June 9, 2022
Accepted: October 10, 2022

BIOGENOST RIZOSFERNOG SLOJA ZEMLJIŠTA RAZLIČITIH GENOTIPOVA PŠENICE POD UTICAJEM TRETMANA ĐUBRENJA

Svetlana M. Roljević Nikolić[1*], Željko K. Dolijanović[2], Dušan Đ. Kovačević[2], Snežana I. Oljača[2] i Helena J. Majstorović[1]

[1]Istraživačko-razvojni institut Tamiš,
Novoseljanski put 33, Pančevo, Srbija
[2]Univerzitet u Beogradu, Poljoprivredni fakultet,
Nemanjina 6, Beograd, Srbija

R e z i m e

Rizosfera je dinamična sredina na čiju biogenost utiču brojni činioci, među kojima i snabdevenost zemljišta hranljivim materijama i biljka domaćin. Cilj ovog rada bio je da utvrdi brojnost osnovnih grupa mikroorganizama u rizosferi četiri podvrste, odnosno sorte pšenice, u okviru tri tretmana đubrenja u sistemu organske proizvodnje. Poljski ogled je realizovan po metodi potpuno slučajnog blok sistema u četiri ponavljanja, na zemljištu tipa izluženi černozem. Uočena je značajna varijabilnost brojnosti proučavanih fizioloških grupa mikroorganizama između podvrsta, odnosno sorti pšenice, kao i tretmana đubrenja. U rizosferi sorte obične pšenice pronađen je najveći broj mikoriznih gljiva (24,37 x 10^3 g^{-1}). Zemljište uzorkovano u zoni rizosfere kompaktum pšenice odlikovalo se najvećim brojem oligonitrofilnih bakterija (361,47 x 10^5 g^{-1}) i amonifikatora (119,27 x 10^5 g^{-1}). Nije bilo značajnih razlika u brojnosti aktinomiceta između sorti obične, kompaktum i tvrde pšenice, ali je njihov najmanji broj utvrđen kod sorte krupnika (11,25 x 10^3 g^{-1}). Na tretmanu kombinovane primene biofertilizatora i organskog đubriva, utvrđen je značajno veći broj gljiva (28,2%), amonifikatora (56,6%) i oligonitrofilnih bakterija (14,6%) u poređenju sa kontrolom. Dobijeni rezultati ukazuju na to da genotip domaćina, uz primenu odgovarajućih formulacija đubriva, može uticati na brojnost mikroorganizama, što je naročito važno u uslovima organske proizvodnje, koja se u potpunosti oslanja na prirodne resurse i procese.

Ključne reči: pšenica, organska proizvodnja, biohumus, biofertilizator, gljive, aktinomicete, amonifikatori, oligonitrofilne bakterije.

Primljeno: 9. juna 2022.
Odobreno: 10. oktobra 2022.

[*]Autor za kontakt: e-mail: roljevic@institut-tamis.rs

Journal of Agricultural Sciences (Belgrade)
Vol. 67, No. 4, 2022
Pages 381-393

https://doi.org/10.2298/JAS2204381O
UDC: 636.4.087.2:663.918.3
Original scientific paper

HAEMATOLOGY AND SERUM BIOCHEMISTRY OF PIGS FED GROWER FEED FORTIFIED WITH COCOA (*THEOBROMA CACAO*) SEED TESTA

Akinduro V. Olabisi[1*], Asaniyan E. Kehinde[2], Osunkeye O. Jacob[1], Fakolade P. Olusola[1] and Adeosun J. Mojijolajesu[1]

[1]Department of Animal Science, College of Agriculture, Osun State University, Osogbo, Osun State, Nigeria
[2]Department of Animal Production and Health, Ondo State University of Science and Technology, Okitipupa, Ondo State, Nigeria

Abstract: The on-farm attempt to cut down feed costs through bulk fortification of standard animal feed with available cheap conventional feed ingredients or wastes had been a normal practice without a known empirical impact on livestock health and wellbeing. Therefore, this study determined the haematological parameters and serum biochemistry of pigs fed cocoa (*Theobroma cacao*) seed testa at varying inclusion levels in the formulated standard grower pig feed; T_1:0% CST, T_2:25% CST, T_3:50% CST, T_4:75% CST and T_5:100% CST. Thirty (30) 8-week-old pigs were randomly assigned to the five treatments of six pigs each and replicated thrice, with two pigs per replicate in a completely randomised design (CRD). The experiment lasted for 10 weeks. At the end of the feeding trial, blood samples were collected randomly from three pigs per treatment through the jugular vein using hypodermic needle and syringe for haematological analysis and serum biochemistry. This study recorded significant differences ($P<0.05$) in most of the haematological parameters, except for white blood cell (WBC), platelet and heterophils; apart from high-density lipoprotein, all serum biochemical indices were also significantly different ($P<0.05$). Haematological parameters and serum indices were within the normal range for the healthy pig, except for lymphocytes. However, based on the results of the haematological indices and the serum biochemical parameters, it could be concluded that cocoa seed testa at the 25% inclusion level in the standard grower pig feed had the optimum support for the wellbeing and healthy performance of pigs.

Key words: blood parameters, serum indices, pig health, pig diets, cocoa seed testa.

[*]Corresponding author: e-mail: victor.akinduro@uniosun.edu.ng

Introduction

Feed constitutes about 75–80% of the production cost of pigs (Tewe, 1997). This is presently evident from the high cost of conventional feedstuffs like soybean meal, groundnut cake and fishmeal (Amaefule et al., 2019). This is consequent upon the fact that both legume and cereal grains that are available are keenly competed for by man for either direct consumption and/or for industrial uses (Emiola et al., 2011). Hence, the high cost of such ingredients results in a level that their ideal usage in feed formulation for livestock becomes almost uneconomical. Keeping costs within reasonable margins demands alternative uses of non-conventional feed ingredients. This resulted in searching for alternative feed stuffs of almost zero competition with human nutritional needs. However, the on-farm attempt to cut down feed costs through bulk fortification of standard animal feed with available cheap conventional feed ingredients or wastes had been a normal practice without a known empirical impact on livestock health and wellbeing.

There are several reports on both energy and protein-based under-utilised feed resources for feed ingredients in pig and poultry nutrition. These reports include tigernut (Ukpabi et al., 2015), palm oil sludge (Esonu et al., 2006), wild variegated cocoyam (Agbabiaka et al., 2006). Additionally, Makinde et al. (2019) reported that cocoa and its by-products show great potential as an alternative feed resource that can replace conventional feed ingredients in animal nutrition. Most agro and industrial by-products are now being used with little or no processing in feeding livestock. Cocoa by-products show great potential as an alternative feed resource that can partially replace conventional feed ingredients used in animal nutrition (Olayinka et al., 2019). Cocoa bean testa/shell is the seed coat covering the cocoa cotyledon, and it constitutes nearly 10% of the bean weight, resulting in problems of disposal for the cocoa processing factories in Nigeria. Moreover, Magistrelli et al. (2016) noted that the pig stands above other monogastrics in the use of cocoa testa in their nutrition; with a positive effect on the balance of the intestinal microbial ecosystem, subsequently reducing intestinal inflammatory diseases. The presence of theobromine, an anti-nutrient, in cocoa bean shells brought a lot of setbacks to cocoa bean testa utilisation in livestock feeding. However, theobromine remediation strategies like physicochemical treatments such as the boiling of cocoa bean testa/shell or hydrotropic extraction have been proposed (Makinde et al., 2019). Oduro-Mensah et al. (2018) also proposed fungi fermentation treatments for cocoa bean testa/shell. Caffeine and theobromine are purine alkaloids widely consumed as stimulants and snacks in coffee and cocoa-based foods and drug ingredients (Emiola et al., 2011).

A recent body of knowledge on these two alkaloids, however, is centered on their potential reproductive toxicities. Evidential in the prominent effects of increasing concentrations of dietary theobromine were anorexia, decreases in body

weight of mature rats, growth retardation in immature rats and atrophy of the thymus glands in rats of both sexes and testicular atrophy in male rats as reported by Emiola et al. (2011). However, the level of the inherent negative impact of the alkaloids in the cocoa bean testa/shell can be established through haematological indices of the animal fed the diet containing cocoa bean testa/shell. The opportunity derived from blood examination during investigations revealed the presence of some metabolites in addition to other constituents in the body of animals as it plays a vibrant role in the physiological, nutritional and pathological status of an organism (Nse Abasi et al., 2014). Findings by Olafedehan et al. (2010) have revealed that important information for the diagnosis and prognosis of diseases is embedded in the blood composition of animals. Haematological studies are useful in the diagnosis of many diseases as well as the investigation of the extent of damage to blood (Emiola et al., 2011). A haematological study is of ecological and physiological interest in helping to understand the relationship of blood characteristics to the environment (feed/feeding inclusive) and so could be useful in the choice of animals that are genetically resistant to a certain form of diseases and environmental conditions as revealed by Isaac et al. (2013). Amusa et al. (2015) have made us understand that haematological components are very important in monitoring feed toxicity, particularly of feed constituents that have direct effects on blood formation. Therefore, the study was designed and structured to determine the effects of graded levels of cocoa (*Theobroma cacao*) seed testa/shell incorporated into pig grower feed on the haematological indices and serum biochemistry of pigs.

Material and Methods

Experimental site

The experiment was conducted at the Piggery Unit of the University Teaching and Research Farm, College of Agriculture, Osun State University, Osogbo, Ejigbo Campus, Ejigbo, Osun State. The site is located on latitude 7º54N and longitude 4º18E at an altitude 426 m above sea level. Ejigbo is strategically located in the middle of the region, 35 km north-east of Iwo, 30 km from Ogbomoso in the north and 24 km from Ede in the south-east. It is about 40 km north-west of Osogbo, the capital of Osun State and about 95 km north-east of Ibadan. The mean annual rainfall in Ejigbo is 52.35mm and there were variations from the mean value from year to year (Ejigbo - Wikipedia, 2020).

Experimental materials

Sun-dried cocoa seed testa was sourced from a reputable Cocoa Processing Industry in Akure, Ondo State, Nigeria. The proximate composition of the cocoa

seed testa was as reported by Rojo-Poveda et al. (2020) in Table 1. The formulated grower pig feed is presented in Table 2.

Table 1. The proximate composition of cocoa seed testa.

Composition	Value (g/100g)
Protein	10.30–27.40
Crude fat/oil	1.50–8.49
Ash	5.96–11.42
Moisture	3.60–13.13
Crude fibre	39.25–66.33
Carbohydrate	7.85–70.25
Energy	122.0 kcal/100g

Source: Rojo-Poveda et al. (2020).

Table 2. The gross composition of the standard grower pig feed.

Ingredients	Composition (%)
Maize	42.23
Rice bran	14.97
Wheat bran	14.97
Fish meal	3.28
Soya bean meal	19.97
Groundnut cake	2.43
Bone meal	1.58
Salt	0.32
Premix	0.25
Total	100
Calculated analysis	
Metabolisable energy (Kcal/kg)	3435
Crude protein (%)	18

Experimental diet

The five experimental diets as shown in Table 3 comprised the 100% formulated standard grower pig feed with 0% cocoa seed testa (T_1), a mixture of the 75% standard grower pig feed and 25% cocoa seed testa (T_2), a mixture of the 50% standard grower pig feed and 50% cocoa seed testa (T_3), a mixture of the 25% standard grower pig feed diet and 75% cocoa seed testa (T_4) and 100% cocoa seed testa with the 0% standard grower pig feed (T_5). The formulated standard grower pig feed and the bulk mixtures of the cocoa seed testa and the grower pig feed were milled at the University Teaching and Research Feed Mill, Ejigbo Campus, College of Agriculture, Osun State University, Osogbo, Ejigbo. The experiment lasted for ten weeks. The five experimental diets (treatments) were assigned to the

pigs (experimental units) in a completely randomised design experiment. The facilities allowed for continuous access to water and feed by the pigs ad-libitum.

Table 3. Experimental diets.

Diets	Composition (%)	
	Standard grower pig feed	Cocoa seed testa
T_1	100	0
T_2	75	25
T_3	50	50
T_4	25	75
T_5	0	100

Experimental design, duration and management

A total of thirty (30) 8-week-old weaner pigs were divided into five groups of six pigs per treatment and housed in an individual pen with intensive and conventional managements. Each pen (containing a group of the animals) was assigned to each of the five dietary treatments in a completely randomised design (CRD) to give five treatments with six replicates each. Feed and water were supplied ad-libitum. Before the arrival, the pen was cleaned and disinfected. Routine management operations as applicable to the study area were carried out in the course of the experiment. The experiment lasted for 10 weeks.

Experimental sample collection and preparation

At the termination of the experiment, feed was withdrawn for about 15 hours prior to blood collection; three pigs were selected randomly from each treatment. Samples of blood were drawn from each pig through the jugular vein using a hypodermic needle and a syringe. Ten mm of blood samples were taken early in the morning aseptically through the jugular vein from each animal.

Each blood sample (4ml) meant for the determination of haematological parameters was kept inside an anticoagulant sample bottle containing ethylenediaminetetracetic acid (EDTA) and was gently shaken to prevent coagulation while the sera (6ml) were collected in non-EDTA bottles and preserved in an ice-pack for further transfer to the laboratory, where they were analysed to determine the haematological indices and serum biochemistry parameters using standard laboratory procedures.

Data analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) using the SAS software (SAS version 9:1, 2008). If significant differences were found, then the means were separated using the Duncan's Multiple Range Test (DMRT) of the same statistical package.

Results and Discussion

Haematological indices of pigs fed cocoa seed testa fortified diets

Results of the haematological parameters as presented in Table 4 revealed that six out of nine haematological variables investigated showed a level of significant difference (P>0.05). They include: packed cell volume (PCV), haemoglobin concentration (HBC), red blood cell (RBC), lymphocytes, monocytes and basophils. There were no significant (P>0.05) differences in white blood cell counts (WBC), blood platelet (thrombocytes) and heterophil values. Treatments T1 (0%) (38.00± 0.58%), T2 (25%) (39.50± 0.87%) and T4 (75%) (37.50± 1.44%) were similar in PCV values with T3 (50%) (26.00± 0.58%) and T5 (100%) (31.50± 0.87%) being significantly (P<0.05) different from one another. However, T3 (50%) and T5 (100%) were significantly different from treatments T1 (0%), T2 (25%) and T4 (75%). PCV recorded the highest value in T2 (25%), closely followed by T4 (75%) and then T1 (0%); similarities were seen in the three treatments.

Table 4. The effect of cocoa seed testa (CST) at various inclusion percentages on haematological indices of weaner pigs.

Parameters	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)
PCV (%)	38.00±0.58^{a}	39.50±0.87^{a}	26.00±0.58^{c}	37.50±1.44 ^{a}	31.50^{b}±0.87
HBC (g/dl)	11.30±0.40^{bc}	12.65±0.38 ^{a}	8.90±0.23^{d}	11.05±0.32^{c}	12.15^{ab}±0.26
WBC (x10^{3}/µl)	5200.00±202.07	5700.00±317.54	4725.00±245.37	5175.00±129.90	5375.00±534.05
RBC (x10^{6}/mm^{3})	5.15±0.25^{b}	6.38±0.10^{a}	4.07±0.60^{c}	5.18±0.60^{b}	4.87±0.09^{b}
Platelet (x10^{3}/µl)	76500.00±2020.73	78500.00±5484.83	7300.00±1732.05	80750.00±1299.04	81000.00±3752.78
Lymphocytes (%)	59.50±1.44^{bc}	65.00±1.15^{a}	57.50±0.87^{c}	62.50±1.44^{ab}	62.50±0.87^{ab}
Heterophils (%)	31.00±0.58	32.00±1.73	35.00±1.73	34.50±1.44	35.50±1.44
Monocytes (%)	1.50±0.29^{ab}	1.00±0.00^{b}	2.00±0.00^{a}	1.00±0.00^{b}	1.50±0.29^{ab}
Basophils (%)	0.50±0.29^{b}	2.00±0.58^{a}	0.50±0.29^{b}	0.50±0.29^{b}	1.50±0.29^{ab}

^{abc}means in the same row with different superscripts are significantly different (P<0.05). PCV – packed cell volume, HBC – haemoglobin concentration, RBC – red blood cell, WBC – white blood cell.

The significant difference recorded for haemoglobin concentration (HBC) among the treatments followed no definite trend. However, similarities were noticed across the treatments for HBC except for T3 (50%) (8.90±0.23g/dl). Treatment T2 (25%) (12.65±0.38g/dl) with the highest HBC value had a similar value with T5 (100%) (12.15±0.26g/dl) but significantly different (P<0.05) from others. T3 (50%) had the least HBC value (8.90±0.23g/dl) and was significantly

different from all the treatments. T2 (25%) had the highest RBC value (6.38±0.10 $x10^6/mm^3$) and was significantly ($P<0.05$) different from the other treatments. Treatments T1 (0%) (5.15±0.25 $x10^6/mm^3$), T4 (75%) (5.18±0.60 $x10^6/mm^3$) and T5 (100%) (4.87±0.09 $x10^6/mm^3$) which were similar ($P>0.05$) regarding RBC values were significantly ($P<0.05$) different from T3 (50%) (4.07±0.60 $x10^6/mm^3$). Treatment T2 (25%) had the highest lymphocyte value (65.00±1.15%) and was significantly ($P<0.05$) different from treatments T1 (0%) (59.50±1.44%) and T3 (50%) (with the least value, 57.50±0.87%) but similar to treatments T4 (75%) and T5(100%), which were equally similar to treatment T1 (0%), which had a similar value with treatment T3 (50%). T3 (50%) (2.00±0.00%) had the highest monocyte value and significantly ($P<0.05$) different from T2 (25%) (1.00±0.00%) and T4 (75%) (1.00±0.00%). Treatment T2 (25%) (2.00±0.58%) had the highest basophil value, which was significantly ($P<0.05$) different from treatments T1 (0%) (0.50±0.29%), and T3 (50%) (0.50±0.29%).

The effects of the diets on the haematological parameters are good indicators of the physiological and health status of the animals as reported by Etim et al. (2013). The higher the percentage of PCV, RBC and HB, the better the haematological profile of the animal (Akinduro, 2016). T2 (25%) (39.50± 0.87%) had the highest PCV, which made it the most preferred among the treatments, though other treatments met the standard percentage required according to Eze et al. (2010). Serum indices are always a reflection of animal responsiveness to their internal and external environment (Akinduro, 2016). Haematological components are those parameters that are related to the blood and blood-forming organs. Blood acts as a pathological reflector of the status of exposed animals to toxicants and other conditions (Olafedehan et al., 2010).

The significant difference indicated different effects of the treatments (percentage levels of cocoa testa in the standard grower pig feed) on the haematological parameters (Olumide et al., 2017). However, all the haematological indices except lymphocytes had their values within the normal range for healthy pigs (Research Animal Resources [RAR], 2009; Etim et al., 2013). Consequently, the non-significant effects of the treatments on WBC, blood platelet (thrombocytes) and heterophil values reflected their sustained physiological contributions in the wellbeing of the pigs.

The slightly higher values recorded for lymphocyte above the normal range in pigs (40–60%) as reported by Etim et al. (2013) showed that the fed diets i.e., T2 (25%), T4 (75%) and T5 (100%) (65.00±1.15%, 62.50±1.44% and 62.50±0.87%) respectively, showed that the pigs could be susceptible to lympho-proliferative neoplasm, notably occurring in viral and bacterial infections, often seen in infectious mononucleosis (Epstein-Barr virus) and whooping cough (*Bordetella pertussis*) (Mania et al., 2018), as a result of higher values for lymphocyte above the normal range. These results contradict that of John et al. (2020) stating that

lymphocyte counts were not significantly affected by the use of a related by-product (cocoa placenta meal [CPM]) supplemented with exogenous enzyme complex. Packed cell volume is involved in the transport of oxygen and absorbed nutrients, whose increased value results in an increased primary and secondary polycythemia (Isaac et al., 2013). However, no symptoms of polycythemia were found in the pigs since their PCV values fell within the normal range.

The pigs under treatment T2 (25% cocoa seed testa) significantly had the highest value similar to those observed in treatments T1 (0% cocoa seed testa) and T4 (75% cocoa seed testa). Similarly, the haemoglobin concentration (HBC) value of the pigs under T2 (25% cocoa seed testa) had the highest value of 12.65±0.35g/dl within the normal range of HBC for pigs (Research Animal Resources [RAR], 2009; Etim et al., 2013). These tend to show that 25% cocoa seed testa in the standard pig grower feed enhanced the best transport of oxygen, nutrient absorption and carbon dioxide exchange in the animal. Also, the RBC values for all the treatments were within the normal range of 4.5–6.3(x 10^6/mm^3) reported by RAR (2009) for healthy pigs. Hence, indicating that the feed was of high quality in terms of protein content, the level of digestibility and tolerable level of anti-nutrients, the optimum carriage of oxygen to the tissues as well as the level of carbon dioxide returned to the lungs would have been realised (Isaac et al., 2013). White blood cell (WBC) and platelet (thrombocytes) counts were not significantly ($P>0.05$) affected by the treatments. Animals in T1 (0%, control), T2 (25%) and T5 (100%) maintained the normal WBC range, which corroborated a report by Brockus et al. (2005) 5.2–17.9 (x10^3/µl), while T3 (50%) and T4 (75%) fell below the normal range.

However, this result contradicted that of RAR (2009) range of 7–20 (x10^3/µl). Nonetheless, the inclusion of cocoa seed testa above 25% suggests a negative effect on the blood. This is similar to a report made by Ogunsipe et al. (2017), which showed that 20% was the optimum biological level of cocoa shells when used as an energy substitute for maize in a pig diet. Most importantly, animals fed 50% and 75% cocoa seed testa indicated that they would have a less defensive mechanism against any infection as a result of low WBC. However, a high value of WBC has been associated with the toxicity of diets or the poor detoxification process which led to the increased production of WBC to fight foreign substances in the body. However, a low value suggests susceptibility to infection (Nwakolor, 2001). Moreover, this might be the reason for disease condition (central nervous system depression, restlessness, diarrhea recorded in the treatments). Cocoa seed testa has been used in laying birds by Olumide et al. (2017) and found to be useful in the replacement of maize up to 10% in the diet of commercial laying birds without any harmful effects on the egg quality indices, performance and haematology. This tends to create the possibility of specifically replacing either energy or protein-based feed ingredients with cocoa seed testa in pig diets. Protein

and energy have been found to be two major components of feed that are determinants of the performance and productivity of farm animals (Amaefule et al., 2019).

Serum biochemistry of pigs fed diets fortified with cocoa seed testa

The effect of cocoa seed testa on serum biochemistry was evident as all the tested blood indices measured for serum analysis such as alanine transaminase (ALT), aspartate transaminase (AST), cholesterol, alkaline phosphatase, triglyceride, low-density lipoprotein, globulin, total protein and albumin showed significant ($P<0.05$) differences except for high-density lipoprotein. The ALT content increased across the treatments, which means the significant changes could have resulted from the inclusion of cocoa seed testa in the diets of the animals, with T5 (100%) (containing the highest inclusion, being the highest inclusion level (6.71±0.01 U/L), followed by T4 (75%) (5.44±0.34 U/L), T3 (50%) (3.97±0.03 U/L) and T1 (0%) (2.00±0.23 U/L). However, T2 (25%) (1.77±0.74 U/L) had the lowest value. Treatment T2 (25%) (45.47±3.05 U/L) had the highest AST value, which made it significantly ($P<0.05$) different from other treatments. The similarity was noticed between T4 (75%) (29.77±2.77 U/L) and T5 (100%) (32.34±4.33 U/L) in the AST content of the serum, unlike T1 (0%), T2 (25%) and T3 (50%); T3 was similar to treatments T4 (75%) and T5 (100%). T1 (0%) had the least AST value. Cholesterol was significantly ($P<0.05$) different across the treatments. Treatments T2 (25%) and T3 (50%) had the highest and the lowest cholesterol values, respectively. Treatment T4 (75%) was next to the highest, followed by T1 (0%) and T5 (100%). ALP was the highest in treatment T1 (0%), followed by T5 (100%) and T2 (25%). Treatments T3 (50%) and T4 (75%) had similar values. Treatments T2 (25%) (101.82±0.03mg/dl) and T1 (0%) (72.73±1.05mg/dl) had the highest and lowest values of triglyceride, respectively. Similarities were noticed between T3 (50%) and T4 (75%) and between T3 (50%) and T5 (100%). Regarding low-density lipoprotein, the similarity was observed between T1 (0%) and T5 (100%) (with the lowest value, 9.14±1.54 mg/dl), and T3 (50%) recorded the highest value of 30.44±0.79mg/dl.

Equally, there were significant differences ($P<0.05$) among the treatments for globulin. Namely, T4 (75%) (2.85±0.23 mg/dl) had the highest value, which was significantly ($P<0.05$) different from T2 (25%) (1.85±0.17g/dl), and T3 (50%) (2.05±0.29g/dl). T5 (100%) (1.49±0.21g/dl) had the lowest value, but similar to T1 (0%) (2.35±0.02g/dl). Also, treatments T2 (25%) and T3 (50%) were similar in values, with both having shared similarities with treatments T1 (0%) and T5 (100%). Treatment T1 (0%) (6.36±0.21g/dl) significantly ($P<0.05$) had the highest total protein value similar to treatment T4 (75%) (6.29±0.02g/dl) but different from treatments T2 (25%) (5.04±0.24g/dl), T3 (50%)(4.83±0.29g/dl) and T5 (100%) (The lowest value, 4.81±0.27g/dl) that were similar in values. The albumin was

significantly (P<0.05) different among the treatments with treatment T1 (0%) having the highest value (4.01±0.01g/dl, significantly (P<0.05) different from other treatments. Treatments T4 (75%) (3.44±0.01g/dl) and T5 (100%) (3.32±0.06g/dl) had similar values that were significantly (P<0.05) different from treatments T2 (25%) (3.19±0.07g/dl) and T3 (50%) (the lowest value, 2.78±0.00g/dl) that were as well different significantly (P<0.05) in values.

Table 5. The effect of the varying dietary inclusion of cocoa seed testa (CST) at various percentages levels on the serum biochemistry of pigs.

Parameters	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)
Alanine transaminase ALT (U/L)	2.00±0.23^{c}	1.77±0.74^{c}	3.97±0.03^{b}	5.47±0.85^{ab}	6.71±0.01^{a}
Aspartate transaminase (AST) (U/L)	25.32±0.59^{c}	45.47±3.05^{a}	35.61±0.03^{b}	29.77±2.77^{bc}	32.34±4.33^{bc}
Cholesterol (mg/dl)	105.10±2.55^{c}	135.71±0.20^{a}	61.91±0.79^{e}	111.33±2.61^{b}	73.47±1.57^{d}
Alkaline phosphatise (U/L)	69.90±1.35^{a}	16.72±0.39^{c}	5.19±0.48^{d}	5.44±0.34^{d}	22.66±1.31^{b}
Triglyceride (mg/dl)	72.73±1.05^{d}	101.82±0.03^{a}	88.64±1.31^{bc}	85.00±1.31^{c}	90.91±2.10^{b}
High-density lipoprotein (mg/dl)	31.41±3.55	32.29±0.11	29.75±2.15	27.44±0.07	27.50±2.55
Low-density lipoprotein (mg/dl)	11.15±0.62^{d}	15.04±1.25^{c}	30.44±0.79^{a}	19.20±1.09^{b}	9.14±1.54^{d}
Globulin (g/dl)	2.35±0.02^{ab}	1.85±0.17^{bc}	2.05±0.29^{bc}	2.85±0.23^{a}	1.49±0.21^{c}
Total protein (g/dl)	6.36±0.02^{a}	5.04±0.24^{b}	4.83±0.29^{b}	6.29±0.21^{a}	4.81±0.27^{b}
Albumin (g/dl)	4.01±0.01^{a}	3.19±0.07^{c}	2.78±0.00^{d}	3.44±0.01^{b}	3.32±0.06^{b}

^{abcde}means in the same row with different superscripts are significantly different (P<0.05).

