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## TABLE OF CONTENTS

### Agriculture

H. SZAJŚNER, U. PROŚBA-BIAŁCZYK, E. SACAŁA, A. KOSZELNIK-LESZEK, B. SZUBZDA – <i>The effect of pre-sowing seed stimulation on the germination and pigment content in sugar beet (<i>Beta vulgaris</i> L.) seedlings leaves</i> .....	207
R. WEBER, K. NOWOSAD, H. BUJAK, E. GACEK – <i>Grain yield variability of winter wheat cultivars in post-registration tests in Lower Silesia</i> .....	223
M. ZALEWSKA-KORONA, W. RADZKI, E. JABŁOŃSKA-RYŚ, A. SŁAWIŃSKA – <i>Evaluation of selected physicochemical parameters of twenty-one determinate varieties of tomato fruits (<i>Lycopersicon esculentum</i> Mill.) grown in Poland</i> .....	237
P. ZAPOTOCZNY, K. ŻUK-GOŁASZEWSKA, E. ROPELEWSKA – <i>Physical properties of fenugreek seeds (<i>Trigonella foenum-graecum</i> L.) in multifactorial agricultural experiments</i> .....	245

### Animal Breeding and Husbandry

M. ADAMSKI, J. KUŹNIACKA, N. MILCZEWSKA – <i>Preferences of consumers for choosing poultry meat</i> .....	261
T. DASZKIEWICZ, D. KUBIAK, E. HOŁDYŃSKA, N. PIASKOWSKA – <i>The comparison of meat quality from different carcass cuts of male fallow deer (<i>Dama dama</i> L.)</i> .....	273

### Biology

B. FOJCIK, M. CHRUŚCIŃSKA, A. NADGÓRSKA-SOCHA – <i>Epiphytic habitats in an urban environment; contamination by heavy metals and sulphur in the barks of different tree species</i> .....	283
D. KUBIAK, A. BIEDUNKIEWICZ, A. BALCZUN – <i>Diversity of lichens in forest communities of the “Pupy” nature reserve in the Puszcza Piska Forest (ne Poland)</i> ...	297

### Food and Nutrition Sciences

E.J. BOROWSKA, B. PIŁAT, A. NARWOJSZ, P. URBAN – <i>The effect of drying methods on the content of selected bioactive compounds and fibre in carrot pomace</i> .....	311
--	-----

D. KALICKA, A. ZNAMIROWSKA, M. BUNIEWSKA, M.J. ESTEVE MÁ S, A.F. CANOVES – <i>Effect of stevia addition on selected properties of yoghurt during refrigerated storage</i> .....	323
T. OLEJNICZAK – <i>Post-purchase dissonance – a difficult area of research in Poland</i>	335

### **Environmental Protection**

M. PTA K, B. NOWAK – <i>Thermal-oxygen conditions in Lakes Roś and Rospuda Filipowska (North-Eastern Poland) in the summer half-year 2005–2014</i> ....	343
---	-----

### **Fishery**

I.M. BATYK, M. WOŹNIAK – <i>Effect of socioeconomic characteristics of consumers on preferences of purchase and consumption of freshwater fish</i> .....	357
A. SKRZYPCZAK, <span style="border: 1px solid black; padding: 0 2px;">A. SPRINGER</span> , A. GOŹDZIEJEWSKA, G. FURGAŁA-SELEZNIOW – <i>How to effectively collect zooplankton in illuminated cages for fish rearing?</i> .....	371

### **Humans and Environment**

G. FURGAŁA-SELEZNIOW, A. LEWKOWSKA, M. JANKUN, P. WOŹNICKI, P. RUTKOWSKA, A. SKRZYPCZAK – <i>Analysis of the visitor flow and forest education actions in Kudypy Forest District</i> .....	389
--	-----

### **Veterinary Medicine**

I. BABIŃSKA, E. KUCZEWSKA, J. KONKIEL, M.Z. FELSMANN, J. SZAREK, K. POPLAWSKI, A. SNARSKA – <i>Selected aspects of humane animal protection in Polish law</i> ..	407
--	-----

## **SPIS TREŚCI**

### **Rolnictwo**

H. SZAJSNER, U. PROŚBA-BIAŁCZYK, E. SACAŁA, A. KO SZELNIK-LESZEK, B. SZUBZDA – <i>Wpływ przedsewnej stymulacji na kiełkowanie i zawartość barwników w liścieniach buraka cukrowego (Beta vulgaris L.)</i> .....	207
R. WEBER, K. NOWOSAD, H. BUJAK, E. GACEK – <i>Zmienność plonowania odmian pszenicy ozimej w doświadczeniach porejestrowych na Dolnym Śląsku</i> .....	223

M. ZALEWSKA-KORONA, W. RADZKI, E. JABŁOŃSKA-RYŚ, A. SŁAWIŃSKA – <i>Ocena wybranych parametrów fizykochemicznych owoców 21 odmian samokończących pomidora gruntowego (<i>Lycopersicon esculentum</i> Mill.) uprawianego w Polsce</i>	237
P. ZAPOTOCZNY, K. ŻUK-GOŁASZEWSKA, E. ROPELEWSKA – <i>Zmiany właściwości fizycznych nasion kozieradki pospolitej (<i>Trigonella foenum-graecum</i> L.) uzyskane w wieloczynnikowych eksperymentach agrotechnologicznych</i>	245

### **Chów i hodowla zwierząt**

M. ADAMSKI, J. KUŹNIACKA, N. MILCZEWSKA – <i>Preferencje konsumentów przy wyborze mięsa drobiowego</i>	261
T. DASZKIEWICZ, D. KUBIAK, E. HOŁDYŃSKA, N. PIASKOWSKA – <i>Porównanie jakości mięsa pochodzącego z różnych elementów tuszy daniela (<i>Dama dama</i> L.)</i>	273

### **Biologia**

B. FOJCIK, M. CHRUSCIŃSKA, A. NADGÓRSKA-SOCHA – <i>Siedliska epifityczne w środowisku miejskim; zanieczyszczenie kory różnych gatunków drzew metalami ciężkimi i siarką</i>	283
D. KUBIAK, A. BIEDUNKIEWICZ, A. BALCZUN – <i>Różnorodność porostów w zbiorowiskach leśnych rezerwatu „Pupy” w Puszczy Piskiej</i>	297

### **Nauka o żywności i żywieniu**

E.J. BOROWSKA, B. PIŁAT, A. NARWOJSZ, P. URBAN – <i>Wpływ sposobu suszenia wytlóków z marchwi na wybrane składniki bioaktywne i błonnik</i>	311
D. KALICKA, A. ZNAMIROWSKA, M. BUNIOWSKA, M.J. ESTEVE MÁS, A.F. CANOVES – <i>Wpływ zastosowania stewii na wybrane cechy jogurtu przechowywanego chłodniczo</i>	323
T. OLEJNICZAK – <i>Dysonans pozakupowy – trudny obszar badań w Polsce</i>	335

### **Ochrona środowiska**

M. PTAK, B. NOWAK – <i>Warunki termiczno-tlenowe jeziora Roś i Rospuda Filipowska (północno-wschodnia Polska) w półroczu letnim 2005–2014</i>	343
---	-----

### **Rybactwo**

I.M. BATYK, M. WOŹNIAK – <i>Wpływ cech socjoekonomicznych konsumentów na preferencje zakupu i spożycia ryb słodkowodnych</i>	357
--	-----

- A. SKRZYPCZAK, A. SPRINGER, A. GOŹDZIEJEWSKA, G. FURGAŁA-SELEZNIOW – *Jak efektywnie pobierać zooplankton w sadzach oświetlonych do podchowu ryb . .* 371

### **Człowiek i środowisko**

- G. FURGAŁA-SELEZNIOW, A. LEWKOWSKA, M. JANKUN, P. WOŹNICKI, P. RUTKOWSKA, A. SKRZYPCZAK – *Analiza ruchu odwiedzających i działań związanych z edukacją leśną w Nadleśnictwie Kudypy . . . . .* 389

### **Medycyna weterynaryjna**

- I. BABIŃSKA, E. KUCZEWSKA, J. KONKIEL, M.Z. FELSMANN, J. SZAREK, K. POPLAWSKI, A. SNARSKA – *Wybrane aspekty humanitarnej ochrony zwierząt w prawodawstwie polskim . . . . .* 407

**THE EFFECT OF PRE-SOWING SEED STIMULATION  
ON THE GERMINATION AND PIGMENT CONTENT  
IN SUGAR BEET (*BETA VULGARIS* L.)  
SEEDLINGS LEAVES**

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**Key words:** pigments, sugar beet, germination, laser radiation, magnetic field.

**Abstract**

Two methods were used in a laboratory experiment to assess the effect of stimulation of sugar beet seed clusters. Four duration times of exposure to magnetic field (MF) were used:  $P_1$  (10 s),  $P_2$  (30 s),  $P_3$  (60 s) and  $P_4$  (300 s) and two different doses of semiconductor laser radiation (LR) applied (five- and ten-fold irradiation by the basic dose of  $0.25 \text{ J cm}^{-2}$ , respectively). Subsequently, the sowing value of the seeds, the morphological traits of the seedlings and pigment content in their leaves were assessed. Stimulation of the seed germination energy and capacity was recorded. Seed vigor, their germination speed and germination time improved significantly under the influence of MF. The radicle elongated after both treatments. The pigment condensation in seedlings was stimulated most intensively by the lower of the two LR doses used. The obtained results allow the statement that magnetic field and laser radiation modify the germination process of sugar beet.

**WPLYW PRZEDSIĘWNEJ STYMULACJI NA KIELKOWANIE I ZAWARTOŚĆ  
BARWNIKÓW W LIŚCIENIACH BURAKA CUKROWEGO (*BETA VULGARIS* L.)**

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**Słowa kluczowe:** barwniki, burak cukrowy, kiełkowanie, promieniowanie laserowe, pole magnetyczne.

**A b s t r a k t**

W doświadczeniu laboratoryjnym oceniano efekty stymulacji kłębków buraka cukrowego dwiema metodami. Zastosowano cztery ekspozycje pola magnetycznego (MF)  $P_1$  (10 s),  $P_2$  (30 s),  $P_3$  (60 s) i  $P_4$  (300 s) oraz dwie zróżnicowane dawki promieniowania lasera półprzewodnikowego (5-krotną i 10-krotną, dawka podstawowa naświetlenia wynosiła  $0,25 \text{ J cm}^{-2}$ ). Oceniano wartość siewną nasion, cechy morfologiczne siewek oraz zawartość barwników w liściach siewek. Obserwowano wpływ badanych czynników na stymulację energii i zdolności kiełkowania. Wigor nasion, szybkość i czas kiełkowania istotnie się poprawiły pod wpływem działania pola magnetycznego. Korzeń zarodkowy wydłużył się po zastosowaniu obu sposobów traktowania nasion. Kondensację badanych barwników w największym stopniu stymulowało 5-krotne naświetlanie promieniami lasera.

**Introduction**

The high seed quality is decisive on the yield quantity and quality (ZAHOORET al. 2007). Therefore seed refining became a commonly applied, indispensable treatment, that aims at enhancement of the seedsvigour and reduction of the variability of their physical and chemical characteristics. At the present time, the chemical, as well as physical and physiological methods of seed refining are known (ANDREOLI and KHAN 2000, GRZESIK at al. 2012, JAKUBOWSKI 2015, JALALI and SALEHI 2013) The physical methods were developed by the end of the last century) (LIPSKI et al. 1996, ALADJADJYAN 2007, DELIBALTOVA and IVANOVA 2006).

The most important among them are: the irradiation by the ionizing and microwave radiation (WÓJCIK et al. 2004) application of the constant and alternating magnetic field (PIETRUSZEWSKI 1992, PROKOP et al. 2002a, 2002b) and treating of the seed material with laser radiation (GŁADYSZEWSKA 2011, HERNANDEZ et al. 2008, SZAJNSNER 2009 SZAJNSNER et al. 2014).The application of these methods is legitimate on the merits of pro-ecological actions: although

the magnetic field is no hazard to the environment, it may improve the seed vigour through the enhancement of the physiological processes in plant, and may as such be used as an auxiliary factor, supplementing other methods of seed refining (ROCHALSKA 2002a) or cultivation especially under stress conditions (PEJIC et al. 2011). Magnetic fields, especially those of low frequency, affect positively plant development as they stimulate transport of chemical substances in a plant. According to ROCHALSKA (2002a) this effect takes place through the alteration of the opening patterns of ion channels. The influence of magnetic field was investigated in many species of agricultural and horticultural plants, including vegetables and arboricultures. Pre-sowing treatment of the seed material with laser radiation exerts positive effect on the seed germination, the initial plant development and on the quantity and quality of yield in cereal plants (SZAJSNER 2009), as well as in vegetables (DROZD and SZAJSNER 2007), root crops (SACAŁA et al. 2012) and in legumes (PODLEŚNY 2002), with no concurrent detrimental effects on the natural environment.

First research in Poland on the seed treating with MF was conducted by KOPEĆ (1985). The materials in that study were the seeds of the sugar beet cultivar (cv.) PN Mono 3. The Early Plant Development (EPD) technology is a new method in seed refining of the sugar beet. Treating seeds with the EPD method makes it possible to obtain fast and regular emergence of plants and their accelerated development, both of which positively affect the plant yield. As demonstrated in many experiments with sugar beet seeds, the seed stimulation resulted in an increase of the mass of the store root and of the sugar content in it. It also enhanced the technological quality of the yield, as the water-soluble ash content was observed to be lower (WÓJCIK 2001).

The aim of the study was to investigate the effect of different doses of semiconductor laser radiation (LR) and magnetic fields (MF) on the initial growth and pigment content in sugar beet (*Beta vulgaris* L.) seedlings leaves.

## Material and Methods

Seed clusters of sugar beet cv. Alegria and cv. Sporta (Syngenta Sp. z o.o. Poland) were analysed in a laboratory experiment. The clusters were subject to two methods of stimulation: laser radiation (LR) at the doses  $D_5$  and  $D_{10}$  (five- and ten-fold irradiation by the basic dose of  $0.25 \text{ J cm}^{-2}$ , respectively) and magnetic field (MF) of 30 mT induction. Four duration times of exposure to MF were used:  $P_1$  (10 s),  $P_2$  (30 s),  $P_3$  (60 s) and  $P_4$  (300 s). Laser stimulation was carried out using semiconductor laser (model CTL-1106MX, CTL – Centrum Techniki Laserowej – Laser Instruments Sp. z o.o. Poland), of 200 mW

power and 670 nm wavelength. The irradiated fragment of the seed surface was determined by the CTL 1202 S scanner, coupled with the laser.

The irradiated as well as the non-treated (reference) seed clusters were sown in plastic trays. Cuvette lined with filter paper moistened with distilled water and placed in climate chamber MLR-351 H (SANYO Electric Co., Ltd. Japan). The experiment was established in four replicates of 50 seeds each.

The assessment of the seed parameters affecting their sowing quality: the germination energy (as % of normal seedlings – 4 days after sowing) and capacity (14 days after sowing) was carried out in laboratory conditions, according to the method proposed by ISTA (2008). The vigor index of the control and stimulated seed clusters was calculated as the product of the mean sprout length (cm) multiplied by the mean germination capacity [%] according to PANASIEWICZ (2008). The speed germination index was calculated according to Maguire, (JAKUBOWSKI 2015) as the sum of the quotients of the number of normally germinated seeds to the number of days elapsed since the sowing date until the removal of the seeds from the tray.

$m$  – number of the normally germinated seeds;

$d$  – number of days elapsed since the sowing date until the removal of the seeds from the tray

$$\text{Maguire index} = \frac{m_1}{d_1} + \frac{m_2}{d_2} + \dots + \frac{m_n}{d_n}.$$

The index of the average germination time of one seed according to Pieper (JAKUBOWSKI 2015) was calculated as the quotient of the sum of products of the number of normally germinated seeds on a particular day and the number of days elapsed since the sowing date until the removal of the seeds from the tray (*i.e.* the numerator in the formula), to the sum of these days (*i.e.* the denominator in the formula).

$$\text{Pieper index} = \frac{(m_1d_1 + m_2d_2 + \dots + m_nd_n)}{(m_1 + m_2 + \dots + m_n)}$$

The mean number of seeds germinated per day, according to Kotowski, (JAKUBOWSKI 2015) was calculated as the quotient of the sum of normally germinated seeds, to the sum of products of the number of normally germinated seeds on a particular day and the number of days elapsed since the sowing date until the removal of the seeds from the tray.

$$\text{Kotowski index} = \frac{(m_1 + m_2 + \dots + m_n)}{(m_1d_1 + m_2d_2 + \dots + m_nd_n)}$$

Furthermore, the morphological traits of the seedlings such as the length of the radicle, the length of the epigeal portion of the seedling and that of cotyledon, were compared, and the alterations in carotenoids and chlorophyll pigment content in the seedling leaves were assessed.

Photosynthetic pigments extraction and determination.

Plant material (0.2 g of cotyledons) was homogenized in chilled 80% (v/v) acetone and then centrifuged at  $5000 \times g$  for 10 min. at 4°C. The obtained supernatant was collected and put to a flask a 25 cm<sup>3</sup> and filled by 80% acetone to a final volume 25 cm<sup>3</sup>. The absorbance of obtained extracts was recorded at 470, 647, 663 nm (Cecil Aurius Series CE 2011 Visible Spectrophotometer (Cecil Instruments Limited, Milton, Cambridge, Great Britain)) and the concentrations of chlorophyll a, of chlorophyll b, total chlorophyll (chlorophyll a + chlorophyll b) and carotenoids were calculated using LICHTENTHALER (1987) and LICHTENTHALER and BUSCHMAN (2001) equations.

The study was carried out according to commonly recognized standards of laboratory experiments. The data obtained were analysed by two-way ANOVA (The statistical analysis of the results was performed with the STATISTICA 10.0 software (Stat Soft®, Inc., USA), with cultivars and the methods of stimulation applied to seed clusters as two experimental factors. The significance of the two sources of variation was tested with Snedecor *F* test, whereas the homogenous groups were determined using Duncan's multiple comparison test.

## Results

The control plants of both varieties which were tested did not differ significantly from each other with regard to energy and germination capacity (Figure 1).

The LR and the MF applied in the experiment enhanced significantly the values of the germination energy and capacity, compared to the reference seeds. Although the longest exposure to MF, P<sub>4</sub>, resulted in a significant reduction of germination energy compared to reference (Figure 2a). For germination all variants caused a stimulating effect (Figure 2b). Using P<sub>1</sub>, P<sub>2</sub> and P<sub>3</sub> exposure times caused an increase in that same parameter value in Alegria cv. On the other hand, in Sporta cv. such stimulation was observed as the effect of the MF exposure defined as P<sub>2</sub>, P<sub>3</sub> and P<sub>4</sub> (Figure 3). Moreover, the P<sub>1</sub>, P<sub>2</sub>, P<sub>3</sub> and P<sub>4</sub> exposures resulted in a significant enhancement of the vigor in the examined sugar beet (both varieties) seeds (Figure 4).

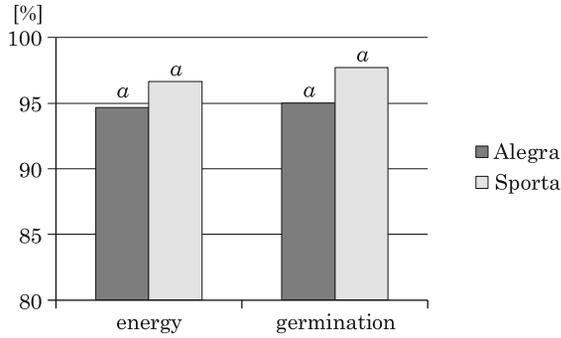


Fig. 1. Energy and germination capacity for control:  $LSD_{\alpha = 0.05}$ ; energy = 3.75; germination = 4.15

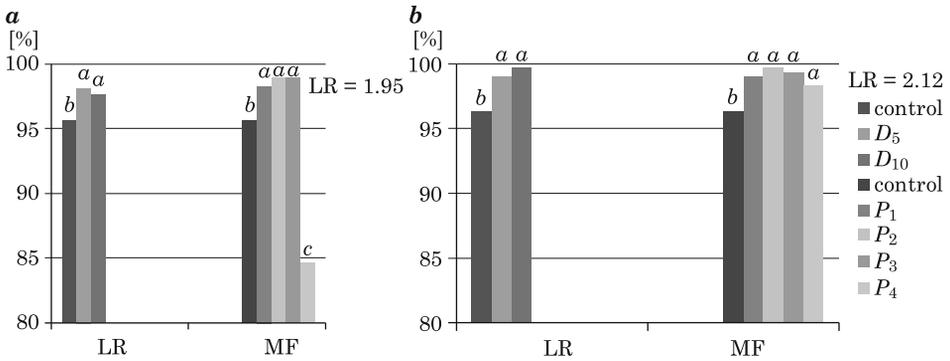


Fig. 2. The energy (a) and germination capacity (b) – average value.:  $LSD_{\alpha = 0.05}$ ; MF = 1.71

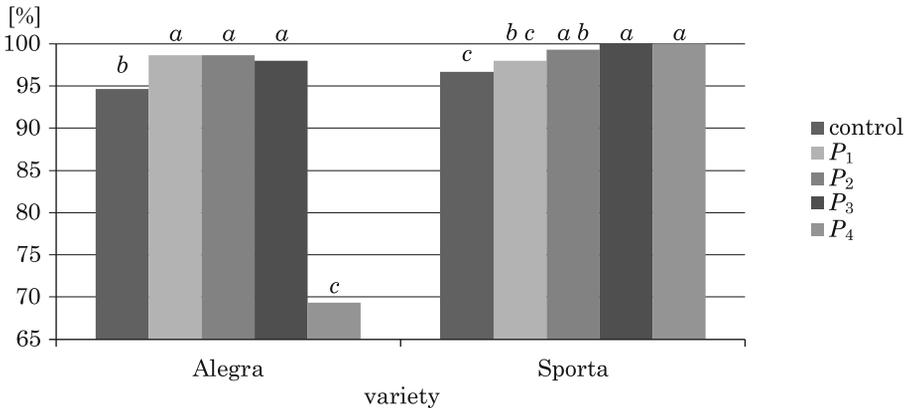


Fig. 3. The effect of stimulation (MF) on germination energy – interaction (variety x doses):  $LSD_{\alpha = 0.05} = 2.41$

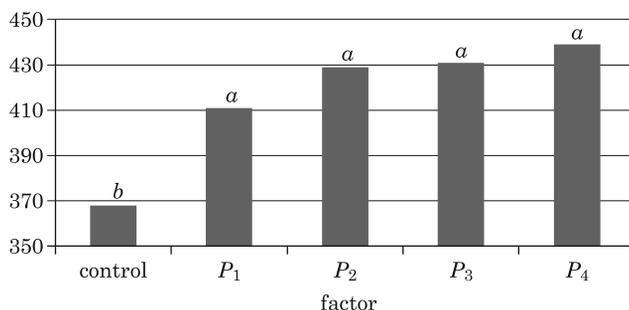


Fig. 4. The effect of stimulation (MF) on vigor – average value:  $LSD_{\alpha = 0.05} = 37.4$

Germination indices were calculated as well for both varieties. Pieper index, giving the average germination time per one seed indicates that the dose of  $D_{10}$ , seeds need longer time to germinate but a dose  $D_5$  significantly shortens the time. Based on the indices of Kotowski and Maguire it may be concluded that, the  $D_{10}$  dose decreased the average number of germinated seeds, and  $D_5$  germination rate significantly (Figure 5).

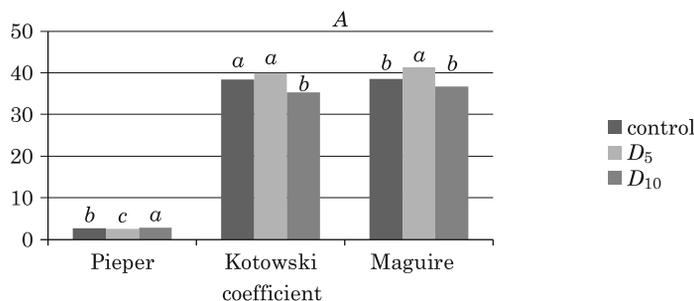


Fig. 5. The coefficient of germination – average value:  $LSD_{\alpha = 0.05}$ ; Pieper = 0.129; Kotowski = 1.969; Maguire = 2.662

The interaction calculated for Pieper index allowed for conclusion that, use of magnetic field  $P_4$  resulted in elongation whereas application  $P_3$  shorter germination Alegry cv., Maguire index showed a significant increase in the average number of seeds germinated after application fields  $P_2$  and  $P_3$ . In Sporta cv. no significant effects were observed of none of the applied pre-sowing seed stimulation techniques (Figure 6).

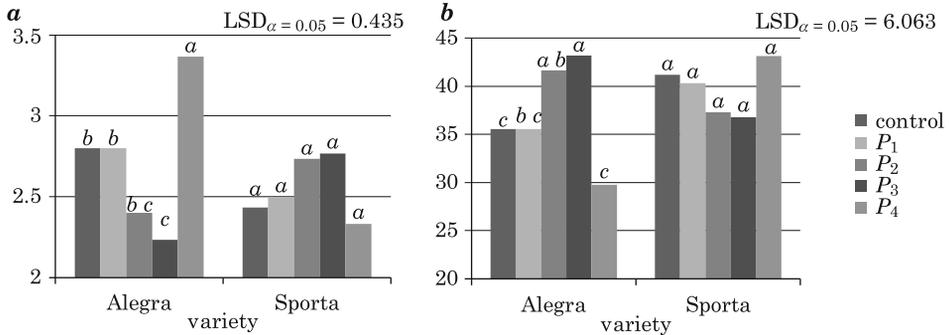


Fig. 6. The coefficient of germination to Pieper (a) and Kotowski (b) (MF) – interaction (variety x doses)

The seedlings of Alegra cv. produced shorter radicles and cotyledons. Significant stimulation (for two varieties) was observed in radicle length trait after each one of the treatments applied: the effect oscillated between the length enhancement by 20.2 mm after the  $P_1$  duration of MF exposure and the enhancement by > 20.4 mm after the  $D_{10}$  dose of LR (Figure 7a).

The seeds of Sporta cv. showed higher sensitivity, the LR applied, with respect to the length of radicle by 34.8 mm. Alegra cv. did not react to the laser light (Figure 8).

When compared to the cotyledons of the reference seedlings, the seedlings grown from the seeds exposed to  $D_5$  dose of LR and to the MF through the  $P_1$ ,  $P_3$  and  $P_4$  durations, had shown significant stimulation of the cotyledon length (Figure 7b). The length of the seedlings (both varieties) was extended only under the influence of the MF –  $P_2$ ,  $P_3$  and  $P_4$  (Figure 7c).

Statistical analysis indicated significant variation of the content of particular pigments in the leaves of the examined seedlings, after application of the pre-sowing seed stimulation techniques. The highest content of carotenoid pigments, compared to the reference plants, was found in the seedlings grown from the  $D_5$  – and  $P_1$  treated seeds (LR and MF respectively). An increase in the carotenoid content was also observed after  $P_3$  duration of MF (Figure 9).

In Alegra cv., the stimulating effect of the five-fold LR ( $D_5$ ). In Sporta cv., an increase in carotenoid content was observed after five- and ten- folddose of LR (Figure 10).

Chlorophyll a content increased significantly too as the effect of  $D_5$  dose of LR and MF applied over  $P_3$  and  $P_4$  exposure times. Analysis of the data obtained also revealed an interaction of cultivars and treatments. The content of chlorophyll a increased under the influence of all the factors used with the exception of MF  $P_4$  (Figure 9).

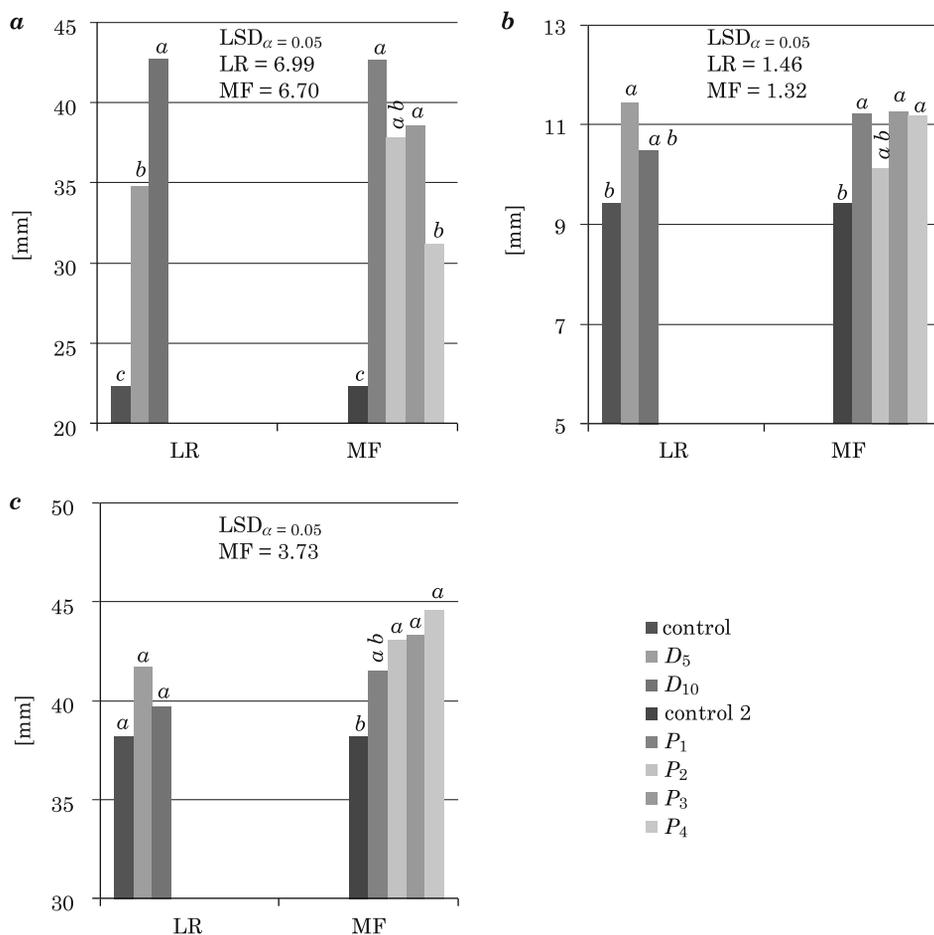


Fig. 7. Influence of seeds treatment for the root (a), cotyledon length (b) and seedling length (c) - average value

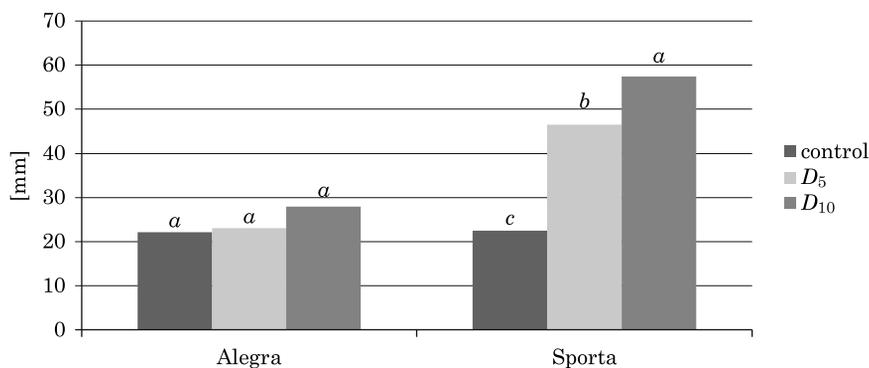


Fig. 8. The influence of laser radiation on root length - interaction (variety x doses): LSD $_{\alpha} = 0.05$ ; LR = 9.88

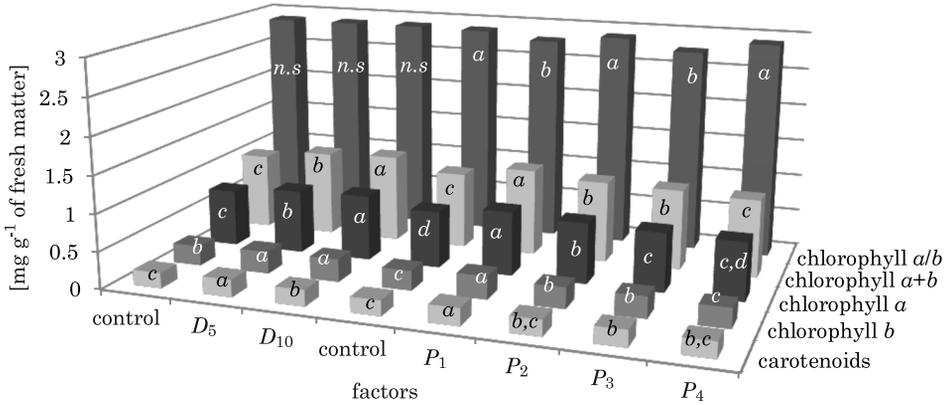


Fig. 9. The influence of pre-sowing stimulation on pigments content – average value:  $LSD_{\alpha} = 0.05 = LR$ ;  $chl\ a\ b^{-1} = n.s.$ ,  $MF = 0.0844$ ;  $chl\ a + b = 0.0250$ ,  $MF = 0.0295$ ;  $chl\ a = 0.0179$ ,  $MF = 0.0170$ ;  $chl\ b = 0.0126$ ,  $MF = 0.0121$ ; carotenoids =  $0.0039$ ,  $MF = 0.0121$ ; a, b, c – homogeneous groups

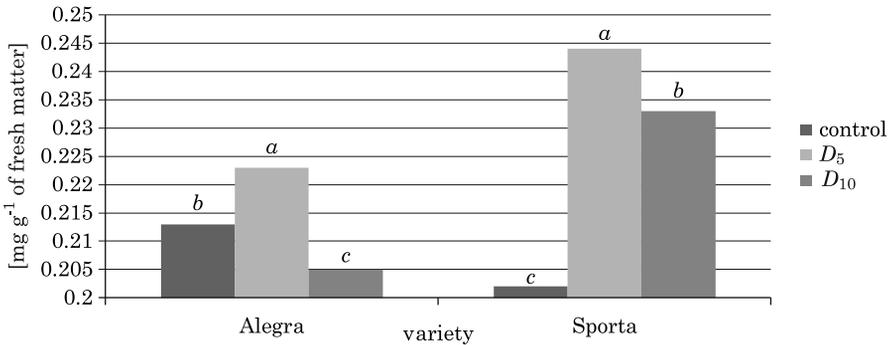


Fig. 10. The influence of pre-sowing stimulation on carotenoids pigments content – interaction (variety x doses):  $LSD_{\alpha = 0.05} = 0.0056$

Based on the interaction observed in a Alegra cv. increase in carotenoids under the influence of dose  $D_5$ , in Sparta cv. highest condensation observed under the influence of dose  $D_5$ , less affected dose  $D_{10}$  (Figure 10). The content in seedlings of the chlorophyllb increased after the seeds were treated with the  $D_5$  and  $D_{10}$  dose of LR and MR –  $P_1, P_2, P_3$  (Figure 9). In seedlings grown from the seeds treated with both doses of LR and exposed to MF over all of the duration times except  $P_4$ , significant enhancement of chlorophyll  $a + b$  content was observed. The highest stimulation of the chlorophyll  $a + b$  content was obtained after  $D_{10}$  dose of LR and after  $P_1$  of MR (Figure 9).

The interaction between cultivars and treatments demonstrated statistically significant stimulation of chlorophyll  $a$  content in Alegra cv., resultant from each one of the experimental factors applied, at all rates and over all of the exposure times.

In Sporta cv., the longer of the four MF exposure times:  $P_3$  and  $P_4$ , significantly enhanced the concentration of chlorophyll *a* in the leaves of the seedlings.

The content of chlorophyll *a* + *b* was stimulated in Alegra cv. compared to the reference plants, as the effect of all the applied treatments except  $P_4$  exposure to MF. In Sporta cv. after treating the seeds with MF –  $P_3$  and  $P_4$ .

The quotient of chlorophyll *a* to chlorophyll *b* oscillated between 2.64 and 2.96 depending on the applied treatment and the examined cultivar (Figure 11).

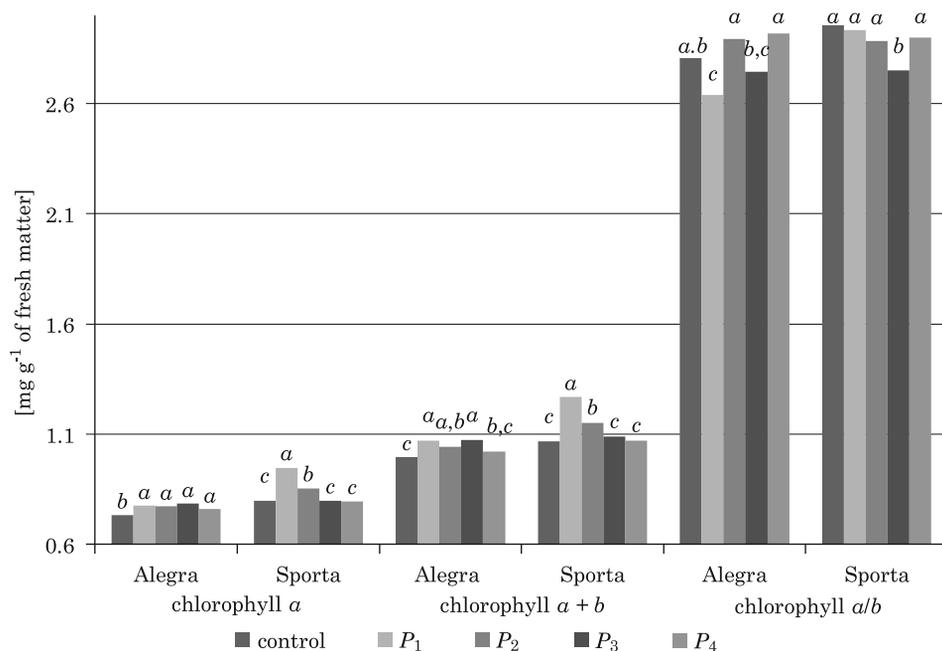


Fig. 11. The influence of pre-sowing stimulation on chlorophyll *a*, chlorophyll *a* + *b* and chlorophyll *a*/*b* content – interaction (variety x doses):  $LSD\alpha = 0.05$ ;  $chl a = 0.025$ ;  $chl a + b = 0.042$ ;  $chl a/b = 0.119$

## Discussion

Refining seed and other sowing materials by with LR treatment results in an enhancement of the treated material and an increase in quantity and quality of the yield obtained (PROŚBA-BIAŁCZYK et al. 2012) The experiments with sugar beet showed higher sugar content, and higher technological value: lower content of water-soluble ash and of melassigenic substances, in the roots of plants grown from the stimulated seeds (ROCHALSKA 2007).

Constant and alternating magnetic fields stimulate the process of seed germination, and plant development, as well as improve the yield and its

quality (NAWROZ and HERO 2010). MARTINEZET al. (2001) have demonstrated that low intensity MF positively affects seed germination and early plant development. In contrary, high intensity MF may adversely affect plants, reducing the germination capacity of seeds and inhibiting plant growth.

The laser radiation and magnetic field used in the present experiment have, in general, increased germination energy and capacity significantly. Only the longest duration of the MF ( $P_4$ ) was proved to be a reducing factor, as it had negatively affected seed germination energy. This last observation corresponds to and confirms the results of MARTINEZET al. (2001). Furthermore, the values of Pieper index also show extending of the germination time, after  $P_4$  exposure to MF, in the seeds of one of the examined cultivars. A contrasting effect – the shortening of the seed germination time – was seen when the seeds were exposed to MF through a shorter period of time ( $P_3$ ).

Both of the applied refining factors used in the experiment stimulated the length of the radicle, compared to the reference plants, by as much as 35 mm in the case of  $D_{10}$  LR dose. The cotyledons elongated after using  $D_5$  LR and  $P_1$ ,  $P_3$  and  $P_4$  MF exposure times. In an earlier research PROŚBA-BIAŁCZYK et al. (2013) had observed stimulation of the morphological traits in seedlings grown from the LR-stimulated seeds and originated in energy hill technology. Moreover, the higher content of carotenoid and chlorophyll pigments was obtained in the seedlings from stimulated seeds, and laser irradiation positively altered chlorophyll a to chlorophyll b ratio. While studying the effects of laser irradiation in the grain of maize, PODLEŚNY and STOCHMAL (2005) had observed the seed mass during imbibition stage being higher in the LR-exposed seeds compared to reference ones. The result of this phenomenon was germination that was earlier and more uniform in time, and faster growth and development of the seedlings. When compared to the seedlings grown from the non-treated seeds, those grown from the irradiated grain were characterized by longer sprout and roots. Data available in literature indicate that also magnetic field impels the germination process and is doing so by amplifying the enzymatic activity in the stimulated seeds. Seedsexposed to MF imbibe faster and start germinating more rapidly, which leads to the earlier and more regular plant emergence (PODLEŚNY et al. 2004). In the seedlings grown from the stimulated seeds longer stem and roots are observed, as well as higher plant vigour compared to the reference plants (PODLEŚNY 2004). In the experiments with a coupled application of LR and MF in the seeds of buckwheat, CIUPAK et al. (2007) reported the 7% increase in the number of seeds germinated at the initial germination stages.

The results obtained by GARCIA et al. (2013) concerning the application of MF in soybean seed refining evidenced the increase in the percentage of the germinated seeds and in the root length in the seedlings grown from the

stimulated seeds. Seeds stimulated with the MF of 16 Hz and 50 Hz frequency and 5 mT induction through the exposure time of 2 h, while germinating under stress conditions in the temperature 5°C, had shown their germination capacity higher by ca. 20% and a considerable, by 1 day, shortening of the average germination time (ROCHALSKA 2002a). Exposing seeds of wheat to MF also increased their field emergence capacity, accelerated their development and enhanced the yield. This effect was particularly apparent in the case of older grain of the reduced qualitative parameters (ROCHALSKA 2002b). In the experiment on the wheat grain stimulation with MF, KORNARZYŃSKI and PIETRUSZEWSKI (2005) had found the greatest germination speed and the average relative germination speed in the seeds exposed to 15 mT induction magnetic field. Also CARBONELL et al. (2000) had found, in their research concerning rice and barley, that the germination of rice was faster and the yield of barley – higher – after the grain stimulation using MF.

PIETRUSZEWSKI (1993) had used alternating MF of 50 Hz frequency to stimulate spring wheat grain and determined an optimal dose at 30 mT induction with 4 sec. and 8 sec. exposure times. He had obtained yield increase by 24% and 20% for 4 sec. and 8 sec. exposures respectively in Henika cv., whereas the respective values in Jara cv. were 36% and 25%. Moreover, he had observed an increase in the ear and grain number, as well as in grain mass, compared to the reference (PIETRUSZEWSKI 1993). In maize search (ALADJADJIYAN 2010) a more intensive stem growth or a yield increase compared to the reference plants were observed after 150 mT or 60–200 mT MF stimulation, respectively.

Biologically active light becomes absorbed by respective pigments. Carotenoids are photolabile, i.e. their content in a plant is dependent on the presence of light. They absorb light within the part of spectrum that is not absorbed by chlorophylls and subsequently convey the absorbed energy to chlorophyll molecules. They therefore have an auxiliary function in photosynthesis and play a protective role in the processes of photooxidation.

The five-fold laser irradiation of the seeds, used in the present experiment, has resulted in an increase of the content of all the examined pigments: carotenoids, chlorophyll a, chlorophyll b, as well as that of chlorophyll  $a + b$ , like in the study of seedlings of maize (HERNANDEZ et al. 2008). The magnetic field in the  $P_3$  and  $P_4$  exposure durations also affected the content of all the examined pigments, except for the chlorophyll b. While investigating the use of MF in sugar beet independently or along with the seed cluster abrasion and conditioning ROCHALSKA (2005) had found an increased chlorophyll content in plant leaves. In another study, using weak (0.5 mT) constant MF resulted in the increased leaf dimensions and enhanced the protein and chlorophyll content in the seedlings of onion (NOWITSKY et al. (2001).

Experimenting with the chick pea, NAWROZ and HERO (2010) had shown a positive influence of short-lasting seed exposures to MF on a number of morphological traits of seedlings and on the content of photosynthetically active pigments. The stimulated traits included stem and root length, the fresh and dry plant mass, and the content of chlorophyll *a* and *b* as well as that of carotenoids. These results comply well with the ones obtained by DHAWI and AL-KHAYRI (2001) in the study on the date palm (*Phoenix dactylifera* L.). These authors found that the content of chlorophyll and carotenoid pigments in plants had increased considerably as the effect of exposure to the constant MF, while it decreased after using the alternating MF. The content of photosynthetically active pigments was increasing as the effect of low MF doses, whereas the high MF doses triggered the opposite, negative effect. Furthermore, a greater variation in the condensation of chlorophyll *a* and of carotenoids was observed, than of chlorophyll *b*.

## Conclusions

1. Each of the two examined methods of pre-sowing seed stimulation significantly increased the values of germination energy and germination capacity of the seed clusters.

2.  $P_1$ ,  $P_2$ ,  $P_3$  and  $P_4$  exposures to magnetic field significantly improved seed vigour, whereas the germination speed (Maguire index) and germination time (Pieper index) were stimulated only by the  $D_5$  dose.

3. The radicle length was stimulated significantly by each of the seed treating methods examined.

4. The highest concentration of the examined plant pigments was observed after five-fold laser irradiation ( $D_5$ ). Magnetic field at  $P_3$  exposure duration enhanced the content of carotenoids and chlorophylla.

5. The five-fold laser irradiation ( $D_5$ ) and  $P_3$  exposure time to magnetic field proved to be the treatments most advantageous to the examined seed clusters.

6. The use of magnetic field and laser radiation may be the method used successfully in order to offset the rising and accelerate the growth of seedlings.

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**GRAIN YIELD VARIABILITY OF WINTER WHEAT  
CULTIVARS IN POST-REGISTRATION TESTS  
IN LOWER SILESIA**

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**Key words:** winter wheat, cultivars, genotype x environment interaction.

**A b s t r a c t**

The paper provides an analysis of the variability in grain yield by a number of the winter wheat cultivars in Lower Silesia based on Post-Registration Variety Testing experiments carried out in 2010, 2011 and 2013. The study analyzes the yield values of nine winter wheat cultivars obtained in trials performed within the frames of the Post-Registration Variety Testing Agrotechnical System (PDO) in Lower Silesia. The experiments were established at four localities characterized by diverse soil conditions. Among the cultivars under research, Askalon was found to produce higher yields than the remaining cultivars, both in the standard and intensive tillage mode. A lower grain yield in both tillage modes was characteristic of Figura. The significant interaction of the cultivars tested with the localities indicates their varied performance at particular locations. The considerable differences in the winter wheat yield values in some of the localities as compared with average yields in the Lower Silesian area point out to essential influence of microregionalization in the territory concerned.

## ZMIENNOŚĆ PLONOWANIA ODMIAN PSZENICY OZIMEJ W DOŚWIADCZENIACH POREJESTROWYCH NA DOLNYM ŚLĄSKU

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Słowa kluczowe: pszenica ozima, odmiany, interakcja genotypowo-środowiskowa.

### Abstrakt

Celem pracy była analiza zmienności plonowania kilku odmian pszenicy ozimej na Dolnym Śląsku na podstawie wyników doświadczeń porejestrowych z lat 2010, 2011 i 2013. W badaniach wykorzystano plony dziewięciu odmian pszenicy ozimej uzyskane z doświadczeń prowadzonych w ramach systemu Porejestrowego Doświadczalnictwa Odmianowego (PDO) na Dolnym Śląsku. Doświadczenia założono w czterech miejscowościach odznaczających się zróżnicowanymi warunkami glebowymi. Spośród analizowanych odmian Askalon charakteryzował się istotnie wyższymi plonami w porównaniu z pozostałymi zarówno w wariancie standardowej, jak i intensywnej agrotechniki. Niższym plonowaniem w obu wariantach uprawy odznaczała się Figura. Istotna interakcja odmian z punktami doświadczalnymi wskazuje na zróżnicowane plonowanie odmian w poszczególnych miejscowościach. Znaczne różnice w plonach odmian pszenicy ozimej w niektórych miejscowościach, w porównaniu ze średnimi plonami na Dolnym Śląsku, wskazują na duże znaczenie mikrojeronizacji na obszarze tego regionu.

### Introduction

The winter wheat occupies the largest area under cultivation in Poland. The acreage where this cereal is sown has recently been incessantly increasing, now reaching 2 mln ha. The species is grown both on the soils reckoned among the black soils but also at locations characterized by less advantageous water-air proportions. Cultivation of the winter wheat on light soils is linked with high danger of declining yields, particularly in years of temporarily insufficient precipitation during the vegetation season. Water accessibility from the soil represents a fundamental factor that influences the plants' performance. The drought-caused stress can result in reduction of all yield components, which – to a varied degree – depend on the plant cultivar (GONZALES et al. 2007). The majority of cultivars registered in Poland are characterized by low resistance to water stress (DRZAZGA et al. 2011), whereas the cultivars distinguishable by high yield stability display significant tolerance to water deficiency during vegetation (AHMAD et al. 2003). Cultivars searched for should be characterized by a high and stable grain yield in defined

macro- or microregions of growing (SALOMON et al. 2008, MOHAMMADI et al. 2012). Wide adaptation of a given cultivar to defined environmental conditions is defined as its capability of high productivity in varied environmental conditions of the region, different years or tillage modes (MADRY and IWAŃSKA 2011). Consequently, narrow adaptation of a cultivar denotes its ability to produce a substantial yield in subregions of a cultivation area (for instance within areas of certain communes having different environmental conditions). Cultivars which exhibit stable performance in macroregions are distinguishable by remarkable tolerance to the biotic and abiotic types of stress (ALBRIZIO et al. 2010). Pertinent research has revealed that the wheat cultivars grown within the Polish territory are characterized by low grain yield stability (BUJAK and TRATWAL 2011, BUJAK et al. 2013, WEBER et al. 2011). However, the significant genetic diversity of the newly registered cultivars combined with the climatic changes occurring in Europe simply force the necessity to search for genotypes distinguished by good adaptation within the area of a given voivodeship or microregion. The purpose of the present study was to analyze grain yield variability of several winter wheat varieties in Lower Silesia based on results of the post-registration trials from years 2010, 2011 and 2013.

## **Material and Methods**

The study on grain yield variability made use of yield data concerning nine winter wheat cultivars obtained in experiments carried out within the Post-registration Variety Testing Agricultural System (PDO) in Lower Silesia. Of the PDO trials, four localities had been selected which varied with respect to edaphic conditions (Table 1). The analysis covered three-years of the wheat cultivars growing: 2010, 2011 and 2013. The area of a single study plot in each experiment equalled 15 m<sup>2</sup>. The significant factor which was responsible for the diversification of the studied cultivars' yields in the years mentioned was the uneven precipitation at the localities under analysis (Table 2). The trials were set up by the method of incomplete block design in two replications, the standard and the intensive tillage mode compared. At the intensive level nitrogen fertilization applied was by 40 kg ha<sup>-1</sup> higher than in the standard mode. Also, complete chemical protection against fungal diseases, usage of an anti-logging factor, and foliar feeding of the plants with multi-component nutrient distinguished the intensive tillage mode from the standard, where these agronomic measures were not taken. Manuring with the remaining macroelements and other agronomic endeavours were performed at the same extent at all the plots of the analyzed experiments. Calculations were carried out with the average yield obtained from all the plots at the four locations in

the three years serving as the basic value. In order to estimate the grain yield variability in the analyzed wheat cultivars at particular localities, statistical analysis as proposed by CALIŃSKI et al. (1987) was employed. The calculations were made with the help of the Sergen 4 software (CALIŃSKI et al. 2003). The calculations have been carried out for the intensive and the standard tillage mode separately.

Table 1  
Soil type of the localities and selected tillage measures applied in 2010, 2011 and 2013

Specification – denotations	Zybiszów (ZYB)	Tarnów (TAR)	Naroczyce (NAR)	Tomaszów (TOM)
Soil complex	2	3	5	5
Soil bonitation class	II	IIIa	IVa	Ivb
P [mg kg <sup>-1</sup> ] content in the soil	high	average	average	high
K [mg kg <sup>-1</sup> ] content in the soil	high	high	average	high
Mg [mg kg <sup>-1</sup> ] content in the soil	average	average	high	average
pH of the soil	6.3	6.0	6.1	6.0
Nitrogen rates at a <sub>1</sub> [kg ha <sup>-1</sup> ]	114	110	80	135
Nitrogen rates at a <sub>2</sub> [kg ha <sup>-1</sup> ]	154	150	120	175
Phosphoric rates [kg ha <sup>-1</sup> ]	60	67	60	50
Potassium rates [kg ha <sup>-1</sup> ]	90	101	90	75
Seed dressing	Oxafun T	Funaben Plus	OxafunT	Sarfun T
Herbicide	Legato Plus 1.25 l	Boxer 2l + HelmTribi	Legato Plus 1.5l	Legato Plus 1.5l
Fungicides at a <sub>2</sub>	Alert 375 1l	Dobromir 0.6 l	Alert 375 1l	Alert 375 1l
Foliar fertilization at a <sub>2</sub>	Basfoliar 8 l	Basfoliar 5 l	Basfoliar 6l	Basfoliar 8l

Table 2  
Atmospheric conditions at different localities

Localities	Zybiszów	Tarnów	Naroczyce	Tomaszów
Sum of precipitation [mm] in 2009 (X–XII)/2010 (I–VII)	588	620	559	585
Sum of precipitation [mm] in 2010 (X–XII)/2011 (I–VII)	507	587	663	664
Sum of precipitation [mm] in 2012 (X–XII)/2013 (I–VII)	635	637	740	667
Mean temperature [°C] in 2009 (X–XII)/2010 (I–VII)	9.2	8.6	8.4	8.6
Mean temperature [°C] in 2010 (X–XII)/2011 (I–VII)	9.8	9.2	9.2	8.8
Mean temperature [°C] in 2012 (X–XII)/2013 (I–VII)	8.7	8.3	8.2	7.8

## Results and Discussion

A preliminary analysis for each locality has revealed significant diversification of the cultivars' yields both in the intensive and standard tillage mode (Table 3).

Table 3  
Mean grain yields of the tested cultivars [ $t \text{ ha}^{-1}$ ]

Localities – standard tillage mode					
Cultivar	Zybiszów	Tarnów	Naroczyce	Tomaszów	Average
Figura	7.58	7.09	6.92	5.90	6.87
Muszelka	8.31	7.18	6.87	6.20	7.14
Bogatka	8.26	6.90	6.74	5.98	6.97
Mulan	8.60	7.96	7.67	6.17	7.60
Ostroga	8.26	7.62	7.29	5.61	7.20
Askalon	8.42	8.10	7.57	5.95	7.51
Bamberska	8.62	7.40	7.25	5.83	7.28
Natula	8.62	7.61	7.52	6.02	7.45
Smaragd	9.03	7.62	7.16	6.00	7.45
Mean	8.41	7.50	7.22	5.96	7.27
Intensive tillage mode					
Figura	8.52	7.72	7.74	6.79	7.69
Muszelka	9.29	7.71	8.11	6.94	8.01
Bogatka	9.71	7.85	8.08	6.80	8.11
Mulan	9.67	8.33	8.68	7.05	8.43
Ostroga	9.33	8.05	8.01	6.71	8.02
Askalon	9.72	8.53	8.51	7.05	8.45
Bamberska	10.00	7.96	8.42	7.18	8.39
Natula	9.64	8.37	8.52	6.90	8.36
Smaragd	10.30	8.35	8.45	7.14	8.56
Mean	9.58	8.10	8.28	6.95	8.22

An analysis of variance for the synthesis of many years' period (Table 4) enabled assessment of the variability in respect of years, localities and cultivars, as well as verification of the following hypotheses:

- about lack of interaction of the cultivars with the localities,
- about lack of the cultivars interacting with the years,
- about lack of interaction of the cultivars with the environments.

Table 4

Mean square variation in the overall analysis of variance

Specification	Number of degrees of freedom	Standard tillage mode	Intensive tillage mode
Source of variation		Mean square	Mean square
Years	2	1319.30*	1588.5*
Localities	3	2764.78**	3113.79*
Environments	6	1698.04**	2264.12**
Genotypes	8	75.92*	95.54*
Genotype x year	16	26.59*	30.07*
Genotype x locality	24	20.34*	15.04*
Genotype x environment	48	17.32**	17.46**
Regression on explanatory variable	8	22.26	30.81*
Regression deviation	40	16.33**	14.79**
Experimental error	423	1.97	1.54

\* – significant at the significance level of  $\alpha = 0.05$ ; \*\* – significant at the significance level of  $\alpha = 0.01$

In the considered standard and intensive tillage modes, significant influence of atmospheric conditions on the performance by the studied cultivars in the period 2010–2013 was found. The mean yields of the analyzed cultivars at particular localities also displayed wide variability. However, on the good wheat complex the cultivars' yield values were higher than at the localities having soils of class III or IV. The performed variance analysis has shown that the mean yields of the cultivars at particular localities were substantially diverse. The high value of the cultivar x environment interaction also points out to widely varying reaction of particular cultivars to the diverse edaphic and atmospheric conditions prevailing at the localities under research in each year of study. Diverse environmental conditions, and particularly the varying amount of total precipitation at each locality during the plants' vegetation season, significantly affected the cultivars' performance. The varied response of the varieties to changes in the environmental conditions cannot be explained by their linear regression relative to the environmental effects. The significant deviations from regression in both tillage modes under consideration indicate that the interaction of cultivars with the environments studied cannot be described by simple regression relationship.

