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GAS EXCHANGE INTENSITY OF SPRING WHEAT AND UNDERSOWN PERSIAN CLOVER UNDER CONDITIONS OF DIVERSIFIED DENSITY OF PLANTS

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K e y w o r d s: stomatal conductance, photosynthesis, transpiration, water use efficiency, undersown crop.

A b s t r a c t

The influence of competitive interactions between spring wheat and the undersown Persian clover, as well as the diversified density of plants, on the stomatal conductance and intensity of the processes of photosynthesis and transpiration in both species was evaluated during a pot experiment conducted between 2010 and 2012. The spring wheat and Persian clover cultivation methods – pure sowing, cultivation in a mixture of the species and the density of plants – higher (consistent with recommendations of agricultural technology) and lower (decreased by 20% of the recommended density) were the factors of the experiment. The gas exchange processes were analysed during 5 periods determined by the spring wheat development rhythm (leaf development, tillering, stem elongation, inflorescence emergence, ripeninig). Based on the quotient of the photosynthesis intensity and transpiration intensity, the water use efficiency (WUE) index was computed. It was shown that wheat cultivated with the undersown Persian clover was characterised by lower stomatal conductance, CO_2 assimilation and transpiration. Water use efficiency in the process of photosynthesis did not change under the influence of the sowing method almost throughout most of the experimental period. In the mixture, the Persian clover photosynthesis intensity was lower than in the pure sowing during the stages of the cereal tillering and ripening. In this sowing method, the lower stomatal conductance, transpiration and water use efficiency were recorded during the generative development of the cereal.

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INTENSYWNOŚĆ WYMIANY GAZOWEJ PSZENICY JAREJ I WSIEWKI KONICZYNY PERSKIEJ W WARUNKACH ZRÓŻNICOWANEGO ZAGĘSZCZENIA ROŚLIN

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S ł o w a k l u c z o w e: przewodność szparkowa, fotosynteza, transpiracja, wskaźnik efektywności wykorzystania wody, wsiewka.

A b s t r a k t

W doświadczeniu wazonowym, zrealizowanym w latach 2010–2012, oceniano wpływ oddziaływań konkurencyjnych między pszenicą jarą i wsiewką koniczyny perskiej oraz zróżnicowanego zagęszczenia roślin na przewodność szparkową oraz intensywność procesów fotosyntezy i transpiracji u obu gatunków. Czynnikami doświadczenia były: sposób uprawy pszenicy jarej i koniczyny perskiej – siew czysty, uprawa we wzajemnej mieszance; zagęszczenie roślin: większe (zgodne z zaleceniami agrotechniki), mniejsze (zmniejszone w stosunku do poprzedniego o 20%). Procesy wymiany gazowej analizowano w pięciu okresach wyznaczonych przez rytm rozwojowy pszenicy jarej (wschody, krzewienie, strzelanie w źdźbło, kłoszenie, dojrzałość). Na podstawie ilorazu intensywności fotosyntezy i transpiracji obliczono współczynnik wykorzystania wody (WUE). Wykazano, że pszenica uprawiana z wsiewką koniczyny odznaczała się mniejszą przewodnością szparkową, asymilacją CO_2 oraz transpiracją. Efektywność wykorzystania wody w procesie fotosyntezy prawie w całym badanym okresie nie zmieniała się pod wpływem sposobu siewu. W mieszance intensywność fotosyntezy koniczyny perskiej była mniejsza niż w siewie czystym w fazie krzewienia i dojrzałości zboża. W tym sposobie siewu mniejszą przewodność szparkową, transpirację oraz efektywność wykorzystania wody odnotowano w okresie rozwoju generatywnego zboża.

Introduction

In caring for the balance of agricultural systems, increasing attention is paid to environmentally friendly farming methods. Increasing the diversity of crops by, among other things, application of undersown crops, plays an important role in those systems (JASKULSKA and GAŁĘZEWSKI 2009). The undersown crops vegetate in the field together with the main crop until its harvest and then continue their development in the autumn and are subsequently harvested for feed or (which has become the most frequent practice recently) they are ploughed down as so-called *green manure*. Undersown crops cultivation has a positive influence on the sanitary status of the field by limiting the occurrence of weeds and pathogens. It prevents water erosion, when reduce leaching of mineral compounds (mainly nitrates) to the deeper layers of the soil. It increases the content of humus and mineral components in the soil, stimulates biological life of the soil and supports its biological balance (HOLLAND 2004, BLACKSHAW 2005, KÄNKANEN and ERIKSSON 2007, JASKULSKA

and GAŁĘZEWSKI 2009, GAUDIN et al. 2013). Several studies highlight, the positive influence of undersown crops on the physical characteristics of the soil (particularly its structure and humidity) (RAIMBAULT and VYN 1991, UNGER and MERLE 1998). The importance of the undersown crops in the main crop yield formation is smaller and unclear. The role of the undersown crop in that aspect differs depending on the main crop and the undersown crops species, level of agricultural technology applied, and weather and soil conditions, as well as the yield accomplished by those crops (MICHALSKA et al. 2008, TREDER et al. 2008, SOBKOWICZ and PODGÓRSKA-LESIAK 2009, PICARD et al. 2010, WANIC et al. 2013, WANIC et al. 2016a).

Spring wheat is classified as a very good plant for cultivation with undersown crops because of poor tillering, small height and no excessively abundant foliage (ZAJĄC 2007). In addition, clovers are considered valuable undersown crop plants mainly because of their well-developed root system, extensive above-ground biomass and symbiotic fixation of atmospheric nitrogen. The most extensive collection of studies available concerns the red and the white clover as well as their mixtures with grasses (KÄNKÄNEN and ERIKSSON 2007). Little information however can be found concerning the Persian clover although it is a plant, which under favourable habitat conditions (particularly humidity conditions), generates high yields of biomass abundant with nitrogen. Its suitability as the undersown crop was confirmed by PŁAZA et al. (2013).

In mixed crop stand, diversified interactions occur between the main crop and the undersown crop. In most cases, those interactions assume the form of competition for environmental resources (water, light, nutrients and space); however, that competition may also take place by means of various chemical compounds excreted into the environment. Such competition results in a reduction of the plants' population, change of the development rhythm, morphological characteristics and fertility (SHEAFFER et al. 2002, SOBKOWICZ 2003, SOBKOWICZ and PODGÓRSKA-LESIAK 2009). This results in obtaining yields different from those expected. The interactions between plants may also influence their physiological processes. The intensity and direction of competition depend on the choice of component species (and cultivars), their development period, density of plants and abundance of resources in the habitat (THORSTED et al. 2006, MICHALSKA et al. 2008, TREDER et al. 2008).

The literature offers little information on the influence of competition on CO_2 assimilation, transpiration and activity of the stomata of both species. Given the above, the research hypothesis was formulated assuming that competitive interactions would occur between the spring wheat and Persian clover and that they would influence the progress of the above-indicated processes while their intensity would depend on the development stage and density of the plants.

Evaluation of the influence of spring wheat cultivation with Persian clover and of the plant density on the stomatal conductance, CO_2 assimilation and transpiration during the entire period of common vegetation of both species was the aim of the studies.

Material and Methods

The studies were based on a pot experiment conducted in three series in the greenhouse laboratory of the University of Warmia and Mazury in Olsztyn. The experiments were conducted during the following periods: series I: from 12 April until 19 July 2010, series II – from 24 March until 30 June 2011, and series III – from 26 March until 28 June 2012. Spring wheat (cultivar Nawra) and Persian clover (cultivar Gobry) were cultivated in pure and mixture stands in two density variants: the recommended density and density decreased by 20% from the recommended values.

The factors of the experiment were:

I. cultivation method of spring wheat and Persian clover:
   – pure sowing,
   – mixed sowing,

II. plant density:
   – higher (according to the recommendations of agricultural technology) referred herein as the "recommended density",
   – lower (decreased as compared to the recommended by 20%).

The experiment was established according to the additive pattern whereby the number of plants in the mixture was the sum of their numbers in pure sowing. This pattern allowed the study of the competition between the spring wheat and Persian clover from the very beginning of the vegetation and levelled the influence of intraspecific competition on the development of that process (SEMERE and FROUD-WILIAMS 2001).

The experiment consisted of 120 pots (two species in pure and mixture x two sowing densities *x* 5 development stages *x* 4 replicates). Kick-Brauckmann type pots 22 cm in diameter and 25 cm deep were used for the experiment. The seeds were sown in the pots at equal distance from one another (thanks to patterns) and placed in the soil at the depth of: 3 cm (spring wheat) and 1 cm (Persian clover). In the pots with the recommended density, for both sowing methods, 19 grains of spring wheat and 12 grains of Persian clover were planted. In the lower density pots, those numbers were 15 and 9 respectively. This corresponded to the numbers of plants per 1 m^2: spring wheat – recommended density – 500, lower density – 400; Persian clover – 300 and 240 respectively.

The pots were filled with substrate composed of Eutric Cambisol (Humic),which had the following percentage of the fractions: 64% of grains less than 0.02 mm (clay), 12% of silt (0.1–0.02 mm) and 24% of sand (> 1 mm). The soil was slightly acidic (pH in 1 M KCl from 5.6 to 6.2), and had the content of organic carbon from 13.2 to 14.4 g kg^{-1}, the content of nitrogen from 0.69 to 0.74 g kg^{-1}, a high content of phosphorus (9.2–11.6 mg 100 g^{-1} of soil) and magnesium (8.8–9.1 mg 100 g^{-1} of soil), and a medium content of potassium (12.9–14.5 mg 100 g^{-1}). The soil was taken from the depth of 0–25 cm.

The mineral NPK fertilisation was applied once, one week before the sowing date. Water solutions of urea, monosodium phosphate and potassium sulphate were prepared. They were applied in the appropriate doses to the soil, mixed well and transferred to the pots. Identical fertilisation with phosphorus and potassium was applied to all the plants [g pot^{-1}]: P – 0.200 and K – 0.450. The dose of nitrogen was diversified depending on the species and sowing method and it was [g pot^{-1}]: for spring wheat in pure sowing – 0.500, for the mixture of spring wheat with Persian clover – 0.300 and for Persian clover as pure crop – 0.125.

During the plants' vegetation, the greenhouse temperature was maintained within 20–22°C. Only during the leaves formation stage it was decreased to 6–8°C to allow the wheat to undergo the process of vernalisation. Soil humidity during vegetation was maintained at a constant level of 60% of the maximum water capacity of the soil and the shortages were replenished daily as necessary. The vegetation of plants took place under conditions of natural illumination.

The gas exchange measurements were conducted during 5 periods determined by the development rhythm of spring wheat cultivated in pure crop in those objects with the recommended density of plants, that is the stages (BBCH) of: leaves development (12–14), tillering (21–23), stem elongation (31–32), inflorescence emergence (54–56) and ripening (87–89). Measurements on the spring wheat plants were started at the leaf development stage and concluded at the stage of inflorescence emergence. Measurement of Persian clover plants started at the tillering stage and during the inflorescence emergence stage of the spring wheat (an earlier time was impossible because of the leaves being too small for analysis) and ended at the stage of cereal ripening. The gas exchange was measured using the compact photosynthesis testing system Eijkelkampl LCi. Stomatal conductance, CO_2 assimilation and transpiration were measured on three random selected stems during each measurement period. The measurement was taken on the youngest fully developed leaf in ten replicates. The results obtained served computation of the water use efficiency – WUE index: assimilation/transpiration.

The experiment results are presented in the form of average values for three series of tests.

They were processed statistically by means of variance analysis at the significance level of $p = 0.05$. The Tukey's test (HSD) was used for evaluation of differences between objects. The computations were conducted using the *Statistica* computer software.

Results

The stomatal conductance in the leaves of wheat cultivated in the mixture with Persian clover was lower than in case of pure crop cultivation throughout the entire vegetation period (Table 1). The largest differences between the mixture and pure sowing were recorded during the stem elongation stage (more than 50%), and the smallest during the inflorescence emergence (almost 30%). No significant influence of plant density on the activity of stomata was found from the leaf development stage until the stem elongation stage. During the inflorescence emergence stage, higher conductance characterised leaves of wheat in objects with the recommended density (by 23.1%). During the leaf development stage, mixture limited conductance in the leaves of wheat cultivated at the recommended density more than in the case of cultivation at the lower density. During the inflorescence emergence, no significant differences between the mixture and the pure stand were found in the objects with the recommended density. On the other hand, significantly lower activity of stomata was recorded in case of mixture. During the other periods, the sowing method differentiated the studied characteristics in a similar way in objects of both densities.

Table 1

Stomatal conductance of spring wheat [mol H_2O m^{-2} s^{-1}]

Plant density (A)	Sowing method (B)	Growth stages of spring wheat (BBCH)			
		leaf development (12–14)	tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)
A_1	B_1	0.29^a	0.27^a	0.18^a	0.34^a
	B_2	0.15^c	0.18^b	0.09^b	0.29^a
Average for A_1		0.22^a	0.23^a	0.14^a	0.32^a
A_2	B_1	0.33^a	0.32^a	0.17^b	0.20^a
	B_2	0.08^b	0.33^a	0.19^c	0.21^b
Average for A_2		0.27^a	0.25^a	0.14^a	0.26^b
Average for A	B_1	0.31^a	0.30^a	0.19^a	0.34^a
	B_2	0.18^b	0.18^b	0.09^b	0.24^b

A – plant density: A_1 – recommended, A_2 – lower
B – sowing method: B_1 – pure crop, B_2 – cultivation as mixture with Persian clover
a, b, c – value marked with the same letter do not differ significantly ($p \leq 0.05$)

Persian clover cultivated in the mixture with spring wheat was characterised by significantly lower stomatal conductance during the stages of wheat tillering and inflorescence emergence by 58.0 and 40.6% respectively (Table 2).

Table 2

Stomatal conductance of Persian clover [mol H_2O m^{-2} s^{-1}]

Plant density (A)	Sowing method (B)	Growth stages of spring wheat (BBCH)			
		tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)	rripening (87–89)
A_1	B_1	1.12^a	0.98^a	2.55^a	0.18^{bc}
	B_2	0.38^b	0.72^b	1.22^b	0.41^a
Average for A_1		0.75^a	0.85^b	1.89^a	0.30^a
A_2	B_1	0.87^a	1.17^a	2.13^a	0.12^c
	B_2	0.46^b	0.92^{ab}	1.56^b	0.23^b
Average for A_2		0.67^a	1.05^a	1.85^a	0.18^b
Average for A	B_1	1.00^a	1.08^a	2.34^a	0.15^b
	B_2	0.42^b	0.82^a	1.39^b	0.32^a

A – plant density: A_1 – recommended, A_2 – lower
B – sowing method: B_1 – pure crop, B_2 – cultivation as mixture with spring wheat
a, b, c – value marked with the same letter do not differ significantly ($p \leq 0.05$)

The opposite situation was recorded at the end of the vegetation period. Higher stomatal activity (by more than twofold) was recorded in the mixture than in the pure sowing. Sowing density had significant influence on the studied characteristic during the stem elongation and ripening stages. During the first of those periods, the leaves of clover growing at the lower density were characterised by higher activity than those of the clover cultivated at the higher density. During the latter period, the situation was the opposite. Plant density had similar influence on the magnitude of the differences between the sowing methods during the stages of tillering, inflorescence emergence and ripening. During the stem elongation period, the stomata of plants in pure sowing were characterised by significantly higher activity than in the plants in mixture solely for those objects with the recommended density of plants.

