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TABLE OF CONTENTS

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Original scientific paper

INVESTIGATION ON THE NUMBER AND SIZE OF THE LEAVES IN SOME OLD DOMESTIC AND NEW TOBACCO VARIETIES IN THE REPUBLIC OF MACEDONIA

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Abstract

Investigations were carried out with five old domestic oriental tobaccos of the types: Prilep (P 10–3/2 and P 12–2/1), Djebel (Dj №1) and Yaka (YK 7–4/2 and KY)and five commercial oriental varieties of Prilep tobacco (P–23, P–84, NS–72, P–66–9/7 and P–79–94), to study the number, length, width and area of the middle belt leaves per stalk. The trial was set up inthe Experimental field of Tobacco Institute–Prilep in 2013 and 2014, in randomized block design with three replications, using traditional agricultural practices. The aim of the investigation is to study some autochthonous varieties and new commercial varieties for the stated quantitative traits, and with analysis of variance to estimate the significance of differences by varieties and years, which will improve our knowledge on stability of the traits, genotypical homogeneity and progress in selection of oriental tobacco in the Republic of Macedonia. Differences in leaf the number and size among genotypes in the two-year investigations are highly significant, which is genetic indicator of their mutual differences. The error of the mean value is low, indicating stability and homozygosity of the genotypes. The variety P–66–9/7 is characterized with the highest number of leaves ($\bar{x}$ = 60) and it has 33 leaves more than YK 7–4/2 and Dj №1. The largest leaf size was measured in P–79–94 ($\bar{x}$ = 23,3cm – length, 12,1 cm – width, 179 cm^2–area). It has 4,8 cm longer, 3,2 cm wider and 74,5 cm^2 larger leaves then YK 7–4/2, which is characterized by the smallest leaves. These data point out to a successful breeding activity of the Tobacco Institute in the selection of oriental aromatic tobaccos. Through evaluation of stability of varieties, the breeder improves its knowledge ontheir homozygosity and higher security in the choice of parental pairs for implementing selection programs.

Keywords: tobacco (*Nicotiana tabacum L.*), old varieties, commercial varieties, quantitative traits, analysis of variance.

Introduction

Tobacco is a crop that is grown for its leaf and all breeding activities are directed to increasing its yield and quality. Scientific Tobacco Institute - Prilep dates from 1924 and its main activity is the selection of oriental, small-leaf, aromatic tobaccos.

The aim of the study is to investigate and compare the number of leaves per stalk and sizeof the middle belt leaves in some old domestic varieties and new commercial varieties, in order to get a better knowledge and more successful selection of oriental tobacco in Macedonia.

Material and methods

Two-year investigations were made on the number of number, length, width and area of the middle belt leaves in five old domestic

varieties of the types: Prilep (P 10–3/2 and P 12–2/1), Djebel (Dj № 1) and Yaka (YK 7–4 / 2 and KY –Kishinska Yaka), and five new commercial varieties of the type Prilep: P–23, P–84, NS–72, P–66–9/7 and P–79–94. The traitswere measured during tobacco growth in the field. The leaf area (relative area) was calculated by multiplying the length by the width, using the coefficient k = 0,6354 (Gornik, 1973). Each amount represents the arithmetic average for the examined property that is accompanied by error of the mean value (Najceska, 2002).

During tobacco vegetation in field (May - September) in 2013, mean monthly temperature was 19,4^{0}C, number of rainy days 34 and total precipitation amount 153 mm. In the same period in 2014mean monthly temperature was 18,3^{0}C, number of rainy days 33 and total precipitation amount 223 mm.

General characteristics of the old domestic tobacco varieties

The cultivation of old tobacco varieties in this region began long ago, during the Ottoman Empire. The centuries–long presence led to their adaptation to the present agro–ecological conditions. Through successive natural selection they have acquired resistance to drought and diseases and can rightly be called autochthonous. Today, the old varieties make a valuable material for breeding activity in the Institute.

Prilep P 10–3/2– characterized by cup-like habitus, average stalk height 50cm, with 30-36 sessile leaves, dry mass yield averages 1200 kg/ha.

Prilep P 12–2/1– characterized by cup-like habitus, average stalk height 55cm, with 34-38 sessile leaves, dry mass yield averages 1500 kg/ha.

P 10-3/2 and P12-2/1 are put into production in the 30-ies of the last century; phenotypic and genotypic are very similar; originating from the local tobacco variety Djumaj–bale from Gorna Djumaja – Bulgaria.

Djebel Dj№ 1–released in the first half of the last century; Originated from the local variety Xanthian Yaka grown in the Djebel tobacco producing region in Bulgaria; characterized by a cylindrical habitus, average stalk height 80cm, 26–30 sessile leaves erected toward the stalk, dry mass yield averages 1000 kg/ha.

Yaka YK 7–4/2–released in mass production in 1932. Originated from Xanthian Yaka originating from Xanthy – Greece; a plant with narrow, spindle shaped–elliptic habitus; average stalk height 100cm, with 26–32 sessile leaves, dry mass yield averages 1000 kg/ha.

These four domestic varieties were created in the Tobacco Institute–Prilep by Rudolf Gornik (Gornik, 1973) by individual selection(Borojević, 1981).

KY (KishinskaYaka), it is believed to be transmitted from Moldova (Uzunoski, 1985). Environmental conditions had a great influence on the morphology and chemistry of this genotype and with multi–decades selection auniform and stable variety was formed.It is characterized by elongated–elliptic habitus with about 40 sessile leaves.

General characteristics of the new commercial tobacco varieties

The new commercial oriental sun–cured varieties created in the Scientific Tobacco Institute–Prilep present a high quality raw material for the domestic and world market. Due to their pleasant aroma and harmonious chemical composition they enter in the mixtures of the highest–quality cigarette brands.

Prilep P–23 – created by Kostadin Nikoloski and Milan Mitreski, through hybridization and selection in Tobacco Institute – Prilep; recognized by the Ministry of Agriculture, Forestry and Water Management of the Republic of Macedonia in 1995 (Korubin – Aleksoska A., 2004). It has elliptical–conical habitus, average stalk height 65 cm, with about 55 densely arranged leaves, dry mass yield 2000–2500 kg/ha.

Prilep P–84 – created by Kiril Naumovski and Ana Korubin–Aleksoska, through hybridization and selection; recognized in 1988 in former Yugoslavia, as one of the first

varieties of the type Prilep. Characterized by cylindrical – elliptical habitus, average stalk height 65 cm, with approximately 40 – 42 sessile leaves, elliptical in shape, dry mass yield 2500–3200 kg/ha.

Prilep NS–72 –created by Dushko Boceski and Simeon Karayankov; recognized in 1984 in former Yugoslavia as one of the first varieties of the type Prilep obtained by crossing (Korubin – Aleksoska A. et al., 2012); characterized by cylindrical–elliptical habitus, average stalk height 75 cm, with approximately 50 sessile leaves, elliptical in shape, dry mass yield 2800–3300 kg/ha.

Prilep P–66–9/7 –created in Tobacco Institute–Prilep by Miroslav Dimitrieski and Gordana Miceska; recognized by the Ministry of Agriculture, Forestry and Water Management of R. Macedonia in 2004 (Korubin – Aleksoska A. et al., 2012); characterized by elliptical–conical habitus, with 54–60 ovate leaves, sessile and evenly distributed on the stem, dry mass yield 3000–3600 kg/ha. It has been the most represented tobacco variety in our country in recent years.

Prilep P–79–94 – created in Tobacco Institute–Prilep by Milan Bogdanceski; recognized by the Ministry of Agriculture, Forestry and Water Management of R. Macedonia in 2001 (Korubin – Aleksoska A., 2004); characterized by cylindrical–elliptical habitus, average stalk height 75 cm, with about 55 sessile leaves densely distributed, especially in the upper part of stem, dry mass yield 2500–3000 kg/ha.

Results and discussion

The highest leaf number among the old varieties was found in Kishinska Yaka – KY (36–2013, 38–2014, i.e. $\bar{x}$ = 37) and the lowest in YK 7–4/2 (26–2013, 28–2014, i.e. $\bar{x}$ = 27) and Dj № 1 (26–2013, 29–2014, i.e. $\bar{x}$ = 27.5). Among the new commercial varieties, the highest leaf number was found in P–66–9/7 (58–2013, 62–2014, i.e. $\bar{x}$ = 60) and the lowest in P–84 (40–2013, 42–2014, i.e. $\bar{x}$ = 41). The variety P–66–9/7 has 33 leaves more than YK 7–4/2 and Dj № 1 (Table 1).

Analysis of variance for the number of leaves per stalk showed highly significant differences among the varieties. Only in the combination Dj № 1 – YK7–4/2 in 2013 the difference was not significant, and in 2014 its significance was 0,05. Differences in leaf number per years showed no significance. This is an indication of different genotypes and proves that this is a highly heritable trait and varietal characteristic.

The highest leaf length among the investigated varieties were measured in P–79–94 (23,2 cm –2013, 23,4 cm – 2014, i.e. $\bar{x}$ = 23,3 cm) and the lowest in YK 7–4/2 (18,2 cm –2013, 18,7 cm – 2014, i.e. $\bar{x}$ = 18,45 cm). The difference in length of the middle belt leaves between the two varieties is 5 cm (Table 1). The comparison of the middle belt leaf length of the semi–oriental variety Otlja O 9–18/2 (32,5 cm – 2013, 33 cm – 2014, i.e. $\bar{x}$ = 32,75 cm), with that of the variety P–79–94 shows that leaves of O 9–18/2 are 9,5 cm longer compared to P–79–94 and 14,3 cm longer compared to YK 7–4/2.

The analysis of variance for the length of the middle belt leaf shows highly significant differences among varieties in 82,2% (2013) and 77,8% of the combinations (2014) and 0,05 significance in 6,7% (2013) and 8,9% (2014). No significance was observed in 11,1% (2013) and 13,3% (2014). The significance of differences in about 87% of the combinations indicates that this trait is varietal characteristic.

The highest leaf width among the varieties was measured in P–79–94 (11,9 cm – 2013, 12,3 cm – 2014, i.e. $\bar{x}$ = 12,1 cm), and the lowest width in YK 7–4/2 (8,7 cm – 2013, 9,1 cm – 2014, i.e. $\bar{x}$ = 8,9 cm). The difference in width of the middle belt leaves between these two varieties is 3,2 cm (Table 1). Comparison of width in the middle belt leaves shows that the

semi–oriental variety O 9–18/2 (17,5 cm – 2013, 18 cm – 2014, i.e. $\bar{x}$ = 17,75 cm) has 5,65 cm wider leaves than P–79–94 and 8,85 cm wider compared to YK 7–4/2.

The analysis of variance for the width of the middle belt leaves shows highly significant differences among varieties in 88.9% (2013) and in 82.2% (2014) and 0.05 significance in 4.4% (2013) and 8.9% (2014), while in 6.7% (2013) and in 8.9% (2014) no significance was observed. The significance of differences in about 93% of the combinations indicates that the investigated trait is varietal characteristic.

The largest area of the middle belt leaves was measured in variety P–79–94 (175,9 cm^2– 2013, 183 cm^2 – 2014,т.е. $\bar{x}$ = 179 cm^2), and the lowest in YK 7–4/2 (101 cm^2 – 2013, 108 cm^2 – 2014, i.e. $\bar{x}$ = 104,5 cm^2). This means that P–79–94 has about 74,5 cm^2 larger leaves than YK 7–4/2 (Table 1). Still, this difference is within the allowed limits for oriental tobaccos. Comparison of the area of middle belt leaves in the semi-oriental variety Otlja O 9–18/2 (361 cm^2 – 2013, 377 cm^2– 2014, i.e. $\bar{x}$ = 369 cm^2) shows that it has 190 cm^2 larger leaves than P–79–94 and 264 cm^2 larger than YK 7–4/2, which indicates that they are two different categories oftobacco.

The analysis of variance for the area of the middle belt leaves in 2013 shows highly significant differences among varieties in 66,7%, 0,05 significance in 20% and no significance in 13,3% of the combinations, while in 2014 high significance was assessed in 62,2%, 0,05 significance in 20% and no significance in 17,8%. The significance of differences in about 84% of the combinations indicates that the investigated trait is varietal characteristic. The differences in leaf area by years are small but highly significant and they appear as a result of various meteorological factors during the growing season.