Table 5 provides the outcome of a detailed analysis of the studied cultivars in respect of their yield performance and interaction with the environment. In the standard tillage mode, significantly lower yields at the four localities under analysis were characteristic of cultivars Bogatka and Figura. Mulan and Askalon were distinguishable by positive main effects. In the analyzed environ-

ments, the latter cultivars mentioned produced significantly higher yields as compared with the overall mean for all the objects studied. The remaining cultivars did not display any significant deviations in their yields from the general mean. Remarkable stability in performance at the analyzed sites was typical of cultivar Askalon, whereas the rest of the cultivars were characterized by significant interaction with the environments. In particular, these were cultivars Muszelka and Ostroga that responded with significant variability in grain yield to the environmental changes bound with soil and atmospheric conditions.

Table 5  
Testing of particular cultivars and their interactions

Denotations	Standard tillage mode			Intensive tillage mode		
	estimate for the main effect	<i>F</i> statistics for the main effect	<i>F</i> statistics for the interaction with environments	estimate for the main effect	<i>F</i> statistics for the main effect	<i>F</i> statistics for the interaction with environments
Figura	-4.03	18.41	6.06*	-5.32	55.66*	4.46*
Muszelka	-1.35	0.76	16.49*	-2.16	2.24	18.24*
Bogatka	-3.05	13.94	4.58*	-1.15	0.71	16.54*
Mulan	3.25	6.29	11.54*	2.07	3.05	12.34*
Ostroga	-0.78	0.31	13.60*	-2.02	3.08	11.69*
Askalon	2.38	18.40	2.12	2.27	4.20*	10.76*
Bamberska	0.01	0.00	8.45*	1.65	2.17	11.03*
Natula	1.76	2.12	10.09*	1.31	2.06	7.39*
Smaragd	1.80	3.54	6.30*	3.35	10.42*	9.47*
Critical values $\alpha = 0.05$	-	3.78	2.12	-	3.78	2.12

Also at the intensive tillage mode, Figura performed significantly poorer than the other cultivars, whereas Askalon and Smaragd, when under conditions of diverse complexes of agricultural usefulness of soils, were distinguishable by a higher grain yield than the remaining objects. Significant grain yield instability at the intensive tillage mode was characteristic of Muszelka and Bogatka. Assessment of the analyzed localities with regard to the genotype x environment (G x E) interaction was carried out through division of statistics *F* of this interaction into components corresponding with particular contrasts (comparisons) between cultivars. A corresponding *F* statistics, expressed as a proportion of statistics *F* for the G x E interaction from the overall variance analysis, shows what part of this interaction accounts for a given contrast. In

order to graphically illustrate the environments on a plane, the first two principal components, which represent estimates of contrasts between cultivars calculated for particular localities, were used. Figure 1 and Figure 2 depicts distribution of localities on a plane in the system of principal components. A locality with a large share in the G x E interaction is distinguishable by a long distance from the origin of the co-ordinate system. The yields of cultivars in this environment-locality differ significantly from the mean yields obtained during the study years under analysis.

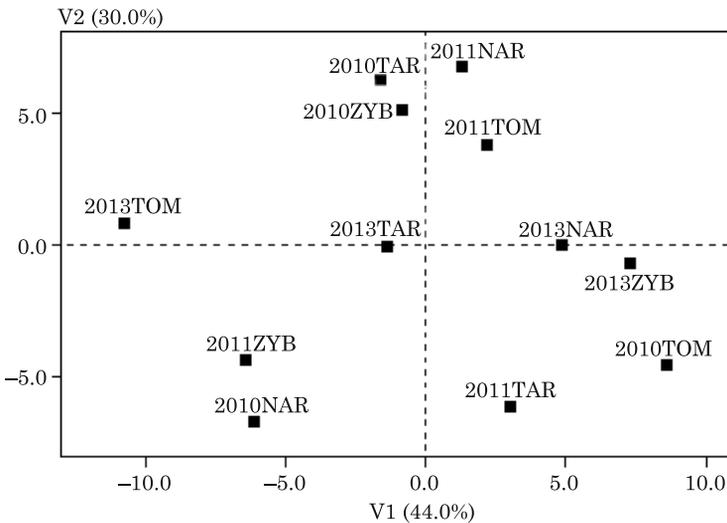


Fig. 1. Distribution of localities in the system of principal components – standard tillage mode; for denotations see Table 1

An analysis of the standard tillage mode (Figure 1) allows a statement that the most distant from the origin of the system are localities 2010NAR and 2011NAR, and also 2010TOM and 2013TOM, all representing Naroczyce and Tomaszów. These localities are characterized by the mean grain yield values significantly deviating from the averages for the cultivars tested in Lower Silesia. The substantial differences in the cultivars' performance at the localities under research – as compared with the average grain yields of the studied objects in the analyzed macroregion – result from changeable atmospheric conditions during the three years of investigations. This is evidenced by long distances between the analyzed localities in particular years (2010NAR, 2011NAR and 2013NAR, or 2010TOM, 2011TOM and 2013TOM).

Figure 2 presents the localities in the system of the first two principal components for the intensive tillage mode. Also in this variant, points

2010TOM and 2013TOM as well as 2011ZYB and 2013ZYB, denoting Tomaszów and Zybiszów, display a considerable distance from the origin of the co-ordinate system, which indicates substantial differences in the cultivars' grain yields at these localities in comparison with their performance at the other sites. The localities mentioned exhibit also considerable differences between the wheat yield values in particular study years, which is testified by long distances between the points of 2010TOM, 2011TOM, 2013TOM, 2010ZYB, 2011ZYB and 2013ZYB.

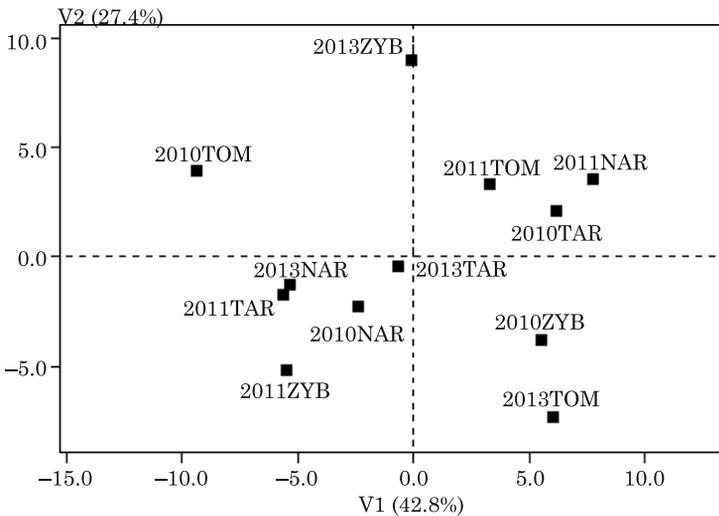


Fig. 2. Distribution of localities in the system of principal components – intensive tillage mode; for denotations see Table 1

Applying an analysis of dual components, the structure of the G x E interaction in respect of cultivars can be analyzed. Figure 3 shows the cultivars in the system of principal components for the standard tillage mode. The magnitude (share) of the interaction of particular cultivars with the environments is depicted by the section (vector) of the  $F$  statistics value drawn from each point to the origin of the system. The largest shares in the sum of deviation squares for the G x E interaction are those of cultivars Mulan, Ostroga and Muszelka. These particular cultivars are distinguishable by more variable performance in the studied localities than the remaining objects. On the other hand, higher grain yield stability is characteristic of Smaragd, Askalon and Bogatka.

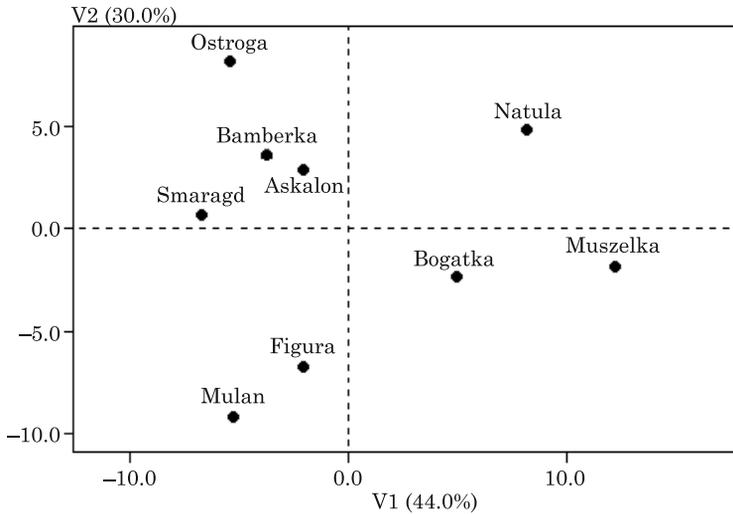


Fig. 3. Representation of cultivars in the system of principal components – standard tillage mode

At the intensive tillage mode (Figure 4), Muszelka and Bogatka were characterized by significant grain yield variability within the studied localities, whereas more stable performance was characteristic of Smaragd and Figura. A comparison of distances between particular cultivars when under intensive and standard tillage mode reveals significant differences. This points out to the cultivars variedly responding to the doses of nitrogen fertilizer and plant protection preparations applied and to atmospheric conditions. The investigations by PODOLSKA (2009) show that the Polish cultivars can be divided into two groups. The first one comprises ones that need an average dose of nitrogen, whereas the other group is composed of cultivars distinguishable by the grain yield growing at high-dose nitrogen fertilization.

Cultivar Askalon was characterized by substantially higher grain yields as compared with the remaining cultivars both at the standard and intensive tillage mode, and therefore this particular cultivar deserves to be recommended for Lower Silesia. Poorer performance at both tillage modes was typical of Figura. It should be emphasized, however, that at the intensive agronomy level this cultivar exhibited significant grain yield variability in particular study years. Good performance by cultivars Smaragd and Askalon at the intensive tillage mode depends to a great extent on favourable distribution of precipitation in the vegetation season, which guarantees optimum soil moisture in critical phases of the plants' development. The main factor responsible for lower grain yields of the studied cultivars on light soils was water deficit during the period of the wheat vegetation, which finds confirmation in the research

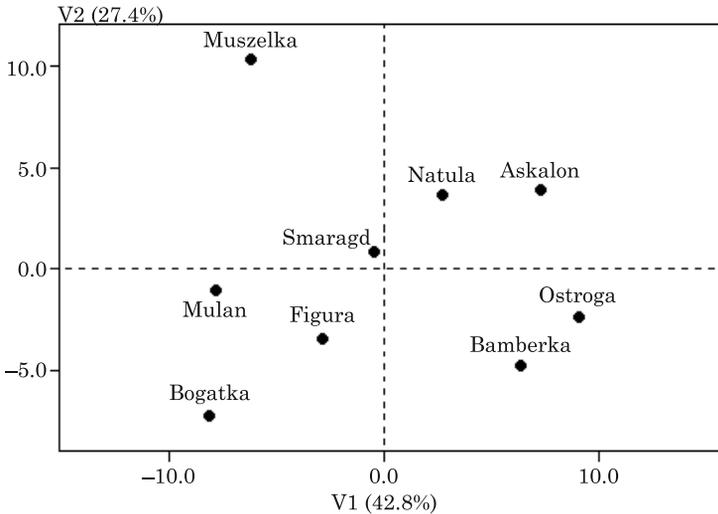


Fig. 4. Representation of cultivars in the system of principal components – intensive tillage mode

by MITTLER (2000). And thus, the wheat cultivars more resistant to water stress during the critical developmental phases are distinguishable by significantly better performance in comparison with other cultivars (GUPTA et al. 2001, FOULKES et al. 2001). Certain breeding programmes are aimed at acquisition of such genotypes that would be characterized by high tolerance to soil salinity and water deficiencies during vegetation (FAROOQ and AZAM 2007). If the genotype variance exceeds the variance of the effects of the interaction (genotype x experimental station), then a breeder can attain good efficacy of selection and obtain breeding lines characterized by high stability in a given region. In a reverse situation, selection of cultivars of narrow adaptation can prove to be a better strategy (GRÜNBERG et al. 2005). The remarkable diversity of environmental conditions in Lower Silesia contributed to occurrence of high interaction of the studied cultivars with the localities both at the intensive and standard tillage mode. A desirable wheat cultivar should be distinguishable by stable performance in all types of environments within the target region and to display high grain yield stability through years (NAVABI et al. 2006). A cultivar which is dynamically stable in each environment produces a grain yield that differs by a constant quantity from the average yield value for all cultivars in a given environment. On the other hand, a statically stable cultivar maintains a constant grain yield level at any location. Frequently, also those cultivars are acknowledged as desirable which are narrowly adapted to a defined environment and exhibit high repeatability of production through years (ANNICCHIARICO et al. 2006).

The advancement in breeding during the last twenty-year span is the main factor behind growing yields. At present, such wheat cultivars are recommended which are characterized by capability of wide adaptation to changeable environmental conditions (the so-called universal) or ones distinguishable by high and stable grain yields within a small acreage. Yet, the significant diversification of climatic conditions within particular regions makes micro-regionalization (often pertaining to a poviat or even commune territory) gaining in importance. A good choice of localities for wheat cultivars PDO testing in particular voivodeships contributes to more precise delineation of regionalized cultivation. Based on PDO results, it is possible to distinguish cultivars that are recommendable not only at a given tillage mode but also to define their macro- or microregionalization.

## Conclusions

1. The significant interaction of the cultivars with the localities indicates their diverse performance.

2. The changeable atmospheric conditions in the three years of study, and particularly water deficit on lighter soils during the plants' vegetation season, brought about lower stability in grain yield by the wheat cultivars in the localities under analysis.

3. Due to the varied number of cultivars studied in the post-registration experiments in each year, the genotype x environment interaction underwent significant changes. Therefore, the number of experimental localities, characterized by changeable soil and atmospheric conditions, should not be reduced substantially.

4. The considerable differences in performance by the cultivars at some locations in comparison with the mean values for Lower Silesia point out to great significance of microregionalization in the acreage of this voivodeship.

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**EVALUATION OF SELECTED PHYSICOCHEMICAL  
PARAMETERS OF TWENTY-ONE DETERMINATE  
VARIETIES OF TOMATO FRUITS  
(*LYCOPERSICON ESCULENTUM* Mill.)  
GROWN IN POLAND**

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**Key words:** tomato varieties, colour, chemical composition, carotenoids, vitamin C.

**Abstract**

The aim of the research was to assess the quality of 21 soil-grown determinate tomato varieties. All of them are recommended for crop production in Polish climate conditions. The colour of fruits and the contents of dry mass, extract, total sugars, vitamin C and carotenoids were analysed. It is not feasible to select one single variety with high levels of all the studied parameters. The varieties with the highest content of total carotenoids (Ricoco and Progress) are characterized by the low content of vitamin C. Malinowy Kujawski variety is characterised by the high content of dry mass and the highest content of total extract and sugars. However, it has the lowest value of  $\alpha^*$  parameter and low content of carotenoids. Due to small size of fruits, high contents of dry matter, total extract, sugars and total carotenoids Progress variety can be recommended both for the fresh market and processing.

**OCENA WYBRANYCH PARAMETRÓW FIZYKOCHEMICZNYCH OWOCÓW 21 ODMIAN  
SAMOKOŃCZĄCYCH POMIDORA GRUNTOWEGO (*LYCOPERSICON ESCULENTUM*  
Mill.) UPRAWIANEGO W POLSCE**

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**Słowa kluczowe:** odmiany pomidora, barwa, skład chemiczny, karotenoidy, witamina C.

## A b s t r a k t

Celem badań była ocena jakości owoców 21 odmian samokończących pomidora gruntowego, polecanych do uprawy w polskich warunkach klimatycznych jako przydatne do przetwórstwa, jak również do spożycia w stanie świeżym. Oznaczano barwę owoców i zawartość suchej masy, ekstraktu ogółem, cukrów, witaminy C i karotenoidów. W badaniach wykazano, że nie można jednoznacznie wytypować odmiany o wysokiej wartości wszystkich badanych parametrów. Odmiany o najwyższej całkowitej zawartości karotenoidów (Ricoco i Progress) charakteryzują się niską zawartością witaminy C. Owoce odmiany Malinowy Kujawski charakteryzują się wysoką zawartością suchej masy i najwyższą zawartością ekstraktu ogółem i cukrów. Jednocześnie stwierdzono dla tej odmiany najniższą wartość parametru  $\alpha^*$  i niską zawartość karotenoidów ogółem. Ze względu na niewielkie rozmiary owoców, wysoką zawartość suchej masy, cukrów ogółem, ekstraktu oraz karotenoidów ogółem owoce odmiany Progress można polecić zarówno na rynek warzyw świeżych, jak i do przetwórstwa.

**Introduction**

Tomato plant (*Lycopersicon esculentum* Mill.) is grown in many countries around the world. The biggest producer of tomato is China with 28.7% contribution, followed by United States, India, Turkey, Egypt, Italy, Iran, Spain, Brazil and Mexico. In Poland tomato crop covers the area of approximately 10.9 thousands hectares and its production reached 261 thousands tons in 2012 (GUS 2013). However, tomato consumption in Poland is rather modest (approximately 9.6 kg per capita), comparing to the average consumption in European Union (31.1 kg per capita) (FILIPIAK and MACIEJCZAK 2010).

Creative breeding of tomato provides every year new varieties for both direct consumption and processing. These varieties have to satisfy the increasing consumers demands for both appearance and the content of nutrients and biologically active compounds. Tomato varieties differ greatly in their physical and chemical parameters which affects the usefulness for processing. Fruits which are produced for the fresh market are characterized by fleshy texture, rather large mass and good taste. On the contrary, for the industrial purposes dwarf and hard texture varieties, which are resistant to damaging during transport, are recommended. Polish producers of industrial tomatoes prefer varieties which give bigger fruits and are convenient for manual harvest. Additionally, they should possess intense colour which results from the presence of high amounts of carotenoids (ZALEWSKA-KORONA and JABŁOŃSKA-RYŚ 2012). European register of cultivated plants from 2014 contains 3746 tomato varieties and the choice of appropriate variety depends on local climatic conditions and the purpose of the use. Moreover, producers pay attention to the factors like the shape of the plants, pest and infection resistance, and other factors connected with climate. Producers tend to choose those varieties which bring in the highest crop (NAIKA et al. 2005). The content of different

physically important chemical compounds depends on variety but also on crop and climate conditions, and the state of fruits maturity.

The aim of the research was to assess the quality of 21 soil-grown tomato varieties (17 Polish, 2 Dutch, 1 Italian and 1 French) all of which are recommended for crop production in Polish climate conditions. These varieties are proposed for both processing and direct consumption.

## Materials and Methods

The studies were conducted in 2012 and 2013 in Milejów, near Lublin (35°23' N, 22°89' E). The region has a moderately continental climate. The average annual temperature both in 2012 and 2013 was 8.1°C., while the average annual rainfall was 503 mm and 650 mm respectively. It was unevenly distributed throughout the year. Detailed weather data are given in Table 1.

Table 1  
Mean temperatures and total precipitation over the experimental period – data provided by the Meteorological Station in Lublin

Temperature [°C]					
Year	Month				
	V	VI	VII	VIII	IX
2012	14.7	16.8	20.8	18.5	14.4
2013	14.8	17.8	18.5	18.6	11.5
Long-term average (1981–2010)	13.5	16.1	18.2	17.6	12.9
Precipitation [mm]					
2012	34.0	68.0	58.0	45.0	38.0
2013	106.0	113.0	88.0	17.0	41.0
Long-term average (1981–2010)	59.9	66.5	80.6	58.9	61.2

The plants were grown in arable soil (class II according to the Polish systematics). The level of N-NO<sub>3</sub>, P and K in 2012 reached 18.9; 35 and 58 mg dm<sup>-3</sup> respectively, while in 2013 reached 15; 49 and 58 mg dm<sup>-3</sup> respectively. The soil was supplemented with YaraMila Complex fertilizer (N – 150 mg dm<sup>-3</sup>, P – 80 mg dm<sup>-3</sup>, K – 250 mg dm<sup>-3</sup>). The seeds were sown in the first week of April. The production of tomato seedlings was conducted in a heated tunnel. After the proper leaves had appeared, the seedlings were transferred into seeding pallets (dimensions of a single pot was 6 x 6 cm). The tomato seedlings were transplanted into the field on the 23<sup>rd</sup> of May.

The plants grew in the spacing of 70 x 60 cm. All the varieties used in the experiment were determinate type of growth. The experiment was established as one-factor, in the randomized block system in two repetitions and the size of a single plot was 8.4 m<sup>2</sup>. The protection of plants against weeds, diseases and pests was conducted in accordance with "Vegetable Plant Protection Program". The fruits samples (3 kg for each variety) were harvested manually between 17 to 21<sup>st</sup> of September 2012 and 10 to 16 of September 2013 at the state of maturity when skin colour was intense. An average weight of fruits for every harvested sample was determined. The colour determination was done with X-Rite 8200 (Czech Republic), based on the CIE-Lab standard, values of the absolute parameters  $L^*$  (lightness),  $a^*$  (green/red shade) and  $b^*$  (blue/yellow shade) were recorded. The contents of dry mass, extract, total sugars, vitamin C and carotenoids were analysed according to the methods described in Polish Norms (*Przetwory owocowe...* PN-90/A-75101.03; *Przetwory owocowe...* PN-90/A-75101.02; *Przetwory owocowe...* PN-A-75101-07:1990; *Produkty spożywcze...* PN-A-04019:1998; PN-A-75101-12:1990).

Chemical analyses were performed in 3 replicates, while the colour determination was done in 15 replicates for each year. The tables show the average results for the two years. Statistical difference was determined with Tukey test with the level of significance set at  $p < 0.05$ .

## Results and Discussion

The results of biometrical and colour measurements are shown in Table 2. Statistically important differences between varieties are reported. The size of the fruits is rather not crucial in processing. However, this feature is important for the manual harvesting. The weight of the fruits varied from 66 g (Progress F<sub>1</sub>) to 244 g (Mirsini). One variety of tomato used in the experience was type of cherry (Ricoco F<sub>1</sub>). Fruits of this variety had an average weight of 18 g.

Colour is one of the factors that affect the appearance of the fruits. During ripening the lightness ( $L^*$ ) parameter decreases and is inversly proportional to the intensity of the colour. The values of  $L^*$  parameter ranged from 37.35 (Betalex) to 48.99 (Beta). Similar results were obtained by LOPEZ CAMELO and GOMEZ (2004) and TOOR and SAVAGE (2006). Other authors noted lower values ranging from 34.5 to 37.6 (RADZEVIČIUS et al. 2008). The contribution of red colour ( $a^*$ ) reflects the state of fruits ripeness. The highest value of this parameter was observed for Dual Plus F<sub>1</sub> (33.32), while the lowest for Malinowy Kujawski (14.62). BRANDT et al. (2006) noted this parameter ranging from 28.5 to 32.1, whereas ODRIZOLA-SERRANO et al. (2008) reported values reaching from 11.9 to 21.8. Tomatos that are intended for processing should

Table 2

Average weight and colour of tomato fruits

Variety	Average fruit weight [g]	$L^* \pm SD$	$a^* \pm SD$	$b^* \pm SD$
Allflesh 1120 F <sub>1</sub>	84	38.18 <sup>a,b</sup> ± 0.78	29.99 <sup>h,j</sup> ± 1.19	24.39 <sup>f,i</sup> ± 1.67
Beta	77	48.99 <sup>d</sup> ± 2.96	20.15 <sup>b,c</sup> ± 2.38	18.97 <sup>b-d</sup> ± 3.17
Betalux	115	37.35 <sup>a</sup> ± 1.43	26.69 <sup>e,g</sup> ± 1.40	23.45 <sup>e-h</sup> ± 3.37
Bohun	156	37.78 <sup>a</sup> ± 1.41	26.22 <sup>e,f</sup> ± 1.34	24.92 <sup>f,j</sup> ± 3.11
Dual Plus F <sub>1</sub>	71	39.28 <sup>a-c</sup> ± 1.43	33.32 <sup>k</sup> ± 0.82	28.74 <sup>j,l</sup> ± 2.86
Duty F <sub>1</sub>	173	38.51 <sup>a,b</sup> ± 1.79	29.65 <sup>g,j</sup> ± 2.81	26.79 <sup>b-k</sup> ± 2.64
Etna F <sub>1</sub>	76	38.28 <sup>a,b</sup> ± 1.15	23.62 <sup>d,e</sup> ± 2.38	21.56 <sup>d-g</sup> ± 1.18
Gigant	207	46.31 <sup>d</sup> ± 3.95	20.59 <sup>b-d</sup> ± 2.87	19.34 <sup>c-e</sup> ± 3.66
Hubal	127	47.58 <sup>d</sup> ± 1.50	20.07 <sup>b,c</sup> ± 2.66	20.72 <sup>c-f</sup> ± 3.60
Malinowy Kujawski	138	46.56 <sup>d</sup> ± 3.94	14.62 <sup>a</sup> ± 1.93	10.23 <sup>a</sup> ± 1.96
Malinowy Rodeo	232	38.68 <sup>a,b</sup> ± 1.10	20.89 <sup>b-d</sup> ± 2.02	14.87 <sup>b</sup> ± 2.42
Mieszko F <sub>1</sub>	79	41.69 <sup>c</sup> ± 1.54	27.16 <sup>f,h</sup> ± 1.42	32.92 <sup>l</sup> ± 3.05
Mirsini	244	38.03 <sup>a,b</sup> ± 0.86	27.42 <sup>f,i</sup> ± 1.16	24.95 <sup>g,j</sup> ± 1.24
Progress F <sub>1</sub>	66	39.40 <sup>a-c</sup> ± 1.96	30.58 <sup>k</sup> ± 1.13	24.60 <sup>f,j</sup> ± 1.27
Promyk	77	46.37 <sup>d</sup> ± 1.57	18.00 <sup>b</sup> ± 3.88	18.30 <sup>b-d</sup> ± 5.86
Rejtan F <sub>1</sub>	122	40.78 <sup>b,c</sup> ± 2.95	21.55 <sup>c,d</sup> ± 1.24	23.65 <sup>f-h</sup> ± 2.39
Riccoco F <sub>1</sub> (ch)	18	39.62 <sup>a-c</sup> ± 1.82	31.58 <sup>j,k</sup> ± 2.43	25.71 <sup>g,j</sup> ± 2.60
Sabała	102	47.13 <sup>d</sup> ± 2.47	18.25 <sup>b</sup> ± 3.10	17.15 <sup>b,c</sup> ± 3.74
Sokal F <sub>1</sub>	79	42.21 <sup>c</sup> ± 1.88	29.85 <sup>g,j</sup> ± 3.10	30.77 <sup>h,l</sup> ± 2.68
Ulan	101	47.66 <sup>d</sup> ± 1.44	20.93 <sup>b-d</sup> ± 2.39	22.22 <sup>d-g</sup> ± 2.20
Veloz (Chelsea)	103	40.10 <sup>a-c</sup> ± 1.13	31.24 <sup>i,k</sup> ± 1.25	28.43 <sup>i-k</sup> ± 2.16

ch – variety type of cherry

possess possibly high red colour contribution. This criterion is fulfilled by Dual Plus, Velos, Progress F<sub>1</sub> varieties. Fruits of cherry tomato variety (Riccoco) was also characterized by high value of the parameter  $a^*$  (31.58). Higher participation of redness is connected with lower brightness, the correlation coefficient for these parameters was -0.720. High contribution of yellow colour may result from not even colour distribution or unripeness of fruits. The yellowness parameter ( $b^*$ ) showed values varying from 10.23 (Malinowy Kujawski) to 32.92 (Mieszko F<sub>1</sub>). BRANDT et al. (2006) reported values of  $b^*$  parameter ranging from 23.9 to 30.2 while ODRIZOLA-SERRANO et al. (2008) demonstrated values reaching from 21.6 to 33.1.

One of the most important parameters which influence the usefulness for processing is the content of dry mass and extract (Table 3). The main direction of tomato processing is puree production and the higher dry mass content is, the variety is more suitable for the production. The lowest dry mass and

extract content was reported for Betalux and amounted of 3.69% and 2.85%, correspondingly. The highest value of dry matter was noted for Progress F<sub>1</sub> (6.01%) and highest value of total extract was noted for Malinowy Kujawski variety (5.33%). TOOR and SAVAGE (2006) reported dry mass content ranging from 5.1% to 6.2%, while other authors observed lower values (4.6–5.3%). It was shown a high correlation coefficient between the content of total extract and the dry matter (0.821). The sugars and total extract are also correlated (0.810).

Table 3

Average content of the analyzed chemical parameters

Variety	Dry matter [%] ± SD	Total extract [%] ± SD	Total sugars [%] ± SD	Ascorbic acid [mg 100 g fw. <sup>-1</sup> ] ± SD	Total carotenoids [mg 100 g fw. <sup>-1</sup> ] ± SD
Allflesh 1120 F <sub>1</sub>	5.84 <sup>h,i</sup> ± 0.11	4.73 <sup>i,j</sup> ± 0.24	2.33 <sup>i,k</sup> ± 0.08	8.30 <sup>a-d</sup> ± 1.57	3.41 <sup>f,g</sup> ± 0.12
Beta	5.46 <sup>e-g</sup> ± 0.28	4.43 <sup>g-i</sup> ± 0.10	2.25 <sup>h-j</sup> ± 0.10	18.93 <sup>b</sup> ± 2.05	2.23 <sup>b,c</sup> ± 0.06
Betalux	3.69 <sup>a</sup> ± 0.10	2.85 <sup>a</sup> ± 0.10	1.43 <sup>a</sup> ± 0.09	9.10 <sup>b-e</sup> ± 1.42	2.27 <sup>b,c</sup> ± 0.11
Bohun	4.48 <sup>b,c</sup> ± 0.13	4.25 <sup>f,g</sup> ± 0.19	1.84 <sup>c-e</sup> ± 0.15	6.39 <sup>a,b</sup> ± 1.09	3.05 <sup>e,f</sup> ± 0.19
Dual Plus F <sub>1</sub>	5.17 <sup>d,e</sup> ± 0.17	4.42 <sup>g-i</sup> ± 0.17	1.97 <sup>d-h</sup> ± 0.10	11.24 <sup>d,f</sup> ± 1.61	3.21 <sup>f</sup> ± 0.09
Duty F <sub>1</sub>	5.20 <sup>d-f</sup> ± 0.30	4.35 <sup>f-h</sup> ± 0.21	2.01 <sup>e-i</sup> ± 0.08	8.35 <sup>a-d</sup> ± 1.62	3.80 <sup>g,h</sup> ± 0.13
Etna F <sub>1</sub>	4.23 <sup>b</sup> ± 0.07	3.85 <sup>b-d</sup> ± 0.05	1.69 <sup>a-d</sup> ± 0.08	6.25 <sup>a,b</sup> ± 0.89	2.58 <sup>c,d</sup> ± 0.09
Gigant	5.56 <sup>e-h</sup> ± 0.14	5.25 <sup>h,l</sup> ± 0.21	2.61 <sup>k,l</sup> ± 0.17	8.17 <sup>a-c</sup> ± 1.11	2.76 <sup>d,e</sup> ± 0.09
Hubal	4.43 <sup>b</sup> ± 0.07	3.75 <sup>b,c</sup> ± 0.14	1.65 <sup>a-c</sup> ± 0.07	12.02 <sup>e,f</sup> ± 1.13	2.49 <sup>c,d</sup> ± 0.06
Malinowy Kujawski	5.65 <sup>g-i</sup> ± 0.24	5.33 <sup>l</sup> ± 0.19	2.67 <sup>l</sup> ± 0.19	9.41 <sup>c-e</sup> ± 1.87	1.57 <sup>a</sup> ± 0.11
Malinowy Rodeo	4.86 <sup>d</sup> ± 0.10	4.18 <sup>d-g</sup> ± 0.10	1.74 <sup>b-e</sup> ± 0.06	5.95 <sup>a</sup> ± 0.71	2.29 <sup>b,c</sup> ± 0.27
Mieszko F <sub>1</sub>	4.83 <sup>c,d</sup> ± 0.13	4.83 <sup>l</sup> ± 0.20	1.66 <sup>a-c</sup> ± 0.17	13.25 <sup>f,g</sup> ± 1.47	2.28 <sup>b,c</sup> ± 0.09
Mirsini	5.28 <sup>e-g</sup> ± 0.17	4.75 <sup>i,j</sup> ± 0.05	1.85 <sup>c-f</sup> ± 0.18	7.89 <sup>a-c</sup> ± 1.42	3.87 <sup>h</sup> ± 0.22
Progress F <sub>1</sub>	6.01 <sup>i</sup> ± 0.11	4.67 <sup>h,j</sup> ± 0.35	2.31 <sup>i,j</sup> ± 0.16	8.36 <sup>a-d</sup> ± 1.70	4.06 <sup>h</sup> ± 0.52
Promyk	4.48 <sup>b,c</sup> ± 0.20	3.67 <sup>b</sup> ± 0.05	1.77 <sup>c-e</sup> ± 0.12	9.17 <sup>b-e</sup> ± 1.36	1.99 <sup>b</sup> ± 0.10
Rejtan F <sub>1</sub>	4.37 <sup>b</sup> ± 0.08	2.88 <sup>a</sup> ± 0.13	1.45 <sup>a,b</sup> ± 0.13	9.65 <sup>c-e</sup> ± 1.88	2.50 <sup>c,d</sup> ± 0.21
Ricoco F <sub>1</sub>	5.52 <sup>e-h</sup> ± 0.25	4.30 <sup>f,g</sup> ± 0.09	2.14 <sup>f,j</sup> ± 0.14	7.50 <sup>a-c</sup> ± 1.22	5.35 <sup>i</sup> ± 0.29
Sabała	4.89 <sup>d</sup> ± 0.13	4.20 <sup>e-g</sup> ± 0.09	2.21 <sup>g-j</sup> ± 0.15	22.20 <sup>i</sup> ± 1.33	1.32 <sup>a</sup> ± 0.15
Sokal F <sub>1</sub>	4.40 <sup>b</sup> ± 0.28	3.87 <sup>b-e</sup> ± 0.12	1.62 <sup>a-c</sup> ± 0.20	11.99 <sup>e,f</sup> ± 1.77	2.01 <sup>b</sup> ± 0.07
Ułan	4.45 <sup>b,c</sup> ± 0.13	4.05 <sup>c-f</sup> ± 0.16	1.91 <sup>c-g</sup> ± 0.14	16.20 <sup>g,h</sup> ± 0.93	2.29 <sup>b,c</sup> ± 0.13
Veloz (Chelsea)	5.53 <sup>e-h</sup> ± 0.24	4.97 <sup>j,k</sup> ± 0.08	2.19 <sup>g-j</sup> ± 0.24	15.23 <sup>g</sup> ± 0.52	3.81 <sup>h</sup> ± 0.18

Sugars represent major constituent of tomato extract. The highest amount of total sugars was observed for Malinowy Kujawski (2.67%), while the lowest for Betalux (1.43%). This data were in agreement with the results obtained by HALLMANN and REMBIAŁKOWSKA (2007).

The level of vitamin C ranged from  $5.95 \text{ mg} \cdot 100 \text{ g fw.}^{-1}$  for Malinowy Rodeo variety to  $22.20 \text{ mg} \cdot 100 \text{ g fw.}^{-1}$  for Sabała variety. According to HALLMANN and REMBIAŁKOWSKA (2007) the mean content of vitamin C in fruits grown organically amounted of  $8.04 \text{ mg} \cdot 100 \text{ g fw.}^{-1}$  and in fruits grown traditionally amounted of  $6.77 \text{ mg} \cdot 100 \text{ g fw.}^{-1}$ . Other author demonstrated the content of vitamin C ranging from  $6.96\text{--}21.23 \text{ mg} \cdot 100 \text{ g fw.}^{-1}$  (ODRIZOLA-SERRANO et al. 2008) and  $23\text{--}28 \text{ mg} \cdot 100 \text{ g fw.}^{-1}$  (EJAZ et al. 2012).

The concentration of total carotenoids varied considerably ( $1.32\text{--}5.35 \text{ mg} \cdot 100 \text{ g fw.}^{-1}$ ). The highest amount was noticed in Ricoco F<sub>1</sub> and Progress F<sub>1</sub> varieties (above  $4.0 \text{ mg} \cdot 100 \text{ g fw.}^{-1}$ ). In a previous study, LISIEWSKA and KMIECIK (2000) observed average concentration of carotenoids amounting of  $4.97 \text{ mg} \cdot 100 \text{ g fw.}^{-1}$ . Carotenoids content depends on the climatic conditions. ABUSHITA et al. (2000) compared 12 tomato varieties grown in Hungary and reported values ranging from  $6.27$  to  $9.83 \text{ mg} \cdot 100 \text{ g fw.}^{-1}$ , while LENUCCI et al. (2009) observed values ranging from  $9.57$  to  $20.63 \text{ mg} \cdot 100 \text{ g fw.}^{-1}$  in tomatoes grown in Italy.

The concentration of total carotenoids in tomato fruits determines their red color. It was shown a high correlation coefficient between the  $a^*$  parameter and the content of total carotenoids (0.739).

The content of biologically active compounds in tomato fruits is influenced by the way of cultivation, climate and soil conditions, along with the state of ripeness. In the current experiments all applied conditions were the same. Therefore, it can be assumed the obtained changes are caused by the differences between analyzed varieties.

## Conclusions

The analyses have shown that it is not feasible to select one single variety with high levels of all the studied parameters. The varieties with the highest content of total carotenoids (Ricoco F<sub>1</sub> and Progress F<sub>1</sub>) are characterized by the low content of vitamin C. Malinowy Kujawski variety is characterised by the high content of dry mass and the highest total extract and sugars. However, it has the lowest value of  $a^*$  parameter, and it contains low amount of carotenoids. Due to small size of fruits, high contents of dry matter, total extract, sugars and total carotenoids Progress F<sub>1</sub> variety can be recommended both for the fresh market and processing. The contents of sugars, vitamin C, carotenoids in varieties cultivated in Poland are lower than those reported in other countries like Hungary or Italy. This difference can be attributed to more favourable weather conditions.

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**PHYSICAL PROPERTIES OF FENUGREEK SEEDS  
(*TRIGONELLA FOENUM-GRAECUM* L.)  
IN MULTIFACTORIAL AGRICULTURAL  
EXPERIMENTS**

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**Key words:** thousand seed weight, density, porosity, geometrical and optical properties, correlation.

**Abstract**

The aim of this study was to identify changes in the physical properties of fenugreek seeds (*Trigonella foenum-graecum* L.), observed in agricultural experiments with multifactorial designs. The thousand seed weight, true density, bulk density, color and spectral composition, linear dimensions, shape factors and textural properties of fenugreek seeds were determined. The results were processed statistically by analysis of variance, correlation analysis. True density was from 1.283 to 1.318 g cm<sup>-3</sup>, one thousand seed weight from 10.83 to 14.37 g, and the length (*L*) and width (*S*) contained in the range of 3.78 to 4.01 and 2.65 to 2.84 mm, respectively, depending on the experimental group, respectively. The influence of inoculation and agricultural treatments for one thousand seed weight has been demonstrated. Statistically significant correlations were noted for bulk density (from -0.809 to 0.772) and porosity (-0.754 to 0.762) with shape factors *W*<sub>3</sub>, *W*<sub>9</sub> and *W*<sub>12</sub>.

**ZMIANY WŁAŚCIWOŚCI FIZYCZNYCH NASION KOZIERADKI  
POSPOLITEJ (*TRIGONELLA FOENUM-GRAECUM* L.) UZYSKANE  
W WIELOCZYNNIKOWYCH EKSPERYMENTACH AGROTECHNOLOGICZNYCH**

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**Sł o w a k l u c z o w e:** masa 1000 nasion, gęstość, porowatość, właściwości geometryczne i optyczne, korelacja.

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## A b s t r a k t

Celem pracy było zidentyfikowanie zmian właściwości fizycznych kozieradki pospolitej (*Trigonella foenum-graecum* L.) obserwowanych w eksperymentach rolniczych z wieloczynnikowymi układami. Oznaczono masę 1000 nasion, gęstość rzeczywistą, gęstość w stanie zsypanym oraz barwę, w tym skład spektralny, wymiary liniowe, współczynniki kształtu i właściwości tekstualne nasion kozieradki. Wyniki poddano obróbce statystycznej z wykorzystaniem analizy wariancji i analizy korelacji. Gęstość rzeczywista wynosiła od 1,283 do 1,318 g cm<sup>-3</sup>, masa 1000 nasion od 10,83 do 14,37 g, natomiast długość (*L*) i szerokość (*S*) – od 3,78 do 4,01 i od 2,65 do 2,84 mm w zależności do grupy doświadczalnej. Stwierdzono wpływ inokulacji oraz zabiegów agrotechnicznych na masę 1000 nasion. Statystycznie istotne współczynniki korelacji zanotowano dla gęstości zsypanej (od -0,809 do 0,772) oraz porowatości (-0,754 do 0,762) ze współczynnikami kształtu  $W_3$ ,  $W_9$  oraz  $W_{12}$ .

**Introduction**

The health benefits and environmental impacts of leguminous plants have been long recognized. Fenugreek (*Trigonella foenum-graecum* L.), also known as Alholva and Greek hayseed, is an annual plant of the family *Fabaceae*. The species is widely cultivated in West, South and Southeast Asia, North Africa, regions of Europe, Australia and North America (BASU et al. 2014, MCCORMICK et al. 2006, WIERZBOWSKA and ŻUK-GOŁASZEWSKA 2014). Fenugreek is used in human and animal nutrition, and it has been long recognized for its medicinal properties (MAKAI et al. 2004, POI et al. 1991). The plant is farmed mainly for its highly nutritional seeds which contain 25–30% of proteins with high biological value, lipids, saponins, flavonoids, choline, trigonelline, minerals (potassium, sulfur, calcium, magnesium), vitamins PP, H, F and B<sub>1</sub>, carotene and bioelements (iron, phosphorus, potassium, calcium) (AKBARI et al. 2012, BASU et al. 2014, EL NASRI and EL TINAY 2007, LAMFON 2012, SHIRANI and GANESHARANEE 2009, ŻUK-GOŁASZEWSKA et al. 2015). Fenugreek seeds contain 7.5–10% lipids (DM), mostly neutral lipids, including diglycerides (6.3%), triglycerides (86.1%) and glycolipids (5.4%), phospholipids (10.5%), small amounts of monoglycerides, sterols and free fatty acids (HEMAWATHY and PRABHAKAR 1988).

Fenugreek extract has estrogenic effects, and it is recommended for the treatment of impotence and relieving symptoms of menopause. In pharmaceutical research, there is ongoing interest in fenugreek which contains diosgenin, an active compound that reduces cholesterol levels in the human body. Fenugreek is also used in the production of oral hormones and steroids (ONCINA et al. 2000). In traditional medicine, fenugreek treatments were available in the form of infusions, tinctures, extracts, meads, antidepressant and psychoanaleptic tonics, and muscle building extracts. Fenugreek seeds and extracts aid digestion and absorption of nutrients, in particular amino acids.

They contribute to muscle growth, weight gain and have restorative properties. Fenugreek products are recommended for convalescents, anemia patients and athletes, in particular bodybuilders (HOJDEN 2000). The plant is also widely used in cosmetology as an ingredient of facial masks and treatments for seborrhea, acne and skin inflammations.

In addition, fenugreek is an environmentally friendly plant that fixes atmospheric nitrogen into compounds available for plants. Indicators of the nitrogen-fixing ability of legumes, including fenugreek, can be used to determine the nitrogen balance in plant production and to promote the cultivation of leguminous plants (PIETRZAK 2011). Fenugreek's total nitrogen-fixing ability was determined at  $100 \text{ kg ha}^{-1}$ , and it was similar to that observed in lentils (MCCORMICK et al. 2006). Species-specific variations are noted due to differences in the biomass of leguminous plants. KRISTENSEN (2002) demonstrated that peas and faba beans fixed 41 and  $54 \text{ kg N t}^{-1}$  DM seeds, respectively. Similar results were reported by SCHMIDTKE (2008). Fenugreek is also used to reclaim land in former sulfur mines covered with calcium from the float-sink separation process (KLIMONT et al. 2013).

The quality of fenugreek seeds is determined by their chemical composition and physical parameters (SHIRANI and GANESHARANEE 2009). An in-depth knowledge of the physical properties of seeds is required for the construction of threshing, transport, reloading, separation and drying machines to minimize seed damage during post-harvest handling, processing and storage, and to extract biologically active compounds from the seeds. The relevant knowledge is highly useful in the design and construction of farming machines and devices. The physical parameters of many seed varieties are influenced by the applied agrotechnical practices, local climate (BENÍTEZ-RODRÍGUEZ et al. 2014), seed moisture content and storage conditions (ALTUNTAS et al. 2005, AREMU et al. 2014, BLAHOVEC and LAHODOVÁ 2015).

The objective of this study was to identify changes in selected physical attributes, including geometric parameters and color of fenugreek seeds (*Trigonella foenum-graecum* L.). The experimental factors were agrotechnical practices, chemical protection and seed inoculation. The thousand seed weight, true density, bulk density, porosity, color and geometric parameters (linear dimensions, shape factors) of fenugreek seeds were determined.

## Material and Methods

Fenugreek seeds were grown in experimental fields at the Research Station in Tomaszkowo, Poland ( $53^{\circ}43' \text{ N}$ ,  $20^{\circ}24' \text{ E}$ ), of the University of Warmia and Mazury in Olsztyn. The experiment was established on typical brown soil of

quality class IVa with a light loam overlay. The soil was characterized by a slightly acidic pH, moderate content of phosphorus and potassium, and low levels of magnesium. Nitrogen concentration was determined at  $1.13 \text{ g kg}^{-1}$  of soil. Mineral fertilization consisted of  $30 \text{ kg N ha}^{-1}$  (urea),  $30.5 \text{ kg P ha}^{-1}$  (46% granular triple superphosphate) and  $83 \text{ kg K ha}^{-1}$  (60% potassium salt). The field experiment described in the study BIEŃKOWSKI et al. (2016). Harvested seeds were cleaned, dried to 12% moisture content ( $\pm 0.5\%$ ), transported to the laboratory and stored at  $7^\circ\text{C}$ . The moisture content of seeds was measured repeatedly before the experiment. All of the analyzed physical parameters were determined at 12% seed moisture content. The experimental designs with a division into plots are presented in Table 1.

Table 1  
Experimental set containing the division into groups and number of groups

Number of experimental group (sample)		Experimental design				
I	II	A	B	C	D	E
29 (no = 1021)	B_0	0	0	0	0	0
6 (no = 1026)	A_0	0	1	2	1	2
41 (no = 1077)		0	2	0	1	0
42 (no = 1026)		0	1	1	1	2
52 (no = 1041)		0	2	2	0	2
16 (no = 1026)	A_1	1	1	1	1	1
22 (no = 1059)		1	0	2	1	1
23 (no = 1033)		1	2	1	1	0
24 (no = 1059)		1	2	2	0	2
43 (no = 1036)		1	1	0	1	2

A – seed inoculation: (0) without inoculation, (1) with *Rhizobium meliloti* inoculation

B – sowing date: (0) very early, (1) delayed by 10 days, (2) delayed by 20 days

C – row spacing: (0) 15 cm, (1) 30 cm, (2) 45 cm

D – weed control: (0) mechanical, (1) chemical

E – chemical protection against pathogens: (0) without seed treatment, during growing season – Penncozeb 80WP, (1) seed treatment, without protection during growing season, (2) Dithane M45 seed treatment, during growing season – Penncozeb 80WP

The statistical analysis covered two experimental designs. In the first setup, the plots were divided into 10 experimental groups. In the second stage of the analysis, selected experimental groups were combined into a single data set (Table 1). It was assumed that unlike in uninoculated seeds (A\_0), inoculation (A\_1) combined with various types of chemical protection would differentiate the physical parameters of seeds. The third group (B\_0) consisted of seeds without *Rhizobium* inoculation or chemical protection (group 29).

The initial moisture content of fenugreek seeds was determined with the use of a laboratory weighing scale and SUP-3 general-purpose dryer according to standard PN-ISO 712:2002. Bulk density  $\rho_b$  [ $\text{g cm}^{-3}$ ] was measured accord-

ing to modified standard PN-EN ISO 7971- 3:2009. The measuring vessel was a 200 cm<sup>3</sup> graduated cylinder, and measurements were read to the nearest 0.01 g. True density  $\rho_t$  [g cm<sup>-3</sup>] was determined with a pycnometer according to standard PN-EN 1097-6:2002. Thousand seed weight [g] was determined according to standard PN-68/R-74017. Porosity was calculated from the following formula:

$$\varepsilon = \frac{\rho_t - \rho_b}{\rho_t} \cdot 100 [\%] \quad (1)$$

The color parameters of fenugreek seeds were determined in the MiniScan XE Plus spectrophotometer. A seed sample was placed in a transparent container, and measurements were performed in the bulk of the seeds. Color parameters were described in the *Lab*\* color space for 10° observer and standard illuminant D65. Spectral coefficients were determined in the range of 400 to 700 nm at 10 nm intervals.

The image analysis work station consisted of the EPSON PERFECTION 4490 Photo flatbed scanner and a graphics processing unit with the Intel Pentium D 830 processor. SILVERFAST EPSON v 6.4.3 scanning software supported full control of scanned images. The images were analyzed with the use of modified MaZda v 4.7 software (SZCZYPIŃSKI et al. 2009). An image segmentation algorithm was developed in the first stage of computer-aided analysis. Fenugreek seeds were spread on a contrasting background to facilitate the determination of the binarization threshold. The binarized image was superimposed onto the original image and scaled with calipers to the spatial resolution of the original image. The measurement error was computed as the sum of image scaling error (0.01 mm) and binarization result as a systematic error. A set of measured linear dimensions and shape factors was developed according to the method proposed by ZAPOTOCZNY (2011).

Fenugreek seeds are characterized by significant differences in their physical properties, including geometric parameters (Table 2), density and optical parameters. High phenotypic variation could obstruct the identification of differences induced by environmental conditions (experimental factors).

The data set was prepared for analysis according to the procedure described by ZIELIŃSKA et al. (2012). The results were processed in several stages involving various statistical procedures: (i) one-way ANOVA, to determine the presence of significant differences between mean values of selected physical attributes describing seeds subjected to various treatments, (ii) correlation analysis, to determine functional relationships between groups of physical attributes. Data were processed in Statistica 12.0 (StatSoft Inc., Tulsa, USA), Matlab Statistics Toolbox (MathWorks Co., USA).

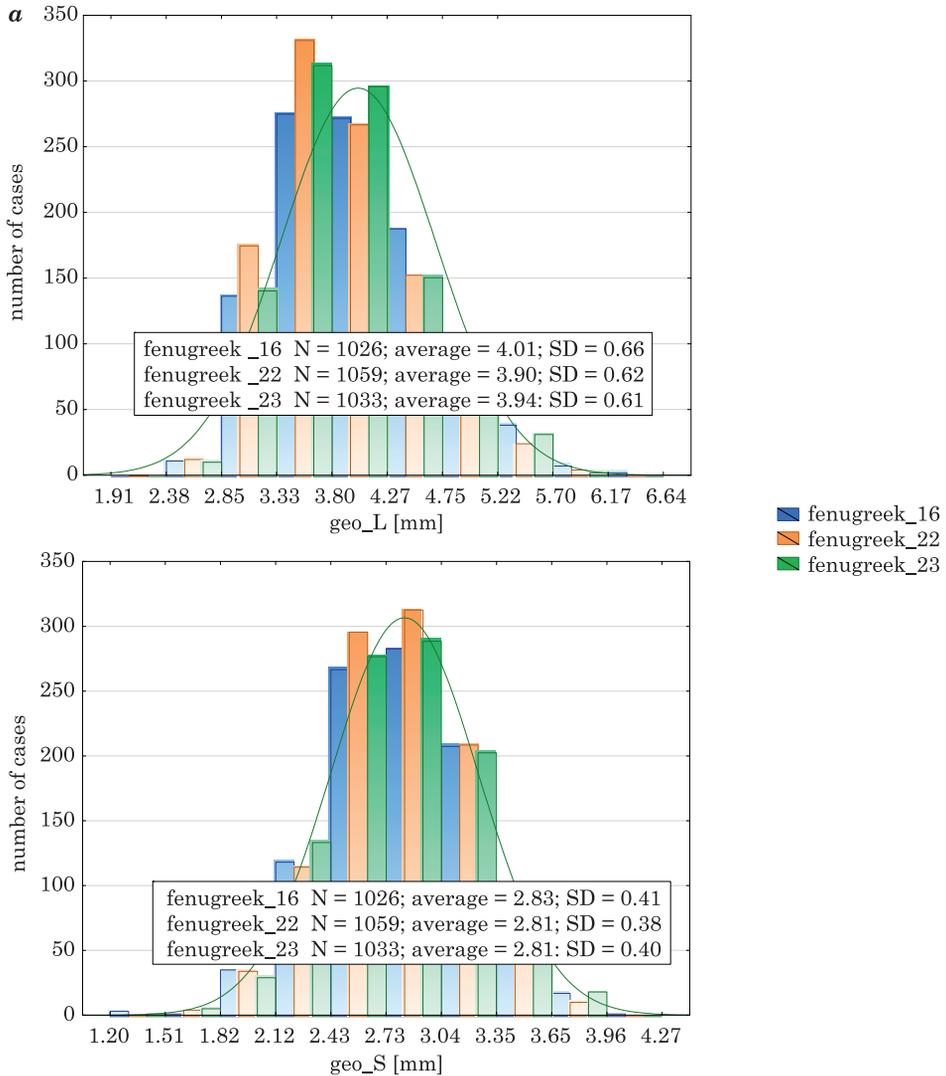
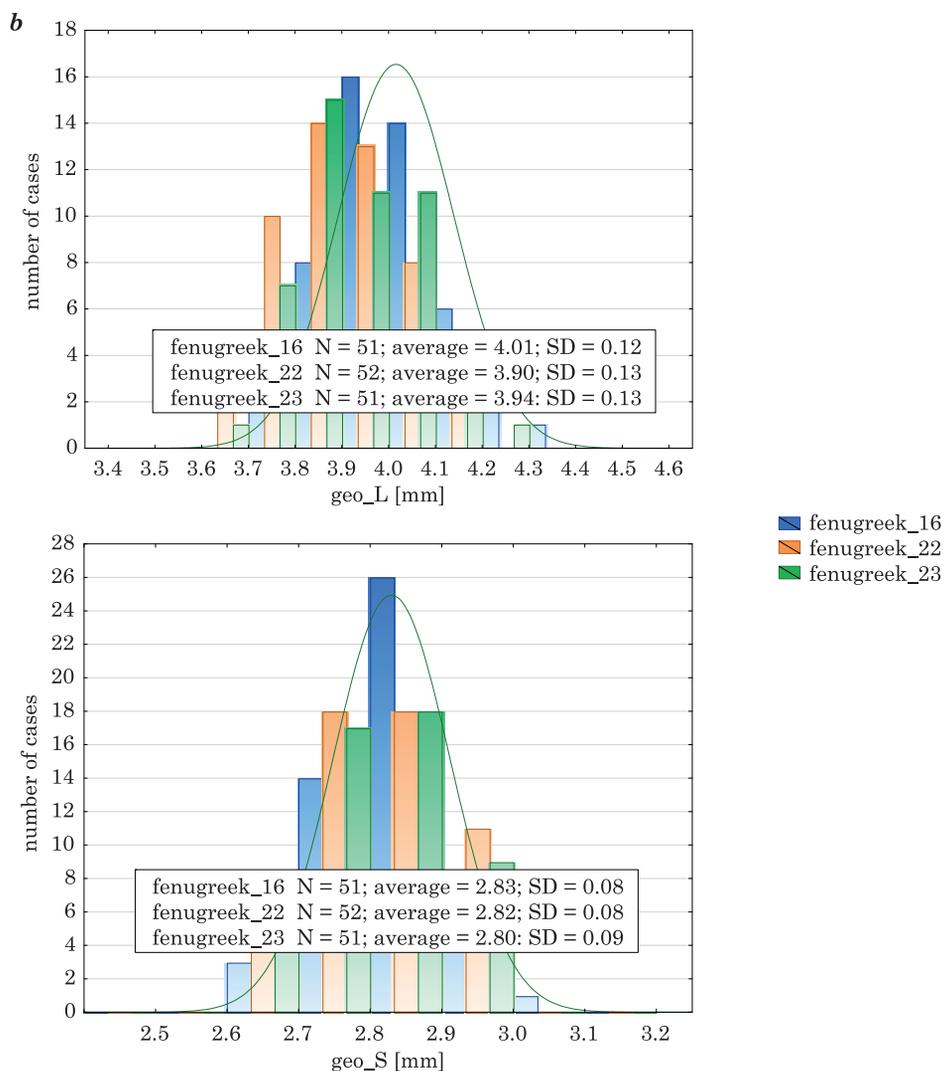


Fig. 1. Histograms of the distribution of selected linear dimensions (a) before and (b) after the cases averaging

Geometric parameters were randomly averaged before analysis because they were characterized by significant individual variation in the studied population, which could hinder the determination of the influence of applied agrotechnical practices on these values. The measured parameters were averaged in initial stages of the analysis, and data sets containing average values were used in successive statistical procedures such as correlation analysis. The above approach was adopted to minimize variation within



groups. Histograms of the distribution of selected variables before and after averaging are presented in Figure 1.

One-way ANOVA was carried out with the involvement of a post-hoc test with Studentized range statistics which analyzes the null hypothesis of the equality of means (Newman-Keuls test) at a significance level of  $P < 0.05$ . The aim of the analysis was to determine whether combinations of various agrotechnical practices applied before sowing and in the growing season influence the mean values of variables in experimental groups.