Addition of Persian clover limited CO_2 assimilation by spring wheat from the leaf development stage until the inflorescence emergence stage (Table 3).

The negative influence of clover on carbon dioxide assimilation was the most pronounced during the leaf development and stem elongation stages. In the mixture, it was lower than in pure sowing by 36.8 and 27.4% respectively. That addition had the smallest limiting influence on the process of photosynthesis by wheat during the inflorescence emergence stage (the difference between the pure and the mixture was 6.6%). Lower density of plants

influenced that process negatively during the leaf development stage (by 25.5%) and tillering (by 14.0%). The influence was positive during the stem elongation stage (by 17.9%) and the inflorescence emergence stage (by 6.6%). Poorer CO_2 assimilation by the wheat plants in the mixture than in the pure crop was found in both objects with different densities of plants.

Persian clover in the mixture assimilated significantly less CO_2 than in the pure sowing during the tillering stage (by 21.9%) and the ripening stage (by 41.2%) – Table 4.

Table 3

Photosynthetic rate of spring wheat [μm CO_2 m^{-2} s^{-1}]

Plant density (*A*)	Sowing method (*B*)	Growth stages of spring wheat (BBCH)			
		leaf development (12–14)	tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)
A_1	B_1	6.72^a	3.94^a	3.92^{ab}	3.81^{ab}
	B_2	4.73^b	3.07^b	2.78^c	3.50^b
Average for A_1		5.73^a	3.51^a	3.35^b	3.66^b
A_2	B_1	5.52^b	3.25^b	4.53^a	4.00^a
	B_2	3.01^c	2.78^c	3.36^c	3.79^b
Average for A_2		4.27^b	3.02^b	3.95^a	3.90^a
Average for *A*	B_1	6.12^a	3.60^a	4.23^a	3.91^a
	B_2	3.87^b	2.93^b	3.07^b	3.65^b

A – plant density: A_1 – recommended, A_2 – lower
B – sowing method: B_1 – pure crop, B_2 – cultivation as mixture with Persian clover
a, b, c – value marked with the same letter do not differ significantly ($p \leq 0.05$)

Table 4

Photosynthetic rate of Persian clover [μm CO_2 m^{-2} s^{-1}]

Plant density (*A*)	Sowing method (*B*)	Growth stages of spring wheat (BBCH)			
		tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)	rripening (87–89)
A_1	B_1	5.69^b	6.25^a	6.99^a	6.70^a
	B_2	4.44^c	5.61^a	6.16^{ab}	3.39^b
Average for A_1		5.07^b	5.93^a	6.58^a	5.05^a
A_2	B_1	7.80^a	6.66^a	5.66^b	4.42^b
	B_2	6.09^b	5.56^a	5.39^b	3.14^b
Average for A_2		6.95^a	6.11^a	5.53^b	3.78^b
Average for *A*	B_1	6.75^a	6.46^a	6.33^a	5.56^a
	B_2	5.27^b	5.59^a	5.78^a	3.27^b

A – plant density: A_1 – recommended, A_2 – lower
B – sowing method: B_1 – pure crop, B_2 – cultivation as mixture with spring wheat
a, b, c – value marked with the same letter do not differ significantly ($p \leq 0.05$)

During the stages of stem elongation and inflorescence emergence, the differences between the sowing methods were smaller and assumed the character of a trend. In those objects with lower plant density, photosynthesis progressed more intensively than in the objects with the higher plant density during the tillering stage (by 37.1%). During the stem elongation stage, photosynthesis was similar at both objects (no significant differences). Then during the stages of inflorescence emergence and ripening of the wheat, it showed lower values (by 16.0 and 25.1% respectively). The negative influence of wheat on assimilation was visible in both levels of plant density during the tillering stage, while during the ripening stage it was visible in the objects with the recommended density.

During the leaf development stage, taking the average for plant density, the sowing method did not significantly diversify the transpiration from the spring wheat leaves (Table 5). During the further development stages, that cereal transpired less water with the presence of the undersown crop than in pure sowing. The highest influence of the undersown crop on the development of that process was visible during the stem elongation stage (when the difference between the mixture and pure sowing was 46.1%). No clear influence of plant density on water transpiration from wheat plants was established. During the leaf development stage, it was similar in objects of both densities. During tillering and stem elongation, transpiration was more intensive amongst the objects with lower density (by 25.8 and 46.3% respectively). During the inflorescence emergence, the situation was the opposite and higher transpiration was recorded for those objects with the recommended density (by 25.0%). The interaction between the sowing method and plant density showed that

Table 5

Transpiration rate of spring wheat (mmol H_2O m^{-2} s^{-1})

Plant density (A)	Sowing method (B)	Growth stages of spring wheat (BBCH)			
		leaf development (12–14)	tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)
A_1	B_1	2.91^{c}	1.79^{ab}	2.13^{b}	2.98^{b}
	B_2	3.65^{ab}	1.47^{b}	1.06^{c}	2.52^{a}
Average for A_1		3.28^{a}	1.63^{b}	1.60^{b}	2.75^{a}
A_2	B_1	4.10^{a}	2.18^{a}	2.99^{a}	2.25^{bc}
	B_2	3.10^{bc}	1.92^{a}	1.69^{b}	2.14^{c}
Average for A_2		3.60^{a}	2.05^{a}	2.34^{a}	2.20^{b}
Average for A	B_1	3.51^{a}	1.99^{a}	2.56^{a}	2.62^{a}
	B_2	3.38^{a}	1.70^{b}	1.38^{b}	2.33^{b}

A – plant density: A_1 – recommended, A_2 – lower
B – sowing method: B_1 – pure crop, B_2 – cultivation as mixture with Persian clover
a, b, c – value marked with the same letter do not differ significantly ($p \leq 0.05$)

during the leaf development stage, plants with the recommended density and mixture transpired more water than in case of pure sowing. In the case of the lower density, the situation was the opposite and they transpired less water. During tillering and stem elongation, the sowing method at both densities changed that characteristic in a similar way and during the inflorescence emergence stage, larger differences between the mixture and the pure sowing occurred with the objects with the recommended plant density.

Transpiration from the Persian clover leaves during the stages of wheat tillering, inflorescence emergence and ripening was more intensive in the pure sowing than in the mixture by 26.3, 34.1 and 65.3% respectively (Table 6). During the tillering stage, this process was 11.7% greater in the objects with the lower than recommended plant density. During stem elongation, the process developed similarly with both density objects. During the period of generative development of the cereal, more water transpired plants in pots with the recommended density (during the inflorescence emergence stage by 15.7% and during the ripening stage by 50.3%). Interaction of the experimental factors showed that during the tillering stage, mixture limited transpiration in a similar way in objects of both densities. During the stem elongation stage, mixture did not diversify its intensity depending on the experimental factors, while during the inflorescence emergence and ripening stages it limited water transpiration from the plants in objects with lower density more than pure sowing.

Table 6

Transpiration rate of Persian clover [mmol H_2O m^{-2} s^{-1}]

Plant density (A)	Sowing method (B)	Growth stages of spring wheat (BBCH)			
		tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)	rripening (87–89)
A_1	B_1	7.20^a	6.29^a	7.81^a	6.24^a
	B_2	6.11^b	6.26^a	6.48^b	4.88^a
Average for A_1		6.66^b	6.28^a	7.15^a	5.56^a
A_2	B_1	8.53^a	6.54^a	7.45^a	5.30^a
	B_2	6.35^b	5.86^a	4.90^c	2.10^c
Average for A_2		7.44^a	6.20^a	6.18^b	3.70^b
Average for A	B_1	7.87^a	6.42^a	7.63^a	5.77^a
	B_2	6.23^b	6.06^a	5.69^b	3.49^b

A – plant density: A_1 – recommended, A_2 – lower
B – sowing method: B_1 – pure crop, B_2 – cultivation as mixture with spring wheat
a, b, c – value marked with the same letter do not differ significantly ($p \leq 0.05$)

The index values of water use efficiency for the spring wheat photosynthesis process provide information that during the leaf development stage it managed water better in case of pure sowing than in mixture (by 60.5%) and

during the stem elongation – worse (by 35.2%) – Table 7. During the tillering and inflorescence emergence stages, no significant differences between the sowing methods were found. Wheat managed water more economically in the objects with the recommended density than those with lower density from the leaf development stage until the stem elongation stage. The role of sowing density in development of water usage economy during the analysed period decreased systematically, however, with the passage of time. The result was that during the inflorescence emergence stage the wheat managed water in a more economical way in the objects with the lower density. During the entire period studied, the sowing method differentiated the values of water usage in a similar way in objects of both plant densities.

Table 7

Water use efficiency (WUE) of spring wheat [μm CO_2 mmol H_2O]

Plant density (A)	Sowing method (B)	Growth stages of spring wheat (BBCH)			
		leaf development (12–14)	tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)
A_1	B_1	2.31^a	2.20^a	1.84^b	1.28^c
	B_2	1.30^c	2.09^a	2.62^a	1.39^c
Average for A_1		1.81^a	2.15^a	2.23^a	1.34^b
A_2	B_1	1.35^c	1.49^c	1.52^b	1.78^b
	B_2	0.97^d	1.45^c	1.99^a	1.77^b
Average for A_2		1.16^b	1.47^b	1.76^b	1.78^a
Average for A	B_1	1.83^a	1.85^a	1.68^b	1.53^{ab}
	B_2	1.14^b	1.77^a	2.31^a	1.58^a

A – plant density: A_1 – recommended, A_2 – lower
B – sowing method: B_1 – pure crop, B_2 – cultivation as mixture with Persian clover
a, b, c – value marked with the same letter do not differ significantly ($p \leq 0.05$)

In case of the Persian clover, the sowing method's influence on the water use efficiency in photosynthesis was manifested at the inflorescence emergence stage (Table 8). The clover managed water better in the mixture than in the pure sowing (by 24.1%). During the tillering stage, clover used water more effectively in the objects with the lower density. During the other periods studied, the WUE index experienced no significant changes influenced by the density of plants. Interaction of the experimental factors showed that during the inflorescence emergence stage, Persian clover used water in more economical way in the mixture than in the pure stand at the object with lower plants density. During the ripening stage, water use in the photosynthesis process was more effective in the object with the recommended plant density in pure sowing than in the object with lower density in the mixture.

Table 8

Water use efficiency (WUE) of Persian clover (μm CO_2 mmol H_2O)

Plant density (A)	Sowing method (B)	Growth stages of spring wheat (BBCH)			
		tillering (21–23)	stem elongation (31–32)	inflorescence emergence (54–56)	rripening (87–89)
A_1	B_1	0.79^b	0.99^a	0.90^a	1.07^b
	B_2	0.73^b	0.90^a	0.95^a	0.69^c
Average for A_1		0.76^b	0.95^a	0.93^a	0.88^a
A_2	B_1	0.91^a	1.02^a	0.76^c	0.83^{bc}
	B_2	0.96^a	0.95^a	1.10^a	1.50^a
Average for A_2		0.94^a	0.99^a	0.93^a	1.17^a
Average for A	B_1	0.85^a	1.01^a	0.83^b	0.96^a
	B_2	0.85^a	0.93^a	1.03^a	1.10^a

A – plant density: A_1 – recommended, A_2 – lower
B – sowing method: B_1 – pure crop, B_2 – cultivation as mixture with spring wheat
a, b, c – value marked with the same letter do not differ significantly ($p \leq 0.05$)

Discussion

The response of plants to common cultivation was manifested through a change in their development rhythm, morphological characteristics, productivity and yield. This was the consequence of changes in the physiology of plants (YIN et al. 2009), including stomatal conductance, photosynthesis and transpiration. In the analysed experiment, strong competition between spring wheat and Persian clover in the mixture was documented (WANIC et al. 2016 b). Competition for the limited growth factors may decrease the intensity of photosynthesis, which was confirmed by the studies by JASTRZĘBSKA et al. (2015) as well as by our own studies. The studies showed that CO_2 assimilation by spring wheat and the undersown of Persian clover in mixture was lower than in the case of the pure sowing. In the case of wheat, the negative influence of the undersown on the process was most clearly visible during the leaf development and stem elongation stages. In Persian clover, the influence was the most pronounced during the stages of the cereal tillering and ripening. During the leaf development stage, poorer CO_2 assimilation by the wheat did not result from development of leaves with smaller area (it was even larger than in the case of pure sowing) and darkening by the partner in the mixture (the plants were small) but from other influences that disrupted that process (maybe allelopathy) (HARKOT and LIPIŃSKA 1995). This is also confirmed by lower activity of the wheat stomata during that period. Starting with the tillering stage, the wheat in the mixture with clover absorbed less nitrogen than in the pure sowing, which influenced the photosynthesis process nega-

tively because the photosynthetic capacity of the leaves is correlated closely with their nitrogen (EVERS et al. 2010). OLSZEWSKA (2008) obtained different results. She recorded positive influence of white clover on the development of photosynthesis in *Festulolium braunii*, resulting from the absorption of atmospheric nitrogen by the clover and making a part of it available to the grass. Moreover, in our own studies, in the presence of the clover undersown the wheat developed the leaves with a smaller surface area and lower stomata activity (particularly during the stem elongation stage). ZHOU and PENG (2012) also reported restriction of the photosynthesis resulting from limitation of the stomatal conductance. Lower assimilation of CO_2 by the wheat did not result, however, from limiting light access to the leaves of the cereal because during the entire vegetation period its plants were higher than the clover plants. Persian clover in the mixture was subject to strong wheat pressure from the very beginning of vegetation. According to NIELS et al. (2001), in the mixture the dominant species uses the light more effectively in photosynthesis than the subordinated one but only under conditions of good nitrogen supplies. Shortage of nitrogen in the leaves (see our earlier work – WANIC et al. 2016b) decreased photosynthesis. Similar conclusions can be found in the work by ŻUK-GOŁASZEWSKA (2008). In the analysed experiment, absorption of nitrogen by both species was lower in the pure sowing, which meant that the effective use of sunlight by both species was relatively lower. Smothered by the cereal, which was taller, of greater mass and better equipped with the leaves, the clover's assimilation apparatus were less developed than in the case of pure sowing. Already during the tillering stage, the mass of its stems was lower by more than a half than in pure sowing (WANIC and MYŚLIWIEC 2014). In addition, LÜSCHER et al. (2001) showed that competition reduced the mass of stems and carbon content in them significantly. During the entire period of common cultivation, the clover was effectively shaded by the cereal, which limited its access to the sun. This, in combination with lower absorption of nitrogen than in pure sowing and limitation of stomatal activity during certain periods, disrupted photosynthesis.

EVERS et al. (2010) also link the decrease in the photosynthesis rate with increased shading by the leaves of the neighbouring plant, the decrease of nitrogen content and the resulting lower photosynthetic capacity in the leaves. In a situation where the plants compete for light, reduction in the surface area of their leaves decreases light absorption and simultaneously increases light absorption by the neighbour (HIKOSAKA et al. 2012). This is confirmed by our own studies as well as LÜSCHER et al. (2001), who showed reduction of photosynthesis in clover leaves in the lower parts of the stand where the light availability was less than for ryegrass leaves due to the clover being shaded by the grass.