Table 1. Number and size of the middle belt leaves in old domestic and new commercial tobacco varieties from the Republic of Macedonia

Tobacco varieties	Quantitativetraits							
	Number of leaves per stalk		Lengthof the middlebelt leaves(cm)		Widthof the middlebelt leaves(cm)		Area of the middlebelt leaves(cm^2)	
	$\bar{x} \pm s_{\bar{x}}$		$\bar{x} \pm s_{\bar{x}}$		$\bar{x} \pm s_{\bar{x}}$		$\bar{x} \pm s_{\bar{x}}$	
	2013	2014	2013	2014	2013	2014	2013	2014
Old domestic tobacco varieties								
1. P 10-3/2	32 ± 0,09	34 ± 0,08	22,1 ± 0,10	22,4 ± 0,11	10,2 ± 0,05	10,5 ± 0,04	143 ± 1,65	149 ± 1,63
2. P 12-2/1	34 ± 0,10	36 ± 0,11	22,5 ± 0,12	23,3 ± 0,11	10,4 ± 0,07	11,1 ± 0,06	149 ± 1,83	164 ± 1,70
3. Dj № 1	26 ± 0,16	29 ± 0,14	19,4 ± 0,11	20,2 ± 0,10	11,5 ± 0,04	11,8 ± 0,04	142 ± 1,55	151 ± 1,49
4. YK7-3/2	26 ± 0,17	28 ± 0,15	18,2 ± 0,08	18,7 ± 0,09	8,7 ± 0,08	9,1 ± 0,07	101 ± 1,59	108 ± 1,52
5. KY	36 ± 0,15	38 ± 0,15	21,8 ± 0,12	22,1 ± 0,11	11,6 ± 0,07	11,8 ± 0,05	161 ± 1,93	166 ± 1,84
New commercial tobacco varieties								
6. P-23	45 ± 0,20	48 ± 0,18	20,2 ± 0,13	22,1 ± 0,12	10,7 ± 0,04	11,1 ± 0,03	137 ± 1,85	156 ± 1,74
7. P-84	40 ± 0,14	42 ± 0,15	20,4 ± 0,10	21,3 ± 0,09	10,5 ± 0,05	10,7 ± 0,03	136 ± 1,77	145 ± 1,59
8. NS-72	43 ± 0,21	45 ± 0,17	21,8 ± 0,17	22,9 ± 0,15	10,8 ± 0,07	10,9 ± 0,06	149 ± 2,17	158 ± 2,03
9. P-66-9/7	58 ± 0,15	62 ± 0,15	20,9 ± 0,12	21,5 ± 0,14	11,2 ± 0,05	11,2 ± 0,04	149 ± 1,58	153 ± 1,54
10. P -79-94	52 ± 0,14	54 ± 0,15	23,2 ± 0,12	23,4 ± 0,11	11,9 ± 0,06	12,3 ± 0,02	158 ± 1,69	183 ± 1,62
$LSD_{0,05}$ $LSD_{0,01}$	0,581 1,053		0,420 0,756		0,163 0,293		5,655 10,179	

Conclusions

Two-year investigations of some old domestic oriental varieties (P 10–3/2, P 12–2/1, Djebel Dj № 1, Yaka YK 7–4/2, KY – Kishinska Yaka) and new commercial varieties (P–23 P–84, NS–72, P–66–9/7, P–79–94) showed a small error of the mean value forthe traits: number, length, width and areaof the middle belt leaves per stalk, which is an indication of correct setting of the experiment, high genetic

stability of the traits and homozygosity of the varieties.

The highest leaf number per stalk was obtained in P–66–9/7 (60), which is 33 leaves more than YK 7–4/2 and Dj № 1 – characterized by the lowest number of leaves. The aanalysis of variance showshighly significant differences between varieties and no significantdifferences between the years, which is indication that these are different genotypes and that the investigated trait is highly heritable and varietal characteristic.

The highest leaf length and width was measured in P–79–94 ($\bar{x}$ = 23,3 cm length, $\bar{x}$ = 12,1 cm –width), and the lowestlength and width inYK 7–4/2 ($\bar{x}$ = 18,45 cm length, $\bar{x}$ = 8,9 cm - width), showing that the leaves of P–79–94 are 5 cm longer and 3,2 cm widercompared to YK 7–4/2. The significance of 87% for the leaf length and 93% for the width indicates the differences betweenvarieties, suggesting thatthese traits arevarietal characteristics.

The largest area of the middle belt leaves was measured in P–79–94 ($\bar{x}$ = 179 cm^2), and the lowest in YK 7–4/2 ($\bar{x}$ = 104,5 cm^2), which means that P–79–94 has 74,5 cm^2larger leaves than YK 7–4/2. The significance of differences between the varieties in about 84% of the combinationsconfirms that the investigated trait is a varietal characteristic.

Compared to the semi–oriental variety Otlja O 9–18/2 it can be concluded that differences in leaf size among theten genotypes iswithin allowed limits for oriental aromatic tobaccos.

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EVALUATION OF BREAD WHEAT GENOTYPES WITH DIFFERENT PHENOLOGICAL CHARACTERS IN TERMS OF RESISTANCE TO SUNN PEST DAMAGE

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Abstract
The research was conducted with 23 different varieties in field condition and closed area in 2010 and 2011. Twenty three bread wheat varieties were grown in open fields and closed areas in Faculty of Agriculture, University of Namik Kemal experimental area. The nifm number, sunn pest damage ratio, black point, protein ratio, moisture ratio, sedimentation rate, retarded sedimentation rate, gluten rate and index in the bread wheat varieties were examined. The highest sunn pest damage rate in the samples grown in field condition was found from Tekirdağ sample with 3,08 % and followed by Alga, Renan, Sadova, Geya and Krasnodarskaya-99 samples. The lowest damage rate was obtained from Enola and Dropia with 1,60 %; Krasunia with 1,63 %. In bread wheat samples grown in closed areas, the sunn pest damage rate increased considerably and measured between 4,93-12,75%. According to the average of two seasons, the highest sunn pest damage rate was obtained respectively from Geya, Tekirdağ, Renan and Sadova samples. The lowest damage rates were obtained from Gelibolu, KateA-1, Krasunia and Dropia samples. When the quality features were analysed, gluten, gluten index, zeleny sedimentation and retarded sedimentation values reduced substantially in comparison with values obtained outdoors. Retarded sedimentation values in all samples were at a quite low level that making the crop useless.
Key words: Sunn pest, quality, sunn pest rate, sedimentation, gluten.

Introduction

Wheat is an important agricultural crop in terms of total cultivation area and production quantity in our Thrace region and Turkey . 21.8 million ton of 35.2 million ton cereal production in 2011 year and 20.1 million ton of 33.4 million ton cereal production in 2012 in our county was obtained from wheat (TUIK 2013). The quality characters of wheat varieties are affected by various factors such as cultural practices, climate, soil conditions, the amount of seed, fertilizers, growing conditions, pests and diseases, storage conditions and seed quality.

Sunn pest is the most important factor which is showing negative effects on wheat quality and quantity in our country. The sunn pest in Southeastern Anatolia Region and Thrace Region makes significant damage on the wheat quality and is currently making significant losses in the region. Damage rates of sunn pest in areas, which are untreated by chemical control, was reported to cause significant reduction on the wheat quantity and quality according to the instructions (Özkaya ve Özkaya 1993, Köse ve ark. 1997, Talay 1997, Hariri ve ark. 2000, Köksel ve ark. 2002, Kınacı ve Kınacı 2004, Erbaş 2005, Koçak ve Babaroğlu 2005, Olanca ve ark. 2008, Gözüaçık ve Yiğit 2011).

In the study, sunn pest damage rate, moisture rate in the grain, test weight, protein rate, gluten, gluten index, sedimentation, retarded sedimentation and black point rate in bread wheat varieties were investigated. The aim of this study was to provide valuable information

on the effects of sunn pest damage on bread wheat quality characters.

Material and Methods

Material

In the study, three bread wheat varieties having different properties as different maturity groups, long and short plant height, alternative and winter type, awned and awness, soft and hard grain structure, red and white grain structure were used as experimental material. It was aimed to determine the effects of sunn pest damage on grain quality characters, sunn pest damage rate and grain yield in bread wheat cultivars.

Methods

In this study, 23 different bread wheat varieties was grown in the closed area and open field condition of Agricultural Faculty of Namik Kemal University, Tekirdağ, Turkey (Figure 1). Both trial was established in the same field and left a 2 meter space between trials.

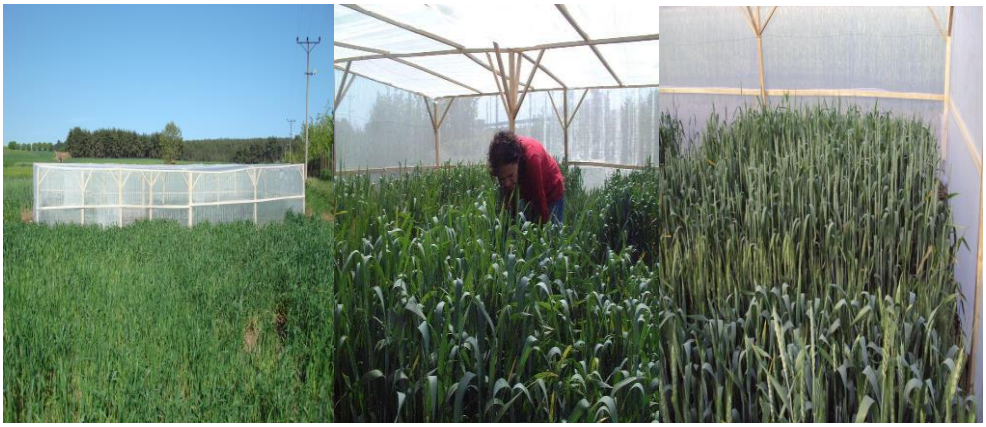

Figure 1. Plant growth and observations in experimental areas

Twenty three bread wheat cultivars in both trials were sown an on 2 meter length and 2 rows with 3 replications. It was used to 500 seeds/m^2 in sowing. The total of 150 adult's sunn pest including 75 male and 75 female were released in the closed area in the dates of 30 April 2010 and 02. May 2011. The harvesting and threshing of all the cultivars in the trials were made separately. Sunn pest damage rate, black point rate, protein rate, moisture rate, gluten, gluten index, sedimentation and retarded sedimentation values on harvested grains of bread wheat varieties were determined. The data obtained from the study was analyzed using Tarist statistical package software according to randomized complete block design. Differences between mean values were controlled by Duncan test.

Results and Discussion

Plants Grown in the Open Field

Twenty three bread wheat cultivars were grown both in open and closed field conditions to determine the ratio of sunn pest damage. The variance analysis was made to determine effects of the sunn pest damage on moisture rate, protein rate, sedimentation, retarded sedimentation, gluten, gluten index and black point rate in grains. The differences between the all mean values except the grain moisture rate were found statistically significant at the 0.01 level. The results of Duncan test, mean values and significance groups were given in Table 1.

While the highest sunn pest damage rate was obtained from Tekirdag with 3.08, and alsoAlga with 2.95 %, Renan with 2.88 % and Sadova with 2.85 % were found same statistically group with Tekirdag. The lowest sunn pest damage rate beetween bread wheat varieteis grown open field condition was obtained from Dropia and Enola with 1.60 % sunn pest damage rato. Krasunia and Kate A1 with 1.63 %, Gelibolu with 2.15% and

Guadralupe with 2.22%, Pehlivan with 2.25% and Selimiye with 2.28 % followed Selimiye. There are significant variations between bread wheat genotypes in respect to resistance of sunn pest damage rate and the the variations can be used in plant breeding (Kınacı and Kınacı 2004).

The unique properties of wheat reside primarily in its gluten-forming storage proteins. Their intrinsic viscoelastic behavior is responsible for the characteristics of different wheat-based foods and for the use of wheat gluten proteins in different food products. While gluten rateof fourteen bread wheat varieties was found 28 % or higher, the gluten rate of nine bread wheat varieties was below 28%. Rates of sunn pest damage in the bread wheat varieties did not cause a significant decrease in gluten content. The highest gluten content was obtained from Sadova with 37%. Saraybosna variety with 36.5%, Pobeda with 36%, Sana with 33%, Geya with 32.67% and Za 75 with 32.50 % were followed this variety for gluten contents. The lowest gluten content was obtained from Krasnadoskaya 99 and Golia with 22%, Krasunia with % 23 and Guadralupe with 23.50 % gluten content was statistically took place after this genotype.

Table 1. The average values and the significance groups of bread wheat varieties in the open field conditions during 2010-2011 years.

Varieties	Sunn pest damage rate (%)	Moisture rate (%)	Gluten value (%)	Gluten index (%)	Sedim. value (ml)	Retarded sedimentation (ml)	Black point (%)	Protein content (%)
Tekirdağ	3.08 a	11.53	28.00 g	92.00 c	53.00 f	38.50m	3.50 c	12.90 c-f
Alga	2.95 ab	11.55	30.50e	94.50 ab	55.00 de	60.00 a	2.50 de	12.50 gh
Renan	2.88 abc	11.55	29.00 f	93.50 b	46.50 k	45.50 g	3.50 c	3.05 bcd
Sadova	2.85 a-d	11.23	37.00 a	85.00 e	57.00 a	51.00 d	5.00 b	13.73 a
Geya	2.80 bcd	11.45	32.67 c	65.00 k	39.50 o	36.50 n	3.00 cd	13.25 bc
Krasnodak.99	2.80 bcd	11.80	21.50 l	87.00 d	40.83 m	35.00 o	3.50 c	11.85 kl
Nina	2.78 bcd	11.72	27.00 h	92.00 c	56.50 ab	50.00 d	2.00 e	12.60 fgh
Yubileyneya	2.73 bcd	11.52	29.00 f	95.15 a	53.00 f	60.00 a	2.50 de	12.80 d-g
Za 75	2.73 bcd	11.72	32.50 cd	87.00 d	53.50 f	38.50 m	5.00 b	13.40 ab
Saraybosna	2.68 cde	11.80	36.50 ab	80.50 g	56.00 bc	50.00 d	3.00 cd	13.70 a
Sana	2.65 def	11.52	33.00 c	67.50 i	40.00 no	21.50 s	2.50 de	13.00 b-e
Golia	2.50 efg	11.72	22.00 l	94.33 ab	34.00 r	34.00 p	3.00 cd	12.05 ik
Tina	2.50 efg	11.53	29.25 f	83.50 f	55.50 cd	46.50 f	3.00 cd	12.95 c-f
Pobeda	2.50 efg	11.70	36.00 b	77.50 h	49.83 g	31.00 r	2.50 de	13.85 a
Odeska-226	2.45 fgh	11.33	25.00 i	94.00ab	54.50 e	59.50 a	7.00 a	12.80 d-g
Selimiye	2.28 ghi	11.62	25.25 i	77.50 h	44.50 l	43.50 hi	3.00 cd	12.40 hi
Pehlivan	2.25 hi	11.75	30.33 e	82.50 f	37.83 p	43.17 i	2.50 de	12.50 gh
Guadralupe	2.22 i	11.45	23.50 k	93.50 b	40.50mn	45.00 g	3.00 cd	11.50 l
Gelibolu	2.15 i	11.47	26.50 h	87.00 d	44.00 l	39.50 l	5.50 b	12.50 gh
Kate A1	2.15 i	11.77	32.00 d	60.00 l	48.83 hi	42.00 k	3.50 c	12.83 d-g
Krasunia	1.63 k	11.67	23.00 k	95.00 a	49.50 gh	52.50 c	6.50 a	11.85 kl
Dropia	1.60 k	11.62	25.00 i	95.00 a	53.50 f	57.00 b	5.00 b	12.65 e-h
Enola	1.60 k	11.55	29.00 f	82.50 f	48.50 i	44.00 h	3.50 c	13.00 b-e
HKO	0.0.25		1.406	1.896	1.833	1.415	0.200	2.290

The quantity and quality of gluten are considered the most important quality parameters of wheat flour. Gluten Index Method (GIM) is a rather new method for determining gluten quantity and quality in wheat semolina and flour. All bread wheat

varieties except Kate A1 in terms of gluten index was showed appropriate values. The obtained results indicated that the effects of sunn pest damage on gluten content and gluten index were not significant. The highest gluten index value was obtained from Yubileyneya variety with 95.15%. Krasunia and Dropia varieties with 95 % gluten index were followed this variety. Alga with 94.50 %, Golia with 94.33 %, and Odeskaya 266 with 94% gluten index was statistically took place after. While the lowest gluten index was obtained from Kate A1 with 60%, Geya with 65 % and Sana with 67% gluten index were ranked after from this variety.