Table 2

Selected examples of fenugreek seeds with different dimensions

Geometric parameters	Fenugreek				
					
Length [mm]	3.66	3.40	3.66	3.12	5.06
Width [mm]	3.33	2.64	2.06	2.73	2.01
Surface area [mm <sup>2</sup> ]	9.61	7.67	6.15	6.92	8.57
Circuit [mm]	31.04	26.30	25.49	25.50	30.85

A correlation analysis was carried out to determine linear relationships between variables (Pearson's correlation coefficient,  $r$ ). Correlations were identified between the geometric properties of the analyzed seeds vs. their true density, bulk density, porosity and thousand seed weight. The presence of correlations between optical parameters (surface texture, color, spectral composition) was not analyzed because those variables are not logically interconnected.

## Results and Discussion

The mean values of the analyzed parameters and the results of comparison of mean values are presented in Tables 3–7. In the group of physical properties (Table 3), thousand seed weight was most influenced by seed inoculation,

Table 3  
Means and standard deviations obtained from one-way ANOVA for selected physical features – experimental set I

Number of plot	Thousand seed weight [g]	Bulk density [g cm <sup>-3</sup> ]	True density [g cm <sup>-3</sup> ]	Porosity [%]
29	13.61 (0.20) <sup>c</sup>	0.762 (0.011) <sup>b</sup>	1.298 (0.006) <sup>a</sup>	41.8 (0.4) <sup>ab</sup>
42	10.83 (0.30) <sup>a</sup>	0.730 (0.002) <sup>a</sup>	1.318 (0.020) <sup>a</sup>	44.7 (0.7) <sup>a</sup>
6	13.13 (0.41) <sup>c</sup>	0.748 (0.006) <sup>ab</sup>	1.300 (0.004) <sup>a</sup>	42.7 (0.3) <sup>ab</sup>
41	11.64 (0.18) <sup>ab</sup>	0.729 (0.006) <sup>a</sup>	1.297 (0.019) <sup>a</sup>	44.0 (1.1) <sup>a</sup>
52	10.83 (0.40) <sup>a</sup>	0.743 (0.012) <sup>ab</sup>	1.304 (0.009) <sup>a</sup>	42.8 (1.5) <sup>ab</sup>
22	13.12 (0.29) <sup>c</sup>	0.788 (0.024) <sup>c</sup>	1.293 (0.008) <sup>a</sup>	40.0 (1.8) <sup>b</sup>
43	11.59 (0.29) <sup>ab</sup>	0.744 (0.011) <sup>ab</sup>	1.299 (0.005) <sup>a</sup>	43.2 (0.1) <sup>ab</sup>
16	14.37 (0.54) <sup>d</sup>	0.731 (0.015) <sup>a</sup>	1.283 (0.005) <sup>a</sup>	43.5 (1.4) <sup>ab</sup>
23	11.95 (0.48) <sup>b</sup>	0.748 (0.008) <sup>ab</sup>	1.316 (0.009) <sup>a</sup>	43.5 (0.1) <sup>ab</sup>
24	11.87 (0.48) <sup>b</sup>	0.757 (0.002) <sup>ab</sup>	1.315 (0.003) <sup>a</sup>	42.4 (0.3) <sup>ab</sup>

Standard deviation is given in parentheses; <sup>a, b, c</sup> denote homogeneous groups;  $P < 0.05$

Table 4  
Means and standard deviations obtained from one-way ANOVA for selected color parameters – experimental set I

Number of plot	$L^*$ [-]	$a^*$ [-]	$b^*$ [-]	YI E313 [-]	550 [nm]	600 [nm]	650 [nm]	700 [nm]
29	47.65 (0.53) <sup>abc</sup>	8.12 (0.25) <sup>a</sup>	18.83 (0.76) <sup>a</sup>	64.53 (1.25) <sup>a</sup>	16.16 (0.60) <sup>abc</sup>	21.05 (0.54) <sup>ab</sup>	25.89 (0.54) <sup>ab</sup>	33.53 (0.79) <sup>bc</sup>
42	47.84 (0.56) <sup>bc</sup>	7.64 (0.61) <sup>a</sup>	18.28 (1.18) <sup>a</sup>	62.38 (3.08) <sup>bc</sup>	16.34 (0.49) <sup>bc</sup>	21.01 (0.78) <sup>ab</sup>	25.87 (0.98) <sup>ab</sup>	33.22 (1.26) <sup>abc</sup>
6	45.78 (0.72) <sup>d</sup>	6.31 (0.62) <sup>b</sup>	13.82 (2.10) <sup>c</sup>	50.92 (5.81) <sup>c</sup>	14.86 (0.50) <sup>d</sup>	18.40 (0.85) <sup>d</sup>	22.08 (1.66) <sup>c</sup>	27.77 (1.81) <sup>d</sup>
41	46.61 (0.38) <sup>abd</sup>	7.24 (0.30) <sup>a</sup>	17.57 (0.65) <sup>ab</sup>	61.20 (1.42) <sup>bc</sup>	15.43 (0.20) <sup>abd</sup>	19.76 (0.41) <sup>ac</sup>	24.02 (0.96) <sup>a</sup>	31.49 (0.90) <sup>ab</sup>
52	47.12 (0.97) <sup>abc</sup>	7.37 (0.58) <sup>a</sup>	18.38 (1.53) <sup>a</sup>	62.80 (3.10) <sup>bc</sup>	15.91 (0.80) <sup>abc</sup>	20.33 (1.14) <sup>abc</sup>	24.76 (1021) <sup>a</sup>	32.27 (1.66) <sup>ab</sup>
22	48.33 (0.86) <sup>c</sup>	8.13 (0.57) <sup>a</sup>	19.16 (1.25) <sup>a</sup>	64.68 (2.93) <sup>a</sup>	16.81 (0.62) <sup>c</sup>	21.66 (1.03) <sup>b</sup>	26.98 (1.46) <sup>b</sup>	34.74 (1.56) <sup>c</sup>
43	47.26 (0.57) <sup>abc</sup>	7.87 (0.87) <sup>a</sup>	17.45 (1.13) <sup>ab</sup>	61.24 (2.86) <sup>bc</sup>	15.78 (0.48) <sup>abc</sup>	20.40 (0.64) <sup>abc</sup>	25.84 (1.05) <sup>ab</sup>	32.57 (1.05) <sup>abc</sup>
16	46.48 (1.04) <sup>ad</sup>	7.53 (0.55) <sup>a</sup>	16.20 (1.39) <sup>b</sup>	58.31 (3.30) <sup>b</sup>	15.22 (0.89) <sup>ad</sup>	19.57 (1.11) <sup>c</sup>	24.61 (1.39) <sup>a</sup>	31.10 (1.85) <sup>a</sup>
23	47.62 (0.58) <sup>abc</sup>	7.66 (0.30) <sup>a</sup>	18.03 (0.76) <sup>ab</sup>	62.01 (1.32) <sup>bc</sup>	16.03 (0.55) <sup>abc</sup>	20.74 (0.65) <sup>abc</sup>	25.92 (1.00) <sup>ab</sup>	33.12 (0.89) <sup>abc</sup>
24	47.44 (0.59) <sup>abc</sup>	7.41 (0.34) <sup>a</sup>	18.09 (0.68) <sup>ab</sup>	61.96 (1.26) <sup>bc</sup>	16.04 (0.56) <sup>abc</sup>	20.62 (0.57) <sup>abc</sup>	25.26 (0.97) <sup>ab</sup>	32.67 (0.99) <sup>abc</sup>

Standard deviation is given in parentheses; <sup>a, b, c</sup> denote homogeneous groups;  $P < 0.05$

sowing date, row spacing and plant protection treatments. The highest thousand seed weight (14.37 g) was noted in plots where seeds were inoculated with *Rhizobium meliloti*, sowing was delayed by 10 days, the distance between rows was 30 cm, weeds were chemically controlled, seeds were dressed, and no chemical protection was applied in the growing season. The next analyzed parameter was bulk density whose values were correlated with the applied treatments. The variants were split into three groups, and two groups were identified when porosity was taken into account. The lowest value of the variable (40.0%) was observed in the plot with inoculated seeds, early sowing and chemical plant protection. The applied agrotechnical practices did not influence the mean value of true density (Table 3). In a study by ALTUNTAS et al. (2005), seed density was determined in the range of 1240.36–1165.25 kg m<sup>-3</sup>.

In the group of optical parameters the smallest difference in mean values between treatments was noted for parameter  $a^*$  (Table 4). Two homogeneous groups were identified. The first group contained the variant without seed inoculation, sowing delayed by 10 days, 30 cm row spacing, chemical weed control, comprehensive fungicide treatment (seed dressing with Dithane M45

and with Penncozeb 80WP in the growing season), and the lowest value of the analyzed parameter. The second group comprised the remaining variants. Parameter  $L^*$  was characterized by the highest variation of mean values. An analysis of spectral mean values at 400–430 nm, 480 nm and 500 nm did not reveal differences between the analyzed production technologies. Several homogeneous groups were identified for each of the remaining wavelengths.

Image analysis is used to assess the geometric parameters of grain (EMAD-ZADEH et al. 2010, ZAPOTOCZNY 2012). The geometric parameters of fenugreek seeds are shown in Table 5. In ANOVA, the smallest differences between means were noted for seed width  $S$  (3 groups) and surface area  $F$  (4 groups). The highest number of subgroups was identified for circumference  $Ul$  and shape factor  $W_5$  (Table 5). In a study by ALTUNTAS et al. (2005) who found that seed length ranged from 4.01 to 4.99 mm, and seed width – from 2.35 to 2.61 mm.

Statistically significant differences in the mean values of selected variables were also observed between plots in the second experimental design (Table 6–7). Inoculation and chemical plant protection influenced thousand seed weight.

Table 5  
Means and standard deviations obtained from one-way ANOVA for selected geometrical parameters – experimental set I

Number of plot	$F$ [mm <sup>2</sup> ]	$S$ [mm]	$L$ [mm]	$Ul$ [mm]	$W_5$ [-]	$W_8$ [-]	$W_9$ [-]
29	8.95 (1.87) <sup>a</sup>	2.84 (0.38) <sup>a</sup>	3.85 (0.61) <sup>be</sup>	30.32 (3.43) <sup>ab</sup>	1.276 (0.261) <sup>be</sup>	0.752 (0.141) <sup>d</sup>	1.224 (0.049) <sup>b</sup>
42	8.72 (2.17) <sup>b</sup>	2.76 (0.43) <sup>b</sup>	3.91 (0.67) <sup>ab</sup>	30.25 (4.01) <sup>ab</sup>	1.212 (0.269) <sup>cd</sup>	0.721 (0.149) <sup>abc</sup>	1.244 (0.061) <sup>d</sup>
6	9.06 (2.02) <sup>a</sup>	2.83 (0.39) <sup>a</sup>	3.94 (0.65) <sup>ac</sup>	30.82 (3.77) <sup>cd</sup>	1.234 (0.268) <sup>ad</sup>	0.734 (0.142) <sup>bc</sup>	1.235 (0.056) <sup>a</sup>
41	8.53 (2.04) <sup>d</sup>	2.74 (0.43) <sup>b</sup>	3.83 (0.58) <sup>de</sup>	29.81 (3.71) <sup>f</sup>	1.225 (0.262) <sup>acd</sup>	0.728 (0.140) <sup>abc</sup>	1.237 (0.060) <sup>a</sup>
52	8.75 (1.99) <sup>b</sup>	2.74 (0.41) <sup>b</sup>	3.91 (0.65) <sup>ab</sup>	30.19 (3.65) <sup>a</sup>	1.248 (0.261) <sup>ab</sup>	0.719 (0.150) <sup>ab</sup>	1.228 (0.055) <sup>bc</sup>
22	8.97 (1.88) <sup>a</sup>	2.81 (0.38) <sup>a</sup>	3.90 (0.62) <sup>ab</sup>	30.40 (3.45) <sup>ab</sup>	1.284 (0.264) <sup>e</sup>	0.739 (0.145) <sup>c</sup>	1.223 (0.047) <sup>b</sup>
43	9.04 (1.92) <sup>a</sup>	2.79 (0.39) <sup>a</sup>	4.01 (0.64) <sup>c</sup>	30.91 (3.52) <sup>cd</sup>	1.246 (0.268) <sup>ab</sup>	0.712 (0.144) <sup>a</sup>	1.243 (0.056) <sup>d</sup>
16	9.15 (2.07) <sup>a</sup>	2.83 (0.41) <sup>a</sup>	4.01 (0.66) <sup>c</sup>	31.09 (3.72) <sup>d</sup>	1.249 (0.275) <sup>ab</sup>	0.721 (0.144) <sup>abc</sup>	1.244 (0.055) <sup>d</sup>
23	9.01 (1.94) <sup>a</sup>	2.81 (0.40) <sup>a</sup>	3.94 (0.61) <sup>ac</sup>	30.65 (3.48) <sup>bc</sup>	1.267 (0.274) <sup>be</sup>	0.727 (0.143) <sup>abc</sup>	1.231 (0.051) <sup>ac</sup>
24	8.19 (2.07) <sup>c</sup>	2.65 (0.42) <sup>c</sup>	3.78 (0.63) <sup>d</sup>	29.19 (3.88) <sup>e</sup>	1.202 (0.278) <sup>c</sup>	0.716 (0.145) <sup>ab</sup>	1.233 (0.057) <sup>ac</sup>

Standard deviation is given in parentheses; <sup>a, b, c</sup> denote homogeneous groups;  $P < 0.05$

This parameter was highest (13.61 g) in the group without seed inoculation or chemical protection in the growing season (*B\_0*). Chemical protection (*A\_0*) lowered thousand seed weight (11.66 g). Significant differences in mean bulk density, porosity, parameter  $b^*$  and yellowness index *YI E313* were observed between the control group (*B\_0*) and the group subjected to chemical protection without inoculation (*A\_0*). The applied treatments also influenced parameter  $a^*$ . Two homogeneous groups were identified: the first; contained the control group and the group with inoculation and chemical treatment, and the second; comprised the group with chemical protection only. Analyses of true density, parameter  $L^*$  and spectral values in the range of 400–480 nm and 500–570 nm did not reveal statistically significant differences between experimental factors (Table 6).

Table 6  
Means and standard deviations obtained from one-way ANOVA for selected physical features and colour parameters – experimental set II

Experimental group	Thousand seed weight [g]		Bulk density [g cm <sup>-3</sup> ]		True density [g cm <sup>-3</sup> ]			Porosity [%]
	$L^*$ [-]	$a^*$ [-]	$b^*$ [-]	YI E313 [-]	490 [nm]	600 [nm]	700 [nm]	
<i>B_0</i>	13.61 (0.20) <sup>c</sup>		0.762 (0.011) <sup>b</sup>		1.298 (0.006) <sup>a</sup>			41.8 (0.40) <sup>a</sup>
<i>A_0</i>	11.66 (0.99) <sup>a</sup>		0.739 (0.010) <sup>a</sup>		1.304 (0.018) <sup>a</sup>			43.3 (1.2) <sup>b</sup>
<i>A_1</i>	12.51 (1.12) <sup>b</sup>		0.749 (0.020) <sup>ab</sup>		1.300 (0.014) <sup>a</sup>			42.4 (1.9) <sup>ab</sup>
<i>B_0</i>	47.65 (0.53) <sup>a</sup>	8.12 (0.25) <sup>a</sup>	18.83 (0.76) <sup>b</sup>	64.53 (1.25) <sup>b</sup>	10.84 (0.21) <sup>a</sup>	21.05 (0.54) <sup>b</sup>	33.53 (0.79) <sup>a</sup>	
<i>A_0</i>	46.84 (1.00) <sup>a</sup>	7.14 (0.72) <sup>b</sup>	17.01 (2.35) <sup>a</sup>	59.32 (6.07) <sup>a</sup>	10.99 (0.38) <sup>ab</sup>	19.87 (1.25) <sup>a</sup>	31.18 (2.51) <sup>b</sup>	
<i>A_1</i>	47.42 (0.92) <sup>a</sup>	7.72 (0.60) <sup>a</sup>	17.78 (1.40) <sup>ab</sup>	61.63 (3.10) <sup>ab</sup>	11.20 (0.35) <sup>b</sup>	20.59 (1.02) <sup>ab</sup>	32.83 (1.69) <sup>a</sup>	

Standard deviation is given in parentheses; <sup>a, b, c</sup> denote homogeneous groups;  $P < 0.05$

Table 7  
Means and standard deviations obtained from one-way ANOVA for selected geometrical parameters – experimental set II

Group	$F$ [mm <sup>2</sup> ]	$S$ [mm]	$L$ [mm]	Ul [mm]	$R_D$ [-]	$W_5$ [-]	$W_8$ [-]	$W_9$ [-]
<i>B_0</i>	8.95 (1.87) <sup>b</sup>	2.84 (0.38) <sup>b</sup>	3.85 (0.61) <sup>b</sup>	30.32 (3.43) <sup>a</sup>	11547559 (4935198) <sup>a</sup>	1.276 (0.261) <sup>c</sup>	0.752 (0.141) <sup>b</sup>	1.224 (0.049) <sup>b</sup>
<i>A_0</i>	8.76 (2.06) <sup>a</sup>	2.77 (0.42) <sup>a</sup>	3.90 (0.64) <sup>a</sup>	30.26 (3.80) <sup>a</sup>	11589547 (5676832) <sup>a</sup>	1.232 (0.265) <sup>a</sup>	0.725 (0.145) <sup>a</sup>	1.236 (0.059) <sup>a</sup>
<i>A_1</i>	8.87 (2.01) <sup>ab</sup>	2.78 (0.40) <sup>a</sup>	3.93 (0.64) <sup>a</sup>	30.43 (3.67) <sup>a</sup>	11756438 (5475035) <sup>a</sup>	1.249 (0.273) <sup>b</sup>	0.723 (0.144) <sup>a</sup>	1.235 (0.054) <sup>a</sup>
–	–	–	–	–	$R_D = \frac{F^3}{(\sum_i l_i)^2}$	$W_5 = \frac{F}{L_{sz}}$	$W_8 = \frac{L}{S}$	$W_9 = \frac{L \cdot S}{F}$

Standard deviation is given in parentheses; <sup>a, b, c</sup> denote homogeneous groups;  $P < 0.05$

$l_i$  = minimum distance between pixel of object and outline of object;  $L_{sz}$  – length of the skeletonized object

Chemical protection alone or combined with inoculation contributed to significant differences between the mean values of  $S$ ,  $L$  and shape factors  $W_8$  and  $W_9$  in comparison with the control group (Table 7). In seeds produced by chemically treated fenugreek plants grown from uninoculated material ( $A_0$ ), the mean surface area differed significantly from the control group ( $B_0$ ). Three homogeneous groups were identified for shape factor  $W_5$ . The differences in the mean values of  $Ul$  and  $R_D$  between groups were not statistically significant.

The significant correlation coefficients demonstrating the presence of linear relationships between geometric parameters and the remaining physical properties of seeds are presented in Table 8. Significant correlations were observed only between bulk density ( $r = -0.809$  to  $0.772$ ), porosity ( $r = -0.754$  to  $0.762$ ) and selected shape factors. The correlations between thousand seed weight, true density and geometric parameters were not statistically significant, which could be attributed to the absence of significant differences in the mean values of a given attribute between experimental groups despite the application of various treatments.

Table 8  
Analysis of correlation between physical properties and selected geometrical parameters

Geometrical parameters	Physical properties			
	thousand seed weight [g]	bulk density [g cm <sup>-3</sup> ]	true density [g cm <sup>-3</sup> ]	porosity [%]
$Geo\_W_3 = \frac{U_1^2}{F}$	-	-0.809	-	0.762
$Geo\_W_9 = LSF^{-1}$	-	-0.805	-	0.738
$Geo\_W_{12}$	-	0.772	-	-0.754

$P = 0.05$

Scatter plots and regression equations are shown in Figure 2. Groups 22 and 29 are distinguished from others. They include the uninoculated seeds. This fact confirms that inoculation influenced density, porosity and geometrical parameters.

## Conclusions

1. The results of this study revealed that agrotechnical practices influenced thousand seed weight, and they supported the identification of four homogeneous groups of fenugreek seeds. Thousand seed weight ranged from 10.83 to 14.37 g across the evaluated groups. The applied agrotechnical practices,

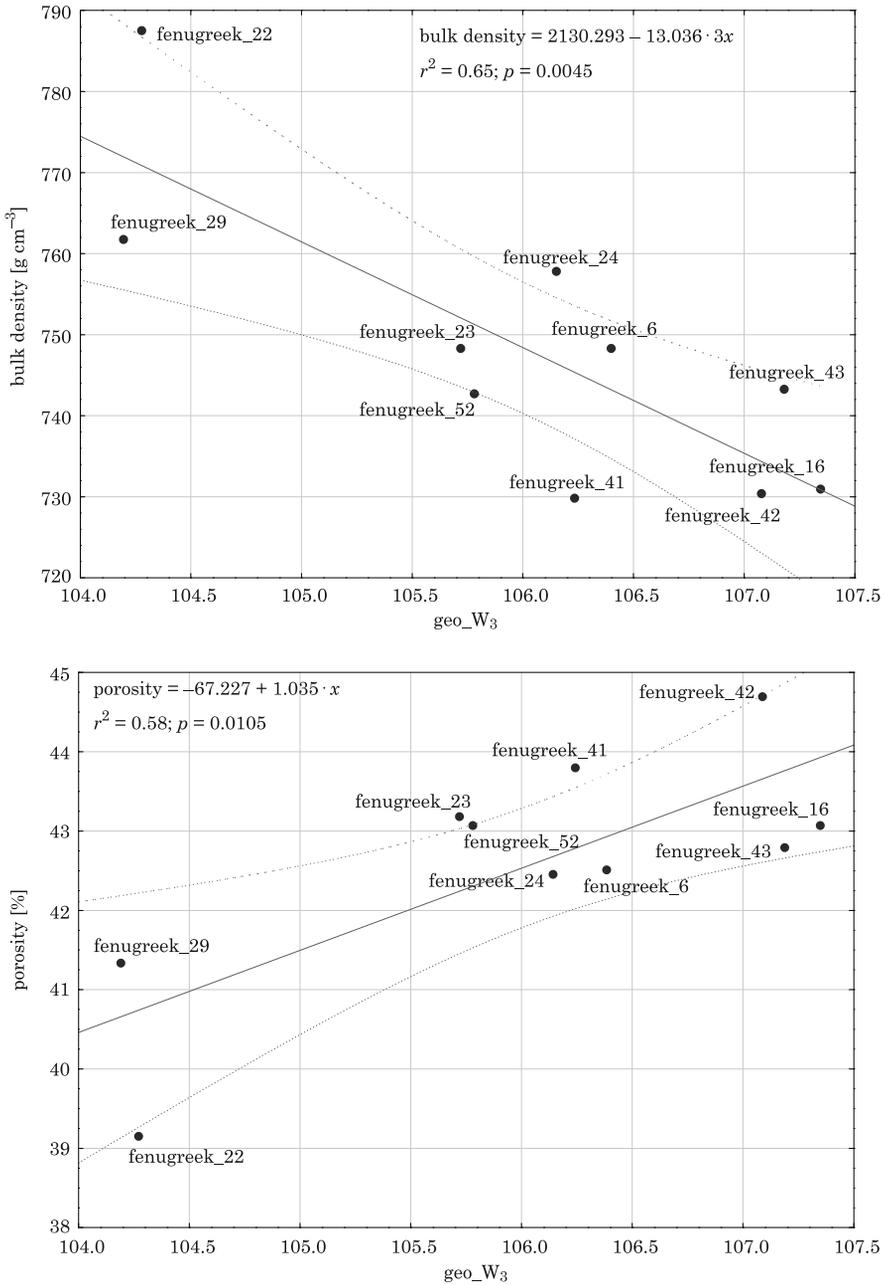


Fig. 2. Scatter plots for selected physical properties relative to shape factor  $Geo\_W_3$

chemical treatments and inoculation had no effect on true density. The impact of the above factors on porosity and bulk density was ambiguous. In an analysis of bulk density, three homogeneous groups were identified, where the control group (29) was a separate set. A porosity analysis produced two homogeneous groups.

2. Optical properties constituted the second group of the analyzed physical parameters. The highest variation was noted for color parameter  $L^*$  (lightness) and wavelengths of 600 and 700 nm. Uninoculated seeds from group 6 were characterized by the lowest  $L$  value of 45.

3. No significant variations in geometric properties were observed. Four to five homogeneous groups were identified based on surface area ( $F$ ), circumference (UI), shape factors  $W_9$ ,  $W_8$ , and  $R_D$ . Seed length ranged from 3.78 to 4.01 mm, and seed width was determined at 2.65 to 2.84 mm. Inoculation influenced thousand seed weight and shape factor  $W_5$ . Seeds that were not inoculated or chemically protected were characterized by higher thousand seed weight than seeds subjected to chemical protection only (13.61 and 11.66 g, respectively).

4. The significant correlation coefficients were noted for bulk density (-0.809 to 0.772) and porosity (-0.754 to 0.762) with shape factors  $W_3$ ,  $W_9$  and  $W_{12}$ .

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## PREFERENCES OF CONSUMERS FOR CHOOSING POULTRY MEAT

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Key words: poultry, meat, quality, consumer, preferences.

### Abstract

Poultry meat, with regard to lower fat content than the one in other animal species, is considered by consumers as dietetic. It also constitutes a source of complete protein, iron and minerals. It is also cheaper than red meat. In recent years, national poultry meat market has been developing very dynamically, and Poland with poultry meat production at the level of 2,420,000 tons belongs to significant EU producers. With such a large production and unlimited access to fresh poultry meat as well as a growing care of health, consumer searches for raw materials which are distinguished by a very good quality. The aim of the study was analysis of consumer knowledge on the subject of poultry meat quality, and of criteria which consumers use while purchasing it, based on surveys carried out on 100 respondents. From the research it follows that most of the surveyed people consume poultry meat usually two or three times a week, mostly in the form of a breast fillet and quarter, or wings. Consumers prefer meat derived from broiler chickens (84%), while least frequently they buy goose meat (1%). Consumers first of all are guided by meat freshness and then by its price. For more than half of the surveyed, it does not matter if meat comes from a conventional or organic production. Only 26% of consumers prefer meat produced in a guaranteed quality system, QAFP. When buying poultry meat of high quality, consumers first of all expect a higher health safety, higher nutritional value and better palatability. When buying poultry meat, the surveyed are mostly concerned about antibiotics and genetically modified plants used in poultry nutrition.

### PREFERENCJE KONSUMENTÓW PRZY WYBORZE MIĘSA DROBIOWEGO

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Słowa kluczowe: drób, mięso, jakość, konsument, preferencje.

### Abstrakt

Mięso drobiowe, ze względu na mniejszą zawartość tłuszczu niż mięso innych gatunków zwierząt, uważane jest przez konsumentów za dietetyczne. Stanowi także źródło pełnowartościowego białka oraz żelaza i składników mineralnych. Jest tańsze od mięsa czerwonego. Krajowy rynek mięsa drobiowego w ostatnich latach rozwija się bardzo dynamicznie, a Polska z produkcją mięsa drobiowego na poziomie 2 420 000 ton należy do znaczących producentów UE. Przy tak dużej produkcji i nieograniczonym dostępie do świeżego mięsa drobiowego oraz coraz większej dbałości o zdrowie konsumenci poszukują surowców, które wyróżnia bardzo dobra jakość. Celem badań była analiza rozeznania konsumentów na temat jakości mięsa drobiowego oraz kryteriów, którymi kierują się przy jego zakupie. Materiał stanowiły wyniki badań ankietowych przeprowadzonych na 100 respondentach. Z badań wynika, że większość ankietowanych spożywa mięso drobiowe, zwykle dwa lub trzy razy w tygodniu, najczęściej w postaci filetu z piersi i ćwiartki czy skrzydeł. Konsumenci preferują mięso pochodzące od kurcząt brojlerów (84%), a najmniej kupują gęsiny (1%). Kierują się w pierwszej kolejności świeżością mięsa, a w drugiej jego ceną. Dla ponad połowy respondentów nie ma znaczenia czy mięso pochodzi z produkcji konwencjonalnej czy ekologicznej. Jedynie 26% konsumentów preferuje mięso wyprodukowane w gwarantowanym systemie jakości QAFP. Kupując mięso drobiowe wysokiej jakości, przede wszystkim oczekują większego bezpieczeństwa zdrowotnego, większej wartości żywieniowej i lepszej smakowitości. Podczas zakupu mięsa drobiowego ankietowani najbardziej obawiają się stosowania w żywieniu ptaków antybiotyków oraz pasz z wykorzystaniem roślin genetycznie modyfikowanych.

## Introduction

Poultry production is one of the best developing sectors of animal production. Polish poultry market is influenced not only by preferences of Polish consumers, but also by foreign trade, prices and markets of other types of meat. Poland is a significant producer of poultry meat in the European Union. Currently, the total poultry meat production in Poland is 2,420,000 tons. Taking into consideration only meat from broiler chickens, Poland takes the first position in the European Union. The most important recipients of poultry meat in the European Union include Germany, the Czech Republic, Great Britain and France. Polish chicken meat is also exported to Benin, Hong Kong, China and African countries (ADAMSKI and WENCEK 2012).

Currently, in Poland meat consumption per person annually equals 27.0 kg, which is a value similar to the mean in the European Union. An increase in poultry meat consumption is affected by an increasing care among modern consumers of their own health. White meat may be consumed even several times a week. It is cheaper than red meat, contains less fat, thus it is dietetic, and additionally it is a very good source of protein, iron and minerals. It is also often chosen as it is easily separated, and easily and quickly prepared for consumption. Besides, it is distinguished by a high coefficient of protein digestion, a very favorable conversion of feed protein into animal protein, used in the production of various delicatessen food products and high taste qualities.

It is also important for consumers that poultry fats contain a lot of mono- and polyenoic fatty acids, triglycerides and phospholipids. Hence, the percentage of undesirable saturated fatty acids in poultry meat is lower than in the fat of pork, mutton or beef (ADAMSKI 2010, ADAMSKI and WENCEK 2012, NOWAK and TRZISZKA 2010).

Consumers evaluate meat first of all based on visual and smell impressions. One of such traits is meat fattiness, if it is too high it causes discouragement in the consumer to purchase it, as poultry meat is considered as a dietetic product. From among the traits which are visually evaluated, which a consumer pays attention to, the color of meat and carcass is of great importance. Consumers first of all prefer meat of red color, as well as carcasses of a natural yellow color, which they connect with meat origin from rearing with unconventional methods and proper animal nutrition. The important sensory traits which may be evaluated after thermal treatment and meat consumption include palatability, which includes taste and smell of the meat, as well as succulence that is dryness or moisture and meat tenderness, which is one of the meat texture parameters (CASTELLINI et al. 2008, DOKTOR 2007, GRABOWSKI and KIJOWSKI 2004, POŁOM and BARYŁKO-PIKIELNA 2004, ZDANOWSKA-SĄSIADK et al. 2013).

Poultry meat consumers are more and more interested in the conditions of birds' housing and production system from which the birds come, as well as in the animal welfare and quality and safety of the raw materials and products offered on the market (ADAMSKI 2010, ARAL et al. 2013, DOKTOR 2007). In Europe, as well as in Poland, obtaining poultry meat in accordance with the certified principles of organic rearing becomes more and more popular, although it is more expensive than with conventional methods, if only because of a longer period of bird rearing or higher utilization of feed per growth of 1 kg of body weight, and also because of other principles of organic rearing which are more or less restrictive (CASTELLINI et al. 2002, CASTELLINI 2005, HERBUT and KORELESKI 2004, MIKULSKI et al. 2011, ELEROĞLU et al. 2013, PIETRZAK et al. 2013).

An increase in the poultry meat production through its intensification, as a response to an increase in the demand for this type of meat, caused that consumers attach more and more importance to the quality of raw materials and products being on the market. Growing requirements of Polish and foreign consumers relate not only to the good quality of poultry, but also to a higher health safety, that is raw materials free of pathogenic microorganisms, substances and physical contaminations. With regard to such preferences on the consumer market in mid-2010, an initiative was undertaken to introduce the generally understood guaranteed quality, whose example is the elaborated, introduced and coordinated by the National Poultry Council – Chamber

of Commerce in Warsaw, QAFP system (Quality Assurance for Food Products). The main goal of the QAFP system is distinguishing production which would introduce higher quality products into the market, and maximization of their health safety through continuous veterinary and production supervision (ADAMSKI and WENCEK 2012, BRZOZOWSKI 2012).

With such a high production scale and unlimited access to fresh poultry meat as well as with an increasing care of health, consumer market searches for raw materials, which are distinguished by a very high quality. Thus, conducting surveys on consumer preferences for choosing poultry products and raw materials seems to be a really up-to-date and significant issue. The aim of the study was an analysis of consumer knowledge on the quality of poultry meat, and criteria which consumers use when purchasing it.

## **Material and Methods**

The present research was carried out based on a survey. The answers of respondents were obtained through direct filling of questionnaires, but also through posting the survey on a website, which enabled carrying out research in various regions of Poland. The survey included 16 questions, five of which concerned general information about consumers such as sex, age, education, place of residence, range of monthly salary, 11 questions concerned consumer's preferences for choosing poultry meat. From the total number of 100 respondents, 54 people filled questionnaires posted on the website, while 46 people took part in a direct survey.

## **Results and Discussion**

Data concerning characteristics of the studied group of respondents (Table 1) shows that among the surveyed, women constituted 64%, while men 36%. The lowest percentage (6%) among respondents constituted young people below 18, whereas the most numerous group surveyed (63%) was represented by people between 19 and 30. The percentage of the surveyed within the age group from 31 to 50 and above 50, was 18% and 13%, respectively. The surveyed included 63% of people living in a town and 37% of people living in a village. Based on an analysis of the financial status of the surveyed, it may be concluded that the two most numerous groups are people whose monthly salary range per person was from 1001 to 1500 PLN, and from 1501 to 2000 PLN. The percentage of these groups was 28% and 23%, respectively. The smallest group among the respondents, constituting 13%, were consumers, whose

monthly income per person was up to 1000 PLN gross. The most numerous surveyed group (33%) turned out to be people with undergraduate education. The percentage of respondents with graduate education and with secondary education was similar (27% and 28%, respectively). The lowest percentage of those who filled the survey were people with primary education (7%) and vocational training, being 5%.

Table 1  
Characteristics of the surveyed group of consumers

Specification	Responses	Share [%]
Sex	woman	64
	man	36
Age	under 18	63
	19 to 30	18
	31 to 50	13
	over 50	
Place of residence	town	63
	village	37
Range of monthly income PLN, gross	up to 1000	13
	1001 to 1500	28
	1501 to 2000	23
	2001 to 3000	15
	over 3000	21
Education	primary	7
	vocational training	5
	secondary	28
	undergraduate	33
	graduate	27

Table 2  
Consumption of meat and preferred type

Specification	Responses	Share [%]
Declaration of meat consumption	yes	94
	no	6
Frequency of meat consumption	almost every day	5
	two, three times a week	46
	once a week	35
	once every two weeks	10
	less than once a month	4
Preferred type of meat	chicken	84
	turkey	5
	duck	10
	goose	1
	other species	0

Consumption of poultry meat was declared by 94% of the surveyed (Table 2). Polish consumers definitely preferred meat of broiler chickens, as was reported by 84% of the respondents. Duck meat was chosen by 10% of those surveyed, while turkey and goose meat by only 5% and 1% of consumers, respectively. Majority of the people consumed poultry meat two or three times per week, most preferably in the form of breast fillet and quarter, or wings (Table 3). Taking into consideration poultry species, preferences of Polish consumers for purchasing meat of broiler chickens were similar as in foreign studies (KWADZO et al. 2013, SALAWU et al. 2014), from which it follows that up to 94.7% of respondents decided to purchase this type of meat.

Table 3

Choice of culinary elements\*

Responses	Share [%]
Whole carcass	11
Brest fillet	86
Quarter	39
Leg	7
Second thigh	14
Thigh	21
Wings	22
Neck	1
Carcass remainder for broth	14
Giblets	5

\* The total of responses exceeds 100%, as respondents picked maximum three culinary elements

From the surveys carried out by NOWAK and TRZISZKA (2010) concerning habits of Polish consumers on the poultry market it follows that slightly more people declared consuming poultry meat than in our studies, approximately 97%. However, similarly to our studies, the most numerous group of the surveyed were those who consumed poultry meat twice a week (approx. 41%), and most often in the form of a chicken breast fillet (42.3%). Similar results concerning frequency of poultry meat consumption were obtained by KOSICKA-GĘBSKA and GĘBSKI (2014), who analyzed Polish statistical data from 2012. The authors indicated that the most numerous group among the surveyed constituted those who consumed poultry meat several times a week (39.9%), while the smallest group several times a year (5.3%).

From an analysis of responses to questions concerning place of purchase and criteria deciding about choosing the meat (Table 4) it follows that more than half of the surveyed (56%) purchased poultry meat in butcher shops, while the second place was a supermarket (23% of the respondents). The smallest group chose hypermarkets when purchasing poultry meat (6%), or

local shops only 4%. Most of the surveyed first of all took meat freshness as their criteria (77%), and then its price (49%). According to consumers such criteria as expiration date (29%) and dietary values (25%) also have their significance when purchasing poultry meat. Promotion in the media, other people’s opinion, country of origin and producer’s information for most of the surveyed were not a significant criterion when buying meat. Percentage of the respondents taking into consideration these latter criteria was within the range from 1 to 4%.

Place of purchase and factors determining meat type

Table 4

Specification	Responses	Share [%]
Place of meat purchase	butcher shop	56
	local shop	4
	marketplace	11
	supermarket	23
	hypermarket	6
Criterion for choosing meat*	price	49
	cleanness	22
	freshness	77
	dietary value	25
	number of pieces per package	5
	expiry date	29
	color	17
	smell	23
	brand/producer	7
	easiness of preparation	8
	country of origin	3
	promotion in the media	1
	information from the producer	1
others' opinion	4	
Purchase of poultry meat from:	conventional production (farm)	25
	organic production	19
	I don't pay attention to the production system	56

\* The total of responses exceeds 100%, as respondents picked maximum three factors for choosing meat

The studies carried out by NOWAK and TRZISZKA (2010) partly confirm the results of our research, as the mentioned authors also indicated that Polish consumers mostly buy poultry meat in butcher shops (31.0% of the surveyed), and less frequently in local shops (21.3%). From these studies, however, it follows that for most consumers (71%) the main criterion when choosing poultry meat was its taste, while the price was given by only 28.3% of the surveyed, which is less than in our studies. 33.3% of the surveyed, which is less than in our studies, thought that freshness is an important quality trait of poultry meat. On the other hand, similarly as in our studies, purchasing

meat of a particular brand (producer) was not that significant. The studies on Polish statistical data analyzed by KOSICKA-GEBSKA and GEBSKI (2014) indicated that most, 62.2%, of Polish consumers made their decision to buy poultry meat based on its general appearance, and then based on its color (50.1% of the surveyed), and its effect on health. The least significant for consumers was meat smell, as was reported by approx. 13% of the respondents.

When asked about preferences for buying meat from production with conventional (farm) or organic methods, over half of the respondents (56%) answered that it has no significant meaning for them (Table 4). A higher percentage among other surveyed, 25%, constituted those who prefer meat produced with conventional rather than organic methods. Low percentage of consumers (19%) buying meat from organic rearing may be affected by the higher price of the product. First of all this results from a longer period of birds; rearing and a significantly higher feed intake per 1 kg of increase in body weight, with lower effectiveness, that is lower body weight at the end of rearing (HERBUT and KORELESKI 2004, ELEROĞLU et al. 2013, PIETRZAK et al. 2013).

The results of the surveys concerning the form of purchased meat and its quality (Table 5) show that a vast majority of the surveyed (93%) chose fresh poultry meat. Frozen products were bought by merely 7%, while processed meat was not purchased by respondents taking part in the study. When asked if they prefer poultry meat produced in the system of guaranteed quality, QAFP, consumers answered in 66% that it has no significant meaning for them, and only 26% preferred this type of meat. This may indicate lack of knowledge among the surveyed on the subject of food produced in the guaranteed quality system, QAFP, in which health safety of meat is being maximized through continuous veterinary and production supervision (ADAMSKI and WENCEK 2012). When choosing meat of high quality, almost half of the surveyed (48%) appreciate its higher health safety. Approximately of the surveyed appreciated higher nutritional value of the product, while higher palatability was expected by 20% of the respondents. Longer shelf life was significant for merely 9% of the surveyed.

From Table 5 it also follows that the greatest concerns among consumers when purchasing meat were related to suspicions about the use of substances such as antibiotics (64% of respondents) or genetically modified plants used in poultry nutrition (43% responses). Meat origin from a production farm caused concerns in 14% of the surveyed, while only 14% of the respondents had no concerns at all. Respondents asked about meat defects, most often mentioned hemorrhages, 56% of responses, while 28% of the surveyed thought that meat is 'sinewy'. Dry and tough meat was the rarest defect mentioned by consumers.

After an analysis of the data presented in Table 4, concerning meat purchase from a particular rearing system and after comparing it with the data

Table 5

Form and quality of meat

Specification	Responses	Share [%]
Preferences in meat form	fresh	93
	frozen	7
	processed	0
Preferences for meat produced in QAFP system	yes	26
	no	8
	It doesn't matter to me	66
Expectations about high quality meat	better health safety	48
	higer nutritional value	23
	higher palatability	20
	Longer expiry date	9
Concerns connected with meat purchase*	concerns about the use of antibiotics in bird nutrition	64
	concerns about the use of GMO in bird nutrition	43
	type of production used	14
	other	1
	I have no concerns	14
Most frequent meat defects**	dry	17
	sinewy	28
	watery	22
	tough	10
	hemorrhages	56
	I didn't encounter any	11

\* The total of all responses exceeds 100%, as respondents picked maximum three factors

\*\* The total of responses exceeds 100%, as respondents picked maximum two meat defects

from Table 5, about consumer concerns it may be concluded that those surveyed are not fully aware of the conditions in an organic rearing of poultry. Since there oblige, among other things, bans on using antibiotics or genetically modified organisms in poultry nutrition (HERBUT and KORELESKI 2004), constituting main concerns when buying poultry meat, only 19% of the surveyed decided to buy meat produced with organic methods. However, more than half of the respondents do not pay attention to the origin of the meat when buying it (Table 4).

### Conclusions

1. Majority of the surveyed (46%) declare consumption of poultry meat two or three times a week, and most frequently in the form of a breast fillet and quarter, or wings. Consumers prefer meat from broiler chickens (84%), while they buy goose meat least frequently, 1% of the respondents. Most of the

consumers take meat freshness as the most important criterion, and only then its price.

2. For more than half of the respondents (56%) it does not matter if the meat comes from a conventional (farm) or organic production. A higher percentage among other surveyed, 25%, were those who prefer meat produced with conventional rather than organic methods (19%).

3. Only 26% of the consumers prefer meat produced under guaranteed quality system, QAFP. When buying poultry meat of high quality, consumers first of all expect higher health safety, higher nutritional value of products, as well as their higher palatability. When purchasing poultry meat, the surveyed are mostly concerned about the use of antibiotics and genetically modified feeds in bird nutrition.

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**THE COMPARISON OF MEAT QUALITY FROM  
DIFFERENT CARCASS CUTS OF MALE FALLOW DEER  
(*DAMA DAMA L.*)**

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**Key words:** fallow deer, carcass cuts, meat quality.

**A b s t r a c t**

The aim of this study was to compare the quality of meat from four carcass cuts (neck, saddle, shoulder and leg) in 10 male fallow deer (*Dama dama L.*) aged between 4 and 5 years. The animals were hunter-harvested in the forests of north-eastern Poland.

Lower moisture content in meat from the saddle and neck resulted from a higher protein and fat content, as compared with meat from the shoulder and leg. The meat from the saddle had the lowest pH and the darkest color. Meat from the shoulder and neck had a lower water-holding capacity (WHC) and higher values of  $a^*$  (redness) than meat from the leg and saddle. The highest value of  $b^*$  (yellowness) was noted in meat from the neck, and the lowest in meat from the saddle.

**PORÓWNANIE JAKOŚCI MIĘSA POCHODZĄCEGO Z RÓŻNYCH ELEMENTÓW  
TUSZY DANIELA (*DAMA DAMA L.*)**

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**Słowa kluczowe:** daniel, elementy tuszy, jakość mięsa.

**A b s t r a k t**

Celem badań było porównanie jakości mięsa z czterech zasadniczych elementów tuszy (karkówki, combra, łopatki i udźca) 10 byków daniela (*Dama dama L.*) w wieku 4–5 lat, odstrzelonych przez myśliwych w lasach północno-wschodniej Polski.

Mniejszy udział wody w mięsie z combra i karkówki wynikał ze stwierdzonej w nim większej, niż w mięsie z łopatki i udźca, zawartości białka i tłuszczu. Mięso z combra charakteryzowało się najniższą wartością pH i najciemniejszą barwą. Z kolei mięso z łopatki i karkówki odznaczało się mniejszą zdolnością utrzymywania wody własnej oraz większą wartością  $a^*$  (czerwoność) w porównaniu z mięsem z udźca i combra. Najwyższą wartość  $b^*$  (żółtość) stwierdzono w mięsie z karkówki, a najniższą w mięsie z combra.

## Introduction

Game meat is of great interest to both researchers and producers. Recent years have witnessed an increasing demand for high-quality, easily digestible meat products characterized by a high nutritional value and superior sensory properties (RESURRECCION 2003). Modern consumers often show a preference for organic and welfare-labeled meat from animals raised under conditions that most closely resemble their natural environment (WIKLUND et al. 2005). Available literature (HOFFMAN and WIKLUND 2006) data show that venison meets this “naturalness” requirement and high consumer expectations. Venison has a high content of protein, vitamins and bioactive compounds (SAMPLES et al. 2006, PURCHAS et al. 2010), a low content of fat with a desirable fatty acid profile (VOLPELLI et al. 2003, POLAK et al. 2008), and a specific taste and aroma referred to as sour, grassy or simply “wild” or “gamey” (HOFFMAN and WIKLUND 2006).

Despite the unquestionable advantages of venison, it should be noted that the quality of meat obtained from different parts of game carcasses may vary with respect to, among others, its basic chemical composition and physicochemical properties (pH, color, water-holding capacity) (PAULSEN et al. 2005, DASZKIEWICZ et al. 2011, 2013). This is an important consideration for meat processing plants and consumers as it will affect the processing suitability and culinary use of meat. In view of the above, the objective of this study was to compare the quality of meat from different carcass cuts of male fallow deer (*Dama dama* L.) hunter-harvested in north-eastern Poland.

## Materials and Methods

### Materials

The experimental materials comprised the carcasses of 10 fallow deer (*Dama dama* L.) bucks aged 4 to 5 years, supplied to a meat processing plant. The animals were hunter-harvested (ambush tactics, a heart shot) in the forests of north-eastern Poland (Sępopol Plain, Region of Warmia and Mazury)

during one hunting season (in November and December). The age of bucks was estimated by hunters, based on the appearance of their antlers and the wear of mandibular premolars and molars. The animals were bled and eviscerated in the field immediately after shooting. After bleeding and evisceration, the beheaded carcasses (with the skin and legs) were transported by hunters (within 2–3 h of harvest) to a cold store where they were stored at 2–4°C, and next they were transported in a refrigerator truck to the meat processing plant. In the meat processing plant carcasses were stored at a temperature ranging from 0°C to 2°C.

The time that passed from the harvest of animals to carcass cutting was 48 to 54 hours (time of harvest was determined based on hunter harvest reports). Each carcass was divided into primal cuts in the meat processing plant, in accordance with Polish industry standard *Mięso z dziczyzny...* BN-84/9241-10. Four cuts from each carcass, i.e. the saddle, neck, shoulder and leg, were then trimmed to obtain lean meat. Meat from each cut was cut into small pieces (weight ca. 10 g), and thoroughly mixed. Average samples (approx. 300 g) of meat from each cut (from each carcass) were collected (forty samples of meat: 4 cuts of each animal × 10 animals). The samples were packaged in polyethylene bags and transported in containers with ice to the laboratory where they were deep-frozen at –26°C and stored until analysis.

### Preparation of meat samples

Prior to meat quality evaluation, the samples were thawed at 2°C until their internal temperature reached –1°C. The samples were put through a laboratory mincer with a 3 mm diameter mesh plate three times. Minced meat was mixed thoroughly, and the obtained samples were analyzed to determine the basic chemical composition and physicochemical properties of meat.

### Research methods

The analysis of the basic chemical composition of meat included the determination of moisture content (samples were dried at 105°C to constant weight), total protein content – by the Kjeldahl method, fat content – by the Soxhlet method and ash content (by incineration at 550°C to obtain a constant weight) (AOAC 1990). The water to protein ratio of samples was calculated as  $W/P$ , where  $W$  = average moisture percentage of the sample and  $P$  = average protein percentage of the sample.

The energy value of meat (in conversion per 100 g of meat) was calculated with the use of individual energy factors for protein – 16.78 kJ g<sup>-1</sup> and fat – 37.62 kJ g<sup>-1</sup> (JANKOWSKA et al. 2005).

The pH of samples was measured in the water homogenates of 10 g meat (meat and distilled water ratio of 1:1) using a combination Polilyte Lab electrode (Hamilton) and a 340i pH-meter equipped with a TFK 325 temperature sensor (WTW).

The instrumental color analysis was based on measurement (three times) of light reflected from different points of meat surface and later transformed into values in the CIE (1978)  $L^*$ ,  $a^*$ ,  $b^*$  color system. An automated HunterLab MiniScan XE Plus spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA, USA) was used to register  $L^*$  (lightness),  $a^*$  (redness) and  $b^*$  (yellowness) values. Prior to the measurement, samples wrapped in oxygen-permeable and water-impermeable foil were stored for 0.5 h at 4°C. The values of  $C^*$  (chroma) were calculated from the following formula:  $C^* = (a^{*2} + b^{*2})^{1/2}$ .

The water-holding capacity (WHC) of meat was determined by the Grau and Hamm method (VAN OECKEL et al. 1999).

## Statistical analysis

The data were processed statistically by one-way ANOVA using Statistica ver. 10 software (StatSoft, Inc. 2011). The significance of differences between means in groups was estimated by Duncan's multiple range test.

## Results

### Basic chemical composition of meat

An analysis of the basic chemical composition of meat obtained from four primal cuts of fallow deer carcasses (Table 1) revealed that meat from the shoulder had a higher ( $P \leq 0.01$ ) moisture content than meat from the neck, leg and saddle. Meat from the saddle had the lowest moisture content, and the noted differences were statistically significant ( $P \leq 0.01$ ) relative to meat from the shoulder and leg, as well as from the neck ( $P \leq 0.05$ ). Lower moisture content in meat from the saddle and neck resulted from higher protein and fat content, as compared to meat from the shoulder and leg. Meat from four primal cuts had similar average concentrations of mineral compounds in ash form. The differences between mean values determined for meat from the saddle

vs. from the shoulder and leg were significant ( $P \leq 0.05$ ), but very small (0.06 and 0.05 percentage points, respectively). The significance of differences between means in groups could be due to the low variability of the analyzed trait.

Table 1  
Basic chemical composition and energy value of muscle tissue from different carcass cuts of male fallow deer (means  $\pm$  SEM)

Traits [%]	Carcass cuts			
	shoulder ( $n = 10$ )	neck ( $n = 10$ )	leg ( $n = 10$ )	saddle ( $n = 10$ )
Moisture	77.18 $\pm$ 0.22 <sup>A</sup>	75.84 $\pm$ 0.20 <sup>Ba</sup>	76.06 $\pm$ 0.24 <sup>C</sup>	75.25 $\pm$ 0.07 <sup>Bb</sup>
Fat	0.60 $\pm$ 0.08 <sup>A</sup>	1.19 $\pm$ 0.07 <sup>B</sup>	0.66 $\pm$ 0.06 <sup>A</sup>	1.04 $\pm$ 0.08 <sup>B</sup>
Protein	20.99 $\pm$ 0.19 <sup>A</sup>	22.40 $\pm$ 0.21 <sup>BCa</sup>	21.83 $\pm$ 0.21 <sup>Bb</sup>	22.68 $\pm$ 0.06 <sup>C</sup>
Ash	1.02 $\pm$ 0.02 <sup>a</sup>	1.04 $\pm$ 0.01 <sup>ab</sup>	1.03 $\pm$ 0.02 <sup>a</sup>	1.08 $\pm$ 0.01 <sup>b</sup>
Water/protein ratio (W/P)	3.68 $\pm$ 0.04 <sup>A</sup>	3.39 $\pm$ 0.04 <sup>B</sup>	3.49 $\pm$ 0.04 <sup>B</sup>	3.32 $\pm$ 0.01 <sup>C</sup>
Energy value [kJ 100 g <sup>-1</sup> ]	375 $\pm$ 3.84 <sup>A</sup>	421 $\pm$ 4.34 <sup>B</sup>	391 $\pm$ 3.89 <sup>C</sup>	420 $\pm$ 3.42 <sup>B</sup>

SEM – the standard error of the mean.

<sup>ABC</sup> – differences between values with different letters in the same rows are significant ( $P \leq 0.01$ ).

<sup>ab</sup> – differences between values with different letters in the same rows are significant ( $P \leq 0.05$ ).

The differences in the content of moisture, protein and fat in meat were reflected in the differences in the water to protein (W/P) ratio and the energy value of meat (Table 1). The W/P ratio was highest in meat from the shoulder, and lowest in meat from the saddle. Meat from the neck and saddle was characterized by the highest ( $P \leq 0.01$ ) energy value, and the lowest ( $P \leq 0.01$ ) from the shoulder.

### Physicochemical properties of meat

The analyzed meat was characterized by high average pH levels (Table 2). Meat from the saddle had the lowest pH value (5.77), which was lower ( $P \leq 0.01$ ) than the pH value determined in meat from the other three cuts.

Meat from the saddle had the darkest color ( $P \leq 0.01$ ) – Table 2, followed by meat from the leg, shoulder and neck. The color of meat from the neck was lighter ( $P \leq 0.01$ ) than from the saddle, leg and shoulder. Meat from the shoulder and neck had a significantly higher values of parameter  $a^*$  (redness) in comparison with meat from the saddle and leg (Table 2). Differences ( $P \leq 0.01$ ) were observed in average values of  $b^*$  (yellowness) between meat from the analyzed primal cuts (Table 2). The highest value of  $b^*$  was noted in meat from the neck, and the lowest from the saddle.

Table 2  
Physicochemical properties of muscle tissue from different carcass cuts of male fallow deer  
(means  $\pm$  SEM)

Traits	Carcass cuts			
	shoulder (n = 10)	neck (n = 10)	leg (n = 10)	saddle (n = 10)
pH	5.92 $\pm$ 0.03 <sup>A</sup>	5.90 $\pm$ 0.02 <sup>A</sup>	5.87 $\pm$ 0.02 <sup>A</sup>	5.77 $\pm$ 0.01 <sup>B</sup>
L* (lightness)	34.06 $\pm$ 0.50 <sup>A</sup>	38.95 $\pm$ 0.78 <sup>B</sup>	33.14 $\pm$ 0.36 <sup>A</sup>	30.48 $\pm$ 0.35 <sup>C</sup>
a* (redness)	17.04 $\pm$ 0.41 <sup>A</sup>	16.55 $\pm$ 0.42 <sup>ABa</sup>	15.49 $\pm$ 0.39 <sup>BCb</sup>	14.46 $\pm$ 0.23 <sup>B</sup>
b* (yellowness)	14.38 $\pm$ 0.25 <sup>A</sup>	15.85 $\pm$ 0.24 <sup>B</sup>	13.05 $\pm$ 0.29 <sup>C</sup>	11.81 $\pm$ 0.24 <sup>D</sup>
C* (saturation)	22.31 $\pm$ 0.43 <sup>Ab</sup>	22.93 $\pm$ 0.43 <sup>A</sup>	20.26 $\pm$ 0.47 <sup>Ba</sup>	18.67 $\pm$ 0.32 <sup>Bb</sup>
Water – holding capacity (WHC) – Grau and Hamm method [cm <sup>2</sup> g <sup>-1</sup> ]	22.49 $\pm$ 1.09 <sup>Aa</sup>	22.09 $\pm$ 0.99 <sup>Aa</sup>	17.74 $\pm$ 1.19 <sup>B</sup>	18.78 $\pm$ 0.82 <sup>b</sup>

SEM – the standard error of the mean.

<sup>ABCD</sup> – differences between values with different letters in the same rows are significant ( $P = 0.01$ ).

<sup>ab</sup> – differences between values with different letters in the same rows are significant ( $P \leq 0.05$ ).

Differences in the mean values of parameters  $\alpha^*$  and  $b^*$  were reflected in differences in color saturation ( $C^*$ ) between the groups (Table 2). Meat from the neck and shoulder was characterized by the highest color saturation, and the lowest from the saddle ( $P \leq 0.01$ ). The difference ( $P \leq 0.05$ ) in color saturation between meat from the leg and saddle was also noted.

In our experiment, muscle tissue from the shoulder and neck had a lower ( $P \leq 0.01$ ) WHC (Table 2), compared to meat from the leg and saddle.

## Discussion

### Basic chemical composition of meat

The results of the present study were similar to these obtained by VOLPELLI et al. (2003) who also stated a high protein and a low fat content in male fallow deer meat from different muscles. The above mentioned authors reported that total protein and fat content ranged from 21.56 up to 21.78% and 0.56–0.72%, respectively (*m. longissimus thoracis et lumborum*) as well as from 20.46 up to 20.90% and 0.55–0.78%, respectively (*m. semitendinosus*). DAHLAN and NOR-FARIZAN HANOON (2008) reported that protein content of the *longissimus dorsi*, *psaos major* and *biceps femoris* muscles of farmed fallow was 22.77, 21.16 and 20.91%, respectively, and fat content 6.13, 9.23 and 9.39%, respectively. The results of studies involving other wild cervid species (PAULSEN et al. 2005, DASZKIEWICZ et al. 2011) also revealed differences in the protein and fat content of meat obtained from different carcass parts.