Competition limited the volume and quality of the light penetrating into the stand and consequently worsened the functioning of the clover leaves (LÜSCHER et al. 2001). Their mass was lower by more than 70% from that in pure sowing (WANIC and MYŚLIWIEC 2014). According to LÜSCHER et al. (2001) the response of clover to shading in the dense stands manifests through elongation of stems and leaf petioles. This growth utilises a large proportion of assimilates at the expense of the leaves. This causes slowdown in the clover growth rate resulting from a decrease in the surface area of the leaves and consequently lower photosynthesis intensity. This was not confirmed by TREDER et al. (2016), who recorded reduction in the height of red clover plants in a stand of spring barley. In the analysed experiment, during the cereal ripening stage, the decrease in CO_2 assimilation by the clover resulted less from shading by the wheat and more from the shortage of biogens, particularly the poor binding of nitrogen.

In our own studies, wheat cultivated in the mixture transpired less water than in the case of pure sowing from the tillering stage until the end of the inflorescence emergence stage, while for the Persian clover this lasted almost throughout the entire vegetation period. JASTRZĘBSKA et al. (2015) also recorded lower water transpiration from spring barley leaves during the stages of stem elongation and inflorescence emergence. OLSZEWSKA (2008) presented different results. She showed that grasses in the mixture with white clover transpired more water than in pure sowing. In the analysed experiment, poorer water evaporation from plants in the mixture was associated with decreased stomatal activity in that object. The stomata allow plants regulation of water circulation and CO_2 assimilation as well as adjustment of those processes to environmental changes (YAN et al. 2012, CORDOBA et al. 2015). They react to changes in environmental conditions by altering their activity and adjusting assimilation and transpiration accordingly. In the analysed experiment, plants in the mixture growing at twice the higher density than in the pure sowing had less growth factors available to them (consequently they were under stress conditions). The reaction to this situation was a decrease in the surface and density of stomata and their activity (VARONE et al. 2012). BLAIKE et al. (1988) report that white clover, in response to the stress resulting from water shortage, reacted by closing the stomata, thus limiting transpiration on the one hand and CO_2 assimilation on the other. Lower water transpiration from the Persian clover leaves also resulted from shading by wheat that was higher and had better foliage as well as a formation of the leaves with much smaller mass and surface than in case of the pure sowing (MYŚLIWIEC et al. 2014). Decreased transpiration, however, may be favourable to the plant because it improves its water balance (RABHI et al. 2012).

The WUE expressed by the ratio of assimilation to transpiration is an important indicator, which informs about adjustment of the plants to stressful situations in the environment. During drought, the plants generally close the stomata, which prevents water loss, decreases photosynthesis and leads to a general increase in water use efficiency (XING and WU 2012). Thus, this indicator tells us whether under changing environmental conditions the plant leaves optimise the CO_2 assimilation rate relative to the water loss (SWARTHOUT et al. 2009). HAFID et al (1997) highlighted strong positive correlation of the WUE index with CO_2 assimilation, transpiration and osmotic regulation. In own studies, the WUE index reached higher values in the wheat than in the clover, which indicates more effective water management by that cereal (LUCERO et al. 2000, JASTRZĘBSKA et al. 2015). It showed that in the mixture, the wheat and the clover managed water in a similar way as in the pure sowing throughout almost the entire vegetation period. Lower stomatal conductance limited to a similar degree the CO_2 assimilation and transpiration, which did not change the water balance of the plant. LUCERO et al. (2000) also did not find any influence on the WUE by mutual interactions of white clover and Italian ryegrass. According to FARQUHAR et al. (1989), during a slow build-up of stress (in the case of those authors, the water stress) photosynthesis and transpiration decrease at a similar pace and hence, the WUE is subject to no evident changes.

In own studies, the influence of plant density on gas exchange differed depending on the species and development stage. Generally, in case of the wheat, the decrease in the CO_2 assimilation was observed in those objects with the higher density during the stages of stem elongation and inflorescence emergence. The decrease in transpiration was observed during the stages of tillering and stem elongation. Higher evaporation of water from that species was found, however, during the tillering stage in the objects with the higher plant density. In the clover, higher density influenced the assimilation increase during the stages of generative development of the cereal while the rate of transpiration showed no recordable related with the density of plants. Dense sowing may lead to shading of plants as the photosynthetic system shows high sensitivity to changing characteristics of the environment, particularly the light intensity (BRESTIC and OLSOVSKA 2001, PAYNTER et al. 2001). On the other hand, excessively sparse sowing may increase ventilation of the standing crop and thus increase transpiration. THORSTED et al. (2006), based on the studies concerning winter wheat and white clover, claim that in a stand of lower density the competition between species (mainly for the light) is weakened, which has positive influence on the assimilation process. In the studies by GALON et al. (2013), it was shown that with the increasing competition (resulting from an increase in plant density) from *Brachiria*

brizantha, reduction in assimilation and stomatal conductance in sugar cane leaves was recorded. This matches our own studies. The referenced authors, however, obtained different results in the case of transpiration.

Conclusions

1. The stomatal conductance of spring wheat in the mixture with Persian clover was lower than in the pure sowing throughout the entire vegetation period. The Persian clover was characterised by lower stomatal activity in the mixture during wheat tillering and inflorescence emergence and higher activity during wheat ripening stage.

2. The higher plant density increased spring wheat stomatal conductance during its inflorence emergence only. In case of the Persian clover no clear influence of the plant density on that characteristic was recorded.

3. The spring wheat in the presence of the Persian clover undersown assimilated less CO_2 than in pure sowing. The CO_2 assimilation by the Persian clover in the mixture with wheat progressed less efficiently than in the pure sowing during the stages of tillering and ripening of that cereal.

4. The intensity of spring wheat photosynthesis in the objects with lower density was higher during the stages of stem elongation and inflorescence emergence. During the stages of leaf development and tillering, it was lower. In case of the Persian clover, the process progressed more efficiently under conditions of higher plant density during the stages of inflorescence emergence and ripening of the cereal.

5. In the mixture, the spring wheat transpired less water than in pure sowing from the stage of tillering until inflorescence emergence. The Persian clover transpired less water during the stages of the cereal tillering, inflorescence emergence and ripening.

6. In the objects with the lower plant density, spring wheat transpired more water during the stages of tillering and stem elongation and less water during the inflorescence emergence stage. Transpiration from the leaves of Persian clover was more intensive in the objects with the recommended density during the stages of inflorescence emergence and ripening of wheat.

7. Wheat managed water more effectively in pure sowing than in the mixture during the leaf development stage while Persian clover was more effective in mixture during the inflorescence emergence stage. Until the stem elongation stage, wheat used water better in the objects with the recommended plant density and worse during the inflorescence emergence stage. Persian clover used water more effectively in the object with the density

lower than recommended during the tillering stage only. During the remaining vegetation period the density of plants had no influence on the examined characteristic.

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SOME PROPERTIES OF SOIL CONTAMINATED WITH FUEL OIL AFTER APPLICATION OF DIFFERENT SUBSTANCES

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K e y w o r d s: heating oil contamination, nitrogen, compost, bentonite, zeolite, calcium oxide, soil properties.

A b s t r a c t

The study has been undertaken in order to determine the influence of different substances (nitrogen, compost, bentonite, zeolite and calcium oxide) on selected properties of soil contaminated with fuel oil. The analyzed properties of soil proved to be dependent on the fuel oil contamination and application of different substances. The experiment was set up on acid soil which was contaminated with fuel oil in the following amounts: 0, 5, 10, 15 and 20 g kg^{-1} d.m. of soil. Fuel oil raised the soil's pH but depressed its hydrolytic acidity, total exchangeable bases and cation exchange capacity. Among the substances applied to soil in order to neutralize the effect of contamination with fuel oil, bentonite and calcium oxide had the strongest influence on the soil's properties. They raised the soil's pH, total exchangeable bases and cation exchange capacity but lowered its hydrolytic acidity. The influence produced by the remaining tested substances, and nitrogen or compost in particular, on the examined characteristics of soil was relatively weak.

WYBRANE WŁAŚCIWOŚCI GLEBY ZANIECZYSZCZONEJ OLEJEM OPAŁOWYM PO APLIKACJI RÓŻNYCH SUBSTANCJI

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S ł o w a k l u c z o w e: zanieczyszczenie olejem opałowym, azot, kompost, bentonit, zeolit, tlenek wapnia, właściwości gleb.

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A b s t r a k t

Celem badań było określenie wpływu różnych substancji (azotu, kompostu, bentonitu, zeolitu i tlenku wapnia) na wybrane właściwości gleby zanieczyszczonej olejem opałowym. Doświadczenia przeprowadzono na glebie kwaśnej zanieczyszczonej rosnącymi dawkami oleju opałowego: 5, 10, 15 i 20 g kg^{-1} gleby. Badane właściwości gleby wykazywały uzależnienie od zanieczyszczenia olejem opałowym i aplikacji do gleby różnych substancji. Olej opałowy spowodował zwiększenie pH gleby, a zmniejszenie kwasowości hydrolitycznej, sumy wymiennych kationów zasadowych i całkowitej pojemności wymiennej. Spośród substancji zastosowanych w celu łagodzenia wpływu zanieczyszczenia gleby olejem opałowym najsilniej na właściwości gleby działały bentonit i tlenek wapnia, które spowodowały wzrost pH gleby, sumy wymiennych kationów zasadowych i całkowitej pojemności wymiennej oraz obniżenie kwasowości hydrolitycznej gleby. Wpływ pozostałych substancji, a zwłaszcza azotu i kompostu, na badane właściwości gleby był stosunkowo niewielki.

Introduction

Intensive growth of industry and agriculture necessities constant use of large quantities of fuels, which must be transported and stored to be used for different purposes (WYSZKOWSKI et al. 2004). Fuel transport and storage may cause contamination of the natural environment, including soils and the ground. Petroleum substances are responsible for extensive changes in soil properties, both biological (WYSZKOWSKA and WYSZKOWSKI 2010, XU et al. 1996) and physicochemical ones (CARAVACA and RODÁN 2003), often inhibiting (WYSZKOWSKI et al. 2004) or – when the contamination is very heavy – halting the growth and development of plants (MCGRATH 1992, OGBOGHODO et al. 2004). Such contaminated soils must be submitted to neutralization. Heavy soil pollution resulting from some breakdowns during fuel transport means that contaminated soils must be *ex-situ* reclaimed, which requires high financial outlays. When small quantities of petroleum substances permeate into soils, much less expensive *in-situ* reclamation technologies are applicable. Unfortunately, they are less effective (ZIÓŁKOWSKA and WYSZKOWSKI 2010), although can be successful when small-scale soil contamination is treated.

Therefore, a study has been undertaken in order to determine the influence of different substances on selected properties of soil contaminated with fuel oil.

Material and Methods

Experiment design. The experiment was set up in a greenhouse at the University of Warmia and Mazury in Olsztyn, on acid soil which was contaminated with fuel oil in the following amounts: 0, 5, 10, 15 and 20 g kg^{-1} d.m. of soil. The soil tested in the experiment had the following characteristics: pH in 1 mol KCl dm^{-3} – 4.52; hydrolytic acidity (HAC) – 25.4 mmol(+) kg^{-1}; total exchangeable bases (TEB) – 85.3 mmol(+) kg^{-1}; cation exchange capacity

(CEC) – 110,7 mmol(+) kg^{-1}; base saturation (BS) – 77.1%; $C_{org.}$ content – 11.3 g kg^{-1}; content of available forms of phosphorus – 71.9 mg P kg^{-1}; potassium – 118.6 mg K kg^{-1} and magnesium – 104.2 mg Mg kg^{-1}. The experiment was run in five series: without any soil amending substances and with the application of nitrogen (200 mg N kg^{-1} of soil), compost (270 g kg^{-1} of soil), bentonite and zeolite (180 g kg^{-1} of soil) and 50% of calcium oxide in a rate corresponding to one full hydrolytic acidity (11.7 g kg^{-1} of soil). In addition, all the pots were enriched with macro- and micronutrients in the following quantities [in mg kg^{-1} of soil]: N – 100 $CO(NH_2)_2$, P – 30 (KH_2PO_4); K – 100 (KH_2PO_4 + KCl); Mg – 50 ($MgSO_4 \cdot 7H_2O$); Mn – 5 ($MnCl_2 \cdot 4H_2O$); Mo – 5 [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$]; B – 0.33 ($H_3BO_3$). The petroleum substances, compost, bentonite and lime as well as the macro- and micronutriens in the form of aqueous solutions were mixed with 9 kg of soil when the experiment was set up and placed in polyethylene pots. Next, maize (*Zea mays* L.) cv. Reduta was sown. During the experiment, the soil relative moisture was maintained at 60% of capillary water capacity. Soil samples for analyses were taken during the harvest of maize in the intensive stem elongation phase.

Analysis of samples. The sampled soil was dried and passed through a 1 mm mesh sieve. The following determination were made: soil reaction (pH) with the potentiometric method in an aqueous solution of KCl in the concentration of 1 mol dm^{-3}, hydrolytic acidity (HAC) and total exchangeable bases (TEB) – by Kappen's method (LITYŃSKI et al. 1976). From the hydrolytic acidity (HAC) and total exchangeable bases (TEB), the cation exchange capacity (CEC) and base saturation (BS) were computed according to the following formulas: $CEC = TEB + HAC$; $BS = TEB \cdot CEC^{-1} \cdot 100$. Additionally, before the experiment was set up, the soil was tested for its content of organic carbon ($C_{org.}$) with Tiurin's method (LITYŃSKI et al. 1976), as well as the content of available phosphorus and potassium with Egner-Riehm's method (LITYŃSKI et al. 1976) and available magnesium with Schachtschabel's method (LITYŃSKI et al. 1976). The results underwent statistical processing with the two-factorial analysis of variance tests, using for that purpose the software Statistica (StatSoft, Inc. 2014). Dependences between oil contamination with fuel oil and the analyzed soil's attributes were also tested with Pearson's simple correlation tests.

Results and Discussion

Fuel oil contamination of soil and its amendment with different substances had significant influence on the analyzed soil properties. Fuel oil raised the soil's pH (to 10 g of fuel oil per 1 kg of soil) but depressed its hydrolytic acidity

(Table 1). In the series without any substances added to soil, the range of soil's pH increase (r = 0.738) and decrease in its hydrolytic acidity (r = -0.904) were comparable. In the series without soil amending substances, fuel oil depressed the total exchangeable bases (r = -0.932) and the cation exchange capacity (r = -0.937) but did not cause any large changes in the base saturation (Table 2). The biggest changes of the total exchangeable bases and the cation exchange capacity were caused by the rate of 15 g of fuel oil per 1 kg of soil. Both the total exchangeable bases and cation exchange capacity declined by the same percentage (11%) under the influence of this rate of the contaminant.