Sedimentation test was considered in comparison with other quality tests, it is a relatively low-cost, less time-consuming test that requires low manpower, is inexpensive and requires no elaborate laboratory equipment. It has proved to be a reliable, highly reproducible quality test that generally gives a good indication of the end-use quality of wheat (Blackman and Gill, 1980 and Carter etal., 1999).

The variations for sedimentation values of bread wheat varieties were found significant levels. Sedimentation value should be 28 ml or more. Zeleny sedimentation value of bread wheat varieties was ranged from 34-57 ml. When bread wheat varieties are compared for this quality character, the highest value in the varieties was obtained from Sadova variety with 57 ml, Nina with 56.50 %, Saraybosna with 56.00% and Tina with 55.50% sedimentation value were followed by this variety. The values obtained indicated that the sunn pest damage rates which are not high level are not significant impact on the sedimentation value. The lowest sunn pest damage rate was obtained from Golia with 34 %, Pehlivan with 37.83% and Geya with 39.50%.

Retarded sedimentation value in bread wheat genotypes was decreased significantly according to sedimentation values in some varieties. This reduction showed that sunn pest damage was reduced significantly by retarded sedimentation value. When bread wheat varieties are compared for retarded sedimentation value, the highest value was obtained from Alga and Yubileyneya varieties with 60 ml value. Odeskaya 226 with 59.50 ml and Dropia with 57 ml were found second place for sunn pest damage. The lowest retarded sedimentation values were observed from Sana with 21.50 ml, Pobeda with 31ml and Golia with 34 ml. The retarded sedimentation value in the majority of examined varieties was found under the sedimentation value. According to the results obtained, sunn pest damage had significant negative effect on retarded sedimentation value of bread wheat varieties.

The black point rate in grain of bread wheat varieties was ranged from 2.0-7.0 %. While the highest balck point rate was found from Odeska 226 with 7%, Krasunia with % 6.5 and Dropia and Za 75, Pobeda with 5% were statistically second place.

The protein content in bread wheat should be between 11-13%. The protein content of bread wheat varieties in the study was found within the desirable limits. The highest protein content among bread wheat varieties was obtained from Pobeda with 13.85%. Sadova with 13.73 %, Saraybosna with 13.70 % were followed this variety. While the lowest protein rate was obtained from Guadraluppe with % 11.50, protein content in Krasunia and Krasnadorskaya with 11.85 % was ranked later. The obtained results indicate that the effect of sunn pest damage on protein content was statistically insignificant.

Plants Grown in the Closed Area in the Field Conditions

The combined variance analysis was made by data obtained from twenty three bread wheat varieties grown in closed areas in field conditions in both years. Differences between the mean values were found statistically significant at the 0.01 level and the results of the significance test (DUNCAN) are given in Table 2.

Sunn pest damage rates were found quite high level and bread wheat varieties showed different responses to sunn pest damage. Sunn pest damage rate in bread wheat varieties was changed between 4.93-12.75 %. Sunn pest damage higher than 10 % makes negative effect on the quality of wheat flour. It has been reported that production of bread from flour damaged by % 20 or higher sunn pest is impossible (Hariri et al., 2000).

Table 2. Mean values and significance groups of bread wheat varieties grown in the closed area in 2010-2011

Varieties	Sunn pest damage rate %	moisture rate %	Gluten value	Gluten index	Sedim. value (ml)	Retarded sedim. value (ml)	Black point %	Protein content %
Geya	12.75 a	11.60	21,50 i	40,00 h	21,50m	5,00 k	5,03 b	11,57 ik
Tekirdağ	12.75 a	11.75	25,50 e	70,00 b	28,50 h	5,17 k	5,00 bc	12,40 c
Saraybosna	12.30 ab	12.20	25,50 e	70,00 b	28,50 h	5,50 ik	3,50 cd	12,40 c
Sana	12.00 ab	11.85	29.00 b	45,00 fgh	29,50 g	9,83 c	3,33 cde	12,32 cd
Za-75	11.50 abc	11.60	26,17 e	40,00 h	24,50 l	6,67 g	4,00 cd	12,31cd
Krasnodak.99	11.25 a-d	11.70	25,50 e	50,00 def	26,50 k	6,50 gh	2,50 ef	12,07 fg
Enola	11.10 b-e	11.70	28.50 bc	44,00 fgh	31,50 e	7,00 fg	4,02 c	12,35 cd
Odeskaya-226	10.75 b-f	11.50	23,00 h	48,33 efg	30,50 f	7,00 fg	8,52 a	12,28 cd
Guadralupe	10.25 c-g	11.50	19,00 k	50,00 def	24,00 l	5,00 k	3,00 de	11,27 l
Renan	10.10 c-h	11.40	23,50 gh	70,00 b	34,50 b	11,50 b	3,50cd	12,27 cd
Yubileyneya	9.75 d-ı	11.70	25,50 e	90,00 a	30,50 f	5,17 k	3,00 de	11,92 h
Nina	9.60 e-ı	11.65	23,00 h	60,00 c	30,50 f	5,00 k	2,00 f	11,87 h
Popeda	9.40 f-i	11.75	24,00 fg	55,00 cd	24,00 l	6,50 gh	2,50 ef	11,63 i
Sadova	9.40 f-i	11.80	28.00 cd	45,00 fgh	27,00 ik	10,50 c	2,00 f	11,93 gh
Tina	9.10 g-k	11.55	24,50 f	72,50 b	30,00 fg	8,00 de	2,00 f	12,22 de
Alga	8.75 g-k	11.50	29.00 b	42,50 gh	33,50 c	5,50 ik	2,00 f	12,68 b
Dropia	8.600h-k	11.65	24,50 f	45,00 fgh	30,50 f	7,50 ef	3,50 cd	11,55 ik
Krasunya	8.517ıik	11.40	23,00 h	75,00 b	38,50 a	12,50 a	6,00 b	12,08 ef
Selimiye	8.250ıik	11.70	23,17 h	52,50 de	31,83 e	8,00 de	3,50 cd	11,65 i
Pehlivan	8.000ik	11.40	27.67 d	45,00 fgh	29,50 g	5,00 k	3,00 de	12,28 cd
Golia	7.850k	11.35	30.18 a	73,00 b	32,67 d	9,83 c	2,50 ef	13,40 a
Katea-1	7.750k	11.40	23,50 gh	45,00 fgh	27,50 i	6,00 hi	3,50 cd	11,47 k
Gelibolu	4.933l	11.45	25,50 e	70,00 b	32,00 de	12,00 ab	5,00 bc	11,90 h
HKO	1.167		2.486	23.567	1.667	1.899	0.877	0.036

Sedimentation values among bread wheat varieties were showed a significant variation and ranged from 21.50-38.50 ml. The sedimentation values obtained from the closed area were relatively lower than varieties grown in the open area. These results showed that sunn pest damage rate, which was above a certain level, was decreased significant level the sedimentation values. The highest sedimentation value among bread wheat varieties was obtained from Krasunia with 38.50 % and Renan with 34,5 %. However, the lowest sedimentation value was observed from Geya with 21.50 %, Pobeda with 24.00 %, Za 75 with 24.50, which were placed after this variety.

The retarded sedimentation values of bread wheat varieties grown in the closed area were very low. The retarded sedimentation values were changed to 5.0-12.50 ml. While Krasunia variety with 12.50 ml sedimentation value had the highest retarded sedimentation value, Gelibolu with 12.00 %, Guadraluppe with 11.50 ml and Sadova with 10.50 % was took second place. Retarded sedimentation values were found low than 5 ml in many varieties. When sunn pest damage rate in bread wheat varieties is % 8 or the higher,

consumption of grains as food will be quite low.

Sunn pest damage rates in the wheat varieties grown in the closed area were found higher than the varieties grown in the open area in field conditions. According to the two-year average values, Geya variety with 12.75 % among bread wheat varieties was found the first line for sunn pest damage rate. Tekirdağ with 12.75 %, Saraybosna with 12.30 % and Sana with 12.00% were ranked same statistical group with this variety. The lowest sunn pest damage rate among bread wheat varieties grown in the closed area was obtained from Gelibolu with 4.93 %, Kate A1 with 7.75 %, Golia with 7.85 % and Pehlivan with 8.0 %.

Gluten proteins promote formation of a strong and visco-elastic dough structure, and this also affects kneading extension and development of dough (Wade, 1970; Finney *et al*., 1978; Boyacıoğlu, 1994). Gluten content of bread wheat varieties grown in the closed area was quite lower than the varieties grown in the open area. This results indicated that sunn pest damage ratio, which is above a certain value, are affected negatively on gluten content. Gluten content in bread wheat varieties was ranged between 19.00-30.18%. The highest value was obtained from Golia variety with 30.18%. Sana and Alga varieties with 29.00% gluten content were ranked after this variety. Gluten contentin bread wheat varieties grown in the closed area was decreased significantly and the lowest values were obtained from Guadralepe with 19.00 %, Geya with 21.50 %, Odeskaya 226, Krasunia and Selimiye with 23.00 %.

Gluten index in bread wheat shoul be between 60-90%. While the eight varieties amongthe examined varieties had gluten index between these values, the fifteen bread wheat varieties had lower values than these values. When gluten index values in bread wheat varieties are compared, Yubileyneya variety had the highest gluten index value with 90 %. Krasunia with 75.0%, Golia with 72.50 %, Gelibolu, Renan, Tekirdağ ve Saraybosna with 70 % were followed this variety. The lowest gluten index value was observed from Geya and Za 75 variety with 40 %. While gluten index values in some bread wheat varieties were changed between the desirable values, gluten index values in the most of bread wheat varieties were low in this study. According to obtained results, high sunn pest damage ratio had negative effects on gluten index values.

The black point rates on grains of bread wheat varieties were found as high as 2-8%. The highest black point rate in grains was observed from Odeskaya 226 with 8.52%. Krasunia with 6.0%, Geya with 5.03%, Gelibolu and Tekirdağ with 5.0% were followed this variety.

The highest protein content in bread wheat varieties grown in the closed area was obtained from Golia with 13.40 %, Alga with 12.68 %. Tekirdağ and Saraybosna with 12.40 % protein content were followed these varieties. The lowest protein content was found from Guadraluppe variety with 11.47 %. Kate A1 with 11.47%, Dropia with 11.55 % and Geya with 11.57 % were ranked after this variety. The suitable protein content in bread wheat is between 11-13% for food products. Protein content of bread wheat varieties damaged by sunn pest was not show a significant change. It showed that direct effect of sunn pest on the protein content is statistically insignificant.

Accoding to the obtained results, the effect of increased sunn pest damage rates on quality characters of bread wheat varieties grown in the closed area was found negative and statistically significant. Gluten content, gluten index, sedimentation and retarded sedimentation values were the most affected characters by sunn pest damage. When sunn pest damage rates in bread wheat varieties grown in the open field conditions compared withbread wheat varieties grown in the closed area, resistance rates of bread wheat varieties to sunn pest damage was not show differences. The highest values for resistance to sunn pest damage in both experiment were obtained from Gelibolu, Dropia, Krasunia and Kate A1. The most varieties affected by sunn pest

damage were Tekirdag, Geya and Krasnadorska 99. The obtained data demonstrated that genotypic structure of varieties in terms of resistance to sunn pest damage in wheat breeding is highly important. Bread wheat varieties, which are show high resistance to sunn pest damage, are developed in a short time by well organized wheat breeding program.