## Physicochemical properties of meat

In the present study meat from all the cuts had a high pH values. BRODOWSKI and BEUTLING (1991) noted lower average pH value (5.58) for the *semimembranosus* muscle of fallow deer shot by hunters. VOLPELLI et al. (2003) and HUTCHISON et al. (2012) reported pH values 5.5–5.6 in meat (*m. longissimus dorsi* and *m. semimembranosus*) from farmed fallow deer. High average pH values of meat (*m. longissimus dorsi*) from the carcasses of farm-raised fallow deer were noted by WIKLUND et al. (2004) (54.5% of samples had  $\text{pH} \geq 6.2$ ). SHAW (2000) reported that pH value of *m. semimembranosus* and *m. longissimus dorsi* from farm-raised fallow deer was 5.86 and 5.70, respectively. The noted differences in the ultimate pH of the meat of both wild and farmed fallow deer could be attributed to pre-slaughter stress factors and the animals' reproductive cycle.

Differences in the color of meat obtained from different carcass parts of fallow deer bucks may be due to the fact that the muscles that work harder and are used in motor activities contain higher amounts of myoglobin (LAWRIE 1998). This explains the higher values of  $a^*$ ,  $b^*$  and  $C^*$  parameters of meat from the neck and shoulder, compared to meat from the saddle and leg. Our results do not confirm the well-known relationship between the pH value and meat color (WARRISS 2010). Meat from the saddle, which had the lowest pH (5.77), had the lowest value of  $L^*$  parameter too. The differences in average pH values between groups were statistically significant, but small (the largest difference reached 0.15 pH units). Therefore, the possible influence of pH levels on the results of instrumental measurements of lightness ( $L^*$ ) could have been masked by the effects of other factors such as the proportions of adipose and connective tissues and the content of residual blood (hemoglobin), which depends on the degree of carcass bleeding.

An increase pH value of meat is usually accompanied by higher WHC (WARRISS 2010), what was not observed in the present study. It seems that the lower WHC of meat from the shoulder depended from a low content of protein that is responsible for water binding, whereas the lower water-holding of meat from the neck was due to a high content of fat that prevents direct contact between protein with water, thus reducing water binding.

## Conclusions

To sum up, the results of our study confirm that fallow deer meat has a high nutritional value (high total protein content, low fat content). The present results indicate also that the quality of meat from four primal cuts of fallow deer carcasses (saddle, neck, shoulder, leg) differs significantly in regard

of basic chemical composition, pH value, color ( $L^*$ ,  $a^*$ ,  $b^*$ ) and water-holding capacity. The differences in the quality of meat from cuts of fallow deer carcasses should be taken into account by game meat processing plants and consumers.

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**EPIPHYTIC HABITATS IN AN URBAN ENVIRONMENT;  
CONTAMINATION BY HEAVY METALS AND SULPHUR  
IN THE BARKS OF DIFFERENT TREE SPECIES**

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**Key words:** epiphytes, bark contamination, monitoring, urban ecology.

**Abstract**

Air pollution is mentioned as one of the major factors that limit the occurrence of epiphytes in urban areas. The purpose of the investigation was to analyze any dissimilarity in the amount of heavy metals and sulphur to the bark of different tree species that had been subjected to bark pollution (pH values were also analyzed). In order to estimate the differences in the amount of pollutants that had reached the bark, the “moss-bag” method was also used.

The present study confirms that the barks of tree species differ in the level of pollutants (such as heavy metals and sulphur). The higher pH values of the bark appears to be an important buffer for the adverse effects of air pollution and determines the recolonization processes of epiphytic species in urban areas.

**SIEDLISKA EPIFITYCZNE W ŚRODOWISKU MIEJSKIM; ZANIECZYSZCZENIE  
KORY RÓŻNYCH GATUNKÓW DRZEW METALAMI CIĘŻKIMI I SIARKĄ**

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**Słowa kluczowe:** epifity, zanieczyszczenie kory, monitoring, ekologia miasta.

## A b s t r a k t

Zanieczyszczenie powietrza jest jednym z głównych czynników ograniczających występowanie epifitów na obszarach zurbanizowanych. Celem badań było określenie zróżnicowania zawartości metali ciężkich i siarki w korze różnych gatunków drzew występujących na terenie zanieczyszczonym (analizowano także poziom pH). Różnice w ilości zanieczyszczeń docierających do kory porównano również za pomocą metody woreczkowej z wykorzystaniem mchu (metoda „moss-bag”).

W prezentowanych badaniach potwierdzono, że kora różnych gatunków drzew różni się poziomem zanieczyszczenia (zarówno metalami ciężkimi, jak i siarką). Wyższy poziom pH kory wydaje się istotnym czynnikiem buforującym niekorzystne oddziaływanie zanieczyszczeń powietrza i warunkującym procesy rekolonizacji epifitów na terenach miejskich.

**Introduction**

Epiphytes are an ecological and physiological specialized group among the mosses. They depend on precipitation from which they also obtain nutrients (they are physiologically active only in a hydrated state) (VANDERPOORTEN and GOFFINET 2009). The local distribution of epiphytes is the result of mutual relationships between various habitat factors, like phorophyte species, the size of tree trunks and environmental conditions (SEAWARD 1979, ZECHMEISTER and HOHENWALLNER 2006, DYMYTROVA 2009). In urban areas one of the crucial element is also air pollution, because toxic substances negatively influence their essential physiological processes (RAO 1982, BATES et al. 2004, ZECHMEISTER and HOHENWALLNER 2006). Heavy metals (lead, zinc, copper) and sulphur compounds can disturb the development of spores and the growth of protonema, which makes the colonization of habitats in polluted areas more difficult (KRZESŁOWSKA et al. 1994, BASILE et al. 1995).

Bark and epiphytes are exposed to air pollutants either directly from the atmosphere or from stemflow (liquid that penetrates the canopy and flowing down the branches and stem of tree) (BERLIZOV et al. 2007, CATINON et al. 2012). Stored dust deposit formed on tree crown is washed out during rainfall, therefore concentrations of nutrients and metals in stemflow is higher compared with rainwater (PRYOR and BARTHELMIE 2005, XIAO and MCPHERSON 2011, CATINON et al. 2012). Percentages of rain water reaching the bole and epiphytes as stemflow is different in various tree species (from 0.8 to 22% of bulk precipitation) (PRYOR and BARTHELMIE 2005, XIAO and MCPHERSON 2011). Factors influencing stemflow are mainly: type of canopy, bark texture and leaf surface morphology (BARKMAN 1958, RASMUSSEN 1978, PRYOR and BARTHELMIE 2005).

The way in which the type of phorophyte (species of tree) influences the amount of pollutants that reach the bark, and inhabiting epiphytes, is interesting. The purpose of the investigation was to analyze any dissimilarities in the amount of heavy metals and sulphur as well as the pH values of different tree

species that had been subjected to bark pollution. In order to estimate the amount of pollutants that had reached the bark and epiphytes (both from wet and dry deposition) the chemistry of bark were analyzed (amount of heavy metals and sulphur). The use of tree bark as indicator in biomonitoring of air pollution in urban areas is still common (e.g. FUJIWARA et al. 2011, ŠKRBIĆ et al. 2012, BARBEŞ et al. 2014, DOĞAN et al. 2014, MOREIRA et al. 2016). To estimate current levels of emission the “moss-bag” method were used in this study (with *Pleurozium schreberi* (WILLD. ex BRID.) MITT.). It is in general also standard method used in biomonitoring (SUN et al. 2009, TRACZEWSKA 2011).

## Materials and Methods

The investigation was carried out in Bolina” park (about 4.7 ha) in the Janów district of Katowice town (GPS N: 50°13’55”, E: 19°05’18”). The quantity of heavy metals, especially zinc and cadmium, in Janów is high, which is mainly the result of the close proximity of the metalworks “Huta Żelaza Ferrum” and “Huta Metali Nieżelaznych Szopienice S.A.” (since 2008 in liquidation) – counted among the main sources of emission in Katowice (*Raport z realizacji...* 2014).

To estimate the amount of pollutants in the bark of different trees, bark from ten tree species was sampled (about 50 g), both deciduous and coniferous (*Acer platanoides* L., *Acer pseudoplatanus* L., *Betula pendula* Roth, *Fraxinus excelsior* L., *Picea pungens* Engelm., *Pinus sylvestris* L., *Populus tremula* L., *Quercus rubra* L., *Robinia pseudoacacia* L., *Tilia cordata* Mill.). The ages and sizes of the trees were approximate (were planted at the same time), only the specimen of *Picea pungens* was smaller. Bark samples, which were around 3 mm thick, were collected from a height of approximately 1.5 m in 30 August 2011.

To estimate the amount of pollutants, that had reached the epiphytes, modified “moss-bag” method were used (SUN et al. 2009, TRACZEWSKA 2011). *Pleurozium schreberi* samples were gathered in a large forest complex in the vicinity of the village of Panoszów (west of Częstochowa; GPS N: 50°48’13”, E: 18°40’23”), an area far from the larger emission sources. In the laboratory *Pleurozium* samples were dried in room temperature. After cleaning they were divided into portions of 15 g. Each sample was packed into a polyethylene net bag (30 x 30 cm) with a 1 mm stitch (produced by the Tenax Company). Bags with *Pleurozium* were then hung on the bark of trees at a height of about 3 m. In order to expose the *Pleurozium* samples in a similar way as the epiphytes and to create similar water supply conditions, the bags were very closely adhered to the tree trunks. The exposure lasted for three months (from the end of May to the end of August 2011).

The samples of bark and moss (after exposition) were dried at 50°C for 24 hours and they were then crushed using a blender. In order to determine the pH, 1 g of each sample of powdered bark was taken and suffused in 5 ml of distilled water. After 48 hours, acidity was measured with a pH-meter. The pH analysis was repeated five times. For the chemical analysis of heavy metal content in bark and moss, 250 mg of each sample was mineralized. The material was digested in 5 ml of concentrated HNO<sub>3</sub> at a temperature up to 120°C for approximately one week (until a clear solution was obtained). The solution was filtered into measuring flasks and diluted with distilled water until the volume reached 10 ml. These solutions were analyzed for heavy metal content (Fe, Cd, Cu, Pb, Zn) using the Atomic Absorption Spectrometry method with a “Unicam 939 Solar” spectrometer. The analysis was repeated five times. The content of heavy metals in the initial examination moss material equaled (mg kg<sup>-1</sup>): Pb – 66.3; Zn – 57.27; Cd – 2.76; Cu – 20.12; Fe – 316.82.

Sulphur content in *Pleurozium* samples were also studied. For this purpose 300 mg of a homogenous sample was put into a ceramic crucible into which 150 mg of tungsten trioxide was added. The content was analyzed using a Vario Max CNS microanalyzer (the analysis was repeated three times). The content of sulphur in the initial examination of the moss material equaled 630 mg kg<sup>-1</sup>.

Values of Spearman rank correlation coefficient (with  $p < 0.05$ ) were computed using STATISTICA software (version 10).

## Results

The content of heavy metals in the bark and exposed moss varied among the tree species that were analyzed (Figures 1–5). Levels of contaminations in bark were varied more significantly (in moss samples the spread between the maximum and minimum values were lower).

In most of the samples the amount of lead was below 80 mg kg<sup>-1</sup>. The highest content was detected in the bark of *Robinia pseudoacacia* (135.7 mg kg<sup>-1</sup>) and *Tilia cordata* (102.18 mg kg<sup>-1</sup>). A higher amount of Pb was registered in the material that was exposed on *Populus tremula* and *Quercus robur* (above 80 mg kg<sup>-1</sup>), while the lowest level was found in the material from *Picea pungens* (55.02 mg kg<sup>-1</sup>) and *Tilia cordata* (55.28 mg kg<sup>-1</sup>).

The content of zinc in bark fluctuated a great deal and ranged from 16.31 mg kg<sup>-1</sup> in *Quercus rubra* to more than 160 mg kg<sup>-1</sup> in *Betula pendula*, *Picea pungens*, *Populus tremula* and *Robinia pseudoacacia*. In most cases of moss material the content of Zn fluctuated in the range of 50–71 mg kg<sup>-1</sup>; significantly higher values were noted only in the material that was exposed on *Populus*

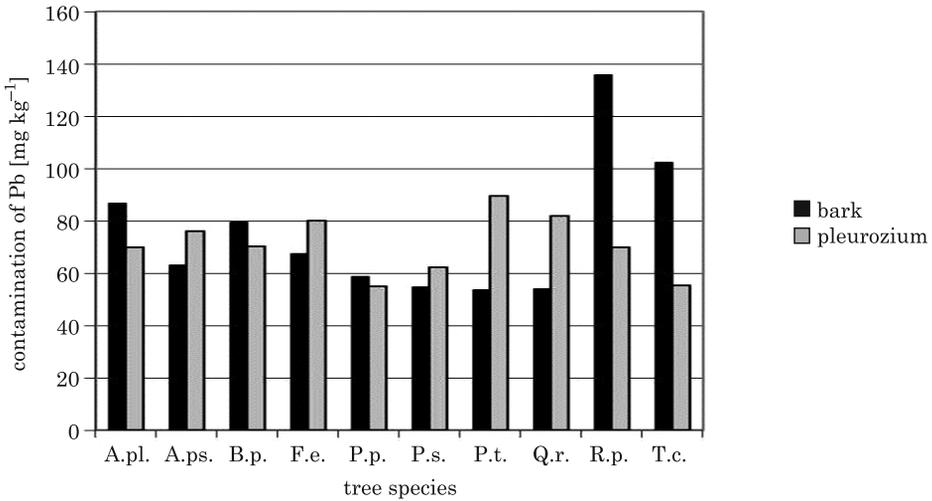


Fig. 1. The content of Pb [mg kg<sup>-1</sup>] in bark of different tree species and *Pleurozium schreberi* exposed on their bark: A.pl. – *Acer platanoides*; A.ps. – *Acer pseudoplatanus*; B.p. – *Betula pendula*; F.e. – *Fraxinus excelsior*; P.p. – *Picea pungens*; P.s. – *Pinus sylvestris*; P.t. – *Populus tremula*; Q.r. – *Quercus rubra*; R.p. – *Robinia pseudacacia*; T.c. – *Tilia cordata*

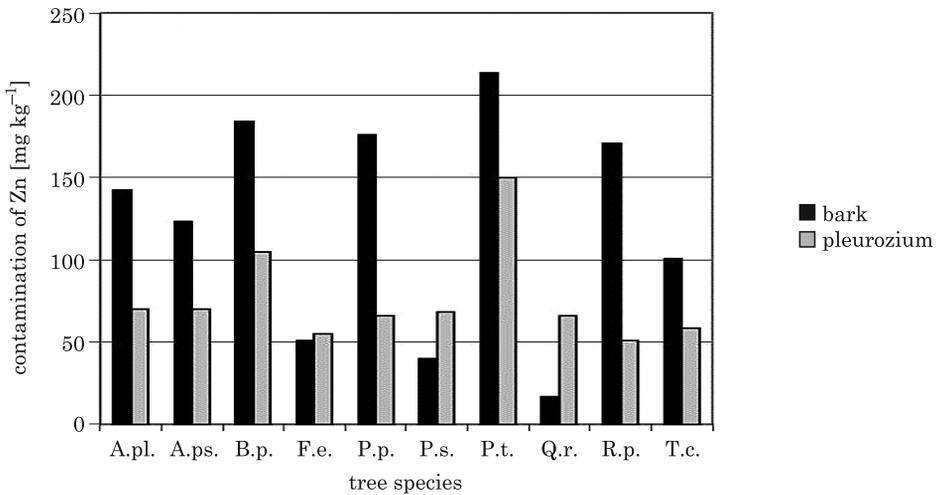


Fig. 2. The content of Zn [mg kg<sup>-1</sup>] in bark of different tree species and *Pleurozium schreberi* exposed on their bark: A.pl. – *Acer platanoides*; A.ps. – *Acer pseudoplatanus*; B.p. – *Betula pendula*; F.e. – *Fraxinus excelsior*; P.p. – *Picea pungens*; P.s. – *Pinus sylvestris*; P.t. – *Populus tremula*; Q.r. – *Quercus rubra*; R.p. – *Robinia pseudacacia*; T.c. – *Tilia cordata*

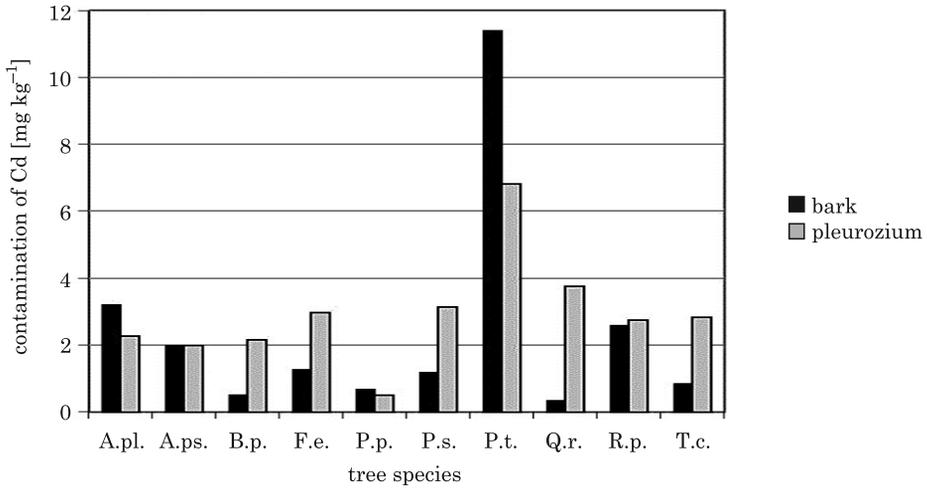


Fig. 3. The content of Cd [mg kg<sup>-1</sup>] in bark of different tree species and *Pleurozium schreberi* exposed on their bark: A.pl. – *Acer platanoides*; A.ps – *Acer pseudoplatanus*; B.p. – *Betula pendula*; F.e. – *Fraxinus excelsior*; P.p. – *Picea pungens*; P.t. – *Populus tremula*; Q.r. – *Quercus rubra*; R.p. – *Robinia pseudacacia*; T.c. – *Tilia cordata*

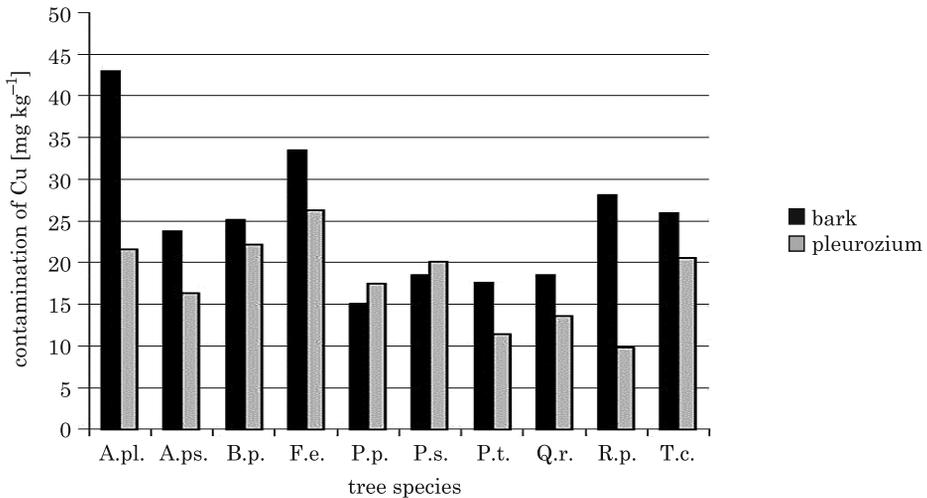


Fig. 4. The content of Cu [mg kg<sup>-1</sup>] in bark of different tree species and *Pleurozium schreberi* exposed on their bark: A.pl. – *Acer platanoides*; A.ps – *Acer pseudoplatanus*; B.p. – *Betula pendula*; F.e. – *Fraxinus excelsior*; P.p. – *Picea pungens*; P.s. – *Pinus sylvestris*; P.t. – *Populus tremula*; Q.r. – *Quercus rubra*; R.p. – *Robinia pseudacacia*; T.c. – *Tilia cordata*

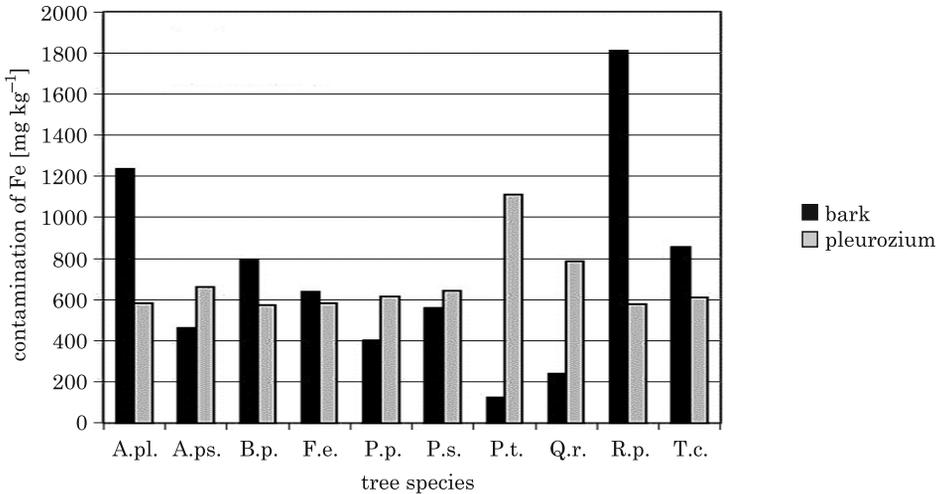


Fig. 5. The content of Fe [mg kg<sup>-1</sup>] in bark of different tree species and *Pleurozium schreberi* exposed on their bark: A.pl. – *Acer platanoides*; A.ps. – *Acer pseudoplatanus*; B.p. – *Betula pendula*; F.e. – *Fraxinus excelsior*; P.p. – *Picea pungens*; P.s. – *Pinus sylvestris*; P.t. – *Populus tremula*; Q.r. – *Quercus robur*; R.p. – *Robinia pseudoacacia*; T.c. – *Tilia cordata*

*tremula* (149.8 mg kg<sup>-1</sup>) and *Betula pendula* (104.66 mg kg<sup>-1</sup>). The tendency to significantly higher content of zinc in the bark vs. moss samples prevails.

The quantity of cadmium in the bark of most of the trees did not exceeded 4 mg kg<sup>-1</sup> and only in the case of *Populus tremula* amount is much higher (11.39 mg kg<sup>-1</sup>). The highest content of Cd was registered in the moss material that was exposed on *Populus tremula* (6.81 mg kg<sup>-1</sup>), while the lowest level was found in the material that was exposed on *Picea pungens* (0.61 mg kg<sup>-1</sup>). In the other moss samples the amount of cadmium fluctuated in the range of 1.97 (*Picea pungens*) to 3.74 mg kg<sup>-1</sup> (*Quercus robur*).

The largest content of copper was registered in the bark of *Acer platanoides* (42.89 mg kg<sup>-1</sup>), while the lowest levels of this metal were registered in bark of *Picea pungens* (15.02 mg kg<sup>-1</sup>). The content of Cu in moss samples varied widely and ranged from 9.75 mg kg<sup>-1</sup> (in material from *Robinia pseudoacacia*) and 11.37 mg kg<sup>-1</sup> (material from *Populus tremula*) to 26.29 mg kg<sup>-1</sup> (material from *Fraxinus excelsior*).

A higher content of iron was detected in the bark of *Robinia pseudoacacia* (1812.6 mg kg<sup>-1</sup>), whereas the lowest content was found in the bark of *Populus tremula* (122.52 mg kg<sup>-1</sup>). The amount of iron was similar and in most cases it had a level of 600 mg kg<sup>-1</sup>; however, a significantly higher content was detected in the material that was exposed on *Populus tremula* (1109.36 mg kg<sup>-1</sup>).

The results show that none of tree species that were analyzed had the highest levels of all of the heavy metals. According to the type of element, the

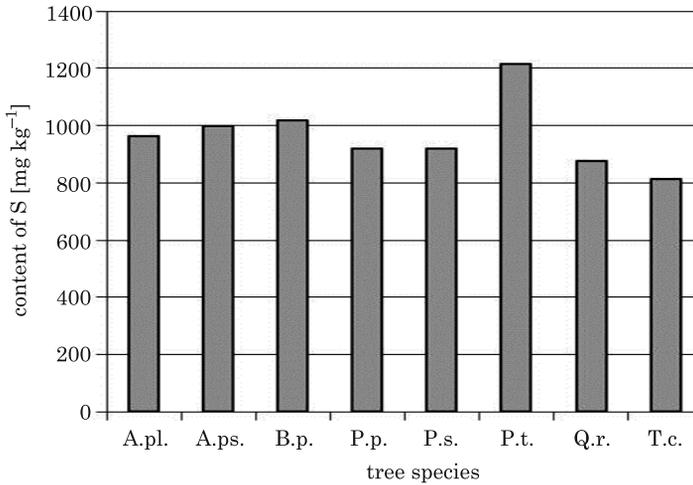


Fig. 6. The content of S [mg kg<sup>-1</sup>] in *Pleurozium schreberi* exposed on different tree species: A.pl. – *Acer platanoides*; A.ps. – *Acer pseudoplatanus*; B.p. – *Betula pendula*; F.e. – *Fraxinus excelsior*; P.p. – *Picea pungens*; P.s. – *Pinus sylvestris*; P.t. – *Populus tremula*; Q.r. – *Quercus rubra*; R.p. – *Robinia pseudacacia*; T.c. – *Tilia cordata*

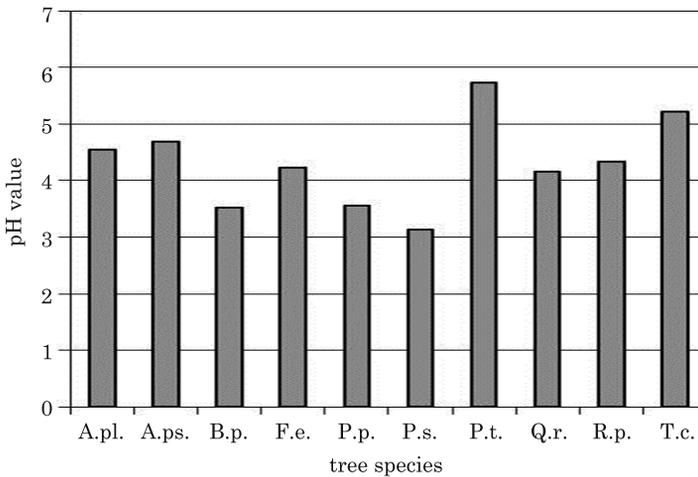


Fig. 7. The pH values of bark of different tree species: A.pl. – *Acer platanoides*; A.ps. – *Acer pseudoplatanus*; B.p. – *Betula pendula*; F.e. – *Fraxinus excelsior*; P.p. – *Picea pungens*; P.s. – *Pinus sylvestris*; P.t. – *Populus tremula*; Q.r. – *Quercus rubra*; R.p. – *Robinia pseudacacia*; T.c. – *Tilia cordata*

most polluted bark occurred in *Robinia pseudoacacia* (Pb and Fe), *Populus tremula* (Cd and Zn) and *Acer platanoides* (Cu). The lowest levels of Pb, Zn and Cd and relatively low levels of Cu and Fe were noted for *Quercus rubra*. Comparing the level of contamination in the bark of different species of trees

and in exposed moss samples – only for Zn and Cu clear tendency to higher values in the bark has been demonstrated.

There are only few highly significant correlation coefficients between the content of metals in the bark of trees and between the bark and the exposed moss samples. A positive relationships were demonstrated between Cu vs. Fe and Pb ( $r = 0.854545$  and  $0.793939$ , respectively), Fe vs. Pb ( $r = 0.951515$ ) – in bark of trees, as well as between S in tree bark and Zn in bark ( $r = 0.79043$ ) and exposed moss samples ( $r = 0.99403$ ). A negative correlations were observed with respect to Fe in bark and moss samples ( $r = -0.851068$ ), as well as between Fe in moss samples vs. Cu and Pb in bark of trees ( $r = -0.70517$  and  $-0.832831$ , respectively).

The level of sulphur in exposed *Pleurozium* varied among the tree species and ranged from 813 (*Tilia cordata*) to 1214 mg kg<sup>-1</sup> (*Populus tremula*) (Figure 6). Moreover, a significant differentiation in the bark pH values of different phorophytes was confirmed (Figure 7). The lowest pH was noted for coniferous trees – *Pinus sylvestris* (3.13) and *Picea pungens* (3.55). The pH of *Betula pendula* bark (3.52) was at a similar level. In other cases pH exceeded 4 and the bark of *Populus tremula* had the highest value (5.72).

## Discussion

According to different authors normal value for Cu, Pb and Zn in bark and *Pleurozium schreberi* tissue is at a level of a few mg kg<sup>-1</sup> (only in *Pleurozium* zinc reaches tens of mg kg<sup>-1</sup>), for Cd – below 1 mg kg<sup>-1</sup>, and relatively varied in the case of Fe – from a few to a few hundred mg kg<sup>-1</sup> (SAMECKA-CYMERMAN et al. 2006, DOGAN et al. 2007, CELIK et al. 2010, SAWIDIS et al. 2011, OKLO and ASEMAVE 2012, MOREIRA et al. 2016). The level of contaminations in analyzed bark of trees and exposed moss samples is relatively high. This high content of heavy metals is possibly a result of metalworks “Huta Żelaza Ferrum” and “Huta Metali Nieżelaznych Szopienice S.A.” being situated nearby.

The investigation confirms the essential differentiation of the chemistry of bark (epiphytic habitat) that result from contamination by heavy metals and sulphur. These differences are mainly affected by the bark quality, type of canopy and stemflow (SCHULZ et al. 1999, PRYOR and BARTHELMIE 2005). The bark and leaf surface morphology contributed to the trapping and retention of contaminations – the rougher the surface, the greater the accumulation of air dust particles. Also old leaves are more contaminated than the young (SAWIDIS et al. 1995). Stemflow washes away contaminations from the canopy (along a tree trunk) is one of the major sources of pollution that reaches epiphytes and may differ significantly in comparison with precipitated water (SKRIVAN et al.

1995). According to BARKMAN (1958), the percentages of rain water reaching the bole in various trees is from 1% (*Picea*) to 22% (*Fagus*). For instance, spruce has a very dense crown and rainfall flows over its branches to the brink of the crown. Most tree species have a less dense crown and the majority of rainfall flows down the branches (WITTIG 1986).

The level of heavy metals and sulphur in bark is modified not only by the inflow of elements during exposure, but also by the washing out of elements by rainfall. The influence of the quantity and form of precipitation is also important. Intensive rainfall is able to flush out significant amounts of elements, while mild rainfall results in water evaporation, which causes the substances to remain in the bark (ČEBURNIS and VALIULIS 1999, LEVIA 2003). This refers to epiphytes as well – rainfall may wash out up to 20% of elements that are deposited on the surface of mosses (ČEBURNIS and VALIULIS 1999).

No simple relations was found between the content of metals in the barks of trees and in the exposed moss. In many cases the level of heavy metals in moss is lower than in the barks of trees (especially it refers to *Acer platanoides*, *Betula pendula* and *Robinia pseudoacacia*) (Figures 1–5). It follows that the concentration of the elements that were examined in the bark is the result of many years of exposure to emissions and long-term accumulation of air pollution (PACHECO et al. 2002, MANDIWANA et al. 2006). On the other hand content of contaminations in the moss that was exposed for a relatively short period of time seems to reflect the current levels of emission (also the effect of reducing emissions during the restructuring of the steelworks). Other authors have suggested that the concentration of the elements in moss is related more to the chemical composition of the last precipitates than to a long-term accumulation (BROWN and BRUMELIS 1996, REIMANN et al. 1999).

Moreover, a significant differentiation in the bark pH values of different phorophytes was confirmed (Figure 7). The pH of bark is the result of a few factors – the species properties and the age of a tree (which are related to the possibility of buffering acidification), the impact of acidic rainfall and the chemical composition of water flowing on the trunk (containing substances flushed out of the treetop and branches) (BATES et al. 2004, POIKOLAINEN 2004). Acid rain causes drastic changes in the chemical properties of both the bark as the ground layer and epiphytes because it reduces the pH of water flowing on the trunk, which causes a decrease in the capacity of the bark to buffer, a decrease in the internal pH of epiphytes and a loss of chlorophyll by epiphytes (ROBITAILLE et al. 1977). The buffering properties of a habitat play a significant role in areas that have high levels of air pollution. The described above results refer to *Populus tremula* seem to confirm this buffering properties (the highest pH of the bark in spite of the highest inflow of sulphur). High pH allows sensitive species to survive even in the city center by altering

sulphur ions into a less toxic form (GILBERT 1968, 1969). Moreover the higher pH level reduce a mobility of metals ions occurring in bark (BATES and BROWN 1981) – it may diminish their bioavailability. It may be an explanation of attachment of epiphytes to phorophytes with more alkaline bark. The analysis of the preference of epiphytes in Katowice towards inhabited phorophytes reveals that the most commonly and abundantly inhabited species were poplar and willow trees, which show higher pH values of bark (STEBEL and FOJCIK 2016). Similar preferences in other urban areas were observed by ADAMS and PRESTON (1992) in London, DYMYTROVA (2009) in Kiev and FUDALI (2011) in Wrocław. Whereas in this study the highest content of Zn and Cd was detected just in the poplar bark, also high amount of Pb, Zn, Cd and Fe was detected in the material that was exposed on *Populus tremula*.

The distribution of epiphytes in an urban area is largely conditioned by the effectiveness of colonization success, which depends on various factors, including bark chemistry (taking into account natural factors and the influence of pollution). The present study confirms that the barks of tree species differ in the level of pollution (such as heavy metals and sulphur), which may be one of the crucial factors that governs the recolonization processes of epiphytic species in urban areas. Higher pH of bark may buffer the influence of foul habitat factors, which enhances the probability of colonization success of epiphytes (FOJCIK et al. 2015).

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**DIVERSITY OF LICHENS IN FOREST COMMUNITIES  
OF THE “PUPY” NATURE RESERVE  
IN THE PUSZCZA PISKA FOREST (NE POLAND)**

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**Key words:** lichens, primary and secondary forests, old oaks, stenotopic species, Poland.

**A b s t r a c t**

The paper presents the results of lichenological research conducted in the “Pupy” Forest Nature Reserve (NE Poland). The aim of the study was to investigate the species composition of lichens and to determine the role and importance of forest plant communities to the diversity of these organisms. Field studies were carried out at seven study sites (plots), each with an area of 0.1 ha. The plots were selected within the most typical and representative forest communities of the reserve. A total of 95 lichen species were identified. The studies have shown that natural forest communities, in particular oak-linden-hornbeam forest and wet mixed spruce-oak forest are distinguished by a much higher diversity of lichens compared to secondary forest stands (pine and spruce monocultures). This is primarily attributed to the presence of age-differentiated populations of oak trees in these communities. The secondary forest stands do not provide the correct conditions for growth of many stenotopic forest lichens, despite the presence of diaspores of this species in its immediate surroundings. In order to protect the populations of valuable lichen species, associated in a particular way with oak trees, one should consider treatments aimed at securing the continuity of oak generations in the reserve.

**RÓŻNORODNOŚĆ POROSTÓW W ZBIOROWISKACH LEŚNYCH REZERWATU  
„PUPY” W PUSZCZY PISKIEJ**

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**Słowa kluczowe:** porosty, lasy naturalne i wtórne, stare dęby, gatunki stenotopowe, Polska.

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### Abstrakt

W artykule przedstawiono wyniki badań lichenologicznych przeprowadzonych w leśnym rezerwacie przyrody „Pupy” (NE Polska). Celem pracy było poznanie zasobów gatunków porostów oraz określenie roli i znaczenia poszczególnych zbiorowisk leśnych dla różnorodności tych organizmów. Badania terenowe przeprowadzono na siedmiu powierzchniach badawczych, z których każda miała 0,1 ha. Wyznaczono je w najbardziej reprezentatywnych i typowych dla rezerwatu zbiorowiskach leśnych. Łącznie stwierdzono 95 gatunków porostów.

Wykazano, że naturalne zbiorowiska leśne, a w szczególności grąd subkontynentalny oraz bór mieszany wilgotny dębowo-świerkowy, wyróżniają się znacznie wyższą różnorodnością porostów w porównaniu z drzewostanami wtórnymi (monokulturami sosnowymi i świerkowymi). Wynika to głównie z obecności w tych zbiorowiskach populacji zróżnicowanych wiekowo dębów. Wtórne drzewostany nie zapewniają warunków odpowiednich do wzrostu wielu stenotopowym porostom leśnym, pomimo obecności w bezpośrednim ich otoczeniu diaspor tych gatunków. W celu ochrony populacji cennych gatunków porostów, związanych w szczególności sposobem z dębami, należy rozważyć zabiegi mające na celu zapewnienie ciągłości pokoleń dębu w rezerwacie.

### Introduction

The Puszcza Piska Forest is one of the largest and most valuable forest complexes of north-eastern Poland (SOKOŁOWSKI 2006). It is a key element in the regional system of natural environmental protection NATURA 2000 (KRUPA and KRUPA 2009, KRUSZELNICKI 2009). Its unique values were appreciated in 2002 when the “Lasy Mazurskie” (Masurian Forests) Promotional Forest Complex was established across its entire area. The idea of establishing Promotional Forest Complexes is linked with multifunctional forestry which merges educational and social functions with a uniform economic and protective program. One of the basic principles of ecology-based sustainable forest management is the thorough identification of the natural values of a forest. In practice, owing to the vast biodiversity of forest ecosystems (JAROSZEWICZ 2007), the scope of this identification is very narrow (KAPUŚCIŃSKI 2006). It takes no account of many groups of organisms, including those that are highly significant to the functioning of a forest biocenosis like, e.g. lichens (FAŁTYNOWICZ 2006, ELLIS 2012). Considering that the occurrence of these organisms are very closely linked to the environment, they may play a role as universal bioindicators to be used for, e.g., the evaluation of forest phytocenoses (KUBIAK 2013).

So far, 289 species of lichens have been recorded in the area of the Puszcza Piska Forest (ZALEWSKA et al. 2011). Types and scopes of conducted investigations as well as science-based reports appearing on taxa being new to this complex (KUBIAK 2011, MONWID and KUBIAK 2012, KUBIAK and SUCHARZEWSKA 2014), enable the speculation that data in this respect is still incomplete. Further in-depth surveys are therefore necessary, especially for the objects of key significance to the biodiversity of this area. These may include the “Pupy” Nature Reserve located in the southern part of the forest complex.

Considering the above points, this study aimed to inventory the species reservoir of lichens in the “Pupy” Nature Reserve and to determine the role and significance of the forest communities present therein with regards to the biodiversity of these organisms. This aim is consistent with the general tasks of the “Lasy Mazurskie” Promotional Forest Complex that includes, i.e. “thorough recognition of the status of forest biocenosis... and conditions of its functioning as well as directions of proceeding changes” (*Program gospodarczo-ochronny...* 2013).

## Study Area

The “Pupy” Nature Reserve was established in 1995 in order to “preserve – from the scientific and educational points of view – the mature forests with spruce, pine, oak and beech introduced on the historical experimental areas” (*Centralny rejestr...* 2016). The protected area covers forest complexes of divisions 184 and 211 of the Spychowo Forest District, with a total area of 58.12 ha. The nature reserve is located on the Masurian Plain, in the mesoregion predominated by sandur areas with large contributions of waterlogged and peat-covered areas. Despite limited diversity in the topographic profile, this area is characterized by high geological and habitat diversity. Twelve plant communities have been distinguished therein (*Plan...* 2006). The area of the nature reserve is predominated by humid mixed oak-spruce forest (*Quercus-Piceetum*), the main component of which is common spruce (*Picea abies*), which forms a multi-generation population. Considerably less numerous and little diversified in terms of age is the population of English oak (*Quercus robur*), represented by among others a few 300-year-old trees. Among the remaining communities, noteworthy is the subcontinental oak-lime-hornbeam forest (*Tilio cordatae-Carpinetum betuli*). On the area colonized by this community, there are clumps of common beech (*Fagus sylvatica*) – a species which occurs outside its geographical range limit here. The oak-lime-hornbeam forest communities occupy larger areas of the nature reserve than it appears from the contemporary character of the forest cover; they are however colonized by pine stands formed as a consequence of artificial planting after clear cutting. Forest communities consistent with the occupied habitat may additionally include humid pine forest (*Molinio careulae-Pinetum*) and alder swamp forest (*Ribeso nigri-Alnetum*). In total, barely 60% of forest stands of the nature reserve are more or less natural communities. The remaining part includes substitute communities, being at the early stage of regeneration towards mixed coniferous forests and oak-lime-hornbeam forests (*Plan ochronny...* 2006).

## Materials and Methods

A field study was conducted in 2012 on seven circular research plots with an area of 0.1 ha each. Research areas were determined within the most typical forest communities representative of the nature reserve (Table 1). Inventory of lichen species was conducted at each plot considering all substrates colonized by these organisms. For specimens difficult to be identified in the field, small fragments were collected for further laboratory analyses. In justified cases, standard taxonomic diagnosis was complemented with results of chromatographic analyses (TLC), differentiating secondary lichen metabolites (KUBIAK and KUKWA 2011). The collected material was deposited in the Herbarium of the Department of Mycology, University of Warmia and Mazury in Olsztyn (OLTC). Species nomenclature was adopted from FAŁTYNOWICZ and KOSSOWSKA (2016). Threat categories of lichens were provided by CIEŚLIŃSKI et al. (2006). Protected species were identified based on the current Regulations of the Ministry of the Natural Environment (Rozporządzenie Ministra Środowiska... Dz.U. 2014, poz. 1408), whereas indicators of lowland old-growth

Table 1  
Characteristics of the research plots in the reserve

Study site no.	Forest subarea no.	Geographical coordinates	Forest site type	Dominated tree stand (species/age)	Admixed species	Forest community
I	184 k	53°33'56.1"N/ 21°21'13.2"E	LŚW	Q/121	<i>B, F, Pa, Ps</i>	<i>Tilio-Carpinetum</i>
II	211 f	53°33'55.1"N/ 21°21'13.3"E	LMW	<i>Pa</i> /79 Q/350	<i>B</i>	<i>Quercus-Piceetum</i>
III	211 g	53°33'51.3"N/ 21°21'05.9"E	LMW	A/36	–	<i>Ribeso nigri-Alnetum</i>
IV	211 m	53°33'37.2"N/ 21°21'06.9"E	BMW	<i>Ps</i> /186	<i>Pa</i>	<i>Molinio careulae-Pinetum</i>
V	211 i	53°33'34.1"N/ 21°21'21.5"E	LMW	<i>Pa</i> /46	<i>B</i>	substitute of wet spruce forest
VI	184 i	53°34'07.8"N/ 21°21'27.2"E	BMW	<i>B</i> /61	<i>Pa, Ps</i>	substitute of wet pine forest
VII	184 f	53°34'14.1"N/ 21°21'33.1"E	LMŚW	<i>Ps</i> /51	<i>C</i>	substitute of oak-horn-beam forest

LŚW – fresh broad-leaved forest; LMW – wet mixed broad-leaved forest; BMW – wet mixed coniferous forest; LMŚW – fresh mixed broad-leaved forest

*A* – *Alnus glutinosa*, *B* – *Betula pendula*, *C* – *Corylus avellana*, *F* – *Fagus sylvatica*, *Pa* – *Picea abies*, *Ps* – *Pinus sylvestris*, *Q* – *Quercus robur*

forests were provided by CZYŻEWSKA and CIEŚLIŃSKI (2003). Names of plant communities were adopted from MATUSZKIEWICZ (2007). Tree stand taxation data were presented according to the FOREST DATA BANK (2015). Similarity of lichen biota recorded at particular research areas and biota associated with particular phorophytes was determined based on the cumulative hierarchical classification, made using the MVSP package (KOVACH 2010). A dendrogram was plotted using the Unweighted Pair Group Method (UPGMA), and Euclidian distance was adopted as a measure of similarity.

## Results

In total, 96 species were identified on the area of the “Pupy” Nature Reserve, including 95 species of lichens (lichenized fungi) and one species of a saprobic fungus (*Stenocybe pullatula*), traditionally included in lichenological records (Table 2). There were 34 species that are at risk of extinction on the regional scale and 10 protected species including 3 under strict and 7 under partial protection. Regarding one species, *Thelotrema lepadinum*, according to the binding Regulation of the Ministry of the Natural Environment, it is necessary to establish a protection zone around its locality with a radius of 50 meters. The lichen biota of the nature reserve includes 12 species of stenotopic forest lichens having the status of indicators of the lowland old-growth forests (Table 2).

Table 2

A list of recorded species

Species	Study site*	Substrate*	Status of the species
1	2	3	4
<i>Absconditella lignicola</i> Vězda & Pišut	IV	dw	
<i>Anisomeridium polypori</i> (M.B. Ellis & Everh.) M.E. Barr	II	Q	
<i>Arthonia spadicea</i> Leight.	I, II	Q	
<i>A. vinosa</i> Leight.	I	Q	NT, IN
<i>Bacidia subincompta</i> (Nyl.) Arnold	I, II	Q	EN
<i>Bacidina phacodes</i> (Körb.) Vězda	I	Q	
<i>B. sulphurella</i> (Samp) M. Hauck & V. Wirth	I, VII	C, F, Q	
<i>Biatora efflorescens</i> (Hedl.) Erichsen	I	Q	VU
<i>B. hemipolia</i> (Nyl.) S. Ekman & Printzen f. <i>pallida</i> Czarnota & Coppins	I	Q	
<i>Bryoria fuscescens</i> (Gyeln.) Brodo & D. Hawksw.	I, II	Q	VU, PP
<i>Buellia griseovirens</i> (Turner & Borrer ex Sm.) Almb.	I, III	A, F, Q	
<i>Calicium glaucellum</i> Ach.	II	Q	VU
<i>Chaenotheca chrysocephala</i> (Ach.) Th. Fr.	I, IV	Pa, Q	
<i>Ch. ferruginea</i> (Turner ex Sm.) Mig.	I, III, IV	A, Pa, Ps	
<i>Ch. furfuracea</i> (L.) Tibell	II, III	A, Q, dw	NT

cont. Table 2

1	2	3	4
<i>Ch. stemonea</i> (Ach.) Mull. Arg.	I, II	B, Ps	EN
<i>Ch. trichialis</i> (Ach.) Th. Fr.	I, III, IV	Pa, Ps, Q, dw	NT
<i>Chrysothrix candelaris</i> (L.) J.R. Laundon	II	Q	CR, SP, IN
<i>Cladonia cenotea</i> (Ach.) Schaer.	V	B	
<i>C. chlorophaea</i> (Florke ex Sommerf.) Spreng.	II, III, IV	A, B, dw	
<i>C. coniocraea</i> auct.	I-VII	A, B, F, Pa, Ps, Q, dw	
<i>C. digitata</i> (L.) Hoffm.	I, II, IV-VI	B, Ps, dw	
<i>C. fimbriata</i> (L.) Fr.	III, VI, VII	A, Ps	
<i>C. glauca</i> Flörke	V	B	
<i>C. macilenta</i> Hoffm.	III-VII	A, B, Ps, dw	
<i>C. ochrochlora</i> Flörke	II, III, VI	A, B, Ps	
<i>C. parasitica</i> (Hoffm.) Hoffm.	IV	Ps	EN, IN
<i>Coenogonium pineti</i> (Schrad.) Lucking & Lumbsch	I-V, VII	A, B, F, Pa, Ps, Q, dw	
<i>Evernia prunastri</i> (L.) Ach.	I-III	A, F, Pa, Q	NT
<i>Fellhanera gyrophorica</i> Sérus. et al.	I	Q	LC, IN
<i>Fellhaneropsis vezdae</i> (Coppins & P. James) Serus. & Coppins	I	Q	LC, IN
<i>Fuscidea arboricola</i> Coppins & Tønsberg	I	F	
<i>F. pusilla</i> Tønsberg	I, II-VI	A, B, Pa, Q	
<i>Graphis scripta</i> (L.) Ach.	I	F	NT
<i>Hypocenomycce scalaris</i> (Ach.) Choisy	I-IV	A, B, Pa, Ps, Q	
<i>Hypogymnia physodes</i> (L.) Nyl.	I-VII	A, B, F, Ps, Q, dw	
<i>H. tubulosa</i> (Schaer.) Hav.	II, IV	Pa, Q	NT, PP
<i>Imshaugia aleurites</i> (Ach.) S.L.F. Meyer	IV	Ps	PP
<i>Lecania cyrtella</i> (Ach.) Th. Fr.	I	Q	
<i>L. naegelii</i> (Hepp) Diederich & van den Boom	I, II	Q	
<i>Lecanora compallens</i> van Herk & Aptroot	VII	C	
<i>L. expallens</i> Ach.	I, II	Q	
<i>L. farinaria</i> Borrer	I	F	
<i>Lecanora persimilis</i> (Th. Fr.) Nyl.	I	Q	DD
<i>L. pulicaris</i> (Pers.) Ach.	I, III, V	A, B, F	
<i>L. sarcopidoides</i> (A. Massal.) A.L. Sm.	IV, VI	Ps	NT
<i>L. thysanophora</i> R.C. Harris	I	Q	
<i>Lecidea nylanderii</i> (Anzi) Th. Fr.	II, III, VI	A, Ps, Q, dw	
<i>Lepraria elobata</i> Tønsberg	I-VII	A, B, C, F, Pa, Q, dw	
<i>L. incana</i> (L.) Ach.	I-VII	B, C, F, Pa, Ps, Q, dw	
<i>L. jackii</i> Tønsberg	I-IV, VI, VII	A, B, F, Pa, Ps, dw	
<i>L. finkii</i> (B. de Lesd. ex Hue) R.C. Harris	I, II, IV	B, F, Q, dw	
<i>L. rigidula</i> (B. de Lesd.) Tønsberg	I	F	
<i>L. vouauxii</i> (Hue) R.C. Harris	I, II	F, Q	
<i>Lichenomphalia umbellifera</i> (L.) Redhead & al.	II, V	B, dw	NT
<i>Loxospora elatina</i> (Ach.) A. Massal.	V	F	EN, IN
<i>Melanelixia fuliginosa</i> (Fr. ex Duby) O. Blanco et al.	I, II, VII	C, Pa, Q	
<i>Melanohalea exasperatula</i> (Nyl.) O. Blanco & al.	II	Pa	
<i>Micarea denigrata</i> (Fr.) Hedl.	IV	Ps, dw	
<i>M. elachista</i> (Korb.) Coppins & R. Sant.	IV	Ps	EN, IN

cont. Table 2

1	2	3	4
<i>M. melaena</i> (Nyl.) Hedl.	IV	Ps	NT, IN
<i>M. misella</i> (Nyl.) Hedl.	I, IV	dw	
<i>M. prasina</i> s. lat.	III	A	
<i>Ochrolechia bahusiensis</i> H. Magn.	I, III	A, F	VU
<i>Ochrolechia microstictoides</i> Räsänen	I, III	A, Q	
<i>Parmelia sulcata</i> Taylor	I-III, V	A, B, F, Pa, Q	
<i>Parmeliopsis ambigua</i> (Wulfen) Nyl.	II, IV, VI	B, Ps	
<i>Pertusaria amara</i> (Ach.) Nyl.	I-III	A, F, Q	
<i>Pertusaria albescens</i> (Huds.) M. Choisy & Werner	I, II	Q	
<i>P. coccodes</i> (Ach.) Nyl.	I	Q	NT
<i>P. coronata</i> (Ach.) Th. Fr.	II	Q	VU, IN
<i>P. flavida</i> (DC.) J.R. Laundon	II	Q	EN, IN
<i>P. leioplaca</i> DC.	I	Q	NT
<i>Phlyctis argena</i> (Ach.) Flot.	I-III, VII	A, C, F, Q	
<i>Physcia tenella</i> (Scop.) DC.	I, II	Pa, Q	
<i>Placynthiella dasaea</i> (Stirt.) Tønsberg	I, II, IV	dw	
<i>P. icmalea</i> (Ach.) Coppins & P. James	I, II, IV, V, VII	B, Ps, dw	
<i>Platismatia glauca</i> (L.) W.L. Culb. & C.F. Culb.	I-V	A, B, Pa, Q	
<i>Porina aenea</i> (Wallr.) Zahlbr.	I	F	
<i>Pseudevernia furfuracea</i> (L.) Zopf	I, II, IV, V	B, Pa, Q	
<i>Pycnora sorophora</i> (Vain.) Hafellner	IV	dw	
<i>Ramalina farinacea</i> (L.) Ach.	I	Q	VU
<i>Ropalospora viridis</i> (Tønsberg) Tønsberg	I, III	A, F	
<i>Scoliosporum sarothamni</i> (Vain.) Vezda	I, II	B, F, Pa	
+ <i>Stenocybe pullatula</i> (Ach. ex Sommerf.) Stein	III	A	
<i>Thelotrema lepadinum</i> (Ach.) Ach.	II	Q	EN, IN
<i>Trapeliopsis flexuosa</i> (Fr.) Coppins & P. James	I, III-V, VII	A, B, Ps, dw	
<i>T. glaucolepidea</i> (Nyl.) Gotth. Schneid.	I	dw	DD
<i>T. granulosa</i> (Hoffm.) Lumbsch	I, II	dw	
<i>Tuckermanopsis chlorophylla</i> (Willd.) Hale	II, IV, V	B, Pa	VU, PP
<i>Usnea dasopoda</i> (Ach.) Rohl.	I	B	VU, PP
<i>U. hirta</i> (L.) Weber ex F.H. Wigg.	II	B, Q	VU, PP
<i>U. subfloridana</i> Stirt.	III	A	EN, SP
<i>Violella fucata</i> (Stirt.) T. Sprib.	I, III, IV	A, F, dw	
<i>Xanthoria parietina</i> (L.) Th. Fr.	I	Q	
<i>Zwackhia viridis</i> (Ach.) Poetsch & Schied.	I, II	Q	VU, IN

+ – saprobic fungus; dw – dead wood; CR – critically endangered, EN – endangered, VU – vulnerable, LC – least concern, NT – near threatened, DD – data deficient, SP – strict protection, PP – partial protection; IN – indicator of lowland old-growth forest

\* denotes as in Table 1

The lichen biota in the reserve consisted of two ecological groups – epiphytic (corticolous) and lignicolous species. Predominating were the epiphytic species, represented by 90 taxa (including 66 obligatory epiphytes), of which the highest number of taxa – 52 – was recorded on oak bark. The lichens associated with this tree represented the most specific group covering the highest number of exclusive species – 26 (Figure 1). It was confirmed by the outcome of the cumulative hierarchical classification (Figure 2). Oaks

encompassed the highest number of protected species – 6, species threatened with extinction – 21, and taxa with the status of indicators of lowland old-growth forests – 8. The role and significance of the remaining phorophytes as substrates for the rare, stenotopic forest lichens were significantly fewer. The conducted classification demonstrated the similarity of the species composition of lichen biota of pine and birch, spruce and hazel, and to a lesser extent – beech and alder (Figure 2).

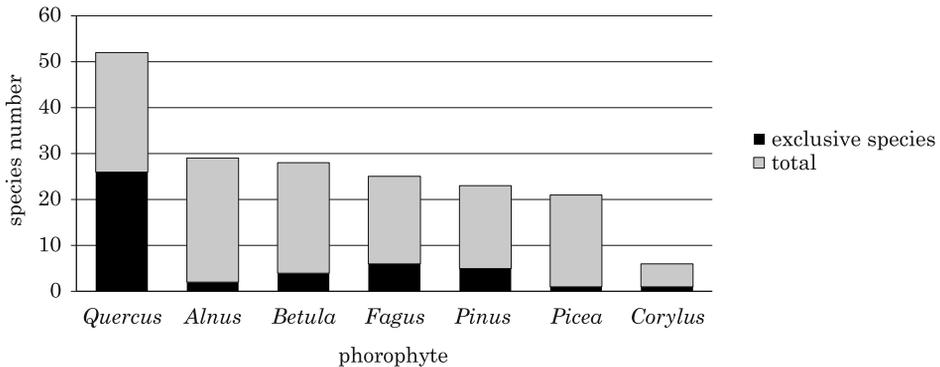


Fig. 1. Species of lichens recorded on particular phorophytes

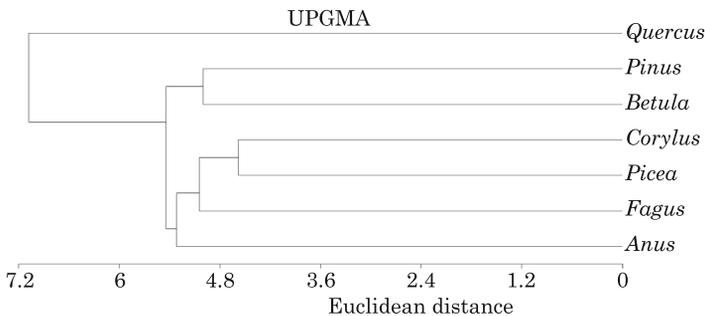


Fig. 2. Similarity dendrogram of the lichen biota's associated with particular phorophytes

The second ecological group of the nature reserve, in terms of taxonomic diversity, was the lignicolous lichens. It contained 23 species, however only six of them were obligatory lignicolous. The highest number of lichen species on wood was noted in the humid mixed pine forest – 18, oak-lime-hornbeam forest – 15, humid mixed coniferous forest – 14, and alder swamp forest – 12.