Data obtained for total protein and albumin indicated that the control diet had the highest value. The low values of total protein and albumin under the different treatments containing the cocoa seed testa were also noticed by Olumide et al. (2017), and Olorode et al. (1996) confirmed that the significant reduction in the total protein of birds fed 10% and 15% shea butter cake was an indication of poor protein utilisation. This is most likely associated with the theobromine content in cocoa seed testa, suggesting that theobromine remediation strategies as proposed by Makinde et al. (2019) and Oduro-Mensah et al. (2018) can be employed to boost the quality of cocoa seed testa in realising better utilisation. Aspartate transaminase (AST) for all the treatments was found to be within the normal range reported by RAR (2009). Hence, the animals were free from myocardial infarction and skeletal muscle disorders and they had liver and other organs functioning well. Moreover, the pigs under experimental diets containing levels of cocoa seed testa have improved triglyceride when compared with the control diet. The value recorded for

triglyceride falls within the normal range as reported by RAR (2009) which is equally an added advantage to the test ingredients used and at the same time falls within the normal range reported by RAR (2009).

Conclusion

Based on the analysed data, it could be concluded that the haematological indices and serum biochemistry parameters recorded supported the general health and wellbeing of the pigs fed cocoa seed testa-based diets without compromise. Hence, the 25% inclusion of CST in the standard grower pig feed had the optimum support for the wellbeing and healthy performance of the pigs.

References

Agbabiaka, L.A., Odoemenam, S.A., & Esonu, B.O. (2006). Preliminary investigation on the potentials of wild variegated cocoyam (*Caladium hortulanum*) replacement for maize in diets of catfish (*Heterobranchus bidorsalis*) fingelings. *International of Agriculture and Rural Development, 7* (1), 138-142.

Akinduro, V.O. (2016). Phytochemical constituents of selected spices in Nigerian Pepper soup and their effects on quality characteristics of broiler chickens. PhD thesis: Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria.

Amefule, R.A., Daniel-Igwe, G., Amaefule, K.U., & Oguike, M.A. (2019). Relative organ weights of four-week old broilers fed low protein diets supplemented with multi-enzyme in humid tropics. *Journal of Veterinary Medicine*, *9* (2), 14-20.

Amusa, H.O., Kehinde, R.A., Atoyebi, R.O., & Abu, O.A. (2015). Haematology, serum biochemistry, organ weight changes of Wistar rats fed processed dehulled jack bean (*Canavalia ensiformis*). *Nigerian Journal of Animal Production, 42* (1), 71-78.

Brockus, C.W., Mahaffey, E.A, Bush, S.E., & Krupp Despain, W. (2005). Haematologic and serum biochemical reference intervals for Vietnamese potbellied pigs (*Sus scrofa*). *Complete Clinical Patholology, 13* (4), 162-165.

Ejigbo - Wikipedia (2020). http://en.m.wikipedia.org/wiki/Ejigbo.

Emiola, I.A., Ojebiyi, O., & Akande, T.O. (2011). Performance and organ weights of laying hens fed diets containing graded levels of sun-dried cocoa bean shell (CBS). *International Journal of Poultry Science*, *10*, 987-990.

Esonu, B.O., Ogbonna, U.D., Anyanwu, G.A., Emenalom, O.O., Uchegbu Etuk, E.B., & Udedibie, A. B.I. (2006). Evaluation of performance, organ characteristics and economic analysis of broiler fed dried rumen digester. *International Journal of Poultry Science, 5* (2), 1116-1118.

Etim, N.N., Enyenihi, G.E., Williams, M.E., Udo, M.D., & Offiong, E.E.A. (2013). Haematological parameters: Indicators of the physiological status of farm animals. *British Journal of Science*, *10*, 33-45.

Eze, J.I., Onunkwo, J.I., Shoyinka, S.V.O., Chah, F.K., Ngene, A.A., Okolinta, N., Nwanta, J.A., & Onyenwe, I.W. (2010). Haematological profiles of pigs raised under intensive management system in South-Eastern Nigeria. *Nigerian veterinary Journal, 31* (2), 115-123.

Isaac, L.J., Abah, G., Akpan, B., & Ekaette, I.U. (2013). Haematological properties of different breeds and sexes of rabbits (pp. 24-27). *Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria.* (ASAN) Abuja, Nigeria.

John, D.D., Michael, B., Kwame, O., Yaw, O.F., & Daniel, B.O. (2020). The response of pigs to diets containing varying levels of cocoa placenta meal (CPM) supplemented with an exogenous enzyme complex. *African Journal of Agricultural Research, 15* (4), 538-545.

Magistrelli, D., Zanchi, R., Malagutti, L., Galassi, G., Canzi, E., & Rosi, F. (2016). Effects of cocoa husk feeding on the composition of swine intestinal microbiota. *Journal of Agriculture and Food Chemistry*, *64*, 2046-2052.

Makinde, O.J., Okunade, S.A., Opoola, E., Sikiru, A.B., Ajide, S.O., & Elaigwu, S. (2019). Exploration of cocoa (*Theobroma cacao*) by-products as valuable potential resources in livestock feeds and feeding systems. *Theobroma Cacao-Deploying Science for Sustainability of Global Cocoa Economy.* (pp. 1-14). Pub: IntechOpen.

Mania, M., Alessandra, C., Enrica, C., & Tiziano, B. (2018). Lymphoproliferative disorders in Patients with chronic myeloproliferative neoplasms: A Systemic review. *American Journal of Hematology, 93* (5), 698-703.

Nse Abasi, N.E., Mary, E.W., Uduak, A., & Edem, E.A.O. (2014). Haematological Parameters and Factors Affecting Their Values. Agricultural Science *Published by Science and Education Centre of North America*, *2* (1), 37-47.

Nwakolor, I.M. (2001). Crossing breeding of large white and Nsukka local pigs: genotype and age are related changes in haematological parameters. *Tropical Journal of Animal Science, 4* (2), 85-96.

Oduro-Mensah, D., Ocloo, A., Lowor, S.T., Mingle, C., Okine, L.K.A., & Adamafio, N.A. (2018). Bio-detheobromination of cocoa pod husks: reduction of ochratoxin. A content without change in nutrient profile. *Microbiology Cell Factories,* 17, 79.

Ogunsipe, M.H., Balogun, K.B., Oladepo, A.D, Ayoola, M.A., & Arikewuyo, M.T. (2017). Nutritive value of cocoa bean shell meal and its effect on growth and haematology of weanling rabbits. *Nigerian Journal of Agriculture, Food and Environment*, *13* (1), 23-28.

Olafedehan, C.O., Obun, A.M., Yusuf, M.K., Adewumi, O.O., Olafedehan, A.O., Awofolaji, A.O., & Adeniji, A.A. (2010). Effects of residual cyanide in processed cassava peal meals on haematological and biochemical indices of growing rabbits. *Proceedings of 35th Annual Conference of Nigerian Society for Animal Production.* (pp. 212 -216). Ibadan, Nigeria.

Olayinka E. T., Ore, A., Adeyemo, O. A.,Ola, O. S. (2019). The role of flavonoid antioxidant, Morin in improving procarbazine induced oxidative stress on testicular function in rat. *Porto Biomedical Journal*, *4,* 1, (e28).

Olorode, B.R., Onifade, A.A., Okpara, A.O., & Babatunde, G.M. (1996). Growth, Nutrient Retention, Hematology and Serum chemistry of Broiler Chickens fed Shea butter cake or Palm Kernel Cake in the Humid Tropics. *Journal of Applied Animal Resources, 10,* 173-180.

Olumide, M.D., Hamzat, R.A., Bamijoko, O.J., & Akinsoyinu, A.O. (2017). Effects of Treated Cocoa (Theobroma cacao) Bean Shell Based Diets on Serum Biochemistry and Haematological Indices of Laying Hens. *International Journal of Livestock Research, 7* (1), 1-13

Research Animal Resource [RAR]. (2009). Reference values for laboratory animals: Normal haematological values. RAR Websites, RAR, University of Minnesota. Retrieved from http://www.ahc.umn.edu/rar/refvalues.html in August, 2022.

Rojo-Poveda O., Barbosa-Pereira L., Zeppa G., & Stevigny C. (2020). Cocoa Bean Shell – A By-Product with Nutritional Properties and Biofunctional Potential. *Nutrients* 2020, 12, 1123.

SAS (2008). Statistical Analysis System Institute Inc. *User's guide, Version 9.1 for window*. Cary, North Carolina.

Tewe, O.O. (1997). Sustainability and development paradigm from Nigeria's livestock industry. Inaugural lecture delivered on behalf of Faculty of Agriculture and Forestry, University of Ibadan.

Ukpabi, U.H., Salawa, L.A., Mbachu, C.L., & Oguike, B.C. (2015). Haematological responses of weaner pigs fed raw tigernut (*Cyperus esculentus*) based diets. *Proceeding of 20th Annual Conference of Animal Science Association of Nigeria.* (ASAN) (pp. 6-10). Abuja, Nigeria.

Received: August 19, 2021
Accepted: October 18, 2022

HEMATOLOGIJA I BIOHEMIJA SERUMA SVINJA HRANJENIH SMEŠOM ZA PORAST OBOGAĆENOM OPNOM SEMENA KAKAOA (*THEOBROMA CACAO*)

Akinduro V. Olabisi[1*], Asaniyan E. Kehinde[2], Osunkeye O. Jacob[1], Fakolade P. Olusola[1] i Adeosun J. Mojijolajesu[1]

[1]Odsek za stočarstvo, Poljoprivredni koledž, Državni univerzitet u Osunu, Osogbo, Država Osun, Nigerija
[2]Odsek za stočarstvo i zdravstvenu zaštitu životinja, Državni univerzitet za nauku i tehnologiju u Ondu, Okitipupa, Država Ondo, Nigerija

R e z i m e

Pokušaji da se u praktičnim uslovima smanje troškovi ishrane životinja, korišćenjem dostupnih jeftinijih konvencionalnih hraniva ili sporednih proizvoda, pored standardne hrane za životinje, predstavljaju široko zastupljenu praksu bez poznatog empirijskog uticaja na zdravlje i dobrobit životinja. Ovim istraživanjem su utvrđeni hematološki parametri i biohemija seruma prasadi koja su kao komponentu smeše za porast konzumirala opnu semena kakaoa (*Theobroma cacao*) pri različitim nivoima uključivanja: T1:0% CST (engl. *cocoa seed testa*), T2:25% CST, T3:50% CST, T4:75% CST i T5:100% CST. Trideset (30) osmonedeljnih prasadi nasumično je raspoređeno u pet tretmana od po šest prasadi (tri puta po dva praseta) po modelu slučajnog blok sistema. Eksperiment je trajao 10 nedelja. Na kraju hranidbenog ogleda, uzorci krvi su uzimani od po tri slučajno izabrana praseta, po tretmanu, iz jugularne vene, korišćenjem podkožne igle i siringe za hematološku i biohemijsku analizu seruma. Ovom studijom su utvrđene značajne razlike ($P<0,05$) u većini hematoloških parametara, osim leukocita, trombocita i heterofila; pored lipoproteina visoke gustine, svi biohemijski indikatori seruma su takođe bili značajno različiti ($P<0,05$). Hematološki parametri i parametri seruma bili su u normalnom opsegu za zdrave svinje, osim limfocita. Međutim, na osnovu rezultata hematoloških parametara i biohemijskih parametara seruma, može se zaključiti da uključivanje opne semena kakaoa na nivou od 25% u standardnu smešu za porast prasadi ima optimalan efekat na dobrobit i zdravlje svinja.

Ključne reči: parametri krvi, parametri seruma, zdravlje svinja, ishrana svinja, opna semena kakaoa.

Primljeno: 19. avgusta 2021.
Odobreno: 18. oktobra 2022.

[*]Autor za kontakt: e-mail: victor.akinduro@uniosun.edu.ng

Journal of Agricultural Sciences (Belgrade) https://doi.org/10.2298/JAS2204395H
Vol. 67, No. 4, 2022 UDC: 664.844:635.64
Pages 395-414 Original scientific paper

THE OPTIMISATION OF THE COLOUR ANALYSIS OF MICROWAVE-DRIED TOMATOES APPLYING THE TAGUCHI TECHNIQUE

Jelili B. Hussein[1*], Moruf O. Oke[2], James A. Adeyanju[2] and Oluseye O. Abiona[3]

[1]Department of Food Science and Technology, Modibbo Adama University, Yola, Adamawa State, Nigeria
[2]Department of Food Engineering, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria
[3]Department of Chemical Sciences, Osun State University, Osogbo, Osun State, Nigeria

Abstract: Food processors and consumers frequently worry about the inconsistent colour of dried tomatoes. To minimize detrimental color changes, process parameters need to be optimized. Thus, digital imaging with the Photoshop software and optimization with the Taguchi technique were explored to determine the surface color of microwave-dried tomato slices. The tomato sample was pretreated with water blanching (WB), ascorbic acid (AA) and sodium metabisulphite (SB). In addition, the sample was cut into 4-mm, 6-mm and 8-mm thicknesses and dried at 90W, 180W and 360W microwave power levels following the Taguchi experimental design. Color characteristics (*L*, a*, b*,* change in color, browning index, hue, and chroma) of the dried tomato slices were determined. The *L*, a*,* and *b** values of fresh tomatoes were 56.73, 44.51, and 38.38, respectively. The optimum processing conditions for the color characteristics varied significantly ($p< 0.05$). Pretreatment is the prime significant processing parameter controlling the *L*, b**, ΔE, BI, and hue values. At the same time, the slice thickness considerably influenced the *a** value, the ratio of *a*/b** and chroma values. The digital imaging color measurements of dried tomato slices provide a suitable method for non-destructive color analysis. The ability to upgrade and modify tomato processors so they can accommodate bulk color characteristics will be made possible by this knowledge.

Key words: color analysis, microwave drying, tomato slice, Adobe Photoshop, Taguchi technique.

[*]Corresponding author: e-mail: jbhussein01@mautech.edu.ng

Introduction

The colour of any material is the human perception of light waves as reflected from that material surface. It was considered a key role in food choice and preference acceptability. It could influence taste thresholds, sweetness perception and pleasantness (Kulanthaisami et al., 2010). Thus, it is a good quality indicator of fresh and dried tomato products that influences consumer acceptability. The colour change in tomatoes during the drying operation was reportedly caused by the reactions taking place inside it, such as pigment degradation (carotenoids) and browning reactions (Maillard reaction and oxidation of ascorbic acid) (Ashebir et al., 2009). Therefore, the extent of the effects of drying on tomato quality can be evaluated by the final values of colour parameters.

The determination of the colour of food materials can be done either subjectively by visual (human) inspection or objectively through colour measuring instruments. The subjective systems involve comparison with coloured references under controlled illumination. Colour standards are often used as reference materials and require more specialised trained observers. Unfortunately, it implies a slower inspection and varies from observer to observer (Afshari-Jouybari and Farahnaky, 2011). Based on these reasons, the objective method of determining colour through colour measuring instruments is preferred. The commonly used devices for food colour analysis are the Hunter lab colorimeter, Minolta colorimeter and Dr Lange colorimeter. However, these instruments can only measure small samples and are often unsuitable for large food products like fruits and vegetables (Tarafdar et al., 2018). Yam and Papadakis (2004) have reported that these colorimeter instruments are designed mainly for quality control, providing average measurement values. It would be difficult and time-consuming if used for point-by-point measurement at many locations to obtain colour distribution, thereby making them not well suited for food engineering research. Also, some of these colorimeter instruments require destructing the food samples before measurements; that is, the food samples need to be homogenised using a grinder or a blender to achieve uniform colour distributions. This grinding or blending takes time and renders the food sample no longer usable for other purposes.

Food sample colour parameters are usually measured in RGB (Red, Green and Blue) or Commission Internationale d'Eclairage (CIE) Lab colour space. The RGB colour space consists of a three-dimensional rectangular coordinate system with R, G and B axes (Figure 1a). These three components per pixel represent a colour image in RGB format with the range 0–255 and their intensity is electronically combined to produce a digital colour picture. Different proportions and intensities of RGB colours are used to create cyan, magenta, yellow, and white (Yam and Papadakis, 2004). The available hardware for colour image processing, such as

colour sensors, monitors and digital cameras, are geared to RGB colour space. The CIE, in 1976, developed the *L*, a*, b** model for colour measurement, which consists of a lightness or luminance component (*L** value, ranging from 0 to 100), along with two chromatic components (ranging from – 120 to +120) (Figure 1b). The *a** component changes from *–a* (greenness) to *+a* (redness), while the component *b** changes from *-b* (blueness) to *+b* (yellowness). The *L*, a*, b** colour is device independent, thereby giving coherent colour despite the input or output devices like a digital camera, a scanner, a monitor, and a printer (Yam and Papadakis, 2004). This characteristic gives it an advantage over other modes of colour measurement. The *L*, a*, b** values are often used in food research studies (Al-Sulaiman, 2011; Idris et al., 2013; Talih et al., 2017; Ilter et al., 2018; Komolafe et al., 2019).

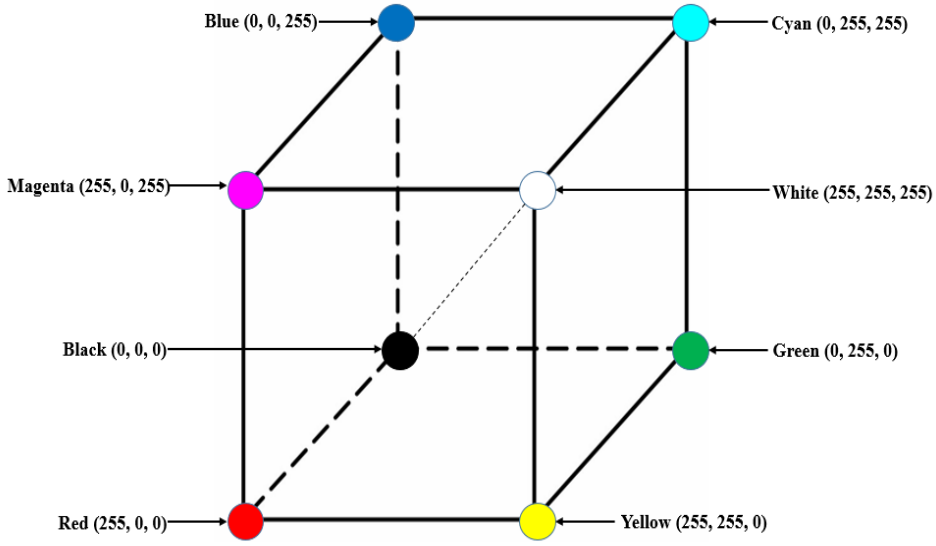

Figure 1a. The RGB colour model.

Nowadays, digital imaging, computer vision, and spatial image acquisition methods have been used in the food processing industries for quality evaluations, identifications, detection of defects, grading and sorting of fruits and vegetables and other prepared goods (Afshari-Jouybari and Farahnaky, 2011). However, most of these methods utilise sophisticated instruments, control systems, and algorithms that are not easily accessible or expensive and sometimes unavailable at the research level (Tarafdar et al., 2018). These have led to the search for a simple, low-cost and robust imaging technique capable of detecting colour values in food samples. Several researchers carried out studies to analyse the visual characteristics

of food samples with the use of a digital camera along with image analysis software like Adobe Photoshop and computer vision (Yam and Papadakis, 2004; Mendoza et al., 2006; Afshari-Jouybari and Farahnaky, 2011; Makino et al., 2016; Tarafdar et al., 2018). However, applying these widely used methods in image analysis to optimise complex food processes like microwave drying to produce good products with acceptable colour characteristics still needs to be explored.

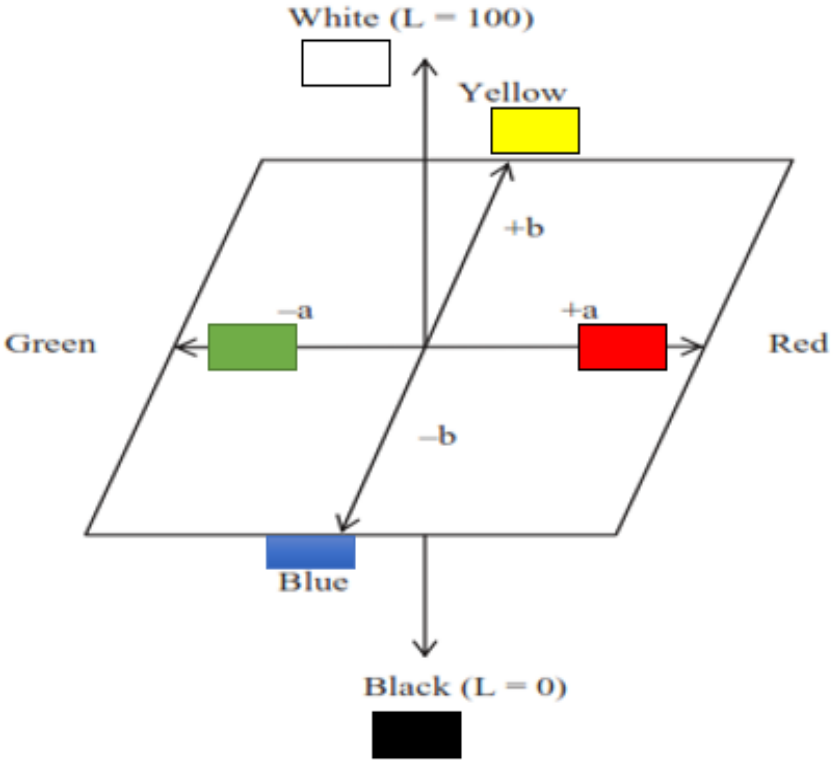

Figure 1b. The CIELAB L^* a^* b^* colour system.

Microwave drying works on the principle of electromagnetic energy, which travels in high-frequency waves that range between 300 GHz and 300 MHz. When a dielectric material is placed in an electromagnetic field, the material becomes polarised and stores electric energy through polarisation. The level and mechanism of polarisation available to materials depend on the state and composition of the material and the frequency of the applied electric field (Adu et al., 1995). The transformation of electromagnetic energy into kinetic molecular energy generates heat within the material, making heat migration to the core of the product and mass migration of water out of the material more accessible. This volumetric heating makes microwave drying perform more uniformly and faster than conventional

drying systems. Temperature gradients do not govern microwave drying, but the heat arises from the oscillation of molecular dipoles and the movement of ionic constituents, respectively, in response to alternating electric fields at high frequencies. The resulting energy is absorbed throughout the volume of the wet material. The rise in internal pressure drives out the moisture from the interior to the material surface (Sanga et al., 2000). Microwave drying is caused by dielectric heating alone, but most microwave drying joins microwave and conventional heating. The heating might be separate or simultaneous (Hussein et al., 2019).

Microwave drying offers several benefits, including developing desirable characteristics in dry products due to the volumetric heating, a high drying rate, and effective heat distribution throughout the material (Sanga et al., 2000). However, microwave drying induces changes in the physical and chemical compositions of the materials. These include the changes in colour, aroma, rehydration ratios and the loss of water-soluble vitamins (mainly ascorbic acid). Therefore, processing parameters are optimised to control the quality, productivity, and drying conditions to minimise detrimental quality changes during processing. Also, determining a good design requires using a strategically designed experiment that exposes the process to various levels of design parameters.

The experimental design technique of Genichi Taguchi that was explicitly devised to improve the quality of Japanese manufactured goods in the post-war period in conjunction with analysis of variance (ANOVA) has been highly successful (Karna and Sahai, 2012). The technique optimises any complex process like drying to produce high-quality dried products at subsequently low cost. The Taguchi technique uses a particular set of arrays known as orthogonal, which means not mixed or separated factors (Taguchi, 1990; Dash et al., 2016). These orthogonal arrays give a minimum number of experimental runs providing a representative of a whole variable. The signal-to-noise (SN) ratio in the Taguchi technique measures the effect of noise factors on performance characteristics and quantifies the variability. Several researchers have successfully applied the Taguchi technique to optimise some complex processes: optimisations of process factors in the production of ready-to-eat peanut chutney (Chandrasekar et al., 2015), frying of potato slices in the microwave (Naik et al., 2017), microwave drying of tomato slices (Hussein et al., 2019), milling quality of brown rice (Sanusi et al., 2020) and drying of tomato slices in the hot-air dryer (Hussein et al., 2020). Despite these applications of the Taguchi technique in optimisation, there needs to be more information available on applying the Taguchi technique in investigating the effect of processing parameters on the colour of microwave-dried tomato slices. Therefore, this study used the Taguchi technique to determine the optimum drying conditions to preserve the colour of microwave-dried tomato slices.

Material and Methods

The UTC varieties of tomatoes used in this study were obtained from the Teaching and Research Farm of the Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria. The appearance, firmness, and size uniformity served as the basis for selection from the lot.

Sample preparation

The ripeness and maturity tests of the tomatoes were determined as described by Choi et al. (1995). Colour measurement was performed on the surface of the tomatoes with the aid of a Minolta Chroma Meter CR-200 (Minolta Camera Co., Ltd., Osaka, Japan) tristimulus colour analyser, this was replicated four times. The readings were obtained in the CIE L^* a^* b^* colour space and the coordinates L^*, a^*, and b^* were determined with the D65 standard observer at a visual angle of 10°C. Tomatoes with similar coordinates were selected to use fruit with homogeneous ripeness. The maturity stage of the tomato was determined by checking the total soluble solid (% Brix) with an Abbe Refractometer (AOAC, 2016). A Kenwood blender (Philips HR 2001, China) was used to blend the fresh tomatoes. The resulting blended tomato was then centrifuged at 1500 rpm for 10 minutes, and the supernatant was used. The refractometer prism was rinsed, zeroed with distilled water, and wiped dry with cotton wool. Two drops of supernatant were placed on the refractometer prism and viewed through the eyepiece. The determination was replicated three times, and the average result was 6.67 ± 0.26 Brix. The titratable acidity was 0.33, and the maturity index (ratio of total soluble solid to titratable acidity) was 20.21.

The pretreatment of tomatoes

Tomatoes were thoroughly cleaned by washing under tap water. After that, they were rinsed with distilled water and wiped with a tissue towel, as described by Hussein et al. (2016). Twelve (12) kg of tomatoes were subjected to each water blanching (WB) for 1 minute, 5% w/v of ascorbic acid treatment (AA), and 5% w/v of sodium metabisulphite treatment (SM) for 5 minutes. The ratio of tomatoes to dipping solution – 1:10 (w/v), as used by Hussein et al. (2020), was adopted. After the pretreatment process, each portion was sliced to a thickness of 4, 6, and 8 mm, respectively, with the aid of a Tomato Slicer (NEMCO 56610-13/16" Roma).

The Taguchi experimental plan

The Taguchi experimental plan was designed with the Minitab 16 (Minitab, Inc. Coventry, UK) software for three factors at three levels, having an array of L9 (3x3). The outline that gave nine experimental runs was obtained and used to evaluate the responses of the colour changes with the interactions between pretreatment, slice thickness, and microwave power, as presented in Table 1.

The drying procedure

The pretreated samples were dried at three levels of microwave powers of 90, 180, and 360 W with thicknesses of 4, 6, and 8 mm according to the experimental runs in Table 1. The superficial fluid on the surface of the pretreated samples was blotted with a paper towel. A pretreated tomato slice of 200 g was uniformly spread in a single layer on a 30-cm diameter glass dish with about 1 cm depth and was placed at the centre of the microwave. The glass dish was rotated in the microwave chamber during operation to allow the even absorption of microwave energy by the drying samples. The microwave oven was operated for a 5-minute ON cycle and a 25-minute OFF cycle for the first hour and 1-minute ON and 5-minute OFF intervals for the subsequent drying times, as described by Hussein et al. (2019). The oven output power and processing time adjustment were made with a digital control facility in the microwave oven.