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ECONOMIC ANALYSIS OF FRESHWATER FISH PRODUCTION IN THE REPUBLIC OF SERBIA

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Abstract
The authors present the basic characteristics of the fish production in the Republic of Serbia. In the case of the fishpond "OZZ Despotovo", in the municipality of Bačka Palanka, the main economic indicators achieved in the production of freshwater fish (carp, grass carp, silver carp, catfish) are analyzed. The observed production requires very high investment per unit area (€ 4,381.39 ha^{-1}). Despite significant investment, achieved economic results are relatively modest (the contribution margin € 892.15 ha^{-1}; the cost-effectiveness ratio 1.09; the profit rate 8.10%). System support of country in the modernization of existing fishponds and raising the new ones, it can significantly contribute to the improvement of production and economic results in the fish production on the territory of the Republic of Serbia.
Key words: freshwater fish production, costs, economic analysis.

Introduction

Limited resources in terms of fishing the saltwater fish species, as well as the growing need for freshwater fish, have led to the expansion of the area under the ponds in many countries around the world (*Trbović et al., 2013*). The potential of aquaculture seems to be even higher when one is considering that the catches of undomesticated fish have been constant or even declining slightly in recent years. Many waters are overfished and cannot provide the quantity of fish needed to satisfy the rising demand. This demand is driven by the growing world population and the overall increase in prosperity (*Subasinghe, 2006*). The emerging supply gap could be covered with products from aquaculture farms (*de Silva, 2001; Delgado et al., 2003*). Also, on the world market, fish from the pond is more valuable, since it is considered as healthier and safer than fish from free fishing. The reason lies in the fact that the ponds, in most cases, are away from the big polluters (*Đinović et al., 2010*).

Consumption of fish per capita is growing constantly worldwide, which is in accordance with the recommendations of nutritionists and doctors. This fact suggests that this type of meat is very important component of a healthy diet (*Ljubojević et al., 2013*). Fish provides the human organism with a sufficient amount of proteins, free amino acids, minerals and vitamins (*Ackman, 2000*), as well as sufficient quantities of polyunsaturated fatty acids (PUFA) (*Kminková et al., 2001*). Previous studies have shown that polyunsaturated fatty acids lower cholesterol levels in the blood, and may be significant in the prevention of cardiovascular disease (*Conor and Conor, 2010*). On the other hand, it also affects on the reduction of mortality among patients with coronary heart diseases (*Kris-Etherton et al., 2002*).

Despite of all these facts, the average annual consumption of fish, in R. of Serbia, according to data of the fishing, ponds production and fish import, is only 5 kg per capita (*Milijašević et al., 2012*). The reasons for the small use of fish in the diet are reflected in the low

purchasing power of the population, limited and inadequate offer on the market and lack of habit of using fish in the diet.

Fisheries consists of fish farming in ponds, open water fishing and fish processing. Aquaculture is defined as the controlled breeding of aquatic organisms such as fish, crustaceans, molluscs and plants (*Schulz et al., 2005*). Fish farming over time has become an important area of industrial production, and agriculture with fisheries takes a very important place in the R. of Serbia's economy. It is still constantly being developed and improved in order to satisfy as many growing nutritional needs of the population as possible. In our country, the main objective of fish farming is the production of consumable fish for the market, but also providing spawn for the needs of fish production and restocking the water out of the pond, in order to maintain fish stocks and contribute to the development of sport fishing (*Hristić and Bunjevac, 1991*).

Unfortunately, in the absence of a fisheries development's strategy in the Republic of Serbia, this industry is left to the uncontrolled and uncontrollable development. This is indicated by the fact that in the last few years the fishing farms, out of fear that they will not sell the produced fish, lowered prices below the production one, and thus create the basis for their material failure. On the other hand, development opportunities are great because the total fish production is only 30% of total consumption in the country, according to data of the Statistical Office of the Republic of Serbia and the Serbian Chamber of Commerce.

Development of freshwater fish production, as well as any other economic activity, should be also followed with adequate development of research in this area. Although R. of Serbia has a long tradition of warm-water fish ponds, as well as a large number of proven experts in practice, there is not enough research on the economics of fisheries (*Čanak, 2012*). For that reason, this paper aims to contribute to complementing the research and literature about the economics of freshwater fisheries. Also, the task of this paper is to provide answers to the most important questions about the cost-effectiveness of dealing with this branch of animal husbandry in agricultural production, based on the specific example of the pond "OZZ Despotovo", where the fundamental economic indicators achieved in the production of freshwater fish, particularly carp, grass carp, catfish and silver carp, were analyzed.

Materials and methods

This paper analyzes the main economic indicators of success that are realized in the fish production in R. of Serbia. The research is based on an analytical calculation of production, and data from the pond "OZZ Despotovo" is used for making the calculation. Calculation is primarily based on the calculation of the direct costs of production and cover margins as the main results. The additional indicators of success (profits, cost-effectiveness ratio and profit rate) will be calculated, in order to get more reliable assessment of the cost-effectiveness of this production. Also, sensitivity analysis method, examines the movement of financial results depending on changes in yield and / or market price, as well as changes in the most important direct inputs.

The paper presents the basic tendencies in fish production in R. of Serbia. For this purpose, we used data from the Statistical Office of the Republic of Serbia, as well as data from FAO base (for the period from 2005. to 2014.), which were analyzed with the classical statistical methods.

Results and discission

Fish production and turnover in the Republic of Serbia

According to the Statistical Office of the Republic of Serbia, in the last decade the area of carp ponds in R. of Serbia significantly increased. Looking at 2014, carp ponds were spread on 8,724 hectares, which is two times

more compared to ten years ago, when the total exploited area were 4,374 ha (2005). The above mentioned growth is accomplished at a rate of 7.89% per annum. The most common fish species are carp (83%), followed by silver and white carp (10.68%), grass carp (3.12%) and at the end catfish (0.30%).

In the same time period, the yield of freshwater fish is at the average level of 748 kg ha^{-1}, but at the same time it should be emphasized that the movement of yields, from year to year, is very unstable, as it is shown by the variation interval (Tab.1). There is a space for improvement of production techniques and technologies, which is also shown by the fact that in the analyzed period a negative rate of change in the yield per unit area is recorded. The total production of the most common fish species in the R. of Serbia is around 6,000 tonnes per year, with the annual growth of 6%. However, it is still only 0.009% of the world total.

Table 1. Areas, yields and production of consumable fish in Serbia (2005-2014)

Indicators	Average value	Coefficient of variation (%)	Variation interval		Rate changes (%)
			Min.	Max.	
Area (ha)	8.079	16,89	4.374	8.940	7,89
Yield (t ha^{-1})	0,75	13	0,59	0,87	-1,82
Production (t)	6.000	19,27	3.806	7.322	6

Based on the data from Table 2, it can be concluded that fish export from R. of Serbia is still symbolic, and it is an average of 126 tons per year. On the other hand, it is encouraging that carp export records growth rate of 15.56% per annum, while export of other types of fish has registered a growth too, but this growth was much more modest.

Table 2. Fish export from R. of Serbia (2005-2014)

Indicators	Average value	Coefficient of variation (%)	Variation interval		Rate changes (%)
			Min.	Max.	
Carp (t)	89,86	67,16	15,50	237,61	15,56
Other fish (t)	36,28	44,07	26	62,64	1,20

Although fish production increased in the last decade, but still together with the amount of fish catched from lakes and rivers, covers less than 30 percent of the domestic demand for fish, while more than 70 percent is imported. However, fish import in R. of Serbia, in the last decade, is characterized by a negative growth rate, 17.52% of carp, and 12.55% for other types of fish respectively (Tab. 3).

Table 3. Fish import in R. of Serbia (2005-2014)

Indicators	Average value	Coefficient of variation (%)	Variation interval		Rate changes (%)
			Min.	Max.	
Carp (t)	628	66	167,90	1.517	-17,52
Other fish (t)	391,72	54,98	61,73	696,64	-12,55

Economic analysis of freshwater fish production

For the analysis of profitability of freshwater fish production in the mixed pond "OZZ Despotovo", total costs are primarily classified into direct and general (Tab. 4). The total cost per unit area are very high, € 4,381.39 ha^{-1}. Such high costs, place aquaculture in rank of highly intensive productions.

Direct costs of this complex production include the following items: the cost of materials (spawn – one-year, two-year, food, hydrant lime, fuel and lubricants, other material), labor costs and direct services. In the framework of the direct costs, costs of pelleted food have the largest share (€ 1,661.78 ha^{-1}, or 37.93%). Nutrition with pelleted complete food allows a higher yield in all the categories of analyzed cyprinid fish (*Ljubojevic et al., 2012*). However, in many systems of fish farming in R. of Serbia, nutrition is still consisted of maize, wheat and barley, which reduces yield, and therefore the quality of produced fish. Also, significant cost represents spawn, which takes more than 1/3 of the share of total production costs. Together with the food cost, they include 72.89% of total costs. The remaining cost of materials (hydrant lime, fuel and lubricants, other materials) have no significant participation, so they take a share of less than 4%. Necessary expenses for labor in the analyzed semi-intensive pond amount to € 500.53 ha^{-1}, or 11.42% of total costs. In intensive production systems, these costs are much lower, so their reduction in the analyzed pond, can significantly affect the level of production economy. Direct services include the maintenance of the pond and do not represent a significant element of the cost. General costs are covered by the corresponding part of the depreciation of buildings and equipment, various overhead expenses, and interest on current assets, since it was assumed that the 1/4 variable investment was financed from borrowed sources of financing.

On the other hand, the value of production is mostly conditioned by the height of the achieved yields of certain fish categories. In R. of Serbia, yields in the fisheries sector, are modest in comparison with the yields which are realized in the world (*Markovic et al., 2014*). This is one of the key causes of low profitability of domestic aquaculture. On the analyzed pond, there is a polyculture, in which the dominant share in income have a three-year and two-year carp (88.66%). The rest of the revenue is generated by the sale of one-year carp, grass carp, silver carp and catfish. The total value of production per unit area, which is accomplished in analyzed pond, is € 4,767.36 ha^{-1}.

Looking at absolute indicators of success (the contribution margin, profit) which are achieved in the analyzed pond, it can be noted that in R. of Serbia relatively modest results are achieved in this production. Height of realized contribution margin (€ 892.15 ha^{-1}) and profit (€ 385.98 ha^{-1}) can not be considered as quality indicators for such an intensive production, which is characterized by high investments per unit of capacity. In almost all branches of agriculture, in R. of Serbia, better results are achieved in relation to the analyzed production, and analyzed pond respectively. Analyzing the relative success indicators, we also come to the same conclusion. The cost-effectiveness ratio, obtained by analyzing the mentioned production is only 1.09, and shows that on € 1 of the total costs, it is achieved € 1.09 of production value. The profit rate shows us that in the observed polyculture € 8.10 of profit is achieved at € 100 of production value. Both indicators have a low value, although this is a production that requires high investments of variable factors per unit of capacity.

Table 4. Calculation of freshwater fish production (2014)

		215 ha	215 ha	215 ha	1 ha	
No.	Costs	Quantity	Price (€ u.m.$^{-1}$)	Value (€)	Value (€ ha^{-1})	Structure (%)
1	Carp yearlings	12.794	2,73	34.927,62	162,45	3,71
2	A two-year carp fry	99.112	2,73	270.575,76	1.258,49	28,72
3	A two-year grass carp fry	2.910	2,05	5.965,50	27,75	0,63
4	A two-year silver carp fry	4.172	1,64	6.842,08	31,82	0,73
5	A two-year catfish fry	2.690	4,09	11.002,10	51,17	1,17
6	Pelleted food 25/7	533.562	0,47	250.774,14	1.166,39	26,62
7	Pelleted food 30/7	208.841	0,51	106.508,91	495,39	11,31
8	Hydrant lime	131.729	0,07	9.221,03	42,89	0,98
9	Fuel and lubricants			21.398,47	99,53	2,27
10	Other materials			6.709,78	31,21	0,71
O	**Cost of materials**			723.925,39	3.367,09	76,85
11	Labor costs			107.614,51	500,53	11,42
12	Direct services			1.630,27	7,58	0,17
A	***Direct costs***			***833.170,17***	***3.875,21***	88,45
13	Depreciation of buildings and equipment, overheads ...			101.226,45	470,82	10,75
14	Interest on working capital (1/4)	180.981,35	4,20%	7.601,22	35,35	0,81
B	***Overheads***			***108.827,67***	***506,18***	11,55
C	**TOTAL COSTS**			**941.997,84**	**4.381,39**	100,00
	The achieved results					
15	Carp 1	22.498	2,73	61.419,54	285,67	
16	Carp 2	74.594	2,73	203.641,62	947,17	
17	Carp 3	296.260	2,38	705.098,80	3.279,53	
18	Grass carp	8.351	1,88	15.699,88	73,02	
19	Silver carp	11.503	1,53	17.599,59	81,86	
20	Catfish	6.312	3,41	21.523,92	100,11	
D	**PRODUCTION VALUE**			**1.024.983,35**	**4.767,36**	
E	**CONTRIBUTION MARGIN (D-A)**			**191.813,18**	**892,15**	
F	**PROFIT (D-C)**			**82.985,51**	**385,98**	
G	**COST-EFFECTIVENESS RATIO (D/C)**				**1,09**	
H	**PROFIT RATE (F/D)*100**				**8,10**	

All absolute and relative indicators of success clearly testify that fish production in R. of Serbia is not at a high level of development, and therefore financial results are not sufficiently good and they are significantly below potential opportunities.

Most branches of agricultural production is inclined to fluctuations in achieved financial results in individual years. Therefore, sensitivity analysis was performed in the analyzed case, wherein tested changes of variables on which the financial result in the freshwater fish production is the most sensitive

(production value, value of food costs, value of expenditures of spawn).