The study demonstrated that forest communities of the nature reserve were of greatly different significance to the species diversity of lichens (Figure 3). The greatest diversity of these organisms was observed in the oak-lime-

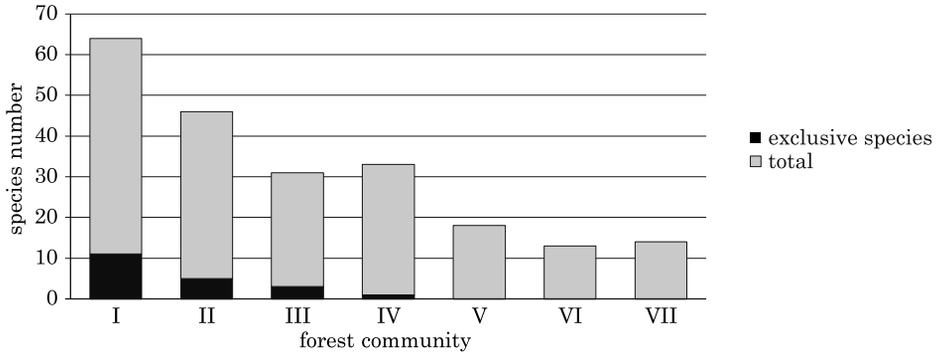


Fig. 3. Species of lichens associated with particular forest communities

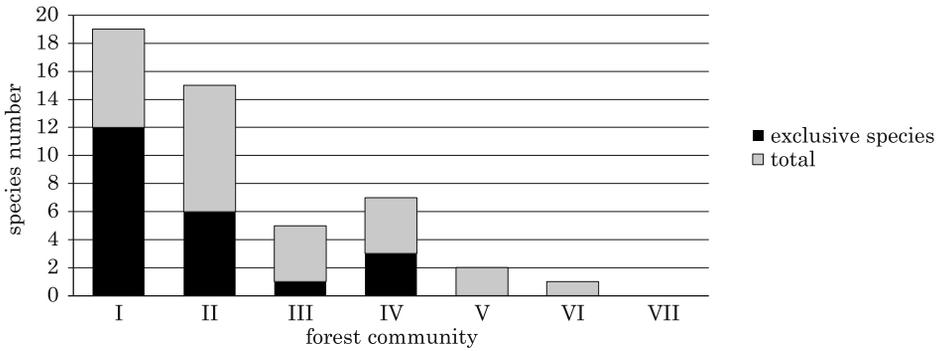


Fig. 4. National red listed species in particular forest communities

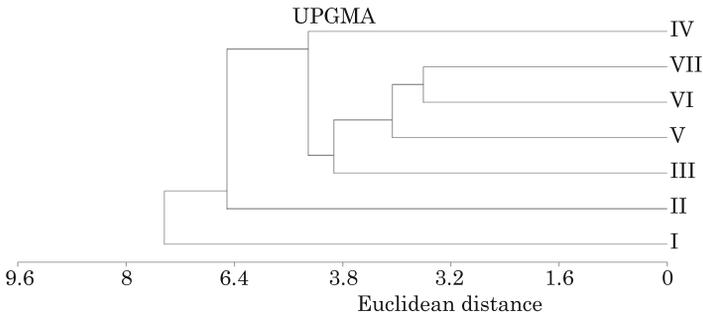


Fig. 5. Similarity dendrogram of the lichen biota's associated with particular forest communities

-hornbeam forest – 64 species, of which 11 were obligatory taxa associated with this community. In addition, oak-lime-hornbeam forest was characterized as having the highest number of taxa threatened with extinction – 19, and of indicators of lowland old-growth forests – 5. Considering the quantitative and qualitative aspects of lichen biota diversity, considerably lesser significance

should be ascribed to communities in the following order: humid mixed coniferous forest, humid pine forest, and alder swamp forest. The other substitute communities were characterized by the lowest total number of species, lack of exclusive species, lack or very low diversity of threatened lichens (with low threat categories) as well as the lack of indicator species typical of old-growth forests (Figure 4). The assessment of similarity of lichen biota associated with individual communities revealed three main groups (Figure 5). The first was represented by the best preserved association with the oldest forest stand – oak-hornbeam forest and mixed coniferous forest, the second – by humid pine forest, and the third one – by alder swamp forest and substitute communities.

## Discussion

The contribution of lichens to particular types of forest communities in Poland is poorly recognized (CZYŻEWSKA 2003). Especially valuable communities colonized by numerous species of lichens, particularly the epiphytic ones, include the natural and self-regenerating meso- and eutrophic deciduous forests (CIEŚLIŃSKI et al. 1995, CIEŚLIŃSKI 2003, CZYŻEWSKA and CIEŚLIŃSKI 2003). Old forest stands of this type, oak-hornbeam forests in particular, are accompanied by the highest number of stenotopic species including those acknowledged as indicators of the old-growth forests (CZYŻEWSKA and CIEŚLIŃSKI 2003). The exceptional character of the lichen biota of typically-formed oak-hornbeam forest results mainly from diversification of the species and the spatial structure of the forest stand. The presence of appropriate phorophytes is one of the key determinants of lichen diversity in forests (ULICZKA and ANGELSTAM 1999, LIE et al. 2009, NASCIBENE et al. 2009, THOR et al. 2010, BRUNIALTI et al. 2013). Among the main tree species predominating in forest communities in Poland, irrespective of the spatial scale of reference, the richest biota of lichens are usually associated with oak (CIEŚLIŃSKI and TOBOLEWSKI 1988, CIEŚLIŃSKI et al. 1995, CIEŚLIŃSKI 2003, 2008). The exceptional abundance of its epiphytes is determined by a few factors. On the one hand, it results from a long life span of individual specimens and the occurrence of oak in various types of natural forest communities (DANIELEWICZ and PAWLACZYK 2006, CIEŚLIŃSKI 2008), but on the other hand it is also determined by the specific structure of periderm and its physicochemical properties (BARKMAN 1969). Oak bark is hard and solid. It has a moderately eutrophic character, low pH (3.5–6.0) and excellent capacity for water absorption from air. The characteristic relief of the bark of old trees with multiple cracks and grooves reaching 10 cm in depth, creates the diversity of micro-

habitats exploited by various groups of epiphytes. In addition, mineral compounds and humus deposit in cracks of the periderm, which facilitates the occurrence of some species (e.g. *Cladonia* spp.). The high diversity of lichens associated with oak in the “Pupy” Nature Reserve is influenced by its contribution in the structure of two various forest communities – oak-hornbeam forest and mixed coniferous forest. These communities are characterized by various specificity of the internal microclimate (*Eco-climatological studies...* 1995), which ensures diversified conditions suitable for a wide group of epiphytes. In addition, the age span of trees of this species is exceptionally large in the scale of the whole reserve. The size (breast height diameter) of a tree is not always positively correlated with the total number of epiphytes associated with it, however it is of great significance to many stenotopic lichens, threatened with extinction in the country (MEŽAKA et al. 2008, JOHANSSON et al. 2009). Unfortunately, most of the old oaks in the reserve show disease symptoms or are partly dying. Furthermore, there is no generation continuity in the population of this species, which is a significant threat to many obligatorily-associated epiphytes (MONWID and KUBIAK 2012). A similar situation afflicts the pine, as the age difference between the oldest, the ageing trees in the humid pine forest, and substitute tree stands reaches over 120 years.

The conducted study demonstrated that at the current stage of development the substitute tree stands do not provide appropriate conditions for the growth of most lichen species recorded in the investigated nature reserve. Especially tangible was the lack of stenotopic taxa having the status of indicators of lowland old-growth forests. Noteworthy is that this lack occurs despite the presence of diaspores of these organisms in the closest environment. The number of 12 species of this type, recorded in the “Pupy” Nature Reserve, is significantly lower compared to the highest values reported for nature reserves in north-eastern Poland (e.g.: “Budzisk” in the Puszcza Knyszyńska Forest – 34 species, “Las Warmiński” in the Puszcza Napiwodzko-Ramucka Forest – 30 species, “Starożyn” in the Puszcza Augustowska Forest – 29 species, or “Borki” in the Puszcza Borecka Forest – 29) (CZYŻEWSKA and CIEŚLIŃSKI 2003, KUBIAK and SUCHARZEWSKA 2012). It needs to be emphasized, however, that for a few lichens of this group, the “Pupy” Nature Reserve constitutes one of the very few (e.g. *Loxospora elatina*) or the only (e.g. *Thelotrema lepadinum*) site of occurrence in the area of the Puszcza Piska Forest. It is, therefore, extremely important to preserve these localities and to monitor populations of these species in the nature reserve.

## Conclusions

The “Pupy” Nature Reserve protects populations of many lichen species that are rare in Poland. Apart from threatened species and those at risk of extinction, it encompasses a relatively large group of lichens having the status of old-growth forest indicators. Forest communities of the analyzed nature reserve differ significantly in terms of their importance for lichen biodiversity. The key impact in this respect is ascribed to the subcontinental oak-lime-hornbeam forest and humid mixed oak-spruce forest. The high biodiversity of lichens in these communities is determined, apart from the relatively good preservation status, also by the presence of old trees, oak trees in particular. Despite the presence of diaspores of many stenotopic forest lichens in the closest environment, today the substitute forest stands do not ensure the appropriate conditions for their growth. Considering the dynamic structure of forest stands and deteriorating conditions of the oldest trees (oaks in particular), it is necessary to monitor the effect of processes ongoing in the reserve on lichen biota. This should particularly pertain to the population of a protected species *Thelotrema lepadinum* which requires establishing a protection zone. In order to protect populations of valuable lichen species, especially those related to the oak tree, actions should be undertaken with the aim of preserving the generation continuity of the oak tree in the nature reserve.

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**THE EFFECT OF DRYING METHODS  
ON THE CONTENT OF SELECTED BIOACTIVE  
COMPOUNDS AND FIBRE IN CARROT POMACE**

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**Key words:** carrot pomace, drying, carotenoids, polyphenols, DPPH radical, fibre.

**A b s t r a c t**

The aim of the study was to analyse the content and profile of carotenoids, polyphenols content, the capacity to scavenge DPPH radical, the content of neutral detergent fibre, acid detergent fibre and hemicellulose, cellulose and lignin fractions in carrot pomace dried with different methods. Fresh carrot pomace obtained as a by-product during the extraction of juice from Nantejska carrot was used in tests. Portions of pomace were frozen, dried by convection or lyophilized. The study demonstrated a significant loss of total carotenoids in convective dried (CPC) and lyophilized (CPL) carrot pomace. The greatest decrease was found for  $\beta$ -carotene. The content of phenolics increased by ca. 2.5% for CPC and by ca. 20% for CPL. The capacity to scavenge DPPH radicals was also higher in dry pomace. Significantly higher content of neutral detergent fibre, including hemicellulose fraction, was found in CPC. The analysis did not clearly indicate the optimal drying method. The choice of drying method should depend on the type of final product in which carrot pomace will be used.

**W P Ł Y W S P O S O B U S U S Z E N I A W Y T Ł O K Ó W Z M A R C H W I N A W Y B R A N E S K Ł A D N I K I  
B I O A K T Y W N E I B Ł O N N I K**

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**S ł o w a k l u c z o w e:** w y t ł o k i m a r c h w i o w e, s u s z e n i e, k a r o t e n o i d y, p o l i f e n o l e, D P P H, b ł o n n i k.

### Abstrakt

Celem badań była ocena wpływu metody suszenia wyłoków z marchwi na zawartość i profil karotenoidów, zawartość polifenoli, aktywność przeciwutleniającą oznaczoną jako zdolność wiązania rodnika DPPH, zawartość błonnika pokarmowego (NDF), błonnika kwaśnego detergentowego (ADF) oraz frakcji hemiceluloz, celulozy i lignin. Materiał badawczy stanowiły wyłoki uzyskane jako produkt uboczny przy tłoczeniu soku z marchwi odmiany Nantejska. Część wyłoków świeżych zamrożono, a część poddano suszeniu konwekcyjnemu i liofilizacji.

Wykazano, iż zarówno proces suszenia konwekcyjnego, jak i liofilizacji spowodował duże obniżenie zawartości karotenoidów ogółem w wyłokach, odpowiednio o 31,8% i 34,5%. Największy spadek, na poziomie 41%, odnotowano dla  $\beta$ -karotenu. Stwierdzono także większą zawartość polifenoli ogółem w wyłokach suszonych niż w świeżych. Wzrost zawartości polifenoli wyniósł ok. 2% w przypadku konwekcji i ok. 20% dla liofilizacji. Oznaczona wyższa zawartość polifenoli może wynikać m.in. z lepszej dostępności analitycznej tych związków z materiału suszonego. Aktywność wiązania rodnika DPPH była również większa dla wyłoków suszonych. Badane wyłoki są dobrym źródłem błonnika. Pod względem zawartości błonnika pokarmowego (NDF), w tym frakcji hemiceluloz, wyróżniały się wyłoki suszone konwekcyjnie. Analizując uzyskane wyniki, trudno jednoznacznie wskazać wariant optymalny. Wybór w znaczącym stopniu zależał będzie od rodzaju produktu końcowego, do którego wyłoki będą aplikowane.

## Introduction

Fruit and vegetable processing generates a significant amount of waste and by-products, which sometimes account for 10–35% of processed raw material (TARKO et al. 2012). Pomace is a particularly important by-product, and is obtained during the industrial production of wine, juice and beverages (CYBULSKA et al. 2013, HERNÁNDEZ-ORTEGA et al. 2013). In Poland, which is a major producer of fruit and vegetable juices, ca. 260,000 t of fruit pomace and ca. 100,000 t of vegetable pomace is created each year. Pomace consists mainly of cell wall polysaccharides, which constitute dietary fibre (pectin, cellulose and hemicellulose) (NAWIRSKA and KWAŚNIEWSKA 2004, BORYCKA 2012, GAZALLI et al. 2013, HERNÁNDEZ-ORTEGA et al. 2013). Studies have indicated that pomace is also a rich source of bioactive compounds with health-related benefits, such as vitamins, minerals and polyphenols (SHARMA et al. 2012, TARKO et al. 2012, HERNÁNDEZ-ORTEGA et al. 2013). Dry pomace can be a valuable additive in many food products, including beverages, breads, biscuits, extruded products, etc. (STOLL et al. 2003, GULLÓN et al. 2007, UPADHYAY et al. 2010, KUMAR and KUMAR 2011, GAYAS et al. 2012, KOHAJDOVÁ et al. 2012, CYBULSKA et al. 2013).

Carrot pomace is a particularly valuable residue. To date, it has mainly been utilised through composting and processing into animal feed. Because of its high water content (ca. 70%) fresh pomace is susceptible to a rapid increase in microbial contamination (TARKO et al. 2012). Drying is one of the major methods to prevent this process. The removal of a significant amount of water from pomace reduces its weight by several times, which also reduces

the cost of transport and storage. Major factors determining the quality of dry pomace include pre-treatment, drying method and drying parameters. Convective drying is the most popular method. Other, rarely used methods, mainly because of greater costs, are lyophilisation and microwave drying (MARMO 2007, AL-HARAHSEH et al. 2009, KUMAR et al. 2012, HERNÁNDEZ-ORTEGA et al. 2013, JANISZEWSKA et al. 2013). High temperatures can cause darkening of pomace, and the degradation of carotenoids, polyphenols, aromatic compounds, etc. (GAWAŁEK 2005). Because pomace is mainly used in the food industry, an increasing number of studies are being carried out to design drying conditions that could produce dry pomace characterised by good sensory, nutritional and physical parameters (CIURZYŃSKA et al. 2011).

The study attempts to determine the influence of the applied drying method of carrot pomace – the convection and freeze drying – on changes of biologically active substances and fiber. The aim of the study was to identify more favorable method of drying carrot pomace.

## **Material and Methods**

### **Preparation of pomace**

Analyses were carried out on fresh carrot pomace (CPF), convective dried carrot pomace (CPC), and lyophilized carrot pomace (CPL). Fresh carrot pomace was obtained as a by-product during the extraction of juice from Nantejska carrot in a laboratory hydraulic press (Biowin, Poznań, Poland). Carrot used in the study was harvested in 2014 near Olsztyn, Poland. Fresh carrot pomace was divided into three portions. The first portion was frozen at  $-25^{\circ}\text{C}$ , and two other portions were dried either by convection in an oven (KBC 200, Warszawa, Poland) (for 5 hours at  $40^{\circ}\text{C}$ ), or freeze-dried in a lyophilizer (Heto CD 13-2, Danmark) (for 48 hours, condenser temp.  $-60^{\circ}\text{C}$ , pressure 1 mbar). Fresh pomace before freezing and dry pomace were closed in plastic bags after evacuation of air from inside the package under vacuum. Fresh carrot pomace for chemical analysis was blended in a homogenizer (BRAUN 2096, KRONBERG, Germany), and dry pomace was blended in a laboratory mill (IKA M-20, Germany).

### **Chemical reagents**

2,2-Diphenyl-1-picrylhydrazyl (DPHH), catechin,  $\text{AlCl}_3$ , Folin-Ciocalteu's reagent, alpha-amylase, tert butyl methyl ether, acetone, ethanol, hexane,

sulphuric acid, hydrochloric acid, methanol, toluene, sodium carbonate and sodium hydroxide were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Acros Organics (Morris Plains, New Jersey, USA). All other reagents were of analytical purity and supplied by POCh (Gliwice, Poland).

### **Determination of total phenolics**

The total phenols content of fresh pomace and dry pomace were determined by the Folin-Ciocalteu method (AOAC 1974). The extraction of phenols was performed a mixture of methanol : water 80:20 (v/v) solution in an ultrasonic bath for 30 minutes. The extraction process was repeated 3 times, each time separating the supernatant by centrifuging (Eppendorf 5810R, Germany). An aliquot of the extract (1 ml) was mixed with 0.5 ml of Folin-Ciocalteu's reagent and 2 ml of sodium carbonate (20%). After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 750 nm (FLUOstar Omega, BMG LABTECH GmbH, Germany). Gallic acid (GA) was chosen as a reference standard. The data were expressed as mg of gallic acid equivalents (GAE) per 100 g of dry matter (d.m.) pomace.

### **Determination of antioxidant activity using DPPH (2,2-Diphenyl-1-picrylhydrazyl)**

The DPPH radical-scavenging activity of methanol extracts (prepared according to the above procedure) from fresh and dry carrot pomace was determined by using the method described by MOURE et al. (2001). Extracts from pomace samples were mixed with a methanol solution of DPPH. Absorbance was measured at the beginning of the test and after 16 minutes of reaction against the blank at 515 nm (FLUOstar Omega, BMG LABTECH GmbH, Germany). Results were expressed in  $\mu\text{M}$  DPPH scavenged by 1 mg of phenolics.

### **Determination of carotenoids**

Carotenoids were determined using high-performance liquid chromatography (HPLC). Extracts were prepared according to the procedure proposed by CHEN and YANG (1992), using 30 mL a mixture of hexane : acetone : absolute

ethyl alcohol : toluene in a ratio of 10:7:6:7 (v/v/v/v) to which an internal reference standard ( $\beta$ -Apo-8'-carotenal) was added. Saponification, 40% methanol solution of potassium hydroxide was added to the extract and left to stand for 16 hours. The released carotenoids were extracted 3 times with hexane. After the evaporation of hexane using nitrogen, samples were dissolved in 1 mL of mixture containing methanol: dichloromethane (45:55, v/v) and the supernatant was separated by centrifuging (Eppendorf model 5417R Hamburg, Germany), and then analysed chromatographically. Compounds were separated using HPLC and a procedure proposed by EMENHISER et al. (1995) modified by CZAPLICKI (2006). Modification involved the use of methanol-MTBE (methyl tert-butyl ether) gradient and simultaneous different flow rates of the mobile phase. This ensured high resolution of the process and minimised the time of analysis. Samples were analysed using a high-performance liquid chromatography (HPLC) apparatus, series 1200 from Agilent Technologies (Santa Clara, CA, USA), with a fitted diode array detector (DAD) from the same supplier. Compounds were separated at 30°C on a YMC C<sub>30</sub>, 3  $\mu$ m, 150 mm  $\times$  4.6 mm column from YMC Europe GmbH (Germany). Carotenoids were detected at 450 nm. The content of carotenoids was expressed as mg per 100 g of dry matter.

### Determination of fibre

The content of neutral detergent fibre (NDF) and acid detergent fibre (ADF) in the obtained material was determined by the VAN SOEST procedure (1963, 1967) as modified by MCQUEEN (1979). This method consists in the hot extraction of samples using acidic or inert detergent solutions. The content of hemicellulose was calculated from the difference between NDF and ADF; the content of cellulose was calculated from the difference between ADF and lignin content. Results concerning the contents of the fibre were expressed as g per 100 g of dry matter. The content of fibre was measured using a Fibertec System I (Tecator, Sweden).

### Determination of dry matter

Dry matter in fresh and dry carrot pomace was determined by the weighing method according to the Polish standard *Przetwory owocowe...* PN-A-75 101-03:1990).

## Statistical analysis

The results of all analysis (performed in triplicate) were statistically analyzed using Statistica 12.0 PL software (StatSoft, Kraków, Poland). The differences between the means were determined using analysis of variance (ANOVA) with Duncan test at  $P < 0.05$  significance level.

## Results and Discussion

### Bioactive compounds and antioxidant activity

The analysed fresh (CPF), convective dried (CPC) and lyophilized carrot pomace (CPL) differed for the content of bioactive compounds and antioxidant activity (Table 1). Differences between most samples were statistically significant ( $P < 0.05$ ). The drying process caused qualitative and quantitative changes in the level of carotenoids. The total content of carotenoids in fresh, convective dried and lyophilized carrot pomace was 120.14 mg/100 g d.m., 81.93 mg/100 g d.m. and 78.66 mg/100 g d.m., respectively (Table 1). The loss of carotenoids was 31.8% for convective drying and 34.5% for lyophilisation. Smaller changes in carotenoid content during drying were reported by HERNÁNDEZ-ORTEGA et al. (2013) (a 29% decrease for convective dried and a 20% decrease for microwave dried carrot pomace). In plant tissues, carotenoids exist in cis and trans forms and during thermal processing some of the trans forms are either lost or converted to cis and their derivatives (SHARMA et al. 2012).

All analysed samples, regardless of the drying method, contained the highest amount of  $\beta$ -carotene, followed by  $\alpha$ -carotene (Table 1). Considering total carotenoid content,  $\beta$ -carotene accounted for 62.3% (CPF), 53.5% (CPC) and 55.3% (CPL). The predominant share of  $\beta$ -carotene has been reported in studies by SHARMA et al. (2012) and LEONG and OEY (2012). The content of  $\beta$ -carotene in both types of dry carrot pomace was similar, and about 41% lower than in fresh pomace (Table 1). The lowest loss of  $\beta$ -carotene, not exceeding 14%, was reported by LEONG and OEY (2012) for freeze-dried carrot. The loss of  $\alpha$ -carotene in our study was lower than that for  $\beta$ -carotene and did not exceed 22%. The analysis also demonstrated the presence of small amounts of  $\beta$ -cryptoxanthin and lutein, as well as traces of carotene isomers, with slightly greater levels detected in dry pomace.

The content of phenolics also changed after drying (Table 1). The highest level of total phenolics (1188.04 mg/100 g d.m.) was found in CPL, and the lowest (987.88 mg/100 g d.m.) in CPF. To summarize, the total phenolics content in the analysed pomace was ca. 20% higher in lyophilized samples

Table 1  
Carotenoids, total phenols, dry matter contents, and DPPH scavenging activity of carrot pomace

Component	Fresh pomace	Convective dried pomace	Lyophilized pomace
Carotenoids [mg/100 g d.m.]:	–	–	–
Lutein	0.54 ± 0.05 <sup>a</sup>	0.15 ± 0.04 <sup>b</sup>	0.19 ± 0.03 <sup>b</sup>
β-cryptoxanthin	1.72 ± 0.06 <sup>c</sup>	1.06 ± 0.13 <sup>a</sup>	1.40 ± 0.02 <sup>b</sup>
α-carotene	42.97 ± 1.64 <sup>c</sup>	36.91 ± 1.76 <sup>b</sup>	33.57 ± 2.24 <sup>a</sup>
β-carotene	74.91 ± 1.31 <sup>b</sup>	43.80 ± 2.86 <sup>a</sup>	43.50 ± 2.13 <sup>a</sup>
Isomers of carotenoids	–	traces	traces
Total	120.14 ± 2.72 <sup>b</sup>	81.93 ± 2.47 <sup>a</sup>	78.66 ± 1.84 <sup>a</sup>
Total phenols [mg/100 g d.m.]	987.88 ± 45.03 <sup>a</sup>	1013.02 ± 28.14 <sup>a</sup>	1188.04 ± 57.26 <sup>b</sup>
DPPH scavenging activity [μmol DPPH/mg phenolics]	5.75 ± 0.55 <sup>a</sup>	6.80 ± 0.78 <sup>a</sup>	13.74 ± 1.56 <sup>b</sup>
Dry matter [%]	16.54 ± 0.33 <sup>a</sup>	94.43 ± 0.14 <sup>b</sup>	98.43 ± 0.27 <sup>c</sup>

d.m. – dry matter

Values are expressed as mean ± standard deviation,  $n = 3$

Means in the same row with different letters differ significantly ( $P < 0.05$ )

and ca. 2% higher in convective dried samples as compared to fresh pomace. It is possible that the extractability of phenolics from dry material is greater than from fresh material under the conditions of the method used in our study for the determination of phenolics (AOAC 1974). Studies by HERNÁNDEZ-ORTEGA et al. (2013) revealed a greater content of phenolics in fresh, convective dried and microwave dried carrot pomace, which may be attributed to the specific variety of polyphenol-rich carrot used for analyses, or different parameters of juice extraction and then pomace drying. As with the phenolic content HERNÁNDEZ-ORTEGA et al. (2013) reported 1841 mg gallic acid equivalents/100 g d.m. for fresh carrot pomace vs 1505 mg gallic acid equivalents/100 g d.m. for convective dried pomace and 1412 mg gallic acid equivalents/100 g d.m. for microwave dried pomace.

The antioxidant activity of carrot is associated with the presence of phenolics and carotenoids in the plant (SHARMA et al. 2012). In our study the antioxidant activity expressed in μM DPPH scavenged by 1 mg of phenolics was in the range of 6.8–13.74 and was the highest for CPL and the lowest for CPF (Table 1). There may be many reasons explaining such a significant difference in antioxidant activity. One potential reason for the increased antioxidant activity of dry pomace is the formation of compounds during the drying of fresh pomace. Maillard's reaction, facilitated by high temperature, may also contribute to this increase in the case of convective drying (TAMANNA and MAHMOOD 2015). Another reason may be the different stability of phenolics characterised by different capacity to scavenge DPPH radicals during

the used drying processes. Antioxidant activity was determined in methanol extracts of pomace, and thus a diversified selective extractability of antioxidants from individual samples cannot be ruled out, either.

## Fibre

As with carotenoids and phenolics, the study demonstrated differences between the analysed samples in the content of different forms of fibre depending on the drying method (Figure 1). Statistically significant differences were found for most of the samples ( $P < 0.05$ ). Interestingly, high fibre content was found for fresh carrot pomace and dry carrot pomace. The highest content of neutral detergent fibre (NDF) (28.23 g/100 g d.m.) was found in CPC, and the lowest (19.86 g/100 g d.m.) in CPL (Figure 1). The content of acid detergent fibre (ADF) also varied, and was from 16.93 g/100 g d.m. for CPL to 24.67 g/100 g d.m. for CPC. Cellulose fraction was dominant in all analysed samples, with the highest content also found in CPC. The content of lignin was lower, especially in lyophilized pomace. The content of hemicellulose was the lowest (from 1.39 g/100 g d.m. for CPF to 3.57 g/100 g d.m. for CPC). The study demonstrated an increase in hemicellulose after drying. Other authors (D'ARCHIVIO et al. 2010, PALAFOX-CARLOS et al. 2011) reported that the increased content of NDF after heat treatment may result from the formation

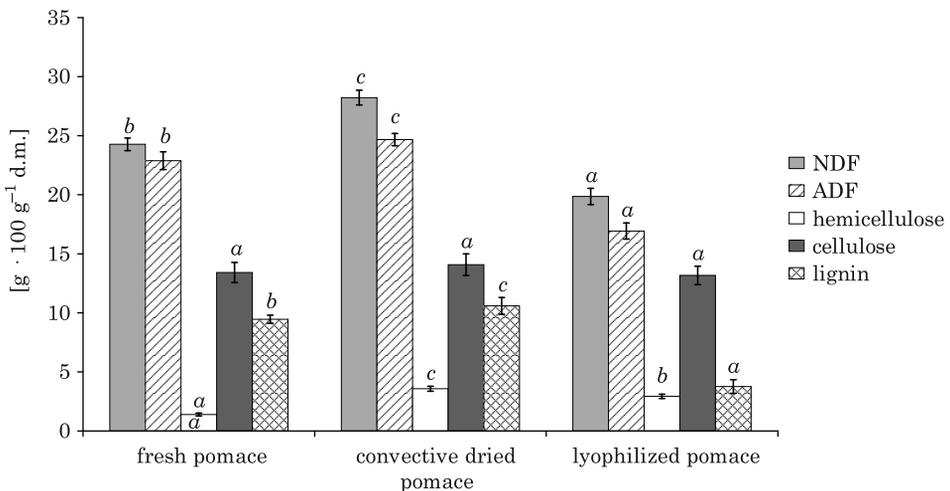


Fig. 1. Content of different fractions of fibre in carrot pomace

Values are expressed as mean  $\pm$  standard deviation,  $n = 3$ ;  $a-c$  - bars with different letters for different fractions differ significantly ( $P < 0.05$ )

NDF - Neutral Detergent Fibre, ADF - Acid Detergent Fibre

of polysaccharide complexes with other compounds, e.g. phenolics. This was confirmed, for example, in a study by KOMOLKA et al. (2012), who found a significant 58–59% increase in NDF content after boiling Brassicaceae vegetables in water. The reported increase in ADF content was even greater (78–112%). Studies by KOMOLKA et al. (2012) revealed that changes in the components of fibre fraction depend on the heat treatment method, and also vegetable species.

The lower levels of individual fibre fractions in CPL found in our study may result from the degradation of fibre structure, promoted by rapid freezing in liquid nitrogen (at -195.8°C), followed by exposure to vacuum. A significant effect of the drying method on changes in the content of fibre in carrot pomace was also demonstrated by ALAM et al. (2013), who found the highest fibre content in samples after convective drying (at 65°C) compared to sun drying and solar drying.

## Conclusions

Carrot pomace obtained as a by-product during juice extraction is a rich source of important nutrients, such as carotenoids, polyphenols and fibre. It also has good antioxidant properties. Drying used for the preservation of fresh (moist) pomace caused qualitative and quantitative changes in the content of the analysed components, and the differences were statistically significant in most cases ( $P < 0.05$ ). The study demonstrated significant changes determined by the drying method. A relatively low stability was found for carotenoids, especially  $\beta$ -carotene, and the greater loss in the content of this substance was caused by lyophilisation. It is worth noting that the used analytical method indicated a greater content of total phenolics and higher capacity to scavenge DPPH radicals for dry carrot pomace compared to fresh pomace. Particular differences were found for lyophilized pomace. Potential reasons explaining the significant differences are presented in the discussion. Interestingly, the drying process, particularly convective, had a positive effect on the increased content of neutral detergent fibre, including hemicellulose fraction. However, the analysis of results did not clearly identify the optimal drying method. It can be assumed that the choice of method should depend on the type of final product in which carrot pomace will be used.

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## **EFFECT OF STEVIA ADDITION ON SELECTED PROPERTIES OF YOGHURT DURING REFRIGERATED STORAGE**

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**Key words:** stevia, steviol glycoside, yoghurt, texture.

### **Abstract**

The aim of this study was to investigate the effect of replacing sucrose 25%, 50%, 75% and 100% of the addition of stevia on yoghurt properties, during refrigerated storage. Reference sample was yoghurt sweetened with 8% added sucrose, the addition of stevia was 0,04%. The yoghurt was analysed for pH, titratable acidity, dry matter, and texture. It was also sensory assessed. With the increase in the concentration of stevia sweetener, the mixture pH decreased, and the increased acidity of the yoghurt. Despite the lower dry matter content of yoghurt with stevia exhibited higher curd hardness values, but the cohesiveness was slightly lower. Springiness and chewiness in yoghurt sweetened with stevia decreased remarkably after 7 days of storage. Yoghurt with stevia were less intense sweet flavor and considered to be more pronounced sweetness and persistent. The best was the yoghurt sweetened with sucrose, and the worst yoghurt with stevia. Mixtures of these sweeteners showed a higher degree of acceptance.

## WPLYW ZASTOSOWANIA STEWII NA WYBRANE CECHY JOGURTU PRZECHOWYWANEGO CHŁODNICZO

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Słowa kluczowe: stewia, glikozydy stewiolowe, jogurt, tekstura.

### Abstrakt

Celem pracy było zbadanie wpływu zastąpienia sacharozy 25%, 50%, 75% i 100% dodatkiem stewii na właściwości jogurtów w czasie przechowywania chłodniczego. Próbę odniesienia stanowił jogurt słodzony 8% dodatkiem sacharozy, dodatek stewii wynosił 0,04%. W jogurtach oceniono pH, kwasowość, suchą masę, parametry tekstury oraz poddano je ocenie sensorycznej. Wraz ze wzrostem stężenia stewii w mieszance słodzącej malało pH, a wzrastała kwasowość jogurtów. Pomimo niższej zawartości suchej masy jogurty ze stewią wykazywały wyższe wartości twardości skrzepu, jednak kohezynność była nieznacznie niższa. Sprężystość i żujność w jogurtach słodzonych stewią spadła znacznie po 7. dniu przechowywania. Jogurty ze stewią wykazywały mniej intensywną słodycz, jednak bardziej wyraźną i uporzczą. Najlepszy okazał się jogurt słodzony sacharozą, a najgorszy jogurt ze stewią. Mieszanki tych środków słodzących wykazywały lepszy stopień akceptacji.

## Introduction

For centuries, people have used various means of suitable sweet taste. A honey has been used for this purpose for a long time. Which over time has been replaced with sucrose produced industrially, and in recent years the glucose-fructose syrup (CICHOSZ et al. 2011, SZAJER 2011). However, it began to appear that eating sugar causes atherosclerosis and influences the increasing rates of type 2 diabetes. In addition, excessive intake of sugar and lack of physical activity triggers medical conditions such as obesity and tooth decay (CICHOSZ et al. 2011). A clear trend of replacing sugar with low-calorie sweeteners is being observed. These are synthetic ingredients or received by technological modification of saccharides characterised by high sweetened activity and low energy. The so-called sweeteners are increasingly at the no-or low-calorie alternative to natural sugars. Products based on them are a response to market demand for sweet, but sugar-free products (SIKORA 2012). Although sweeteners before being released for use in foods were subjected to a thorough evaluation, it still arouses a lot of controversy among scientists and consumers, in relation to information about their possible carcinogenic effects (WASZKIEWICZ-ROBAK et al. 2007).

In the face of the growing demand for low-energy products, food manufacturers are looking for natural substances that could reflect the sweet taste of sugar without causing adverse effects on health. A plant – *Stevia rebaudiana* Bertoni, commonly called stevia, can be a new solution. This plant belongs to the family *Asteraceae* used for centuries by South Americans as a sweetener, as well as the drug substance. Stevia owes its sweet taste to steviol glycosides contained in its leaves. Over eight glycosides had been identified, two of which (stevioside and rebaudioside A) are the most important. Stevioside is present in the leaves in the largest amounts (4–13% DM). It is 150–300 times sweeter than sucrose. However, there is more bitter and licorice flavor than rebaudioside A. It is the less well soluble in water as well. Rebaudioside A is present in smaller amounts than stevioside (2–4% DM). It is the sweetest of steviol glycosides (200–400 times sweeter than sucrose) and has the best taste. It dissolves in water better than stevioside and it is resistant to various pH environments (CARAKOSTAS et al. 2008, LEMUS-MONDACA et al. 2012).

In December 2008, the U.S. Food and Drug Administration (FDA) stated that steviol glycosides can be considered GRAS (Generally Recognized As Safe). The introduction of stevia to the European market took much longer because, according to Regulation (EC) No 258/97 it required an opinion on the use of this plant as a new food additive. The European Food Safety Authority (EFSA) in the process of stevia toxicological assessment stated, that the glycosides found in the stevia are not genotoxic or carcinogenic, and that the plant is safe and can be used as an additive in food and beverage (KUŹMA et al. 2012). The acceptable daily intake (ADI) was set at 4 mg kg<sup>-1</sup> body weight/day (JUSZCZAK 2012). In Europe, steviol glycosides have been approved for use by Regulation No 1131/2011 of 11 November 2011, the new food additive has been marked with number E 960. Stevia is currently used in many countries, including Japan, Brazil, Australia, Switzerland, the United States, Mexico and the European Union. It can be used in the production of reduced-calorie meals, in addition to drinks, sweet dishes and desserts. The high sweetening power steviol glycosides in comparison to sucrose makes it to achieve the desired sweetness with using small amount of this additive.

Among the arguments in favor of allowing the use of steviol glycosides most important are their natural origin, non-toxic, high solubility, stable in aqueous solution over a wide range of pH values and temperatures, non-fermentative, safety for diabetics and people with phenylketonuria (JUSZCZAK 2012). Having the potential of the sweetener it can be expected that the market will demand more and more products with stevia as ingredient. Also in the dairy industry it had been allowed to be used in flavored fermented milk, which can improve the image of health-promoting these products.

The aim of this study was to investigate the effect of substitution of sucrose with different ratios of steviol glycosides, on the physicochemical, textural and sensory properties of set style yoghurt, during 21 days of refrigerated storage.

## Material and Methods

The study was based on white sugar (sucrose from sugar beets) and Truvia<sup>®</sup>, stevia produced by Cargill (min. 95% Rebaudioside A). Yoghurt produced by thermostatic method from pasteurized milk containing 2% fat, concentrated skim milk powder, up to about 11.5% non-fat dry matter. The prepared milk was divided into five parts and added to each the sweeteners, according to Table 1. Reference sample was the yoghurt sweetened with sucrose. In subsequent trials 25%, 50%, 75% and 100% sucrose was replaced stevia, wherein the total addition of sucrose was 8%, and stevia due to the high sweetness of 0.04%. After addition of sweeteners, milk was repasteurised in 85°C for 10 minutes and then cooled down to 45°C. Yoghurt cultures FD-DVS YC-X16-YoFlex<sup>®</sup> Chr. Hansen (*Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*) were incubated in 2% fat UHT milk at 43°C, 4,5h. Each sample was inoculated with 5% of starter culture, dispensed into containers with a capacity of 100 ml and incubated at 43°C to about pH 4.7, and then cooled to 5°C. Assessment of the effect of the addition of sweeteners to the parameters of the yoghurt conducted immediately after the fermentation, designated as the “0 days storage”, and at 3, 7, 14 and 21 days of refrigerated storage. The yoghurts were determined titratable acidity in °SH, pH and conductivity measured by microcomputer pH/conductivity meter Elmetron CPC-411, equipped with a combination electrode type OSH 12-00 and 60 EC conductivity sensor. The dry matter was determined by drying yoghurt at 130°C. Analysis was performed in duplicate, the mixed samples, at a temperature of 20°C +/- 1°C. The texture was determined with the analyser Brookfield CT3 equipped with Brookfield Texture Pro CT software. For determination there was selected the TPA test with the following settings: sample – cylinder 66.00 mm x 33.86 mm, force 0.1 N, head speed 1 mm/s, table TA-BT-KIT, probe TA3/100. The test was performed on intact curd yoghurt, immediately after taking it from the cold, in triplicate. Texture qualities like hardness [N], chewiness [mJ], springiness [mm] and cohesiveness were assessed. The results are presented as arithmetic mean and standard deviation.

Sensory analysis was performed by a team of 21 people trained in the evaluation profile yoghurt, at 3, 7, 14 and 21 days of storage. Evaluators

Addition of the sweeteners during yoghurt manufacture

Table 1

Sweetener content [%]	Addition of sweetener [%]
Sucrose 100%	Sucrose 8%
Sucrose 75% + stevia 25%	Sucrose 6% + stevia 0.01%
Sucrose 50% + stevia 50%	Sucrose 4% + stevia 0.02%
Sucrose 25% + stevia 75%	Sucrose 2% + stevia 0.03%
Stevia 100%	Stevia 0.04%

ranked coded samples of yoghurt from the best to the worst (JĘDRYKA 2001). The sensory profile of yoghurt during storage was compared. The study consisted of nine descriptors selected in accordance to *Sensory analysis...* PN-ISO11035:1999, consistency, color, aroma (sweet, sour, stranger), taste (sweet, sour, cream, off-flavor). Evaluators made their assessment on nine-point scale, with markings edge: 1 point “very characteristic” (for color and consistency) or “undetectable” (in the case of evaluation of taste and aroma) and 9 points: accordingly “very characteristic” or “very intense”.

## Results and Discussion

The basis for the production of yoghurt fermentation process is taking place with the participation of lactic acid bacteria, where the metabolic activity largely contributes to the development of organoleptic and rheological characteristics. Cooling the yoghurt after production limits such activity, but does not inhibit it entirely, because during storage of yoghurt refrigerant still occur changes in its composition and structure. Yoghurts with added stevia were characterised by slightly lower pH values (Figure 1) and higher titratable acidity (Figure 2) compared to yoghurt sweetened with sucrose. With increasing concentration of stevia the pH values of yoghurts decreased and titratable acidity increased more quickly during storage. Yoghurt sweetened with stevia also showed higher electrical conductivity. Therefore, the last day of storage, yoghurt with stevia had the lowest pH (4.2) and the highest titratable acidity (54.2°SH). GUGGISBERG et al. (2011) were examining yoghurt with sucrose and stevia in the 7<sup>th</sup> day of storage, and tests showed a slightly lower pH and higher content of organic acids in yoghurt sweetened with sucrose, however, the content of aromatic compounds formed during fermentation was at a similar level and in the case of diacetyl even higher in stevia sweetened yogurt. PRAKASH et al. (2008) reported that rebaudioside A is stable during the manufacturing process and during storage of yoghurt containing live micro-

flora. So it is not fermented by lactic acid bacteria. Similarly, in the human digestive tract, bacteria from the lower gastrointestinal tract convert it to steviol, which is then excreted (CARAKOSTAS et al. 2008). Studies have reported on the impact of anti-bacterial extracts of stevia in relation to pathogens of the digestive system, as well as its potential antioxidant properties (LEMUS-MONDACA et al. 2012). In light of these reports, it can be hypothesized that steviol glycosides stimulate the yoghurt bacteria, but it has to be examined further.

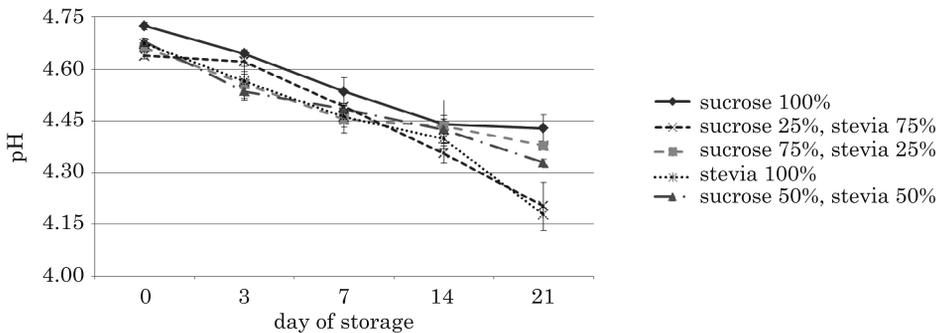


Fig. 1. pH of yoghurts with sweeteners during storage ( $n = 2$ ,  $\bar{x} \pm SD$ )

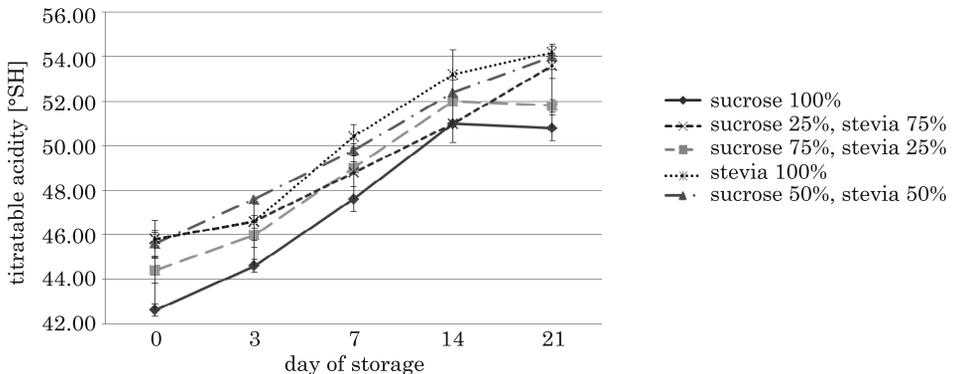


Fig. 2. The titratable acidity ( $^{\circ}\text{SH}$ ) of yoghurts with sweeteners during storage ( $n = 2$ ,  $\bar{x} \pm SD$ )

For a consumer products, texture is an important parameter of the quality, it, makes an impact on the pleasure of eating. After fermentation of yoghurt texture was not formed yet, because the products were not fully mature and chilled. They reached their final form after 72 hours. The research of ŽBIKOWSKI (2012) showed that changes in texture of yoghurt occur most

intensively in the first week of storage. This causes an increase in viscosity or thickening of the curd. These changes may result from post acidification of yoghurt occurring during storage and microbial activity leading to the strengthening of the protein matrix by increasing of lactic acid and bacterial exopolysaccharides production. In this study, yoghurt sweetened with stevia, despite a significantly lower dry matter content (Figure 3) showed higher curd hardness values (3.23 N – 14 days ) than yoghurt sweetened with sucrose (2.97 N – 14 days ) during the entire storage period (Figure 4). However chewiness (Figure 5) and springiness (Figure 6) in yoghurt sweetened with stevia alone decreased significantly after 7 days of storage. Another texture parameter of product containing stevia, that showed lower values than the reference sample, was cohesiveness (Figure 7). GUGGISBERG et al. (2011) believe that the addition of stevia is too small to affect the texture. In the studies showed that yoghurts with sucrose at 14th day of storage had higher hardness (2.79 N) than yoghurts with stevia (2.41 N) but only in the case of higher fat content (3.5%), while non-fat yoghurt hardness was at a similar level.

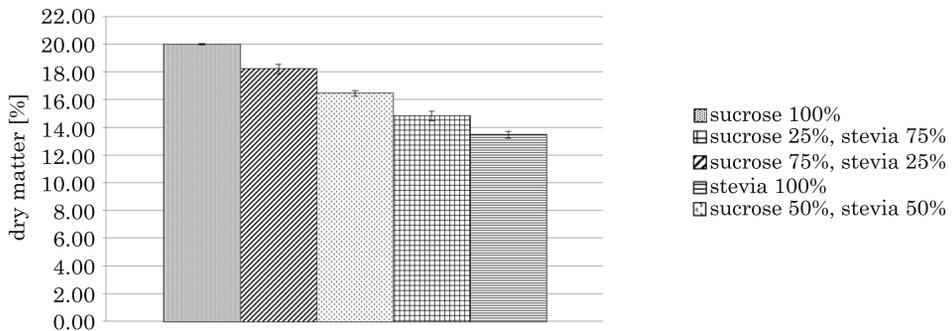


Fig. 3. Average dry matter [%] of yoghurts with sweeteners ( $n = 10$ ,  $\bar{x} \pm SD$ )

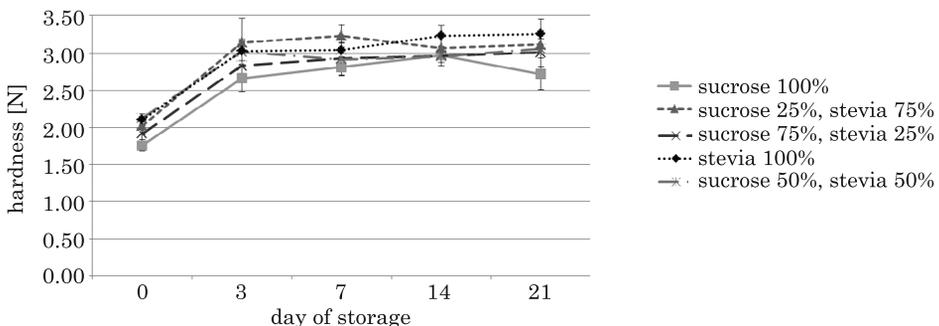


Fig. 4. Hardness [N] – the force necessary to compress the sample in the first compression cycle, in yoghurts with sweetening agents

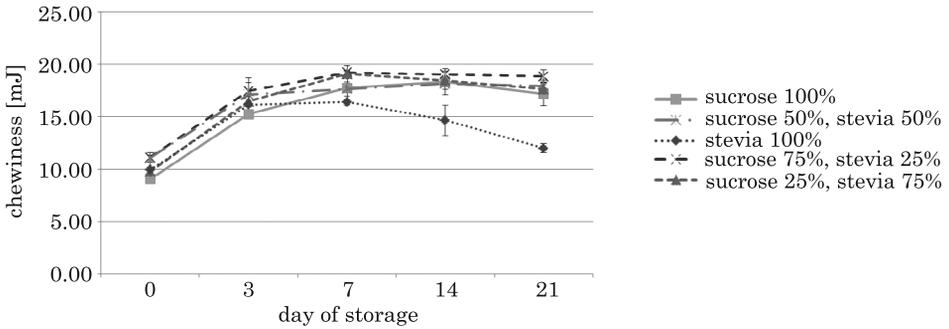


Fig. 5. Chewiness [mJ] energy required to chew yoghurts with sweetening agents during storage ( $n = 3, \bar{x} \pm SD$ )

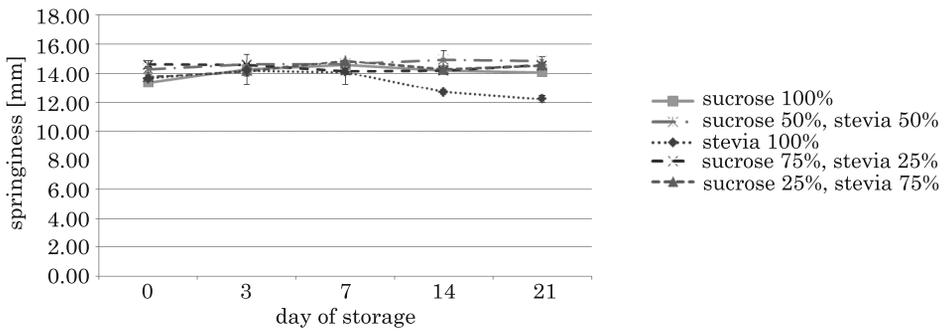


Fig. 6. Springiness [mm] expressed as a difference between the height of sample before and after compression cycle in yoghurts with sweetening agents during storage, ( $n = 3, \bar{x} \pm SD$ )

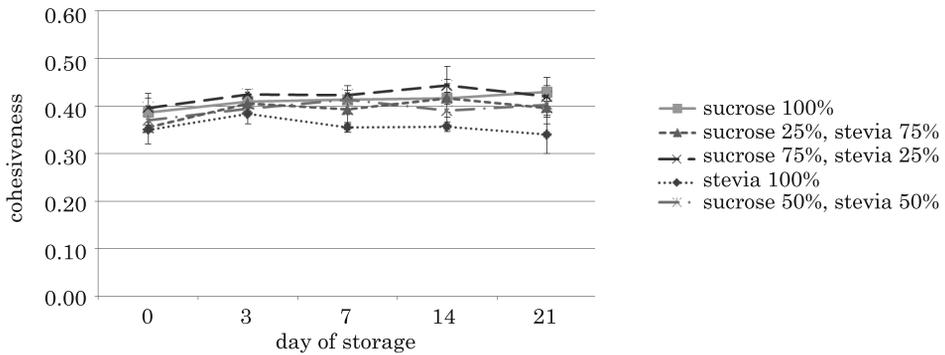


Fig. 7. Cohesiveness – The strength of internal bonds making up the body of the yoghurts with sweetening agents during storage ( $n = 3, \bar{x} \pm SD$ )

Addition of stevia did not cause changes in the color and noticeable changes in the consistency of yogurt, which has been assessed in all trials as a very characteristic. Stevia did not affect the smell of the products, which were characterized by natural, sweet and sour flavor. Truvia® stevia Cargill is a white or off-white powder, very finely ground, odorless, with an intensely sweet taste. During storage of yoghurt, both sweet and acidic aroma were enhanced (Figures 8–10). The evaluators concluded that stevia sweetened products were characterized by a sweet aroma weaker when compared with the reference (Figure 11). Decreasing pH and progressive acidity of yoghurt (called post acidification), observed during storage can lead to modified physical and chemical interactions, which can modify fragrances as a result and also the reduction of release of the aroma and flavor ingredients in yoghurt. Changes in odor intensity of refrigerated yoghurts are also explained by hypothesis of transformation of some of aroma components by bacterial enzyme system (ŻBIKOWSKI 2012). The sweet taste of yogurt was the strongest during the entire period of storage and a sour taste in all samples was average and not very noticeable (Figure 11). The evaluators found that yoghurt sweetened with stevia do not differ in the intensity of sweetness from the other, but the sweet taste of stevia was felt to be more persistent, and considered as less natural. According to ŻBIKOWSKI (2012) sensory profile of stored yoghurt does not depend directly on changes in pH and noticeable acidity, but the balance

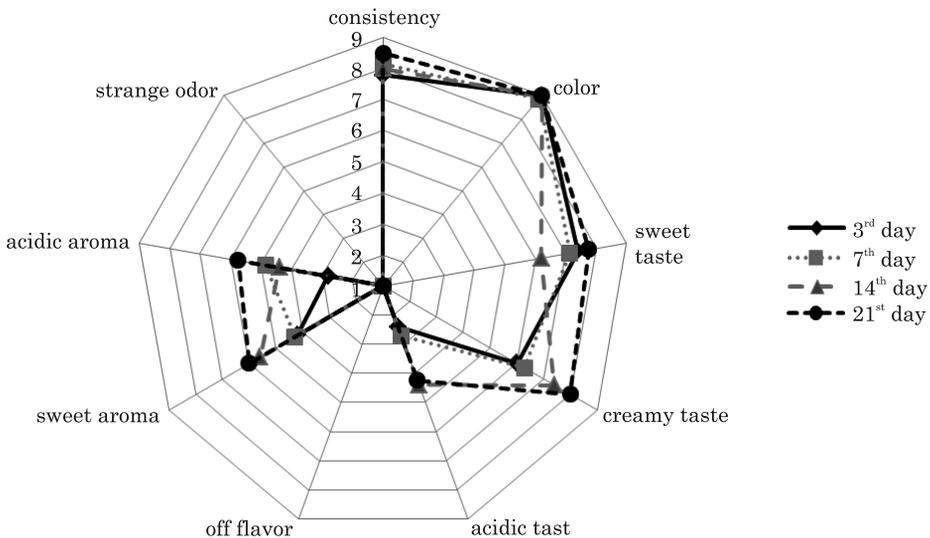


Fig. 8. Sensory evaluation of yoghurt with sucrose during storage in nine-point scale, with markings edge: 1 point “very characteristic” (for color and consistency) or “undetectable” (for taste and aroma) and 9 points: respectively “very characteristic” or “very intense”

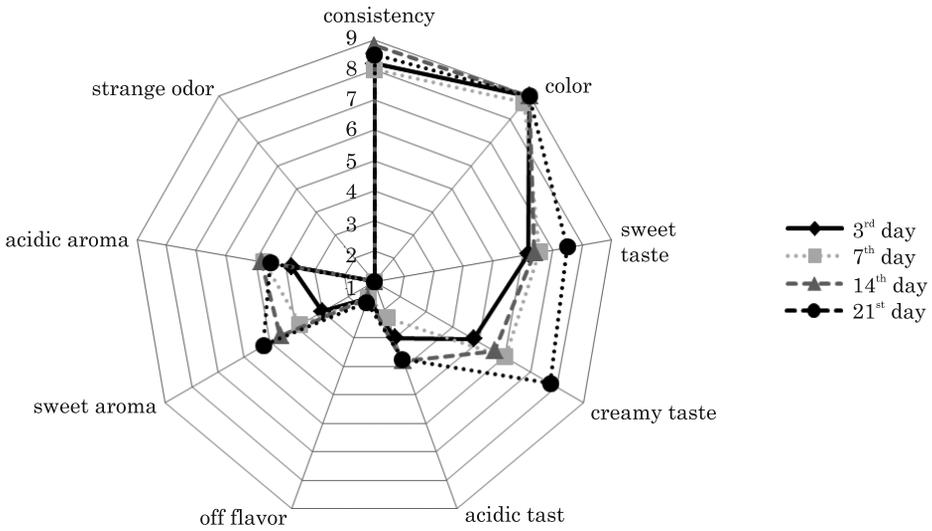


Fig. 9. Sensory evaluation of yoghurt with stevia during storage in nine-point scale, with markings edge: 1 point “very characteristic” (for color and consistency) or “undetectable” (for taste and aroma) and 9 points: respectively “very characteristic” or “very intense”

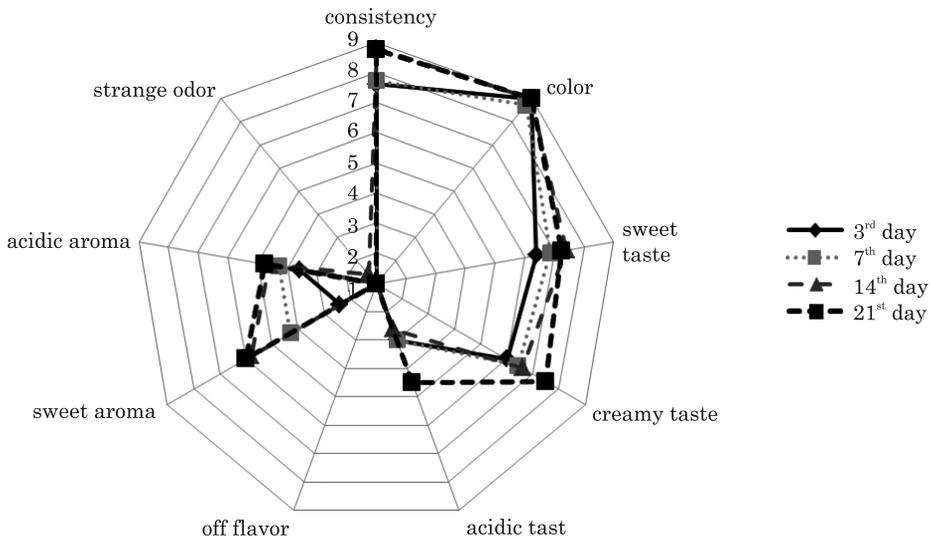


Fig. 10. Sensory evaluation of yoghurt with 50% stevia addition, during storage in nine-point scale, with markings edge: 1 point “very characteristic” (for color and consistency) or “undetectable” (for taste and aroma) and 9 points: respectively “very characteristic” or “very intense”

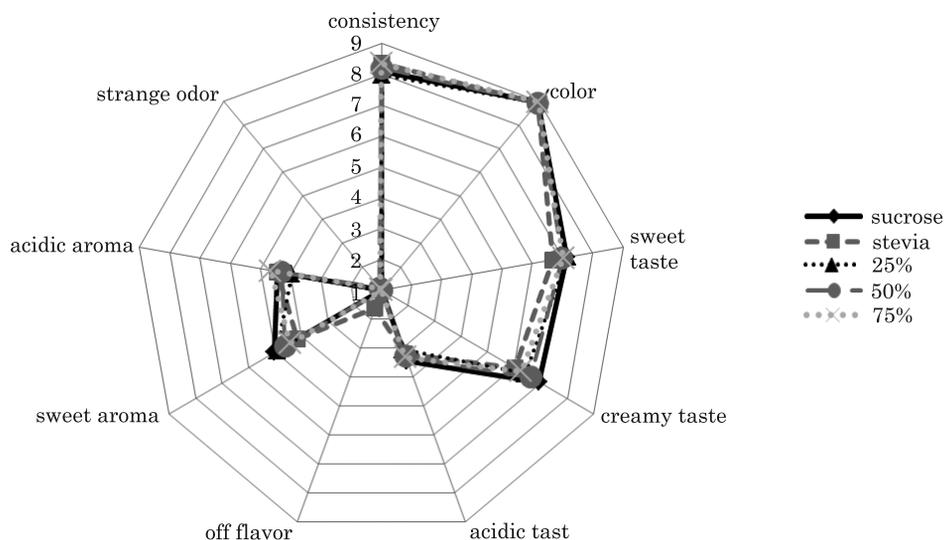


Fig. 11. Average assessment in sensory evaluation of yoghurts with sweeteners in nine-point scale, with markings edge: 1 point “very characteristic” (for color and consistency) or “undetectable” (for taste and aroma) and 9 points: respectively “very characteristic” or “very intense”

between sweet and sour flavors. There were no off-flavor or it was not defined in any of yoghurts during the entire period of storage (Figure 11). The other observation was made by GUGGISBERG et al. (2011) using the same amount of additives sweeteners, observed that yoghurt sweetened with sucrose have been recognized as sweeter than sweetened with stevia. Author also stated that bitterness was significantly higher in the sample containing stevia alone which was also the sample showing a significantly higher off-flavour described by the panel as “artificial”, “cardboard” and “metallic”. In this test, however, stevia contained purified stevioside, not a rebaudioside A, and the sweetening power of the first glycoside is lower and it taste more bitter and licorice (LEMUS-MONDACA et al. 2012).