Table 1

pH and hydrolytic activity (HAC) in soil after maize harvest

Dose of fuel oil in [g kg^{-1} of soil]	Kind of substance neutralizing effect of heating oil						
	without additions	nitrogen	compost	bentonite	zeolite	CaO	average
pH in KCl							
0	4.62	4.57	5.02	6.71	5.33	6.59	
5	4.91	5.33	5.06	6.96	5.63	7.00	
10	5.32	5.10	5.25	7.00	5.58	7.24	
15	5.26	5.02	5.51	6.91	5.44	7.20	
20	5.11	5.05	5.37	6.94	5.67	7.02	
r	0.738**	0.372	0.880**	0.572	0.547	0.650*	
LSD	a – 0.02**, b – 0.02 **, a · b – 0.04**						
Hydrolytic activity (HAC), [mmol(+) kg^{-1} of soil]							
0	22.9	25.2	23.4	12.2	22.7	16.5	20.5
5	22.8	19.4	23.1	14.4	25.3	15.0	20.0
10	21.7	21.0	20.8	16.9	27.4	15.6	20.6
15	20.0	22.2	19.4	15.0	30.4	13.0	20.0
20	20.6	24.3	20.6	11.4	27.5	15.1	19.9
Average	21.6	22.4	21.5	14.0	26.7	15.0	20.2
r	-0.904**	0.067	-0.854**	-0.071	0.812**	-0.590	-0.585
LSD	a – n.s., b – 0.9**, a · b – 2.1**						

LSD for: a – heating oil dose, b – kind of neutralizing substance, $a \cdot b$ – interaction; significant for: ** – p = 0.01, * – p = 0.05, n.s. non-significant; r – correlation coefficient

Soil pollution with petroleum substances causes many changes in soil quality (OGBOGHODO et al. 2004, WYSZKOWSKA and WYSZKOWSKI 2010, ZIÓŁKOWSKA and WYSZKOWSKI 2010). Their actual effect on soil attributes depends on the type and degree of contamination with petroleum substances. Diesel oil generally causes larger changes than other petroleum products, e.g. petrol. BARAN et al. (2002) determined elevated pH, total base cations and cation exchange capacity near point sources of contamination with petroleum substances on the premises of a military airfield in Dęblin, compared to less

Table 2
Total exchangeable bases (TEB), cation exchange capacity (CEC) and base saturation (BS) in soil after maize harvest

Dose of fuel oil in [g kg^{-1} of soil]	Kind of substance neutralizing effect of heating oil						
	without additions	nitrogen	compost	bentonite	zeolite	CaO	average
Total exchangeable bases (TEB), [mmol(+) kg^{-1} of soil]							
0	104.9	95.3	100.0	125.8	88.7	108.7	103.9
5	100.0	99.8	93.3	136.4	99.9	107.7	106.2
10	99.4	95.4	93.9	140.3	90.9	122.2	107.0
15	93.3	103.5	95.3	132.1	92.3	127.0	107.2
20	94.5	104.7	99.8	130.7	100.7	112.1	107.1
Average	98.4	99.7	96.5	133.0	94.5	115.5	106.3
r	-0.932**	0.809**	0.078	0.157	0.475	0.480	0.843**
LSD	*a* –1.7**, *b* –1.8**, *a* · *b* – 4.1**						
Cation exchange capacity (CEC), [mmol(+) kg^{-1} of soil]							
0	127.8	120.5	123.4	138.0	111.4	125.2	124.4
5	122.8	119.2	116.4	150.8	125.2	122.7	126.2
10	121.1	116.4	114.7	157.2	118.3	137.8	127.6
15	113.3	125.7	114.7	147.1	122.7	140.0	127.2
20	115.1	129.0	120.4	142.1	128.2	127.2	127.0
Average	120.0	122.2	117.9	147.0	121.2	130.6	126.5
r	-0.937**	0.729*	-0.316	0.095	0.750**	0.432	0.781**
LSD	a – 1.9**, b – 2.1**, *a* · *b* – 4.6**						
Base saturation (BS), [%]							
0	82.1	79.1	81.0	91.2	79.6	86.8	83.3
5	81.4	83.7	80.2	90.4	79.8	87.8	83.9
10	82.1	82.0	81.9	89.2	76.8	88.7	83.4
15	82.3	82.3	83.1	89.8	75.2	90.7	83.9
20	82.1	81.2	82.9	92.0	78.5	88.1	84.1
Average	82.0	81.7	81.8	90.5	78.0	88.4	83.7
r	0.450	0.256	0.847**	0.145	-0.544	0.607*	0.766**
LSD	*a* – 0.6**, *b* – 0.6**, *a* · *b* – 1.4**						

LSD for: *a* – heating oil dose, *b* – kind of neutralizing substance, *a* · *b* – interaction; significant for: ** – p=0.01, * – p=0.05, n.s. non-significant; *r* – correlation coefficient

polluted soils. In contrast, KUCHARSKI and JASTRZĘBSKA (2005) observed depressed pH, total exchangeable bases, cation exchange capacity and base saturation, a finding which is largely confirmed by the present study.

Application of the tested substances to soil, except nitrogen, favoured higher soil pH, with the strongest and comparable effects produced by bentonite and calcium oxide (Table 1). Bentonite and calcium oxide also caused highly significant decrease in the hydrolytic acidity, reaching 35 and 31%, respectively, in comparison to the series with no soil amendments. Zeolite produced a much weaker (24%) and reverse effect. The application of compost and

nitrogen as urea to soil did not result in any significant alterations of the soil's hydrolytic acidity.

Bentonite and calcium oxide had the strongest effect on the total exchangeable bases and cation exchange capacity of all the substances applied in order to alleviate the impact of soil contamination with fuel oil (Tables 1–2). They caused an increase in the total exchange bases, cation exchange capacity and – to a much smaller degree – the base saturation. Compared to the non-amended series, the total exchangeable bases and cation exchange capacity in soil rose by 35 and 23% under the influence of bentonite and by 17 and 9% when soil was neutralized with calcium oxide. Bentonite and calcium oxide also raised the base saturation by 9 and 6%, respectively. The other substances, and nitrogen (urea) or compost, had a much weaker neutralizing effect. Lime have positively effect on many soil properties (KACZOR et al. 2009). In research by WYSZKOWSKI and SIVITSKAYA (2015) and WYSZKOWSKI and ZIÓŁKOWSKA (2013), bentonite and calcium oxide had the strongest and most positive effect on the analyzed soil properties, especially hydrolytic acidity.

The application of neutralizing substances to soil may prove to be an effective measure in alleviating the effects of soil contamination with fuel oil. Studies completed by other researchers (BARAN et al. 2004, RIFFALDI et al. 2006, QUINTERN et al. 2006) suggest that organic substances, including compost, may have a beneficial effect on properties of soil contaminated with small amounts of fuel oil or petrol. They not only positively affect soil characteristics (BARAN et al. 2004, CZEKAŁA 1997) but also improve the growth and development of crops growing on such soil (KMEŤOVÁ and KOVÁČIK 2014, WYSZKOWSKI and ZIÓŁKOWSKA 2009). However, the above is true only when the soil contamination degree is small. More severe soil pollution with petroleum substances eliminates any possibility of crop cultivation (MCGRATH 1992, OGBOGHODO et al. 2004). Compost and other organic substances improve oxygenation of soil, thus accelerating the microbial decomposition of petroleum substances, besides, by producing a beneficial effect on the soil's sorptive properties, they improve the cycling of elements in the soil environment (ZIÓŁKOWSKA and WYSZKOWSKI 2010).

Conclusions

1. The analyzed properties of soil proved to be dependent on the fuel oil contamination and application of different substances.

2. Fuel oil raised the soil's pH but depressed its hydrolytic acidity, total exchangeable bases and cation exchange capacity.

3. Among the substances applied to soil in order to neutralize the effect of contamination with fuel oil, bentonite and calcium oxide had the strongest influence on the soil's properties. They raised the soil's pH, total exchangeable bases and cation exchange capacity but lowered its hydrolytic acidity.

4. The influence produced by the remaining tested substances, and nitrogen or compost in particular, on the examined characteristics of soil was relatively weak.

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IMPACT OF NUTRITION ON REARING RESULTS AND METABOLIC PROFILES OF KAZAKH WHITE HEAD BREED HEIFERS AND BREEDING BULLS

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K e y w o r d s: nutrition, metabolic profile, body weight, urea, protein, alkaline phosphatase.

A b s t r a c t

This study analysed the feeding of young breeding cattle of the Kazakh White Head race, from birth to the age of 450 days at a Kazakh farm called "Dinar's Ranch". In the final stage of the research, the animal health condition was assessed, based on the biochemical blood parameters. The level of nutrition in the first period of life of the animals significantly influenced the growth and development of young breeding cattle. 1,250 liters of milk, besides solid feed, is recommended for calves from birth to the age of 240 days. Applied nutrition in subsequent life periods, including hay, silage, concentrated feed and mineral additives ensure that the assumed daily body weight increases were real. The average daily dry weight absorption by the heifers was from 5.3 to 7.1 kg, whereas bulls absorbed from 6.5 to 8.7 kg. The daily increase of heifers at the age of 361–450 days was 833 g, whereas for bulls it was 1055 g/day. The metabolic profile parameters of bulls (hepatic enzymes – AST and ALT, urea, protein, alkaline phosphatase – ALP, as well as Ca, Na, K, Mg and P content) were in the range of the reference standards, which reflected the good health status of the animals.

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WPŁYW ŻYWIENIA NA WYNIKI ODCHOWU I PROFIL METABOLICZNY JAŁÓWEK I BUHAJKÓW HODOWLANYCH RASY KAZACHSKIEJ BIAŁOGŁOWEJ

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S ł o w a k l u c z o w e: żywienie, profil metaboliczny, masa ciała, przyrosty dobowe, mocznik, białko, fosfataza alkaliczna.

A b s t r a k t

Celem pracy była analiza żywienia młodego bydła hodowlanego rasy kazachskiej białogłowej od urodzenia do wieku 450 dni w jednym z gospodarstw Kazachstanu o nazwie „Ranczo Dinara". Na końcowym etapie doświadczenia oceniono zdrowie zwierząt na podstawie biochemicznych parametrów krwi. Poziom żywienia w pierwszym okresie życia zwierząt istotnie oddziaływał na przebieg wzrostu i rozwoju młodzieży hodowlanej. Cielętom od urodzenia do wieku 240 dni zaleca się podanie 1250 litrów mleka poza paszami stałymi. Zastosowane żywienie w kolejnych okresach życia oparte na sianie, kiszonce, paszy treściwej i dodatkach mineralnych zapewniło osiągnięcie zakładanych przyrostów dobowych masy ciała. Średnie dzienne pobranie suchej masy przez jałówki wynosiło od 5,3 do 7,1 kg, a buhajki pobierały od 6,5 do 8,7 kg. Przyrosty dobowe jałówek w wieku 361–450 dni wynosiły 833 g, zaś buhajków – 1055 g/dobę. Parametry profilu metabolicznego buhajów (enzymy wątrobowe – AST i ALT, mocznik, białko, fosfataza zasadowa – ALP, jak również zawartość Ca, Na, K, Mg i P) mieściły się w zakresie norm referencyjnych, świadcząc o dobrym statusie zdrowotnym zwierząt.

Introduction

Grassland is a purveyor of cheap feed. Feed manufactured from grasslands can be fed only when fresh and on a pasture or preserved as silage, haylage or hay (HUUSKONEN et al. 2009). Pure-bred herds of meat calves born in the early spring can stay with their mothers, using the pasture until the end of October. Further rearing of calves takes place in the alcove (BADIEJEVA 2012). In Kazakhstan, most beef is derived from dual purpose animals, however White Head race meat breed, Kazakh Herefordand imported pure-bred Hereford have also a large share of beef production. Both genetic and environmental factors affect the quality of beef (AMANZHOLOV et al. 2012, BADIEJEVA 2012). The influence of the breed cattle for fattening is also very important (DYMNICKA et al. 2004, POGORZELSKA et al. 2013). Cattle of different races or genotypes are characterized by diverse physiological features (i.e. early ripening, growth and sex) which consequently affect the quality of meat (WHEELER

1994, BINDON and JONES 2001, BURROW et al. 2004, ISABJEKOV and MALCZEWSKI 2012,). The main factors affecting beef quality, among environmental factors,are the feeding and housing system (POGORZELSKA 1999, POGORZELSKA et al. 2013), the age at slaughter, pre-slaughter trading (stress resulting from transport, residing in a slaughter warehouse, starvation) and meat treatment after slaughter (*The new national*... 2012). Fodder is the main cost component in both breeding and production (fattening) herds. Nutritional strategy is a factor used as a tool for monitoring bulls and heifers reared in breeding herds, as well as to improve and control cattle fattening, animal welfare, safety, nutritional value and the nutritional and technological quality of meat (STENN 1995). Research conducted on the effects of nutrition on the course of rearing has covered not only the diversity of cow breeds, but also the types and availability of used feed (JELMANOWET al 1983). Research into which feeding affects the muscle and slaughter efficiency growth and allows the introduction of components and chemical compounds into the feed is extremely valuable. Such compounds could be potentially absorbed from the gastrointestinal tract and subsequently incorporated into cellular structures or by accumulating in tissues and improving meat nutritional or biological qualities (MINKIEWICZ et al. 2013). An example of this is modifying the feed composition (GRANIT et al. 2001, WOOD et al. 2004). WARREN et al. (2008) found that bovine meat originating from cattle fattened with silage from green forage has a 2–3 day longer shelf life, due to slow progressive changes in lipid oxidation and, thus, has a more stable color compared to the meat of cattle fed with concentrated feeds. LEE et al. (2008) demonstrated that the addition of sulfur and vitamin E to feedincreases the stability of lipids and myoglobin during meat storage. Vitamin E effectively slows down the oxidation of lipids and sulfur preferably affects the stability of oxymyoglobin. PEDREIRA et al. (2003) supplemented feed with Vitamin D3 and found that it affects the improvement of meat tenderness, which is inherently hard, whereas it is irrelevant for animals which are the source of such meat (ANDERSEN et al. 2005). The results of both tests show the high efficiency of such a feeding system and encourage further scientific research in this field (MAKULSKA and WĘGLARZ 2001).

Aim of the work. The aim of this study was an analysis of the nutritional impact of Kazakh White Head race heifers and bulls, which achieved optimal growth and development indicators for young breeding cattle in the period from birth to 450 days of life and to assess health status based on selected biochemical blood parameters.

Material and Methods

The research was conducted on the "Dinara's Ranch" farm near Almaty, Kazakhstan. The material consisted of a beef Kazakh White Head race cattle herd. The first stage of the study determined the details of the nutrition of cows and bulls born in the early spring and staying with their mothers in the pasture up to the age of 7–8 months old. The second stage involved the following months of life in two periods, i.e. from 240 to 360 days and from 361 to 450 days of life, specifying the two feeding periods – summer and winter. The types of feed were specified. Feed doses in subsequent months of heifers and bulls lives were also specified, taking gender into account. Nutrition was based mainly on roughage produced on grasslands, i.e. green forage or silage from grass or corn silage, hay and concentrated feed addition. The total consumption of each feed used in nutritional doses for heifers and bull feeding, from birth to 15 months of their lives, was also presented. Daily gains in body weight at certain ages were used as indicators of growth and development of farm animals.

During alcove feeding, the basic *ad libitum* feed was corn silage supplemented with hay in an amount of 2.5–6 kg (heifers) and 3–7.5 kg (bulls). The addition of roughage is as follows: 1.5–2.5 kg (heifers) and 2.5 kg bulls. Daily doses of silage were increased in 30-day intervals, not to exceed 10% of unused feed per dose.

Young cattle nutrition in the specified periods of breeding in the summer proceeded as follows: heifers (aged 240–360 days) consumed 20 kg of fodder and 1kg of concentrated feed supplemented with microelements, whereas bulls during this period consumed 20 kg of fodder and 3 kg of concentrated feed. In the next period (361–450 days), there was an increase to 22 kg in forage intake by heifers (concentrated feed remained the same, while bulls consumed 23 kg of fodder and 4 kg of concentrated feed during this period).