The sample was turned over at 15-minute intervals. The weight loss was taken at 5-minute intervals with the digital electronic scale (OEM, Freebang-SKU323367). Based on the preset microwave output power and schedule, the experiment was replicated three times, and the data gave the average results. The point when subsequent weight reduction was less than 0.001 g was taken as the final drying stage. After drying, the samples were packed and sealed in black polythene to prevent light and stored until further analyses.

Table 1. The outline of the Taguchi experimental design L_9 (3x3) for microwave oven drying.

Experimental runs	Independent variables in coded form			Experimental variables in their natural units		
	A	B	C	Pretreatment	Thickness (mm)	Microwave power (W)
1	1	1	1	WB	4	90
2	1	2	2	WB	6	180
3	1	3	3	WB	8	360
4	2	1	2	AA	4	180
5	2	2	3	AA	6	360
6	2	3	1	AA	8	90
7	3	1	3	SM	4	360
8	3	2	1	SM	6	90
9	3	3	2	SM	8	180

Colour measurements

The simple digital imaging method described by Yam and Papadakis (2004) and Al-Sulaiman (2011) was adopted. A high-resolution digital camera (Canon XUS105, 12.0 MegaPixel, 4 digital zooms) was used to snapshot the dried tomato sample images under two 40 W fluorescent lamps. The images were captured in a semi-controlled environment. The camera was staged perpendicular at a distance of 60 cm. At the same time, the dried samples were illuminated at 45^o above and 45^o

to the right or left of the viewing axis. They were rendered with and without cast shadows. The colour was then analysed quantitatively using Photoshop (Adobe-Systems, 2015). The histogram window of Photoshop was used to estimate the colour distributions along the x-axis and the y-axis. The histogram window displays the statistics (mean, standard deviation, median, percentage, etc.) of the colour value and lightness (L) of a selected area in the image of dried tomato samples. The statistics for the two other colour values (a and b) were also displayed by the histogram window by choosing a and b under the channel drop-down menu (Figure 2). The colour values for L, a, and b that were determined from the histogram window are not standard colour values. Thus, they were converted to the standard colour values (L^*, a^*, and b^*) using the formulas 1, 2, and 3 (Yam and Papadakis, 2004). The lightness is the L^*, the redness is the a^*, while the yellowness is the b^*.

$$L^* = \frac{lightness}{255} \times 100 \quad (1)$$

$$a^* = \frac{240a}{255} - 120 \quad (2)$$

$$b^* = \frac{240b}{255} - 120 \quad (3)$$

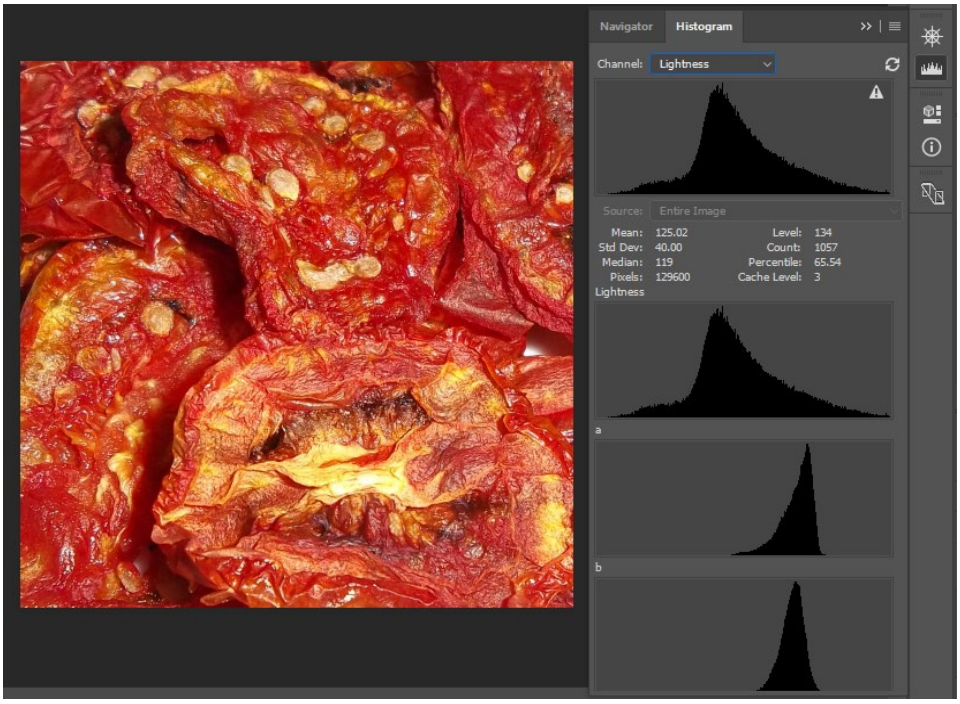

Figure 2. The histogram window displays for the statistics of the microwave-dried tomatoes.

The determination of *a*/b**, chroma (C) and hue angle (α)

The ratio of a^* to b^*, which is an indicator of tomato ripeness, was determined as described by Al-Sulaiman (2011). The chroma and hue angle were also estimated and used to describe the tomato colour changes after drying (Dadali et al., 2007; Karaaslan and Tuncer, 2008).

$$Ratio\ of\ a^* to\ b^* = \frac{a^*}{b^*} \tag{4}$$

$$C = \sqrt{(a^*)^2 + (b^*)^2} \tag{5}$$

$$\alpha = tan^{-1}(\frac{a^*}{b^*}) \tag{6}$$

The determination of the total colour change (ΔE)

The fresh tomato was used as the ideal sample, while the total colour change (ΔE) was estimated as described by Dadali et al. (2007):

$$\Delta E = [(L^* - L^{**})^2 + (a^* - a^{**})^2 + (b^* - b^{**})^2]^{0.5} \tag{7}$$

where;

ΔE = the total colour change of the dried tomato slices;

L^* and L^{**} = the lightness of fresh and dried tomato samples;

a^* and a^{**} = the redness of fresh and dried tomato samples;

b^* and b^{**} = the yellowness of fresh and dried tomato samples.

The determination of the browning index (BI)

The browning index (BI) gave the brown colour purity and was estimated as described by Dadali et al. (2007).

$$BI = \frac{[100(x-0.31)]}{0.17} \tag{8}$$

where; $x = \frac{(a^{**}+1.75L^{**})}{(5.645L^{**}+a^{*}-3.012b^{**})}$

The Taguchi orthogonal array optimisation technique

The Taguchi method suggests the production processes have minor variability as the optimal condition. The variability is expressed by the signal-to-noise (S/N) ratio. The S/N ratio represents quality characteristics for the observed data in the Taguchi technique. The S/N ratio serves as an index to estimate the quality of the production processes. The 'signal' stands for the desired value, the 'noise' stands for the undesirable value, and the signal-to-noise ratio represents the scatter around the expected values (Hussein et al., 2019). The smaller, the better S/N ratio was used to optimise data obtained for BI and ΔE, while the larger, the better S/N ratio was adopted for *L*, a*, b*, a*/b**, chroma, and hue by using equations 9 and 10, respectively.

The smaller, the better S/N ratio property

$$S/N = -10log\frac{1}{n}(\sum y^2) \quad (9)$$

The larger, the better S/N ratio property

$$S/N = -10log\frac{1}{n}(\sum \frac{1}{y^2}) \quad (10)$$

where: n = the number of experimental runs, y = the response data.

The average mean of the response and the S/N ratio for each level of the factors were calculated by taking the average of the mean values of the response of the treatments. The optimal level of the processing parameters (pretreatment, slice thickness and microwave power) and the adequate processing combinations within the experimental realm were identified.

Results and Discussion

The optimisation of the effect of the drying conditions on the colour of the microwave-dried tomato slice

The colour of the fresh tomato was determined, and the average values of L^*, a^*, and b^* were 56.73, 44.51 and 38.38, respectively. The photograph of the pretreated microwave-dried tomatoes is depicted in Figure 3. The changes in the colour of the pretreated microwave dried tomato slices are shown in Tables 2 a and 2 b. It is shown that the brightness (L^*) ranged from 31.06 to 48.83. It was observed that the dried slices were slightly darker (L^* decreased) when compared to fresh tomatoes. The brightness of the dried tomato slices decreased at each drying microwave power examined. This trend shows that the luminance of the tomato slices reduced after drying. Similar decreases in the brightness of dried tomato slices were reported by Izli and Isik (2015). The higher the S/N ratio, the better it was chosen to optimise the L^* value. The largest S/N ratio (33.77) was acquired for the WB pretreated and 4-mm thick slice at 90 W microwave powers. In comparison, the smallest S/N ratio (29.85) was acquired for the AA pretreated and 6-mm-thick slice at 360 W microwave power. This showed that the pretreatment with WB and the 4-mm-thick tomato slice at 90 W microwave power were the prime processing combination in the experimental realm.

The effect of each processing factor on the dried tomato brightness was established by calculating the desired factor levels by the S/N ratio from the Taguchi analysis. The response means of the S/N ratio for each level of the controlling factors are shown in Tables 3a and 3b. The response means of the S/N ratio show that the pretreatment used had the largest delta value; thus, the 1st rank was allocated to it, followed by the thickness (the 2nd rank) and microwave power (the 3rd rank). This shows that pretreatment was the prime significant processing parameter controlling the L^* of the tomato slice. At the same time, the

thickness of the slice and the microwave power followed, respectively. The redness (a^*) and yellowness (b^*) slightly decreased when compared to fresh tomatoes (Table 2a). Namely, there was a slight degradation of the pigments of microwave-dried tomatoes. This little pigment degradation was consistent with the report by Qadri and Srivastava (2014) for microwave oven drying of tomatoes. Izli and Isik (2015) also reported pigment degradation in the microwave, convective, and microwave-convective oven drying of tomatoes. The decrease in the pigment of dried tomatoes compared to fresh ones could be linked to the reactions between the reducing sugars and amino acids in the tomato during drying (Abano et al., 2011).

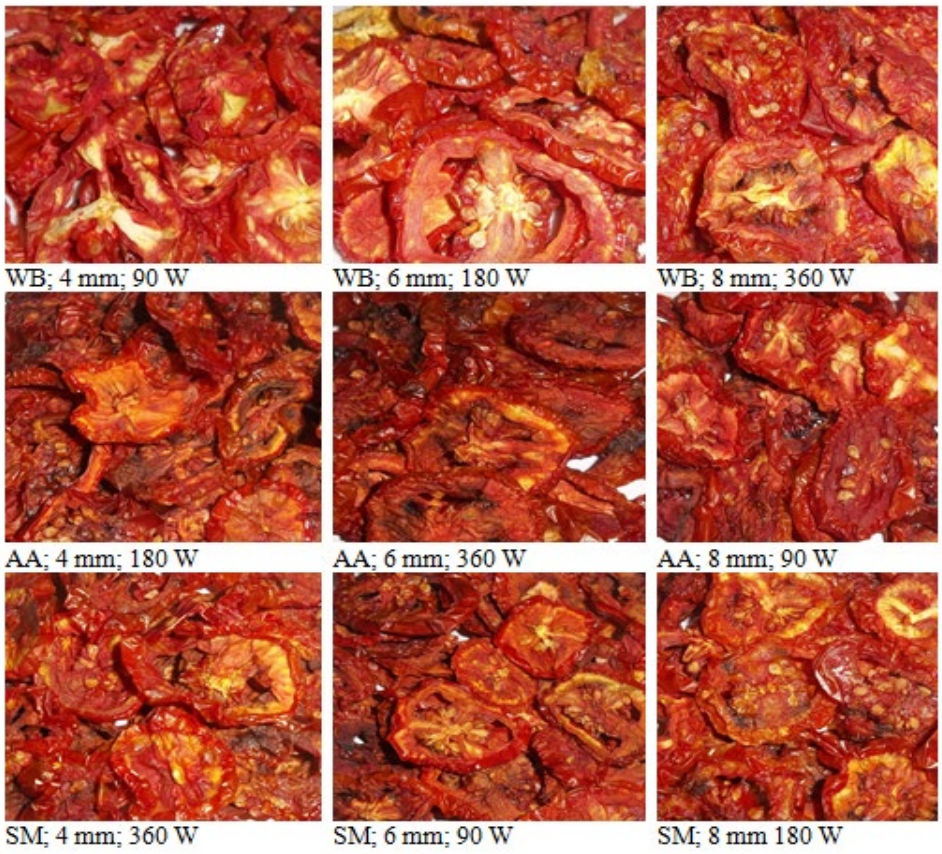

Figure 3. Microwave-dried tomatoes.

The larger the S/N ratio, the better was chosen to optimise the a^* and b^* values of the dried tomatoes. The largest S/N ratio (32.98 for a^* and 32.12 for b^*) was obtained for the WB pretreated and 4-mm-thick slice at 90 W microwave

power. This showed that the pretreatment with WB and the 4-mm-thick tomato slice at 90 W microwave power were the prime processing combination to preserve the red and yellow pigments of the tomato. The response means of the S/N ratio for each level of the controlling factors in *a** and *b** are shown in Table 2a. Regarding *a**, the response means of the S/N ratio indicate that the thickness of the slice had the largest delta value; thus, the 1st rank was allocated to it, followed by pretreatment (the 2nd rank) and microwave power (the 3rd rank). This indicates that the slice thickness was the prime significant processing parameter controlling the *a** of the tomato slice, while pretreatment and the microwave power used followed, respectively. Regarding *b**, the response means of the S/N ratio indicate that pretreatment had the largest delta value; thus, the 1st rank was allocated to it, followed by the thickness of the slice (the 2nd rank) and microwave power (the 3rd rank). This indicates that pretreatment was the prime significant processing parameter controlling the *b** of the tomato slice. In addition, the thickness of the slice and the microwave power used followed, respectively.

Table 2a. The effect of drying conditions on the colour parameters of pretreated microwave-dried tomatoes using the Taguchi technique.

Experimental runs	*L**	S/N *L**	*a**	S/N *a**	*b**	S/N *b**	*a*/b**	S/N *a*/b**
Fresh	56.73 ± 3.08^{a}		44.51 ± 3.14^{a}		38.38 ± 2.00^{ab}		1.16 ± 0.02^{ab}	
WB; 4 mm; 90 W	48.83 ± 1.66^{b}	33.77	44.56 ± 0.94^{a}	32.98	40.34 ± 1.06^{a}	32.12	1.11 ± 0.01^{bc}	0.91
WB; 6 mm; 180 W	48.65 ± 0.34^{b}	33.74	41.23 ± 0.47^{ab}	32.30	38.67 ± 0.23^{ab}	31.75	1.07 ± 0.01^{c}	0.59
WB; 8 mm; 360 W	47.77 ± 1.62^{bc}	33.58	42.02 ± 0.37^{ab}	32.47	38.43 ± 0.82^{ab}	31.69	1.09 ± 0.01^{bc}	0.75
AA; 4 mm; 180 W	41.62 ± 0.38^{bcd}	32.39	40.47 ± 0.27^{abc}	32.14	37.86 ± 0.42^{ab}	31.56	1.07 ± 0.02^{c}	0.59
AA; 6 mm; 360 W	38.91 ± 1.45^{d}	29.85	36.81 ± 0.48^{c}	31.32	31.03 ± 0.98^{c}	29.84	1.19 ± 0.02^{a}	1.51
AA; 8 mm; 90 W	41.53 ± 2.30^{bcd}	32.37	42.14 ± 1.07^{ab}	32.49	35.38 ± 1.34^{b}	30.98	1.19 ± 0.02^{a}	1.51
SM; 4 mm; 360 W	42.08 ± 2.25^{bcd}	32.48	41.60 ± 0.88^{ab}	32.38	38.69 ± 1.82^{ab}	31.75	1.08 ± 0.03^{c}	0.67
SM; 6 mm; 90 W	40.97 ± 3.72^{bc}	32.25	39.79 ± 0.80^{bc}	32.00	37.56 ± 2.06^{ab}	31.50	1.06 ± 0.04^{c}	0.51
SM; 8 mm; 180 W	36.70 ± 3.42^{d}	31.29	38.56 ± 1.52^{bc}	31.72	35.46 ± 1.98^{b}	30.99	1.09 ± 0.02^{c}	0.75

Mean values in the same columns bearing the same superscript are not significantly different ($p<0.05$).

The ratio of *a*/b** of tomatoes indicates the end product redness. It is used as an index for colour and it is positively correlated with the lycopene content (Zanoni et al., 1999; Idris et al., 2013). Idris et al. (2013) reported that the ratio of *a*/b** value of more than 1 indicates an excellent red colour, while lower values denote immaturity or deterioration in colour. Table 2a shows that the value of *a*/b** decreased significantly ($p>0.05$) in both WB and SM pretreated tomatoes compared to the fresh sample. However, an increase in the value of *a*/b** was observed for AA pretreated tomatoes and it was slightly higher than the fresh sample. A decrease in the *a*/b** value after air-drying of tomato was reported by

Kerkhofs et al. (2005). However, an increase in the value of *a*/b** was reported by Ashebir et al. (2009).

The larger the S/N ratio, the better was chosen to optimise the value of *a*/b** of the dried tomatoes. The largest S/N ratio was acquired for the AA pretreated and 6-mm thick slice at 360 W microwave powers and the AA pretreated and 8-mm thick slice at 90 W microwave powers. These were the prime processing combinations to preserve the brightness of the tomato. The response means of the S/N ratio for each level of the controlling factors in *a*/b** (Table 2a) show that the pretreatment had the largest delta value; thus, the 1st rank was allocated to it, followed by microwave power used (the 2nd rank) and slice thickness (the 3rd rank). This indicates that the pretreatment used was the prime significant processing parameter controlling the brightness of the dried tomato slice. The microwave power used and the slice thickness followed, respectively.

The overall colour change (ΔE) was calculated to find the difference between the dried tomato sample colour and the fresh ones. ΔE was significantly higher at higher microwave power and slice thickness than at lower microwave power and slice thickness (Table 2b). This implies that an increase in microwave power and slice thickness increases the degradation rate faster, resulting from high electromagnetic energy absorption by the tomatoes. An increase in ΔE value was observed for the AA pretreated (15.67 to 27.80) followed by the SM pretreated (15.24 to 21.24) and the WB pretreated (8.43 to 9.38) tomatoes. This showed that more pronounced changes were observed in the colour of AA treated samples than in SM and WB treated samples. Gnanasekharan et al. (1992) reported that the colour of tomato changes could be linked to the gradual changes in the three coordinates (lower '**L*' and '**b*'; less negative '**a*'). Talih et al. (2017) have also reported that a dried black carrot pulp brightness value decreases with increasing microwave powers and decreasing the sample thickness. The degradation of the pigments or browning reactions may be related to pigment destruction and may cause colour loss (**L*).

The smaller the S/N ratio, the better was chosen to optimise the ΔE value (Table 2b). The largest S/N ratio (-18.52) was acquired for the WB pretreated and 4-mm-thick slice at 90 W microwave power. In comparison, the smallest S/N ratio (-28.88) was acquired for the AA pretreated and 6-mm-thick slice at 360 W microwave power. This indicates that the pretreatment with the WB and 4-mm thick tomato slice at 90 W microwave power were the prime processing combination. The response means of the S/N ratio for each level of the controlling factors in ΔE (Table 2b) show that the pretreatment of the slice had the largest delta value; thus, the 1st rank was allocated to it, followed by thickness of the slice (the 2nd rank) and microwave power used (the 3rd rank). This suggests that the pretreatment used was the most critical processing factor influencing the overall

colour change of the dried tomato slice. The microwave power used and the thickness of the slices followed, respectively.

Table 2b. The effect of drying conditions on the colour parameters of pretreated microwave-dried tomatoes using the Taguchi technique.

Experimental runs	ΔE	S/N ΔE	BI	S/N BI	*C*	S/N *C*	hue(α)	S/N hue(α)
Fresh	-		-		58.77 ± 3.68^{a}		0.86 ± 0.01^{ab}	
WB; 4 mm; 90 W	8.43 ± 1.52^{d}	-18.52	32.01 ± 3.76^{ab}	-30.11	60.11 ± 1.40^{a}	35.58	0.84 ± 0.01^{bc}	-1.51
WB; 6 mm; 180 W	8.74 ± 0.47^{d}	-18.83	39.82 ± 0.81^{a}	-32.00	56.51 ± 0.48^{ab}	35.05	0.82 ± 0.01^{c}	-1.72
WB; 8 mm; 360 W	9.38 ± 1.66^{cd}	-19.44	36.26 ± 2.87^{a}	-31.19	56.94 ± 0.82^{ab}	35.11	0.83 ± 0.01^{bc}	-1.62
AA; 4 mm; 180 W	$15.67 \pm 0.31b^{cd}$	-23.90	21.50 ± 1.40^{bcd}	-26.65	55.42 ± 0.26^{ab}	34.87	0.82 ± 0.01^{c}	-1.72
AA; 6 mm; 360 W	20.77 ± 1.76^{a}	-28.88	31.56 ± 2.57^{ab}	-29.98	48.15 ± 1.01^{c}	33.65	0.87 ± 0.01^{a}	-1.21
AA; 8 mm; 90 W	15.76 ± 2.61^{bcd}	-23.95	22.36 ± 3.70^{bc}	-26.99	55.02 ± 1.68^{ab}	34.81	0.87 ± 0.01^{a}	-1.21
SM; 4 mm; 360 W	15.24 ± 2.15^{bcd}	-23.66	19.50 ± 2.5^{c}	-25.80	56.82 ± 1.89^{ab}	35.09	0.82 ± 0.01^{c}	-1.72
SM; 6 mm; 90 W	16.75 ± 3.76^{bc}	-24.48	20.30 ± 7.16^{bc}	-26.15	54.74 ± 1.99^{ab}	34.76	0.82 ± 0.02^{c}	-1.72
SM; 8 mm; 180 W	21.24 ± 3.85^{ab}	-26.55	11.78 ± 5.56^{c}	-21.42	52.39 ± 2.45^{bc}	34.39	0.83 ± 0.01^{c}	-1.62

Mean values in the same columns bearing the same superscript are not significantly different ($p<0.05$).

The browning index (BI) was evaluated as the extent of browning in the dried tomatoes. Cernisev (2009) has reported that the browning reaction significantly causes tomato quality degradation during the drying process. The BI ranged from 11.78 to 39.82, with experiment 2 (WB; 6 mm; 180 W) having the highest value while experiment 8 (SM; 6 mm; 90 W) had the lowest value. These results (Table 2b) clearly show that the processing conditions significantly affected tomato browning reactions. The rate of colour formation increased as the intensity of the drying process increased. This observation corroborated the BI of microwave-dried black carrot pulp, as Talih et al. (2017) reported. It was observed that the pretreatment used reduced the BI considerably, and the BI reduced as the slice thickness and the drying time reduced.

The smaller the S/N ratio, the better was chosen to optimise the BI value (Table 2b). The largest S/N ratio (-21.42) was acquired for the SM pretreated and 6-mm thick slice at 90 W microwave power. In comparison, the smallest S/N ratio (-32.00) was acquired for the WB pretreated and the 6-mm thick slice at 180 W microwave power. This indicates that the pretreatment with SM and 6-mm tomato thick slice at 90 W microwave power were the prime processing combination. The response means of the S/N ratio for each level of the controlling factors in BI (Table 3b) show that the pretreatment of the slice had the largest delta value; thus, the 1st rank was allocated to it, followed by thickness of the slice (the 2nd rank) and microwave power used (the 3rd rank). This indicates that the slice pretreatment was the prime significant processing parameter controlling the browning change in the

dried tomato slice. The thickness of the slice and the microwave power used followed, respectively.

Table 3a. The response means of the signal to noise (S/N) ratio for colour parameters of microwave oven drying.

	Pretreatment	Thickness	Microwave power
*L** (using larger the better)			
Level 1	33.70	32.88	32.80
Level 2	31.18	32.60	32.47
Level 3	32.01	32.41	32.62
Delta	1.69	0.47	0.32
Rank	1st	2nd	3rd
*a** (using larger the better)			
Level 1	32.58	32.50	32.49
Level 2	31.99	31.87	32.06
Level 3	32.03	32.23	32.06
Delta	0.60	0.63	0.43
Rank	2nd	1st	3rd
*b** (using larger the better)			
Level 1	31.85	31.81	31.53
Level 2	30.79	31.03	31.44
Level 3	31.41	31.22	31.09
Delta	1.06	0.78	0.43
Rank	1st	2nd	3rd
a/b** (using larger the better)			
Level 1	0.75	0.72	0.98
Level 2	1.20	0.87	0.64
Level 3	0.64	1.00	0.98
Delta	0.56	0.28	0.34
Rank	1st	3rd	2nd

Key: Level 1 = Water blanched, 4-mm thickness and 90 W microwave power; Level 2 = Ascorbic acid, 6-mm thickness and 180 W microwave power; Level 3 = Sodium metabisulphite, 8-mm thickness and 360 W microwave power.

Chroma (*C*) values (Table 2b) were observed to decrease and closely followed the same reduction pattern with *b** values. The *C* value indicates the level of colour saturation and is proportional to the strength of the colour. Slight changes were observed in the *C* values of WB pretreated samples compared with the fresh tomato. However, they differed significantly ($p<0.05$) from each other. This indicates the stability of the red colour of the dried pretreated tomato. Similar observations were reported by Barreiro et al. (1997). The larger the S/N ratio, the better was chosen to optimise the *C* value (Table 2b). The largest S/N ratio (35.58) was acquired for the WB pretreated and 4-mm thick slice at 90 W microwave powers.

In comparison, the smallest S/N ratio (33.65) was acquired for the AA pretreated and 6-mm thick slice at 360 W microwave power. This indicates that the pretreatment with WB and the 4-mm thick tomato slice at 90 W microwave power were the prime processing combination. Ilter et al. (2018) reported that the *C* value increased slightly with microwave power. The higher the *C* value, the higher the colour intensity of the dried tomato perceived by the consumers (Pathare et al., 2013). The response means of the S/N ratio for each level of the controlling factors in *C* (Table 3b) show that the pretreatment had the largest delta value; thus, the 1st rank was allocated to it followed by the thickness of the slice (the 2nd rank) and microwave power used (the 3rd rank). This indicates that the pretreatment used was the prime significant processing parameter controlling the chroma value of the dried tomato slice. The thickness of the slice and the microwave power followed, respectively.

Table 3b. The response means of the signal to noise (S/N) ratio for colour parameters of microwave oven drying.

	Pretreatment	Thickness	Microwave power
ΔE (using smaller the better)			
Level 1	-18.93	-22.03	-22.32
Level 2	-24.73	-23.22	-23.09
Level 3	-24.89	-23.31	-23.15
Delta	5.96	1.29	0.83
Rank	1st	2nd	3rd
BI (using smaller the better)			
Level 1	-31.10	-27.52	-27.75
Level 2	-27.87	-29.38	-26.69
Level 3	-24.46	-26.53	-28.99
Delta	6.64	2.84	2.30
Rank	1st	2nd	3rd
C (using larger the better)			
Level 1	35.24	35.18	35.05
Level 2	34.45	34.49	34.77
Level 3	34.75	34.77	34.62
Delta	0.80	0.69	0.44
Rank	1st	2nd	3rd
α (using larger the better)			
Level 1	-1.62	-1.65	-1.48
Level 2	-1.38	-1.55	-1.69
Level 3	-1.69	-1.48	-1.52
Delta	0.31	0.17	0.21
Rank	1st	3rd	2nd

Key: Level 1 = Water blanched, 4-mm thickness and 90 W microwave power; Level 2 = Ascorbic acid, 6-mm thickness and 180 W microwave power; Level 3 = Sodium metabisulphite, 8-mm thickness and 360 W microwave power.