The taste of control sample with sucrose was the best and 53% of the evaluators gave it the first place. The worst was the yoghurt contains only stevia, 38% put it on the last place. But even a small addition of sugar to the sweetening mixture caused the increased acceptability of yoghurt. Most second preferences were yoghurt with 75% addition of stevia (33%).

## Conclusion

1. The acidity of the products with the addition of stevia were higher than the reference yoghurt.

2. Yoghurts with stevia had lower dry matter content compared to yoghurt with sucrose, which has not affected the consistency.

3. Hardness of yoghurts with stevia added was higher than yoghurt base in the entire period of storage. Other texture parameters, such as cohesiveness, springiness and chewiness of curd decreased after 7 days of storage in yoghurt containing stevia.

4. Yoghurt sweetened with stevia did not differ in the intensity of sweetness from the other, but the sweet taste of stevia was felt more by what it considered to be less natural than the taste of sucrose.

5. Yoghurt with stevia were considered worse than control yoghurt, but yoghurt contains 75% addition of stevia in the mixture, was usually selected as a second choice.

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## **POST-PURCHASE DISSONANCE – A DIFFICULT AREA OF RESEARCH IN POLAND**

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**Key words:** consumer behavior, post-purchase dissonance, marketing research.

### **A b s t r a c t**

The qualitative and quantitative increase in market offerings has led to an increased significance of purchasers in the market and has changed their expectations over a short period of time. Consumer's role has changed from a suppliant to a strong entity with high demands addressed at producers and service providers. The strong position of consumers has spawned an increased research interest in consumer market behavior, especially after purchasing. The decision about purchasing a specific product or service is not the last stage in the consumer decision making process on the market. Many consumer behavior models distinguish two post-purchase types of reaction in consumers – satisfaction (when the expectations have been met), or post-purchase dissonance (when the expectations have not been met and the consumer has doubts concerning the purchase). The aim of the present paper is to make an attempt at specifying the reasons why the issue of post-purchase dissonance has generated so little interest among Polish researchers.

## **DYSONANS POZAKUPOWY – TRUDNY OBSZAR BADAŃ W POLSCE**

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**Słowa kluczowe:** zachowania konsumenckie, dysonans pozakupowy, badania marketingowe.

### **A b s t r a k t**

Wzrastająca ilościowo i jakościowo oferta rynkowa produktów i usług w szybkim czasie doprowadziła do wzrostu znaczenia nabywcy na rynku i zmieniła jego oczekiwania. Konsument z roli petenta stał się silnym podmiotem o wysokich wymaganiach kierowanych do producentów i usługodawców. Silna pozycja konsumenta spowodowała większe zainteresowanie obszarem badań

jego postępowania na rynku, szczególnie po dokonaniu zakupu. Decyzja dotycząca nabycia określonego produktu lub usługi nie jest ostatnim etapem w procesie decyzyjnym konsumenta na rynku. W wielu modelach zachowań konsumenckich wyodrębnia się bowiem dwa rodzaje odczuć pozakupowych konsumenta – zadowolenie (gdy oczekiwania zostały spełnione) lub dysonans pozakupowy (wątpliwości co do zakupu, sytuacja gdy oczekiwania konsumenta nie zostały spełnione). Celem artykułu jest próba charakterystyki trudności badawczych polskich naukowców w procesie pozyskiwania wiedzy o dysonansie pozakupowym odczuwanym przez konsumentów.

## Introduction

The establishment of market economy in Poland led to significant changes in many social and economic areas. The economic transformation process initiated in mid-1989 opened the door to the change of economy – marketization, namely enabling the market to function as the regulating mechanism. The activities undertaken (among others, privatization, economic entities' self-reliance, free price mechanism, creating competitiveness fostering conditions) induced creating consumer freedom to purchase the preferred goods and services. The sufficient quantity and variability of products on the market enabled Polish consumers to cater for their own needs in a more broad and attractive way (GRZEGA 2008). The qualitative and quantitative increase in market offerings has led to an increased significance of purchasers in the market and has changed their expectations. A consumer, whose role used to be rather suppliant, became a strong entity with high demands addressed at producers and service providers.

The decision about purchasing a specific product or service is not the last stage in the consumer decision making process in the market. Many consumer behavior models distinguish two post-purchase reaction types of consumers – satisfaction (when the expectations have been met), or post-purchase dissonance/ dissatisfaction/ discontent (when the expectations have not been met and the consumer has doubts concerning the purchase). According to some sources, the post-purchase dissonance is a phase that precedes the feeling of dissatisfaction with the product or service.

In comparison to such issues as customer satisfaction and loyalty, the post-purchase dissonance is rarely discussed in the literature. Polish sources are especially scarce here, and if the problem is addressed at all, it usually concentrates on analyzing the psychological aspects of cognitive dissonance (on the basis of the feelings experienced by people when making various decisions). In the world's literature this topic is gaining popularity due to its huge impact on the companies (the market competitiveness aspect) and lack of a unified approach to the issue. The aim of the article is to make an attempt at specifying the reasons why the post-purchase dissonance has spawned so little interest

among Polish researchers and the managers of the companies operating on the Polish market.

## **Materials and Methods**

The article presents an overview; the author used EMERALD and JSTOR databases of economics articles as well as domestic marketing journals (e.g. *Marketing i Rynek*) to conduct a review of literature from recent years in order to describe the state of knowledge on consumer behavior among those experiencing post-purchase dissonance. The article therefore presents the current state of knowledge on the process of creation and reduction of post-purchase dissonance in consumer behavior.

The dynamic changes in the market environment made the consumers' purchasing process highly emotional. The high emotionality of and thus difficulty in the purchasing process are determined by such various factors as the increase in market offerings (which makes the consumers feel insecure and the decisions they take more impulsive), multivariate decision making (e.g., household decision making, seeking information and purchasing in various retail outlets), multitude of decision making criteria (often conflicting/mutually exclusive) (GUTKOWSKA 2007), diverse sources of information (formal and informal), the speed of information flow (negative or positive feedback about the product/service), increased consumer demands, and increased purchase risk. All these features of the decision making process make the consumers emotionally distressed. Uncertainty and risk become natural features of consumer purchasing processes. The risk range concerning customer product purchase nowadays is markedly larger than just the decision itself, and is connected with the danger of making a wrong decision.

The importance of post-purchase dissonance is also growing in the face of the development of large-area facilities, which encourage emotional purchasing by customers. Fast, impulse purchases can lead to dissatisfaction with the decisions made. With the growing competitiveness, consumer dissatisfaction leads to economic losses of retail outlets (a dissatisfied customer changes the purchasing place), as well as the producers (negative post-purchase opinions). Research on customer reaction to post-purchase dissatisfaction also indicates that only 4% of the dissatisfied customers file complaints (GRIFFIN 2007), and 91% among the ones who do not file any complaints do not return to purchasing either (OTTO 1998).

Dissonance is a subjective feeling of tension and anxiety which a customer may experience after making a purchase; the anxiety caused by uncertainty as to the appropriateness of the purchasing decision (SMYCZEK 2002). The concept

of *post-purchase dissonance* derives from the theory of the so called *cognitive dissonance*, formulated by Leon Festinger (FESTINGER 2007). He made an assumption that people have a need for an inner harmony (consonance) between their beliefs, attitudes, and values. When there is a discord between at least two of these elements, an unpleasant inner tension occurs (a dissonance). Because a dissonance acts as a punishment, it is natural to strive for the elimination or reduction of the unpleasant state and for regaining harmony (balance). For this reason, people try to avoid situations which could cause a dissonance (HAMER 2005).

The theory of cognitive dissonance was soon applied in explaining some aspects of consumer behavior, especially the post-purchase behavior. According to this concept the majority of purchases (with the obvious exception of routine purchasing) cause a certain degree of anxiety concerning the decision. This anxiety and doubts (post-purchase dissonance) occur in a situation when a customer made a choice from among products with both positive and negative features. In such a situation the chosen item has some drawbacks, and at the same time the rejected alternative has some advantages. The negative features of the purchased product and the positive features of the one not purchased lead in consequence to the post-purchase dissonance. It is worth mentioning that the probability of the occurrence of post-purchase doubts, as well as their intensity depend on a variety of factors (Table 1).

Table 1  
Factors increasing the probability of the rise of post-purchase dissonance

- <b>the importance of the decision to the consumer</b> – the more important the decision, the greater the chance that dissonance will arise
- <b>the degree of irreversibility of the decision</b> – the easier it is to change the decision, the smaller the risk of the appearance of dissonance
- <b>the complexity of the decision</b> – the more complex the decision (i.e. the more difficult it is to make a choice), the greater the dissonance. Complexity – a large number of similar products generates many comparative features
- <b>the number of positive features noticed in the rejected products or the number of negative features in the purchased product</b> – the increase in this number corresponds to the increase in the chance that the dissonance will arise
- <b>the effort and time needed to purchase the product</b> – as the effort increases, the dissonance increases as well
- <b>the type of product</b> – complex – the difficulty in noticing all attributes of the product – increases the chance that the feeling of dissonance will subsequently arise
- <b>the price of product</b> – the higher the price, the more frequently the dissonance will arise
- <b>personality of the buyer</b> – the correlation between self-confidence and the ability to feel dissonance
- <b>social reception of the purchase</b> – the greater the social visibility of the product, the bigger the chance that anxiety about the appropriateness of the purchase will appear

Source: SMYCZEK (2002) as well as RUDNICKI (2004)

The issue of post-purchase dissonance is extremely significant and relevant from both theoretical and practical point of view. In the existing literature the question of post-purchase dissonance as seen from the perspective of economics has received a very general treatment, confined essentially to the explanation of the basic concepts – here among others (RUDNICKI 2004, DULINIEC 1986, GAJEWSKI 1994, FALKOWSKI and TYSZKA 2011, SMYCZEK 2002). It needs to be emphasized, however, that none of the mentioned sources presents any results of the studies conducted on the Polish market. The research on post-purchase dissonance has been conducted in Poland among others by SMYCZEK (2008) ODERKIEWICZ (2012). Theoretical considerations in the marketing area are presented in SMYCZEK (2002) and ANKIEL-HOMA and OLEJNICZAK (2006).

In the foreign literature the first attempt at the transfer of the problem of dissonance from the field of psychology to that of economics was made in the 70s. (ANDERSON 1973, COHEN and GOLDBERG 1970). Subsequently, occasional references to the consumers' decision making process have appeared (GILAD et al. 1987, HOSSEINI 1997, SMITH 2009, CAO and JUST 2010, COOPER and FAZIO 1984). However, what has been much more often analysed are instances of consumer dissatisfaction when the satisfaction level in particular branches was researched (COGHLAN 2012, NAHID and MOJGAN 2012, WU 2013, JUNG and YOON 2013). The interest in the issue of consumer dissatisfaction increased considerably after the year 2000, when, by applying innovative approaches, newly formulated hypotheses were verified in the analyzed area (SWEENEY et al. 2000, LINDSEY-MULLIKIN 2003, SPANGENBERG et al. 2003, O'NEILL and PALMER 2004, WICHARDT 2012, GBADAMOSI 2009).

## **Discussion**

Given the increase in the significance of the study of dissonance on the one hand, and little explanation of the problem offered in the context of economics on the other, it seems justified to pose the question why this topic has been largely neglected in the Polish economic literature. The relatively limited treatment of the question of post-purchase dissonance in the academic and commercial research is an effect of, among others, the difficulty in conducting research in the discussed field, and, apparently, the underestimation of the problem by managers of the enterprises operating on the Polish market.

The major reason for the limited interest in the research on post-purchase dissonance is a broadly-understood difficulty in the preparation and implementation of the research. The preparation of the study entails determining its scope and when it comes to dissonance in particular, many open questions

arise, including such as whether to investigate the consumers' formed lack of satisfaction/dissatisfaction, or only the doubts related to the purchased product or service. Another element inducing a dilemma is the choice of adequate methodology (according to the dissonance theory, it is caused by the doubts arising when the expectations are not met) – in this case, the study should consist in a number of successive stages: determining consumer expectations about the product/service, defining the level of satisfaction with the purchase, identifying the respondents reporting post-purchase doubts, as well as a detailed analysis of the selected group of subjects.

This entails the necessity of triangulation on the part of the researcher, i.e. the application of various testing methods and various types of analyses of the results (quantitative and qualitative). At least personal interview and in-depth individual interview, or group interview. Other methods described in the foreign literature include observation, experiment and the use of a panel of respondents. The complex study of the post-purchase dissonance would require applying the *mixed method*, which involves conducting the tests, analysis, the integration of the results, their interpretation and drawing conclusions, using both quantitative and qualitative methodology (MAZUREK-ŁOPACIŃSKA 2011).

Aside from the mentioned difficulty in capturing the study group, an essential element subject to academic discussion may be the choice of the criterion for measuring dissatisfaction, be it objective (e.g. the number of complaints, statements, opinions on Internet forums) or subjective (customer impressions/opinions). Another reason for limited interest into the question of post-purchase dissonance seems to be its underestimation among the company managers operating on the Polish market.

The development of individual markets, and relatively lower intensity of competition when compared to foreign markets contribute to the fact that company managers are more interested in offering services to the existing customers, increasing their shopping cart value (in terms of quantity and quality) and building their loyalty, rather than seeking opportunities to regain the dissatisfied customers. Naturally, the criticism directed at the Polish managers may be rejected by them on the grounds of the number of studies on customer satisfaction level, however, it must be emphasized that these customer satisfaction studies serve only to identify the scale of the problem (dissatisfaction), but entirely disregard the causes and behaviors of dissatisfied customers.

## Conclusions

In the era of dynamic changes of macro-environmental factors (particularly in the economic and socio-demographic spheres) as well as of the boost in the intensity of market competition, analyzed in terms of both subjective and objective system, companies need to undertake intensified actions aimed at guaranteeing the consumer satisfaction with the purchased products and services. The problem of identification of psychological, economic and socio-cultural determinants of post-purchase dissonance is significant from the perspective of both the supply (economic entities) and the demand (individual consumers) of the market. Aside from identifying the determinants of the post-purchase dissonance, it is of particular significance from the perspective of the suppliers of consumer products, to determine the possibilities and potential instruments to alleviate the post-purchase dissonance. This is an important indicator of the need to increase the significance of the issue of post-purchase dissonance in the economic studies.

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**THERMAL-OXYGEN CONDITIONS IN LAKES ROŚ  
AND ROSPUDA FILIPOWSKA  
(NORTH-EASTERN POLAND) IN THE SUMMER  
HALF-YEAR 2005–2014**

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**Key words:** thermal profile, oxygen, lakes, wind, Mazurian Lakeland.

**A b s t r a c t**

Thermal and oxygen conditions of lake ecosystems are important for their functioning. The availability of detailed information is of particular importance in the context of the observed environmental changes. The paper presents thermal-oxygen conditions in Lakes Roś and Rospuda Filipowska in north-eastern Poland in the years 2005–2014 for the summer half-year (May – October). Both of the lakes are classified as stratified lakes. The mean water temperature throughout the water column in Lake Roś was 11.3°C, and in Lake Rospuda 9.4°C. The temperature distribution was evidently affected by wind. In the case of Lake Roś with better conditions for water mixing due to the orientation of the lake's axis in relation to the direction of dominant winds, the epilimnion covered approximately 25% of the water column (in July), whereas in the case of Lake Rospuda (with axis not aligned with the direction of dominant winds), the zone covered approximately 18%. In both of the lakes, a correlation was recorded between oxygen dissolved in water and water temperature. It was more evident in the case of Lake Roś, where the oxygen curve in all months was similar to that of temperature. In Lake Rospuda, no such situation was recorded for May and June. No statistically significant changes in both of the parameters were recorded in the analysed multiannual.

**WARUNKI TERMICZNO-TLENOWE JEZIORA ROŚ I ROSPUDA FILIPOWSKA  
(PÓLNOCNO-WSCHODNIA POLSKA) W PÓLROCZU LETNIM 2005–2014**

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**Słowa kluczowe:** profile termiczne, tlen, jeziora, wiatr, Pojezierze Mazurskie.

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### Abstrakt

Istotne dla funkcjonowania ekosystemów jeziornych są warunki termiczne i tlenowe w nich panujące. Posiadanie szczegółowych informacji na ten temat jest ważne w kontekście obserwowanych zmian środowiska. W pracy przeanalizowano warunki termiczno-tlenowe jeziora Roś i Rospuda Filipowska w północno-wschodniej Polsce w latach 2005–2014 dla półrocza letniego (maj – październik). Oba jeziora należą do stratyfikowanych. Średnia temperatura wody całego pionu jeziora Roś wyniosła 11,3°C, a jeziora Rospuda 9,4°C. Na rozkład temperatury widoczny był wpływ wiatru. W przypadku jeziora Roś, w którym występują lepsze warunki do mieszania wody z uwagi na orientację osi jeziora w stosunku do kierunku dominujących wiatrów, epilimnion obejmował ok. 25% (w lipcu), podczas gdy w przypadku jeziora Rospuda (niezorientowanego osi do kierunku przeważających wiatrów) strefa ta wyniosła ok. 18%. W obu jeziorach odnotowano związek rozkładu tlenu rozpuszczonego w wodzie z jej temperaturą. Był on bardziej wyraźny w przypadku jeziora Roś, gdzie krzywa tlenowa była we wszystkich miesiącach współsztaltna z krzywą temperatury. W Rospudzie takiej sytuacji nie odnotowano dla maja i czerwca. W analizowanym wieloleciu nie wykryto istotnych statystycznie zmian obu parametrów.

## Introduction

Water is one of the basic elements of the natural environment. Its accessibility and appropriate quality are of key importance for the course of life processes. Moreover, water is important for the broadly defined human economic activity, determining industrial and agricultural production etc. Due to its properties, water rapidly responds to any changes in the environment, both of natural e.g. climate changes (CHOIŃSKI et al. 2015) and artificial origin, e.g. melioration works (PTAK et al. 2013). A special role in the hydrosphere is played by lakes which constitute an easily accessible reservoir of drinking water at the global scale. The strongest relations between the environment and lakes occur in lakelands, where high density of lakes influences the water circulation conditions, topoclimate, tourism development, etc. In Poland, areas of the type are particularly located in the northern part of the country, corresponding to the range of the last glaciation.

The basic parameters of lake waters include temperature and dissolved oxygen. Water temperature is of high importance both in biological (HÖÖK et al. 2007, DUPUIS and HANN 2009, PEŁECHATA et al. 2015) and physical-chemical terms (NONAKA et al. 2007, XU et al. 2012, LI et al. 2013). Oxygen is necessary for the transformations of all aerobic organisms, and is of key importance in biogeochemical processes (WITEK and JAROSZEWICZ 2010). Oxygen dissolved in water is one of the primary parameters determining its quality (TERZHEVIK et al. 2009). In extreme cases, lack of oxygen can lead to death of organisms; such cases are quite common (MHLANGA et al. 2006, JONIAK et al. 2013, RAO et al. 2014).

In view of the above, the detailed determination of the distribution of both of the parameters constitutes an elementary set of information on a given lake

ecosystem. Such information is not only important from the scientific point of view. It can provide the basis for undertaking potential reclamation works for degraded lakes. The objective of the paper is to characterise the thermal-oxygen conditions of two lakes: Roś and Rospuda Filipowska (Rospuda) and assessment of their correlations with meteorological conditions.

### Research Area, Materials and Methods

The analysed lakes are located in the Masurian Lakeland in north-eastern Poland (Figure 1).

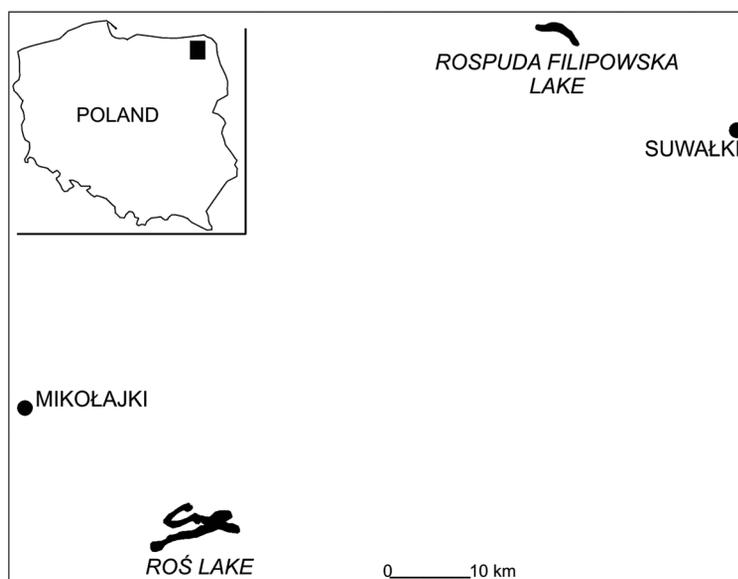


Fig. 1. Location of the studied lakes

Lake Roś with an area of 1,808 ha has a maximum depth of 31.8 m. Its mean depth is 8.1 m. The lake belongs to the land of Great Masurian Lakes, closing their sequence to the south-east (KONDRACKI 2002). It is connected with Lake Śniardwy (the largest lake in Poland) with the Jegliński Channel. The Pisa River (river of III degree), a right tributary of the Narew River, takes its beginning in Lake Roś. Lake Rospuda has an area of 323 ha, maximum depth of 38.9 m. and mean depth of 14.5 m. The Rospuda River flows through the lake. The Area of Protected Landscape “Rospuda Valley” with a total area of 25 250 ha was established for the river in 1991. According to the climatic

division of Poland (WOŚ 2010), Lake Roś is located in region 11 – where mean air temperature amounts to 7°C, the coldest month is January (-3.5°C), and the warmest month is July (17.5°C). The mean annual precipitation total amounts to 552 mm. Lake Rospuda is located in region 12, where mean air temperature amounts to 6.7°C, the coldest month is January (-4.2°C), and the warmest month is July (17.3°C). Mean annual precipitation total amounts to 576 mm.

The paper is based on data collected by the Institute of Meteorology and Water Management – National Research Institute (IMGW-PIB). The data cover measurements of temperature and oxygen dissolved in water in both lakes. The measurements were performed with monthly frequency in the deepest place of each of the lakes every 1 m. This involved the application of a thermal-oxygen sonde YSI Professional and YSI ProOdo. The observations concern the summer half-year (May – October) in the years 2005–2014. Data on air temperatures (daily means) and wind direction for the Suwałki and Mikołajki stations (hourly values) from the years 2005–2014 were also used. The analysis of trends of changes in water temperature, oxygen dissolved in water, and air temperature was performed by means of linear regression in Microsoft Excel software, adopting the significance level of  $p = 0.05$ .

## Results and Discussion

The distribution of water temperature and oxygen dissolved in water in the monthly course is presented in Figure 2.

Due to their considerable depths, Lakes Roś and Rospuda are both stratified lakes. After the period of spring homothermy, thermal layering occurs, and three characteristic zones develop, namely epi-, meta-, and hypolimnion. In both of the cases, such a situation is recorded from the first to the last of the analysed months. The mean water temperature (entire profile) in the analysed multiannual for Lake Roś amounted to 11.3°C, and for Lake Rospuda 9.4°C. The highest difference in temperature in a profile occurs in July for both of the lakes. In the case of Lake Roś, it amounts to 15.6°C, and 15.8°C for Lake Rospuda. The lowest difference in water temperature was recorded in October, and amounted to 2.4°C and 3.7°C, respectively. More evident development of particular zones in the case of Lake Rospuda in all months draws attention. Such a situation should be associated with individual parameters of lakes. Water mixing, generated by wind, is of key importance for heat distribution from the surface zone of water into its deeper sectors. The depth in the lake reached by the wave base is determined not only by the aforementioned individual parameters, but also climatic factors – impact of wind. The analysis of the mean effective length of lake  $D_e$  (determining the average route of wind

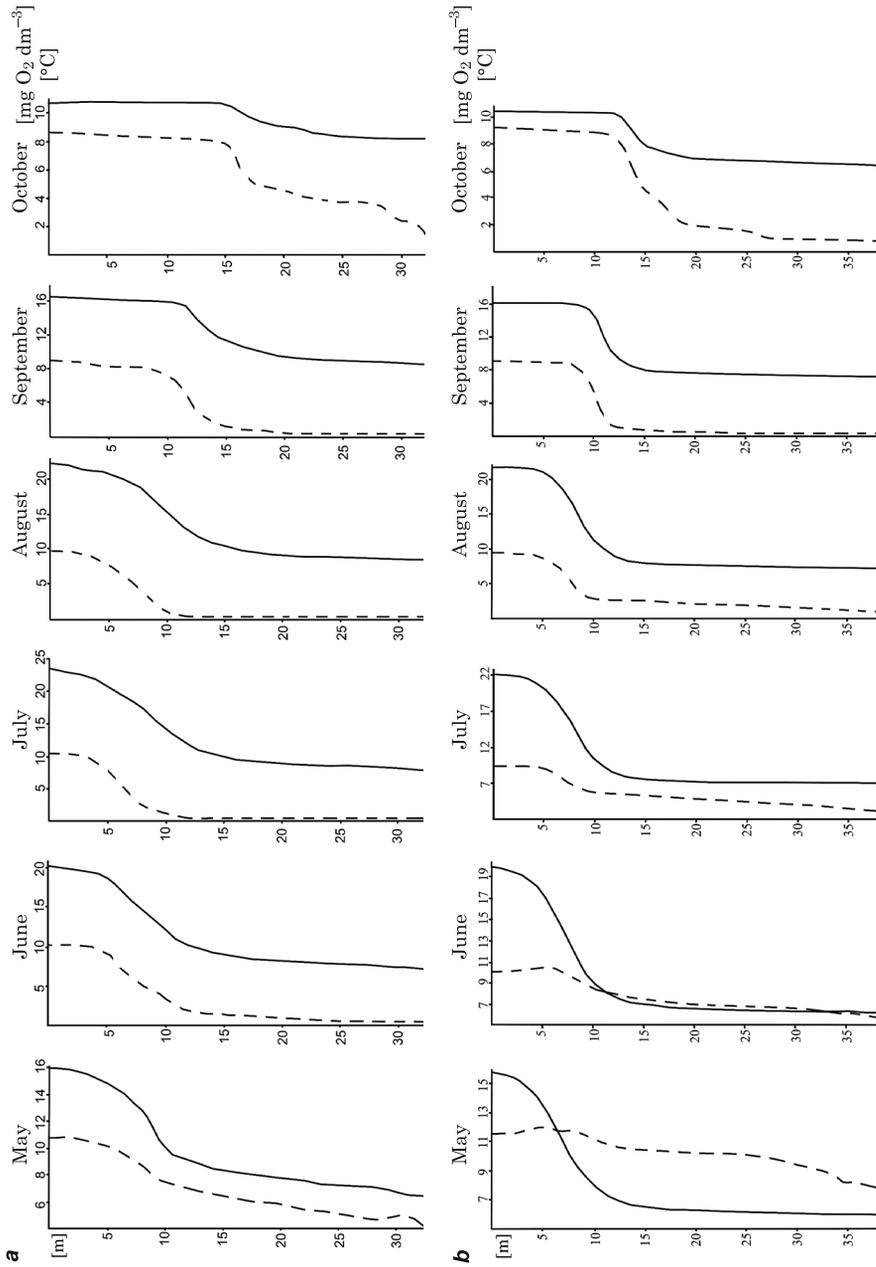


Fig. 2. The distribution of temperatures and oxygen dissolved in water in the analysed profiles (monthly means from the years 2005–2014): *a* – Roś Lake; *b* – Rospuda Lake; dotted line – oxygen, continuous line – temperature

over the water surface on which wind encounters no obstacles) suggests that in the context of mixing of limnic waters, Lake Roś shows better conditions, with the value equalling 6.7 km. For Lake Rospuda, the value amounts to 3.3 km. Therefore, a longer route of wind impact influences the possibility of water mixing in deeper parts of the lake. Moreover, the axis of Lake Roś is parallel to the direction of winds dominant in the region of its location. The performed analysis of the frequency of occurrence of winds from a particular direction (Figure 3) suggests that for the Suwałki station, winds from the western sector were recorded the most frequently (9.6%), and in the case of the Mikołajki station, wind from the south-western direction was dominant (9.1%). In the case of stations Mikołajki and Suwałki, the highest contribution was reached by winds with strength from 1 to 5 m s<sup>-1</sup>. They constituted respectively 85% and 80% of all of the recorded measurements.

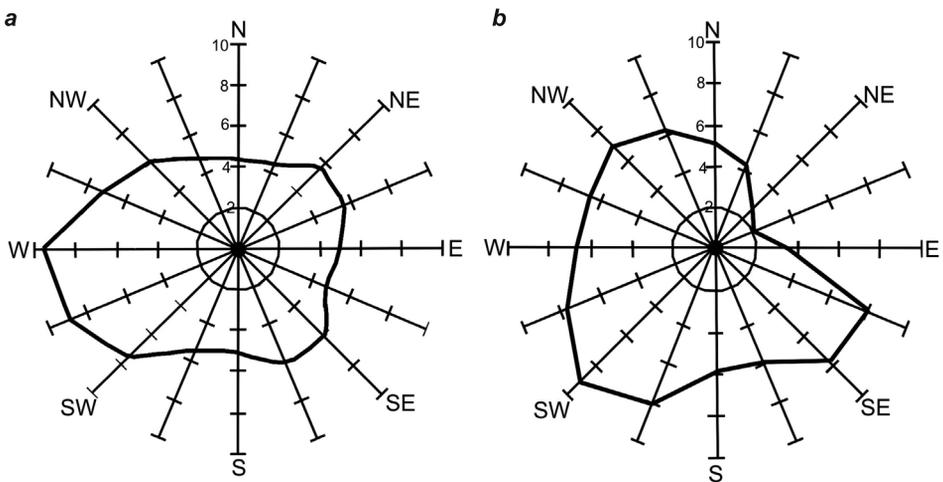


Fig. 3. Wind rose for the Suwałki (a) and Mikołajki (b) stations in the years 2005–2014

In the analysed cases, the above situation is reflected in the distribution of temperature curves taking a milder shape for Lake Roś at the moment of transition of particular zones during thermal stratification. SKOWRON and PIASECKI (2014) point out that the thickness of the epilimnion largely depends on wind. In the case of Lake Roś with better conditions for water mixing in July (complete thermal stratification), the zone covered approximately 25% of the analysed profile, whereas in the case of Lake Rospuda, the zone covered approximately 18%.

Considering the distribution of oxygen dissolved in water, an evident correlation with temperature curves occurs for Lake Roś. In all of the analysed

months, they took the form of a clinograde. Monthly means from the analysed multiannual for the entire profile were variable, and ranged from  $2.1 \text{ mg O}_2 \text{ dm}^{-3}$  in July to  $6.9 \text{ mg O}_2 \text{ dm}^{-3}$  in May. In reference to those values, saturation of water with oxygen was variable. The analysis of the parameter at a level of 50% suggests that in May, water with such (and lower) saturation stagnated at a depth of approximately 18 m. In June and July, the zone expanded reaching a depth of 9 and 7 m, respectively, to decrease again (depths of 8, 12, and 17 m) in the next three analysed months (August, September, October). The anaerobic zone was recorded from June to September, and its range in particular months of the period developed at depths of 21, 12, 11, and 17 m.

The analysis of the distribution of oxygen dissolved in water for Lake Rospuda suggests that it also depends on water temperature, although the correlation is not as evident as in the other lake. In May and June, oxygen curves take the form approximate to an orthograde, whereas temperature curves correspond with a form typical of summer stratification. In the remaining months, the oxygen curve changes shape to clinograde, and is similar to temperature distribution in the profile. Monthly means from the analysed multiannual for the entire profile were variable, and ranged from  $2.6 \text{ mg O}_2 \text{ dm}^{-3}$  in September to  $10.2 \text{ mg O}_2 \text{ dm}^{-3}$  in May. In reference to the values, saturation of water with oxygen was variable. The analysis of the parameter at a level of 50% and higher covered the entire studied profile. In June and July, the zone with water saturation with oxygen below 50% stagnated at a depth below 36 m. In two subsequent months, the zone with such saturation expanded, reaching a depth of 12 m in July, and 9 m in August. The last two months were distinguished by an improvement of oxygen conditions, and the depth at which water was oxygenated at a level of 50% and more reached 11 and 14 m. An anaerobic zone (oxygen saturation below  $1 \text{ mg O}_2 \text{ dm}^{-3}$ ) was recorded in August near the bottom, and in September and October at depths of 14 and 34 m.

The course of water temperature and dissolved oxygen concentration for particular zones developed during full summer stratification (July), and mean air temperature for July (Lake Roś – Miko ajki station, Lake Rospuda – Suwałki station) is presented in Figure 4.

The above curves suggest no occurrence of evident tendencies in the course of temperature in the analysed decade. The distribution of water temperature was variable in particular years. In the case of Lake Rospuda, the temperature amplitude for the epilimnion in the years 2005–2014 amounted to 8, and water temperature for Lake Roś  $4.7^\circ\text{C}$ . In general, temperature of the zone in both of the cases corresponds with air temperature. The two values show a strong correlation (the Pearson's correlation coefficient for Lake Roś is 0.80, and for Lake Rospuda 0.88). The course of air temperature and water temperature

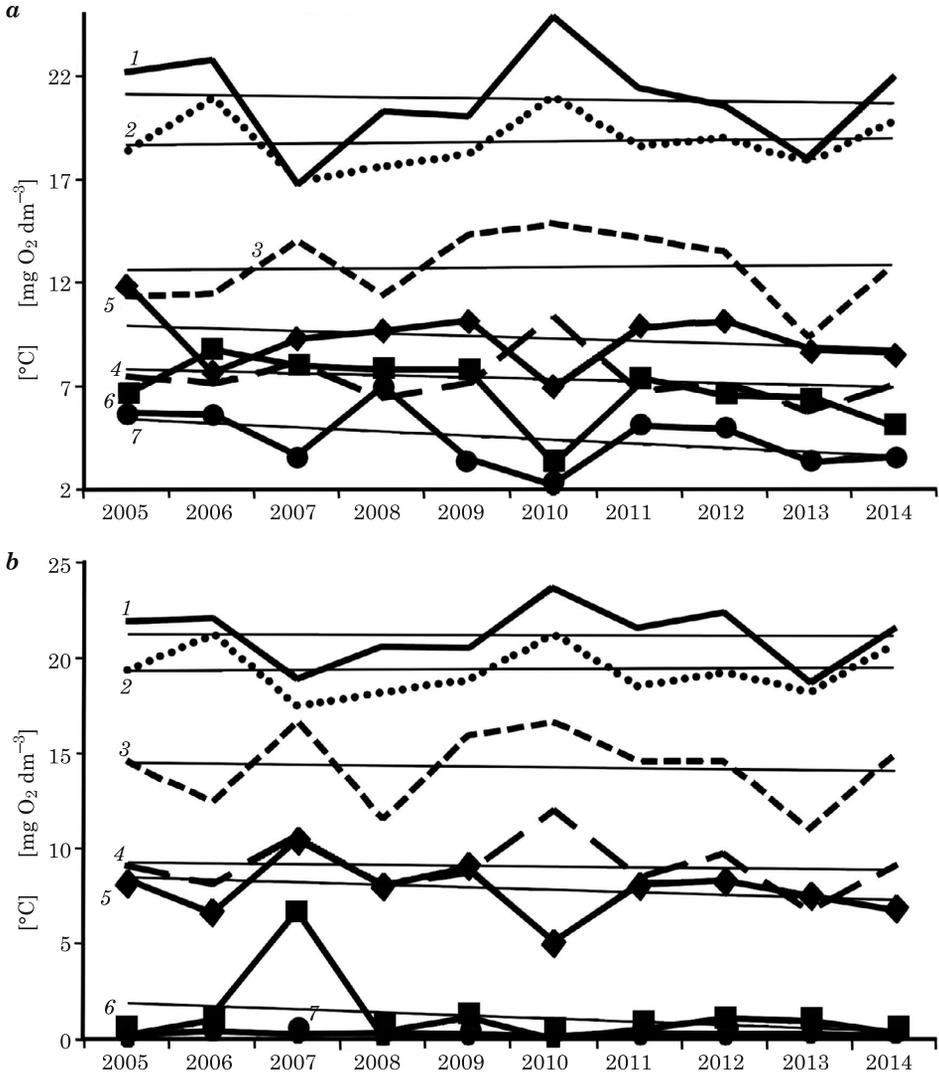


Fig. 4. Changes in water temperature and dissolved oxygen for particular thermal zones during summer stratification (July): *a* – Rospuda Lake, *b* – Roś Lake; 1 – water temperature in the epilimnion; 2 – air temperature; 3 – water temperature in the metalimnion; 4 – water temperature in the hypolimnion; 5 – oxygen concentration in the epilimnion; 6 – oxygen concentration in the metalimnion; 7 – oxygen concentration in the hypolimnion

in the metalimnion is not as synchronic as in the previous case. The variability of the zone was approximate, and amounted to 5.5°C for Lake Rospuda, and 5.6°C for Lake Roś. Similar variability was recorded in the hypolimnion of both lakes, amounting to 4.5°C for Lake Rospuda and 5.2°C for Lake Roś.

An interesting situation was recorded in both of the analysed profiles in July 2010. In the above comparison, this month was distinguished by the highest air temperature, also directly translating into the highest temperature of the near-surface and the deepest water layer. Such a situation was reflected in the second of the analysed parameters, namely concentration of dissolved oxygen. Its lowest value at the highest temperature is related to the Henre's law stating that among others solubility of gases in liquids decreases (concentration of gas decreases) with an increase in temperature. In the monthly scale, the distribution of both of the curves is presented in among others ZEBEK (2009) based on the example of Lake Jeziorak Mały. In July, the highest water temperature corresponds to the lowest values of dissolved oxygen.

The analysis of water temperature fluctuations and changes in oxygen conditions presented in the paper refers to one of the main research trends in limnology. Both of the issues have been discussed in many publications referring to both single cases and larger groups of lakes (BELLA 1970, SMITH and BELLA 1973, STEFAN and FANG 1994, NOWLIN et al. 2004, ESCOBAR et al. 2009, PICCOLROAZ et al. 2015, XU and XU 2016). In the case of Polish lakes, lack of a larger group of publications based on systematic measurements of both of the parameters is observed. There are studies referring to detailed measurements over periods of several months, e.g. a paper concerning Lakes Dgały Wielkie, Dgały Małe, and Warniak with data from the years 2000–2010 (NAPIÓRKOWSKA-KRZEBIETKE et al. 2012), or MAŚLANKA and NOWIŃSKI (2006) analysing the thermocline of Lake Raduńskie Górne in the years 1995–2005, etc. The majority of the papers, however, are based on shorter periods, including papers by OLSZEWSKI (1953), STAWECKI et al. (2004), TROJANOWSKI and PARZYCHA (2004), JAŃCZAK and MAŚLANKA (2006), JAROSIEWICZ and HETMAŃSKI (2009), CHOIŃSKI et al. (2013).

Both of the lakes analysed in the paper are distinguished by quite similar parameters both in terms of temperature and dissolved oxygen concentration in the profile. The recorded variability is determined by local conditions affecting the functioning of both of the ecosystems. As emphasised earlier, the location of the lake in relation to wind is of key importance. In the case of Lake Roś, the dominant wind direction corresponds to the axis of the lake. The situation favours deeper water mixing, as reflected in less evident development of particular thermal layers (Figure 2). The value of oxygen dissolved in water was determined not only by temperature, but also photosynthesis and mineralisation processes.

Oxygen distribution for Lakes Roś and Rospuda suggests low concentration in the hypolimnion. According to (BIEDKA 2012), such a situation can be explained with mineralisation of organic matter deposited from the surface segments, and organic matter contained in bottom sediments in the conditions

of no oxygen supply. According to the *Reports...* (2010, 2013), Lake Rospuda changed its status over a period of several years, and showed susceptibility to eutrophication. It should be also emphasised that the lake was classified as showing good ecological state in 2013 (*Classification...* 2014). According to the classification by CYDZIK et al. (1992), Lake Roś was distinguished by waters of class III, particularly due to the concentration of phosphates above the bottom in the summer period.

As emphasised by ANTONOPOULOS and GIANNIOU (2003), water temperature and oxygen are two of the primary factors of water quality in water ecosystems. In this context, earlier studies suggest that the oxygenation of the hypolimnion has not changed for several decades. Analysing the oxygen conditions of Lake Roś, MARSZELEWSKI (2005) determined that the oxygenation of the hypolimnion in the 1960's was at the level of 32.5%, and at the end of the century (1990's), it already amounted to 0%. Such a situation is maintained until today. The improvement of the state in the near future will be difficult. The first and most important method of protection of lakes is cutting off the supply of pollutants (GROCHOWSKA et al. 2014). In spite of undertaking a broad scope of activities aimed at broadly defined environmental protection, e.g. establishment of new water treatment plants, implementation of the nitrate directive at the moment of Poland's accession to the European Union, etc., the quality of water in many lakes is still not satisfactory. Such a situation should be associated with the internal alimantation of the lakes. In the case of exhausting oxygen resources in the hypolimnion, increased release of phosphorus compounds from bottom sediments is observed, leading to increased production of organic matter in the next season in the surface water layer (BIEDKA 2012). Due to biogene compounds deposited in bottom sediments, even in the case of neutralisation of external pollution sources, the ecological status of a given lake does not always improve. Such a situation is described by SOBCZYŃSKI and JONIAK (2009) in reference to Lake Góreckie. Its trophic status is maintained at a high level in spite of lack of external threats.

## Summary

The presented description of thermal-oxygen conditions of two lakes located in the Masurian Lakeland corresponds with one of the primary research trends of limnology. In the case of Polish lakes, studies concerning the thermal-oxygen structure of lakes based on systematic measurements covering several months are relatively rarely performed. The observations conducted over a decade permitted the determination of the scale of water temperature

fluctuations and the closely correlated oxygen conditions. Both lakes were determined to have similar distributions of the analysed characteristics.

The presented analysis of thermal-oxygen conditions in Lakes Roś and Rospuda does not only constitute a source of detailed information on the subject in reference to the current state. The presented data can be used in the future (in a dozen years or several decades) as material for assessment of the tendency and scale of transformations occurring in both lakes.

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## **EFFECT OF SOCIOECONOMIC CHARACTERISTICS OF CONSUMERS ON PREFERENCES OF PURCHASE AND CONSUMPTION OF FRESHWATER FISH**

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**Key words:** freshwater fish, fish consumption, consumer preferences.

### **A b s t r a c t**

This article contains results of a study the objective of which has been to identify the effect of socio-economic characteristics of consumers on: frequency of fish consumption, preferred form and place of fish purchase. Additionally, the degree to which the above properties affected fish purchase was estimated. Moreover, subjective opinions of consumers on the circumstances of fish consumption were assessed. The empirical research was conducted with a questionnaire-based interview method, and involved 184 randomly selected respondents.

The investigations justify the conclusion that socio-economic features have a statistically significant effect not only on the frequency of fish consumption and occasions when fish are consumed, but also on the preferred form and site of fish purchase as well as the determinants affecting the purchase of freshwater fish.

It has been determined that fish consumption is statistically significantly affected such characteristics as: sex, age and average monthly income of consumers. No statistically significant relationships were determined between the education of respondents and the frequency of fish consumption. The research results point to statistically significant differences in the preferred form and place of purchase related to the following factors: sex, age, education and average monthly income of respondents. The age and education level of consumers were not confirmed to have a statistically significant influence on the occasions on which fish were consumed.

### **WPLYW CECH SOCJOEKONOMICZNYCH KONSUMENTÓW NA PREFERENCJE ZAKUPU I SPOŻYCIA RYB SŁODKOWODNYCH**

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**Słowa kluczowe:** ryby słodkowodne, spożycie ryb, preferencje konsumenckie.

### Abstrakt

W artykule zaprezentowano wyniki badań, których celem było określenie wpływu cech socjoekonomicznych konsumentów na częstotliwość spożywania ryb oraz preferowaną formę i miejsce ich zakupu. Zbadano również stopień wpływu wybranych czynników na zakup ryb. Poznano ponadto subiektywne oceny konsumentów dotyczące okoliczności spożywania ryb. Badania empiryczne zrealizowano metodą wywiadu kwestionariuszowego wśród 184 losowo wybranych respondentów.

Przeprowadzone badania pozwalają stwierdzić, że cechy socjoekonomiczne mają statystycznie istotny wpływ nie tylko na częstotliwość i okoliczności spożywania ryb, ale również na preferowaną formę i miejsce zakupu oraz determinanty wpływające na zakup ryb słodkowodnych.

Stwierdzono, że na spożycie ryb statystycznie istotnie wpływają takie cechy jak: płeć, wiek oraz średni miesięczny dochód. Nie wykazano zależności istotnej statystycznie między wykształceniem respondentów a częstotliwością spożycia ryb. Wyniki badań wskazują na statystycznie istotne różnice między preferowaną formą zakupu ryb oraz miejscem ich zakupu a: płcią, wiekiem, wykształceniem oraz średnim miesięcznym dochodem konsumentów. Nie stwierdzono statystycznie istotnego wpływu wieku i wykształcenia na okoliczności konsumpcji ryb.

## Introduction

In Poland, fish is an underrated type of food and, statistically speaking, it is rarely consumed (HRYSZKO 2015). Fish as food are rich in protein, vitamins and valuable minerals (BORUCKA and WIECZOREK 2003, GAWĘCKI and HRYNIEWIECKI 2005, POLAK-JUSZCZAK 2008a, POLAK-JUSZCZAK 2008b, USYDUS and SZLINDER-RICHERT 2009, ŁUCZYŃSKA et al. 2011). Fish are easily digestible and low in calories (KOŁAKOWSKA and KOŁAKOWSKI 2001, POLAK-JUSZCZAK and ADAMCZYK 2009). Owing to the abundance of important nutrients, especially polyunsaturated fatty acids: eicosapentaenoic acid (EPA) and docosa hexaenoic acid (DHA), fish promote the proper functioning of a human organism. They reduce the risk of myocardial infarct, neoplasms, atherosclerosis, cerebral infarction; they also lower the blood pressure and level of cholesterol (BOURRE 2005, LECERF 2009, PARK and MOZOFFARIAN 2010). Moreover, they improve memory and concentration, and are recommended to pregnant women, partly because they affect the developing brain of a child both before and after birth (CALDLER and GRIMBLE 2002, HIBBELN et al. 2007, MYERS and DAVIDSON 2007)

Compared to other food products, fish prices are constantly rising on the world market. The more noble the fish species, the higher the price. Sometimes, very high prices of certain fish species reflect consumers' preferences in more developed countries, and a considerable decrease in the supply of some species, most often predatory ones, due to overfishing. The existing price tendencies on the global fish market have led to a situation where investment into fishing and aquaculture in developing countries seems to be a particularly profitable option, helping to improve their economies (BROCKI 2009).

The factors that have substantial influence on fish consumption are: wealth of a society, tradition of eating fish, and fish availability. Fish from inland waters are different in nutritional value as well as their taste. These differences stem from environmental conditions, body weight, physiological status, time of fish catch and, above all, freshness of fish meat (PUCHAŁA 2007, STEFFENS and WIRTH 2007, GRELA *et al.* 2010, JABEEN and CHAUDHRY 2011, USYDUS *et al.* 2011, TKACZEWSKA and MIGDAŁ 2012a, TKACZEWSKA and MIGDAŁ 2012b, SKAŁECKI *et al.* 2013).

Recent years have witnessed several campaigns promoting fish as food and fish nutritional values. The Fish Promotion Association has been set up in Poland. The intention behind such promotion of rational nutrition and dissemination of the knowledge about the role of fish as foodstuff has been to raise amounts of fish and fish products sold to consumers, and to encourage them to eat less popular fish species, such as roach or common bream. The most popular promotional campaigns in Poland have been: “Ryby – głosu nie mają zdrowia dodają”, “Ryba wpływa na wszystko”, “Ryby mają głos”, “Mamo jedz ryby”. Many projects, contests and festivals have been held to promote fish, e.g. the Regional Fish Festival, or the Masurian Fish Festival “Rybkę na zdrowie”.

The article presents results of a study which was aimed at investigating the frequency and circumstances of fish consumption, preferred form and site of purchase as well as the influence of fish origin on consumer preferences concerning the determinants guiding the purchase of freshwater fish.

## **Materials and Methods**

The results presented in this article originate from empirical research completed with the use of a questionnaire, delivered to 184 respondents. The respondents selected for the research consisted of people who buy and eat fish. Besides, the questionnaire was addressed to consumers with different socioeconomic characteristics. The data were obtained from respondents different in terms of gender, age, education and the average monthly income per family member.

The questionnaire was specifically designed by the authors and contained alternative questions, disjunctive questions (where one of several answers could be selected) and conjunctive questions (where more than one answer could be selected).

Statistical analysis of the examined characteristics was accomplished with Statistica PL software. It comprised an analysis of frequency.

The respondents consisted of groups characterized by different sex, age, education, place of residence and average monthly income per family member. More details can be found in Table 1.

Characteristics of respondents

Table 1

Socioeconomic characteristics	Specification	Percentage of respondents
Gender	female	64.1
	male	35.9
Age	to 20 years	12.0
	21–30 years	50.5
	31–40 years	18.0
	41–50 years	13.0
	more than 50 years	6.5
Education	basic	0
	vocational	0
	secondary	21.7
	higher	78.3
Place of residence	more than 100 thousand residents	50.4
	city 50–100 thousand residents	8.2
	city 10–50 thousand residents	20.1
	city for 10 thousand residents	4.9
	village	16,3
The average monthly income per person [zł/EUR]	to 800 zł/186 EUR	18.5
	801–1000 zł/186,2–232 EUR	13.6
	1001–1500 zł/233–348 EUR	22.8
	1501–2000 zł/349–465 EUR	29.9
	more than 2000 zł/465 EUR	15.2

In order to assess to what extent differences between the respondents' replies were incidental and to what extent they reflected trends in various periods of the study, appropriate statistical tests were employed. The choice of the tests depended on the nature of analyzed data, and hence comparison of the distribution of variants of answers to individual questions from the questionnaire was supported by the  $\chi^2$  test, assuming the significance level of  $\alpha = 0.05$ . The  $\chi^2$  test allowed us to verify some dependences between socioeconomic characteristics and frequency of fish consumption, purchase site, circumstances of fish consumption, and determinants affecting fresh-water fish purchase.

## Results and Discussion

Nearly half of the respondents (44.6%) declared eating fish 2 to 3 times a month, while as many as 15.2% ate fish only occasionally. One in three of the respondents (30.4%) consumed fish at least once a week (Table 2).

Table 2  
The impact of socioeconomic characteristics on the frequency of fish consumption

Socioeconomic characteristics	The frequency of fish consumption			
	few times a week	once a week	two to three times a month	occasionally
	% of responses			
Total	9.8	30.4	44.6	15.2
Gender				
Women	8.5	31.4	39.8	20.3
Men	12.1	28.8	53.0	6.1
Age				
To 20 years	18.2	0	50.0	31.8
21–30 years	9.7	30.1	41.9	18.3
31–40 years	15.2	42.4	36.4	6.1
41–50 years	0	33.3	58.3	8.3
More than 50 years	0	50.0	50.0	0
Education				
Secondary	10	25.0	47.5	17.5
Higher	9.7	31.9	43.8	14.6
The average monthly income per person				
To 800 zł	11.8	32.4	29.4	26.5
801–1000 zł	0	52.0	40.0	8.0
1001–1500 zł	16.7	19.0	45.2	19.0
1501–2000 zł	12.7	20.0	54.5	12.7
More than 2000 zł	0	46.4	46.4	7.1

The study shows that 8.5% of women and 12.1% of men ate fish several times a week. There was an evident difference regarding occasional fish consumption, which was declared by 20.3% of women and 6.1% of men. Half of those questioned aged up to 20 years consumed fish 2 to 3 times a month, and over 31.8% ate it occasionally. Among the respondents aged 21–30, the largest group declared eating fish 2 to 3 times a month (41.9%) or once a month (30.1%). Among the 31–40-year-olds, the highest percentage consumed fish once a week (42.4%) or 2–3 times a month (36.4%). Over half of the people aged 41 to 50 years ate fish 2–3 times a month (58.3%). The oldest

respondents declared eating fish once a week or 2–3 times a month (50% each, respectively).

Most people with secondary and higher education consumed fish 2–3 times a month. Regarding the respondents' incomes, 52% of those earning from 801 to 1,000 PLN monthly consumed fish once a week. Most persons (54.5%) in the group with an income between 1,501 and 2,000 PLN indicated the frequency of fish consumption as 2–3 times a month. The respondents with incomes above 2,000 PLN consumed fish once a week or 2–3 times a month (46.4% each, respectively).

The study by LEBIEDZIŃSKA et al. (2006) suggests that among the students questioned, 9% of women and 18% of men declared fish consumption several times a week, whereas the survey conducted by KOŁODZIEJCZYK (2008) demonstrated that 45% of the respondents claimed they consumed fish 1–2 times a week. Only one in five students of medicine consumed fish according to rational nutrition recommendations (GAJEWSKA 2009). Nearly the same results were achieved in a study conducted among adolescents aged 14 to 18 years (BORTNOWSKA et al. 2011).

The results show that the frequency of fish consumption by Polish consumers is lower than recommended by the Polish Institute of Food and Nutrition, which in 2012 stated that fish and fish products should be consumed twice a week, including fat fish eaten once a week, to maintain good health (TKACZEWSKA et al. 2014). The analytical report drawn for the Association of Salmonid Fish Producers (2011), most adult Poles (94%) have bought fish or fish products at least once over the past year.