From 450 days of age (±10 days), randomly-selected bull blood was collected from the jugular vein to determine blood biochemical parameters. Blood samples were collected into heparinized tubes and allowed to coagulate. After two hours, the blood was centrifuged for 10 minutes at 3,000 rpm (in an MPW 223e centrifuge) and the resulting serum was collected by pipette and stored in Eppendorf tubes at -18°C until determination. The total protein (TP) [g dl^{-1}] level was determined in blood serum. The content was determined using the LOWRY'S et al. (LOWRY 1951) colorimetric micro-method (Sigma Diagnostic Kits). Further indications of blood serum were performed on a Mindray BS-120 photometer. These included the following biochemical indicators: serum alanine aminotransferase (ALT) [U l^{-1}], alkaline phosphatase (ALP) [U l^{-1}] and urea concentration (UREA) [mg dl^{-1}].

To interpret the results of blood biochemical parameters, the reference levels adopted in developed standards for cattle were used. These standards were as follows: ALT – 25 – 74 U/l, AST – 58–100 U/l, ALP – 41–116 U/l, UREA – 10–45 mg dl^{-1} (ANDREWS 2004, DIRKSEN et al. 2007, WINNICKA 2008) and protein level – 51–71g (WINNICKA 2008). The content of some macroelements, i.e. calcium (Ca), sodium (Na), potassium (K), magnesium (Mg) and phosphorus (P) was also determined in the collected blood.

The achieved results were statistically analyzedusing a one-way analysis of variance in the orthogonal system. The mean (x) and standard deviation (Sd) were determined. The significance of differences was verified using Fisher LSD test (RUSZCZYC 1981). The results were analyzed statistically using Statistica ver. 9.0 (StatSoft 2011).

Results and Discussion

Three groups of factors determine the economic results of beef cattle breeding and production of beef: correct breeding (64–65%), an appropriate system of breeding and production technology (approx. 32%) and the correct choice of race (3–4%). Therefore, the choice of feeding system, grazing and winter maintenance of the basic herd technology, calf rearing method, type of rooms, care of animals, etc. are the most important elements influencing the profitability (DOBICKI 2000).

Race, type of utility, cattle sex, age, conditions of living and (most of all) nutrition, have a decisive impact on the achieved rearing results. Nutrition is one of the most important production factors, constituting a major component of the production cost of animals for slaughter. Since the basic aim of modern cattle feeding methods is to achieve a high rate of daily weight growth, there is a tendency to intensify nutrition. While an increased fattening intensity is possible in almost all production circumstances, which involves the use of large quantities of concentrated feed in rations, in breeding herds such feeding is not recommended.

Nutrition is a major cost factor. It is therefore reasonable to seek the possibility of its reduction. Grasslands are by far the cheapest source of feed, because the production cost of one food unit on pastures is 3 times lower than cereal production (MAKULSKA and WĘGLARZ 2001*)*. WĘGLARZ'S (2010) research on the impact of fattened cattle category on beef quality indicates the impact of slaughter season (winter or summer). In another experiment, bulls from 3 races (Limousine, Hereford and Simmental) were fattened. They were fed with unified rations, composed of corn silage and meadow grass with an addition of hay (1 kg/day) and concentrated feed (1% of body weight/day).

In the experiment, weight was determined, along with the chemical composition of feed, fatty acid profile of the feed, slaughter efficiency and the chemical composition of beef. In terms of fattening and slaughter value, Herefords produced less-favorable results than Limousine and Simmental (CHOROSZY et al. 2006).

A determinant of the nutrition intensity are fodder resources of the farm and feeding systems applied by the manufacturer. However, good results in a breeding herd requires the appropriate rearing of calves. Veal period is a time of the most intense changes taking place in the body (NIWIŃSKA and STRZETELSKI 2005).

Table 1 shows the scheme of calf feeding from birth to 240 days. The basis for calf feeding was milk, but the dose was supplemented with concentrated feed and hay (or green forage in the summer time). The administered doses guaranteed daily increments required to obtain the body weight of calves indicated in Table 1.

Table 1
Nutrition scheme of calves to the age of 240 days – numbers are approximate, regardless of gender

Calf age [days]	Body weight [kg]	Feed consumption [pcs/day]						
		milk [l]	hay [kg]	silage [kg]	green forage [kg]	concentrated feed [kg]	salt [g]	feed Phosph. [g]
5–30	50	5.3	*ad libitum*	–	–	*ad libitum*	–	–
31–60	80	5.3	0.1	–	–	0.4	8	10
61–90	100	6.5	0.3	1.0	–	0.4	12	15
91–120	130	6.5	0.6	–	3.3	0.5	16	20
121–150	150	5.5	–	–	6.0	0.5	18	25
151–180	170	5.5	–	–	12.0	1.0	20	30
181–210	200	3.5	–	–	14.0	1.0	25	35
211–240	220	3.5	2.0	7.0	–	2.3	30	40
Total [kg]		1250	120	240	1060	250	3.90	5.25

These results are comparable with those obtained in other studies (ZWIERZCHOWSKI et al. 2016). The level of nutrition in the first period of animal life significantly affects the growth of muscle tissue. Proper nutrition in subsequent periods of life ensures the achievement of daily weight gains (AMANZHOLOV et al. 2012, BADIEJEVA 2012, *The new national*... 2012).

Table 2 presents the average daily quantity and types of feed absorbed by the heifers and bulls in the subsequent months of their lives. In the period of alcove feeding, the food rations included: hay, corn silage and concentrated feed supplemented with mineral additives. The amount of feed and achieved

body weight affected the dry weight indicator in the dose per 1 kg of animal growth, from birth to an age of 450 days (Table 2). One of the factors affecting the consumption of various types of feed was the sex of animals. Generally, the nutritional needs of young bulls outweigh the needs of heifers. Therefore, in the period from 240 to 360 days of age, hay consumption in a heifer dose ranged from 2.5 kg to 4 kg, while bulls consumed from 3 to 5.5 kg. The increase in silage consumption by heifers was as follows: from 7 kg at the age of 240–270 days to 12 kg at the age of 331–360 days. Bulls of the same age consumed 10kg and 13kg, respectively. The dose was supplemented with concentrated feed in the amount from 1.5 kg to 2 kg for heifers and 2.5 kg for bulls. The feed consumption by animals of both sexes increased significantly after 360 days of life. In the last month of analysis (421–450 days), heifers ate 6kg of hay, 10 kg of silage and 2.5 kg of concentrated feed, while the dose for bulls contained 7.5 kg of hay, 11 kg of silage and 3 kg of concentrated feed.

Table 2

Nutrition scheme of young breeding cattle at the age of 240–450 days (spring births)

Age	Gender	Body weight	Feed consumption pcs/day]				
			hay [kg]	silage [kg]	concentrated feed [kg]	salt [g]	feed phosphate [g]
240–270	heifers	193	2.5	7	1.5	0.03	0.03
	bulls	220	3	10	2.5	0.04	0.04
271–300	heifers	223	2.8	9	1.5	0.03	0.03
	bulls	245	4	10	2.5	0.04	0.04
301–330	heifers	250	3.5	10	1.5	0.03	0.03
	bulls	270	5	11	2.5	0.04	0.04
331–360	heifers	275	4	12	2.0	0.04	0.03
	bulls	305	5.5	13	2.5	0.05	0.04
361–390	heifers	297	5	11	2.5	0.04	0.03
	bulls	335	6	13	2.5	0.05	0.05
391–420	heifers	323	5.5	10	2.5	0.04	0.03
	bulls	365	6.5	11	2.5	0.05	0.05
421–450	heifers	350	6	10	2.5	0.04	0.03
	bulls	400	7.5	11	3.0	0.05	0.05

The global consumption of feed for heifers and bulls in the specified periods of alcove feeding is given in Table 3. It shows the diversity resulting from the size of the rations set for animals of different sexes. The daily consumption of dry matter per dose (Table 3) confirms the increased feed absorption of bulls compared to heifers. Continuous improvements of animal breeds, as well as innovative rearing practices, as well as modifications to the composition of the feed, largely contribute to changes in nutrient concentrations (SCOLLAN et al.

2006). ŁOZICKI et al. (2010) conducted a study on Hereford race bulls fattening from 250 kg of weight to about 550 kg, feeding them with corn silage, hay and concentrated feed, supplemented with a vitamin-mineral mixture. The average daily dry matter intake by bulls amounted from 7.92 to 8.15 kg and the increases exceeded 1300 g/day.

Table 3

Feed consumption by the young breeding cattle in the alcove feeding [kg]

Feed	Age [days]			
	240–360		361–450	
	heifers	bulls	heifers	bulls
Straw/hay	384	525	495	600
Corn silage	1140	1320	930	1050
Concentrated feed	195	300	225	240
Salt	3.9	5.1	3.6	4.5
Feed phosphate	3.6	4.8	2.7	4.5
Dry weight use in dose	5.3	6.5	7.1	8.7

The situation is different for feeding heifers and bulls during the summer using green forage (Table 4). Regardless of gender, the intake of silage was 20–23kg, but heifers were additionally fed only with 1 kg of concentrated feed, while the bulls received it in an amount of 3 kg of 360 days and 4 kg above that age. WAJDA et al. (2006), studied the fattening of bulls from 260 kg of *ad libitum* hay feeding, as well as cereal grits in the amount of 3.5 kg – to a body weight of approximately 350 kg, 4 kg – from the weight of 430 kg (+ mineral additives), and achieved increases in the control fattening (lasting 270 days) exceeding 0.9 kg.

Table 4

Summer type feeding system of the young breeding cattle

Age [days]	Sex	Green forage [kg]	Concentrated feed	Salt [kg]	Feed phosphate [g]
240–360	heifers	20	1	0.03	0.03
	bulls	20	3	0.04	0.03
361–450	heifers	22	1	0.05	0.04
	bulls	23	4	0.05	0.05

The fattening system and nutrition level of cattle has a major impact on the growth rate of animals (O'SULLIVAN 2004). The course of growth and development of animals is best characterized by body weight and achieved daily gains (Table 5).

Table 5

Body weight and daily gains of the young breeding cattle ($\bar{x}$ ± Sd)

Traits	Age [days]	Numbers	
		heifers	bulls
Body weight [kg]	birth	27 ± 1.4	30.0 ± 1.6
	240	193 ± 5.1	221 ± 6.3
	360	275 ± 7.2	305 ± 9.3
	450	350 ± 10.3	400 ± 12.9
Daily gains [g]	birth – 240	692 ± 4.3	796 ± 5.4
	240–360	683 ± 5.8	700 ± 4.7
	360–450	833 ± 4.4	1055 ± 7.2
	birth – 450	718 ± 3.2	822 ± 5.9

The average weight of heifers at birth was 27 ± 1.4 kg and bulls 30 ± 1.6 kg. These are sizes differing significantly from the body weight of calves of specialized meat breeds. Hereford at birth – 33–36 kg, Angus – 26–30 kg, Limousine – 35–40 kg (*Chów bydła...* 2009). The feeding system used in practice influenced the achieved body weight and daily mass gains in specified periods of life. At the age of 360 days, bulls reached weights exceeding 300 kg with increases from the age of 240 days amounting to 700 g/day. Heifers at the same age weighed 275 kg, which was a consequence of the daily gains at 683 g. The next life period is significant, because a daily gain of heifers of over 830 g allows for a 350 kg body weight achievement by the age of 450 days. The daily growth of bulls after 360 days of age exceeded 1050 g. This resulted in a body weight of bulls of 400 kg at the age of 450 days. These results are comparable with those of hybrids fed in a semi-intensive system (NOGALSKI 2014, POGORZELSKA et al. 2013).

To evaluate the metabolic profile of the tested animals, the following indicators were selected: the level of liver enzymes (ALT and AST), urea (UREA), alkaline phosphatase (ALP). Alanine aminotransferase (ALT) (EC 2.6.1.2) and aspartate aminotransferase (AST) (EC 2.6.1.1) are enzymes carrying the amino groups of the amino acids to α-keto acids. Their increased levels can indicate muscle damage or malfunction of the liver (DOORNENBAL et al. 1988, BAIROCH 2000, JACKSON and COCKROFT 2002). WINNICKA (2008) gives a reference range for ALT amounting to 25–74 U/l and AST – 58–100 U/l. The authors' own study has shown that both the level of ALT as well as AST (Table 6) in the serum of bulls is in a range of reference values for the cattle presented by WINNICKA (2008). It can therefore be assumed that the tested animals were characterized by the normal activity of these enzymes. Any significantly increased activity could indicate potential metabolic problems.

Table 6

Biochemical blood parameters of bulls ($\bar{x}$ ± Sd)

Biochemical parameters	Bulls in the age of 450 days
Quantity [pcs]	15
AST [U l]	75.54 ± 2.93
ALT [U l]	25.80 ± 3.43
Ca [mg dl]	2.20 ± 0.20
UREA [mg dl]	5.87 ± 3.43
ALP [U l]	102.63 ± 13.93
Na [mg l]	160.40 ± 16.10
K [mg/l]	3.60 ± 1.20
Mg [mg l]	1.00 ± 0.43
P [mg l]	2.07 ± 0.17
Protein [g dl]	75.63 ± 1.83

Alkaline phosphatase (ALP) (EC 3.1.3.1.) is an enzyme responsible for the release of the phosphorous from esters. It occurs in almost all tissues of the body. In mature animals, ALP is produced in the liver, whereas in maturing animalsit occurs mainly in the bones. Its high level of activity is associated with a rapid growth of bones (BAIROCH 2000, DOORNENBALET al. 1988). When examining the content of alkaline phosphatase in the serum of bulls (Table 6), it was shown to be within a reference standard (WINNICKA 2008). Urea (UREA) is a water-soluble compound formed in the liver during the ornithine cycle, as a derivative of aminoacid changes. Its level indicates the state of protein transitions in the body. An increased concentration is characteristic of dehydration, disease conditions or excessive protein intake in the diet, while a reduced level occurs in the event of a liver malfunction (JACKSON and COCKROFT 2002). The data reported in Table 6 shows the level of urea in serum of bulls was correct and was within the reference standards provided by WINNICKA (2008). A larger supply of proteins in the feed results in more intensive biodegradation carried out in pre-stomachs by bacteria. The amount of ammonia increases which, in turn, is converted to urea (KNOWLES et al. 2000, MOHRI et al. 2007).

The level of total protein in the serum of bulls and, especially, its fluctuations may be an indicator of proper nutrition or the appearance of inflammation. This is an important element in the diagnosis of the state of hydration (JEŽEK et al. 2006, MOHRI et al. 2007, KHAN 2011). In Table 6, the level of TP in the serum of bull samounts to 75.63 g/l. JEŽEK et al. (2006) found that in bulls above the ageof 84 days, the level of total protein was lower and amounted to 56.71 g/l. Inother authors, the level of total protein amounted to: KNOWLES et al. (200) – 62 g/l, NOWAK et al. (2005) – 50.5 g/l, MOHRI et al. (2007) – 63 g/l.

In ŁOZICKI et al. (2010), data from 450 kg Hereford breeding bulls was collected and selected biochemical indicators (glucose, total protein, albumin and urea) were determined in serum, as follows: 51.5 mg/dl, 6.53 g/dl, 2.66 g/dl and 4 mg/dl.