Table 5. The flow of financial results with the change of the most important factors

	The movement of financial results (€ ha^{-1})			
Production value	-40%	-30%	-20%	-10%
	-1.520,97	-1.044,24	-567,50	-90,77
The value of food costs	+40%	+30%	+20%	+10%
	-278,74	-112,56	53,61	219,39
The value of expenditures of spawn	+40%	+30%	+20%	+10%
	-226,69	-73,52	79,64	232,81

It is an obvious lack of tolerance of analyzed production to change in production value. Already with the reduction of this parameter of 9%, the financial result becomes negative. The most important factors on the cost side are the value of food costs and value of expenditures of spawn. The change in their value financial results is more tolerant, since the negative financial result is achieved only with an increase of 24% and 26% respectively.

Conclusions

Based on the analysis it can be concluded that the fish production in R. of Serbia is still underdeveloped, considering that most of the production takes place at the semi-intensive ponds, with outdated supporting infrastructure, while there is a small number od modern fish pond. Production, which is organized in such outdated and semi-intensive ponds, simply is not cost-effective enough, relative to the volume of invested assets, as evidenced by the example of the analyzed fish pond "OZZ Despotovo".

R. of Serbia has a very favorable climatic and soil conditions for freshwater fish production, but the producers face with a subordinate position compared to other branches of agriculture, because there is no adequate stimulative policy for production, processing and marketing of fish. With stimulating support of the state and good fisheries's development strategy, from large importer, Serbia would be able to become an exporter of fish very soon. Export would be based primarily on the European Union's market and Russia, which annually imports about one million tonnes of fish.

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WINE CONSUMER TRAITS

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Abstract
It is essential to attain solidity inside the triangle: experience – satisfaction – and loyalty of wine consumers. However, for this to be achieved, it is necessary to understand wine consumer traits, which is the central objective of this paper. This paper thus lays out the demographic, socio-economic, and psychological traits of wine consumers, followed by the possible categorisations of wine consumers, and finally, types of wine consumer loyalty.
Keywords: wine consumers, wine marketing, strategy of wine development, the Republic of Croatia

Introduction

Consumer behaviour plays an exceptionally important role in the marketing efforts of every business entity. Therefore, knowledge of consumer behaviour for specific products indisputably delivers information of crucial importance for making marketing decisions. It has to be observed foremost by wine makers and retail shops selling wine. However, satisfying the needs of individual consumers and their preferences is in no aspect free of conditions; it is largely made dependent on cultural surroundings, social values, fashion, prestige, available purchasing power and offer. The same goes for the need for wine as a product. A distinction has to be made, however, in the same way, between the need for benefit, the satisfaction of which brings functional advantage, and hedonistic needs related to pleasure and enjoyment or acting self-important before others. It is indisputable that the need for wine is highly propelled by latter needs. Regardless of facts mentioned before, wine consumption in Croatia stands in fierce competition with beer consumption. One should then observe wine consumer traits in view of that fact, so that wine makers and wine distributors in Croatia could intervene with necessary strategic marketing measures in order to achieve possible competitive advantage.

Psychological traits of wine consumers

Two levels of needs can be differentiated when talking about wine consumption. These needs can be answered with two quality levels of wine, although both needs are of the hedonistic kind. So, consumers can consume wine of lower quality and satisfy the simplest hedonistic needs, but they can also consume wines of higher and the highest quality, whereby they not only satisfy hedonistic needs, but also the need for respect and prestige, the need for self-fulfillment. The aforesaid is based on the fact that it is relatively hard to distinguish between need and desire in practice. In the majority of cases, it is extremely difficult to establish exactly when needs turn into desires and vice versa. The easiest way to determine that, is to perceive needs as something that necessarily (in proportions) must be satisfied, while desires mainly indicate the way this satisfaction will be accomplished. Desire, thus, directs us towards the (special) way our needs are going to be satisfied. In other words, desire presents us the way how to attain smaller or greater pleasure along with satisfying our needs. For example,

we can answer our need for wine by drinking a glass of plain table wine, but we may also desire to drink a glass of, say, exquisite French wine of a well-known brand. If we just drink out a glass of plain table wine, the hedonistic need for wine will be satisfied in as much as any other physiological need, but the desire for tasting an exquisite French wine would possibly be left unfulfilled or postponed to the next occasion. Will by satisfying the need for wine at the same time a certain amount of desire also be satisfied, depends on a whole set of different factors, most important among them being: the availability of financial means to buy a certain desired wine, availability of different wines, time availability to buy the wine, surrounding influences, the intensity of the need for wine and so on. Of course, marketing efforts make a certain product seem desirable and attractive to a potential buyer-consumer, and this especially applies to wines of great quality as well as premium wines in the highest price range.

The next psychological consumer traits are motives representing inner forces that govern and instil our behaviour with certain energy to achieve a certain defined goal – the satisfaction of needs, first and foremost. This means that the need turns into a motive when it reaches a high intensity level. Needs are present in coexistence with physiological-psychological and sociological-psychological motives. The first set of motives are innate, the second are acquired. Motives arising from the need for wine undoubtedly stem from acquired motives, especially those coming from reference groups to which the individual is connected. In other words, if the individual has friends who are wine lovers, then he/she shall most probably be a wine lover too, and the other way round, if he/she mingles with beer lovers, he/she shall most probably turn into a beer lover. Motives can be divided into functional and self-expressive motives. Functional motives concern themselves with the attributes of the product, belonging to the lowest levels of need, whereas self-expressive motives look to symbolic attributes of the product, belonging to higher levels of need. When observing the need for wine and the motives which arise from it, we can come to conclude that they agree with the earlier established difference between need and desire. Desires are, in that vein, obviously manifested by self-expressive motives. Functional and self-expressive motives are, of course, intertwined and it is not always easy to keep them strictly apart. For example, drinking a premium wine does not happen merely on grounds of its objective quality attributes, but because of the status symbol it also represents, especially when it is consumed in a restaurant.

It isn't easy to decide which product to buy, since every consumer is at the same time a focal point of a large number of marketing offers, that is to say, a focal point of marketing mixes of different business entities. In the category of wine, this is determined by different brands, wine types, quality attributions of wine at the place of selling, regardless of it being a winery, retail shop or restaurant. The final decision about buying is, in most cases, also additionally influenced by attitudes of other people (amid existence of expected socio-psychological risks) and unforeseen intervening factors in the process of buying. The decision entails a whole range of subdecisions, which only taken together define the final purchasing decision. With regard to wine, these subdecisions are: the choice of the type and brand of wine, the place of buying, the quantity of wine to buy, the time of buying and paying method. The post-purchase consumer behaviour is lastly the result of the achieved level of (dis)satisfaction with the bought wine as opposed to the buyer's expectation prior to buying. It is at best manifested in a repeated purchase, the frequency of purchasing the consumed wine.

Wine customer loyalty

It is, in any case, important to achieve a solidity inside the triangle consisting of experience, satisfaction and wine consumer loyalty. This triangle ought to grow constantly. In other words, this means that each follow up transaction with the wine consumer depends on the level of satisfaction achieved in the transaction preceding it. A segmentation of

wine consumers into groups on the basis of their loyalty should also be carried out, since a marketing mix (product, price, place, promotion) will not address each group in the same way. The question is only, will wine makers address consumer groups they don't find interesting in matters of loyalty. Maintaining a strong connection with wine consumers inspires loyalty which leads to higher customer retention rates. It has long been held crucial to have a good product capable of satisfying consumer's needs. This, however, is merely a necessary assumption like the assumption, among others, to have good raw material for a good product. The greatest asset is to have a good buyer, consumer, and in the long run, to have a client. A good client buys the product of a business entity, stays true to it and will in turn also buy other products by the same business entity. To retain such clients, one should maintain close contact with them, address them personally, get to know them and based on that togetherness aim to satisfy them.Consumer loyalty is manifested by repeated purchase, forgetting of possible mistakes, attracting new customers via "mouth to mouth advertising" by expressing what they like and dislike. Loyalty, however, can be a result of consumer inertia as well as the result of meager competition on the market. It is, in any case, worth to note the practice that many business entities are following, namely, that raising customer retention rates by five percentage points could increase the value of an average customer by 25-100% (Reichheld, 1996).One should bear in mind the following classification of wine consumers based on existing traits of their loyalty:

1. No loyalty
2. Questionable loyalty
3. Potential loyalty
4. True loyalty.

In the first case, wine consumers opt for different wines on different occasions, mostly without any firm reasoning. They view individual wines as similar to others, without gaining any special benefit in buying them from a certain wine maker. To answer this, wine makers came forward with special offers in order to attract wine consumers. In the second case, wine consumers notice small differences between certain wines, brands, but they see no reason to switch between producers. In the third case, a considerable amount of loyalty persists, but it is not that high to include only one wine maker in every purchasing situation. For example, although they claim one specific wine is the best, they fail to order it every time when they, for example, dine at a restaurant. In the end, true wine consumers buy always the same wine brand by the same wine maker.

Wine makers obviously look to retain wine consumers and they view this task as being one of their outmost priorities. By maintaining long-lasting relationships with them, they succeed in customer retention. A high customer retention rate equals a low rate of customer loss**.** Normally, wine customer retention can be defined as the number of consumers doing business with a certain wine maker at the end of the business year in percentage in relation to customers doing business at the beginning of the business year. Let's assume hypothetically that we have 100 wine consumers, each of whom spends 500 € p.a. on wine, that's a total of 50.000 € p.a. An increase in sales revenue of 10% is much easier to attain if each existing consumer spends 50 € more, than to obtain 10 new wine consumers. Every winemaker, thus, has to be capable to satisfy and retain his/her existing wine consumers. Further on, in opposition to satisfaction, which is presented by an attitude, loyalty can be defined in terms of consumer behaviour. A loyal consumer is one who:

- regularly repeats purchase,

- also buys other products of the same wine maker ,
- talks to others about the purchase,
- is immune to the lure of competition.

The simplest formula in this aspect is the following:(Robinette et al., 2001):

loyalty = profit

In other words, the more closer one is to its own consumers, the more successful one is in business. The tighter the bond with your own consumers, the more profitable the business gets. This can be exemplified as shown in figure 1. (Buttle, 2004):

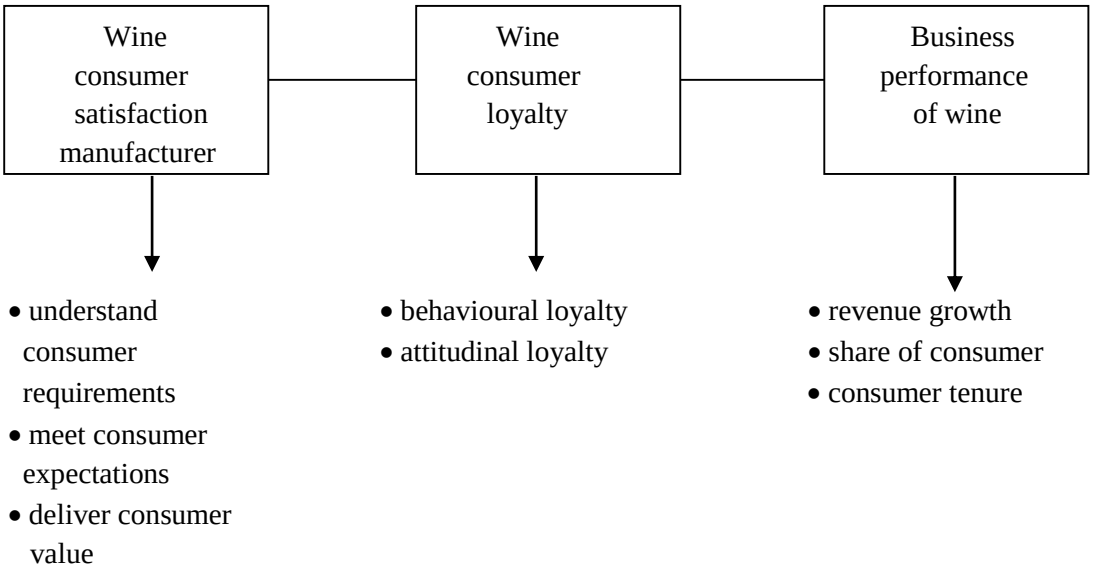

Figure1. Customer satisfaction, loyalty and business performance

In the past three decades wine makers have slowly evolved from focusing on quality to focusing on satisfying consumers and further on, to focusing on loyalty, thereby creating value. Getting the necessary capital, products, employees or even to come up with a product brand isn't the most difficult task for today's wine makers. The hardest part is to accomplish loyalty with wine consumers. The relationship with wine consumers is the fundamental source of value in the new consumer economy. This is why the wine maker shouldn't assume that consumers are going to be loyal (emotion) just because there exists some kind of relationship (behaviour) between them. What represents a challenge in business is how we can convert emotions into transaction, and later on, convert relationship emanating from the transaction into an emotional connection, that is, into unwavering loyalty. The process of establishing loyalty among consumers can ideally be divided into three evolution stages (Rajola, 2003):

1. Knowledge phase: the company must get to know the customer and his expectations. Loyalty is virtually absent from this phase, for the relationship is merely based on the knowledge of products and prices. The customer might very well switch to a competitor boasting a better offer.
2. Emotional or understanding phase: the company watches and analyses the customer's behaviour in all phases leading up to and following the purchase. Loyalty is no longer based only on product and price, for the relationship between customer and provider is getting to be the key factor.

3. Will or loyalization phase: the relationship with the customer must become a personal one. In order to maintain and enhance loyalty, the satisfaction level must be high and reciprocal.