The hue angle (α) (Table 2b) was slightly higher in the dried tomato than in the fresh samples. This indicates that less browning occurred in the dried samples. Hawlader et al. (2006) have reported that a decrease in the α value shows increased brown pigment formation and moving away from yellowness. It was observed that the changes in α values were not significant ($p>0.05$) compared to the drying processes. These results corroborated the previous studies by Maskan (2001) for microwave-dried kiwifruits, Ozkan et al. (2007) for microwave-dried spinach leaves and Arslan and Ozcan (2010) for microwave-dried onion. The larger the S/N ratio, the better was chosen to optimise the α value (Table 2b). The larger the S/N ratio, the better was chosen to optimise the α value (Table 2b). The largest S/N ratio (-1.21) was acquired for the AA pretreated and 6-mm thick slice at 360 W microwave power and the AA pretreated and 8-mm thick slice at 90 W microwave power. These were the prime processing combinations influencing the microwave-dried tomato hue value. The response means of the S/N ratio for each level of the controlling factors in α (Table 3b) show that the pretreatment of the slice had the largest delta value; thus, the 1st rank was allocated to it, followed by the microwave power used (the 2nd rank) and the thickness of the slice (the 3rd rank). This indicates that the slice pretreatment was the prime significant processing parameter controlling the hue value of the dried tomato slice. The microwave power used and the thickness of the slice followed, respectively.

Conclusion

The use of the Adobe Photoshop software for image analysis, followed by the optimisation of the processing conditions applying the Taguchi technique, was experimented. The results of the digital imaging colour measurements of dried tomato slices offer an adequate means of non-destructive colour analyses. The Taguchi technique provided optimum processing variables that produce the smallest change in the colour characteristics of microwave-dried tomato samples compared with fresh samples.

The pretreatment used was the prime significant processing parameter controlling the L^*, b^*, ΔE, BI, and hue values. At the same time, the slice thickness considerably influenced the a^*, ratio of a^*/b^* and chroma values. These results showed that the Adobe Photoshop software for image analysis could be an alternative to sophisticated colour measurement instruments. This information will benefit tomato processors, which can be improved upon and adapted for bulk colour characteristics.

Acknowledgments

The authors acknowledge the financial support from the Modibbo Adama University of Technology Yola through the TETFund PhD Study Fellowship Grant to undertake this research.

References

Abano, E.E., Ma, H., & Qu, W. (2011). Influence of air temperature on the drying kinetics and quality of tomato slices. *Journal of Food Process and Technology, 2* (123), 1-9. Adobe-Systems (2015). *Adobe Photoshop 7.0. User Guide*. Retrieved from San Jose CA.

Adu, B., Otten, L., Afenya, E., & Groenevelt, P. (1995). Thermodynamics of Microwave (Polarized) System. *Journal of Microwave Power and Electromagnetic Energy, 30*, 90-96.

Afshari-Jouybari, H., & Farahnaky, A. (2011). Evaluation of Photoshop Software Potential for Food Colorimetry. *Journal of Food Engineering, 106* (2), 170-175.

Al-Sulaiman, M.A. (2011). Prediction of Quality Indices During Drying of Okra Pods in a Domestic Microwave Oven using Artificial Neural Network Model. *African Journal of Agricultural Research, 6* (12), 2680-2691.

AOAC (2016). *Official Method of Analysis of AOAC International* (20th ed.): Association of Official Analytical Chemist.

Arslan, D., & Ozcan, M.M. (2010). Study the Effect of Sun, Oven and Microwave Drying on Quality of Onion Slices. *LWT – Food Science and Technology, 43*, 1121-1127.

Ashebir, D., Jezik, K., Weingartemann, H., & Gretzmacher, R. (2009). Change in Color and Other Fruit Quality Characteristics of Tomato Cultivars after Hot-Air Drying at Low final-Moisture Content. *International Journal of Food Sciences and Nutrition, 60* (S7), 308-315.

Barreiro, J., Milano, M., & Sandoval, A. (1997). Kinetics of Colour Change of Double Concentrated Tomato Paste during Thermal Treatment. *Journal of Food Engineering, 33*, 359-371.

Cernisev, S. (2009). Effects of Conventional and Multistage Drying Processing on Non-Enzymatic Browning in Tomato. *Journal of Food Engineering, 96*, 114-118.

Chandrasekar, V., Kannan, K., Priyavarshini, R., & Gayathri, R. (2015). Application of Taguchi Method in Optimization of Process Factors of Ready to Eat Peanut (*Arachis hypogaea*) Chutney. *International Food Research Journal, 22* (2), 510-516.

Choi, K., Lee, G., Han, Y., & Bunn, J. (1995). Tomato Maturity Evaluation using Color Image Analysis. *American Society of Agricultural and Biological Engineers, 38* (1), 171-176.

Dadali, G., Apar, D.K., & Ozbek, B. (2007). Color Change Kinetics of Okra Undergoing Microwave Drying. *Drying Technology, 25*, 925-936.

Dash, R.N., Mohammed, H., & Humaira, T. (2016). An integrated Taguchi and Response Surface Methodological Approach for the Optimization of an HPLC Method to Determine Glimepiride in a Supersaturatable Self-nanoemulsifying Formulation. *Saudi Pharmaceutical Journal, 24*(1), 92-103.

Gnanasekharan, V., Shewfelt, R.L., & Chinnan, M.S. (1992). Detection of Color Changes in Green Vegetables *Journal of Food Science, 57* (1), 149-155.

Hawlader, M.N.A., Perera, C.O., & Tian, M. (2006). Properties of Modified Atmosphere Heat Pump Dried Foods. *Journal of Food Engineering, 74*, 392-401.

Hussein, J.B., Filli, K.B., & Oke, M.O. (2016). Thin Layer Modelling of Hybrid, Solar and Open Sun Drying of Tomato Slices. *Research Journal of Food Science and Nutrition, 1*, 15-27.

Hussein, J.B., Oke, M.O., Abiona, O.O., & Adebayo, Q. (2020). Optimization of Processing Parameters for Drying of Tomatoes (*Solanum lycopersicum* L.var) Slices using Taguchi Technique. *Journal of Food Processing and Preservation, e15149*.

Hussein, J.B., Oke, M.O., Adeyanju, J.A., & Sanusi, M.S. (2019). Optimisation of Microwave Drying of Tomatoes (Solanum lycopersicum L.) Slices Using Taguchi Method. *Nigerian Food Journal, 37* (2), 57-71.

Idris, Y.M.A., Ibrahim, Y.A., & Mariod, A.A. (2013). Color of Dehydrated Tomato: Effects of Gum Arabic. *International Journal of Food Properties, 16*, 838-851.

Ilter, I., Akyil, S., Devseren, E., Okut, D., Koç, M., & Ertekin, F.K. (2018). Microwave and Hot Air Drying of Garlic Puree: Drying Kinetics and Quality Characteristics. *Heat and Mass Transfer*, 1-12.

Izli, N., & Isik, E. (2015). Color and Microstructure Properties of Tomatoes Dried by Microwave, Convective, and Microwave-Convective Methods. *International Journal of Food Properties, 18*, 241-249.

Karaaslan, S.N., & Tuncer, I.K. (2008). Development of A Drying Model for Combined Microwave-Fan-Assisted Convection Drying of Spinach. *Biosystems Engineering, 100*, 44-52.

Karna, S.K., & Sahai, R. (2012). An Overview on Taguchi Method. *International Journal of Engineering and Mathematical Sciences, 1* (1), 1-7.

Kerkhofs, N.S., Lister, C.E., & Savage, G.P. (2005). Change in Color and Antioxidant Content of Tomato Cultivars Following Forced-Air Drying *Plant Foods for Human Nutrition, 60*, 117-121.

Komolafe, O.M., Hussein, J.B., Adebayo, Q., Abiona, O.O., & Oke, M.O. (2019). Effect of Pretreatments on the Drying Characteristics and Quality of African Star Apple (*Chrysophyllum albidum*). *Agriculture and Food Sciences Research, 6* (1), 145-154.

Kulanthaisami, S., Rajkumar, P., Raghavan, G.S.V., Venkatachalam, P., Gariepy, Y., Subramanian, P., & Orsat, V. (2010). Drying Kinetics of Tomato Slices in Solar Cabinet Dryer Compared with Open Sun Drying. *Madras Agricultural Journal, 97* (7-9), 287-295.

Makino, Y., Goto, K., Oshita, S., Sato, A., & Tsukada, M. (2016). A Grading Method for Mangoes on the Basis of Peel Colour Measurement Using a Computer Vision System. *Agricultural Sciences, 7*, 327-334.

Maskan, M. (2001). Drying Shrinkage and Rehydration Characteristic af Kiwifruit During Hot Air and Microwave Drying. *Journal of Food Engineering, 48*, 177-182.

Mendoza, F., Dejmek, P., & Aguilera, J.M. (2006). Calibrated Colour Measurements of Agricultural Foods using Image Analysis. *Postharvest Biology and Technology, 41*, 285-295.

Naik, K.M., Bala, K.L., & Srinivas, D. (2017). Study on Optimisation of Microwave Frying of Potato Slices. *International Journal of Pure and Applied Bioscience, 5* (4), 1207-1218.

Ozkan, I.A., Akbudak, B., & Akbudak, N. (2007). Microwave Drying Characteristics of Spinach. *Journal of Food Engineering, 78* (2), 577-583.

Pathare, P.B., Opara, U.L., & Al-Said, F.A. (2013). Colour Measurement and Analysis in Fresh and Processed Foods: A Review. *Food and Bioprocess Technology, 6*, 36-60.

Qadri, O.S., & Srivastava, A.K. (2014). Effect of Microwave Power on Foam-Mat Drying of Tomato Pulp. *Agricultural Engineering International: CIGR Journal, 16* (3), 238-244.

Sanga, E., Mujumdar, A.S., & Raghavan, G.S.V. (2000). Principles and Applications of Microwave Drying. In A.S. Mujumdar (Ed.), *Drying Technology in Agricultural and Food Sciences* (pp. 283-289). Enfield, USA: Science Publishers.

Sanusi, M.S., Akinoso, R., Danbaba, N., & Sunmonu, M.O. (2020). Effect of Processing Parameters on the Milling Quality of Brown Rice using Taguchi Approach. *American Journal Food Technology, 15*, 62-68.

Taguchi, G. (1990). *Introduction to Quality Engineering*. Asian Productivity Organization, Tokyo.

Talih, M., Çaliskan, G., & Dirim, S.N. (2017). Determination of the Drying Characteristics of Black Carrot Pulp During Drying in a Microwave Oven. *Journal of Food Physics, 30*, 22-32.

Tarafdar, A., Shahi, N.C., & Singh, A. (2018). Color Assessment of Freeze-Dried Mushrooms using Photoshop and Optimization with Genetic Algorithm. *Journal of Food Process Engineering, e12920*.

Yam, K.L., & Papadakis, S.E. (2004). A Simple Digital Imaging Method for Measuring and Analyzing Colour of Food Surfaces. *Journal of Food Engineering, 61*, 137-142.

Zanoni, B., Peri, C., Nani, R., & Lavelli, V. (1999). Oxidative Heat Damage of Tomato Halves as Affected by Drying. *Food Research International, 31*, 395-401.

Received: August 1, 2021
Accepted: November 26, 2022

OPTIMIZACIJA ANALIZE BOJE PARADAJZA OSUŠENOG MIKROTALASIMA PRIMENOM TAGUČIJEVE TEHNIKE

Jelili B. Hussein[1*], Moruf O. Oke[2], James A. Adeyanju[2] i Oluseye O. Abiona[3]

[1]Odsek za nauku o hrani i prehrambenu tehnologiju, Univerzitet Modibo Adama, Jola, Država Adamava, Nigerija
[2]Odsek za prehrambeno inženjerstvo, Tehnološki univerzitet Ladoke Akintola, Ogbomoso, Država Ojo, Nigerija
[3]Odsek za hemijske nauke, Državni univerzitet u Osunu, Osogbo, Država Osun, Nigerija

R e z i m e

Prerađivači hrane i potrošači često brinu zbog nedosledne boje sušenog paradajza. Da bi se minimizirale štetne promene boje, parametri procesa trebalo bi da se optimiziraju. Tako je istražena digitalna slika pomoću softvera *Photoshop* i optimizacija Tagučijevom tehnikom da bi se odredila površinska boja kriški paradajza osušenih mikrotalasima. Uzorak paradajza je prethodno tretiran blanširanjem u vodi (VB), askorbinskom kiselinom (AK) i natrijum metabisulfitom (NB). Pored toga, uzorak je isečen na debljine od 4 mm, 6 mm i 8 mm i osušen mikrotalasnima snage 0W, 180W i 360W prema Tagučijevom eksperimentalnom dizajnu. Izmerene su karakteristike boje (*L**, *a**, *b**, promena boje, indeks posmeđivanja, nijansa i hroma) kriški sušenog paradajza. Vrednosti *L**, *a** i *b** svežeg paradajza bile su 56,73, 44,51 odnosno 38,38. Optimalni uslovi prerade za karakteristike boje značajno su varirali ($p<0,05$). Prethodni tretman je najvažniji parametar prerade koji kontroliše vrednosti *L**, *b**, ΔE, BI i nijanse. Istovremeno, debljina kriški je značajno uticala na vrednost *a**, odnos *a*/b** i vrednosti intenziteta hrome. Digitalna slikovna merenja boja kriški sušenog paradajza obezbeđuju odgovarajući metod za nedestruktivnu analizu boje. Ovim saznanjima daje se mogućnost unapređenja i modifikacije prerađivačima paradajza u cilju prilagođavanja boje.

Ključne reči: analiza boja, mikrotalasno sušenje, kriška paradajza, *Adobe Photoshop*, Tagučijeva tehnika.

Primljeno: 1. avgusta 2021.
Odobreno: 26. novembra 2022.

[*]Autor za kontakt: e-mail: jbhussein01@mautech.edu.ng

Journal of Agricultural Sciences (Belgrade) https://doi.org/10.2298/JAS2204415S
Vol. 67, No. 4, 2022 UDC: 637.52.041
Pages 415-431 Original scientific paper

CANNED MEAT PRODUCTS FOR MEMBERS OF THE SERBIAN ARMED FORCES: Na, K, Ca, AND Mg CONTENT AND HEALTH RISKS/BENEFITS

Branislav Đ. Stojanović[1], Zdenka M. Stojanović[2], Sonja S. Marjanović[2], Saša D. Janković[3], Mališa P. Antić[4], Milica R. Balaban[5] and Vesna V. Antić[4*]

[1]Ministry of Defence, Military Health Department, Crnotravska 17, 11000 Belgrade, Serbia
[2]University of Defence, Military Medical Academy, Crnotravska 17, 11000 Belgrade, Serbia
[3]Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia
[4]University of Belgrade-Faculty of Agriculture, Nemanjina 6, 11080 Belgrade-Zemun, Serbia
[5]University of Banja Luka, Faculty of Natural Sciences and Mathematics, Mladena Stojanovića 2, 78000 Banja Luka, Bosnia and Herzegovina

Abstract: Macroelements such as Na, K, Ca, and Mg play a significant physiological role, and their inadequate intake has been linked to severe diseases, such as high blood pressure. Data on risk assessment for human health in Serbia, from the intake of these macroelements through the consumption of canned food, are minimal. Therefore, the content of Na, K, Ca, and Mg in five types of canned meat that members of the Serbian Armed Forces regularly use was examined. Macroelements were determined by inductively coupled plasma mass spectrometry in cans of beef goulash, pork ragout, spam, liver pate, and meatballs in tomato sauce, which were stored from one month to six years. The sodium content was significantly higher than the potassium content in all types of food, so the Na/K ratio below 1, desirable for good health, was not found in any of the analyzed products. Also, a significant number of samples had an unfavorable Ca/Mg ratio above 1. However, due to the low consumption of canned food by members of the Serbian Armed Forces, its contribution to the average daily intake of macroelements is almost negligible. The concentration of macroelements decreased with the shelf life, while a significant source of Ka and Mg, among analyzed ingredients, was ground red pepper.

Key words: macroelements, canned meat products, storage, daily intake, high blood pressure, risk assessment.

[*]Corresponding author: e-mail: vantic@agrif.bg.ac.rs

Introduction

Minerals are nutrients necessary for proper physiological processes and must be taken into the body through food, water, or air. They are divided into macro and microelements. Macroelements (Na, K, Mg, Ca, P, Cl, S) are sometimes required in grams, while microelements (Fe, Zn, Cu, Mn, Se, Cr, Co, Ni, etc.) are usually needed in micrograms for the healthy functioning of the human body. The physiological role of minerals is very diverse. They can play a structural role – as an integral part of bones, teeth, blood, and as structural components of many enzymes (Goff, 2018). Also, minerals participate in synthesizing proteins, hormones, and vitamins. Minerals in lower concentrations have a biological function, while higher concentrations can be harmful to humans (Bogden and Klevay, 2000). The macroelements Na, K, Mg, and Ca are present in the human body in at least 100 mg/(kg BW). The physiological functions of Na and K are multiple: regulation of blood pressure and acid-base balance, muscle contraction, and nerve impulse transmission. Diarrhea, vomiting, or sweating can lead to Na and K loss, which results in hypotension, tachycardia, muscle spasm, and central nervous system failure (EFSA, 2017; National Academies of Sciences, Engineering and Medicine, 2019). Calcium deficiency provokes osteoporosis and can occur due to a poor diet, vitamin D deficiency, and insufficient exposure to sunlight (EFSA Panel on Dietetic Products, 2015a). Symptoms of Mg deficiency are headache, heart problems, muscle pain, etc. (EFSA Panel on Dietetic Products, 2015b).

In contrast, the excessive intake of macroelements can lead to severe adverse health effects. Excessive intake of Na is related to hypertension, while excessive intake of K can lead to weakness, vomiting, and arrhythmias (EFSA Panel on Nutrition, Novel Foods and Food Allergens, 2019; Kogure et al., 2021). High Ca intake is associated with excessive bone calcification, while high Mg intake causes vomiting and diarrhea (EFSA Panel on Dietetic Products, 2015a, 2015b). The European Food Safety Authority (EFSA) has set average requirements (AR) and adequate daily intakes (AI) of macronutrients for adults. AR is an element intake that meets the daily needs of half of the people in a typical healthy population. AI is defined as the average level of element, which is assumed to be sufficient for the population's needs and is used when AR cannot be calculated. Also, the tolerable upper intake level (UL) is defined as the maximum chronic daily intake of minerals estimated to be "unlikely to pose a risk of human side effects". For example, a safe and adequate Na intake is 2000 mg/day, while a K intake is 3500 mg/day. For magnesium, AI amounts to 350 mg/day for males and 300 mg/day for females. On the other hand, the AR for Ca is set at 750 mg/day for the adult population, while UL amounts to 2500 mg/day (EFSA, 2017).

Canned foods have many benefits for consumers, such as convenient use, long shelf life, reasonable price, and short preparation time. The nutritionists do not

recommend a daily intake of canned food, as it often contains higher Na content, either for taste or food safety. The trend of increased Na intake, with an abundant consumption of canned food, is evident (Singh and Chandorkar, 2018). Excessive Na and inadequate K intakes are one of the main factors contributing to hypertension and related cardiovascular diseases. Similar to EFSA, the World Health Organization (WHO) has recommended an optimal safe intake of Na of <2000 mg/day (equivalent to 5 g/day of salt) and of K of at least 3510 mg/day for adults (WHO, 2012a, 2012b). Reducing Na in processed foods is part of a public health strategy worldwide, intending to lower blood pressure and prevent cardiovascular disease in the population. Like the Na/K ratio, the relationship between Ca and Mg in the human body is complex (Sukumar et al., 2019). They participate together in regulating heart function, muscle contraction, and nerve conduction.

Determining macroelement concentration in food is necessary for quality assurance and consumer health protection. Therefore, the main goal of this study was to analyze the content of Na, K, Mg, and Ca in various canned meat products, which are in regular use in the Serbian Armed Forces. The macroelements were determined in beef goulash (BG), pork ragout (PR), spam (SP), liver pate (LP), and meatballs in tomato sauce (MB), stored up to six years, and in individual ingredients, used for their production. The influence of the sterilization process on the concentration of Na, K, Mg, and Ca was also investigated. Macroelements were analyzed by the ICP-MS method. The content of macroelements during the storage period was followed, and soldiers' exposure to Na, K, Ca and Mg by consuming canned meat through the daily intake (EDI) and its contribution to adequate daily intake (AI) were assessed.

Material and Methods

Canned meat products

Cylindrical tinplate cans were used for filling the canned meat products: 1) two-piece cans, Ø 73×29.5, for LP of 100 g; 2) three-piece cans, Ø 73/70×43, for SP of 150 g, and 3) three-piece cans, Ø 99/96×63, for BG, PR, and MB of 400 g. The tinplate quality was corresponding to the European standards, with additional requirements related to the thickness of the sheet, the tin's application, and the application and quality of the varnish (Stojanović et al., 2021). The canned food was produced according to military requirements. After filling and sealing, the cans were sterilized by heating for 30 min at 120 °C (LP); 50 min at 118 °C (SP); 70 min at 120° (BG and PR); or for 105 min at 118 °C (MB). Undamaged cans stored for up to 6 years in typical military facilities that provide appropriate conditions (temperature up to max 25 °C and relative humidity up to max 75%) were analyzed.

ICP-MS

Inductively coupled plasma-mass spectrometry (ICP-MS), with the iCapQ mass spectrometer (Thermo Scientific, Germany), was used for macroelement analysis, as described in our previous work (Stojanović et al., 2021). All samples were measured in duplicate, and metal content was presented as an average. The differences between duplicates were ≤ 7.3%. The limit of detection (LOD) was 5.6, 2.2, 3.1, and 0.1 mg/kg for Na, K, Ca, and Mg, respectively, while the limit of quantification (LOQ) was 7.0, 2.9, 3.6, and 0.4 mg/kg, respectively. Quality control was performed in each sample series by analyzing bovine liver as a reference material (NIST 1577c, from the National Institute of Standards and Technology, USA). Solvents and spiked samples were included in each batch of digestion and analysis. The most abundant isotopes were used for quantification, and the concentrations were within the range of the certified values for all isotopes. Average recoveries from spiked samples ranged between 95.4% and 103.0%.

Dietary intake calculations

The estimated daily intake of particular macroelements through all types of canned food was calculated as the sum of the estimates for that macroelement in a particular food type (EFSA, 2011). For example, EDI for Na through all types of food was calculated as:

EDI (Na) =EDI(Na/BG)+EDI(Na/PR)+EDI(Na/SP)+EDI(Na/LP)+EDI(Na/MB).

The EDI of a particular macroelement (ME, for example, Na) and a particular type of canned food (CF, for example, BG) was obtained as:

EDI [ME/CF, mg/(kg BW) day] = Σ [ME_{conc} (mg/kg) × MC_{conc} (mg/kg)]/30, where ME_{conc} is the concentration of a particular macroelement (mg/kg), and MC is the monthly consumption of the particular type of canned food (kg). MC, determined by the nutrition plan, was as follows: 0.160 kg of BG, 0.400 kg of PR, 0.450 kg of SP, 0.300 kg of LP, and 0.400 kg of MB. In emergency circumstances, consumption was twice as high. Once the EDI value for a particular macroelement is calculated, the percentage of contribution to the guideline value can be obtained:

Contribution (%) = [EDI (mg/day) / (Guideline value)] x 100.

Statistics

The normality of the data distribution was verified by the Shapiro-Wilk test. Data sets are presented in the form MV ± SD (mean value ± standard deviation) with minimum and maximum in a given group. Mean macroelement concentrations were compared with the corresponding daily intakes (AI) for Na, K, and Mg and the average needs (AR) for Ca, using a one-sample t-test, with probabilities less than 0.01, which was considered statistically significant. All analyses were performed using the *IBM SPSS Statistics 19* software package.

Results and Discussion

Macroelement content in meat products

Canned meat occupies an important place in the diet of members of the Serbian Armed Forces. Canned products are of high quality, with preserved nutritional and energy values, sensory properties, and shelf life of at least four years (Stojanović et al., 2021). The content of macroelements during the storage period from one month (m) to six years (y) is shown in Table 1. A concentration decrease with the storage period was noticed in all products, except MB. The content of macroelements decreased in the following order: Na > K >> Ca ≅ Mg. The content of Na was the highest in all samples. In MB samples, Na and Ca concentrations increased, i.e., Mg and K decreased with storage time, but without statistical significance. The Na content in BG samples dropped from 4025.3 mg/kg (11m) to 2449.9 mg/kg (5y/9m), while in the PR samples, it varied from 4161.3 mg/kg (6m) to 2363.2 mg/kg (5y/9m). The maximum Na value in SP samples was 5022.1 mg/kg (1y/1m), while the minimum was 4049.4 mg/kg (6y). The highest Na content in LP samples was 4001.2 mg/kg (7m), while the lowest was 2667.4 mg/kg (6y). A very similar trend was found for the content of other macroelements (K, Ca, Mg) – a pronounced decreasing trend at more prolonged periods, especially after five years of storage. It can be assumed that there is a certain deposition of macroelements in the form of insoluble compounds on the walls of the cans, which causes a decrease in their concentration during storage. Our finding was in agreement with the recent work of Vafaei et al. (2018), where a significant reduction of Na and Ca was found in canned silver carp samples after 7 years of storage.

In fact, more critical for human health than the individual values of Na, K, Ca, and Mg is the ratio of Na/K and Ca/Mg (Iwahori et al., 2017; Sukumar et al., 2019; Morrissey et al. 2020), which is shown in Table 1. Our diet usually includes too high Na/K and Ca/Mg ratios, which leads to a disturbed balance between intracellular (K, Mg) and extracellular (Na, Ca) electrolytes and causes a state of "low-grade metabolic acidosis" (Carnauba et al., 2017). Diseases associated with this condition are cardiovascular and kidney diseases, stroke, and osteoporosis. The imbalance stems from the habits of > 85% of the population who do not eat enough foods rich in potassium and magnesium (Morrissey et al., 2020). All food types from this work had a Na/K ratio above one, with the highest values in the SP, LP, and MB samples. Also, the Ca/Mg ratio was unfavorable (> 1) in the SP and LP samples, while the BG, PR, and MB samples had a Ca/Mg ratio below 1.

The Shapiro-Wilk test showed a normal distribution of macroelement concentrations relative to the mean value in BG, PR, SP, LP, and MB samples during storage. The mean values of concentrations and their standard deviations in BG, PR, SP, LP, and MB samples during storage are shown in Figure 1 (Na and K)

and Figure 2 (Ca and Mg). There was no statistically significant change in the concentration of macroelements concerning the mean value at the significance level of 99% during the storage of analyzed products.

Table 1. The change of macroelement concentration in samples of BG, PR, SP, LP, and MB during the storage period.

Meat product	Storage period (y/m)[a)]	Concentration, mg/kg				Na/K[b)]	Ca/Mg[b)]
		Na	K	Ca	Mg		
Beef goulash (BG)	0/3	3356.8	2427.2	77.9	160.8	1.38	0.48
	0/11	4025.3	2862.2	104.9	194.7	1.40	0.54
	2/2	3303.6	2400.5	88.5	182.4	1.38	0.49
	3/2	3047.7	1894.6	100.4	157.5	1.61	0.64
	4/0	2727.9	2150.3	165.7	231.6	1.27	0.72
	5/1	2762.4	2038.6	104.2	180.2	1.36	0.58
	5/9	2449.9	1855.7	67.7	150.8	1.32	0.49
Pork ragout (PR)	0/3	3371.2	2524.5	64.5	214.5	1.34	0.30
	0/6	4161.3	3103.7	106.3	235.2	1.34	0.45
	2/6	3360.3	2345.9	78.0	152.2	1.43	0.51
	3/2	3097.8	1788.7	81.5	153.2	1.73	0.53
	4/0	3095.8	1795.0	132.6	149.8	1.72	0.89
	5/1	3291.4	1629.6	162.6	147.7	2.02	1.10
	5/9	2363.2	1521.9	88.8	131.2	1.55	0.68
Spam (SP)	0/8	4913.6	2266.0	143.3	152.7	2.17	0.93
	1/1	5022.1	1547.9	155.8	152.7	3.24	1.02
	2/1	4733.3	979.3	315.9	125.1	4.83	2.53
	3/1	4677.5	1053.0	150.2	123.9	4.44	1.21
	4/4	4512.2	1979.9	210.2	139.3	2.28	1,51
	5/0	4284.2	1934.9	256.9	133.3	2.21	1.93
	6/0	4049.4	1228.5	100.8	127.1	3.30	0.79
Liver pate (LP)	0/7	4001.2	1554.2	213.5	121.4	2.57	1.76
	1/1	3983.9	965.9	205.4	120.7	4.12	1.70
	2/6	3274.5	822.3	162.4	84.4	3.98	1.92
	3/0	2872.0	869.9	309.3	116.9	3.30	2.65
	4/4	3657.9	992.3	132.5	94.5	3.69	1.40
	5/0	3558.7	788.8	91.4	79.2	4.51	1.15
	6/0	2667.4	622.3	121.1	80.8	4.29	1.50
Meatballs in tomato sauce (MB)	0/1	4901.2	2370.3	98.0	178.5	2.07	0.54
	1/0	4814.6	2712.3	160.3	190.6	1.78	0.84
	2/8	7122.1	2293.9	87.3	177.7	3.10	0.49
	3/9	5699.1	1992.6	104.1	128.5	2.86	0.81

a) y/m=years/months; b) mg/mg.