Calcium, together with phosphate and magnesium, ensure normal mineralization (AGUILERA and VAUGHAN 2000, RODRIGUEZ 2001) of bones and teeth. Calcium is also responsible for regulation of the nervous and muscular system and is involved in blood clotting. It acts as an activator of certain enzymes such as lipase, ATP-ase (KOLDOVSKY 1989). Phosphorus also plays an important role in numerous metabolic processes, playing a key role in receiving and transporting energy, phosphorylation processes and is important in the metabolism of glucose, fructose and proteins (MWAURA and AKINSOYINU 2010). Sodium and potassium are essential for regulation of osmotic pressure and normal muscle function. Magnesium is involved in many metabolic processes and is present in the nucleus, whose function it supports. The most important function of magnesium in the body is involvement in the synthesis and breakdown of high energy compounds, mainly adenosine triphosphate (ATP).

Magnesium is a co-enzyme or activator of many enzymes, especially those related to the transfer of phosphate groups. It is involved in many metabolic pathways associated with the metabolism of proteins, nucleic acids, lipids and carbohydrates and in the processes of electrolyte transport across cell membranes. It is a factor in living cell regeneration and calcium balance control. In addition, it has a positive effect on blood clotting, regulates the development of the skeletal system, increases the defensive reactions of the body, acts preventively to inflammation of the veins in post-operative situations and strengthens the cardiovascular system. It functions as an anti-stress, anti-anaphylactic and anti-inflammatory factor and lowers the cholesterol level and protects against myocardial damage (TOUYZ 2004). Magnesium and potassium play a regulatory role in the control of blood pressure, reducing the risk of cardiovascular disease.

To sum up, the level of nutrition in the first period of life of the animals significantly influences the growth and development of young breeding cattle. 1,250 liters of milk, in addition to solid feed, is recommended for calves from birth to the age of 240 days. The applied nutrition in subsequent life periods, including hay, silage, concentrated feed and mineral additives ensure that the assumed daily body weight increases were real. The average daily dry weight absorption by the heifers was from 5.3 to 7.1 kg, whereas bulls absorbed from 6.5 to 8.7 kg. The daily increase for heifers at the age of 361–450 days was 833 g, whereas for bulls it was 1,055 g/day. The metabolic profile parameters of bulls (hepatic enzymes – AST and ALT, urea, protein,

alkaline phosphatase – ALP, as well as Ca, Na, K, Mg and P content) were in the range of the reference standards, which reflected the good health status of animals.

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EFFECT OF DIFFERENT ULTIMATE pH RANGE ON MEAT QUALITY OF CROSSBRED POLISH HOLSTEIN × LIMOUSIN HEIFERS

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K e y w o r d s: beef, pH_u value, meat quality.

Abstract

Fifty *longissimus thoracis* muscle samples were obtained from the chilled (48 h, 2–4°C) right carcass side of crossbred Polish Holstein x Limousin heifers (PHF x LIM). Vacuum-packaged samples were chill-stored (0–2°C) for five days, and then the proximate chemical composition, physicochemical properties and sensory attributes of meat were determined. In order to evaluate the influence of pH level on meat quality, the samples were divided into four groups based on their pH_u values: ≤ 5.4, 5.4–5.7, 5.8–6.0 and > 6.0. Meat with pH_u > 6.0 had the lowest dry matter and fat content, and meat with pH_u 5.5–5.7 had the highest dry matter and fat content. Meat with the highest pH_u (> 6.0) was characterized by the highest total water-soluble nitrogen content, the darkest color, the highest water-holding capacity and the highest scores in a sensory evaluation. No significant (p > 0.05) differences in the mean values of the analyzed physicochemical and sensory properties were found between samples of normal quality meat with pH_u of 5.5–5.7 and 5.8–6.0. It can be concluded that there is no need to divide the meat of crossbred PHF x LIM heifers with pH_u 5.5–6.0 into groups based on its technological quality for processing.

WPŁYW RÓŻNEJ WARTOŚCI pH KOŃCOWEGO NA JAKOŚĆ MIĘSA JAŁÓWEK MIESZAŃCÓW POLSKA HOLSZTYŃSKO-FRYZYJSKA ODMIANA CZARNO-BIAŁA × LIMOUSINE

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S ł o w a k l u c z o w e: wołowina, wartość pH_u, jakość mięsa.

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A b s t r a k t

Materiał badawczy stanowiły próbki mięśnia *longissimus thoracis* pobrane z 50 losowo wybranych, wychłodzonych (48 h, 2–4°C) prawych półtusz jałówek mieszańców uzyskanych z krzyżowania krów rasy polska holsztyńsko-fryzyjska odmiana czarno-biała z buhajami rasy limousine (PHF × LIM). Zapakowane próżniowo próbki przechowywano w warunkach chłodniczych (0–2°C) przez 5 dni, a następnie przeprowadzono analizę ich podstawowego składu chemicznego oraz ocenę właściwości fizykochemicznych i sensorycznych. W celu określenia wpływu wartości pH mięsa na jego jakość, próbki podzielono na cztery grupy, w zależności od wartości pH_u: ≤ 5,4; 5,4–5,7; 5,8–6,0; > 6,0. W badaniach wykazano, że zdecydowanie najmniejszą zawartością suchej masy charakteryzowało się mięso o wartości pH_u > 6,0, natomiast największą – mięso o wartości pH_u 5,5–5,7. Zawartość tłuszczu w mięsie kształtowała się podobnie jak zawartość suchej masy. Największą całkowitą zawartość azotu związków rozpuszczalnych w wodzie stwierdzono w mięsie z najwyższą wartością pH_u (> 6,0). Mięso to odznaczało się także najciemniejszą barwą, największą wodochłonnością oraz zdecydowanie najlepszą jakością w ocenie sensorycznej. Nie stwierdzono istotnych różnic ($p > 0.05$) między średnimi wartościami analizowanych cech fizykochemicznych i sensorycznych mięsa „normalnego" z wartością pH_u 5,5–5,7 oraz 5,8–6,0. Tym samym uzyskane wyniki nie wskazują na potrzebę dzielenia mięsa jałówek mieszańców PHF x LIM o wartości pH_u 5,5–6,0 na dodatkowe grupy technologiczne o zróżnicowanej jakości przetwórczej surowca.

Introduction

The pH value is an important indicator of meat quality. It is closely correlated with many other properties of meat, which affect its processing suitability and culinary uses, such as water-holding capacity, color, tenderness and shelf-life (JELENIKOVÁ et al. 2008, KNOX et al. 2008, HAMOEN et al. 2013, GLAMOCLIJA et al. 2015). The meat pH is an easy-to-measure parameter that provides valuable information about post-mortem muscle glycolysis, thus enabling to detect quality defects of meat such as PSE (pale, soft, exudative meat) and DFD (dark, firm, dry meat) (RAMMOUZ et al. 2004).

The rate of post-mortem glycolysis may be too fast, leading to a rapid drop in pH (typical of PSE meat), or too slow, resulting in too high ultimate pH (typical of DFD meat) (KNOX et al. 2008). In both cases, abnormal physicochemical properties of meat (color, water-holding capacity) are developed. Such meat has limited processing suitability and low consumer acceptance, which generates vast economic losses (ADZITEY and NURUL 2011).

Consumer expectations regarding the quality of meat and meat products have risen over the years. Therefore, producers have to select raw materials characterized by the highest technological quality for processing. Differences in the quality of PSE, DFD and normal meat have been extensively documented in the literature (PARK et al. 2007, KNOX et al. 2008, WĘGLARZ 2010, ADZITEY and NURUL 2011, HOLDSTOCK et al. 2014). However, research findings (PURCHAS 1990, DEVINE et al. 1993, SILVA et al. 1999) have shown that the attributes of normal quality meat may also vary, although it remains unknown whether this is a general rule.

The objective of this study was to determine the effect of ultimate pH (pH_u) on the quality of meat from crossbred Polish Holstein-Friesian Black-and-White x Limousin heifers.

Materials and Methods

Materials. The experimental materials comprised samples of *longissimus thoracis* (LT) collected from 50 randomly selected half-carcasses of crossbred heifers produced by crossing Polish Holstein-Friesian (PHF) Black-and-White cows with Limousin (LIM) bulls. All animals were purchased by the same meat processing plant from the same producer. The identification of crossbreeds was based on mating certificates and their characteristic color. The animals rested in lairage for 20–24 hours before slaughter. Carcasses weighing 210 to 300 kg were analyzed.

The carcasses were chilled for approximately 48 hours at 2–4°C, and pH_u was measured in *longissimus dorsi* between 12–13 rib interfaces, on the right half-carcass. The pH of the muscle was measured with the use of a combination Double Pore electrode (Hamilton Bonaduz, Bonaduz, Switzerland) and a pH 340i pH-meter equipped with a TFK 150/E temperature sensor (WTW Wissenschaftlich-Technische Werkstatten, Weilheim, Germany) previously calibrated using two buffers (pH 4 and 7). LT samples were collected from right half-carcasses at the level of the last four thoracic vertebrae. The samples were vacuum-packaged in polyethylene bags polyamide/polyethylene (PA/PE) bags and were chill-stored (0–2°C). Five days post mortem, the samples were analyzed to determine meat quality.

Methods. The samples were taken out from bags, then stored for 0.5 hours at a temperature of 4°C, and meat color (1 point – light, 8 points – dark) and marbling (1 point – invisible, 5 points – very strong) were evaluated. Next some of the samples were used for a sensory analysis, and the remaining 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 proximate chemical composition and physicochemical properties of meat.

Analysis of the chemical composition of meat

The analysis of the proximate chemical composition of meat included the determination of dry matter content (samples were dried at 105°C to constant weight), total protein content by the Kjeldahl method (Kjeltec System 1026

Distilling Unit (Tecator AB, Hoganas, Sweden), fat content by Soxhlet extraction with diethyl ether as the solvent (Soxtec System HT2, Tecator AB, Hoganas, Sweden) and ash content (by incineration at 550°C to constant weight) (AOAC 1990). The content of nitrogen fractions in the water extracts of meat (total nitrogen and non-protein nitrogen) was determined by the Kjeldahl method. The protein nitrogen content of the water extracts of meat was calculated as the difference between total nitrogen and non-protein nitrogen. The water extracts of meat were prepared as described by HERRING et al. (1971).

Physicochemical properties of meat

An analysis of the physicochemical properties of meat included the determination of pH measured in the water homogenates of 10 g muscle tissue (muscle tissue to distilled water ratio of 1:1) using a combination Polilyte Lab electrode (Hamilton Bonaduz, Bonaduz, Switzerland) and a 340i pH-meter equipped with a TFK 325 temperature sensor (WTW Wissenschaftlich-Technische Werkstatten, Weilheim, Germany); color brightness – determined based on the percentage of light reflection against the surface of minced meat (Spekol spectrocolorimeter and remission attachment R45/0, 560 nm wavelength, VEB Carl Zeiss, Jena, Germany); water-holding capacity by the Grau and Hamm method (VAN OECKEL et al. 1999).

Sensory analysis of meat

The sensory attributes (aroma, taste, juiciness, tenderness) of meat were determined after heat treatment at a temperature of 96±2°C, in a 0.6% solution of NaCl (the ratio between meat and solution was 1:2), which lasted until a temperature of 80°C was achieved inside the samples (BARYŁKO-PIKIELNA et al. 1964). Approximately 2 cm *x* 2 cm *x* 2 cm cubes of meat were cut from the middle of each cooked sample and wrapped in aluminium foil. Coded meat samples were presented to the panellists at room temperature. A taste-panel evaluation was made by five trained panelists (*Sensory analysis*... ISO 8586:1993). The panellists used a 5 – point hedonic scale (1 point – the worse, 5 points – the best) to express the intensity and desirability of traits (DASZKIEWICZ et al. 2012). Distilled water was made available to the panelists for mouth cleansing between samples. All sensory attributes of each sample were evaluated during a single session. A maximum of five meat samples were assessed per session.

Statistical analysis

In order to evaluate the influence of acidity on meat quality, the samples were divided into four groups based on their pH_u values (measured in LT 48 hours post mortem): ≤ 5.4 (n=18), 5.4-5.7 (n=20), 5.8-6.0 (n=6), > 6.0 (n=6). The results were processed statistically by one-way analysis of variance (ANOVA) for non-orthogonal designs in the Statistica ver. 10 program (StatSoft, Inc. 2011). The significance of differences between mean values in groups (at $P \leq 0.05$ and $P \leq 0.01$) was estimated by Duncan's test.

Results and Discussion

Chemical composition of meat and marbling

Meat with $pH_u > 6.0$ had the lowest dry matter content, and meat with pH_u 5.5–5.7 had the highest dry matter content (Table 1). The differences in average dry matter content observed in meat with various pH_u levels resulted from significant differences in fat content, which followed an identical pattern. Differences in the fat content of meat were reflected in marbling scores (Table 1). Marbling of LT samples with the highest pH_u was less than meat with $pH_u \leq 5.4$ ($P \leq 0.05$) and 5.5–5.7 ($P \leq 0.01$).

Table 1

Proximate chemical composition [g kg^{-1}] and marbling (points) of meat in relation to pH_u (means ± SD)

Trait	pH_u value of meat			
	≤ 5.4 (n = 18)	5.5–5.7 (n = 20)	5.8–6.0 (n = 6)	> 6.0 (n = 6)
Dry matter	277.53 ± 22.4^{A}	280.42 ± 21.40^{A}	269.45 ± 11.72^{a}	244.95 ± 5.67^{Bb}
Fat	36.39 ± 17.87^{a}	43.68 ± 24.85^{A}	36.38 ± 18.68^{a}	14.65 ± 6.63^{Bb}
Total protein	217.95 ± 8.03	217.76 ± 9.31	221.53 ± 6.64	219.85 ± 1.53
Ash	11.91 ± 1.16	11.50 ± 1.23	11.37 ± 1.35	11.58 ± 1.06
Water/protein ratio (W/B)	3.32 ± 0.13^{a}	3.31 ± 0.14^{a}	3.30 ± 0.08^{a}	3.43 ± 0.04^{b}
Marbling	2.92 ± 1.13^{a}	3.33 ± 1.34^{A}	2.33 ± 0.41	1.67 ± 0.61^{Bb}

Values in the same row with different letters are significantly different, ^{AB} – $P \leq 0.01$; ^{ab} – $P \leq 0.05$

No significant ($P > 0.05$) differences in the percentages of total protein and ash were found between meat samples with different pH_u values (Table 1).

The results of studies investigating relationships between the proximate chemical composition and pH of meat are inconclusive and contradictory. HOLDSTOCK et al. (2014) observed no significant differences in the content

of dry matter, protein and fat between muscles (*longissimus thoracis*) with different average pH_u values (5.57, 5.83, 6.62). In contrast, LAWRIE and GATHERUM (1962), SOBINA (1998), AASLYNG et al. (2003) and DASZKIEWICZ et al. (2009), noted higher dry matter content in meat with low pH. The loweest fat content in meat with the highest pH was observed in our study. It is consistent with the findings of MELLER et al. (1998) and SOBINA (1998), whereas it does not support the previous research of AASLYNG et al. (2003) who noted the opposite relationship. The cited authors (MELLER et al. 1998, SOBINA 1998, AASLYNG et al. 2003,) demonstrated that meat characterized by low acidity had the lowest total protein content, which was not observed in our study.