A loyal wine consumer will buy the wine brand he stays true to, inspite of the fact that the competition has, for example, a cheaper wine of the same kind, out of establishing a more favourable relationship based on the "value-for-money" principle. The consumer will even be prone to buy other wines from the same provider based on the so-called cross-selling principle. Building a relationship is, however, a process that takes a very long time to accomplish. Having this in mind, we should consider two things about the way of satisfying customers: a satisfied customer is by no means a loyal customer, and that satisfying a customer does not mean or guarantee his/her retention.

If we can not be sure enough in the level of customer satisfaction, then we should ask what underlies repeated purchase? What underlies it is the loyalty of a customer. Customer retention and customer loyalty are by no means synonyms. A customer can stay true to a wine maker/seller owing to habit or inertia without feeling loyal to it. This is exactly what we came to conclude earlier.

Wine customer classification

It is also possible to talk about life cycles within relationships with customers bearing importance to wine makers in order to establish an adequate interaction with the customer, thereby arriving at a classification of all customers. At the initial stage of the cycle, it is all about the potential new customer. The wine maker makes an effort to attract the attention of that customer. The interactive stage comes into effect when the potential customer realises his/her needs which can be satisfied with available wines on the market from individual providers. This stage ideally ends with a purchase. In the process of buying (so-called adaptive stage) the potential wine buyer evaluates wines from different providers on the market from the vantage point of their level of satisfying current needs on the one hand, and on the other, considering the readiness to pay the asked price for it. This is how we reach the consumption stage in which the customer directly experiences the functional and quality traits of the wine as well as the capabilities of the provider to offer services before the purchase, at the time of the purchase and after the purchase. If the customer is pleased, it is to assume that this cooperative relationship shall continue in the future, whereby customer loyalty is established. Should this not be the case, the customer will break out of the cooperative relationship and turn to one of the competitors. In any case, the wine maker can recognize which stage his/her target customers are in on the basis of the customer life cycle and see which actions and initiatives are required in each of the stages in order to acquire the largest number of customers. Wine consumers can be divided into:

1. Frequent wine drinkers who drink wine only, several times a week
2. Marginal wine drinkers who enjoy wine, but drink it far less, possibly several times a month
3. People who don't drink wine, but drink other alcoholic drinks
4. People who abstain from wine out of different reasons (religious, health and other reasons)

At this stage we may find it interesting to know how wine consumers evolve from consuming cheap table wines to consuming premium wines. Seen through a dynamic perspective, this evolution is for most consumers truly personal, on the one hand, owing to the evolution of taste, and on the other, owing to the increase of purchase power. It is almost unbelievable to have a wine consumer who started off as a so-called *homo-vinifer*, that is, a wine enthusiast consuming costly premium wines. What wine consumers rate in the course of wine selection are the following: price, the price-quality ratio, qualities, origin, benefits, awards, recommendations of others and so on.

However, they also tend to direct their attention to organoleptic properties like aroma, scent and colour, basing their choice in the end largely on personal experience. Wine purchase is continuously motivated by information about wine origin and vintage year, these two being the components of wine quality.

One can certainly claim that wine is bought mostly because of its grape variety, brand name and price (Barber et al., 2006). Wine consumers are largely educated people, adults, with fairly high incomes. It is worth to note, however, that wine consumer habits change with time. The French, for example, according to past statistics, were drinking 80 liters a year; today they are drinking 55 liters a year. Americans were almost exclusively drinking beer, cocktails and whiskey; they are now drinking 8 liters of wine a year. The Japanese were drinking sake and tea; now they are drinking French classified growths and other European fine wines (Resnick, 2008). Wine drinkers can also be divided into the following groups (Moulton and Lapsley, 2001):

1. Connoisseurs account for perhaps 5 percent of wine consumers. They are knowledgeable about wine and demanding in their requirements. They are quality conscious, but some are egocentric, are prejudiced, and "look down at the others."
2. Aspirants account for perhaps 45 percent of wine consumers. They definitely want to know more about wine. They tend to be curious, open-minded, intellectually honest, and ready to experiment. They may suffer from an inferiority complex relative to those more familiar with wine.
3. Newcomers are not very interested in wine and do not drink much of it. They tend to take whatever advice or product is given to them. They represent probably 35 percent of wine consumers.
4. Simple drinkers consume wine by habit or custom, but have no particular interest in it. They may represent 15 percent of wine consumers.

The first three wine consumer categories are equally spread around the world, whereas the fourth category can be found mostly in countries with wine tradition. What's far more important is the fact that only 5% make out true wine connoisseurs and that there is a fairly serious group accounting for 80% of total wine drinkers, represented by the second and third category that can gradually transform by marketing efforts into the first category. The increase of the living standard and purchase power inspires a stable trend manifested by the increase of the first category of drinkers.

The numbers of true wine aficionados is steadily growing. They know a lot about wines and are pretty demanding and critical at the same time. It should not be surprising then that there are more and more people who read about wine, asking questions about it, showing all the more curiosity, becoming enthusiastic about it, debating about it and lastly truly enjoying it. Apart from demographic socio-economical and psychological traits of wine consumers, it is important to find out on what occasions they consume wine, because this information helps to set up a targeted incentive to increase wine purchase. This is all the more important as wine purchase is an act to a greater extent driven by emotion rather than by reason. Wine consumers differ between each other and they have different expectations, experiencing different levels of satisfaction after consuming wine. Some enjoy wine in the quiet of their homes with their loved ones, others enjoy wine in a restaurant in the company of close friends, while some want to make an impression on their business partners during business lunch and so forth. In order to comprehend the psychology behind wine drinking, it is necessary to point out that wine is unique in relation to other consumer goods, in that, wine consumers can't rate its quality attributes, colour and taste unless they buy and taste it.

Conclusion

It is therefore of utmost importance, based on the previous facts, to know wine consumers and their structure well. This is vital for the development of new wine brands as well as the expansion of existing brands. Knowing wine consumer habits as well as their traits is essential to a complete satisfaction of their need for wine as a product. The same goes for the need to understand that almost every consumer's interest in wine emanates from a simple type of interest in wine as an everyday consumer good whose quality is mainly manifested in its taste. This initial interest will in the end grow into a special honest emotion inspired by the wine. In theory, we can differentiate between interest arising from taste, on one hand, and the interest in wine resulting from a caused emotion, on the other. For example, around 80% of wine consumers enjoy the taste of wine, position wine into price ranks, demand an acceptable and somewhat unpretentious wine list alongside responsive service, whereas 20% of wine consumers are top wine connoisseurs who demand a more extensive, but selected wine list, choosing an individual wine meticulously and enjoying it as only connoisseurs can. Wine consumers are, hence, more prone to select quality wines according the "value for money" principle. They have become more educated with respect to wine and they know how to pair wine with food, the knowledge being the result of numerous specialised TV shows, specialised magazines, contributions in daily newspapers, most importantly social media, blogs and other today's IT channels.

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DIFFERENT APPROACHES IN THE IDENTIFICATION OF MEAT ORIGIN BASED ON PROTEIN PROFILING AND SIMPLE PCR

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Abstract

The meat origin, as a fundamental factor for the quality and the usability of its products, can be determined using DNA or protein analysis. In this study, different techniques are applied to determine the origin of different raw meat samples. The DNA analysis of meat is based on the Polymerase Chain Reaction whereas the techniques involving protein analysis are carried out using electrophoresis. Using the SDS-PAGE technique optimized by changing the running conditions, the amount of materials and the gel concentration, it is possible to differentiate different types of meat. We determine the differences in protein profile of poultry meat compared to the other samples in which are identified two specific fractions between 116 and 200 kD in the zone of myosin heavy chains and one bellow 45 kD in the zone of actin. In the beef samples there is a specific fraction in the zone of tropomyosin, while in pork and beef samples appeared a fraction in the zone of myosin light chain. This technique is suitable and can be only used for internal control in production and processing environments because of its low sensitivity. In practice, there are commercialized kits for the identification of meat and meat products based on DNA analysis. All those kits are dealing with specific primers for different type of meat (beef, pork, poultry, goat, horse, etc). In this case we used the primers for ryanodine receptor gene 1 (RYR1) which protein is involved in calcium pathways of the skeletal muscle cells. We amplify DNA isolated from beef, pork and poultry and DNA analysis based on partial amplification of the RYR1 gene showed the difference between mammalian and poultry meat because there is no amplification on the DNA sample isolated from chicken meat.

Keywords: meat, origin, DNA, protein, identification

Introduction

Meat origin can be determined on protein or DNA level using molecular approach. The proteins in meat can be divided into water soluble or soluble in diluted salt solutions (sarcoplasmic proteins 30-50%); soluble in concentrated salt solutions (myofibrillar proteins 50 -60 %) and insoluble in water or salt solutions (binding tissue proteins 15-20%). From myofibrils, myosin has a globular structure and the ability to retain high amounts of water; actin comes in two forms G-actin and F-actin; actomyosin that is insoluble in water and tropomyosin that is viscous and subunit of myosin. As for sarcoplasmic proteins, most important are: myogen, globulin, myoglobulin which gives the characteristic color of meat and binds the oxygen reversely. Insoluble proteins are

collagen and reticulin. (Du M., McCormick R.J.: (2009).

The identification of meat origin on a protein level could be carried out using ELISA or electrophoresis. Electrophoresis is a commonly used technique in many scientific fields, where charged molecules are separated by using an electrical field. Molecules have different migration rates depending on their total charge, size, and conformation. Most electrophoretic systems use an equal and constant voltage on all of the cross-sectional areas of different matrices employed in the electrophoretic separation. Because many biological compounds have charges and ionisable groups, electrophoresis is frequently utilized in biochemical research, as a tool for separation of complex biological molecules such as proteins, nucleic acids, peptides. The "smiling" pattern often seen on slab gel electrophoresis is result of non-uniform heating of the gel (Gersten D.M. 1996). For separation of proteins, polyacrylamide electrophoresis (PAGE) is commonly used. Sodium dodecyl sulfate (SDS) - an anionic detergent is applied to protein sample to linearize proteins and to impart a negative charge to linearized proteins, and proteins are separated only by length of their polypeptide chains. Gels are formed as acrylamide monomer polymerizes into long chains that are linked together by bis molecules (N,N'-methylenebisacrylamide). In order the polymerization to start, ammonium persulfate (APS) and N,N,N',N'-tetramethylethylenediamine (TEMED) are added to form the free radicals. TEMED accelerates the decomposition of persulfate molecules into sulfate free radicals and these in turn, initiate the polymerization. This study reports optimization of the conditions for identification of the origin of different types of raw meat using SDS-PAGE (Macdonald Wick. 2010).

The analysis and identification of meat origin on DNA level can be done using PCR based methods (Mullis K., Faloona F., Scharf S., Saiki R., Horn G., and Erlich H.: 1986 and Поповски З., Танасковска Б., Мискоска – Милеска Е.: 2013) The basis of molecular identification of meat origin on DNA level is an amplification of specific sequence for appropriate animal genome.

Today, modern kits for identification are available (Ilhak I., Arslan A. 2007), but keeping in mind their price this study shows simple techniques that can be used in teaching purposes and in production management. In that sense we applied SDS – PAGE for identification of meat origin on a protein level and simple PCR for amplification of part of RYR1 gene which is commonly present in mammals (Popovski Z. *et al.* 2002)

Materials and methods

As a starting material for this study were used raw beef, pork and chicken meat samples.

The protein analysis was done by SDS-PAGE, where as a referent point is used mixture of 5 proteins with molecular weight of 200 kD, 116 kD, 97 kD, 66 kD, and 45 kD.

The preparation of the reagents for SDS-PAGE was done according to the procedure described by Macdonald Wick (2010).

The gel used for electrophoresis was divided into an upper stacking gel with lower concentration and lower resolving gel with smaller pores. The stacking gel has a role to deposit the proteins at the top of the resolving gel as a narrow band.

The electrophoresis was performed under the voltage of 185 V for 45 min. The analysis of the gels was done with Gene Tools software using G-box device.

Sample preparation. The samples were prepared using 100 mg meat sample mixed with 100 ml Reducing Buffer (2x). This mixture was denatured on 100°C for 3 minutes.

DNA analysis. Isolation of DNA was made using Promega kit for isolation of DNA from animal tissues. The analysis of DNA integrity was made by 0.8% agarose gel electrophoresis (table 1).

Table 1. AGE-reagents

Reagents	Volume
Agarose	0.24 g
dH_2O	30 mL
Etidium Bromide	1 μL (10g/mL)

Table 2. Reagents for master mix

Reagents	Volume
dH_2O	10.65 μL x number of PCR reactions
10x PCR buffer with Mg	2.5 μL x no. of PCR reactions
25 mM $MgCl_2$	2.0 μL x no. of PCR reactions
1 mM Dntp	2.5 μL x no. of PCR reactions
Forward primer (10 pmol)	1 μL x no. of PCR reactions
Reverse primer (10 pmol)	1 μL x no. of PCR reactions
Taq Gold (5 u/mL)	(2u) 0.4 μL x no. of PCR reactions
Total	**20 μL x no. of PCR reactions**

PCR reaction for amplification of part of RYR1 gene was prepared following the protocol in table 2 (Popovski et al. 2002). In 20 μL of master mix were added 5 μL of DNA sample. The PCR was carried out by the following conditions: 95°C for 3 min (initial denaturation); 95°C for 15 sec (denaturation), 65°C for 15 sec (annealing), 72 °C for 45 sec (polymerization)in 40 cycles and terminal elongation at 72°C for 10 min.

Results and discussion

The protein content is the essence of the analysis that can be used for identification of its origin. The most convenient way for determination of the meat protein profile is electrophoresis. Although there are many factors that can affect these data, such as time of slaughter, way of meat storage, the idea is to find a simpler method that excludes these parameters, but is still reliable.