The highest mean Na and K concentrations were found in MB samples (5634.2 ± 1068.8 mg/kg and 2342.3 ± 295.7 mg/kg, respectively). The BG and PR samples showed similar mean K concentrations, but significantly lower Na mean values than MB samples: 3096.2 ± 523.0 mg/kg (BG) and 3248.7 ± 531.1 mg/kg (PR). The LP samples possessed a mean Na value in the BG and PR sample ranges, while the mean K concentration of LP samples was the lowest by far, amounting to 945.1 ± 295.2 mg/kg. The SP samples possessed a higher mean K concentration than LP (1569.9 ± 503.1 mg/kg) and showed a significantly high mean Na value (4.598.9 ± 344.2 mg/kg). A lower Na/K ratio is desirable for good health; however, none of the analyzed products were found to have the same. Nevertheless, the mean K content in BG, PR, and MB samples was above 2000 mg/kg, which was pretty high, making these products a rich K source. The mean values of Ca were very similar in BG, PR, and MB samples (≅100 mg/kg), while mean Mg values in the same samples were higher and ranged about 170–180 mg/kg. In contrast to BG, PR, and MB samples, the mean values of Ca in SP and LP samples were higher (190.4 ± 74.9 mg/kg and 176.5 ± 73.4 mg/kg, respectively), compared to mean Mg values, which were 136.3 ± 12.4 mg/kg (SP) and 99.7 ± 19.3.0 mg/kg (LP).

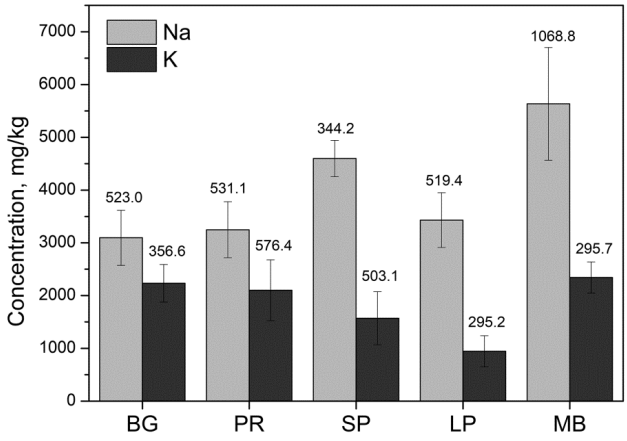

Figure 1. Mean sodium (Na) and potassium (K) concentrations in different kinds of canned meat products (BG – beef goulash; PR – pork ragout; SP – spam; LP – liver pate; MB – meatballs in tomato sauce).

The concentrations of Na, K, Ca, and Mg in the samples from this work were compared with the findings of other authors (Table 2). Other studies show that the results published on canned meat products are minimal compared to fishery products and fresh and processed meat (not canned). Sodium concentrations in samples from other studies were similar to those in our research (Ahuja et al., 2019), even higher in some cases, as in Gillespie et al. (2015), where the highest

Na concentration was 11590 mg/kg. Bilandžić et al. (2021) examined different types of products, including canned meat, where K and Ca concentrations were very similar to those in our work (1932 ± 435 mg/kg and 130 ± 74 mg/kg, respectively). The Mg concentration was slightly higher than the concentration observed in our research (253 ± 313 mg/kg). Djinovic-Stojanovic et al. (2017) found Mg values in canned pork and chicken meat and pate in the range from 117 ± 14.3 mg/kg to 165 ± 34.4 mg/kg, which was almost the same as in our work (99.7 ± 19.3–179.7 ± 27.7 mg/kg).

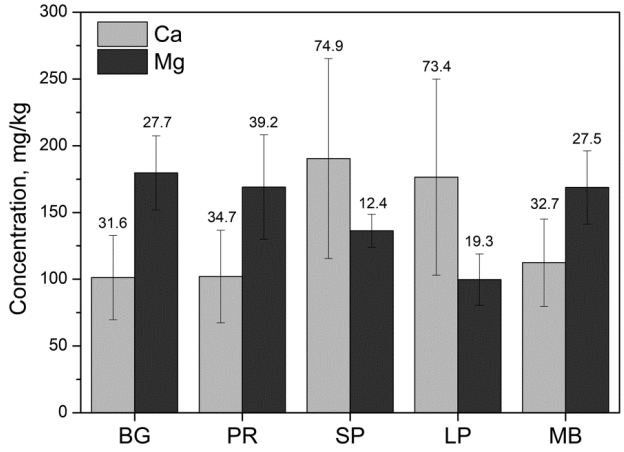

Figure 2. Mean calcium (Ca) and magnesium (Mg) concentrations in different kinds of canned meat products (BG – beef goulash; PR – pork ragout; SP – spam; LP – liver pate; MB – meatballs in tomato sauce).

In canned fishery products, the Na level was mainly between 1600 and 5000 mg/kg (Atanasoff et al., 2013; Rubio et al., 2017; Vafaei et al., 2018; Ahuja et al., 2019; Park et al., 2019). Exceptions were samples of Pacific saury, where a relatively high value of Na, about 7000 mg/kg, was found (Anishchenko et al., 2017). In terms of K, the values in fishery products were very diverse, ranging from about 700 mg/kg (Boufleur et al., 2013) to as much as 7519 mg/kg in dry edible seaweed (Rubio et al., 2017). High values of Ca (2200–4500 mg/kg) were found in samples of Pacific saury (Anishchenko et al., 2017) and dry edible seaweed, which were also extremely rich in Mg (2164 ± 968 mg/kg; Rubio et al., 2017). Non-canned fresh meat, organs, and meat products had significantly lower Na content than canned food (Table 2). Concentrations were below 1000 mg/kg, except in the kidneys, where the Na value was around 1600 mg/kg, and in ham, where the Na values exceeded 1255 mg/kg (Nieto et al., 2018).

Table 2. The data recorded within this study and the results obtained by other authors.

Reference	Macroelements, MV ± SD (mg/kg)			
	Na	K	Ca	Mg
Canned meat products				
Present study	3096.2 ± 523.0– 5634.3 ± 1068.8	945.1 ± 295.2– 2342.3 ± 295.7	101.3 ± 31.6 – 190.4 ± 74.9	99.7 ± 19.3– 179.7 ± 27.7
Gillespie et al., 2015	11590 ± 2270 4720 ± 840	–	–	–
Djinovic-Stojanovic et al., 2017	–	–	–	165 ± 34.4 (pork meat) 117 ± 14.3 (pork pate) 147 ± 32.6 (chicken meat) 142 ± 26.4 (chicken pate)
Ahuja et al., 2019	4490 ± 200	–	–	–
Bilandžić et al., 2021	–	1932 ± 435	130 ± 74	253 ± 313
Canned fishery products				
Boufleur et al., 2013	1596 ± 649– 2635 ± 429	705 ± 105– 1142 ± 213	15.0 ± 5.5– 30.4 ± 15.3	106 ± 23– 202 ± 45
Atanasoff et al., 2013	1950.5 ± 62.9	2447.0 ± 56.0	54.5 ± 1.1	326.9 ± 7.0
Anishchenko et al., 2017	4680.2 ± 433.6 (brand A) 7128.4 ± 565.7 (brand B)	2227.0 ± 72.5 (brand A) 4047.8 ± 150.6 (brand B)	2209.12 ± 431.36 (brand A) 4534.83 ± 785.89 (brand B)	–
Rubio et al., 2017	5044 ± 1760	7519 ± 2692	2205 ± 921	2164 ± 968
Park et al., 2019	2968 ± 366.5– 6041 ± 340.9	672.5 ± 48.14– 3106 ± 65.88	56.55 ± 18.73– 1397 ± 455.0	224.7 ± 7.32– 510.1 ± 47.02
Vafaei et al., 2018	3036.8 ± 137.7– 4004 ± 125.4	–	344.3 ± 62.7– 804.25 ± 40.8	–
Ahuja et al., 2019	2460 ± 580	–	–	–
Meat and processed meat (not canned)				
Tomović et al., 2011	598 ± 141 (muscle) 897 ± 181 (liver)	2940 ± 240 (muscle) 2350 ± 530 (liver)	127 ± 21 (muscle) 209 ± 48 (liver)	276 ± 15 (muscle) 265 ± 44 (liver)
Nikolic et al., 2017	580.44 ± 18.36 (muscle) 956.18 ± 25.77 (liver) 1636.44 ± 38.53 (kidney)	2365.99 ± 32.47 (muscle) 2256.54 ± 20.29 (liver) 1554.63 ± 40.33 (kidney)	65.07 ± 2.57 (muscle) 59.05 ± 4.94 (liver) 164.79 ± 12.26 (kidney)	227.43 ± 4.64 (muscle) 207.59 ± 3.62 (liver) 164.30 ± 3.37 (kidney)
Tomović et al., 2017	635 ± 44 (liver) 1670 ± 90 (kidney) 725 ± 40 (heart)	2910 ± 170 (liver) 2350 ± 190 (kidney) 2770 ± 80 (heart)	142 ± 41 (liver) 166 ± 30 (kidney) 107 ± 19 (heart)	190 ± 10 (liver) 176 ± 11 (kidney) 201 ± 8 (heart)
Nieto et al., 2018	884 ± 204 (sausages) 1027±585 (bacon) 1255±738	–	–	–

Potassium values in fresh meat and organs ranged from 1500 to 3000 mg/kg (Nikolic et al., 2017; Tomović et al., 2011, 2017). The values of Ca and Mg for samples of fresh meat and organs ranged from 60 to 200 mg/kg and from 160 to 270 mg/kg, respectively, which are similar to the values obtained in our study. It can be concluded that fresh meat and organs have lower Na content than canned and processed meat and fishery products.

Macroelement content in raw materials, spices, and additives

The content of macroelements in raw materials, spices, and additives used to produce BG and MB samples and stored for 3 months and 1 month, respectively, was examined to roughly assess their contribution to the final concentration in the analyzed food. The results are presented in Table 3. Regarding the results related to BG, the highest sodium value was found in table salt (389000.0 mg/kg, but a high value was also found in the sample of dry onion –1068.5 mg/kg). High values of potassium were found in beef meat (4089.9 mg/kg) and very high in dry onion (12539.5 mg/kg) and ground red pepper (31774.4 mg/kg). Even though the portion of dry onion and red ground pepper in the BG sample is small, their contribution to the total potassium content in the final product is significant due to the high concentration of K in the mentioned additive and spice. The highest Ca and Mg contents were again found in dry onion and ground red pepper, which indicates that they are a significant source of minerals.

In the case of MB, macroelements were determined in starting raw materials (beef and pork meat and tomato sauce), dry onion, ground red pepper, flour, sugar, dish supplement, food additive, and kitchen salt. Except in kitchen salt, high sodium values were found in dried and minced onions (1155.0 and 1316.9 mg/kg) and tomato sauce (5150.8 mg/kg), while extremely high values were found in minced pepper (83831.8 mg/kg), dish supplement (284383.0 mg/kg) and food additive (363004.8 mg/kg). High potassium values were found in flour (1560.2 mg/kg), dish supplement (2214.5 mg/kg), beef and pork meat (3471.7 and 3477.6 mg/kg), and tomato sauce (4907.4 mg/kg) while extremely high K values (11182.7 and 28360.3 mg/kg) were recorded in dry onion and ground red pepper. Ground red pepper looks like a very desirable kind of spice for the dishes based on meat since it possesses very high K contents and low Na levels. The high Ca content was found in ground red pepper (2082.7 mg/kg), minced onion (2611.3 mg/kg), and dry onion (3221.3 mg/kg), while high Mg content was found in dry onion (995.9 mg/kg) and ground red pepper (1937.8 mg/kg). These results indicate ground red pepper again as a spice representing a significant source of minerals (Table 3). Based on the determined macroelement concentrations, it can be concluded that spices and additives, except for the raw materials, can affect the concentration of macroelements in the finished product. The quality and safety of the raw materials, spices, and additives in terms of the content of macroelements must be constantly monitored to prevent harmful effects on consumer health.

Table 3. Macroelements in raw materials, spices, and additives for the production of BG and MB, and the content of macroelements in BG and MB before and after the sterilization process.

Raw materials, spices and additives	Macroelements, mg/kg			
	Na	K	Ca	Mg
Beef goulash (BG)				
Beef meat	631.4	4089.9	58.3	252.9
Beef tallow	16.9	52.9	24.4	8.4
Dry onion	1068.5	12539.5	3669.0	1265.7
Ground red pepper	260.6	31774.4	2521.8	2224.5
Kitchen salt	389000.0	3.8	< 0.1	< 0.1
BG before sterilization	067.0	789.1	95.6	181.3
BG after sterilization	497.3	583.3	88.1	171.6
Meatballs in tomato sauce (MB)				
Beef meat	564.1	3471.7	59.6	248.7
Pork meat	479.1	3477.6	65.1	216.5
Tomato sauce	5150.8	4907.4	211.2	201.5
Dry onion	1155.0	11182.7	3221.3	995.9
Ground red pepper	218.7	28360.3	2082.7	1937.8
Minced onion	1316.9	28.8	2611.3	112.4
Minced pepper	83831.8	621.1	155.6	74.8
Dish supplement	284383.0	2214.5	539.7	184.8
Additive	363004.8	2.9	1.9	< 0.1
Flour	35.6	1560.2	235.0	312.7
Sugar	32.7	32.4	30.6	3.7
Kitchen salt	391341.8	3.2	< 0.1	< 0.1
MB before sterilization	864.6	755.7	92.8	171.0
MB after sterilization	710.9	632.4	93.7	163.4

The influence of the sterilization process on macroelement content

After testing the starting materials, spices and additives, the next step was to test the contents of GG and MB cans for the presence of macroelements immediately before and after the sterilization process. The values of macroelements in the samples of BG and MB are shown in Table 3. All macroelement concentrations were lower after sterilization (10 to 15%) in both types of canned meat products. This finding again points to the assumption of the deposition of macroelements in the form of insoluble compounds on the walls of the cans.

Dietary intake assessment

Among the dietary factors associated with the current high blood pressure epidemic, sodium, potassium, calcium, and magnesium are particularly interesting. These nutrients play an essential role in controlling blood pressure levels (Karppanen et al., 2005). On the other hand, the body uses an increased blood pressure as the most potent physiological mechanism to prevent Na accumulation

when intake is high. Interestingly, increased sodium excretion significantly improves higher potassium, calcium, and magnesium intakes.

Table 4. The intake of macroelements calculated from the mean concentration data and combined with the dietary information of consumers.

Type of food	Intake of macroelements through canned meat products			
	EMI[a] mg/month		EDI[b] mg/day	
	Regular	Emergency	Regular	Emergency
			Na	
BG	495.4	990.8	16.5	33.0
PR	1299.5	2599.0	43.3	86.6
SP	2069.5	4139.0	69.0	138.0
LP	1029.2	2058.4	34.3	68.6
MB	2253.7	4507.4	75.1	150.2
Total	7147.3	14294.6	238.2	476.4
AI (mg)			2000.0	2000.0
% (AI)			11.9	23.8
			K	
BG	357.2	714.4	11.9	23.8
PR	840.5	1681.0	28.0	56.0
SP	706.5	1413.0	23.5	47.1
LP	283.5	567.0	9.5	18.9
MB	936.9	1873.8	31.2	62.5
Total	3124.6	6249.2	104.1	208.3
AI (mg)			3500.0	3500.0
% (AI)			3.0	6.0
			Ca	
BG	16.2	32.4	0.5	1.0
PR	40.8	81.6	1.3	2.6
SP	85.7	171.4	2.9	5.8
LP	53.0	106.0	1.8	3.6
MB	45.0	90.0	1.5	3.0
Total	240.7	481.4	8.0	16.0
AR (mg)			750.0	750.0
% (AR)			1.1	2.1
			Mg	
BG	28.8	57.6	1.0	2.0
PR	67.6	135.2	2.2	4.4
SP	61.3	122.6	2.0	4.0
LP	29.9	59.8	1.0	2.0
MB	67.5	135.0	2.3	4.6
Total	255.1	510.2	8.5	17.0
AI (mg)			350.0	350.0
% (AI)			2.4	4.9

[a] EMI – estimated monthly intake; [b] EDI – estimated daily intake.

In contrast, Na deficiency during deficient intake or loss due to gastrointestinal causes, sweating, or considerable blood loss can be effectively prevented by lowering blood pressure (Iwahori et al., 2017). Nutrition studies show that Na intake significantly exceeds 2000 mg, a sufficient daily intake that is unlikely to affect blood pressure adversely. In contrast, the current average intake of K, Ca, and Mg is significantly lower than recommended. For example, in the United States, the average intake of these mineral nutrients is only 35–50% of the recommended intake (Bates et al., 2020). Ideally, Na intake values should be less than those of K; however, this is not usually the case. Today, the Na/K ratio is commonly above 0.57, reflecting a much higher intake of sodium and a significantly lower potassium intake than the adequate ones recommended by EFSA (2000 mg for Na and 3500 mg for K). Also, the Ca/Mg ratio attracts great attention because its value above 2 is associated with an increased risk of metabolic, inflammatory, and cardiovascular disorders (Sukumar et al., 2019). The evidence suggests that high Na intake and low K, Ca, and Mg intakes lead to the occurrence and maintenance of high blood pressure and metabolic syndrome in a large portion of the population. This is a consequence of the typical Western diet, which favors calorie-dense foods, usually like canned food.

Mean macroelement concentrations within this study were combined with dietary data to assess soldiers' exposure to Na, K, Ca, and Mg through canned meat consumption. EDI values were used to calculate their contribution to the adequate intake (AI) for Na, K, and Mg and the average requirement (AR) for Ca (EFSA, 2017). According to the nutrition plan, soldiers consume 160 g BG, 400 g PR, 450 g SP, 300 g LP, and 400 g MB monthly in regular conditions. The results are shown in Table 4. The canned food contributes 11.9% to the adequate intake of Na, and only 3.0, 1.1, and 2.4% to the adequate intakes of K, Ca, and Mg. In emergencies, such as staying in the field, the contribution of canned food to the adequate intake of macroelements is twice as high. Thus, the low consumption of canned food contributes very little to the average daily intake of macroelements. Apart from its significant energy value, this food type does not positively contribute to K, Ca, and Mg intakes or negatively to excessive Na intake. However, if individual meals are observed, it can be noticed that the Na content is exceptionally high in SP and MB and exceeds the recommended daily level of 2000 g. Consumers get more Na in just one meal than is needed for normal daily functioning of the body, when SP and MB are on the menu. Further, when SP, PR, or MB are on the menu, K content in one serving satisfies about 20–30% of daily needs because one portion contains 700–1000 mg of potassium. It can be concluded that some products may be reformulated in the future, especially SP and MB, in terms of Na content. Part of the table salt (NaCl) could be replaced by potassium chloride (KCl), significantly affecting the decrease in Na and the increase in K. However, it should be also taken into account that these meals are

prepared for soldiers, often exposed to heavier physical activities that cause increased sweating and more pronounced Na loss. The younger, physically active population will not be affected by the increased Na intake as much as the elderly or sick people. However, long-term exposure to high Na values can lead to physiological changes and diseases in healthy and young people.

Conclusion

The storage period affected reducing macroelement concentration in the canned meat products. The content of Na was the highest among measured macroelements in all types of meat products. The highest mean Na and K contents were found in MB (5634.2 ± 1068.8 mg/kg and 2342.3 ± 295.7 mg/kg, respectively). The mean K content in BG, PR, and MB samples was above 2000 mg/kg, making these products a rich K source. However, none of the analyzed products was found to have a Na/K ratio below 1, which is desirable for good health. In the BG, PR, and MB samples, the mean values of Ca were about 100 mg/kg, while Mg means ranged from about 170 to 180 mg/kg. In contrast, the mean Ca values in SP, and LP samples were higher (≈170–190 mg/kg) than the mean Mg values (≈100–140 mg/kg). Among analyzed spices and additives, ground red pepper represented a significant mineral source, with very high K (≅30000.0 mg/kg) and Mg (≅2000.0 mg/kg) contents. After sterilization, the concentration of all macroelements was lower by approximately 10 to 15%. The rare consumption of canned food, predicted by soldiers' diet, contributes 11.9% to the adequate intake of Na, and 3.0, 1.1, and 2.4% to the adequate intake of K, Ca, and Mg.

Acknowledgements

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (contract No: 451-03-68/2022-14/200116).

References

Ahuja, J.K., Li, Y., Haytowitz, D.B., Bahadur, R., Pehrsson, P.R., & Cogswell, M.E. (2019). Assessing Changes in Sodium Content of Selected Popular Commercially Processed and Restaurant Foods: Results from the USDA: CDC Sentinel Foods Surveillance Program. *Nutrients*, *11* (8), 1754.

Anishchenko, O.V., Sushchik, N.N., Makhutova, O.N., Kalachova, G.S., Gribovskaya, I.V., Morgun, V.N., & Gladyshev, M.I. (2017). Benefit-risk ratio of canned pacific saury (Cololabis saira) intake: Essential fatty acids vs. heavy metals. *Food and chemical toxicology*, *101*, 8-14.

Atanasoff, A., Nikolov, G., Staykov, Y., Zhelyazkov, G., & Sirakov, I. (2013). Proximate and mineral analysis of Atlantic salmon (Salmo Salar) cultivated in Bulgaria. *Biotechnology in Animal Husbandry*, *29* (3), 571-579.

Bates, M., Gunn, J.P., Gillespie, C., & Cogswell, M.E. (2020). The Macronutrient Content of Sodium-Modified Foods Is Unchanged Compared with Regular Counterparts: An Evaluation of Select Categories of Packaged Foods in the United States, 2018. *Journal of the Academy of Nutrition and Dietetics*, *120* (7), 1133-1141.

Bilandžić, N., Sedak, M., Čalopek, B., Đokić, M., Varenina, I., Kolanović, B.S., Božić Luburić, Đ., Varga, I., & Hruškar, M. (2021). Dietary exposure of the adult Croatian population to meat, liver and meat products from the Croatian market: Health risk assessment. *Journal of Food Composition and Analysis*, *95*, 103672.

Bogden, J.D., & Klevay, L.M. (Eds.). (2000). *Clinical nutrition of the essential trace elements and minerals: the guide for health professionals*. Springer Science & Business Media.

Boufleur, L.A., dos Santos, C.E.I., Debastiani, R., Yoneama, M.L., Amaral, L., & Dias, J.F. (2013). Elemental characterization of Brazilian canned tuna fish using particle induced X-ray emission (PIXE). *Journal of Food Composition and Analysis*, *30* (1), 19-25.

Carnauba, R., Baptistella, A., Paschoal, V., & Hübscher, G. (2017). Diet-Induced Low-Grade Metabolic Acidosis and Clinical Outcomes: A Review. *Nutrients, 9* (6), 538.

Djinovic-Stojanovic, J.M., Nikolic, D.M., Vranic, D.V., Babic, J.A., Milijasevic, M.P., Pezo, L.L., & Jankovic, S.D. (2017). Zinc and magnesium in different types of meat and meat products from the Serbian market. *Journal of Food Composition and Analysis*, *59*, 50-54.

EFSA Scientific Report (2011). Overview of the procedures currently used at EFSA for the assessment of dietary exposure to different chemical substances. *EFSA Journal*, *9* (12), 2490.

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (2015a). Scientific opinion on dietary reference values for calcium. *EFSA Journal*, *13* (5), 4101.

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (2015b). Scientific opinion on dietary reference values for magnesium. *EFSA Journal*, *13* (7), 4186.

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Turck, D., Castenmiller, J., de Henauw, S., Hirsch-Ernst, K.I., Kearney, J., & Naska, A. (2019). Dietary reference values for sodium. *EFSA Journal*, *17* (9), e05778.

European Food Safety Authority (EFSA). (2017). *Dietary reference values for nutrients summary report* (Vol. 14, No. 12, p. e15121E). https://doi.org/10.2903/sp.efsa.2017.e15121

Gillespie, C., Maalouf, J., Yuan, K., Cogswell, M.E., Gunn, J.P., Levings, J., Moshfegh, A., Ahuja, J.C.K., & Merritt, R. (2015). Sodium content in major brands of US packaged foods, 2009. *The American journal of clinical nutrition*, *101* (2), 344-353.

Goff, J.P. (2018). Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. *Journal of Dairy Science, 101* (4), 2763-2813.

Iwahori, T., Miura, K., & Ueshima, H. (2017). Time to consider use of the sodium-to-potassium ratio for practical sodium reduction and potassium increase. *Nutrients*, *9* (7), 700. https://doi.org/10.3390/nu9070700

Karppanen, H., Karppanen, P., & Mervaala, E. (2005). Why and how to implement sodium, potassium, calcium, and magnesium changes in food items and diets? *Journal of human hypertension*, *19* (3), S10-S19.

Kogure, M., Nakaya, N., Hirata, T., Tsuchiya, N., Nakamura, T., Narita, A., & Hozawa, A. (2021). Sodium/potassium ratio change was associated with blood pressure change: possibility of population approach for sodium/potassium ratio reduction in health checkup. *Hypertension Research*, *44* (2), 225-231.

Morrissey, E., Giltinan, M., Kehoe, L., Nugent, A.P., McNulty, B.A., Flynn, A., & Walton, J. (2020). Sodium and Potassium Intakes and Their Ratio in Adults (18–90y): Findings from the Irish National Adult Nutrition Survey. *Nutrients*, *12* (4), 938. https://doi.org/10.3390/nu12040938

National Academies of Sciences, Engineering, and Medicine (2019). *Dietary Reference Intakes for sodium and potassium. Washington, DC:* The National Academies Press.

Nieto, C., Tolentino-Mayo, L., Medina, C., Monterrubio-Flores, E., Denova-Gutiérrez, E., & Barquera, S. (2018). Sodium Content of Processed Foods Available in the Mexican Market. *Nutrients*, *10* (12), 2008.

Nikolic, D., Djinovic-Stojanovic, J., Jankovic, S., Stanisic, N., Radovic, C., Pezo, L., & Lausevic, M. (2017). Mineral composition and toxic element levels of muscle, liver and kidney of intensive (Swedish Landrace) and extensive (Mangulica) pigs from Serbia. *Food Additives & Contaminants: Part A*, *34* (6), 962-971.

Park, Y.M., Choi, J.Y., Nho, E.Y., Lee, C.M., Hwang, I.M., Khan, N., & Kim, K.S. (2019). Determination of macro and trace elements in canned marine products by inductively coupled plasma—optical emission spectrometry (ICP-OES) and ICP—mass spectrometry (ICP-MS). *Analytical Letters*, *52* (6), 1018-1030.

Rubio, C., Napoleone, G., Luis-González, G., Gutiérrez, A.J., González-Weller, D., Hardisson, A., & Revert, C. (2017). Metals in edible seaweed. *Chemosphere*, *173*, 572-579.

Singh, M., & Chandorkar, S. (2018). Is sodium and potassium content of commonly consumed processed packaged foods a cause of concern? *Food chemistry*, *238*, 117-124.