Based on the results of our survey, it was found that fish consumption is statistically significantly affected by such characteristics as: sex, age and average monthly income. No statistically significant relationship was identified between the respondents' education and frequency of fish consumption.

The vast majority of the respondents (72.3%) consumed fish at home and associated eating fish with festive occasions. Only 9.2% consumed fish during family celebrations, 7.6% declared fish consumption without any special occasion, 6.5% ordered fish in restaurants (Table 3). Other circumstances (4.4%) indicated by the respondents included travel to another country or region and a wish to consume fish typical of a given geographical area. Irrespective of the socioeconomic characteristics of the respondents, fish are most often consumed at home.

Despite strong culinary traditions of eating fish in Poland, currently fish are rarely found on tables in Polish households. Today, Poles most often eat fish on holidays, during special occasions, or visits to restaurants. Possible reasons are high labour inputs into cooking fish and high prices of fish.

Table 3

The impact of socioeconomic characteristics on consumers' preference of occasions for fish consumption

Socioeconomic characteristics	Occasion of fish consumption				
	at home	family get-togethers	in a restaurant	no special occasion	others
	% of responses				
Total	72.3	9.2	6.5	7.6	4.4
Gender					
Women	73.7	12.7	6.8	5.1	1.7
Men	69.7	3.0	6.1	12.1	9.1
Age					
To 20 years	50.0	18.2	22.7	9.1	0
21–30 years	74.2	6.5	5.4	9.7	4.3
31–40 years	87.9	6.1	6.1	0	0
41–50 years	66.7	12.5	0	12.5	8.3
More than 50 years	66.7	16.7	0	0	16.7
Education					
Secondary	57.5	10.0	12.5	15.0	5.0
Higher	76.4	9.0	4.9	5.6	4.2
The average monthly income per person					
To 800 zł	67.6	14.7	0	17.6	0
801–1000 zł	92.0	0	0	0	8.0
1001–1500 zł	57.1	7.1	19.0	11.9	4.8
1501–2000 zł	74.5	16.4	3.6	5.5	0
More than 2000 zł	78.6	0	7.1	0	14.3

Statistically significant effects of the sex and average monthly income per capita on the circumstances in which fish are consumed by the respondents were determined.

The ever growing pace of life means that people have less time to make dishes from fresh fish, and therefore they use frozen products, which is confirmed by our results (Table 4). Over half of the consumers questioned bought frozen fish (53.3%). The choice is dictated by the ease and speed of making dishes from frozen fish, with no need to remove fish head or viscera. Consumers who buy fresh fish (30.4%) draw attention mostly to the possibility of using all fish parts for making various dishes. The respondents were least interested in fish in gelly – only 1.1% indications, smoked fish (6.5%) and fish products (8.7%). The survey results show the lack of interest in salted fish, which may stem from the fact that an aware consumer sporadically chooses products with high salt content.

Table 4

The impact of socioeconomic characteristics on consumers' preference of the form of purchased fish

Socioeconomic characteristics	Preferred form of purchased fish				
	fresh	frozen	in gelly	smoked	processed fish products
	% of responses				
Total	30.4	53.3	1.1	6.5	8.7
Gender					
Women	27.1	65.3	0	3.4	4.2
Men	36.4	31.8	3.0	12.1	16.7
Age					
To 20 years	18.2	40.9	0	9.1	31.8
21–30 years	29.0	59.1	2.2	2.2	7.5
31–40 years	36.4	57.6	0	6.1	0
41–50 years	37.5	29.2	0	25.0	8.3
More than 50 years	33.3	66.7	0	0	0
Education					
Secondary	32.5	40.0	5.0	5.0	17.5
Higher	29.9	56.9	0	6.9	6.3
The average monthly income per person					
To 800 zł	35.3	64.7	0	0	0
801–1000 zł	24.0	68.0	8.0	0	0
1001–1500 zł	16.7	50.0	0	0	33.3
1501–2000 zł	32.7	49.1	0	18.2	0
More than 2000 zł	46.4	39.3	0	7.1	7.1

Women preferred frozen fish much more, while men more often chose fresh fish. Beside, men bought fish in gelly, smoked fish and fish products more often than women did. Frozen fish were popular among all the investigated age groups, except the 41–50-year-olds, who more often bought fresh fish. Fish in gelly was the least popular option, indicated by just 2.2% of the respondents aged 21–30 years. Smoked fish was preferred by the respondents aged 41–50 years (25%), and fish products were chosen by the youngest persons. Irrespective of their education, the respondents most often bought frozen fish, followed by fresh fish, fish products and smoked fish. All the respondents, except persons with an income over 2,000 PLN, preferred frozen fish the best, while persons with the highest income indicated fresh fish as their most preferred choice.

Also, AMAO and AYANTOYE (2014) concluded that frozen fish is easily available and does not require much labour input to be cooked. On the other hand, consumers in Nigeria preferred smoked fish (32.3%) and, to a lesser

degree, fresh fish (19.2%), while 47.5% claimed they willingly bought both fresh and smoked fish (MOSES at al. 2015).

The research results implicate statistically significant differences between the preferred form of fish purchase and the consumers' gender, age, education and average monthly income.

The questionnaire also revealed that all consumers, regardless of their sex, age, education or average monthly income of a household per capita, most often bought fish in supermarkets (40.2%), which could be stimulated by a wide range of products available in such outlets and frequent special offers. Many respondents also bought fish in discount shops (20.7%) and in fish shops (17.9%). A small percentage of those questioned bought fish directly from street vendors (9.2%), from fish farmers and producers of fish products (4.9%), in restaurants and snack bars (1.1%) – Table 5.

Table 5  
The impact of socioeconomic characteristics on consumers' preference of the place of fish purchase

Socioeconomic characteristics	Preferred place of purchase fish						
	supermarkets	discount	fish shops	street market	fish farms	restaurants	caught by angling
	% of responses						
Total	40.2	20.7	17.9	9.2	4.9	1.1	6.0
Gender							
Women	39.8	21.2	23.7	6.8	5.1	0	3.4
Men	40.9	19.7	7.6	13.6	4.5	3.0	10.6
Age							
To 20 years	68.2	13.6	4.5	4.5	9.1	0	0
21–30 years	40.9	25.8	16.1	9.7	4.3	0	3.2
31–40 years	39.4	12.1	27.3	12.1	3.0	0	6.1
41–50 years	25.0	25.0	25.0	4.2	4.2	8.3	8.3
More than 50 years	16.7	8.3	16.7	16.7	8.3	0	33.3
Education							
Secondary	42.5	15.0	2.5	15.0	15.0	0	10.0
Higher	39.6	22.2	22.2	7.6	2.1	1.4	4.9
The average monthly income per person							
To 800 zł	32.4	32.4	8.8	8.8	17.6	0	0
801–1000 zł	36.0	44.0	12.0	8.0	0	0	0
1001–1500 zł	54.8	11.9	23.8	9.5	0	0	0
1501–2000 zł	30.9	18.2	21.8	10.9	5.5	0	12.7
More than 2000 zł	50.0	3.6	17.9	7.1	0	7.1	14.3

Women much more often than man (23.7% vs 7.6%) bought fish in fish shops. Men twice as often as women (13.6% vs 6.8%) purchased fish on street markets, and three times as often as women – in restaurants. Men much more often than women (10.6% vs 3.4%) declared that the fish they consumed originated from fish ponds and water bodies where they went angling. Fish shops were most often selected by persons aged 31–40 years (27.3%), with higher education (22.2%), and an average monthly income of 1,001 to 1,500 PLN (23.8%). Fish from street vendors were most often bought by respondents over 50 years old (16.7%), with secondary education (15%), and average monthly income from 1,501 do 2,000 PLN (10.9%). In turn, 33.3% of people aged over 50 years and 14.3% of those with an income over 2,000 PLN indicated that the fish they consumed originated mainly from own angling catches. A possible reason for the scanty interest in buying fish from fish farmers could be the lack of knowledge among consumers about direct sale outlets and a low availability of fish, for example on street markets. Unlike the Polish consumers questioned in our study, consumers in Turkey buy fish mostly in local shops (7.8%), on street markets (11.2%), and less often in supermarkets (9%). It is worth noticing that the fish purchased mostly originate from catches in the natural environment (76.7%) (MEHMET *et al.* 2015).

Our results show statistically significant differences between the place where fish is purchased and all the analyzed socioeconomic properties.

The most important factor determining the purchase of fish is its freshness (94%), a finding supported by some earlier studies (KRAFT and ZABROCKI 2010, TKACZEWSKA *et al.* 2014). Other significant determinants of fish purchase are: palatability (91.9%), appearance (81.0%), nutritive value (64.1%), price (52.7%) and habits (50%). The consumers described as less important such characteristics as: ease of cooking (33.7%), special offers (26.6%), origin of fish (16.3%) or fashion (1.1%) – Table 6.

Significant differences were determined between the answers provided by men and women with respect to factors affecting fish purchase. Women ascribed much greater importance to: price (women – 64.4%, men – 31.8%), nutritive value (69.5% vs 54.5%) and appearance (85.6% vs 72.7%). For men, such qualities as easy cooking (men – 45.5%, women – 27.1%) and taste (97% vs 89%) were more important.

Regardless of the level of education and for all respondents aged up to 40 years, the principal determinants of fish purchase were: freshness of fish, their taste and appearance. The age group of 41 to 50 pointed to appearance (100%), taste (91.7%) and freshness (87.5%), while the oldest age group indicted: taste and habits (100% each, respectively) and appearance (83.3%).

Among the respondents with the lowest incomes, the most important determinants were: freshness of fish (91.2%), appearance (85.3%) and price

Table 6  
The impact of socioeconomic characteristics on consumers' preference of the determinants of fish purchase

Socioeconomic characteristics	Level of influence	Factors which determine the purchase of fish									
		1	2	3	4	5	6	7	8	9	10
		% of responses									
Total	strong	52.7	91.8	64.1	94.0	33.7	81.0	50.0	1.1	26.6	16.3
	no impact	7.1	0	3.3	0	11.4	2.2	7.6	84.8	20.1	35.9
Gender											
Women	strong	64.4	89.0	69.5	94.9	27.1	85.6	48.3	0	28.0	18.6
	no impact	4.2	0	0	0	8.5	0	4.2	83.1	16.9	31.4
Men	strong	31.8	97.0	54.5	92.4	45.5	72.7	53.0	3.0	24.2	12.1
	no impact	12.1	0	9.1	0	16.7	6.1	13.6	87.9	25.8	43.9
Age											
To 20 years	strong	18.2	100	72.7	100	31.8	72.7	63.6	0	0	27.3
	no impact	9.1	0	0	0	9.1	9.1	0	90.9	27.3	18.2
21–30 years	strong	61.3	86.0	66.7	96.8	34.4	79.6	28.0	2.2	30.1	16.1
	no impact	6.5	0	2.2	0	16.1	2.2	11.8	76.3	22.6	44.1
31–40 years	strong	48.5	100	72.7	93.9	21.2	75.8	66.7	0	15.2	27.3
	no impact	0	0	0	0	6.1	0	9.1	93.9	9.1	21.2
41–50 years	strong	54.2	91.7	50.0	87.5	54.2	100	75.0	0	45.8	0
	no impact	20.8	0	12.5	0	0	0	0	100	16.7	50.0
More than 50 years	strong	58.3	100	33.3	75.0	25.0	83.3	100	0	41.7	0
	no impact	0	0	8.3	0	16.7	0	0	83.3	25.0	16.7
Education											
Secondary	strong	37.5	100	67.5	87.5	40.0	77.5	52.5	0	12.5	20.0
	no impact	5.0	0	0	0	5.0	5.0	12.5	90.0	20.0	22.5
Higher	strong	56.9	89.6	63.2	95.8	31.9	81.9	49.3	1.4	30.6	15.3
	no impact	7.6	0	4.2	0	13.2	1.4	6.3	83.3	20.1	39.6
The average monthly income per person											
To 800 zł	strong	79.4	76.5	79.4	91.2	29.4	85.3	23.5	0	29.4	5.9
	no impact	5.9	0	0	0	14.7	0	8.8	94.1	11.8	41.2
801–1000 zł	strong	76.0	100	40.0	88.0	44.0	84.0	52.0	8.0	28.0	40.0
	no impact	0	0	8.0	0	8.0	8.0	16.0	84.0	16.0	24.0
1001–1500 zł	strong	42.9	88.1	78.6	100	33.3	66.7	57.1	0	23.8	21.4
	no impact	0	0	0	0	4.8	4.8	2.4	81.0	14.3	26.2
1501–2000 zł	strong	29.1	100	65.5	100	36.4	92.7	52.7	0	21.8	7.3
	no impact	10.9	0	7.3	0	5.5	0	3.6	78.2	23.6	49.1
More than 2000 zł	strong	60.7	92.9	42.9	82.1	25.0	71.4	64.3	0	35.7	17.9
	no impact	17.9	0	0	0	32.1	0	14.3	92.9	35.7	28.6

Key: 1 – price, 2 – taste, 3 – nutritional values, 4 – freshness, 5 – easy to prepare, 6 – appearance, 7 – habits, 8 – fashion, 9 – promotion, 10 – the place of origin of fish

(79.4%). For the persons in the income brackets of 1,001 and 1,500 PLN monthly, the most important qualities of fish were: freshness (100%), taste (88.1%) and nutritive value (78.6%). Respondents with incomes between 1,501 and 2,000 PLN drew attention to: freshness and taste (100% each), as well as appearance (92.7%), while the people earning monthly incomes over 2,000 PLN were mostly interested in: taste (92.9%), freshness (82.1%) and appearance (71.4%).

LEBIEDZIŃSKA et al. (2006) concluded that students making a decision about fish purchase were guided mainly by the fish taste (89% of women and 92% of men) and freshness (66% of women and 70% of men). Subsequently, female students considered the effect of fish on health and its nutritive value. Male students paid attention to the price and appearance of fish

The origin of fish did not play any larger role as a determinant of fish purchase. It would be advisable to emphasize the importance of fish origin in future campaigns promoting fish consumption. It is vital that consumers pay attention to a fish catch area or a fish farm from which the fish they consume originate because research has confirmed that a site of freshwater fish breeding and rearing as well as applied aquaculture techniques have an influence on fish meat quality characteristics (TKACZEWSKA and MIGDAŁ 2012a, 2012b).

The results of our study demonstrate that the vast majority of respondents (73.4%) was ready to pay more for fish originating from Poland.

Statistically significant differences were shown between certain socioeconomic characteristics of consumers and determinants of consumer purchases of freshwater fish (Table 7).

Table 7  
Statistically significant relationship between consumer socio-economic characteristics and determinants affecting the purchase of freshwater fish

Determinants of affecting the purchase of fish	Gender	Age	Education	The average monthly income of person
Price	X	X	X	X
Palatability	-	-	-	X
Nutritional values	X	X	-	X
Freshness	-	-	-	-
Easy to prepare	X	-	-	X
Appearance	X	-	-	X
Habits	X	X	-	X
Fashion	-	-	-	X
Promotion	-	X	-	-
The place of origin of fish	-	X	-	X

X – statistically significantly relationships

## Conclusions

Our analysis of the survey results justifies the claim that the Polish consumer puts fish on the menu far too rarely. The respondents admitted to consuming fish dishes mostly at home, and often on special occasions, like holidays or family celebrations.

Consumers purchased mainly frozen fish and made their purchase in chain shops or discount shops. While buying the fish, the respondents mostly paid attention to: freshness, appearance and taste values. The price was not a key determinant, which may suggest a high awareness of consumers in terms of the pre-defined attributes.

Unfortunately, the respondents did not pay sufficient attention to the place of origin of fish, which to a large extent can affect the perception of a product quality, its freshness and organoleptic characteristics. At the same time, they demonstrated an ethocentric attitude, indicating the preference for buying local, regional and national products with native origin.

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## HOW TO EFFECTIVELY COLLECT ZOOPLANKTON IN ILLUMINATED CAGES FOR FISH REARING?

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**Key words:** lake cages, planktonic organisms, sample collection methods.

### Abstract

The use of a reliable zooplankton sample collection method is important for the quantitative and qualitative assessment of the food source available for juvenile fish. The effectiveness of zooplankton collection in illuminated cages is related to its concentration in the subsurface layer of water around or under the light source. The aim of this study was to compare the effectiveness of three zooplankton sample collection methods in illuminated cages. The experiment was conducted in Lake Maróz, Poland, in illuminated net cages. The light source was an electric bulb (24 V, 60 W), located just above water's surface and switched on 2 hours before sample collection. The zooplankton samples were collected using a bottle sampler and conical tow-net (mesh 30  $\mu\text{m}$ ) hauled at two different speeds. In terms of qualitative and quantitative parameters, the optimal sample collection method was plankton net hauled at a slow vertical rate (0.05  $\text{m s}^{-1}$ ). Average results were obtained using a bottle sampler (5.0  $\text{dm}^3$  volume). Whereas, plankton net hauled vertically at a fast vertical rate (0.10  $\text{m s}^{-1}$ ) was the least effective method, due to the displacement of water outside of the net's inlet.

### JAK EFEKTYWNIJE POBIERAĆ ZOOPLANKTON W SADZACH OŚWIETLONYCH DO PODCHOWU RYB?

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**Słowa kluczowe:** młodociane stadia ryb, sadze jeziorowe, metody poboru prób.

## A b s t r a k t

Zastosowanie miarodajnej metody poboru prób zooplanktonu w środowisku sadzów oświetlonych jest istotne w ocenie ilości oraz jakości pokarmu dostępnego dla ryb. Celem pracy było porównanie efektywności trzech wariantów metodycznych poboru prób zooplanktonu w środowisku sadzów oświetlonych. Eksperyment przeprowadzono w sadzach jeziorowych (jez. Maróz) oświetlonych od zmierzchu żarówką elektryczną (24 V, 60 W), umieszczoną tuż nad powierzchnią wody. Próby zooplanktonu pobierano czerpaczem butlowym oraz siatką planktonową techniką zaciągu pionowego w dwóch wariantach prędkości holu. W analizie porównawczej efektywności poboru prób zastosowano znormalizowany wskaźnik liczebności zooplanktonu. Referencyjnym wariantem metodycznym dla badań jakościowych i ilościowych zooplanktonu był powolny zaciąg pionowy siatką planktonową (prędkość holu  $0.05 \text{ m s}^{-1}$ , teoretyczne tempo filtracji  $0.08 \text{ dm}^3 \text{ dm}^{-2} \text{ s}^{-1}$ ). Odzwierciedlał on największą liczebność zooplanktonu w każdej grupie taksonomicznej ( $p \leq 0.001$  dla Copepoda i Rotifera oraz  $p \leq 0.01$  dla Cladocera). Najmniejszą efektywność poboru prób, tj. liczebność zooplanktonu i wykrywalność taksonów, uzyskano po szybkim zaciągu pionowym siatką (prędkość holu  $0.10 \text{ m s}^{-1}$ , teoretyczne tempo filtracji  $0.16 \text{ dm}^3 \text{ dm}^{-2} \text{ s}^{-1}$ ). Pośrednie wyniki uzyskano po zastosowaniu czerpacza butlowego o objętości  $5.0 \text{ dm}^3$ . Niska efektywność siatki planktonowej przy szybkim zaciągu spowodowana była zjawiskiem wypierania wody poza krawędź otworu wlotowego.

**Introduction**

Zooplankton sample collection for qualitative and quantitative analysis is most often performed in water ecology and aquatic ecosystem biodiversity research (SUTHERS and RISSIK 2009, WILLIAMSON and MCGOWAN 2010). The 1950's and 60's were a time of dynamic development in the research on the effectiveness and standardization of the zooplankton sample collection methods (BOGOROV 1959, MCGOWAN and FRAUNDORF 1966). However, over the last few decades the tools and methods for manual zooplankton sample collection did not change significantly (STARMACH 1955, DE BERNARDI 1984). Plankton nets of various design and parameters, calibrated bottle samplers and traps as well as pumps continue to be the basic tools of zooplankton researchers (DHARGALKAR and VERLECAR 2004, MCGAVIGAN 2012). Certain design features were modified for the purpose of zooplankton research in particular environments (KRŠINIĆ 1990, PAGGI et al. 2001). The developments in optical, electronic and digital technologies brought new methods of measuring zooplankton quantity and biomass (REMSSEN et al. 2004, BROUGHTON and LOUGH 2006). Numerous studies were conducted to compare the quantitative and qualitative parameters of plankton animals based on samples collected in various environments using various tools and methods (SLUSS et al. 2011). Standardization of zooplankton sample collection methods is crucial in order to avoid false interpretation of results (MASSON 2004). Numerous flaws of the zooplankton sample collection methods have been identified. Once submerged, each tool produces turbulent flow near the inlet, which may influence the collected sample size and the reliability of obtained results (DE BERNARDI 1984,

DHARGALKAR and VERLECAR 2004). Simple conical nets have been used for many years with little modification in design. Their major source of error is that the filtration characteristics of conical nets usually are unknown. Filtration efficiency in No. 20 mesh cone nets ranges from 40% to 77% (GREENBERG et al. 1992).

Zooplankton research is also conducted in juvenile fish rearing in illuminated cages. This method of fish rearing was developed and applied in practice in Poland during the mid-1970's (MAMCARZ 1995a) and later adopted in other countries (CHAMPIGNEULLE and ROJAS-BELTRAN 1990). In such cage environment, the main (or only) foodsource for the fish is the naturally-occurred zooplankton. The method is based on the accumulation of zooplankton around lamp placed in a cage with fish (MAMCARZ 1995b).

The use of reliable zooplankton sample collection methods in illuminated cage environments is crucial for assessing the quality and quantity of the food source available to the fish or for the analysis of the effectiveness of attracting zooplankton to the cages of various design. Various research of zooplankton in illuminated cages indicates wide range of its count per 1 liter of volume – from single to tens of thousands of individuals (ŽILIUKIENĖ 2005, CECCUZZI et al. 2010, SICHROVSKY et al. 2013). However, straight comparison of these quantities would be inconclusive, as zooplankton sampling methodologies used in research mentioned above varied. Most popular methods included suction pumps (SKRZYPCZAK et al. 1998, SICHROVSKY et al. 2013), volume samplers (GRAVES and MORROW 1988, FRISCH and WOHLTMANN 2005), and plankton nets with different mesh size ranges from 30  $\mu\text{m}$  to 50  $\mu\text{m}$  (MAMCARZ 1995b, MARTYNOVA and GORDEEVA 2010, FURGALA-SELEZNIOW et al. 2014, SPRINGER and SKRZYPCZAK 2015). Methods of collecting zooplankton must take into account the size of planktonic organisms (eg. freshwater rotifers and immature microcrustacea is 20  $\mu\text{m}$  and more) and the specificity of the environment (GREENBERG et al. 1992). Unfortunately, these methodologies are not standardized, and their effectiveness has not been tested in environment of illuminated cages for fish rearing.

The aim of this study was to compare the effectiveness of three zooplankton sample collection methods in illuminated cages. It should be expected that not all sampling methods are equally effective for objective assessment of food source density in fish rearing cages.

## Materials and Methods

### Sample collection

Experiment was conducted in Lake Maróz, Poland (N: 53°31.6'; E: 20°24.5'; eutrophic type, 332.5 ha, max. depth 41.0 m) in illuminated net cages 1.0 × 1.0 × 2.5 m, square mesh size 1.2 mm. The light source was an electric bulb (24 V, 60 W), located just above water's surface and switched on 2 hours before sample collection. The zooplankton samples were collected from three fishless cages, using three distinct methods, every three nights during May 2013, usually between 22:00–23:00. In two of the methods, the samples were collected by a plankton net (mesh size 30 μm, round inlet with diameter 0.22 m, inlet's area 0.038 m<sup>2</sup>, filtration area 0.24 m<sup>2</sup>, volume 9.0 dm<sup>3</sup>) hauled vertically from the bottom of the cages to the water's surface (2.0 m) using two different tow speeds. Each haul penetrated 76 dm<sup>3</sup> of a water column volume. In the S<sub>1</sub> sampling method, each tow had an average velocity of 0.05 m s<sup>-1</sup> (total hauling time of about 40s) and the estimated filtration rate was about 1.9 dm<sup>3</sup> s<sup>-1</sup>. In the S<sub>2</sub> method, the same plankton net was towed at an average velocity of 0.10 m s<sup>-1</sup> (total towing time of about 20s), and the estimated filtration rate was about 3.8 dm<sup>3</sup> s<sup>-1</sup>. In both methods the plankton net was towed manually and a standard analog stopwatch was used.

In the method *R*, the zooplankton samples were using a TON 2 bottle sampler (made by "MERA-BLONIE" Precision & Mechanical Plants, Gdansk – Poland, 1998) – 5.0 dm<sup>3</sup> volume, 0.14 m diameter of inlet and 0.33 m height. It has features of the Bernatowicz bottle and Friedinger bottle (DE BERNARDI 1984). It minimizes the generation of hydrodynamic whirlpools in front of the sampler as it is lowered and allows the immediate sealing of the inlet and outlet at the desired depth. This device was used to collect samples from the middle part of the water column (0.85–1.20 m under the surface, by lowering it on calibrated rope. Next, analogously to the first two methods (S<sub>1</sub> and S<sub>2</sub>), the sample was filtered using a plankton net at mesh size 30 μm. That mesh nets effectively capture small-bodied and larger-bodied zooplankton (MACK et al. 2012).

The zooplankton samples collected using all three methods were condensed to the volume of 0.1 L, preserved in Lugol's solution and conserved in a 4% formaldehyde solution (RADWAN et al. 2004). The zooplankton identification was performed until the lowest possible taxonomic unit was identified (except juvenile stages of Copepoda) in accordance with methodology KIEFER and FRYER (1978). The quantitative analysis was performed using the Sedgewick-Rafter counting chamber and reported in the volume unit (ind. dm<sup>-3</sup>).

Physical and chemical parameters of water were monitored during zooplankton sample collection in illuminated cages. A multi-parametric probe (YSI Professional Plus Quatro, 2011) was used to measure temperature, dissolved oxygen and pH whereas water transparency was measured using a Secchi disc.

### Abundance index and statistical analysis

Separate qualitative and quantitative analyses of the zooplankton were performed for each sample day and for each sample collection method. The standardized abundance index ( $SAI_{tmi}$ ) was calculated for each identified taxonomic unit. The normalized index was obtained as follows:

$$SAI_{tmi} = \frac{X_{tmi}}{X_{ti}max}$$

where:

$SAI_{tmi}$  – standardized abundance index for taxa  $t$  for the sample collection method ( $m$ ) on the collection date ( $i$ );

$X_{tmi}$  – abundance of taxa  $t$  (ind.  $L^{-1}$ ) for the sample collection method ( $m$ ) on the collection date ( $i$ );

$X_{ti}max$  – maximum abundance of taxa  $t$  (ind.  $L^{-1}$ ) on the collection date ( $i$ );

The  $SAI_{tmi}$  value (range 0–1) was used to compare the effectiveness of zooplankton sample collection. Model  $n = 1$  indicates that the particular sample collection method was the most effective and each day of its use resulted in the highest quantity of the particular taxon in the volume of water. The effectiveness of the sample collection methods was analyzed in terms of three taxonomic groups (Cladocera, Copepoda and Rotifera) and comparing the  $SAI_{tmi}$  values of all species in each of those groups. Non-parametric analysis of variance was applied to assess the general differences in the standardized abundance indices for each sample collection method (Statistica 10.0 for Windows, Statsoft; Tulsa, UK). The results were processed by ANOVA with the non-parametric Kruskal-Wallis test to determine the statistically significant differences ( $P < 0.05$ ). Correlation analysis ( $r$  – Pearson) was used to assess the correlation between the effectiveness of zooplankton collection methods and the physical-chemical parameters of water.

## Results

The analysis of sample collection effectiveness in illuminated cages, was conducted during a time of dynamic quantitative changes in the lake's zooplankton structure. The highest abundance of Cladocera in all three sample collection methods was obtained on May 19<sup>th</sup>: 5 taxa in  $S_1$  and  $R$  methods and 4 taxa in the  $S_2$  method (Figure 1). The dominant species was *Daphnia cucullata* and its abundance in the  $S_1$ ,  $S_2$  and  $R$  methods was 455 ind.  $\text{dm}^{-3}$ , 323 ind.  $\text{dm}^{-3}$  and 379 ind.  $\text{dm}^{-3}$ , respectively.

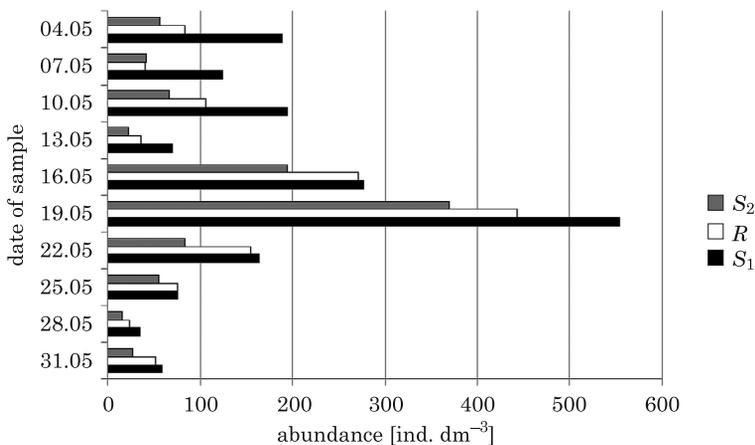


Fig. 1. Dynamics of the Cladocera abundance on the sample collection days using three sampling methods ( $S_1$ ;  $S_2$  and  $R$  – Materials and Methods)

The highest abundance of Copepoda was observed in the sample collected on May 4<sup>th</sup>, resulting from the dominance of two juvenile forms: nauplii and copepodites of *Cyclopoida* (Figure 2). All three sample collection methods yielded 10 Copepoda species and larvae forms. The Copepoda abundance was as follows: 908 ind.  $\text{dm}^{-3}$  (including 78.1% juvenile forms) in the  $S_1$  method, 534 ind.  $\text{dm}^{-3}$  (including 85.2% juvenile forms) and 685 ind.  $\text{dm}^{-3}$  (including 74.4% juvenile forms) in the  $R$  sample collection method.

The highest abundance of Rotifera was confirmed in the sample collected on May 31<sup>st</sup> using methods  $S_1$  and  $R$  (Figure 3). Each of these two methods yielded 8 taxa, whereas the method  $S_2$  yielded 7 taxa. *Pompholyx sulcata* was the dominant species among the Rotifera and its abundance in the sample collection methods  $S_1$ ,  $S_2$  and  $R$  was 756 ind.  $\text{dm}^{-3}$ , 412 ind.  $\text{dm}^{-3}$  and 598 ind.  $\text{dm}^{-3}$ , respectively.

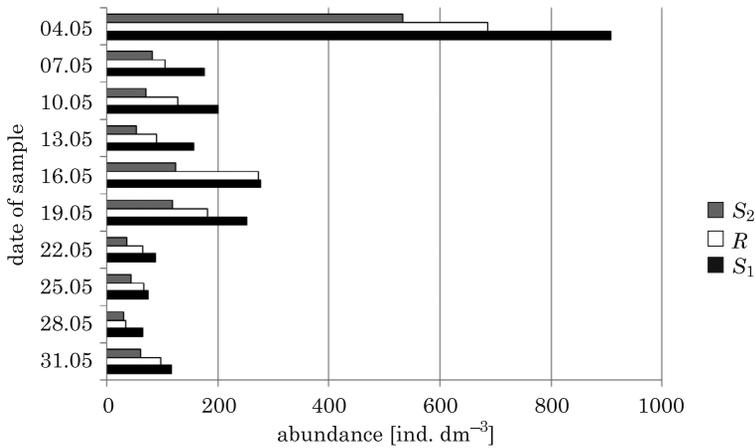


Fig. 2. Dynamics of the Copepoda abundance on the sample collection days using three sampling methods ( $S_1$ ;  $S_2$  and  $R$  – Materials and Methods)

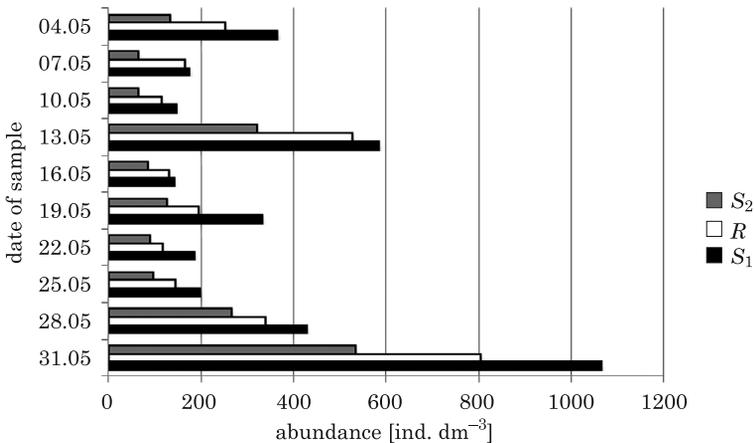


Fig. 3. Dynamics of the Rotifera abundance on the sample collection days using three sampling methods ( $S_1$ ;  $S_2$  and  $R$  – Materials and Methods)

The experiment was conducted in the water ranging from 12.9°C to 18.8°C (Figure 4a). The dissolved oxygen content oscillated from 11.6 mg dm<sup>-3</sup> to 7.5 mg dm<sup>-3</sup>, and the pH ranged from 7.3 to 8.3. The visibility of Secchi disc ranged from 1.9 m to 3.6 m. Independently of the sample collection method, statistically insignificant correlations were noted between the zooplankton abundance and the physical-chemical parameters of water ( $P < 0.05$ ). Significant linear correlations were noted between the water temperature and its transparency and dissolved oxygen quantity. The visibility of the Secchi disc increased together with increasing water temperature ( $r = 0.912$ ). Statistically

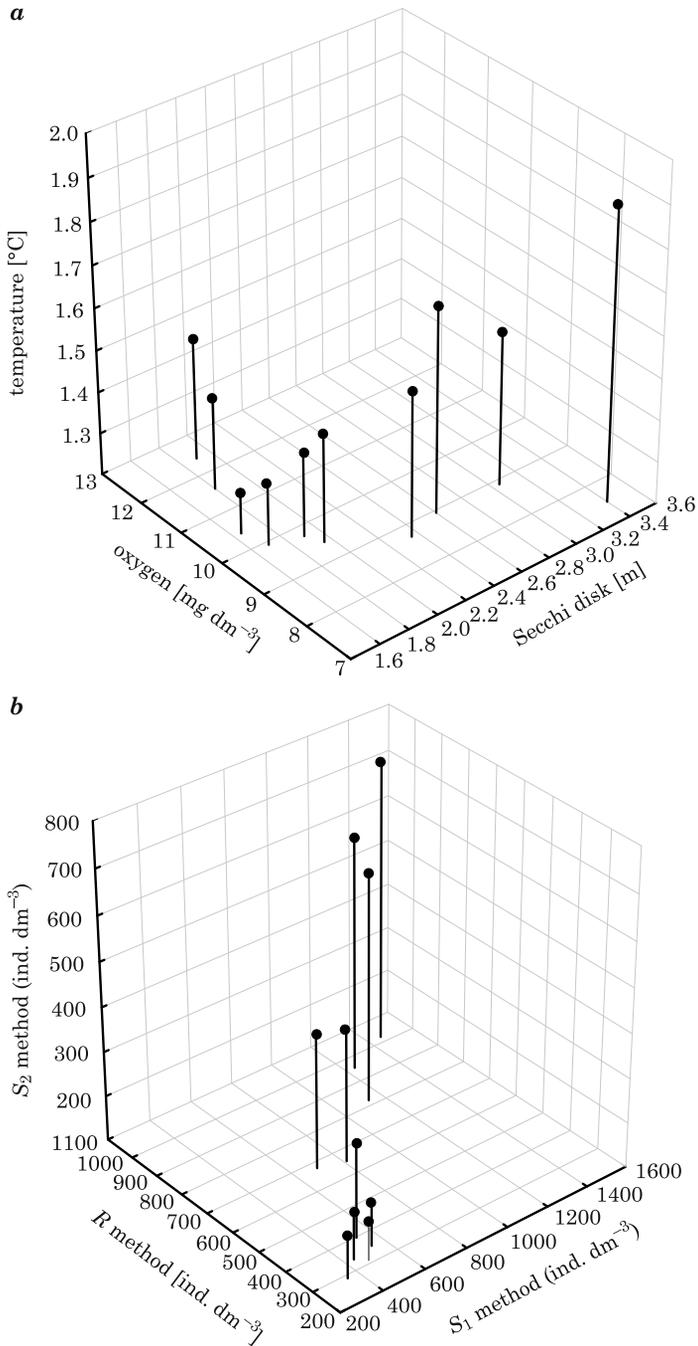


Fig. 4. Correlations between water temperature, dissolved oxygen and Secchi's visibility disc (a) and zooplankton quantities using three different sample collection methods (b)

significant linear correlations were also noted between the zooplankton quantities, which have been shown in all applied methods (Figure 4b). The strongest correlations were noted in the zooplankton abundance in samples collected using the  $R$  and  $S_2$  methods ( $r = 0.982$ ) as well as  $S_1$  and  $S_2$  methods ( $r = 0.979$ ).

A total of 32 zooplankton taxa and forms have been identified on 10 sample collection days (Table 1). The most numerous were the Rotifera (13 species). Crustaceans were represented by 8 species of Cladocera and 11 Copepoda taxa (including 3 juvenile forms). The most frequently found zooplankton taxa in the samples were: 3 Rotifera species (*K. longispina*, *K. cochlearis* and *P. longiremis*), 5 species and forms of Copepoda (*E. graciloides*, *T. crassus*, nauplius forms, copepodit *Calanoida* and copepodit *Cyclopoida*) and 1 Cladocera species (*D. cucullata*). The use of three different sample collection methods yielded variable detection of taxonomic groups and diversification of the average number of individuals. The comparative analysis revealed that the  $S_1$  method was the most effective in that regard. On the contrary, the  $S_2$  method was the least effective as it did not confirm the presence of Cladocera taxon on 9 occasions (twice: *Acroperus harpae*, *Eurycercus lamellatus* *Polyphemus pediculus*; once: *Bosmina coregoni*, *Diaphanosoma brachyurum*, *Leptodora kindtii*) and Copepoda on 5 occasions (twice: *Eucyclops macrurus*; once: *Trichocerca similis*, *Cryptocyclops bicolor*, *Mesocyclops leuckarti*). Whereas on two occasions the  $R$  method did not confirm the presence of Cladocera species (once: *Acroperus harpae*, *Leptodora kindtii*) and Copepoda taxa (once: *Cryptocyclops bicolor*, copepodit *Calanoida*). Only in the case of Rotifera species the  $S_1$  and  $R$  methods had highest detection effectiveness. Samples collected using the  $S_2$  method did not contain Rotifera taxa on three occasions (*Brachionus angularis*, *Euchlanis dilatata*, *Polyarthra longiremis*). The largest average abundance of organisms per volume was obtained using the  $S_1$  method. Whereas the  $S_2$  method indicated the least average density of Rotifera i Copepoda in the rearing cages. In case of 4 Cladocera species (*A. harpae*, *B. coregoni*, *D. brachyurum*, *P. pediculus*), the smallest average density was obtained using the bottle sampler method ( $R$  method).

The highest mean  $SAI_{tmi}$  values for the species in the Cladocera, Copepoda and Rotifera taxonomic groups were noted using the  $S_1$  sample collection method:  $0.98 (\pm 0.04)$ ,  $0.99 (\pm 0.03)$  and  $0.97 (\pm 0.11)$ . The highest maximal  $SAI_{tmi}$  values (1.0) were also noted in the  $S_1$  method, ranging from 91.8% of Copepoda to 83.3% of Rotifera (Table 2). Whereas in the  $S_2$  sample collection method, there was a minimal amount of maximal  $SAI_{tmi}$  only in the Rotifera group (2.4% of all analyzed cases). Using the sample collection method  $R$ , the maximal abundance of Cladocera, Copepoda and Rotifera species in 17.8%, 10.9% and 16.7% of all observations, respectively. The statistical analysis

Table 1  
Structure of the zooplankton and the effectiveness of species identification in the three sample collection methods

Taxon	Average abundance ( $\pm$ SD) in the sample collection method (ind. dm <sup>-3</sup> )			Identification number in the sample collection method		
	<i>S</i> <sub>1</sub>	<i>S</i> <sub>2</sub>	<i>R</i>	<i>S</i> <sub>1</sub>	<i>S</i> <sub>2</sub>	<i>R</i>
<b>Rotifera</b>						
<i>Ascomorpha ovalis</i>	6.0 ( $\pm$ 5.3)	2.7 ( $\pm$ 1.2)	3.0 ( $\pm$ 2.0)	3	3	3
<i>Asplanchna priodonta</i>	36.7 ( $\pm$ 49.0)	21.2 ( $\pm$ 24.7)	28.0 ( $\pm$ 35.4)	6	6	6
<i>Brachionus angularis</i>	7.0 ( $\pm$ 5.9)	2.4 ( $\pm$ 1.9)	4.0 ( $\pm$ 2.7)	5	4	5
<i>Brachionus calyciflorus</i>	28.0 ( $\pm$ 24.0)	7.0 ( $\pm$ 7.1)	20.0 ( $\pm$ 22.6)	2	2	2
<i>Conochilus unicornis</i>	52.1 ( $\pm$ 116.4)	31.6 ( $\pm$ 73.0)	50.5 ( $\pm$ 118.3)	8	8	8
<i>Euchlanis dilatata</i>	3.0 ( $\pm$ 0.0)	1.0 ( $\pm$ 0.0)	1.5 ( $\pm$ 0.7)	2	1	2
<i>Filinia longiseta</i>	14.5 ( $\pm$ 2.1)	3.6 ( $\pm$ 0.9)	9.4 ( $\pm$ 3.7)	2	2	2
<i>Kellicottia longispina</i>	14.1 ( $\pm$ 10.7)	6.9 ( $\pm$ 4.7)	13.2 ( $\pm$ 11.7)	10	10	10
<i>Keratella cochlearis</i>	92.5 ( $\pm$ 79.1)	40.2 ( $\pm$ 33.5)	61.6 ( $\pm$ 47.6)	10	10	10
<i>Keratella quadrata</i>	17.6 ( $\pm$ 11.6)	7.9 ( $\pm$ 6.0)	14.5 ( $\pm$ 8.4)	8	8	8
<i>Polyarthra longiremis</i>	23.0 ( $\pm$ 20.5)	8.9 ( $\pm$ 6.8)	17.1 ( $\pm$ 18.2)	10	9	10
<i>Pompholyx sulcata</i>	234.0 ( $\pm$ 302.6)	129.4 ( $\pm$ 167.1)	185.8 ( $\pm$ 240.8)	5	5	5
<i>Synchaeta sp.</i>	10.3 ( $\pm$ 4.9)	3.7 ( $\pm$ 1.5)	7.7 ( $\pm$ 3.8)	3	3	3
<b>Copepoda</b>						
<i>Trichocerca similes</i>	7.8 ( $\pm$ 6.8)	3.8 ( $\pm$ 1.7)	6.0 ( $\pm$ 5.5)	5	4	5
<i>Cryptocyclops bicolor</i>	8.6 ( $\pm$ 8.1)	1.6 ( $\pm$ 0.5)	4.6 ( $\pm$ 2.6)	7	6	6
<i>Cyclops strenuous</i>	12.0 ( $\pm$ 5.0)	6.0 ( $\pm$ 1.7)	6.3 ( $\pm$ 1.2)	3	3	3
<i>Cyclops vicinus</i>	25.0 ( $\pm$ 17.3)	14.8 ( $\pm$ 11.8)	17.3 ( $\pm$ 14.0)	4	4	4
<i>Eucyclops macrurus</i>	3.0 ( $\pm$ 1.4)	–	2.5 ( $\pm$ 0.7)	2	–	2
<i>Eudiaptomus graciloides</i>	20.5 ( $\pm$ 24.5)	9.8 ( $\pm$ 16.5)	14.2 ( $\pm$ 19.4)	10	10	10
<i>Mesocyclops leuckarti</i>	13.6 ( $\pm$ 11.9)	8.3 ( $\pm$ 5.7)	8.8 ( $\pm$ 5.3)	5	4	5
<i>Thermocyclops crassus</i>	16.7 ( $\pm$ 9.9)	5.6 ( $\pm$ 3.4)	9.9 ( $\pm$ 7.5)	10	10	10
Nauplius	88.3 ( $\pm$ 121.4)	53.3 ( $\pm$ 85.5)	78.2 ( $\pm$ 114.4)	10	10	10
Kopepodit <i>Calanoida</i>	15.0 ( $\pm$ 23.0)	3.9 ( $\pm$ 4.6)	9.6 ( $\pm$ 15.5)	10	10	9
Kopepodit <i>Cyclopoida</i>	58.5 ( $\pm$ 87.2)	28.9 ( $\pm$ 49.8)	42.3 ( $\pm$ 57.0)	10	10	10
<b>Cladocera</b>						
<i>Acroperus harpae</i>	2.2 ( $\pm$ 1.7)	3.0 ( $\pm$ 0.0)	2.5 ( $\pm$ 2.1)	3	1	2
<i>Bosmina coregoni</i>	19.8 ( $\pm$ 16.1)	9.8 ( $\pm$ 6.1)	8.9 ( $\pm$ 7.6)	5	4	5
<i>Bosmina longirostris</i>	41.8 ( $\pm$ 31.2)	14.3 ( $\pm$ 11.3)	21.5 ( $\pm$ 13.6)	8	8	8
<i>Daphnia cucullata</i>	115.1 ( $\pm$ 139.7)	70.8 ( $\pm$ 104.0)	95.9 ( $\pm$ 123.4)	10	10	10
<i>Diaphanosoma brachyurum</i>	7.1 ( $\pm$ 2.6)	6.5 ( $\pm$ 6.4)	5.0 ( $\pm$ 3.0)	3	2	3
<i>Eurycercus lamellatus</i>	3.0 ( $\pm$ 1.4)	–	1.5 ( $\pm$ 0.7)	2	–	2
<i>Leptodora kindtii</i>	10.9 ( $\pm$ 6.1)	5.8 ( $\pm$ 5.1)	9.6 ( $\pm$ 8.1)	9	8	8
<i>Polyphemus pediculus</i>	5.8 ( $\pm$ 3.3)	3.0 ( $\pm$ 2.8)	2.3 ( $\pm$ 1.3)	4	2	4

Sample collection methods: *S*<sub>1</sub> – plankton net (slow vertical haul); *S*<sub>2</sub> – plankton net (fast vertical haul); *R* – bottle sampler

(ANOVA, Kruskal-Wallis test) of mean  $SAI_{tmi}$  values in the  $S_1$  and  $S_2$  collection methods did not reveal statistically significant differences between the taxonomic groups ( $P > 0.05$ ). Such differences ( $H = 6.48$ ;  $P < 0.05$ ) were noted only in the sample collection method  $R$ , appearing only between the taxa Cladocera (mean  $SAI_{tmi}$  value =  $0.59 (\pm 0.27)$ ) and Rotifera (mean  $SAI_{tmi}$  value =  $0.71 (\pm 0.21)$ ).

Table 2

The characteristics of standardized abundance index of the zooplankton in the three sample collection methods

Methodical variant	Parameter	Unit of measure	Cladocera	Copepoda	Rotifera
$S_1$	$SAI_{tmi}$	$\bar{x} (\pm SD)$	$0.98 (\pm 0.04)^A$	$0.99 (\pm 0.03)^A$	$0.97 (\pm 0.11)^A$
	$SAI_{tmi}max$	%	86.7	91.8	83.3
$S_2$	$SAI_{tmi}$	$\bar{x} (\pm SD)$	$0.35 (\pm 0.28)^A$	$0.41 (\pm 0.22)^A$	$0.43 (\pm 0.23)^A$
	$SAI_{tmi}max$	%	–	–	2.4
$R$	$SAI_{tmi}$	$\bar{x} (\pm SD)$	$0.59 (\pm 0.27)^A$	$0.66 (\pm 0.27)^{AB}$	$0.71 (\pm 0.21)^B$
	$SAI_{tmi}max$	%	17.8	10.9	16.7

Sample collection methods:  $S_1$  – plankton net (slow vertical haul);  $S_2$  – plankton net (fast vertical haul);  $R$  – bottle sampler; the mean of  $SAI_{tmi}$  with the same letter index are not statistically different ( $P < 0.05$ )

The analysis of the mean  $SAI_{tmi}$  values obtained using the three sample collection methods, reveals statistically significant differences in each taxonomic group (Figure 5). The highest statistical significance was noted among the Rotifera ( $H = 154.8$ ;  $P < 0.001$ ) and Copepoda ( $H = 142.9$ ;  $P < 0.001$ ) groups. In the Cladocera group, the mean  $SAI_{tmi}$  values were significantly different at the level of  $P < 0.01$ . Using the test statistics value  $H = 80.6$ , the lower statistical significance of the differences among the Cladocera group is a reflection of the relatively high standard deviation values for the sampling methods  $S_2$  and  $R$ :  $0.35 (\pm 0.28)$  and  $0.59 (\pm 0.27)$ , respectively. This phenomenon is directly related to the highest number of undetected species in the Cladocera group using the sample collection method  $S_2$ .

## Discussion

The seasonal and cyclical abundance of zooplankton is typical for all types of waters in various climate zones. This phenomenon is directly related to numerous, previously described abiotic and biotic factors, particularly water temperature and eutrophy (WANG et al. 2007, SUTHERS and RISSIK 2009). Our study was based on two sample methods commonly used in open water plankton research (DHARGALKAR and VERLECAR 2004). Due to the peculiarity

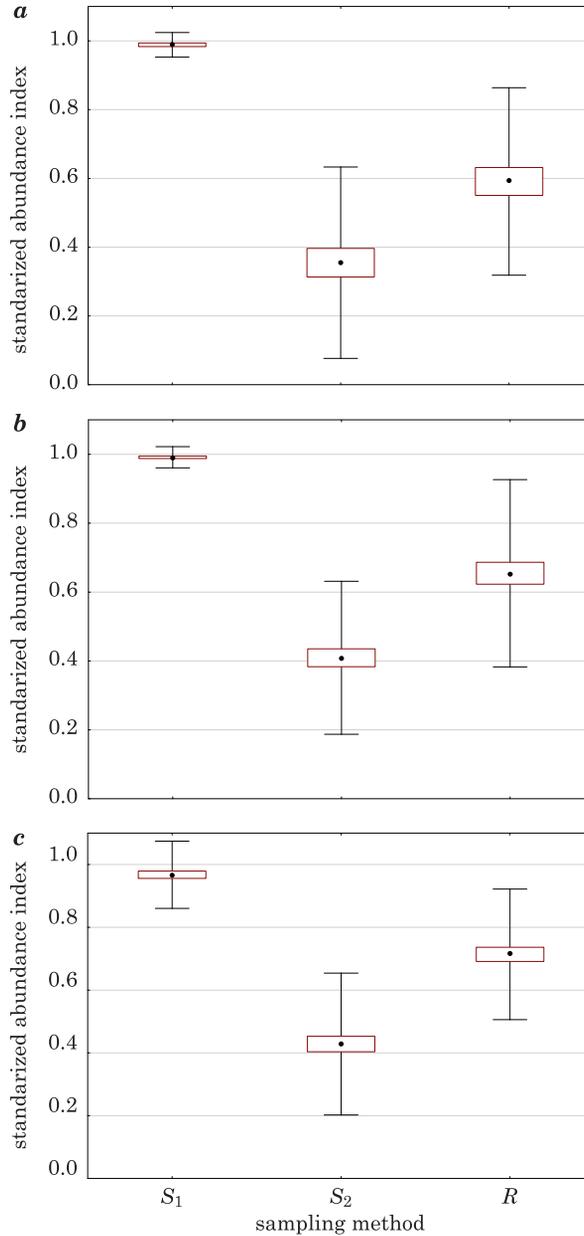


Fig. 5. Mean values of  $SAI_{tmi}$  with SE (frame) and SD (whiskers) for the species in taxonomic group Cladocera (*a*), Copepoda (*b*) and Rotifera (*c*) in the particular collection methods ( $S_1$ ;  $S_2$  and  $R$  – Materials and Methods). The means  $SAI_{tmi}$  values for Copepoda and Rotifera are significantly different at the level of  $P < 0.001$ , and for Cladocera at the level  $P < 0.01$  (ANOVA, Kruskal-Wallis test)

of the illuminated surface cages (volume, zooplankton aggregation, free access, technological manipulation), the use of other tools and methods seems to be unjustified. The experiment was performed while maintaining the fish rearing technology standards in illuminated cages. The mesh size 1.2 mm of net cage has been used in koregonid rearing (MAMCARZ 1995a). This mesh size is not limited penetration of freshwater plankton inside the net cages (CECCUZZI et al. 2010, FURGALA-SELEZNIOW et al. 2014). In experimental cages were identified large planktonic organisms, eg. *Leptodora kindtii*.

The results obtained using the plankton net ( $S_1$  and  $S_2$  methods) demonstrate that despite travelling the same distance (water column), the amount of effectively filtered water was greater in the  $S_1$  method. The expected filtration rate of the  $S_1$  method was about  $1.9 \text{ dm}^3 \text{ s}^{-1}$ , while in the  $S_2$  method was about  $3.8 \text{ dm}^3 \text{ s}^{-1}$ . The slower rate of real filtration in the  $S_2$  method pushed the excess water outside and made hydrodynamic whirlpools. It is not possible to unequivocally determine that the net moved at an optimal speed in the  $S_1$  method. A comparison of the zooplankton abundance on each of the sample collection days suggests that if theoretically, the net filtered  $76 \text{ dm}^3$  of water during slow towing ( $S_1$  method), then in the  $S_2$  method it filtered about  $30 \text{ dm}^3$ . Our direct observations support this thesis. Using the  $S_2$  method in an illuminated cage after dusk, it was observed that water was pushed upwards above the plankton net. Zooplankton forms (visible with the naked eye) were displaced outside the inlet together with the excess water. On the contrary, such escape of the zooplankton was not observed in the  $S_1$  method.

Considering the hydromechanics theory, in an aquatic environment the filtration rate is directly related to the factors determining the extent of hydrodynamic resistance (LANDAU and LIFSZYC 2009). In practice, this means that with constant water density and plankton net porosity, the filtration rate will be inversely proportional to the hauling speed. According to HERON (1968), the effectiveness of this filtration is dependent on the net's material and mesh knots. It has also been observed that during sample collection using the plankton net, the effective filtration declines due to the mesh clogging by the filtered organisms (KNOECHEL and CAMPBELL 1992). Using the same hauling speed, the zooplankton collection can be more effective using a smaller diameter inlet or a larger filtration surface (TRANter and SMITH 1968). Research on the optimal filtration and reduction of water turbulence in front of the plankton net was conducted also in flume tanks (MAHNKEN and JOSSI 1967). However in such controlled conditions it is not possible to account for the numerous factors, including those responsible for the reduced filtration rate during mesh clogging. As shown by research on zooplankton sample collection methods in various environments, many of the solutions designed and tested on models are not effective in the field (DE BERNARDI 1984, MASSON

2004, SEMENOVA 2011). In the riverine zooplankton research by SLUSS et al. (2011), three sample collection methods were used (alpha bottle sampler, manual bilge pump and Schindler trap) and the least abundant samples were collected using the Schindler trap. This tool was the least effective in collecting smaller zooplankton forms (*Bosmina* sp. and *Keratella* sp.).

Many previously published studies note the reduced effectiveness of zooplankton collection using a plankton net in comparison to other methods, particularly the calibrated traps (trap sampler). According to KANKAALA (2007), the plankton net's effectiveness was comparable to 75% of the trap's effectiveness, specifically in the case of Copepoda (41%), Cladocera (51%) and Rotifera (66%). This author does not recommend the use of plankton net in quantitative research due to the probability of larger crustaceans avoiding/escaping the net as well as the inability of the net to contain the small and soft-bodied Rotifera. Lesser effectiveness of the plankton net, as compared to the Ruttner sampler, was also demonstrated in research on the Vistula Lagoon zooplankton (GUTKOWSKA et al. 2012).

The results we obtained using a plankton net hauled at a high speed and a bottle sampler might confirm the previously published conclusions (KANKAALA 2007, GUTKOWSKA et al. 2012). However, it must be noted that in many cases the unsatisfactory effectiveness of the plankton net results from the inability to match the hauling speed to the effective filtration rate. This is demonstrated in our results obtained using slow hauling speed as well as by the previously published report of high effectiveness of the plankton net in comparison to other sample collection methods (WIKTOR 1982). Based on the analysis of a wide range of study results, none of the zooplankton sample collection methods is universally-applicable. On the contrary, each of them is usually dedicated to specific environmental conditions (PAGGI et al. 2001). The effectiveness of sample collection and the reliability of the obtained results continues to be largely dependent on the accurate selection of methods and the researcher's manual abilities (LANGFORD 1953, MACK et al. 2012).

## Conclusions

The results of this study allow an objective comparison of these three sample collection methods' effectiveness. Using each sample collection method in two identical settings allows a comparison using the normalized SAI. In addition, the environment of < 2 m deep cage illuminated at night (above surface 60W bulb) allows the analysis of relatively large samples and direct visual control of the vertical haul with minimal influence of water waving and movement on the zooplankton distribution.