In order to analyze the protein profile, meat samples that contain about 2 μg proteins are shown in figure 1.

Except for the band in the zone under 46 kD at chicken sample, there are two more protein fractions at the same sample between 116 and 200 kD. These bands are also visible in the mixed samples that contain chicken meat (#5,6,8). Taking in consideration the molecular mass of the fractions, it is possible that they stand for isoforms of myosine that vary in these samples.

In order to make distinction between beef and pork, the concentration of the gel was increased from 10% to 15%, so that a better resolution is obtained (figure 2).

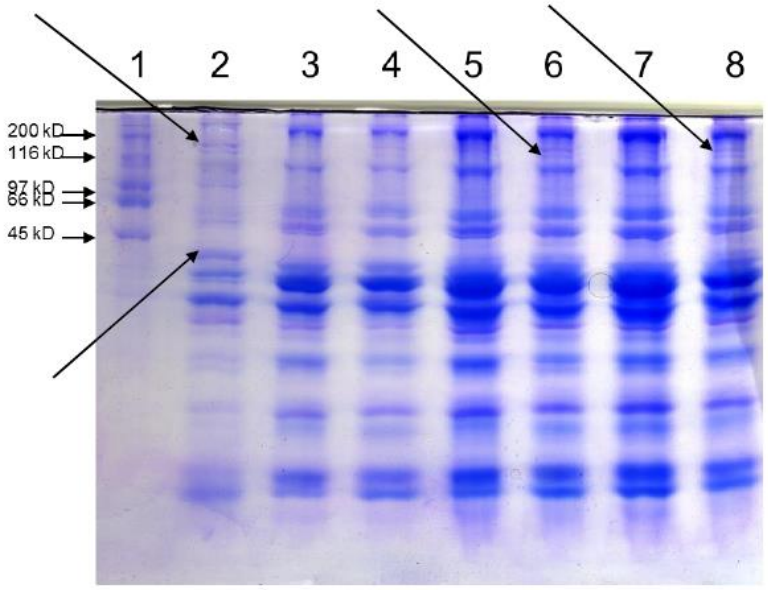

Figure 1. 10% SDS PAGE of meat proteins. #1. 2 μL **standards #2. 2** μL **chicken #3. 2** μL **pork #4. 2** μL **beef #5. 2** μL **chicken + 2** μL **pork #6. 2** μL **chicken + 2** μL **beef #7. 2** μL **beef + 2** μL **pork #8. 0.7** μL **chicken + 0.7** μL **beef + 0.7** μL **pork.**

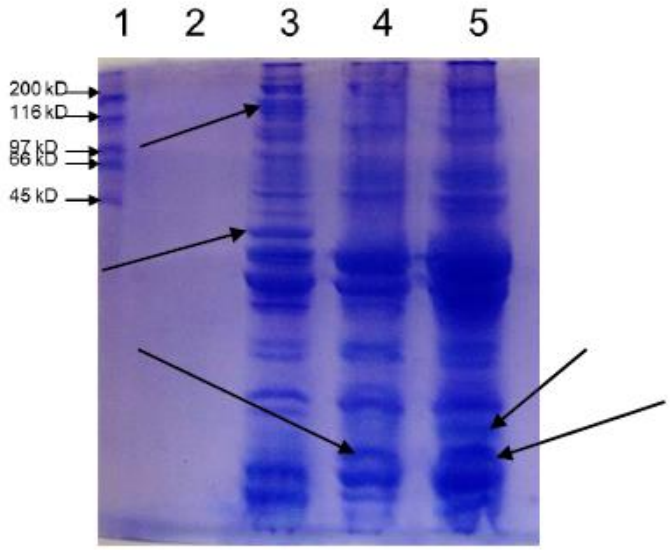

Figure 2. 15% SDS PAGE of meat protein. #1. 4 μL **standards #2. empty #3. 5** μL **chicken #4. 5** μL **pork #5. 5** μL **beef**

Because of the increased gel concentration, more bands can be distinguished in every sample. Besides the presence of two bands in the zone of 116-120 kD and one under 45 kD in the chicken sample compared to the beef sample, another variations are visible that can be used for distinction between pork and beef meat. In the zone of low molecular mass (> 20 kD) at pork and beef samples a protein fractions was showed up that doesn't appear in the chicken sample, whereas at the pork sample, above these fraction, a specific band is visible characteristic only for the pork sample.

There are still issues for the sensibility of this technique. The presence of extremly low amount of meat from different origin is

practicaly impossible to identify by using this method. That's why it is necessary to introduse techniques such as DNA analysis based on PCR.

Apart of the protein analysis that implies identification of protein fractions present or absent in different types of meat, DNA analysis is based on determination of DNA fragment characteristic for appropriate animal genome. The greatest part of the commercial kits are based on target sequences for specific animals for which are designed appropriate primers that are used for detection of the presence certain meat type.

In this study, analysis of the origin of different meat types is done by amplification of RYR1 gene fragment. It is used as marker gene in pig selection because it is part of the intercellular metabolism of calcium, and mutation of its functional segment brings cellular calcium deficiency and presence of pale, watery meat.

The isolated DNA from chicken, pork and beef was amplifyed and analyzed on 3% agarose gel. (figure 3).

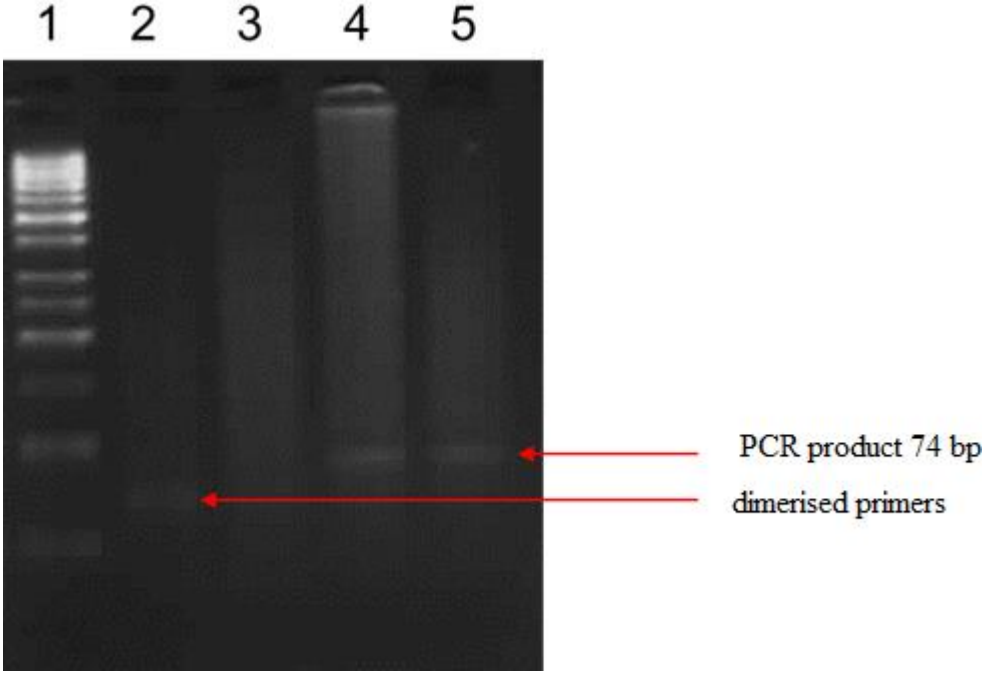

Figure 3. Agarose gel electrophoresis on PCR fragments from RYR1 gene from different meat samples. #1. DNK Ladder 50 -1000 bp # 2. blank. #3. chicken. #4. pork #5. beef.

Based on this electrophoregram can be concluded that there is no amplification of DNA sample from chicken, whereas the specific fragment with 74 bp is present after the amplification of DNA isolated from beef and pork meat.

Conclusion

SDS PAGE of meat protein profile can be used only for screening the meat origin during the production proceses. The sensibility of SDS PAGE could not recommend this method for identifying small amounts of undeclared or forged meat.

DNA analysis based on detection of RYR1 gene fragment is not a good choice for distinguishing different types of meat, because this technique allows only to make difference between poultry and mammal meat.

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THE EFFECT OF APPLYING WITH IMMUNOCYTOPHYTE ON THE CONTENT AND CHEMICAL COMPOSITION OF THE ESSENTIAL OIL FROM COMMON BASIL OF 'TRAKIA' CULTIVAR

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Abstract
The aim of the study was to establish the effect of the leaf treatments with Immunocytophyte on the content, yield and chemical composition of the essential oil distilled from dry leaf and stems biomass and flower spikes of common basil, 'Trakia' cultivar. The experiment was carried out in the period 2007-2009 on the Training-and-Experimental fields of the Agricultural University – Plovdiv, set by the block plot design in four replications, the plot size being 20 m^2. The following variants were studied: 1) Control; 2) 0.5 tablet per 50 m^2, 3) 1 tablet per 50 m^2, 4) 1.5 tablet per 50 m^2, applied three times during the vegetation period before flowering of the second-order branches after basic fertilization with 16 kg/da of nitrogen (applied three times) and irrigation to 80-100% of water holding capacity. The results showed that the application of Immunocytophyte had a negative effect on the essential oil content. Nevertheless, higher essential oil yields were obtained in the treated variants thanks to the higher yield of dry matter. The major components of the essential oil distilled from dry leaf and stem biomass and flower spikes are linalool, limonene, methyl chavicol and methyl cinnamate.
Key words: immunocytophyte, chemical composition trakia cultivar

Introduction

The sweet basil (*Ocimum basilicum L.)* is one of the most ancient medicinal plants and herbs. It is usually grown for the production of ethereal oil and also for obtaining fresh and dry leaves. The most important component of the basil is its ethereal oil. Its content varies from 0.1 to 0.45% depending on the ecological and agro-economic conditions *(Arabaci and Bayram 2004).* The economically significant components of the ethereal oil that are applied in food industry, perfumery and cosmetics are methyl chavicol, methyl cinnamate and linalool. Growth regulators are chemicals applied in small quantities and lead to changes in plant growth, as evidenced by stimulation or inhibition of natural growth. Many researchers establish positive influence of different growth regulators on the vegetative development and extraction of essential oils for different types of basil. According *Mahmod, et al.,* (1996) the application of IAA (indole 3- acetic acid), and kinetin at basil increases increases the content of the essential oil.

Gupta, et al., (1992, 1995) reported that foliar application of triacontanol 10mcg dose has had a positive effect on the yield of fresh herbs and extraction of essential oils in basil.

All these facts allow us to consider that the application of biostimulatorsm stimulates the vegetative growth and the yield of ethereal oil of basil. Considering the aforementioned, the purpose of this survey is to establish the influence of leaf treatments with Immunocytophyte on the content, the yield and the chemical composition of the essential oil distilled from dry leaf-and-stem mass and flower spikes of ordinary basil of the kind Trakiya.

Material and methods

The study was conducted during the period 2007-2009 in the experimental and integration base of the Plant Growing Department at the Agricultural University, town of Plovdiv. We made a field experiment whose main direction was to grow basil as a postharvest crop. The growing of basil starts with the preliminary production of seedlings. The period from the sowing of the seeds to the formation of the seedlings during the years of the survey lasted for 48-54 days. The experiment in the main field was made using the block method in four repetitions as the experimental lot covered an area of 20m^2 located on alluvial (previously marshy) soil. The planting of the seedlings was performed manually using standard seedlings at a distance of 70 cm between the rows and 15 cm within the row itself (96200 plants/ha). It was done on 16.07 during the three years of the survey. The gathering was conducted during the full blooming stage on 06.10 on the 82nd day after planting the seedlings during the years of the experiment. Variants of the factor:

1. Immunocytophyte 0.5 tablet per 50 m^2
2. Immunocytophyte 1 tablet per 50 m^2
3. Immunocytophyte 1.5 tablet per 50 m^2
4- No foliar fertilization (control sample).

*The treatment Immunocytophyte was conducted three times until the blossoming of the offshoots of the second order, using a small sprayer consuming 300 l/ha of solution during background fertilization with N-160 kg/ha (three times until the blossoming of the offshoots from the second order) and also irrigation in order to maintain the limit water absorption of the field – 80-95% in a layer of 0-30 cm.

When conducting the experiment, we examined the following indicators:

- yield of dry mass, content, yield and the chemical composition of the essential oil has been established using the gas chromatograph PYE UNICAM series 204.

The obtained data has been mathematically processed using the programme SPSS.

Results and discussion

During all three years of the experiment, the combination of climatic factors was favourable for the growth of basil. The experimental year 2007 was characterized by the highest average monthly temperature for July and August, which combined with the high relative humidity of the air in August (70%) contributed to the better development of the plants, which resulted in higher yield of dry biomass.

The lowest values of the average monthly relative humidity of the air over the years of the survey were registered in August and early September of the year 2008, which combined with the relatively high temperatures had a negative effect on the growth of the plants, which resulted in lower yield of dry biomass compared to the other two years of the survey.

The variation of the temperature is the main factor affecting the content of essential oil. The registered lower content of oil in the dry leaf-and-stem mass and flower spikes in the year 2007 was due to the sudden drop of the temperature in early September from 28.1 ^{o}C during the third ten days of August to an average temperature of 17.9^{o}C during the first ten days of September. In 2009 the higher temperatures in September and the first ten days of October contributed to the accumulation of a larger quantity of oil in the dry leaf-and-stem mass and flower spikes.