Stojanović, B., Janković, S., Đorđević, V., Marjanović, S., Vasilev, D., Stojanović, Z., Balaban, M., & Antić, V. (2021). Determination of toxic elements in meat products from Serbia packaged in tinplate cans. *Environmental Science and Pollution Research, 28*, 48330-48342.

Sukumar, D., DeLuccia, R., Cheung, M., Ramadoss, R., Ng, T., & Lamoureux, A. (2019). Validation of a Newly Developed Food Frequency Questionnaire to Assess Dietary Intakes of Magnesium. *Nutrients*, *11* (11), 2789.

Tomović, V.M., Petrović, L.S., Tomović, M.S., Kevrešan, Ž.S., & Džinić, N.R. (2011). Determination of mineral contents of semimembranosus muscle and liver from pure and crossbred pigs in Vojvodina (northern Serbia). *Food Chemistry*, *124* (1), 342-348.

Tomović, V.M., Jokanović, M.R., Pihler, I.I., Švarc-Gajić, J.V., Vasiljević, I.M., Škaljac, S.B., & Tomašević, I.B. (2017). Ultimate pH, colour characteristics and proximate and mineral composition of edible organs, glands and kidney fat from Saanen goat male kids. *Journal of Applied Animal Research*, *45* (1), 430-436.

Vafaei, M., Naseri, M., & Imani, A. (2018). Long-term storage effect on some mineral elements of canned silver carp (*Hypophthalmichthys molitrix*) with reference to daily intake changes. *Journal of Food Composition and Analysis*, *66*, 116-120.

World Health Organization. (2012a). *Guideline: Potassium intake for adults and children*. https://www.who.int/publications/i/item/9789241504829

World Health Organization. (2012b). *Guideline: Sodium intake for adults and children*. https://www.who.int/publications/i/item/9789241504836

Received: February 26, 2022
Accepted: November 1, 2022

KONZERVISANI MESNI PROIZVODI ZA VOJSKU SRBIJE: SADRŽAJ Na, K, Ca, I Mg I ZDRAVSTVENI RIZICI/BENEFITI

Branislav Đ. Stojanović[1], Zdenka M. Stojanović[2], Sonja S. Marjanović[2], Saša D. Janković[3], Mališa P. Antić[4], Milica R. Balaban[5] i Vesna V. Antić[4*]

[1]Ministarstvo odbrane, Uprava za vojno zdravstvo,
Crnotravska 17, 11000 Beograd, Srbija
[2]Univerzitet odbrane, Vojnomedicinska akademija,
Crnotravska 17, 11000 Beograd, Srbija
[3]Institut za higijenu i tehnologiju mesa,
Kaćanskog 13, 11000 Beograd, Srbija
[4]Univerzitet u Beogradu-Poljoprivredni fakultet,
Nemanjina 6, 11080 Beograd-Zemun, Srbija
[5]Univerzitet u Banjoj Luci, Prirodno-matematički fakultet,
Mladena Stojanovića 2, 78000 Banja Luka, Bosna i Hercegovina

R e z i m e

Makroelementi kao što su Na, K, Ca i Mg igraju značajnu fiziološku ulogu, a njihov neadekvatan unos se dovodi u vezu sa teškim oboljenjima kao što je visok krvni pritisak. Podaci o proceni rizika po zdravlje ljudi u Srbiji, od unosa ovih makroelemenata preko konzumiranja konzervisane hrane, su minimalni. Zbog toga je ispitan sadržaj Na, K, Ca i Mg u pet vrsta mesnih konzervi koje pripadnici Vojske Srbije redovno koriste. Makroelementi su određeni metodom masene spektrometrije sa induktivno spregnutom plazmom, u konzervama goveđeg gulaša, svinjskog paprikaša, mesnog nareska, jetrene paštete i ćufti u paradajz sosu, koje su skladištene u periodu od mesec dana do šest godina. Sadržaj natrijuma je bio značajno veći od sadržaja kalijuma u svim vrstama hrane, tako da odnos Na/K ispod 1, poželjan za dobro zdravlje, nije pronađen ni u jednom analiziranom proizvodu. Takođe, značajan broj uzoraka je imao nepovoljan Ca/Mg odnos veći od 1. Međutim, zbog niske potrošnje konzervisane hrane od strane pripadnika Vojske Srbije, njen doprinos prosečnom dnevnom unosu makroelemenata je skoro zanemarljiv. Koncentracija makroelemenata opadala je sa rokom trajanja, dok je značajan izvor Ca i Mg, među analiziranim sastojcima, bila mlevena crvena paprika.

Ključne reči: makroelementi, konzervisani mesni proizvodi, skladištenje, dnevni unos, visok krvni pritisak, procena rizika.

Primljeno: 26. februara 2022.
Odobreno: 1. novembra 2022.

[*]Autor za kontakt: e-mail: vantic@agrif.bg.ac.rs

Journal of Agricultural Sciences (Belgrade) https://doi.org/10.2298/JAS2204433M
Vol. 67, No. 4, 2022 UDC:339.166.82:631.147(497.11)"2021"
Pages 433-452 Original scientific paper

THE BASIC FEATURES OF TYPICAL CONSUMERS OF ORGANIC FOOD

Vladimir Č. Mitić[*] and Milica M. Čolović

Singidunum University, Danijelova 32, Belgrade, Serbia

Abstract: The major goal of current research is to determine the general frequency of buying organic food and the elementary demographic features of a typical consumer of organic food. Six hundred participants of different genders, ages, levels of education, material and marital statuses, incomes and living areas were comprised. A Google questionnaire was used, in the period from June to December 2021, and it was sent to 800 people, out of whom 600 people filled out the questionnaire completely. The metric characteristics of the applied questionnaire were, as in previous researches in which this instrument was used, at an appropriate level. SPSS version 26 was used for data processing and analysis. Nonparametric techniques have been used due to the irregular distribution of scores on the measured variables. Descriptive statistics was used to adequately present the sample, and the Mann-Whitney U test and the Kruskal-Wallis test were employed to detect the existing differences between the groups of participants. It has been shown that the frequency of buying organic food in Serbia is still at a very low level. Classic customers of organic food are mostly older women with higher educational levels and incomes. They usually live in urban areas, while their marital status and the number of children have no effects on making their decision to purchase organic food and products.

Key words: organic food, consumer, frequency of organic food purchases, main demographic characteristics, food market.

Introduction

The major goal of current research is to determine the general frequency of buying organic food and the elementary demographic features of typical consumers of organic food. Besides, the potential differences in gender, age, the level of education, material and marital status, income and living area were examined and analyzed.

The value of the organic food market in 2014 was around 80 billion dollars, with the leading countries being the USA, Germany, France and China (Golijanin and Popović, 2018). The largest market for organic food is North America, which

[*]Corresponding author: e-mail: mitkeee018@gmail.com

is estimated at 51 billion US dollars, while Europe is in the second place with 45 billion US dollars (IFOAM, 2020). According to data from 2019, the organic food market amounted to 106 billion dollars (IFOAM, 2021), while in 2022, it amounted to 129 billion dollars, which represents an increase of 15% (IFOAM, 2022). The average annual growth of sales of organic products in the world was over 11% in the year 2020, and the international demand for organic food tends to grow over the next few years (Eberle et al., 2022).

It is interesting that organic food was previously produced mainly in the most developed countries, and that in recent years it has been increasingly produced in developing countries, from which even about one-third of the total produced organic food comes (Yadav, 2016). Most of the organic food produced in these countries is exported, so 80–90% of the total organic food produced in Ukraine is sold abroad. In Serbia, this ratio is even more pronounced, considering that only about 1% of produced organic food is sold on the domestic market, while the rest is exported (Sredojević and Oljača, 2018). According to some results, about 40% of the total produced organic food comes from Asia, while about 90% of total produced organic food is sold on the markets of North America and Europe (Willer and Lernoud, 2016).

In Serbia, the organic food market is not sufficiently developed due to less informed consumers and less developed awareness of the benefits of organic food (Vlahović et al., 2011). In addition to insufficient information on the safety and quality of organic food, the low standard of living is an important obstacle (Golijan, 2016). Therefore, some authors (März et al., 2012) describe the organic food market in Serbia as small and very modest. This is indicated by the growth rate of the organic food market, which is very small (only 2 to 3% per year) if we keep in mind that the organic food market in the EU grows by an average of 10 to 15% per year (Končar et al., 2019). In order to reduce business risk, the largest numbers of producers in Serbia produce conventional food in addition to organic (Simin, et al., 2019). However, a number of other authors (Renko et al., 2011) believe that organic food will be increasingly bought by educated consumers and consumers who care about their health and will become part of their daily diet, so its higher price will not be a barrier for consumers to buy it.

Material and Methods

There are three major questions that provide the basis for current research:

1. What is generally the average frequency of purchasing organic food in Serbia?
2. Are there any differences in these frequencies between people who live in different regions: Vojvodina, Belgrade, Southern and Eastern Serbia and Western Serbia?

3. What are the main features of organic food buyers and what are their main demographic characteristics (gender, education, age, area, marital status, number of children and their average monthly incomes)?

The main purpose of current research is to give an explanation and a wider picture of the main demographic features of typical organic food consumers and estimate the necessity of a healthy lifestyle among people from Serbia in general, and more precisely from different regions of Serbia. This presents an important theoretical contribution to the growing and increasingly important topic in contemporary literature, and on the other hand, it also provides pertinent and practical significance which could be used by various stakeholders like: traders, producers, distributors, policymakers, different interest groups and others. A better understanding of the main characteristics of organic food customers allows producers and sellers of organic food to better adjust products to customers in accordance with their investigated features, as well as to develop appropriate marketing strategies to attract new and retain old customers.

The research was conducted online via a Google questionnaire between June and December 2021 and the final sample included 600 out of 800 subjects, as 200 subjects were excluded from the study, because they had not filled out the questionnaire completely. There were participants of different genders, educational levels, ages, material and marital statuses, numbers of children, as well as the areas and regions in Serbia. In addition to the list of basic data on the respondent, a specially designed questionnaire was used for the needs of current research, which was conducted online via Google Forms. It consisted of 20 items on a five-point Likert scale concerning different variants related to nutrition, the frequency of consumer purchases, motives, barriers and habits. The reliability of this questionnaire in research on this and similar topics (Čolović and Mitić, 2021; Čolović and Mitić, 2022; Čolović et al., 2021) expressed as a measure of internal consistency (Cronbach's alpha values) ranged from 0.71 to 0.81. In the current research, the measure of internal consistency had a value of 0.76 of Cronbach's alpha. In addition to descriptive statistics, the Mann-Whitney U test was used to determine differences between two groups of subjects and the Kruskal-Wallis one-way analysis of variance test was used to examine differences in scores between larger numbers of groups of subjects.

Results and Discussion

The frequency of organic food purchases by regions of Serbia

Based on the obtained results on the frequency of purchasing organic food, it can be seen that, in Serbia, there is still no developed awareness of the importance of healthy nutrition and the benefits it brings. Thus, in the examined sample, only 5.7% of respondents stated that they buy organic food every day, 14.1% – two to

three times a week, while the largest part is made of those who stated that they never buy organic food, even 27.8%. There is a very high percentage of those who rarely buy organic food. Thus, 27.7% stated that they buy organic food once a month, while 24.7% do so once a week (Table 1). It is interesting that the largest number of respondents who do not buy organic food are from southern and eastern Serbia (41.3%) and western Serbia (38.7%).

Table 1. The frequency of organic food purchases.

	Serbia		Southern and Eastern Serbia		Western Serbia		Vojvodina		The city of Belgrade	
	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent
Never	167	27.8	62	41.3	58	38.7	32	21.3	15	10
Once a month	166	27.7	38	25.3	30	20	50	33.3	48	32
Once a week	148	24.7	30	20	35	23.3	38	25.4	45	30
2–3 times a week	85	14.1	16	10.7	22	14.7	21	14	26	17.3
Daily	34	5.7	4	2.7	5	3.3	9	6	16	10.7
Total	600	100.0	150	100.0	150	100.00	150	100.0	150	100.0

One of the possible reasons for such results may be insufficient information for consumers about the importance and significance of quality nutrition, as well as all the benefits it brings. Also, some of the potential reasons that are common in our population can be the unavailability of organic products, distrust in certification, taste, various psychological factors, etc. The biggest obstacle for consumers in buying organic food is certainly the high price, which is not surprising given the lower standard of our population compared to more developed countries (Čolovic and Mitić, 2022). This can be seen in comparison with research conducted in other countries, where the percentage of consumers who do not buy organic food is much lower. Thus, in Italy, only about 11% stated that they never buy organic food (Hamilton and Hekmat, 2018), and in Poland – about 15% (Bryła, 2016). Nevertheless, emphasizing that organic food is of high quality, without additives and harmful substances, and with a good price-quality ratio, will contribute to the further development of this market (Milić et al., 2022). The biggest motivation of customers to buy organic food is a concern for health, so emphasizing that aspect can increase the frequency of buying organic food (Čolović and Mitić, 2021), especially among middle-aged people who are often under stress (Čolović et al., 2022) and in midlife crises (Čolović and Stojković, 2017).

Given that the frequency of buying organic food is the highest in Belgrade and Vojvodina, which are above other regions in Serbia in terms of living

standards and incomes, the price is obviously the main factor that affects the frequency of buying organic food. The obtained results are in line with the research conducted by Dašić et al. (2019), who state that about 55% of customers in Serbia rarely buy or do not buy organic products. In a survey conducted in 2013 in Serbia, on a sample of 300 respondents, almost 72% of them rarely or never bought organic food (Đokić et al., 2014).

It can be concluded that the number of customers in Serbia who do not buy organic food is decreasing. When it comes to customers who regularly and orderly buy organic food in Serbia, that percentage varies depending on the research and is around 14% in the research carried out by Vehapi and Dolićanin (2016), about 20% in the study conducted by Mitić and Čolović (2022), according to Đokić et al. (2014), that percentage is slightly higher and amounts to around 28%, and up to 40% according to research done by Dašić et al. (2019).

Demographic characteristics of organic food consumers

The current research involved 600 respondents, of whom 450 were females and 150 males (Figure 1).

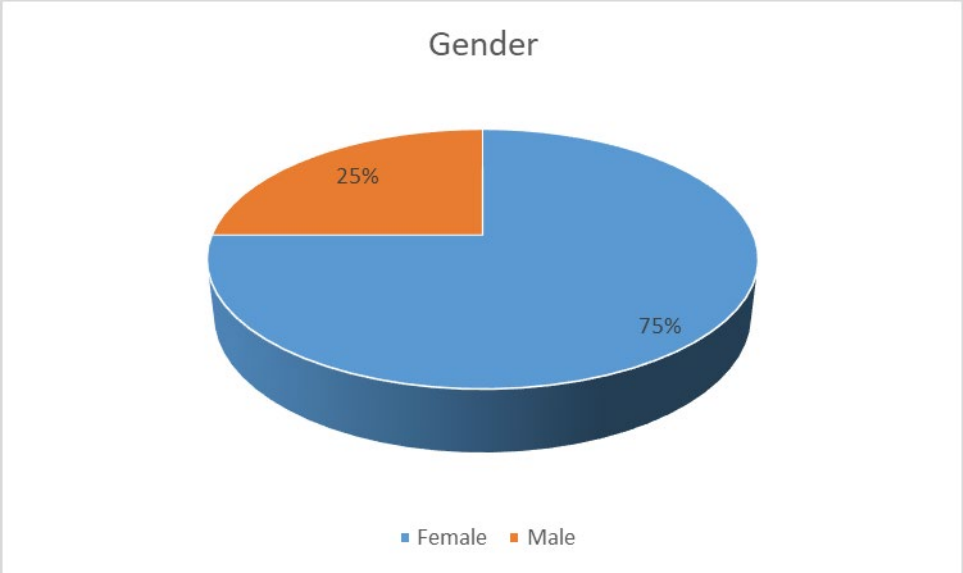

Figure 1. The gender of the respondents.

Statistically significant gender differences in scores were obtained in favor of women, who proved to be more frequent buyers of organic food ($U=25231.000$; $p<0.05$). A possible reason for these results may be greater attention and

commitment to physical appearance by women than men. Also, the reason for the obtained results may be the fact that women go shopping more often and pay more attention to planning purchases and checking the quality of food they intend to buy in order to maintain better self-care as well as the health of their family members (Table 2).

Table 2. Gender differences in the purchase of organic food.

	GENDER	N	MR	∑R
FREQUENCY OF	Men	150	264.35	32254.00
PURCHASING ORGANIC	Women	450	322.64	146872.00
FOOD	*Total*	600		

According to several studies conducted in Serbia (Dašić et al., 2019; Đokić et al., 2014; Mitić and Čolović, 2022), there are statistically significant differences when it comes to gender, and women are ahead of men in terms of the frequency of buying organic food. This is confirmed by research carried out by Kranjac et al. (2017) on a sample of 398 respondents, according to which about 20% of women buy organic food more than men. The obtained results are in line with the research conducted in Brazil (Feil et al., 2020), according to which, buyers of organic food are mostly females (59.3%). Also, this result is consistent with a number of studies (Azzurra et al., 2019; Eisinger-Watzl et al., 2015; Đokić et al., 2014; Hoda et al., 2015; Kranjac et al., 2017; Lea and Worsley, 2005; McCarthy et al., 2016; McFadden and Huffman, 2017; Mohsen and Dacko, 2013; Nandi et al., 2017; Nasir and Karakaya, 2014; Onyango et al., 2007; Petrescu et al., 2016; Rimal et al., 2005; Stojić and Dimitrijević, 2020; Vittersø and Tangeland, 2015), and is not in line with studies according to which gender has no effect on the frequency of organic food purchases (Marreiros et al., 2010; Hashem et al., 2018), nor with the study, stating that men buy organic food more often than women (Perić et al., 2017).

In the current research, there were 15 persons (2.5%), who have completed only primary school, 215 (35.8%) with secondary education, 63 persons or 10.5% completed vocational studies, while 280 persons (46.7%) have higher education and 27 persons have a PhD diploma, which presents 4.5% of the total sample (Figure 2).

According to research conducted among respondents in Novi Sad and Belgrade (Radojević et al., 2021), the level of education is the most important factor influencing the purchase of organic food. This is confirmed by research done by Kranjac et al. (2017), who have stated that as many as 82% of organic food buyers have a university degree, as well as by Renko et al. (2011), who point out that organic food buyers are highly educated people or they are on their way to become so.

As far as education is concerned, the frequency of organic food purchases has been shown to increase with the level of education, which can be related to the increasing awareness of the importance of a healthy nutrition and plenty of advantages of organic food in relation to conventional food and GMO (genetically modified organism) food. A higher level of education is mostly associated with higher income (which proved to be true in the current research), i.e., a higher possibility of buying organic food.

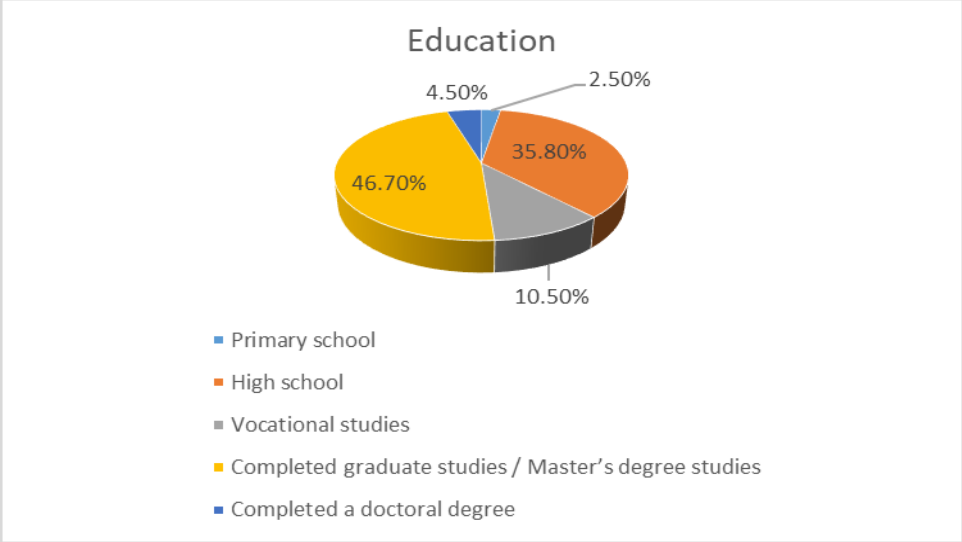

Figure 2. The education of the respondents.

More precisely, people with a PhD are the most frequent buyers of organic food, while people who have only completed primary school are the least likely to buy this type of food (Table 3).

Table 3. Educational differences in the purchase of organic food.

	EDUCATION	N	MR
FREQUENCY OF PURCHASING ORGANIC FOOD	Primary school	15	231.74
	High school	215	261.41
	Vocational studies	63	286.79
	Completed graduate studies/ Master's degree studies	280	302.65
	Completed a doctoral degree	27	341.24
	Total	600	

The magnitude of the obtained differences, measured by the Kruskal-Walis test, was at a statistically significant level ($\chi 2 = 10.32$; $p < 0.05$). These results are consistent with the results obtained in some studies (Baudry et al. al., 2016; Curl et al., 2013; Dettmann and Dimitri, 2010; Đokić et al., 2014; Hashem et al., 2018; Husic-Mehmedovic et al., 2017; Kesse-Guiot et al., 2013; Nandi et al., 2017; Paul and Rana, 2012; Shamsollahi et al., 2013; Singh and Verma, 2017; Tung et al., 2012), while these findings are not in line with other results (Lea and Worsley, 2005; Mohsen and Dacko, 2013; Rimal et al., 2005), according to which, the level of education does not affect the frequency of organic food purchases.

About 80% of respondents in our sample stated that they had an average income (480 respondents), 57 persons rated their income as above average, which represents 9.5%, while 63 persons stated that they had income below average (10.5%) (Figure 3).

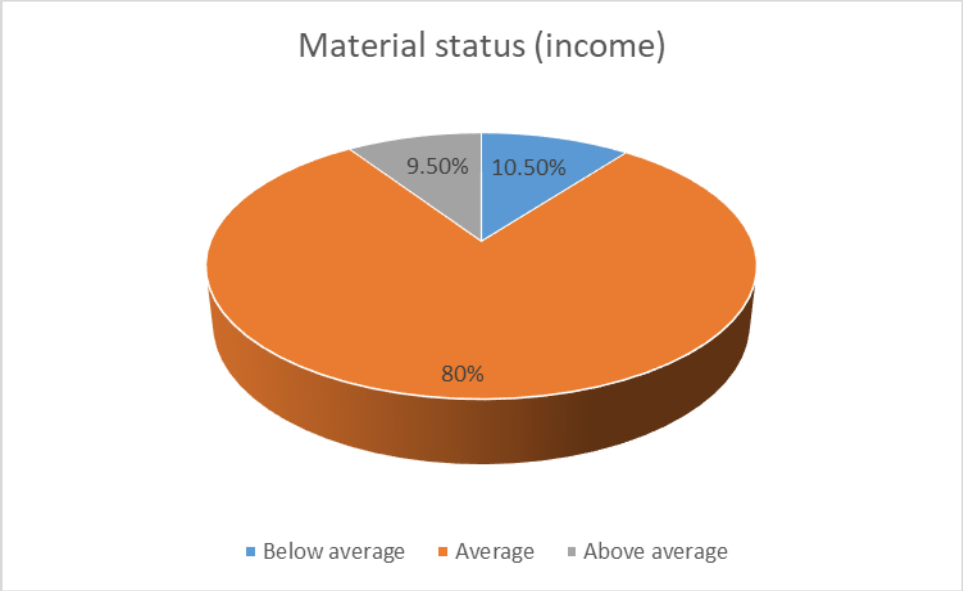

Figure 3. The material status (income) of the respondents.

Research conducted in India has shown that there is no correlation between income and the frequency of buying organic food (Misra and Singh, 2016). The highest frequency of buying organic food is observed among consumers with the highest incomes, according to the results of a large number of studies conducted in Serbia (Ćirić et al., 2020; Đokić et al., 2014; Kranjac et al., 2017). This can be related to the higher price of organic products, and the inability of people with lower material status to afford organic food and products. It has been shown that

with a better material status, that is, with an increase in income, the frequency of buying organic food generally increases. People who reported having an above-average income mostly tended to buy organic food (Table 4).

Table 4. The results of the Kruskal–Wallis test (the significance of differences regarding the material status – income).

	MATERIAL STATUS (INCOME)	N	MR
FREQUENCY OF PURCHASING ORGANIC FOOD	Below average	63	271.17
	Average	480	298.86
	Above average	57	334.04
	Total	600	

The obtained differences in the scores of the respondents were at a statistically significant level (χ^2=5.324, $p<0.05$). The results of the current research are in accordance with the results of other studies (Dumortier et al., 2017; Eisinger-Watzl et al., 2015; McCarthy et al., 2016; Nandi et al., 2017; Rizzo et al., 2020; Singh and Verma, 2017), while they are not in line with Hashem et al. (2018) and Mohsen and Dacko (2013), who have stated that the level of income does not affect the frequency of buying organic food.

In the current sample, the largest number of respondents was aged 25 to 39 years (46%). People between the ages of 40 and 65 were in the second place (30.8%), followed by younger respondents (19%), while older respondents made up 4.2% of the sample (Figure 4).

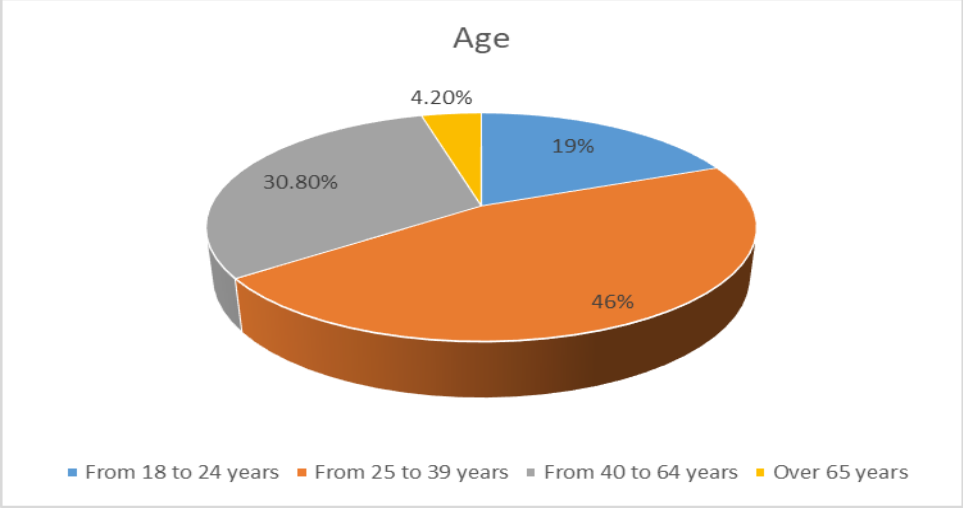

Figure 4. The age range of the respondents.

Research conducted in Serbia through an online questionnaire in April 2020, in which 1022 respondents participated, showed that the most frequent buyers of organic food were in the age category of 25 to 39 years, which is in accordance with the results obtained in the research conducted by Kranjac et al. (2017), who claim that the highest frequency of buying organic food is observed in the population aged 21 to 40. Although buyers of organic food are mostly younger, no statistically significant differences were obtained when it comes to age (Đokić et al., 2014).

According to research conducted in Denmark on a sample of 1.176 respondents, there is a negative correlation between age and buying organic food, which means that older people buy less organic food (Hansen et al., 2018). Different results were obtained in Germany where younger respondents more often buy organic food (Grebitus et al., 2015), as well as in England with a sample of 416 respondents (Hashem et al., 2018), and in China, where the sample size comprised 402 respondents (McCarthy et al., 2016).

The obtained differences in the scores of respondents of different ages are at a statistically significant level (χ^2=5.241; p<0.05). With age, the frequency of buying healthy food increases. Thus, the oldest respondents most often buy this type of food. The turning point in making this decision is probably related to the appearance of certain diseases and the decline of vital functions, which consequently leads to increased care for one's own health and awareness of the importance of a healthy nutrition. Considering the majority of older respondents have a regular income, it becomes clear that this further facilitates and contributes to the purchase of organic food within this age group.