An analysis of water-soluble nitrogen fractions revealed that meat with the highest pH_u (> 6.0) had the highest content of water-soluble protein and non-protein nitrogen and, consequently, the highest total water-soluble nitrogen content (Table 2). Meat with pH_u 5.8–6.0 contained the lowest concentrations of the above nitrogen fractions.

Table 2

Nitrogen fractions in meat in relation to pH_u (means ± SD)

Trait	pH_u value of meat			
	≤ 5.4 (n = 18)	5.5–5.7 (n = 20)	5.8–6.0 (n = 6)	> 6.0 (n = 6)
The ratio between total N of water-soluble compounds and total N in meat [%]	27.58 ± 1.42^{Aa}	26.55 ± 1.59^{A}	25.83 ± 0.82^{Ab}	31.61 ± 2.25^{B}
The ratio between N of water--soluble non-protein compounds and total N in meat [%]	12.27 ± 1.38	12.03 ± 0.77	11.41 ± 1.57^{a}	12.62 ± 0.75^{kb}
The ratio between N of water--soluble protein compounds and total N in meat [%]	15.30 ± 1.20^{A}	14.52 ± 1.66^{A}	14.41 ± 1.75^{A}	18.99 ± 2.52^{B}

Values in the same row with different letters are significantly different, ^{AB} – $P \leq 0.01$; ^{ab} – $P \leq 0.05$

The differences in the content of water-soluble nitrogen in meat with various pH_u values were probably due to different activities of proteolytic enzymes in the early post-mortem period (KEMP et al. 2010, WU et al. 2014). In the initial stage of meat aging during the normal course of glycolysis, non-lysosomal enzymes (calpains) are activated at high pH values. As post-mortem glycolysis progresses, meat acidity increases, the activity of calpains decreases and the activity of lysosomal enzymes (cathepsins) increases as they become involved in autolysis initiated by calpains (LOMIWES et al. 2014). Lower concentrations of nitrogen compounds were noted in the water extracts of meat with pH of 5.8–6.0, because such pH values do not promote the activity of calpains or cathepsins.

Physicochemical properties of meat

An evaluation of the physicochemical properties of meat revealed that meat with the highest pH_u (> 6.0) was characterized by the significantly ($P \leq 0.05$) darkest color and the highest water-holding capacity in comparison with other groups (Table 3). Presented results confirm the literature data. No significant differences in the mean values of the evaluated physicochemical parameters (color and WHC) were noted between samples of normal quality meat and meat with $pH_u \leq 5.4$. However, despite the absence of significant ($P > 0.05$) differences, the values of water-holding capacity varied across samples of normal quality meat. A tendency towards lower water-holding capacity was observed in meat with pH_u 5.8–6.0 relative to meat with pH_u 5.5–5.7.

Table 3

Physicochemical properties of meat in relation to pH_u (means ± SD)

Trait	pH_u value of meat			
	≤ 5.4 (n = 18)	5.5–5.7 (n = 20)	5.8–6.0 (n = 6)	> 6.0 (n = 6)
$pH_{48\ h}$	5.32 ± 0.06^{A}	5.54 ± 0.05^{B}	5.81 ± 0.02^{C}	6.27 ± 0.12^{D}
$pH_{168\ h}$	5.32 ± 0.06^{A}	5.60 ± 0.08^{B}	5.82 ± 0.04^{C}	6.35 ± 0.15^{D}
Color brightness [%]	12.67 ± 1.68^{A}	12.70 ± 2.05^{A}	12.33 ± 1.37^{A}	9.67 ± 1.03^{B}
Color (points)	4.72 ± 0.67^{A}	5.23 ± 0.99^{a}	5.08 ± 0.97^{a}	6.17 ± 0.61^{Bb}
Water-holding capacity [cm^2[	7.21 ± 1.31^{A}	6.92 ± 1.47^{a}	7.65 ± 0.80^{A}	5.22 ± 1.49^{Bb}

Values in the same row with different letters are significantly different, ^{ABCD} – $P \leq 0.01$; ^{ab} – $P \leq 0.05$

The correlations between the pH and color of meat have been widely described in the literature. In meat with high pH_u, fibers are tightly packed and meat structure is closed. Such meat is dark because its surface does not scatter light to the same extent as the more open surface of meat with lower pH_u (SEIDEMAN et al. 1984, LI et al. 2014). In addition, the closed structure of meat reduces the diffusion of oxygen into the muscle from the surface, and any oxygen reaching the interior is used up by the high activity of the cytochrome encouraged by the high pH. This results in a thin surface layer of bright red oxygenated myoglobin (MbO_2) allowing the purple color of the underlying reduced myoglobin (Mb) to show through (WARRISS 2000, ABRIL et al. 2001, MIN et al. 2002, LI et al. 2014).

The differences in the water-holding capacity of meat with various acidity levels result from the fact that at high pH_u, proteins far from their isoelectric points can bind more water and the water-holding capacity of meat increases (PARK et al. 2007). The water-holding capacity of meat is also related to the autolytic degradation of cytoskeletal proteins (HUFF-LONERGAN and LONERGAN

2005, ZHANG et al. 2006, PEARCE et al. 2011). The degradation of cytoskeletal proteins (desmin, talin and vinculin) reduces the connections between the myofibrils and the sarcolemma, and between the myofibrils, which are involved in the transmission of longitudinal and lateral shrinkage of the myofibrils to the entire muscle cell during rigor mortis. Thus, water can move into the extracellular space where it is lost as drip. Proteolysis of the cytoskeletal framework of the muscle cell leads to the weakening of the sarcolemma, followed by expulsion of water from the extracellular space to the muscle cell. As a result, the water-holding capacity of meat increases. Nevertheless, LI et al. (2014) demonstrated that the degradation of muscle proteins (mostly desmin) may be slower at pH 5.8–6.2 than at higher pH values, which can be associated with lower water-holding capacity of meat, as confirmed by our study.

Sensory attributes of meat

Meat with $pH_u > 6.0$ received the highest scores for taste, tenderness and juiciness (Table 4). Meat with pH_u 5.8–6.0 was characterized by the lowest values of the above sensory attributes. The differences between mean values in these groups were significant. There were no significant ($P > 0.05$) differences in the mean values of the analyzed sensory properties between samples of meat with pH_u 5.5–5.7 and 5.8–6.0. However, a tendency towards lower sensory quality was noted in meat with pH_u 5.8–6.0.

Table 4

Sensory properties (points) of meat in relation to pH_u (means ± SD)

Trait	pH_u value of meat			
	≤ 5.4 (n = 18)	5.5–5.7 (n = 20)	5.8–6.0 (n = 6)	> 6.0 (n = 6)
Aroma – intensity	5.00 ± 0.00	4.93 ± 0.24	5.00 ± 0.00	5.00 ± 0.00
Aroma – desirability	4.97 ± 0.11	4.90 ± 0.26	5.00 ± 0.00	5.00 ± 0.00
Taste – intensity	4.17 ± 0.49^{a}	4.33 ± 0.63	3.83 ± 0.75^{A}	4.83 ± 0.26^{Bb}
Taste – desirability	4.25 ± 0.46	4.33 ± 0.65	3.83 ± 0.75^{A}	4.67 ± 0.52^{B}
Tenderness	4.19 ± 0.75	4.08 ± 0.71	3.83 ± 0.68^{a}	4.67 ± 0.26^{b}
Juiciness	4.06 ± 0.48	3.83 ± 0.63^{a}	3.67 ± 0.82^{A}	4.58 ± 0.49^{Bb}

Values in the same row with different letters are significantly different, ^{AB} – $P \leq 0.01$; ^{ab} – $P \leq 0.05$

Our results, which point to higher tenderness of beef with high pH (> 6.0) in comparison with meat with intermediate pH values (5.8–6.0), corroborate the findings of other authors (PURCHAS 1990, DEVINE et al. 1993, JELENIKOVÁ

et al. 2008, DASZKIEWICZ et al. 2009, PULFORD et al. 2009, WU et al. 2014). According to PURCHAS (1990), meat with pH of around 6.0 is characterized by the lowest tenderness.

The results of the sensory evaluation are consistent with the previously described post-mortem changes in meat, affected by endogenous proteolytic enzymes (calpains and cathepsins). Their lower activity at pH 5.8–6.3 as well as slower degradation of myobrillar proteins decrease meat tenderness (SILVA et al. 1999). According to TAKAHASHI (1996), changes in tenderness during post-mortem meat aging result from the direct effect of calcium ions on myofibrils, which is determined by pH values, similarly to the activity of the calpain system. It should be noted that the sensory impressions or sensations experienced during meat consumption are interrelated. For instance, tenderness and juiciness are closely related. The more tender the meat, the more quickly the juices are released by chewing and the more juicy the meat appears, which was observed in our study and reported by other authors (SILVA et al. 1999, BINDER et al. 2004, JAWORSKA and PRZYBYLSKI 2014). High juiciness of meat with high pH results also from water binding which contributes to lower drip loss during thermal processing.

Conclusions

1. The results of the present study revealed differences in the proximate chemical composition, physicochemical properties, sensory attributes and processing suitability of normal quality meat, PSE meat ($pH \leq 5.4$) and DFD meat ($pH > 6.0$).

2. No significant differences were found for the physicochemical properties and sensory attributes of normal quality meat with pH_u in the ranges of 5.5–5.7 and 5.8–6.0. It can be concluded that there is no need to divide the meat of crossbred PHF *x* LIM heifers with pH_u 5.5–6.0 into groups based on its technological quality for processing.

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CHEMICAL COMPOSITION OF RAPESEED PRODUCTS AND THEIR USE IN PIG NUTRITION

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K e y w o r d s: rapeseed meal, rapeseed cake, glucosinolates, pigs.

A b s t r a c t

The development of double low varieties of oilseed rape and the growth of the biofuel sector have contributed to the launching of attractive high-protein feeds into the local market. Rapeseed meal and cake, which have a high content of protein with a desirable amino acid profile, can partially replace soybean meal in pig diets. The use of rapeseed-based feeds in pig nutrition is limited by the presence of antinutritional components: glucosinolates and their breakdown products. Double low varieties of oilseed rape are low in glucosinolates, and the content of antinutritional factors in rapeseed meal and cake can be further reduced by thermal processing. High crude fiber content, which reduces nutrient digestibility, is yet another antinutritional factor in rapeseed meal. A better knowledge of the chemical composition of rapeseed products, including the limitations resulting from the presence of antinutritional factors, will contribute to their wider use in pig feeding. The aim of this study was to determine the nutritional value of rapeseed meal and cake, and to evaluate their potential use in pig nutrition.

SKŁAD CHEMICZNY I WYKORZYSTANIE PRODUKTÓW RZEPAKOWYCH W ŻYWIENIU ŚWIŃ

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Abstrakt

Zintensyfikowanie w uprawie odmian rzepaku „00" oraz rozwój branży biopaliw spowodował, że na krajowym rynku pojawiły się atrakcyjne pasze białkowe. Poekstrakcyjna śruta oraz makuch rzepakowy charakteryzują się wysoką zawartością białka o bogatym składzie aminokwasowym, przez co mogą stanowić częściowe uzupełnienie poekstrakcyjnej śruty sojowej w mieszankach dla trzody chlewnej. O przydatności pasz z rzepaku w żywieniu świń decyduje obecność związków antyżywieniowych: glukozynolanów i produktów ich rozpadu. Rzepak podwójnie ulepszony charakteryzuje się niższą zawartością tych substancji, a dodatkowo obróbka cieplna sprzyja zmniejszeniu ich ilości w śrucie i makuchu rzepakowym. Kolejnym czynnikiem antyżywieniowym w śrucie rzepakowej jest podwyższona zawartość włókna surowego ograniczającego strawność składników pokarmowych. Dzięki znajomość składu chemicznego oraz po uwzględnieniu ograniczeń wynikających z obecności związków antyżywieniowych w paszach rzepakowych możliwe staje się szersze wprowadzenie ich do mieszanek dla trzody chlewnej. Celem opracowania była charakterystyka wartości odżywczej oraz określenie możliwości wykorzystania poekstrakcyjnej śruty rzepakowej i makuchu rzepakowego w żywieniu świń.

Introduction

The global production of rapeseed oil has been growing dynamically in recent years due to increasing demand for vegetable oils on the market (human consumption, animal feed, industrial and non-food uses). According to ROSIAK (2014), the leading producers of rapeseed are the European Union (*Brassica napus L.*), Canada (canola), China and India (mainly *B. juncea*). In 2013–2014, rapeseed production reached 20.9 million tons in the EU, 18 million tons in Canada, 14.2 million tons in China and 7.0 million tons in India. In Poland, winter rapedseed (*B. napus L.*) is the most important oilseed crop, accounting for over 95% of the total area under oilseed crops. In 2015, oilseed rape output reached approximately 2.6 million tonsof seeds. According to the Central Statistical Office, the area under winter and spring rapeseed increased from 789.600 hectares in 2006–2010 to 951.100 hectares in 2014, and decreased by 13% (to approx. 0.8 mln ha) in 2015.

The acreage dedicated to rapeseed is determined mainly by the production costs and profitability of this crop relative to cereals. According to OLEJNICZAK and MIKOŁAJCZAK (2013), the production profitability of rapeseed and its products can be improved by increasing the yield of different varieties. The heterosis effect can be used to increase rapeseed yields far above the best open-pollinated varieties. Attempts should also be made to improve plant resistance to abiotic and biotic stresses, and to modify qualitative traits.

New varieties are developed based on genetic variations in traits. The value of rapeseed produced for human food, animal feed and non-food uses (biofuels, industrial uses, cosmetic industry, pharmaceutical industry) can be improved by increasing their oil content and concentrations of active ingredients such as tocopherols, by modifying the fatty acid profile and by reducing the amount of antinutritional factors.

Intensive breeding programs led to a considerable improvement in the nutritional value of oilseed rape. Conventional varieties of oilseed rape with a high content of erucic acid (48–54%) and glucosinolates (110–160 μM/g seeds) were replaced with single low varieties (low erucic acid) and double low varieties (low erucic acid and low glucosinolate) (KRZYMAŃSKI 2000).

In recent years, extensive research has been done to produce stable lines of yellow-seeded rapeseed (*B. napus L.*) (OCHODZKI 2002, HERNACKI 2007, SMULIKOWSKA et al. 2008). Yellow pigmentation is linked with an 8% increase in the fat content (from 45% to 53%), a 5% increase in the protein content (from 17% to 22%) and an estimated 7% decrease in the fiber content (from 33% to 26.4–25%) of seeds. Yellow seeds have thinner seed coats with lower mechanical resistance, which makes them more difficult to store and less resistant to pathogenic fungi and crop protection agents. Yellow-seeded rapeseed is characterized by lower yields than rapeseed with black seeds (MYSZKA et al. 2011). According to LIPIŃSKI (2003), the nutritional and feed value of yellow-seeded rape *(Brassica rapa L.),* determined based on its chemical composition, content of antinutritional factors, protein quality and nutrient concentrations, exceeds those of winter rapeseed. The cited author demonstrated that when incorporated into the diets of growing pigs, field mustard seeds increase the digestibility of most nutrients, improve the retention and utilization of nitrogen and pig performance without compromising carcass quality parameters. Pig diets containing field mustard seeds were somewhat more effective in improving performance than diets containing winter rapeseeds.