In terms of the abundance and detection of taxa, the results of this study demonstrate that the reference method of zooplankton sample collection in illuminated cages is slow vertical haul using a plankton net. This was determined by the greatest abundance of each taxonomic group at the statistical level  $P < 0.001$  for Copepoda and Rotifera and  $P < 0,01$  for Cladocera. Average effectiveness was observed for the TON bottle sampler. The obtained results indicate that a 5 dm<sup>3</sup> water sample collected using the TON bottle sampler in the water column at the depth of 0.8–1.2 m allows a relatively precise qualitative assessment of the zooplankton in an illuminated cage. However, this method does not fully reflect the true abundance, confirming that the zooplankton is not uniformly distributed in an illuminated environment. The reduced density and underestimated abundance of the zooplankton in the samples results from the insufficient volume of the bottle sampler and the single sample collection point. The results might be more reliable if the samples were collected using the bottle sampler at different depths. Regardless, the water sampler produces turbulent flow during towing and alters the distribution of zooplankton congregated around the light source. This excludes the bottle sampler as an effective method. However, effectiveness of slow vertical haul is a reflection of the filtration efficiency. With a constant mesh size of conical tow-net, it will be dependent on planctonic organisms density and its species' structure, as well as of water temperature.

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## **ANALYSIS OF THE VISITOR FLOW AND FOREST EDUCATION ACTIONS IN KUDYPY FOREST DISTRICT**

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**Key words:** forest education, natural environment protection, forest recreation, forest tourism.

### **A b s t r a c t**

Kudypy Forest District lies in the Province of Warmia and Mazury, in the vicinity of Olsztyn, the capital city of the province. The surface area of this District is almost 18 thousand ha. Kudypy Forest District belongs to the Forest Promotional Complex called the Olsztyn Forests, which covers the areas of two forest districts: Kudypy and Olsztyn. Kudypy Forest District is composed of 13 forestries and the Forest Arboretum of Warmia and Mazury, which is the main educational facility. Arboretum is also the main attraction for visitors. Kudypy Forest District has created three educational trails along key features. There are also jogging and nordic-walking trails set up in Kudypy Forest District. The outdoor classrooms are situated in four forestries. The most popular type of educational activities accomplished by Kudypy Forest District in 2012–2015 were field lessons and guided trips.

The engagement of Kudypy Forest District's employees in forest education generates beneficial outcomes. The interest among children and their participation in various forms of recreation and forest education has risen over the past years in connection with increasing of the number of the Forest District's employees involved in the organization of such recreational and educational activities. The participation of adult persons has remained on a stable and relatively high level.

**ANALIZA RUCHU ODWIEDZAJĄCYCH I DZIAŁAŃ ZWIĄZANYCH Z EDUKACJĄ LEŚNĄ  
W NADLEŚNICTWIE KUDYPY**

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Słowa kluczowe: edukacja leśna, ochrona przyrody, sylwarekrecja, sylwaturystyka.

**Abstrakt**

Nadleśnictwo Kudypy położone jest w bezpośrednim sąsiedztwie Olsztyna – stolicy województwa warmińsko-mazurskiego, a jego powierzchnia wynosi prawie 18 tysięcy ha. Wraz z Nadleśnictwem Olsztyn tworzy ono Leśny Kompleks Promocyjny „Lasy Olsztyńskie”. W skład Nadleśnictwa Kudypy wchodzi 13 leśnictw oraz Leśne Arboretum Warmii i Mazur, które jest jego najważniejszym obiektem turystyczno-edukacyjnym. Nadleśnictwo Kudypy utworzyło trzy ścieżki edukacyjne, wyznaczyło ścieżkę biegową i trasę nordic-walking. W czterech leśnictwach organizowane są zielone klasy. Nadleśnictwo prowadzi różnego rodzaju akcje i imprezy edukacyjne, z których najpopularniejsze w latach 2012–2015 były lekcje terenowe i wycieczki z przewodnikiem.

Uzyskane w pracy wyniki wskazują, że zaangażowanie pracowników Nadleśnictwa Kudypy przynosi pozytywne efekty. Udział dzieci w wieku szkolnym (7–12 lat) w różnych formach rekreacji i edukacji leśnej w badanym okresie wzrósł wraz ze wzrostem liczby pracowników nadleśnictwa zaangażowanych w działalność edukacyjną. Zainteresowanie osób dorosłych sylwarekrecją i edukacją leśną pozostawało na stałym poziomie.

**Introduction**

Nature-based tourism is the fastest growing tourism sector not only nationally, but also globally (KUENZI and MCNEELY 2008). Environmental attitudes of tourists are heterogenous across tourism motivations. According to LUO and DENG (2007), specifically those people who are more supportive of the natural environment protection tend to have a higher desire to be close to nature, to learn about nature, and to escape from routine and issues associated with cities. Forest tourism and recreational activities are a very attractive and unique type of nature-based tourism (MUSZYŃSKI and KOZIOŁ 2012). Provision and management of the forest to fulfil the function of tourism and recreation are important because residents of urban areas show a massive demand for leisure activities in the forest (PIENKOS and KIKULSKI 2005). Forest managers became the people-ecosystem managers in order to meet societie’s demands (*European Forest Recreation and Tourism...* 2009). Socio-economical transition

has brought changes in the recreational use of forests during last 50 years (CIESZEWSKA 2008). Owing to their tourism-related assets, forests attract tourists, with their estimated number in Poland being around 30 million annually (PIEŃKOS and KIKULSKI 2005). Forest tourism and recreational activities need to be put in the context of other uses of the forest in order to assess their complementarity or conflict (FONT and TRIBE 2000).

In Poland, changes in the functions performed by forests from production to social ones began in the second half of the 20<sup>th</sup> century. According to WAŻYŃSKI (1997), the year 1961 marks the beginning of the recreational management of forests. The obligation to separate some wooded areas for recreational purposes was implemented in Poland in the third edition of the Principles of Silviculture in 1969. According to PIGAN (2012), the first educational sites in Polish forests were created in the 1970s. They first appeared in national parks and in some forest districts.

The Act on Forests of 1991 with subsequent amendments ensure public access to forests owned by the State Treasury (Ustawa z 28 września 1991... Dz.U., 1991 poz. 444). This act contains provisions defining the recreational use of forests and forest education of the general public. The Principles of Silviculture (*Zasady hodowli...* 2012) define the guidelines for the recreational management of forests. Article 54 of the Act on Forests (Ustawa z 28 września 1991 ... Dz.U., 1991 poz. 444) states that the State Forests National Forest Holding (in brief State Forests) are eligible to obtain earmarked subsidies to educate the community by creating and managing Promotional Forest Complexes and by setting up educational trails. The State Forests are obligated to make forests available for social purposes (recreation and tourism).

Meanwhile, the State Forests are obligated to protect forest ecosystems from negative consequences of the recreational use of wooded areas (WAŻYŃSKI 1997). Forest education plays an important role in attempts to achieve these aims. The State Forests in Poland undertake many actions in this respect. PIGAN (2011) mentions some cases of breaching the law by individuals pursuing recreational and tourism activities. One of the essential causes is the lack of knowledge about forest ecosystems. Forest education performs an important role in counteracting irresponsible actions, as it contributes to an improved awareness of ecological issues among members of the general public.

Because of their organizational structure, many activities pursued by the State Forests take place in forest districts. This stems from the provisions of the Act on Forests, i.e. Article 35 (Ustawa z 28 września 1991... Dz.U., 1991 poz. 444), which specifies that the head of a forest district has a certain measure of independence in implementing forest management activities, according to the binding forest management plan and is responsible for the condition of a forest in the district (PERLIŃSKA and ROTHERT 2013). The State

Forests strive towards the most convenient forms of making forests accessible to the general public, and protect wooded areas in terms of ecological sustainability and natural conservation (KOZIOŁ and MUSZYŃSKI 2009). Nature-based recreation and tourism make an important contribution to social sustainability.

The social benefits of Kudypy Forest District activities were studied. The main aim of this study was to examine the use of forests for the purposes of tourism and recreation in Kudypy Forest District, based on an analysis of activities carried out by foresters and on a review of the actual visitor management practices.

## Material and Method

### Description of the research area

Kudypy Forest District lies in the Province of Warmia and Mazury, in the vicinity of Olsztyn, the capital city of the province, and is situated in the area subjected to the Regional Directorate of the State Forests in Olszyn. The geographical coordinates are as follows: 53°32'00" – 53°54'00" N, 20°05'00" – 20°30'00" E. The surface area of this district is 17 927.91 ha, of which afforested land represents 88.73% (*Plan urzędzenia...* 2014). The forest district is composed of 13 forestries and the Polish Silviculture Society Forest Arboretum of Warmia and Mazury. In addition to this, the forest district runs a forest nursery and the Bobry Wildlife Breeding Centre. Hydrographically, the area covered by Kudypy Forest District lies in the catchments of the Łyna River and Pasłęka River, drained to the Vistula Lagoon. There are several large lakes situated within the forest district, e.g. Ukiel, Wulpińskie, Limajno, Rentyńskie and Kortowskie. The dominant tree species creating the forest cover is the pine, which constitutes nearly 67% of all tree stands. Other coniferous species are the spruce and the larch, which together overgrow nearly 7% of the district's area. Deciduous species (beech, birch, oak, alder, ash, and others) cover almost 27% of the total surface area (*Plan urzędzenia...* 2014). Kudypy Forest District is dominated by tree stands which belong to the 3<sup>rd</sup> and 2<sup>nd</sup> age class (*Nadleśnictwo Kudypy* 2016).

Kudypy Forest District harvests approximately 75 000 m<sup>3</sup> of timber year, most of which is bought by timber processing companies (*Nadleśnictwo Kudypy* 2016). The foresters allow the general public to take advantage of forest products in a way which ensures the sustainability of woods. There are six hunting associations active in the territory of Kudypy Forest District (oral information provided by Dariusz Zalewski, a member of a hunting association

“Darz Bór”). There are two areas of protected landscape, four areas of the Natura 2000 network and two nature reserves in Kudypy Forest District (Table 1). Furthermore, there are 13 objects designated as monuments of nature in this forest district (*Program ochrony...* 2014). The forms of nature protection and conservation found in this forest district also include plant and animal species protection. There are, for example, 11 protection zones delineated around bird nests, of which seven protect nests of the lesser spotted eagle, one is around a nest of the red kite and three surround nests of the white-tailed eagle (Kudypy Forest District, own data).

Forms of nature protection in Kudypy Forest District

Table 1

Name of the area	Surface area [ha]	Formal status
Protected Landscape Area of the Middle Łyna River Valley	15 307.8	Official Journal of Law of the Province of Warmia and Mazury (Rozporządzenie nr 160... Dz.Urz.Warmi. 2008, nr 201), supervision by the Governor of the Province of Warmia and Mazury
Protected Landscape Area of the Pasłęka River Valley	43 307.3	Official Journal of Law of the Province of Warmia and Mazury (Rozporządzenie nr 147... Dz.Urz.Warmi. 2008, nr 179), supervision by the Provincial Conservator of Nature
Special avian protection area Natura 2000, the Pasłęka River Valley, PLB280002	20 669.9	Official Journal of Law (Rozporządzenie Ministra Środowiska z 21 lipca 2004... Dz.U. 2004, poz. 2313)
Habitat protection area Natura 2000 Jonkowo-Warkaly, PLH280039	226.5	Decision of the European Commission 2011/64/EU
Habitat protection area Natura 2000 the Pasłęka River, PLH280006	8 418.5	
Habitat protection area Natura 2000 „Warmińskie Buczyzny” PLH280033	1 525.9	
Fauna reserve, a refuge of beavers on the Pasłęka River	4 249.2	Zarządzenie Ministra Leśnictwa... M.P. 1970, poz. 21
Forest reserve „Kamienna Góra”	95.14	Zarządzenie Ministra Ochrony Środowiska... M.P. 1995, poz. 83

Source: the authors based on the Forest Management Plan of the Kudypy Forest District (*Plan urządzenia lasu*. 2014), and the Catalogue of Natura 2000 areas (*Katalog obszarów Natura 2000*. 2016)

Kudypy Forest District belongs to the Forest Promotional Complex called the Olsztyn Forests, which covers the areas of two forest districts: Kudypy and Olsztyn, comprising in total 33 894 ha of forests as of 1 January 2011 (*Program gospodarczo-ochronny...* 2012). The objective of this forest complex is to promote sustainable silviculture management, to regenerate nature resources in forests and to provide the general public with education about forests.

## Research Methods

The research was based on an analysis of source documents and other reports and reviews. In addition to this, face-to-face interviews were carried out with employees of Kudypy Forest District and with a member of one of the local hunting associations. Own observations were also taken into consideration.

The following source documents were consulted:

- Act on Nature Conservation of 16 April 2004, Official Journal of Law (Ustawa z 16 kwietnia 2004 r. ... Dz.U. 2004, poz. 880),
- Act of 13 October 1995, the Hunting Law, Official Journal of Law (Ustawa z 13 października 1995 r. ... Dz.U. 1995, poz. 713),
- Act on Forests, 28 September 1991, Official Journal of Law (Ustawa z 28 września 1991 r. ... Dz.U. 1991, poz. 444),
- Regulation of the Minister of the Environment of 6 October 2014, on animal species protection, Official Journal of Law, (Rozporządzenie Ministra Środowiska z dnia 6 października 2014 r. ... Dz.U. 2014, poz. 1348),
- Regulation no 160, of the Governor of the Province of Warmia and Mazury, of 19 December 2008, on the Protected Landscape Area of the Middle Łyna River Valley, (Rozporządzenie nr 160... Dz.Urz.Warmi. 2008, nr 201),
- Regulation no 147, of the Governor of the Province of Warmia and Mazury, of 13 November 2008, on the Protected Landscape Area of the Pasłęka River Valley, (Rozporządzenie nr 147... Dz.Urz.Warmi 2008, nr 179).

The following documents were reviewed:

- Forest Management Plan, Kudypy Forest District, drawn for the period of 1 January 2014 to 31 December 2023, based on the state of the forest as of 1 January 2014 (*Plan urzędzenia...* 2014),
- Nature protection programme, as of 1 January 2014, Bureau for Forest Management and Geodesy, the Olsztyn Branch (*Program ochrony...* 2014),
- The nature management and protection programme for the Promotional Forest Complex called the Olsztyn Forests, 2012, the Regional Directorate of the State Forests in Olsztyn (*Program gospodarczo-ochronny...* 2012),
- Specification of forest recreational management facilities in the territory of the Regional Directorate of the State Forests Olsztyn in 2008, as of 31 May 2008,
- Reports of Kudypy Forest District on tourism and educational activities,
- The website of Kudypy Forest District.

## Results

### The recreational and tourism management of the forests in Kudypy Forest District

The Warmia and Mazury Forest Arboretum in Kudypy is one of the most important facilities in this area dedicated to recreation and tourism. It is a specialist botanic garden, which presents collections of arboreal plants. The employees of the arboretum are also engaged in research and education about nature (*Nadleśnictwo Kudypy* 2016). This garden is one of the seven arboreta owned by the State Forests, and fulfils the requirements set for botanic gardens. It belongs to the Council of Botanic Gardens and Arboreta and in 2005 was issued a permit by the Ministry of the Environment to operate as a botanic garden (KUSZEWSKA and RUTKOWSKA 2014). The main objective of the arboretum is to perform scientific, educational and social roles. Apart from holding collections of plants, the arboretum is dedicated to ecological education. It has suitable infrastructure, such as places designated for relaxation, bonfires and educational exhibitions, e.g. about devices used to control pest insects, breeding boxes for birds and bats, feeding racks for wild animals. In 2013–2015, the arboretum was visited by a total of 28 793 persons, most of whom (17 817) were children (Figure 1).

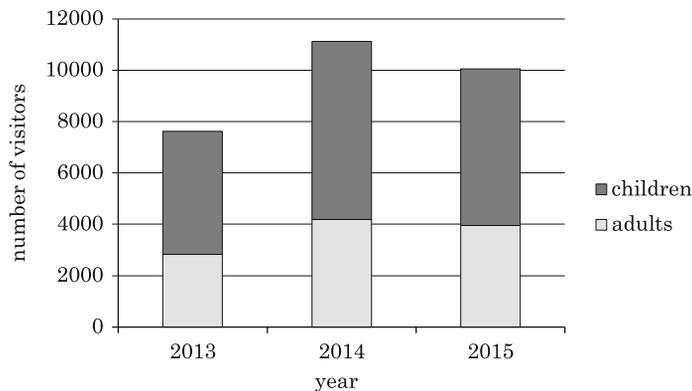


Fig. 1. The number of visitors to the arboretum garden in 2013–2015

The arboretum is divided into three sections: Polish flora, collections, and natural forest. The Polish flora section comprises a collection of trees and shrubs, which comprises around 300 species. The collections section contains over 700 species and varieties of trees and shrubs, of which the most numerous are maples and cotonaesters, as well as honeysuckles and wild roses.

The natural forest is a separated fragment of an old natural forest, with some trees designated the status of a monument of nature (*Program ochrony...* 2014). Moreover, the arboretum has a geological lapidarium, that is a collection of glacial stones and boulders from Warmia and Mazury. The supervision over the research and educational activities carried out in the Polish flora section of the Arboretum is maintained by the Department of Botany and Nature Protection, the University of Warmia and Mazury in Olsztyn, and the collections section is supervised by the head of the SSGW (University of Life Sciences in Warsaw) Arboretum in Rogowo (*Program ochrony przyrody* 2014). At present, the Arboretum functions as a separate forest district. It covers an area of 15.7 ha (as of 1 January 2014). At the Arboretum, there is a Visitor Centre of Forest Education, called Kudypka Polana. The centre has a teaching and conference room equipped with audio-visual equipment and seating 40 persons. There is also a small museum of silviculture and an exhibition room. Next to the building of the Visitor Centre of Forest Education, there is a playground with facilities for sports and recreation. Kudypy Forest District has started a mobile phone application called The Warmia and Mazury Forest Arboretum, which is a mobile device guide showing visitors around the botanic garden.

Kudypy Forest District has created three educational trails along key features (Table 2). The Kudypka Polana educational trail leads along a forest path from the arboretum to a picnic area located near the main building of the Forest District (KUSZEWSKA and RUTKOWSKA 2014). The picnic area features huts, benches, picnic tables and rubbish bins. There is also a place prepared for bonfires, two beach volleyball courts and a stage for outdoor artistic performances. The Arboretum-Dajtki trail can be followed on foot or by bicycle. This trail leads along forest paths from the arboretum in Kudypy to the outskirts of the town of Olsztyn, and its suburb called Dajtki. Along the trails, there are information boards and benches. The trail Uroczysko-Gietrzwałd lies near the

Table 2

Educational trails in Kudypy Forest District

Name of a trail	Key feature (theme)	Length of a trail [km]	Number of boards	Location
Kudypka Polana	selected animal species living in the Kudypy forests	1	9	Kudypki Forestry
Arboretum – Dajtki	silviculture	3	19	Kudypki Forestry
Uroczysko Gietrzwałd	nature protection and management	1	18	Żelazowice Forestry

Source: the authors, based on own field observations and information contained in the Guidebook to the Warmia and Mazury Forest Arboretum in Kudypy (KUSZEWSKA and RUTKOWSKA 2014)

village Gietrzwałd, in the vicinity of the Sanctuary of Virgin Mother. Along this trail, there are tables and benches, and the so-called green classroom (outdoor classroom), where educational classes can be held. Other outdoor classrooms are situated in the forestries Buki, Chmury, Redykajny and Różanka.

Apart from the educational trails, there are also jogging and nordic-walking trails set up in Kudypy Forest District. The jogging trails, 5.7 km and 4.4 km long, run in the vicinity of the arboretum. The trails were created as part of a project performed in collaboration with the Ministry of the Environment and titled "Freedom is in Nature". The nordic-walking trail was set up along the shores of Ukiel Lake, from the beach called Słoneczna Polana in Olsztyn to the village Łupstych (KUSZEWSKA and RUTKOWSKA 2014). Kudypy Forest District built six car parks for visitors pursuing forest recreational activities (Table 3).

Table 3

Car parks created by Kudypy Forest District

Forestry	Area [ha]	Location
Redykajny	0.08	near Redykajny Lake
Redykajny	0.48	near Redykajny Lake
Wrzesina	0.13	off the road Olsztyn–Łukta
Naglady	0.24	off the road Olsztyn–Ostróda
Arboretum	0.39	off the road Olsztyn–Ostróda
Stary Dwór	0.63	off the road Olsztyn–Warszawa

Source: Nature Protection Programme (*Program Ochrony Przyrody* 2014)

### **Analysis of the tourism and educational activities in the Forest District Kudypy**

The number and types of tourism and educational activities accomplished by Kudypy Forest District in 2012–2015 as well as the number of participants are presented in Table 4. In total, 560 different events were organized, which were participated in by 19 193 persons. The most popular were field lessons and guided trips, which together made up nearly 90% of all activities. They were attended by 78% of all participants (Table 4, Figure 2*a, b*). The percentages of participants in age groups can be seen in Figure 2*c*. The number of activities in years ranged from 122 in 2013 to 157 in 2014 (Figure 3). The highest number of participants (5595) was recorded in 2015. In the four years (2012–2015), the most numerous age group attending the events organized by Kudypy Forest District were children aged 7 to 12 years. It is caused by the fact that the primary schools organize trips for their pupils as part of environmental education. The second most numerous group consisted of persons over 19 years of age (Figure 3).

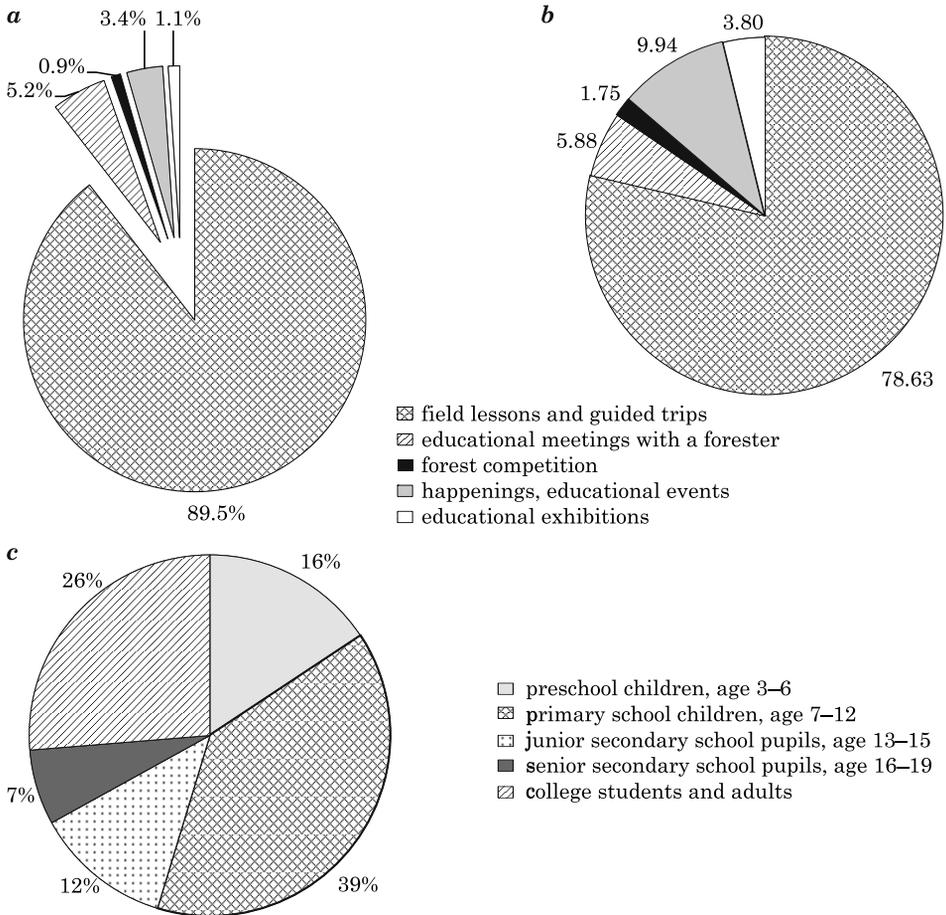


Fig. 2. Tourist and educational offer of Kudypy Forest District in 2012–2015 – structure of events (a), percentages of participants in particular events (b) and age structure of participants (c)

The number of field lessons and guided trips organized by Kudypy Forest District varied from 104 in 2013 to 142 in 2014. The total number of persons attending such activities in 2012–2013 was nearly 3 000 (Figure 4). In 2014, there was a considerable increase in the number of attendees – by over 50%. In 2015, the number of participants remained comparable to that recorded in 2014. The highest increase was noted in the group of primary school pupils and lower secondary school pupils. The number of these participants rose by almost 100% between the years 2012–2013 and 2014–2015.

Table 4  
Forms of the forest education addressed to the general public and carried out by Kudypy Forest District, attendance in 2012–2015

Forms of forest education	Number of events	Number of participants	Preschool children	Primary school children	Junior secondary school pupils	Senior secondary school pupils	College students and adults
Field lessons and guided trips	501	15 092	2 542	5 797	2 145	1 167	3 441
Educational meetings with a forester	29	1 128	293	692	54	0	89
Forest competitions	5	336	0	236	0	0	100
Happenings, educational events	19	1 907	100	510	130	92	1 075
Educational exhibitions	6	730	80	250	30	30	340
<b>Total</b>	<b>560</b>	<b>19 193</b>	<b>3 015</b>	<b>7 485</b>	<b>2 359</b>	<b>1 289</b>	<b>5 045</b>

Source: the authors, based on reports of Kudypy Forest District regarding tourist and educational activities

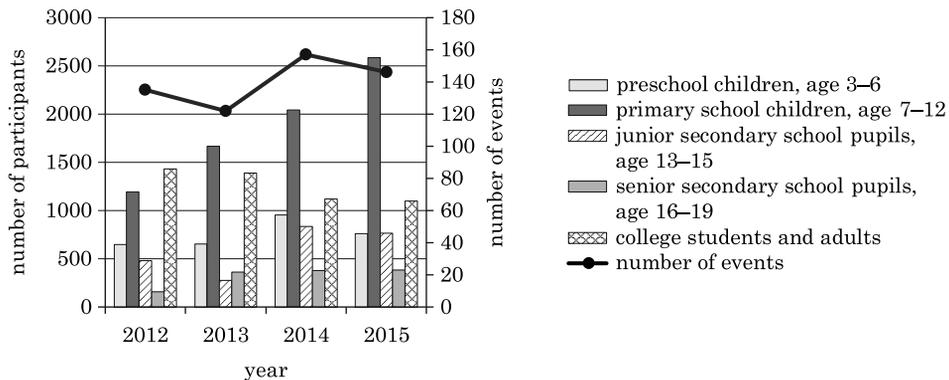


Fig. 3. Tourist and educational activities organized by Kudypy Forest District in 2012–2015, number of participants, divided into age groups

The number of educational meetings with a forester organized by Kudypy Forest District in 2012–2015 ranged from 6 to 9 (Figure 5). Such meetings are mostly held at schools. The highest number of participants was noted in 2012 (350 persons), and the lowest one was in 2013 (219 persons). In 2012, 2013 and 2015, the most numerous group of participants were children aged 7 to 12 years, while in 2014 preschool children were more numerous.

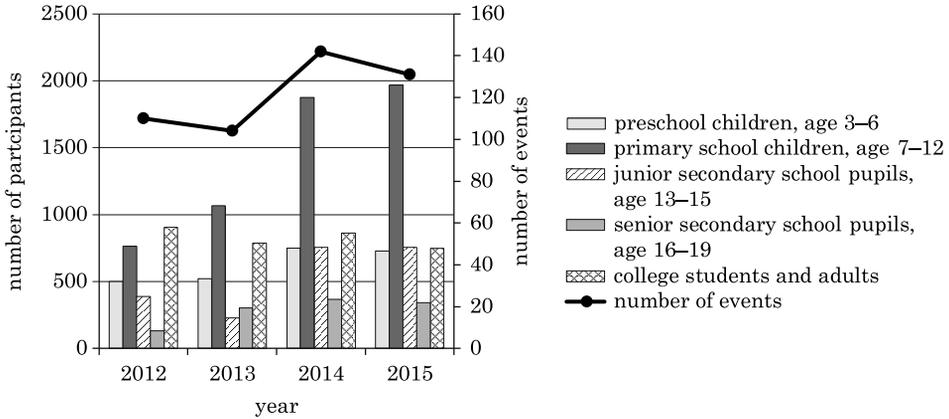


Fig. 4. Field lessons and guided trips organized by Kudypy Forest District in 2012–2015 – number of events and number of participants, divided into age groups

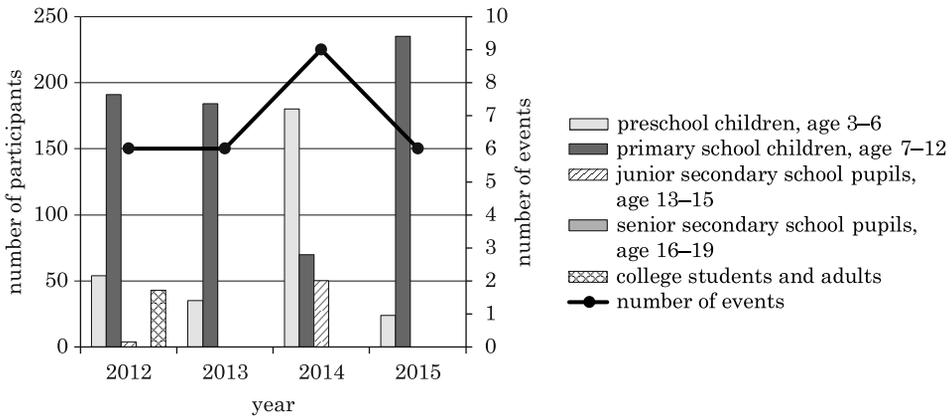


Fig. 5. Educational meetings with a forester organized by Kudypy Forest District – number of events and number of participants, divided into age groups

The number of happenings and educational events organized by Kudypy Forest District in 2012–2015 ranged from 2 in 2012 to 8 in 2013 (Figure 6). The following events were held: “Night of Owls”, “Search for Signs of the Spring in the Arboretum”, “A Big Day of Bees”, and a cross-country run “Freedom is in Nature”. These events were definitely attended most numerously by adults (in total 1075 persons). The number of adult participants in individual years was quite stable (210 to 340 persons). The second most numerous group was composed of children between 7 and 12 years of age (in total 510). In 2012–2014, the number of participants who belong to this age group did not exceed 100 persons a year (from 45 to 85). However, the number of attendees

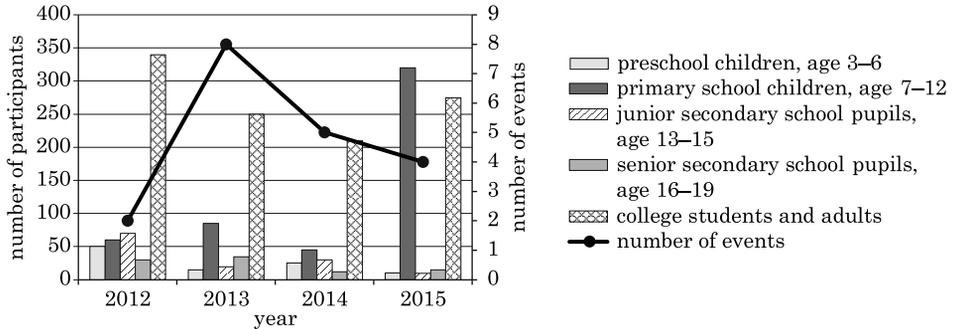


Fig. 6. Happenings and educational events organized by Kudypy Forest District – number of events and number of participants, divided into age groups

of this age category increased by several fold in 2015 (320 persons) – Figure 6. The highest number of participants was observed in 2015 (630), while the lowest one was in 2014 (322).

In 2012–2015, Kudypy Forest District held five forest theme competitions, which were participated by a total of 336 persons. They included quizzes, arts contests and literary competitions. There were two such contests in 2013, while in each of the remaining years there was just one contest. The number of participants ranged from 46 in 2012 to 130 in 2013. The contestants were children aged 7 to 12 and adults. In 2012, 2013 and 2015, there were two educational exhibitions. No such exhibition was organized in 2014.

Promotion and development of tourism were pursued by Kudypy Forest District in collaboration with many other institutions. Their types and the

Table 5  
Institutions cooperating with Kudypy Forest District in promotion and development of tourism in 2012–2015

Institutions	Number
Schools	25
Ecological education centres	7
Civic centres and museums	4
Non-government organizations	10
Churches	1
Radio	2
Television	1
Local governments	3
Scouting organizations	2
Fire brigades	3

Source: the authors based on reports of Kudypy Forest District regarding tourist and educational activities

number are specified in Table 5. The number of employees involved in the forest education of the general public in the years 2012–2015 equalled 19, 18, 21 and 31, respectively.

## Discussion

Benefits of forest services such as satisfying the public's recreational needs and providing opportunities for tourist activities are mostly achieved owing to the involvement of the State Forests National Forest Holding. Kudypy Forest District provides opportunities for nature-based tourism and recreation activities taking place in forests and undertaken by tourists, without having to build accommodation facilities, which add to some deforestation, as implicated by KUVAN (2005). In Turkey, forests are classified into three groups: production forests, conservation forests and national parks and other protected areas (KUVAN 2005). Similar divisions function in most European countries (ELANDS and WIRTH 2010), including Poland (Ustawa z 28 września 1991 r. ... Dz.U. 1991 poz. 444).

Different management actions are undertaken to improve quality of forest recreation and to reduce conflicts. They are usually divided into three main groups (PROBSTL et al. 2010). Restrictive management actions dominated in Central and Eastern Europe until the 1990s (CIESZEWSKA et al. 2010). Such restrictive rules and regulations, barriers and fences as well as prohibitions are still broadly enforced, especially in urban and rural areas. However, since the last decade of the 20<sup>th</sup> century, new infrastructural elements have become more prominent, implicating a change in the approach to forest and recreation management (CIESZEWSKA 2008, CIESZEWSKA et al. 2010). Soft management actions (infrastructure, signposting) and facilitating management actions (guiding, education) are gaining importance. A good example is the foundation of the Warmia and Mazury Forest Arboretum, whose beginning dates back to 1990, when a tree seeding stand damaged by a hurricane was planted with the first seedlings of trees and shrubs.

Kudypy Forest District undertakes actions which can be classified as corresponding to all the three approaches to visitor management mentioned above. Forest education seems to be especially important. Kudypy Forest District provides special infrastructure for forest education. The visitor center, arboretum garden, outdoor classrooms or educational trails are the examples of soft management actions. All facilitating management actions pursued in Kudypy Forest District are mentioned in Table 4, and a software application developed for mobile devices is also connected to forest education.

Forest is an area for leisure and sport activities, too. Furnishing some forest areas with some attractive new infrastructure to facilitate such activities is one of the main management approaches implemented by Kudypy Forest District. Products of the soft management actions connected to this approach are jogging and nordic walking trails, playgrounds, parking spaces, shelters and picnic areas.

Making forests open to the general public in Kudypy Forest District complies with the provisions of the Act on Forests (Ustawa z 28 września 1991 r. ... Dz.U. 1991 poz. 444) and the Silviculture Principles (*Zasady hodowli...* 2012). Examples of restrictive management actions include prohibitions, rules and regulations, as well as fees charged for entrance to the arboretum, protection zones around birds' nests, barriers and fences.

An important role in forest tourism is attributed to sightseeing, where the main motivation is to become familiarized with animate and inanimate nature, unique forms of landscape, monuments of culture and art, other monuments and statues, heritage objects, and other sights endowed with personal sentimental values (KOZIOŁ and MUSZYŃSKI 2009). Another significant component of forest tourism is forest and wildlife education, carried out by the State Forests, which in 2014 attracted over 2.16 million people all over Poland, of which 23.5% were adults (*Leśnictwo* 2015). The aim of these educational activities is the disseminate the knowledge about forests, including the multi-functional and sustainable forest management, and to raise the awareness of the general public about rational and responsible use of all functions performed by a forest (KAPUŚCIŃSKI and ZADURA 2009, KOZIOŁ and MUSZYŃSKI 2009). In 2014, the areas administered by the State Forests comprised the following types of facilities serving educational aims: 58 forest education centres, 548 forest education classrooms, 272 shelters and outdoor classrooms, where the so-called green lessons are conducted, 992 educational trails, 106 dendrological parks and gardens, and 1 904 forest education points as well as 2 771 other objects (*Leśnictwo* 2015).

MUSZYŃSKI and KOZIOŁ (2012) propose a division of forest recreational visitors which is slightly different from the one applied in our article, that is not according to age groups but to interests. In our paper, we focused on a group that MUSZYŃSKI and KOZIOŁ (2012) would identify as visitors not interested in the collection of non-timber forest products. KOZIOŁ and MUSZYŃSKI (2009) claim that the forms of forest tourism which are the most desirable and highly appreciated by the general public are the ones associated with health benefits, recreation and sightseeing, while other forms are either directly or indirectly, to different degrees and in different scopes, related to these three types. In our paper, we focused on forest tourism connected with forest education. Forest education, beside pertinent legal regulations, is essen-

tial for the protection of forest ecosystems from the adverse impact of tourism. Forest education contributes to the awareness of common people regarding forest protection measures. The social functions of forests are difficult to measure and can vary considerably among countries (DWYER et al. 2003). In Poland and other developed, post-industrial societies, the benefits of forests for recreation and amenity values may be most important, while in developing countries, the area of forests available for subsistence activities or the number of people employed in the sector may be a better indication of their social value.

The results obtained in our study suggest that the engagement of Kudypy Forest District's employees in forest education generates beneficial outcomes. The interest among children and their participation in various forms of recreation and forest education has risen over the past years in connection with increasing of the number of the Forest District's employees involved in the organization of such recreational and educational activities. The participation of adult persons has remained on a stable and relatively high level.

Translated by JOLANTA IDŹKOWSKA

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**SELECTED ASPECTS OF HUMANE ANIMAL  
PROTECTION IN POLISH LAW**

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**Key words:** animal welfare, animal rights, forensic veterinary, Polish law, veterinary surgeon, veterinary inspection.

**Abstract**

The history of legal protection of animals in Poland dates back to the early 20<sup>th</sup> century. The legal solutions adopted at that time in the form of regulations of the President of the Republic of Poland and acts of the Parliament were in force for decades. The turn of the 20<sup>th</sup> century brought some dramatic changes in this regard. Parliamentary acts, both newly passed and amended, harmonised Polish law with European standards; many legal acts have been passed in Poland since 1 May 2004 which were adopted throughout the European Union. The most important legal act which protect animals against suffering inflicted by humans is the Animal Protection Act of 1997. The Act, amended many times, together with the law of 2015, which protects laboratory animals, laid the grounds for legal and administrative actions against people who violate its provisions. This law is amended in line with scientific findings regarding the feeling of pain by animals. This paper analyses the flaws in the current laws from a veterinary surgeon standpoint.

**WYBRANE ASPEKTY HUMANITARNEJ OCHRONY ZWIERZĄT  
W PRAWODAWSTWIE POLSKIM**

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**A b s t r a k t**

Historia prawnej ochrony zwierząt w Polsce sięga początków XX w. Przyjęte wtedy normy prawne w postaci rozporządzeń Prezydenta RP i ustaw sejmowych obowiązywały przez dziesięciolecia. Przełom XX i XXI wieku przyniósł radykalne zmiany w tym zakresie. Nowelizowane i uchwalane na nowo ustawy dostosowywały polskie prawo do wymogów europejskich, a od 1 maja 2004 r. w Polsce przyjęto wprost wiele aktów prawnych uchwalonych dla całej Unii Europejskiej. Najważniejszym aktem prawnym chroniącym zwierzęta przed cierpieniem zadawanym przez człowieka jest ustawa z 1997 r. o ochronie zwierząt (Dz.U. z 21. 08., poz. 724). Ten wielokrotnie nowelizowany akt prawny wraz z ustawą z 15.01.2015 r. (Dz.U. 26. 02., poz. 266) chroniącą zwierzęta używane do doświadczeń stanowią podstawę do podejmowania działań prawnych i administracyjnych w stosunku do osób nieprzestrzegających przepisów prawa zawartych w tych aktach normatywnych. Prawo to zmieniane jest zgodnie z osiągnięciami nauki w zakresie odczuwania cierpienia przez zwierzęta. W artykule przeanalizowano wady aktualnego prawa z punktu widzenia lekarza weterynarii.

**Introduction**

People have been interested in animals since time immemorial. This has resulted in a growing body of knowledge about them. Scientific research into animals has revealed anatomical and physiological differences between humans and animals. Findings of studies carried out by ethologists, behaviourists, psychologists, neurobiologists and veterinarian have made it possible to accept that physical and mental suffering is felt by animals in a similar, if not the same, manner as by humans (ELWOOD 2011, ELWOOD et al. 2009, VIÑUELA-FERNÁNDEZ et al. 2007, KIELBOWICZ 2004). It has also been shown that the majority of painkillers used by people have the same effect on animals (ZIMMERMAN 1986). The findings of scientific research, together with growing public awareness, are reflected in legal acts on animal welfare that are increasingly often adopted by many countries and international organisations

(BABIŃSKA et al. 2007a, FELSMANN et al. 2014, LISTOS et al. 2015). In Poland, the issue is regulated in detail by the Animal Protection Act of 21 August 1997 (amended many times). According to its provisions, crimes against animals are penalised; moreover, certain administrative actions can be taken against animal owners and guardians (e.g. seizing an animal from its owner because of improper care). However, cases of cruelty against animals still happen (FELSMANN et al. 2016, RUDY 2011a,b, SZAREK 2005, LISTOS et al. 2015). This has resulted in an increase in the severity of penalties for violating the animal welfare act.

Despite constant changes in the Polish law regarding animal welfare, some people, especially veterinary surgeons, should analyse the effectiveness of the legal regulations in force (FELSMANN et al. 2015). This study is an attempt to analyse the current laws regulating the principles of humane animal welfare, considering the history and solutions applied elsewhere in the world in this regard.

### **International legal regulations**

Increasing social awareness regarding the need to protect animals from suffering has resulted in changes in the law aimed at penalising crimes against animals (BABIŃSKA et al. 2007a,b, RADECKI 2003). In the late 20<sup>th</sup> century, a number of international organisations implemented a range of legal acts with continental and global reach, especially conventions regulating certain aspects of dealing with animals. The World Animal Rights Declaration of 1977, adopted by UNESCO on 15 October 1978 is a prime example of such measures. In Europe, animal protection conventions have been drawn by the European Council. Poland ratified the Convention for the Protection of Animals kept for Farming of 10 March 1976, and the Convention for the Protection of Animals for Slaughter of 10 May 1979 in 2008 (Journal of Laws 1976, item 190, Journal of Laws 1978, item 24, Journal of Laws 1991, item 112, Journal of Laws 1996 item 263, Journal of Laws 2003, item 17, Journal of Laws 2007, item 665, Journal of Laws 2008, item 810). Unified legal regulations on animal welfare are valid throughout the European Union and have been in force in Poland since 1 May 2004.

### **Historical background of animal protection in Poland**

Protection of animals, including their habitats, has been provided for centuries.

Polish rulers implemented laws protecting certain game from the beginning of Polish history, but they did not do it for humane reasons.

Planned species protection, based on scientific grounds, was implemented in Poland after World War I. The main principles in this regard were laid down in the Nature Protection Act of 10 March 1934 (Journal of Laws 1934, item 274). Under the executive regulations for the Act, protection was provided for the pond turtle (from 1935) and the European bison (from 1938). Under the executive regulations to the act which is now in force (Nature Protection Act of 16 April 2004), about 750 animal species are covered by legal protection, with vertebrates accounting for 2/3 of the number (Journal of Laws 2004, item 880).

Implementation of the Regulation of President of the Republic of Poland on Animal Protection of 22 March 1928 (Journal of Laws 1928, item 332) is regarded as a genuine breakthrough in humane animal protection in Poland. This can be seen as progress even from a contemporary perspective. The Regulation contained twelve articles, but it remained in force for 69 years, until the Animal Protection Act of 21 August 1997 was enacted on 24 October 1997 (Journal of Laws 1997, item 724). This Act has been amended many times, and its current text differs significantly from the original version.

## **Contemporary animal protection in Poland**

The principles of legal protection of animals have been laid down in many normative acts. All of them can be regarded as regulations on environmental protection (RADECKI 2003, RADECKI 2007).

The most general legal act which regulates animal protection is the Environmental Protection Act of 27 April 2001, whose Article 127 regards animal welfare as an element of environmental protection. Likewise, the Nature Protection Act of 16 April 2004 points to the need for protecting animals as an element of nature (Journal of Laws 2001, item 627, Journal of Laws 2004, item 880). Many other legal acts contain provisions on selected, specific elements of animal protection, including those that lay down the principles of administrative proceedings: The Inland Fisheries Act of 18 April 1985 (Journal of Laws 1985, item 91), The Act on the Profession of Veterinary Surgeon and on Veterinary Chambers of 21 December 1990 (Journal of Laws 1991, item 27), The Hunting Law Act of 13 October 1995 (Journal of Laws 1995, item 713), The Veterinary Inspection Act of 29 January 2004 (Journal of Laws 2004, item 287), The Act on Animal Health Protection and on Controlling Infectious Diseases in Animals of 11 March 2004 (Journal of Laws 2004, item

625), The Act on Breeding and Reproduction of Farm Animals of 29 June 2007 (Journal of Laws 2007, item 921), The Marine Fisheries Act of 19 December 2014 (Journal of Laws 2014, item 222).

### **Penal and administrative humane animal protection in view of the legal regulations in Poland**

Humane protection of animals is a term which regulates the overall rules governing human attitudes towards animals. In its general principle, it includes protection for animals against human actions which may inflict pain and suffering. It has ethical and humane grounds, forbidding the causing of harm to animals, which are as able to feel pain as humans. Furthermore, legal protection of animals means that animals are looked after by specific institutions and legal entities. Legal regulations are necessary since everyone establishes the limits of protection subjectively and they can change them at any moment as they see fit, not always to the benefit of animals.

All considerations regarding legal, penal and administrative issues related to animal welfare must proceed by reviewing the Animal Protection Act of 21 August 1997 (Journal of Laws 1997, item 724). It regulates a range of important human actions towards animals and establishes penalties for offences or crimes violating its regulations. According to this Act, every animal is a living creature and, therefore, able to suffer. It cannot be regarded and treated as a thing; people must respect it and provide it with protection and care. Every animal must be treated humanely. With a view to implementation of the regulations on animal protection, public administration bodies act in collaboration with the Veterinary Inspection and the veterinary self-government. The latter act for the protection of animals, mainly vertebrates, by collaboration with the relevant institutions and organisations, both at home and abroad.

This law contains a number of definitions which are necessary to understand the legislators. The most frequently used include: humane treatment of animals – treatment in which an animal’s needs are taken into account and which provides it with protection and care; grave negligence – this is understood as keeping animals in a state of starvation, dirt, untreated disease or in an improper room; cruel treatment – these are cases of animal abuse and other human actions which result in pathological changes in an animal’s body or in its death; particular cruelty – this involves inflicting pain on an animal by a person in a slow and drastic manner, such actions are premeditated; overburdening of animals – forcing animals to make excessive physical effort, which is beyond the animal’s physical capabilities; tethering – all mechanical devices which restrict an animal’s movements and devices used by people to direct animal movements.

## **Killing animals and cruelty to animals**

According to the law, killing animals is acceptable only in several specific cases. These include slaughtering for economic reasons (including fishing and hunting), killing animals which pose a threat to people or animals. It is noteworthy that a decision to kill an animal immediately must be taken by an authorised entity, mentioned in Art. 33.3 of the Act. It is significant that the animal's owner is not among such authorised entities.

Cruelty to animals denotes inflicting pain on them or making them suffer. A catalogue of actions which specify this include, *inter alia*, deliberate wounding or mutilating an animal as a result of branding by burning or freezing, each treatment which results in a change of an animal's appearance, whose aim is not to protect its health or life, e.g. clipping dogs' ears and tails. Draught animals must not be overburdened with loads or forced to run excessively fast, animals must not be frightened or teased maliciously, neglected, kept in improper living conditions or in place where they cannot maintain their natural body posture. Cruelty also denotes using sick or old animals for work or for sport or entertainment, forcing them to do things which could cause pain, hitting with hard and sharp objects, especially on the head, lower abdomen, lower parts of the limbs, organising animal fights, having sexual intercourse with animals, abandoning an animal, especially dogs or cats, carrying or moving animals in a way which causes unnecessary suffering and stress. Other forms of cruelty include using cruel methods in rearing or breeding animals, keeping animals without food or water for a longer period than allowed, performing surgeries by unauthorised persons, in a manner which can cause unnecessary pain. The Act provides penalties for an action which can be regarded as cruelty to animals.

## **Seizing an animal from its owner**

In cases of real threats to an animal's health and/or life resulting from cruelty to it, it can be seized from its owner or guardian temporarily by the local administrative body responsible for the place where the animal lives. The animal is then transferred to one of the places suited to keeping various categories of animals: to an animal shelter in the case of pets or laboratory animals, to another farm in the case of farm animals, to a zoological garden in the case of animals used for entertainment, in film production or for sport. Such a decision is taken on the basis of information from the police, town security, a veterinary surgeon or a representative of a social institution whose aim is to provide protection to animals. An animal is transferred to a new place

upon a consent of the person who is to receive it. If such consent is denied, the animal can be handed over free of charge to another legal entity or an institution which will look after it properly. If remaining with the owner or guardian poses a direct threat to the animal's life or health, it can be seized immediately and the fact is reported to a local administrative body which issues a relevant decision. The cost of transport, maintenance and the necessary treatment of the animal is borne by its owner or guardian. Seizing an animal from its owner/guardian initiates legal proceedings against its owner and the animal can be returned if the case is dismissed.

### **Owners' obligations towards their pets**

A person who keeps a pet is obliged to protect it against cold, excessive heat and atmospheric precipitation, to provide it with access to daylight, proper feed and access to water and to keep it in a place which enables it to change the body position freely. Pets cannot be held on a leash in a permanent manner for more than 12 hours a day or in a manner which causes bodily harm or which makes them suffer, without being able to make necessary movements. A leash cannot be shorter than 3 m.

Under the 2011 amendment of the animal protection act, pets cannot be traded or bought at fairs or markets. A dog or a cat can be bought only at a place of breeding, which must be registered with the relevant breeders' association (e.g. Polish Cynological Society, Polish Felinological Society, etc.) and only such breeding farms can reproduce animals for commercial purposes. An exception to this rule are entities which run animal shelters and organisations (such as the Polish National Animal Care Association), which are authorised to trade in dogs and cats outside the breeding sites.

Providing care to homeless animals is another issue which is regulated by provisions of the Animal Protection Act. Catching them and providing them with care is a task of communes, which must prepare a programme of care and prevention of animal homelessness every year (RUDY 2011a, b). Every commune in Poland must have an agreement with an animal shelter, to which it will transfer all the cats and dogs caught in its area, and with a veterinary surgeon in order to provide round-the-clock veterinary care in the case of road accidents with animals. Moreover, the programme provides for looking after stray cats, *inter alia* by feeding them and developing procedures for catching homeless animals. Reducing animal homelessness should include obligatory sterilisation and castration of animals which are brought to animal shelters, finding new owners for them and the possibility for putting down blind litters. Such a programme must also provide for procedures of handling homeless

farm animals, to which it must provide care in an agricultural farm. The cost of care provided to homeless animals is borne by the local commune.

The 2011 amendment of the Animal Protection Act introduced considerable changes concerning the procedure of handling pets which reside unattended in areas of hunting districts. Under previous regulations, hunters could shoot wild dogs and cats at a distance of 200 m from residential or farm buildings. The current act does not provide for such a solution, but it imposes an obligation on leaseholders or holders of hunting districts to take actions aimed at preventing wandering of dogs by instructing the owner to control the animal. Forest service personnel can catch an animal and return it to the owner; if this proves impossible, they must deliver it to an animal shelter.

### **Owners' obligations towards farm animals**

An entity which keeps farm animals is obliged to provide care and proper living conditions for the animals, which cannot cause injury or harm or be a source of suffering. Animals must not be kept in an excessive density resulting from failure to suit the required surface area to the species, age or physiological conditions of the animals. Calves aged above 8 weeks should be kept loose and in groups, except at feeding time and for not longer than 1 hour at a time. For working animals, the conditions in which they are kept must not pose a threat to their life or health or be a source of suffering. It is unacceptable to overburden animals, use sick or undernourished ones, or use any objects to direct them which could mutilate them. Animals pulling a load must not be forced to trot or gallop. Only horses aged 5 years or more can be used for logging. Animals must have a species-specific time for a rest every day to regenerate. Detailed conditions of keeping farm animals, specifying such items as the area size and frequency of giving feed and water, are laid down in two executive regulations to the Act, namely, the Regulation of the Minister of Agriculture and Rural Development of 28 June 2010 on the minimal condition of maintaining farm animals other than those whose protection standards were laid down in the European Union regulations and the Regulation of the Minister of Agriculture and Rural Development of 15 February 2010 on the requirements and handling of farm animals whose protection conditions are laid down in European Union (Journal of Laws 2010, item 344, Journal of Laws 2010, item 778).

One of the latest amendments of the Animal Protection Act introduced regulations regarding the maintenance and handling of broiler chickens. These animals can be looked after by people trained in regulations concerning welfare

of broiler chickens or have qualifications in veterinary medicine, animal husbandry or agriculture.

Both for broiler chickens and the other farm animal species, the regulations obligate their owners to maintain the relevant documentation concerning breeding and veterinary treatment, which must be stored for 3 years and made available to representatives of the Veterinary Inspection.

### **Animals used for entertainment, movie production, sport and for special purposes**

These animals include those subjected to special training. These are most frequently dogs used by police, customs services, border guards, used for looking for explosives, drugs or people. These also include service dogs and animals-actors. Conditions during performances and training and the methods of handling such animals cannot pose a threat to their lives or health, or cause pain; they should also be provided with veterinary care. The organiser of a show must prepare its scenario or programme, which it submits for acceptance to the Chief Veterinary Officer. Such shows or sports events cannot have elements of cruelty; particularly forbidden are bull, dog or cock fights. Animals can be kept, bred and presented for special purposes only in properly adapted stables, circuses or circus schools, supervised by the Veterinary Inspection.

### **Transport of animals**

In regard to a procedure to be followed when transporting vertebrate animals, the Animal Protection Act refers one to the Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC (O.J. L 3, 5 January 2005). It designates the District Veterinary Officer to supervise and control the observing of the regulation in the territory of Poland. His tasks include, *inter alia*, supervising training for carriers and issuing licences to confirm qualifications for transporting animals.

### **Animal treatment**

Veterinary treatment of animals is acceptable only to save their lives or health or to limit their population as part of homelessness prevention measures. They can only be performed by authorised persons – veterinary

surgeons. Each procedure must be carried out with caution, inflicting as little pain and stress on the animal as possible. Painful procedures are carried out with general or topical anaesthesia, except those that are carried out with no anaesthesia, according to the principles of veterinary treatment.

### **Slaughter, killing and reducing animal populations**

Animals can be put down only in a humane manner, while inflicting as little physical and mental suffering as possible (FELSMANN et al. 2016, RUDY and RUDY 2010a). A decision to kill an animal to end its suffering can be taken by a veterinary surgeon, a member of the forest service, a game warden or a policeman. A animal can be kill by a veterinary surgeon by giving the animal an appropriate drug, or, for a wild animal, by a person authorised to use a firearm.

Slaughter animals can be killed after being rendered unconscious and only by a properly qualified person. This applies both to slaughter at a slaughterhouse and to on-farm slaughter (RUDY and RUDY 2010b).

Animals must not be killed in the presence, or with the participation of children. Further flaying of a carcass (e.g. skinning, scalding, etc.) cannot be done until respiratory and muscular reflexes stop. Moreover, with certain exceptions, killing pregnant females or females within 48 hours of delivery is forbidden.

Killing an animal can be done only with the consent of its owner. Environmental protection bodies (e.g. General or Regional Direction of the Environmental Protection Board) are authorised to give such consent for wildlife. However, if there is a suspicion or a threat of an infectious animal disease whose combating is obligatory, animals can be killed without such a consent (RUDY and RUDY 2011).

### **Supervision over observing the animal protection regulations**

The Veterinary Inspection has been designated by the Act to supervise the observance of the animal welfare regulations; the Inspection can cooperate in this regard with NGOs involved in animal protection.

## **Penal regulations**

Failure to observe the animal protection regulations is punishable under the law. The Act defines the type and degree of penalty for committing offences against its provisions. The unjustified killing of an animal or any form of cruelty to animals is punishable by fines, restriction of freedom or imprisonment for a period of up to 2 years, and up to 3 years if the criminal act was done with particular cruelty. Those found guilty of cruelty to animals can be forbidden to possess an animal for a period of up to 10 years. They can also be punished with a fine of up to PLN 10,000, which is used for a purpose associated with animal protection. Any actions aimed at harming an animal are forbidden. People must bear in mind animal welfare, protect it and treat it with proper respect.

The original, 1997 version of the Act, contained a number of provisions related to animal experiments. These provisions were transferred in 2005 to the Animal Experiments Act, amended in 2015. The valid Act on Protection of Animals Used for Scientific or Educational Purposes of 15 January 2015 contains a number of provisions which help to protect animals against unnecessary suffering when they are used in experiments or teaching demonstrations (FELSMANN et al. 2015). The current regulation provides for supervising animal experiments by the Veterinary Inspection, which is a step in the right direction. The Act still leaves the decision of whether to approve procedures with live animals in the hands of local ethics committees for animal experiments. It must be emphasised that such committees do not have to have a veterinary surgeon among its members. Moreover, personnel of the Veterinary Inspection cannot interfere even with the most drastic experimental procedures if they have been approved by an ethics committee. The Act provides for a punishment of up to three years' imprisonment (Art. 66) for its violation, as well as for administrative punishments in the form of fines of up to PLN 50,000 (Art. 70).

## **Conclusions**

The Polish regulations concerning humane animal protection are constantly evolving. Despite certain flaws which are particularly visible to veterinary surgeons, they provide for ever improving and more effective animal protection against suffering inflicted by people.

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