Table 5 shows the magnitude of the obtained differences.

Table 5. Differences in the purchase of organic food regarding the age of respondents.

	AGE	N	MR
FREQUENCY OF PURCHASING ORGANIC FOOD	From 18 to 24 years	114	274.57
	From 25 to 39 years	276	282.68
	From 40 to 64 years	185	297.15
	Over 65 years	25	321.78
	Total	600	

The obtained results are in accordance with the conducted studies (Dumortier et al., 2017; Eisinger-Watzl et al., 2015; Rimal et al., 2005; Roitner-Schobesberger et al., 2008; Singh and Verma, 2017), but are inconsistent with the results of other studies (Fotopoulos and Krystallis, 2002; Hassan et al., 2009; Hughner et al., 2007; Misra and Singh, 2016; Torjusen et al., 2010).

According to research conducted in China, residents of urban areas are more frequent buyers of organic food (McCarthy et al., 2016). When it comes to Serbia, about 85% of organic food buyers live in urban areas, i.e., cities (Kranjac et al., 2017). Most of the people in the sample live in the city, as many as 510 of them, which is 85%. Only 90 respondents are from rural areas – 15% (Figure 5).

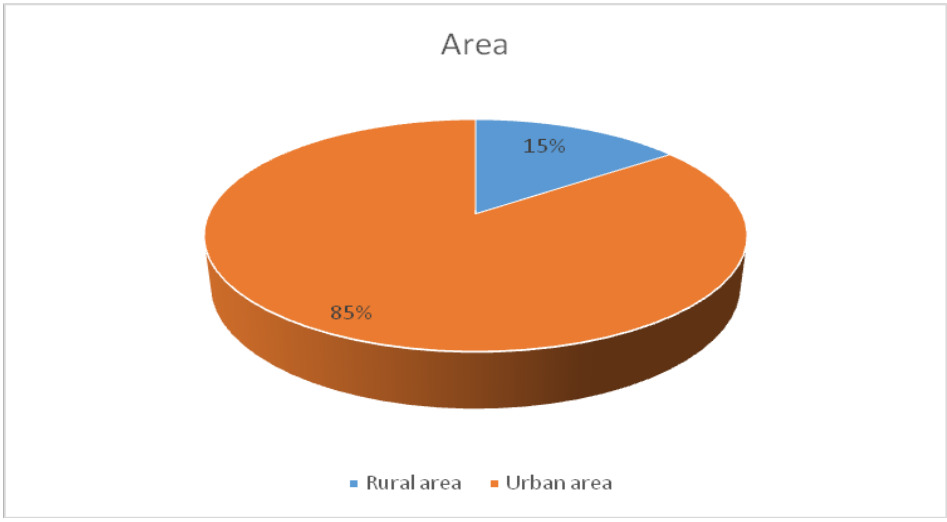

Figure 5. The area of the study.

People who live in urban areas – big cities, tend to often buy organic food (Table 6).

Table 6. Differences in the purchase of organic food regarding the area.

	AREA	N	MR	∑R
FREQUENCY OF PURCHASING ORGANIC FOOD	Rural area	90	253.90	20599.00
	Urban area	510	294.24	161232.00
	Total	600		

One of the possible reasons is the fact that people who have been living in the cities have much more information available and greater availability of organic food stores. The size of the obtained differences is at a statistically significant level ($U=17824.000$; $p<0.05$).

The results of the current research are in line with research according to which customers in urban areas are more likely to buy organic food than customers in rural areas (McEachern and Willock, 2004; Kranjac et al., 2017) as well as with another one according to which customers from the city center tend to buy organic

food more often than those who live in the suburbs (Hamzaoui-Essoussi and Zahaf, 2012).

In a sample of 600 respondents, the largest number of respondents is married (44.7%). They are followed by single persons (26.1%), then persons who are in a relationship (21.4%). Only 6.1% of respondents are divorced, while widows/widowers make up only 1.8% of the total number of respondents (Figure 6).

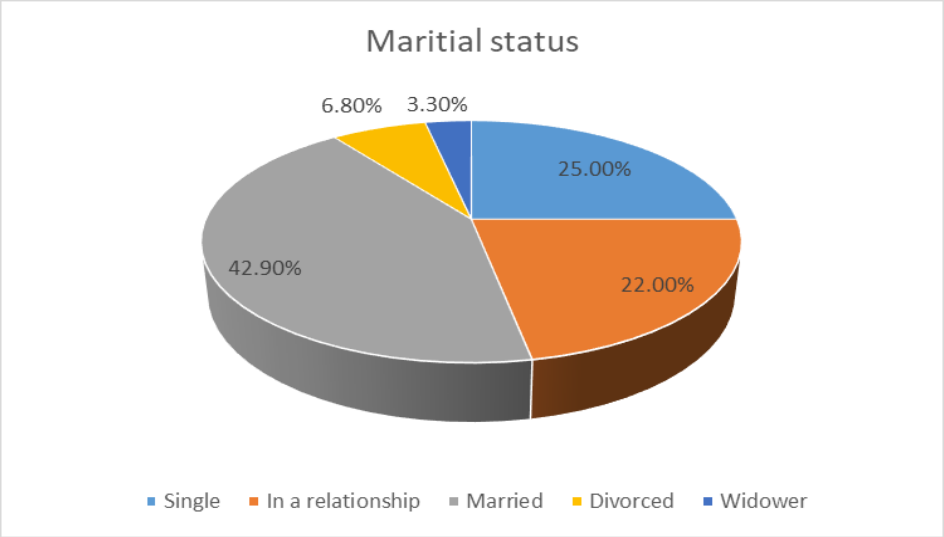

Figure 6. The marital status of the respondents.

No statistically significant differences were obtained in the scores of respondents of different marital statuses (χ^2=6.461; p>0.05) (Table 7). According to research conducted by Đokić et al. (2014), buyers of organic food, except for high income and education, are usually married, have children and live in households with a large number of members. The results of the research coincide with the results of the previous research, except for the number of members in households, which is up to 4 members (Kranjac et al., 2017).

Table 7. Differences in the purchase of organic food regarding the marital status.

	MARITAL STATUS	N	MR
FREQUENCY OF PURCHASING ORGANIC FOOD	Single	150	277.73
	In a relationship	132	313.23
	Married	257	288.75
	Divorced	41	268.23
	Widow/widower	20	341.90
	Total	600	

Nandi et al. (2017) conducted research among organic food consumers in India and came to the conclusion that the most common buyers of organic food are married and have more children. This result is not in accordance with the results of previous research according to which married people are most often buyers of organic food (Dimitri and Dettmann, 2012; Hamzaoui-Essoussi and Zahaf, 2012; Kranjac et al., 2017).

Households with a larger number of members are less likely to decide to buy organic food, with those families with young children having a higher frequency of buying organic food (McCarthy et al., 2016). Nandi et al. (2017) concluded, based on a survey conducted among organic food consumers in India, that the most common buyers of organic food are married and have more children. The results of a study conducted in Canada partially coincide with the previous research, according to which the majority of organic food buyers are married, but have only one child (Hamzaoui-Essoussi and Zahaf, 2012).

The largest number of respondents does not even have children (49%).The approximate number of families with one child (21.7%) or with two children (22.8%) is followed by families with three children (5.5%), while there are the fewest families with more than three children (1%) (Figure 7).

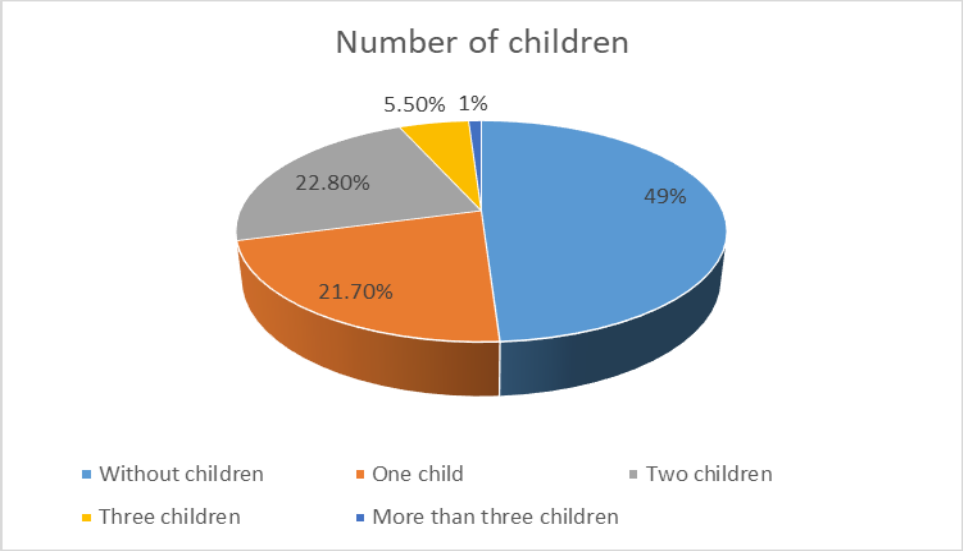

Figure 7. The number of children.

Table 8. Differences in buying organic food in relation to the number of children in the family.

	NUMBER OF CHILDREN	N	MR
FREQUENCY OF PURCHASING ORGANIC FOOD	Without children	294	295.68
	One child	130	338.58
	Two children	137	318.46
	Three children	33	335.23
	More than three children	6	265.32
	Total	600	

The number of children was not statistically significant in the research ($\chi 2=10.752$; $p>0.05$) (Table 8). This result is not in accordance with the results of previous findings according to which married people are most often buyers of organic food (Dimitri and Dettmann, 2012; Đokić et al., 2014; Hamzaoui-Essoussi and Zahaf, 2012; Kranjac et al., 2017). A potential explanation for this is that families with a larger number of children have higher costs, so the higher price of organic food compared to conventional food is a big barrier for them to buy it.

Conclusion

Based on the obtained results, it can be concluded that the awareness of the importance and purchase of organic food in our environment is still at a very low level, due to which the frequency of purchasing this type of food is also very low.

It can be concluded that a certain level of awareness of the importance of healthy nutrition in order to improve and preserve the health and quality of one's own life and the life of their family member existence is most evident in certain groups of our respondents. It can be concluded that the interest of buyers of organic food in Serbia grows year by year, but the high price is still the main barrier to purchase, so the frequency of buying organic food in Serbia is lower than in other countries.

When it comes to gender, statistically significant differences were obtained in favor of women, thus confirming the second hypothesis (H2). Also, statistically significant differences were obtained when it comes to the education of the respondents, which proved that with an increase in the level of education and income, the frequency of buying organic food also increases, thus confirming the third (H3) and fourth (H4) hypotheses. This is not surprising since highly educated people usually have the highest incomes in society. There are statistically significant differences when it comes to age, and with increasing age, the frequency of buying organic food also increases, thus confirming the fifth hypothesis (H5). Buyers of organic food most often live in urban areas, which confirms the sixth hypothesis (H6). When it comes to marital status, no statistically

significant differences were obtained, therefore, the seventh hypothesis (H7) was not confirmed. Also, the number of children, i.e., the number of household members, did not prove to be statistically significant for the purchase of organic food, thus rejecting the eighth hypothesis (H8).

So it turned out that the typical buyers of organic food are mostly female, with higher education and income, from the urban environment, and of older age. The results are not surprising if we keep in mind that females generally spend more time shopping, and that high income makes it easier to include organic food in daily nutrition given the fact that it is more expensive than conventional. Also, more educated consumers who live in urban areas have more information and are better informed about the benefits of organic food, so they more often decide to buy organic food. Over the years, consumers take more and more care of their health, and therefore more often decide to buy and consume organic food in order to contribute better to their health.

The significance of this research is reflected in providing, based on the obtained results, a better insight into the buyers of organic food and their main socio-demographic characteristics. This is very important considering the fact that socio-demographic characteristics have a great influence on consumers in making a decision to purchase organic food. Insights from this study can help family and consumer science professionals redesign their outreach, curricula, and policy recommendations around organic food consumption.

The originality of the paper is reflected in the fact that the respondents are from all regions of Serbia, both urban and rural areas, which achieves a greater representativeness of the sample and greater differences between the respondents in terms of socio-demographic characteristics.

The limitation of current research represents an uneven sample, primarily by gender and area, and it would be useful to equalize the number of respondents according to the mentioned variables in future research. Also, it would be interesting to extend research to other socio-demographic characteristics in order to see the influence they have on making the decision to buy organic food, or even to conduct research in other markets to identify any differences in the most important socio-demographic characteristics of consumers, as well as, examine whether the frequency of purchasing organic food is at an approximate level in the other countries. Another interesting fact that would be good to investigate is the possible change in the frequency of buying an organic food after the Covid-19 Pandemic and whether there was a reassignment of the main demographic characteristics among buyers of organic food.

References

Azzurra, A., Massimiliano, A., & Angela, M. (2019). Measuring sustainable food consumption: A case study on organic food. *Sustainable production and consumption*, *17*, 95-107.

Baudry, J., Touvier, M., Allès, B., Péneau, S., Hercberg, S., & Kesse-Guyot, E. (2016). Typology of eaters based on conventional and organic food consumption: results from the NutriNet-Santé cohort study. *British Journal of Nutrition*, 700-709.

Bryła, P. (2016). Organic food consumption in Poland: Motives and barriers. *Appetite*, *105*, 737-746.

Curl, C., Beresford, S., Hajat, A., Kaufman, J.D., Moore, K., Nettleton, J.A., & Diez-Roux, A.V. (2013). Associations of organic produce consumption with socioeconomic status and the local food environment: Multi-ethnic study of atherosclerosis (MESA). *PLoS One, 8* (7), e69778.

Čolović, M., Nikić, G., & Stamatović, M. (2021). The relation between gender and differences in emotional intelligence of female managers in modern rural tourism, *Ekonomika poljoprivrede*, *68* (1), 69-83.

Čolović, M., & Mitić, V. (2021). Determinant factors influencing organic foods purchase. *Acta agriculturae Serbica*, *26* (51), 89-95.

Čolović, M., & Mitić, V. (2022). The main motives for buying organic food in people of the former Yugoslavia. *British Food Journal*, https://doi.org/10.1108/BFJ-06-2021-0651

Čolović, M., Mitić, V., Nikolić, M., & Milojković, D. (2022). Occupational Safety as One of the Important Factors of Professional Satisfaction and Stress in Regular Conditions and During the COVID-19 Pandemic. (pp. 171-176). *Handbook of Research on Key Dimensions of Occupational Safety and Health Protection Management.* IGI Global, Hershey, Pennsylvania.

Čolović, M., & Stojković, M. (2017). Midlife crisis – external manifestations and internal changes. *Teme*, *42* (2), 315-336.

Ćirić, M.R., Ilić, D.S., Ignjatijević, S.D., & Brkanlić, S.D. (2020). Consumer behaviour in online shopping organic food during the Covid-19 pandemic in Serbia. *Food and Feed Research*, 149-158.

Dašić, G., Radosavac, A., Knežević, D., & Đervida, R. (2019). Preferences of customers and improvement of production and sales of organic products in Serbia. *Ekonomika poljoprivrede*, *66* (1), 127-142.

Dettmann, R.L., & Dimitri, C. (2010), Who's buying organic vegetables?, *Journal of Food Products Marketing*, *16* (1), 79-91.

Dimitri, C., & Dettmann, R.L. (2012). Organic food consumers: what do we really know about them? *British Food Journal*, *114* (8), 1157-1183.

Dumortier, J., Evans, K.S., Grebitus, C., & Martin, P.A. (2017). The influence of trust and attitudes on the purchase frequency of organic produce. *Journal of International Food & Agribusiness Marketing*, *29* (1), 46-69.

Đokić, I., Đokić, N., Pavlović, N., & Znidersic-Kovac, R. (2014). Promotion of organic food in Serbia: Implications from organic food consumers' profile research. *Ekonomika poljoprivrede*, *61* (4), 837-849.

Eberle, L., Milan, G.S., Borchardt, M., Pereira, G.M., & Graciola, A.P. (2022). Determinants and moderators of organic food purchase intention. *Food Quality and Preference*, *100*, 104609.

Eisinger-Watzl, M., Heuer, T., Wittig, F., & Hoffmann, I. (2015). Customers Purchasing Organic Food - Do They Live Healthier? Results of the German National Nutrition Survey II. *European Journal of Nutrition & Food Safety*, 59-71.

Feil, A., Cyrne, C., Sindelar, F.C., Barden, J.E., & Dalmoro, M. (2020). Profiles of sustainable food consumption: Consumer behavior toward organic food in southern region of Brazil. *Journal of Cleaner Production*, *258* (10), 120690.

Fotopoulos, C., & Krystallis, A. (2002). Purchasing motives and profile of the Greek organic consumer: a countrywide survey. *British Food Journal*, *104* (9), 730-765.

Golijan, J. (2016). Motivi koji utiču na kupovinu organskih prehrambenih proizvoda. *Agroekonomika*, *45*, 73-80.

Golijanin, J., & Popović, A. (2018). Basic characteristics of the organic agriculture market. *Proceedings of the Fifth International Conference Competitiveness Of Agro-Food And Environmental Economy.* (pp. 236-244). Bucharest, Romania.

Grebitus, C., Steiner, B., & Veeman, M. (2015). The roles of human values and generalized trust on stated preferences when food is labeled with environmental footprints: insights from Germany. *Food Policy* 52, 84-91.

Hamilton, K., & Hekmat, S. (2018). Organic food and university students: a pilot study, *Nutrition & Food Science*, *48* (2), 218-227.

Hamzaoui-Essoussi, L., & Zahaf, M. (2012). Canadian Organic Food Consumers' Profile and Their Willingness to Pay Premium Prices. *Journal of International Food & Agribusiness Marketing*, *24* (1), 1-21.

Hansen, T., Sørensen, M.I., & Eriksen, M.L.R. (2018). How the interplay between consumer motivations and values influences organic food identity and behavior. *Food Policy*, *74*, 39-52.

Hashem, S., Migliore, G., Schifani, G., Schimmenti, E., & Padel, S. (2018). Motives for buying local, organic food through English box schemes. *British Food Journal*, *120* (7), 1600-1614.

Hassan, D., Monier, S., Nichele, V., & Simioni, M. (2009). Organic Food Consumption Patterns in France. *Journal of Agricultural & Food Industrial Organization*, 1-23.

Hughner, R.S., Prothero, A., Shultz, C.J., & McDonagh, P. (2007). Who Are Organic Food Consumers? A Compilation and Review of Why People Purchase Organic Food. *Journal of Consumer Behaviour*, *62* (2-3), 94 -110.

Husic-Mehmedovic, M., Arslanagic-Kalajdzic, M., Kadic-Maglajlic, S., & Vajnberger, Z. (2017). Live, eat, love: Life equilibrium as a driver of organic food purchase. *British Food Journal*, *119* (7), 1410-1422.

Hoda, A., Karen, J.C., & Nancy, R. (2007). Health Consciousness and Organic Food Consumption. *Journal of Family & Consumer Sciences, 107* (3), 27-32.

IFOAM (2020). The World of Organic Agriculture STATISTICS & EMERGING TRENDS 2020. Research Institute of Organic Agriculture (FiBL) and IFOAM, *Organics International*. Retrieved from https://www.fibl.org/fileadmin/documents/shop/1150-organic-world-2020.pdf

IFOAM (2021). The world of organic agriculture: statistics and emerging trends 2021. Research Institute of Organic Agriculture (FiBL) and IFOAM, *Organics International*. Retrieved from https://www.fibl.org/fileadmin/documents/shop/1150-organic-world-2021.pdf

IFOAM (2022). The world of organic agriculture: statistics and emerging trends 2022. Research Institute of Organic Agriculture (FiBL) and IFOAM, *Organics International*. Retrieved from https://www.fibl.org/fileadmin/documents/shop/1150-organic-world-2022.pdf

Kesse-Guyot, E., Péneau, S., Méjean, C., & Edelenyi, F.S. (2013). Profiles of Organic Food Consumers in a Large Sample of French Adults: Results from the Nutrinet-SantÃ© Cohort Study. *PLoS ONE*, 1-13.

Končar, J.A., Grubor, A.B., Marić, R.M., Vukmirović, G.M., & Đokić, N.S. (2019). Possibilities to improve the image of food and organic products on the AP Vojvodina market by introducing a regional quality label. *Food and Feed Research*, *46* (1) 111-123.

Kranjac, M., Vapa-Tankosić, J., & Knezevic, M. (2017). Profile of organic food consumers. *Ekonomika poljoprivrede*, *64* (2), 497-514.

Lea, E., & Worsley, T. (2005). Australians' organic food beliefs, demographics and values. *British Food Journal*, *107* (11), 855-869.

Marreiros, C., Lucas, M.R., & Röhrich, K. (2010). *Explaining organic food choice on the basis of socio-demographics. A study in Portugal and Germany.* CEFAGE-UE Working.

März, U., Stolz, T., Kalentić, M., & Stefanović, E. (2012). *Organic agriculture in Serbia 2012-At a Glance.* Beograd: National Association "Serbia Organica".

McEachern, M.G., & Willock, J. (2004). Producers and consumers of organic meat: A focus on attitudes and motivations. *British Food Journal*, *106* (7), 534-552.

McFadden, J.R., & Huffman, W.E. (2017). Willingness-to-pay for natural, organic, and conventional foods: the effects of information and meaningful labels. *Food Policy, 68*, 214-232.

McCarthy, B., Liu, H.B., & Chen, T. (2016). Innovations in the agro-food system: Adoption of certified organic food and green food by Chinese consumers. *British Food Journal*, *118* (6), 1334-1349.

Milić, D., Simin, M.T., Trbić, G.D., Radojević, V., & Vukelić, N. (2022). Why I buy organic products – perception of middle income country consumers (Republic of Serbia). *Economics of Agriculture*, *69* (2), 497-515.

Misra, R., & Singh, D. (2016). An analysis of factors affecting growth of organic food: Perception of consumers in Delhi-NCR (India). *British Food Journal*, *118* (9), 2308-2325.

Mitić, V., & Čolović, M. (2022). The main demographic characteristics of customers and the frequency of purchases organic food. *Economics of Agriculture*, *69* (2), 349-364.

Mohsen, M.G., & Dacko, S. (2013). An extension of the benefit segmentation base for the consumption of organic foods: A time perspective. *Journal of Marketing Management*, *29* (15-16), 1701-1728.

Nandi, R., Bokelmann, W., Gowdru, N.V., & Dias, G. (2017). Factors influencing consumers' willingness to pay for organic fruits and vegetables: Empirical evidence from a consumer survey in India. *Journal of Food Products Marketing*, *23* (4), 430-451.

Nasir, V.A., & Karakaya, F. (2014). Consumer segments in organic foods market. *Journal of Consumer Marketing*, *31* (4), 263-277.

Onyango, B.M., Hallman, W.K., & Bellows, A.C. (2007). Purchasing Organic Food in US Food Systems. A Study of Attitudes and Practice. *British Food Journal*, *109* (5), 399-411.

Paul, J., & Rana, J. (2012). Consumer behavior and purchase intention for organic food. *Journal of Consumer Marketing*, *29* (6), 412-422.

Perić, N., Vasić-Nikčević, A., & Vujić, N. (2017). Consumers attitudes on organic food in Serbia and Croatia: A comparative analysis. *Ekonomika Poljoprivrede*, 1049-1064.

Petrescu, D.C., Petrescu-Mag, R.M., Azadi, H., & Burny, P. (2016). A new wave in Romania: organic food. Consumers' motivations, perceptions, and habits. *Agroecology and Sustainable Food Systems*, *41* (1), 46-75.

Radojević, V., Simin, M.T., Trbić, D.G., & Milić, D. (2021). A Profile of Organic Food Consumers-Serbia Case-Study. *Sustainability*, *13* (1), 131. https://doi.org/10.3390/su13010131

Renko, N., Butigan, R., Renko, S. (2011). *WP7 Market data, market trends and consumer behaviour report*. University of Zagreb, Croatia.

Rimal, A.P., Moon, W., & Balasubramanian, S. (2005). Agro-biotechnology and organic food purchase in the United Kingdom. *British Food Journal*, *107* (2), 84-97.

Rizzo, G., Borrello, M., Guccione, G.D., Schifani, G., & Cembalo, L. (2020). Organic Food Consumption: The Relevance of the Health Attribute. *Sustainability*, 595-607.

Roitner-Schobesberger, B., Darnhofer, I., Somsook, S., & Vogl, C.R. (2008). Consumer perceptions of organic foods in Bangkok, Thailand. *Food Policy*, *33* (2), 112-121.

Shamsollahi, A., Chong, C.W., & Nahid, N., (2013). Factors influencing on purchasing behaviour of organic foods. *Humanities and Social Science Research, 1* (2), 93-104.

Singh, A., & Verma, P. (2017). Factors influencing Indian consumers' actual buying behaviour towards organic food products. *Journal of Cleaner Production*, 473-483.

Simin, M.T., Trbić, D.G., & Petrović, M. (2019). Organic production in the Republic of Serbia: Economic aspects. *Ekonomija: teorija i praksa*, *12* (3) 88-101.

Sredojević, Z., & Oljača, S., (2018). *Efikasnost organske proizvodnje – malina, višnja, paprika,* Univerzitet u Beogradu, Poljoprivredni fakultet, Beograd.

Stojić, V., & Dimitrijević, M. (2020). Consumers' intentions to use of organically produced food in the Šumadija region. *Ekonomika poljoprivrede*, *67* (1), 253-267.

Torjusen, H., Brantsaeter, A.L., Haugen, M., Lieblein, G., Stigum, H., Roos, G., Holmboe-Ottesen, G., & Meltzer, H.M. (2010). Characteristics associated with organic food consumption during pregnancy; data from a large cohort of pregnant women in Norway. *BMC Public Health*, *10,* 775.

Tung, S.J., Shih, C.C., Wei, S., & Chen, Y.H. (2012). Attitudinal inconsistency toward organic food in relation to purchasing intention and behavior: An illustration of Taiwan consumers. *British Food Journal*, *114* (7), 997-1015.

Vehapi, S., & Dolićanin, E. (2016). Consumers behavior on organic food: Evidence from the Republic of Serbia. *Ekonomika Poljoprivrede*, *63* (3) 871-889.

Vittersø, G., & Tangeland, T. (2015). The role of consumers in transitions towards sustainable food consumption. The case of organic food in Norway. *Journal of Cleaner Production*, *92*, 91-99.

Vlahović, B., Radojević, V., & Živanić, I. (2011). Istraživanje stavova potrošača o potrošnji organske hrane u Srbiji. *Ekonomika poljoprivrede*, *58* (3), 443-456.

Willer, H., & Lernoud, J. (2016). *The world of organic agriculture. Statistics and emerging trends 2016:* Research Institute of Organic Agriculture FiBL and IFOAM Organics International.

Yadav, R. (2016). Altruistic or egoistic: Which value promotes organic food consumption among young consumers? A study in the context of a developing nation. *Journal of Retailing and Consumer Services, 33*, 92-97.