Influence of the leaf treatments with Immunocytophyte on the yield of dry mass

As regards the yield of dry mass, the obtained results show that the tested the leaf treatments with Immunocytophyte increase the yield of dry mass during each of the experimental years (Table 1). The maximum yield of dry mass during the years of the survey has been registered for the treatments with Immunocytophyte - 1 tablet per 50 m^2 with values and a relative increase compared to the control sample during the period of the survey - 8120.2kg/ha (119.4%). The tested foliar fertilizers do not influence the proportion of fresh and dry mass. There are some insignificant differences during the separate years. For the variants included in the survey, it was within 1: 5.55- 5.83 on average for the period.

Table 1. The effect of the leaf treatments with Immunocytophyte on the productivity of dry biomass

indicators	version	2007y.	%	2008 y.	%	2009 y.	%	Average for the period	% compared to controlledlat
Yield of dry matter kg/ha	0- kontrol	6820,31a	100,0	6580,36a	100,0	7000,22a	100,0	6800,29a	100,0
	0.5 tablet	8130,85b	119,3	7690,42b	116,9	8410,02b	120,1	8040,76b	118,3
	1 tablet	8080,11b	118,4	7800,88b	118,6	8630,62b	123,3	8120,2b	119,4
	1.5 tablet	7230,35ab	106,0	6900,57a	104,9	7550,9ab	108,0	7330,61ab	107,8
LSD 5%		101.98		86.76		77.00		78.10	
Ratio Fresh to dry weight	0- kontrol	1: 6,00		1: 5,53		1: 5,45		1: 5,66	
	0.5 tablet	1: 5,77		1: 5,55		1: 5,36		1: 5,55	
	1 tablet	1: 5,92		1: 5,57		1: 5,33		1: 5,64	
	1.5 tablet	1: 6,09		1: 6,02		1: 5,65		1: 5,83	

Evaluation of the leaf treatments with Immunocytophyte on the content of essential oil in the dry leaf-and-stem mass and flower spikes

The aromatic and medicinal features of basil *(Ocimum basilicum)* are related to the presence of essential oil contained in glandular trichomes located on all overground parts.

The data in Table 2 shows that the foliar fertilizers have a negative influence on the content of essential oil. The obtained results are unidirectional during the separate years of

the survey and there are proven differences from the control sample for the treatments with Immunocytophyte. On average for the period, the dry leaf-and-stem mass decreases maximally for Immunocytophyte – to 0.49% compared to the control sample of 0.53% and in the dry flower spikes it decreases most significantly for Immunocytophyte to 2.80% compared to the control sample of 2.89%.

Table 2. The effect of the the leaf treatments with Immunocytophyte on the content of essential oil in the dry leafs and stems biomass and flower spikes.

indicators	version	2007y.	2008 y.	2009 y.	Average for the period	% compared to controlledlat-0
dry leaf and stem biomass						
content of essential oil %	0- kontrol	0.46b	0.55b	0.59b	0.53a	100.0
	0.5 tablet	0.38a	0.52a	0.55a	0.48a	90.6
	1 tablet	0.38a	0.52a	0.56a	0.49a	92.5
	1.5 tablet	0.40ab	0.54b	0.57ab	0.50a	94.3
LSD 5%		0.04	0.02	0.035	0.29	
dry flower spikes						
content of essential oil %	0- kontrol	2.59b	2.95c	3.14b	2.89a	100.0
	0.5 tablet	2.49a	2.83a	3.01a	2.78a	96.2
	1 tablet	2.47a	2.87b	3.06a	2.80a	96.9
	1.5 tablet	2.51a	2.86ab	3.06a	2.81a	97.2
LSD 5%		0.08	0.05	0.08	0.64	

Evaluation of the leaf treatments with Immunocytophyte on the yield of oil

The yield of ethereal oil is a function of the yield of plant mass and the percentage of essential oil. The analysis of experimental data (Table 3) shows that the highest yield of essential oil from the leaf-and-stem mass and the flower spikes during the separate years has been obtained when applying leaf treatments with Immunocytophyte. The maximum yield of oil from the dry leaf-and-stem mass over the years has been registered for the variant1 tablet per 50 m^2 with an average value for the period for 23 kg/ha (17%) and dry flower spikes- 78.5 kg/ha (120%). On average for the period, the maximum total yield of oil was registered for leaf treatments with Immunocytophyte - 1 tablet per 50 m^2 - 101.5 kg/ ha (119%).

Although the applied leaf treatments with Immunocytophyte decreases the content of essential oil in the dry leaf-and-stem mass and flower spikes, the obtained higher yield of oil is due to the registered higher yield of dry leaf-and-stem mass and flower spikes. Thus, the plants compensate for the lower levels of secondary compounds by means of a higher production of biomass, which leads to a higher yield of oil from unit area.

The output is an indicator showing the necessary quantity of raw material for obtaining 1 kg of essential oil. The data about it (Table 3) shows that the values for the variants with leaf treatments with Immunocytophyte are higher owing to the lower content of essential oil found in them. The largest quantity of leaf-and-stem mass for the yield of 1kg of essential oil during the years of the experiment was registered for Immunocytophyte - 1 tablet per 50 m^2 - with an average value for the period of 211.34 kg and dry flower spikes for the variant-36.11kg.

Table 3. The effect of the leaf treatments with Immunocytophyte on the yield of essential oil distilled from dry leafs and stems biomass and flower spikes

version	2007y.			2008y.			2009y.			Average for the period		
	yield of the essential oil кg/ha	%	output	yield of the essential oil кg/ha	%	output	yield of the essential oil кg/ha	%	output	yield of the essential oil кg/ha	%	output
from the dry leaf and stem biomass												
0- kontrol	14.1a	100	217.39	20.0a	100	181.82	24.8a	100	169.49	19.6a	100	189.57
0.5 tablet	17.9c	127	263.15	22.1b	111	192.31	27.7b	112	181.82	22.6a	115	212.43
1 tablet	17.7c	126	263.15	22.3b	112	192.31	28.9b	111	178.57	23.0a	117	211.34
1.5 tablet	16.6b	118	250.00	20.8ab	104	185.19	25.7ab	104	175.44	21.0a	107	203.54
LSD 5%	0.13			0.11			0.14			1.76		
from the dry flower spikes												
0- kontrol	5.00a	100	38.61	74.1a	100	33.90	73.1a	100	31.84	65.7a	100	34.78
0.5 tablet	58.2b	116	40.46	84.4c	114	35.64	85.2b	117	33.52	75.9a	116	36.54
1 tablet	57.7b	115	40.60	88.0c	119	34.95	89.9c	123	32.79	78.5a	120	36.11
1.5 tablet	51.9ab	104	39.90	74.3a	100	35.01	74.7a	102	33.09	67.0a	102	36.00
LSD 5%	0.20			0.36			0.41			3.49		
total yield												
0- kontrol	64.1a	100		94.1a	100		92.9a	100		85.3a	100	
0.5 tablet	76.1c	119		106.5b	113		112.9b	115		98.5a	116	
1 tablet	75.4c	118		110.3b	117		118.8b	121		101.5a	119	
1.5 tablet	68.5b	107		95.1ab	101		100.4 ab	103		88.0a	103	
LSD 5%	0.43			0.62			1.58			4.87		

Influence of the leaf treatments with Immunocytophyte on the chemical composition of the essential oil obtained from dry leaf-and-stem mass and flower spikes

Essential oils are characterized by two or three comparatively high concentrations (20-70%) of some components compared to the other whose quantity is very small *(Burt, 2004).*

Table 4 shows that the following components have been identified: linalool, limonene, methyl chavicol and methyl cinnamate. The component whose content constitutes the largest percentage in the essential oil distilled from the dry leaf-and-stem mass and the flower spikes is linalool and the other constituents rank as follows in descending order: limonene, methyl chavicol and methyl cinnamate.

Table 4. The effect of the leaf treatments with Immunocytophyte on the chemical composition of the essential oil distilled from dry leafs and stems biomass and flower spikes

chemical ingredients %	version	2007y.	2008 y.	2009 y.	Average for the period	% compared to controlledlat	chemical ingredients %	version	2007y.	2008 y.	2009 y.	Average for the period	% compared to controlledlat
		essential oil distilled from dry leaf and stem							essential oil distilled from dry flower spikes				
linalool **%**	0- kontrol 0.5 tablet 1 tablet 1.5 tablet	58.19a 57.06a 57.19a 57.92a	56.53a 55.41a 55.62a 55.99a	56.41a 53.26a 54.98a 56.27a	57.04a 55.24a 55.96a 56.73a	100,0 96,8 98,1 99,5	linalool **%**	0- kontrol 0.5 tablet 1 tablet 1.5 tablet	72.58a 71.15a 70.18a 72.47a	68.35a 67.58a 67.20a 68.32a	64.35a 63.02a 63.20a 64.43a	68.48a 67.25a 66.86a 68.41a	100,0 98,2 97,6 99,9
LSD 5%		4.70	4.45	5.56	3.05		LSD 5%		3.21	3.56	3.71	9.07	
lemonol **%**	0- kontrol 0.5 tablet 1 tablet 1.5 tablet	10.04a 11.11b 10.99b 10.08a	9.48a 10.26b 11.58c 9.68a	9.20a 9.85b 10.03c 9.73b	9.57a 10.41ab 10.87b 9.83a	100,0 108,8 113,6 102,7	Lemonol **%**	0- kontrol 0.5 tablet 1 tablet 1.5 tablet	4.38a 5.23b 5.45b 4.55a	5.10a 5.35b 5.42b 5.29ab	5.24a 6.86b 6.78b 5.24a	4.91a 5.81a 5.88a 5.03a	100,0 118,3 119,8 102,4
LSD 5%		0.66	0.32	0.28	1.29		LSD 5%		0.24	0.22	0.36	1.55	
methyl chavicol - **%**	0- kontrol 0.5 tablet 1 tablet 1.5 tablet	2.73c 2.40b 1.98a 2.40b	2.11b 2.01ab 1.90a 1.93a	2.06b 1.99ab 1.90a 2.02ab	2.30a 2.13a 1.93a 2.12a	100,0 92,6 83,9 92,2	methyl chavicol - **%**	0- kontrol 0.5 tablet 1 tablet 1.5 tablet	0.9a 0.10ab 0.11b 0.08a	0.10a 0.11a 0.13b 0.10a	0.10a 0.12ab 0.14b 0.11a	0.10a 0.11ab 0.13b 0.10a	100,0 110,0 130,0 100,0
LSD 5%		0.15	0.14	0.14	0.58		LSD 5%		0.019	0.017	0.028	0.028	
methyl cinnamate - **%**	0- kontrol 0.5 tablet 1 tablet 1.5 tablet	0.42b 0.37a 0.38a 0.41b	0.40b 0.38ab 0.37a 0.40b	0.53b 0.48a 0.49a 0.51ab	0.45a 0.41a 0.41a 0.44a	100,0 93,2 93,2 100,0	methyl cinnamate - **%**	0- kontrol 0.5 tablet 1 tablet 1.5 tablet	0.40b 0.37ab 0.36a 0.36a	0.52c 0.45b 0.42a 0.50c	0.64b 0.56a 0.55a 0.59ab	0.52a 0.46a 0.44a 0.49a	100,0 88,5 84,6 94,2
LSD 5%		0.030	0.028	0.032	0.15		LSD 5%		0.033	0.028	0.046	0.24	

The applied leaf treatments with Immunocytophyte does not influence the content of linalool in the oil obtained from the dry leaf-and-stem mass. Its average quantity for the period varies from 55.24 to 57.04% in the individual variants and in the oil obtained from dry flower spikes, its quantity varies from 66.86 to 68.48%.

The content of limonene in the oil distilled from dry racemes varies from 4.91 to 5.88% and is almost twice as large in the oil obtained from dry leaf-and-stem mass – 9.57-10.87%. It has been proven that its content increases in the oil obtained from dry leaf-and-stem mass for the period of the survey as its maximum was registered for 1 tablet per 50 m^2 - 10.87%. There is a tendency to an increase of its content in the oil obtained from dry flower spikes for the leaf treatments with Immunocytophyte. Under the influence of treatments with Immunocytophyte, the content of methyl chavicol in the oil obtained from dry leaf-and-stem mass decreases. On the contrary, in the oil obtained from dry flower spikes it has been proven to increase during the years, reaching its maximum after applying per 1 tablet per 50 m^2. The treatments with Immunocytophyte reduce the content of methyl cinnamate in the oil obtained from dry leaf-and-stem mass and flower spikes.

Conclusions

The threefold application of leaf treatments with Immunocytophyte leads to an increase in the yield of dry mass from unit area (kg/ha) in all treated variants. The strongest effect has been registered for Immunocytophyte1 tablet per 50 m^2.

After the threefold treatment with Immunocytophyte, the percentage of the essential oil in the dry leaf-and-stem mass and the flower spikes decreases. This decrease is the largest after applying Immunocytophyte1 tablet per 50 m^2 with proven difference compared to the control sample over the years.

The leaf treatments with Immunocytophyte has a positive effect on the yield of essential oil from dry leaf-and-stem mass and flower spikes from unit area (kg/ha), which increases the total yield. The maximum yield was registered for Immunocytophyte1 tablet per 50 m^2, which increases the yield of essential oil by 19% on average for the period compared to the control sample. The leaf treatments with Immunocytophyte with have a positive effect on the content of limonene in the essential oil distilled from dry leaf-and-stem mass and flower spikes and also on the content of methyl chavicol in the essential oil obtained from dry flower spikes. The leaf treatments with Immunocytophyte do not influence the content of linalool in the oil obtained from the respective organs. The leaf treatments with Immunocytophyte have a negative effect on the content of methyl cinnamate in the oil obtained from dry leaf-and-stem mass and flower spikes and the content of methyl chavicol in the oil obtained from dry leaf-and-stem mass.

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