Received: September 23, 2021
Accepted: December 1, 2022

BAZIČNE KARAKTERISTIKE POTROŠAČA ORGANSKE HRANE

Vladimir Č. Mitić[*] i Milica M. Čolović

Univerzitet Singidunum, Danijelova 32, Beograd, Srbija

R e z i m e

Osnovni cilj aktuelnog istraživanja je utvrđivanje opšte učestalosti kupovine organske hrane i elementarnih demografskih karakteristika tipičnog potrošača organske hrane. Istraživanjem je obuhvaćeno šest stotina učesnika različitog pola, starosti, stepena obrazovanja, materijalnog i bračnog statusa, prihoda i životnog prostora. Korišćen je Gugl upitnik, u periodu od juna do decembra 2021. godine, koji je poslat na adrese 800 ljudi, od kojih je 600 ljudi u potpunosti popunilo upitnik. Metričke karakteristike primenjenog upitnika bile su, kao i u prethodnim istraživanjima u kojima je korišćen ovaj instrument, na odgovarajućem nivou. Za obradu i analizu dobijenih podataka korišćen je SPSS verzija 26. Korišćene su neparametarske tehnike zbog nepravilne raspodele rezultata na merenim varijablama. Korišćene su: deskriptivna statistika u cilju adekvatnog predstavljanja uzorka, te Man-Vitnejev U test i Kruskal-Volisov test u cilju otkrivanja postojećih razlika između grupa učesnika. Pokazalo se da je učestalost kupovine organske hrane u Srbiji i dalje na veoma niskom nivou. Klasični kupci organske hrane su uglavnom žene, starije životne dobi, višeg obrazovanja i primanja. Obično žive u urbanim sredinama, a njihov bračni status i broj dece ne utiču na donošenje odluke da konzumiraju organsku hranu i proizvode.

Ključne reči: organska hrana, potrošači, učestalost kupovine organske hrane, glavne demografske karakteristike, tržište hrane.

Primljeno: 23. septembra 2021.
Odobreno: 1. decembra 2022.

[*]Autor za kontakt: e-mail: mitkeee018@gmail.com

INSTRUCTIONS FOR AUTHORS

MANUSCRIPT SUBMISSION

By submitting a manuscript authors warrant that their contribution to the Journal is their original work, that it has not been published before, that it is not under consideration for publication elsewhere, and that its publication has been approved by all co-authors, if any, and tacitly or explicitly by the responsible authorities at the institution where the work was carried out.
Authors are exclusively responsible for the contents of their submissions, the validity of the experimental results and must make sure that they have permission from all involved parties to make the data public.
Authors wishing to include figures or text passages that have already been published elsewhere are required to obtain permission from the copyright holder(s) and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.
Authors must make sure that all only contributors who have significantly contributed to the submission are listed as authors and, conversely, that all contributors who have significantly contributed to the submission are listed as authors.
The registration of the authors and the submission of the papers should be done via the following link: http://aseestant.ceon.rs/index.php/jas/user
Manuscripts are to be pre-evaluated at the Editorial Office in order to check whether they meet the basic publishing requirements and quality standards. They are also screened for plagiarism.
Authors will be notified by email upon receiving their submission. Only those contributions which conform to the following instructions can be accepted for peer-review. Otherwise, the manuscripts shall be returned to the authors with observations, comments and annotations.

MANUSCRIPT PREPARATION

Authors must follow the instructions for authors strictly, failing which the manuscripts would be rejected without review.
The manuscript should be written in MS-Word in .doc, .docx, format. Font Times New Roman, font size 12, single spacing, margin 2.5 cm should be used when writing the paper. Page numbering should be avoided.
Original scientific paper - The paper should report the unpublished results of original research. This paper should occupy 6 to 12 pages.
Review article - The article which contains original, detailed and critical review of research problem or area where the author has made a certain contribution, noticed by auto citation (at least 10). This article should occupy 15 to 20 pages.
Preliminary communication - Original research paper of full format, small-scale or preliminary character. It should occupy 2 to 6 pages.

The obligatory parts of each Original scientific paper and Preliminary communication are the following: Title of the paper, Name(s) of author(s), Complete postal address(es) of affiliations, Abstract, Key words, Introduction, Material and Methods, Results and Discussion, Conclusion, Acknowledgements, References and Summary in Serbian (if manuscript is submitted in English and vice versa). The obligatory parts of each Review article are the following: Title of the paper, Name(s) of author(s), Complete postal address(es) of affiliations, Abstract, Key words, Introduction, Analysis-discussion of a certain topic, Conclusion, References and Summary in Serbian (if manuscript is submitted in English and vice versa). If manuscript is written in English British version is preferred.

Title of the paper

The title of the paper should describe the content of the paper as accurately and concisely as possible. Authors are recommended to use words in the title which are suitable for indexing and browsing purposes. The title should be centred and written in capital letters. If the paper has already been announced at certain meeting as an oral presentation, under the same or similar title, the datum should be stated on it at the bottom of the first page, after the data of the corresponding author.

Authors' Names

First name, middle initial(s) and last (family) name of all authors, in the original form, should be provided. The names should be written below the title, in lower-case letters, centred and bolded. If several different affiliations need to be mentioned, using the command "insert footnote", consecutive numerals should be placed as the superscript after the respective author's name. The corresponding author should be designated with an asterisk as the superscript, after the last (family) name, and his/her e-mail address should be given under the line, at the bottom of the first page of the paper.

Authors' Affiliations

The full name and address of the institution where the author is employed should be provided. It should be centred and written immediately after the author's name. If authors belong to different institutions, the numerals should be placed as the superscript before the name of institution to provide information on the institution where each of the stated authors is employed.

Abstract

The abstract is a short informative review of the content of the paper, which should enable the reader to estimate its relevance easily and accurately. It is in the interests of the author that the abstract contains terms used for indexing and browsing purposes. The references should not be given in the abstract. The abstract should include the aim of research, the methods, the results and the conclusion. It should contain between 200 and 250 words and be placed between the name of the authors' affiliations and key words. The title of the abstract should be bolded and indented pressing the tab key. The colon should be used after the title of the abstract, and then the text of the abstract should follow without any indentation.

Key words
Key words are terms or phrases which describe best the content of the article for the needs of indexing and browsing purposes. The number of key words should be 3 to 10. They should appear below the abstract. The title of key words should be bolded and indented by pressing the tab key. The colon should be used after the title, and then the list of key words in lower-case letters should be given with the full stop at the end. Key words should be provided in Serbian and English after abstract on both languages.

Introduction
The introduction should contain all the relevant information on past researches according to the stated problem and what can be achieved by further research. Reviewing the references, the author and the year should be provided, and the mentioned author should be cited in References. The title of the introduction should be centred and bolded, written in lower-case letters, below which using one line spacing, the text of the introduction should follow, justified. Each new paragraph should be indented pressing the tab key. These rules should be applied to all parts of the paper.

Material and Methods
The material and methods should be clearly outlined explaining all applied procedures in the paper. Generally known methods should be presented briefly, and a detailed explanation should be given if there is a deviation from previously published procedures. Papers, which have an experimental character, should provide the way of statistical data processing. This part, as well as the part Results and Discussion, if needed, may comprise certain subparts, too.

Results and Discussion
In the part Results and Discussion data obtained on the basis of observation and conducted experiments should be interpreted. In the comment of the results, references should be quoted at the end of the paper, providing the comparison between the obtained results and previous knowledge of the certain area.

Conclusion
All relevant items achieved in the researched area should be mentioned in the conclusion. Listing of all results with repetition of numbers previously specified in Results and Discussion should be avoided. Conclusion should not contain references.

Acknowledgements
Acknowledgements should contain the title and the number of the project that is the title of the program within which the paper was written, as well as the name of the institution which financed the project or program. It should be placed between the conclusion and references.

References

The References section should contain only papers cited in the main text. The paper cited in the text should contain the last (family) name and the year. If the citation is comprised of one author, it is stated as Jalikop (2010) or (Jalikop, 2010). When the citation is comprised of the two authors it is stated as Sadras and Soar (2009) or (Sadras and Soar, 2009). If more than two authors are cited, after the last (family) name of the first author, the abbreviation "et al." is given, and then the year. This citation is stated as Lehrer et al. (2008) or (Lehrer et al., 2008). If more than one paper are cited simultaneously for a certain problem, they should be listed chronologically. A large number of cited papers out of brackets should be separated by comma (,) and if in brackets, by semicolon (;). If two or more papers of the same author are cited, they must be listed chronologically (1997, 2002, 2006, etc.). If a certain author appears several times for the same year, the letters are added (2005a, b, c, etc.). The citations of personal communication and unpublished papers should be avoided, except that it is an absolute necessity. Such citations should appear in the text only as (Brown, personal communication), and not in the list of References.

The references, cited in the text should be stated in the list of references in the original form, alphabetically, without numbering. If a greater number of publications of the same author is cited, then the papers where the author is the single author should first be cited and then the publications of the same author with one and then with more co-authors. If a considerable number of publications appear in any of the above mentioned categories, they should be listed chronologically (1997, 2002, 2006, etc.), and if a great number of publications is of the same year then the letters are added (2005a, 2005b, 2005c, etc.). References entry should contain: the last (family) name of the author, the first letter of the author's name, the year of publishing in the brackets, the title of the paper, the title of the journal, the volume and the number of pages (the first-the last). When the book is cited, the publisher and place of publishing should be given. The lines of each reference entry should be indented after the first line. APA - Publication Manual of the American Psychological Association citation style is used in this journal.

The examples of listing references are the following:

Periodicals

Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (Helianthus annuus L.). *Journal of Agricultural Sciences*, *54*, 1-10.

Books

Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

Book chapter

Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R. M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits.* (pp. 441-514). New York: John Wiley and Sons, Inc.

Proceedings
Behera, T.K., Staub, J.E., Behera, S., Rao, A.R., & Mason, S. (2008). One cycle of phenotypic selection combined with marker assisted selection for improving yield and quality in cucumber. In M. Pitrat (Ed.), *Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae* (pp. 115-121). Avignon, France.

Thesis
Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

Report
Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

Web site
Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from http://research.amnh.org/entomology/spiders/catalog/index.html

Summary
The summary in Serbian is given at the end of the paper and should comprise 200 to 250 words. Before the main text of the summary, as well as in English, the title of the paper, first name, middle initial(s) and last (family) name of all authors and the names and addresses of affiliations should be given. The title of the summary is centred and written separately. Below the title, the text of the summary should follow, without any indentation, and immediately after the text of the summary, the key words are given with the full stop at the end. The e-mail address of the corresponding author should be given at the bottom of the page.

Tables
Tables numbered with Arabic numerals (1, 2, etc.), followed by the title should be placed in the text using 9 font size and a maximum width of 13 cm. They should be clear, simple and unambiguous. The vertical sections should be avoided, and the number of columns should be limited so that the table is not too wide. Also, an unnecessary usage of horizontal sections should be avoided. The title of the table, single spaced above the table, justified, and with the full stop at the end should be given. The detailed explanation of abbreviations, symbols and signs used in the table should be provided below the table. Each table must be mentioned in the text.

Illustrations
All graphs, diagrams and photographs should be titled "Figure" (1, 2, etc.). They should be placed in the text. Graphs and diagrams should be computer drawn, using 9 font size and a maximum width of 13 cm, so that they can be legible and distinct after the size reduction. The overuse of colours and hues should be avoided for aesthetic reasons. The detailed legend without abbreviations for each graph and

diagram should be given. The photographs must be of high quality so that they can technically be well reproduced. They should be submitted in "TIF" or "JPG" format, and they will be printed in black and white. The title of the illustration should be justified, with a full stop at the end, single spaced from the illustration and given below it. Each illustration should be mentioned in the text.

Abbreviations and units
Only standardised abbreviations should be used in the paper. Measure units should be expressed using International System of Units (SI). The abbreviations can be used for other expressions provided these expressions are stated in the full form when appear for the first time with the abbreviated form in the brackets. Values from 1 to 9 can be written in letters, but others numerically.

Nomenclature
The complete nomenclature (chemical and biochemical, taxonomical, genetic etc.) must be adjusted to international codes and commissions, such as *International Union of Pure and Applied Chemistry, IUPAC-IUB Combined Commission on Biochemical Nomenclature, Enzyme Nomenclature, International Code of Botanical Nomenclature, International Code of Nomenclature of Bacteria* etc.

Formulae
All formulae and equations in the paper should be worked out by means of the programme "WORD Equation". An ample space should be left around the formulae for the sake of visibility. Subscripts and superscripts should be clear. Greek letters and other non-Latin symbols should be explained when they are first used. The meaning of all symbols should be given immediately after the equation where these symbols are first used. Equations should be numbered by Arabic numerals, serially in brackets, at the right-hand side. Each equation must be mentioned in the text as Eq. (1), Eq. (2), etc.

The corresponding author will be sent a free copy of the journal after it has been published.
All future associates are asked to prepare the paper according to the given instructions in order to facilitate the work of the Editorial Board. Unless the paper is prepared according to the given instructions it will not be accepted for the prospective publishing.

Editorial Board of the Journal
Journal of Agricultural Sciences

UPUTSTVO AUTORIMA

SLANJE RUKOPISA

Prilikim podnošenja rukopisa autori garantuju da rukopis predstavlja njihov originalan doprinos, da nije već objavljen, da se ne razmatra za objavljivanje kod drugog izdavača ili u okviru neke druge publikacije, da je objavljivanje odobreno od strane svih koautora, ukoliko ih ima, kao i, prećutno ili eksplicitno, od strane nadležnih tela u ustanovi u kojoj je izvršeno istraživanje.
Autori snose svu odgovornost za sadržaj ponesenih rukopisa, kao i validnost eksperimentalnih rezultata, i moraju da pribave dozvolu za objavljivanje podataka od svih strana uključenih u istraživanje.
Autori koji žele da u rad uključe slike ili delove teksta koji su već negde objavljeni dužni su da za to pribave saglasnost nosilaca autorskih prava i da prilikom podnošenja rada dostave dokaze da je takva saglasnost data. Materijal za koji takvi dokazi nisu dostavljeni smatraće se originalnim delom autora.
Autori garantuju, da su kao autori navedena samo ona lica koja su značajno doprinela sadržaju rukopisa, odnosno da su sva lica koja su značajno doprinela sadržaju rukopisa navedena kao autori. Registracija autora i prijava radova se vrši preko linka: http://aseestant.ceon.rs/index.php/jas/user
Pri prijavi rada autori treba da navedu podatke za kontakt (ime i prezime, ustanovu i E-mail adresu) najmanje tri potencijalna recenzenta. Oni treba da budu eksperti iz date oblasti istraživanja koji će obezbediti objektivnu procenu rada. Predloženi recenzenti ne bi trebalo da budu iz iste institucije iz koje su i autori rada.
Nakon prijema, rukopisi prolaze kroz preliminarnu proveru u redakciji kako bi se proverilo da li ispunjavaju osnovne kriterijume i standarde. Pored toga, proverava se da li su rad ili njegovi delovi plagirani.
Autori će o prijemu rukopisa biti obavešteni elektronskom poštom. Samo oni rukopisi koji su u skladu sa datim uputstvima biće poslati na recenziju. U suprotnom, rukopis će, sa primedbama i komentarima, biti vraćen autorima.

UPUTSTVO ZA PRIPREMU RUKOPISA

Autori su dužni da se pridržavaju uputstva za pripremu radova. Rukopisi u kojima ova uputstva nisu poštovana biće odbijeni bez recenzije.

Za obradu teksta treba koristiti program MS-Word. Rukopise treba slati u jednom od sledećih formata .doc, .docx, koristiti font Times New Roman, veličina 12, jednostruki prored, margine 2,5 cm. Strane ne treba numerisati.
Originalan naučni rad – Rad koji sadrži prethodno neobjavljivane rezultate sopstvenih istraživanja. Obim ovog rada treba da iznosi od 6 do 12 strana.
Pregledni rad – Rad koji sadrži originalan, detaljan i kritički prikaz istraživačkog problema ili područja u kome je autor ostvario određeni doprinos, vidljiv na osnovu autocitata (najmanje 10). Obim ovog rada treba da iznosi od 15 do 20 strana.

Prethodno saopštenje – Originalan naučni rad punog formata, ali manjeg obima ili preliminarnog karaktera (od 2 do 6 strana).
Obavezna poglavlja svakog originalnog naučnog rada i prethodnog saopštenja su sledeća: naslov rada, imena autora, naziv ustanove autora, sažetak, ključne reči, uvod, materijal i metode, rezultati i diskusija, zaključak, zahvalnica, literatura i rezime na srpskom jeziku (ako je rad na engleskom i obrnuto). Pregledni rad mora da sadrži: naslov rada, imena autora, naziv ustanove autora, sažetak, ključne reči, uvod, analizu-diskusiju određene teme, zaključak, literaturu i rezime na srpskom jeziku (ako je rad na engleskom i obrnuto). Ako su radovi na engleskom jeziku, prednost se daje britanskoj varijanti ovog jezika.

Naslov rada
Naslov rada treba što vernije da opiše sadržaj rada i da ima što manje reči. U interesu je autora da se u naslovu koriste reči prikladne za indeksiranje i pretraživanje. Naslov se piše velikim slovima i centrirano. Ako je rad prethodno bio izložen na nekom skupu u vidu usmenog saopštenja, pod istim ili sličnim naslovom, podatak o tome treba navesti pri dnu prve stranice, posle podataka autora za kontakt.

Imena autora
Navodi se puno ime, srednje slovo i prezime svih autora, u originalnom obliku. Imena se pišu ispod naslova, malim slovima, centrirano i boldovano. Ukoliko su autori iz različitih institucija brojčanom oznakom u superskriptu, iza prezimena, označiti ustanovu u kojoj radi svaki autor. Autor za kontakt označava se zvezdicom u superskriptu, iza prezimena, komandom „insert footnote“, a njegova e-mail adresa navodi se ispod crte pri dnu prve stranice članka.

Naziv ustanove autora
Navodi se pun naziv i adresa ustanove u kojoj je autor zaposlen. Ispisuje se neposredno nakon imena autora, centrirano. Ukoliko su autori iz različitih institucija brojčanom oznakom u superskriptu ispred institucije označava se ustanova u kojoj je zaposlen svaki od navedenih autora.

Sažetak
Sažetak je kratak informativni prikaz sadržaja članka koji čitaocu omogućava da brzo i tačno odredi njegovu relevantnost. U interesu je autora da sažetak sadrži termine koji se koriste za indeksiranje i pretraživanje. Sažetak ne sme da sadrži reference. Sastavni delovi sažetka su cilj istraživanja, metode, rezultati i zaključak. Sažetak treba da ima od 200 do 250 reči. Reč „Sažetak“ piše se boldovano i uvlači jednim tabulatorom, nakon čega slede dve tačke, a zatim tekst sažetka.

Ključne reči
Ključne reči su termini ili fraze koje najbolje opisuju sadržaj članka za potrebe indeksiranja i pretraživanja. Broj ključnih reči može biti od 3 do 10. Navode se ispod sažetka. Naslov „Ključne reči“ piše se boldovano i uvlači jednim

tabulatorom. Nakon toga slede dve tačke, a zatim nabrajanje ključnih reči malim slovima, sa tačkom na kraju. Treba izbegavati korišćenje ključnih reči koje se nalaze u naslovu rada. Ključne reči se dostavljaju na srpskom i engleskom jeziku posle sažetaka na oba jezika.

Uvod

Uvod treba da sadrži informacije o dosadašnjim istraživanjima po navedenom pitanju i šta se datim istraživanjem želi postići. Prilikom osvrta na literaturu, navesti autora i godinu, a autora citirati u spisku literature. Naslov „Uvod“ piše se sa prvim velikim slovom, centrirano i boldovano, nakon čega sa jednim razmakom ispod naslova sledi tekst uvoda poravnat po levoj i desnoj margini. Svaki novi pasus uvlači se jednim tabulatorom. Ova pravila važe i za sva ostala poglavlja.

Materijal i metode

Materijal i metode treba izložiti jasno uz objašnjenje svih primenjenih postupaka u radu. Opšte poznate metode izložiti kratko, a detaljnije ih objasniti ukoliko se odstupa od ranije objavljenih postupaka. Za radove eksperimentalnog karaktera obavezno navesti način statističke obrade podataka. U ovom poglavlju, kao i u poglavlju „Rezultati i diskusija“, po potrebi se mogu dati i određena podpoglavlja.

Rezultati i diskusija

U poglavlju „Rezultati i diskusija“ interpretiraju se podaci dobijeni na osnovu zapažanja i izvršenih eksperimenata. U komentaru rezultata treba se pozivati na literaturu koja se navodi na kraju rada, čime se obezbeđuje poređenje dobijenih rezultata sa dosadašnjim saznanjima u toj oblasti.

Zaključak

U zaključku treba ukratko navesti najznačajnije rezultate dobijene u radu. Izbegavati nabrajanje svih rezultata istraživanja sa ponavljanjem brojčanih vrednosti koje su prethodno već navedene u poglavlju „Rezultati i diskusija“. Zaključak ne sme da sadrži reference.

Zahvalnica

Zahvalnica treba da sadrži naziv i broj projekta, odnosno naziv programa u okviru koga je rad nastao, kao i naziv institucije koja je finansirala projekat ili program.

Literatura

Poglavlje „Literatura“ treba da sadrži samo radove citirane u glavnom tekstu. Rad citiran u tekstu treba da sadrži prezime autora i godinu. Ako citat obuhvata jednog autora on se navodi kao Jalikop (2010) ili (Jalikop, 2010). Kada citat obuhvata dva autora on se navodi kao Sadras i Soar (2009) ili (Sadras i Soar, 2009). Ako se u tekstu citiraju više od dva autora posle prezimena prvog autora navodi se skraćenica „et al.“, a zatim godina. Ovakav citat navodi se kao Lehrer et al. (2008) ili (Lehrer et al., 2008). Ako se za određeni problem istovremeno citira više radova onda se oni hronološki nabrajaju. Odvajanje većeg broja citiranih radova van

zagrade vrši se zarezom (,) a u zagradi tačkom i zarezom (;). Ako se citiraju dva ili više rada istog autora oni moraju biti poređani prema hronološkom redu (1997, 2002, 2006, itd.). Ukoliko se određeni autor pojavljuje nekoliko puta u istoj godini, dodaju se slova (2005a, b, c, itd.). Citate ličnih komunikacija i neobjavljenih podataka treba izbegavati, osim ako je to apsolutno neophodno. Takvi citati bi trebali da se pojave samo u tekstu (npr. Brown, lična komunikacija), ali ne i u spisku referenci.
Literatura koja je citirana u tekstu navodi se u spisku referenci u originalnom obliku, po abecednom redu, bez numeracije. Ako se citira veći broj radova istog autora najpre se navode radovi kada je autor sam, a zatim kada su prisutna dva i više autora. Ako se u nekoj od ovih kategorija javlja veći broj radova, treba ih hronološki srediti po godinama (1997, 2002, 2006, itd.), a ako se u istoj godini javlja veći broj radova dodaju se slova (2005a, 2005b, 2005c, itd.). Literaturni podatak treba da sadrži: prezime autora, početno slovo imena, godinu izdanja u zagradi, naslov rada, naziv časopisa, volumen i broj stranica (prva-poslednja). Prilikom citiranja knjiga navodi se izdavač i mesto izdavanja. Redovi svake reference posle prvog reda moraju biti uvučeni. U časopisu se koristi APA - Publication Manual of the American Psychological Association citatni stil.

Primeri navođenja referenci su sledeći:

Periodičan časopis
Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (Helianthus annuus L.). *Journal of Agricultural Sciences*, *54*, 1-10.

Knjiga
Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

Poglavlje u knjizi
Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R.M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits.* (pp. 441-514). New York: John Wiley and Sons, Inc.

Zbornik
Behera, T.K., Staub, J.E., Behera, S., Rao, A.R., & Mason, S. (2008). One cycle of phenotypic selection combined with marker assisted selection for improving yield and quality in cucumber. In M. Pitrat (Ed.), *Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae* (pp. 115-121). Avignon.

Teza
Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

Izveštaj
Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

Veb sajt
Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from http://research.amnh.org/entomology/spiders/catalog/index.html

Rezime
Rezime na srpskom jeziku (za radove napisane na engleskom jeziku) ili na engleskom jeziku (za radove napisane na srpskom jeziku) navodi se na kraju rada i treba da ima od 200 do 250 reči. Ispred osnovnog teksta rezimea, navodi se naslov rada, puno ime, srednje slovo i prezime svih autora i naziv i adresa ustanove autora. Naslov „Rezime“ piše se razmaknuto i centrirano. Nakon naslova sledi jedan razmak, a zatim tekst rezimea, uvučen jednim tabulatorom. Neposredno nakon teksta rezimea, navode se ključne reči, sa tačkom na kraju. E-mail adresa autora za kontakt navodi se ispod crte, pri dnu stranice.

Tabele
Tabele obeležene arapskim brojevima (1, 2, itd.) praćene naslovom treba da se nalaze na odgovarajućem mestu u tekstu, u fontu 9. Maksimalna širina tabela treba da bude 13 cm. One treba da budu jasne, što jednostavnije i pregledne. Treba izbegavati vertikalne crte, a broj kolona ograničiti tako da tabela ne bi bila preširoka. Takođe, treba izbegavati nepotrebnu upotrebu horizontalnih crta. Naslov tabele, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom iznad tabele. Ispod tabele treba dati detaljno objašnjenje skraćenica, simbola i znakova korišćenih u samoj tabeli. Svaka tabela mora biti pomenuta u tekstu.

Ilustracije
Svi grafikoni, dijagrami i fotografije treba da se nazovu „Slika“ (1, 2, itd.). Prilažu se na odgovarajućem mestu u tekstu. Grafikone i dijagrame treba uraditi fontom 9, u crno-beloj tehnici i sa maksimalnom širinom od 13 cm. Voditi računa da oni budu čitki i jasni i nakon redukcije veličine. Za svaki grafikon i dijagram treba obezbediti detaljnu legendu bez skraćenica. Fotografije moraju biti visokog kvaliteta da bi se tehnički mogle dobro reprodukovati. Prilažu se u „TIF“ ili „JPG“ formatu, u crno-beloj tehnici. Naslov ilustracije, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom ispod ilustracije. Svaka ilustracija mora biti pomenuta u tekstu.

Skraćenice i jedinice
U radu treba koristiti samo standardne skraćenice. Merne jedinice treba izražavati u internacionalnom sistemu jedinica (SI). Kod navođenja jedinica posle broja treba da stoji razmak (osim za % i °C). Skraćenice se mogu koristiti i za druge izraze pod

uslovom da se ti izrazi navedu u punom obliku prilikom prvog pominjanja, sa skraćenim oblikom u zagradi. Vrednosti od 1 do 9 mogu se izražavati slovima, a ostali brojevi isključivo numerički.

Nomenklatura

Celokupna nomenklatura (hemijska i biohemijska, taksonomska, genetička itd.) mora biti usklađena sa međunarodnim kodeksima i komisijama, kao što su *International Union of Pure and Applied Chemistry, IUPAC-IUB Combined Commission on Biochemical Nomenclature, Enzyme Nomenclature, International Code of Botanical Nomenclature, International Code of Nomenclature of Bacteria* itd.

Formule

Sve formule i jednačine u radu moraju biti urađene pomoću programa „Word Equation". Pri pisanju formula, radi preglednosti, ostaviti dovoljno praznog prostora oko same formule. Subskripti i superskripti treba da budu jasni. Prilikom pisanja jednačina treba dati smisao svih simbola odmah posle jednačine u kojoj se simbol prvi put koristi. Jednačine treba da budu numerisane arapskim brojevima, serijski u zagradama, na desnoj strani linije. Svaka jednačina mora biti pomenuta u tekstu kao Eq. (1), Eq. (2), itd.

Nakon objavljivanja rada, autoru za kontakt će biti poslat jedan primerak časopisa.
Mole se svi budući saradnici da rad pripreme prema datom uputstvu, kako bi olakšali rad redakcije časopisa. Ukoliko se rad ne pripremi po navedenom uputstvu neće biti prihvaćen za objavljivanje.

Redakcioni odbor časopisa
Journal of Agricultural Sciences

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

63

JOURNAL of Agricultural Sciences / editor-in-chief Snežana Oljača. - [Štampano izd.]. - Vol. 44, no. 1 (1999)- . - Belgrade : University of Belgrade, Faculty of Agriculture, 1999- (Belgrade-Zemun : Faculty of Agriculture). - 24 cm

Tromesečno. - Je nastavak: Review of Research work at the Faculty of Agriculture = ISSN 0354-3498. - Drugo izdanje na drugom medijumu: Journal of Agricultural Sciences (Belgrade. Online) = ISSN 2406-0968
ISSN 1450-8109 = Journal of Agricultural Sciences (Belgrade)
COBISS.SR-ID 169380871