The objective of this study was to determine the nutritional value of rapeseed meal and cake, and to evaluate their potential use in pig nutrition.

Rapeseed products

The by-products of oil production from rapeseed are rapeseed meal and rapeseed cake. The terms “oil cake” and “press cake” are often used interchangeably in the literature and in farming practice. According to some authors, the two products differ in their physical form. Press cake denotes fat-free seed residues that have been shaped into cakes in a press and have to be disintegrated before they are used in animal feed, whereas oil cake comprises friable material. In industrial practice, both products are referred to as rapeseed cake. This term is consistent with the provisions of the Regulation of the Minister of Agriculture and Rural Development of 19 January 2005 on feed materials placed on the market (Journal of Laws of 28 January 2005) which defines rapeseed cake as the by-product of oil production, obtained by

pressing rapeseeds with minimum 94% botanical purity, and defines rapeseed meal as the product of rapeseed extraction. Rapeseed meal is produced by oil extraction from seeds.The seeds are heated to a temperature of around 80°C and are then pressed in a series of presses. Extracted oil is further processed, and the pressed cake, which contains approximately 10–12% of oil, is extracted with organic solvents. The final product, rapeseed meal, contains 2–4% of oil (GÓRECKA et al. 2003).

Glucosinolates

Rapeseeds contain glucosinolates, the main antinutritional factor which prevents the use of oil extraction by-products in pig nutrition. The development of double low varieties of rapeseed, whose glucosinolate and eruic acid content was reduced through genetic modification, made rapeseed and its by-products suitable for use in animal feeds (KRZYMAŃSKI 1993, KAPUSTA 2015).

Plants synthesize glucosinolates from amino acids: methionine, alanine, valine, leucine and isoleucine (aliphatic glucosinolates), thyrosine and phenylalanine (aromatic glucosinolates), and tryptophan (indoleglucosinolates). The biosynthesis of selected glucosinolates is preceded by a series of reactions that elongate the amino acid side chain (WITTSTOCK and HALKIER 2002, BEKAERTET al. 2012).

Approximately 120 glucosinolates have been identified to date (HALLET al. 2014). Glucosinolates are present in highest concentrations in seeds and young seedlings, and they are found in lower concentrations in flowers, siliques, roots and leaves. Indoleglucosinolates become the predominant form with plant aging (CLOSSAIN-BESNARD and LARHER 1991, BROWN et al. 2003, ISHIKAWA et al. 2014).

Glucosinolates have relatively high chemical stability and are tolerant of high temperatures. They are chemical compounds with low levels of biological activity, and they are not toxic to animals. However, they easily undergo enzymatic hydrolysis in the presence of myrosinase, an enzyme found in oilseed rape seeds (RASKET al. 2000, BOHINIC et al. 2012, PATYRA and KWIATEK 2015). This enzyme is activated when seed structure is damaged as a result of chewing, grinding, crushing or pressing, andglucosinolates are easily transformed into highly toxic compounds such as isothiocyanates (ITC), 5-vinyloxazolidine-2-thione (VOT, goitrin), thiocyanate (SCN) and nitrile (CHB). Myrosinase is deactivated during thermal processing of rapeseed meal (BILLE et al. 1983, VERMOREL et al. 1986, THOMKE et al. 1998, TROCZYŃSKA 2005, TRIPATHI and MISHRA 2007, BRZÓSKA et al. 2010a, DINKOVA-KOSTOVA and KOSTOV 2012).

Toxic substances produced during glucosinolate hydrolysis are absorbed from the digestive tract into the bloodstream of animals, and are carried to bodily tissues (SŁOMIŃSKI et al. 1988). Owing to their goitrogenic effects, those substances cause hypertrophy of the thyroid gland and inhibit the production of thyroid hormones: triiodothyronine (T3) and thyroxine (T4) (CHICHŁOWSKA 1990, CAMPBELL and SCHÖNE 1998, RAMEEH 2015). Thyroid hormones regulate biochemical processes in bodily organs, such as protein and phospholipid synthesis (RAJ 2003). Impaired secretion of thyroid hormones has a negative impact on many metabolic processes (ŚLEBODZIŃSKI et al. 1985), and it inhibits growth and development (CAMPBELL and SCHÖNE 1998, LOZANO and TRUJILLO 2012).

Experiments involving pigs revealed their varied tolerance to dietary glucosinolates. Younger animals are more vulnerable to the adverse effects of those compounds (CORINO et al. 1991). Glucosinolatescan reduce feed intake, in particular in piglets and pigs in initial stages of fattening (SCHÖNE et al. 1997, TRIPATHI and MISHRA 2007). This effect is determined mainly by the glucosinolate content of feed rations. According to SCHÖNE et al. (1997), pigs weighing 20 to 50 kg should not receive more than 2.0–2.4 mM kg^{-1} of glucosinolates daily. Larger doses decrease appetite and reduce weight gains. RAJ (2003) demonstrated that complete diets containing 0–2.58 mM kg^{-1} of glucosinolates did not suppress appetite or feed intake. Glucosinolates have no negative influence on the growth rate of pigs if their total dietary inclusion level does not exceed 1 μM/gof the ration (MAWSON et al. 1994a).

When administered to growing-finishing pigs in all stages of fattening, glucosinolates can affect the size of internal organs, including the liver, kidneys and the thyroid gland (BOURDON and AUMAITRE 1990, BUASTO et al. 1991, ZIÓŁKOWSKI et al. 1994, THOMKE et al. 1998, XIE et al. 2012). In a study of growing-finishing pigs, RAJ (2003) demonstrated that an increase in the daily glucosinolate dose from 0 to 6.1 mM kg^{-1} per animal increased liver weight from 1485 to 2001 g. According to BUASTO et al. (1991), the increase in liver and kidney weight induced by glucosinolate doses of up to 17.3 mM kg^{-1} was not accompanied by histological changes.

The weight of the thyroid gland and T3 and T4 serum levels are highly sensitive indicators of the presence of glucosinolates in animal feed and their adverse effects of animals. Thyroid hormones T3 and T4 stimulate protein synthesis. Their biosynthesis is inhibited by glucosinolates and their breakdown products, which increases the weight of the thyroid gland in pigs (MAWSON et al. 1994b, SCHÖNE et al. 1997, ZIÓŁKOWSKI et al. 1995, XIE et al. 2012).

The serum levels of thyroid hormones have been relatively rarely studied. According to CORINO et al. (1991), OPAŁKA et al. (2001) and SCHÖNE et al.

(1990, 1997), changes in hormone secretion are proportional to glucosinolate levels in feed. RAJ (2003) studied growing-finishing pig weighing 25–70 kg and demonstrated that an increase in the glucosinolate content of feed from 0 to 6.1 mM kg^{-1} promoted a gradual decrease in T4 plasma levels from 48.2 to 24.5 ng ml^{-1}. The corresponding decrease in T3 serum levels was significantly less pronounced, from 1.27 to 0.89 ng ml^{-1}. The above results indicate that dietary glucosinolates induce greater changes in the concentrations of T4 than T3. Similar results were reported by BUASTO et al. (1991) in a study of growing-finishing pigs weighing 35 to 67 kg or 108 kg which were administered glucosinolates doses of 0.1–17.3 mM per kg of feed.

The observed changes in the weight of the thyroid gland in pigs weighing 25 to 70 kg (RAJ 2003) revealed a highly significant negative correlation between T4 concentrations and the weight of the thyroid gland ($r = -0.88$), and a far less significant correlation between the weight of the thyroid gland and T3 concentrations ($r = -0.19$). The cited author also compared T3 and T4 levels in young (25–70 kg) and older (60–110 kg) pigs receiving similar daily doses of glucosinolates (4.7 and 4.4 mM/animal/day, respectively). In younger animals, T3 and T4 concentrations decreased by approximately 17% and 40%, respectively ($P \leq 0.01$), whereas in older pigs, the noted differences was two-fold smaller at only 10% and 20%, respectively, and they were not statistically significant. The above results indicate that young pigs are much more sensitive to dietary glucosinolates than older animals.

In double low varieties of rapeseeds, the maximum allowable content of alkenyl and indoleglucosinolates in seeds is set at 15 μM g^{-1}, and erucic acid concentrations may not exceed 0.5% of total fatty acids. The glucosinolate content of rapeseed for industrial applications is limited to 18 μM/g fat-free dry matter, and erucic acid content – to 2.0% of total fatty acids. Those limits guarantee that the seeds, meal and cake of rapeseed are safe for animals and, consequently, humans.

Thermal treatment reduces the glucosinolate content of rapeseed products. Their concentrations are determined by the type of product and conditions of the technological process. The glucosinolate content of rapeseed meal obtained from the seeds of double low varieties ranges from 4.1 to 26.7 μmol g^{-1} fat-free dry matter (KRASUCKI and GRELA 1999, PASTUSZEWSKA and RAJ 2003). Rapeseed cake has a similar glucosinolate content of approximately 23.6 μmol g^{-1} fat-free dry matter (HANCZAKOWSKA and WĘGLARZY 2012). The maximum recommended levelsof glucosinolatesin rapeseed-based animal feeds are15–20 μM g fat-free dry matter (SMULIKOWSKA 2002).

In the European Union and Poland (Regulation of the Minister of Agriculture and Rural Development of 28 June 2004 r., Journal of Laws, nr 162, item 1704) the maximum allowable concentrations of volatile mustard oil (undesir-

able substances) in animal feed expressed as allylisothiocyanates, have been set at: 4000 mg kg^{-1} in rapeseed meal, 150 mg kg^{-1} in complete diets, excluding: complete diets for cattle and sheep, where the limit is 1000 mg kg^{-1}, complete diets for pigs (excluding piglets) and poultry, where the limit is 500 mg kg^{-1}.

The amount of seeds and by-products of rapeseed that can be safely used in complete diets for pigs, poultry and cattle can be calculated based on the concentrations of allylisothiocyanates determined in analyses and provided by manufacturers of rapeseed meal and cake.

Crude fiber

Crude fiber present in seeds is another antinutritional factor that reduces the nutritional value, processing suitability and efficacy of rapeseed products in animal nutrition.The crude fiber content of oilseed rape seeds, cake and meal is approximately 8–9%, 9–12% and 11–14% on a dry matter basis, respectively (PASTUSZEWSKA and RAJ 2003, SMULIKOWSKA 2006, BRZÓSKA et al. 2010a).

Crude fiber is the main component of plant cell walls, and it is resistant to hydrolysis by enzymes in the digestive tract. It is composed mainly of non-starch polysaccharides and polyphenolic lignin. Other non-digestible ingredients include oligosaccharides, tannins and starch (ASPET al. 1987, 1988, SLAVIN 2013).

According to OCHODZKI (1997) and BURACZEWSKA (2001), crude fiber could be regarded as an antinutritional factor due to its inhibitory effect on nutrient digestibility.

The fiber content of the seeds and by-products (meal, cake) of rapeseed can be lowered through breeding and development of new varieties (KRZYMAŃSKI 1993, OGRODOWCZYK and BARTKOWIAK-BRODA 2013 or processing, such as seed husking before oil extraction and dry fractionation of meal and cake (KORNIEWICZ et al. 1995, MIŃKOWSKI 2002).

In rapeseed, the seed coat (hull) accounts for around 15–16% of seed weight and 30% of meal weight. The high weight of the seed coat is responsible for the high crude fiber content of rapeseed meal and cake (MIŃKOWSKI 2002). The seed coat contains 34–42% of crude fiber. The content of hulls and fiber in rapeseed meal and cake can be reduced by selecting large seeds and yellow-seeded varieties with a thinner seed coat (SMULIKOWSKA et al. 1998, LIPIŃSKI 2003).

KORNIEWICZ et al. (1995) demonstrated that screen separation of rapeseed meal enabled to decrease the fiber content of the fine fraction from 13% to 9% and to increase its protein content from 38% to 42%. In a study by MIŃKOWSKI

(2002), husking reduced the crude fiber content of rapeseed meal from 12% to 7%. The metabolizable energy of the resulting meal was 17% higher in comparison with standard rapeseed meal.

The fiber content of rapeseed products can also be lowered with the use of fungal enzymes. In a study by OCHODZKI et al. (1995), enzymes decreased the content of insoluble dietary fiber from 28.9% to 13.0% while increasing the content soluble fiber from 1.6% to 3.7%.

KORNIEWICZ et al. (1999) analyzed complete diets for growing-finishing pigs containing 14% of rapeseed meal or 18% of rapeseed cake, which were supplemented with enzymes produced by *Aspergillusniger* strains during fermentation. The enzymatic preparation increased fiber digestibility from 34.6% to 41.6%, it increased weight gains and decreased feed intake per kg body weight gain by 5%. The noted improvement resulted from an increase in the energy value of feed.

Protein content and amino acid composition

The nutritional value of rapeseed products is affected by the amount of extracted oil. Rapeseed meal contains approximately 36–38% of protein and 2–4% of fat (BRZÓSKA et al. 2010a). The cold pressed cake obtained from double low varieties of rapeseed contains 28–34% of total protein whose amino acid composition is similar to that of whole seeds. Cold pressing involves far less heat, and lysine remains more active than in rapeseed meal (DOROSZEWSKI et al. 1996, PASTUSZEWSKA et al. 1997).

Rapeseed products are high-protein feeds, and they meet the pigs' requirements for sulfur-containing amino acids, threonine and tryptophan when incorporated into cereal-based complete diets. Such diets may be deficient in lysine which is the first limiting amino acid. Protein and lysine digestibility is lower when compared with the majority of other high-protein feeds, but rapeseed protein has high biological value due to increased methionine and cystine content, and therefore may effectively supplement soybean meal in animal diets (BURACZEWSKA et al. 1998, LIPIŃSKI et al. 1998b, FRANKIEWICZ 1999). In comparison with soybean protein, rapeseed protein is characterized by lower nutritional value due to its lower lysine content and lower ileal digestibility (BURACZEWSKA et al. 1999, PARTANEN et al. 2001, CHOI et al. 2015).

PASTUSZEWSKA and OCHTABIŃSKA (1996) analyzed the chemical composition and nutritional value of protein from four cold-pressed rapeseed cakes. Protein digestibility reached 79.9–85.5%, and the biological value of protein determined in a growth trial (80.1–96.7) was largely influenced by

glucosinolate content. Those results suggest that seeds intended for cold pressing should be characterized by minimal concentrations of glucosinolates.

OSEK et al. (1999) evaluated rapeseed oil cake as a substitute for soybean meal in pig nutrition. In the described experiment, growing-finishing pigs were fed diets containing 16% and 20% of oil cake throughout the fattening period. The cited authors demonstrated that rapeseed oil cake had no significant effect on average daily gains. Similar results were reported by DOROSZEWSKI et al. (1997) in a study of growing-finishing pigs where soybean meal was replaced with 18% or 21% of rapeseed cake.

The biological value of protein in rapeseed products is also highly influenced by toasting temperature which often exceeds 120°C. GRALA et al. (1994) found that temperatures higher than 90°C applied for more than 10 minutes lead to protein denaturation and reduce lysine digestibility.

Minerals

Rapeseed meal and cake are relatively rich sources of minerals whose concentrations are comparable in both products. Rapeseed cake contains considerable amounts of phosphorus (10–11 g kg^{-1}), but phytic phosphorus accounts for approximately 40–60% of the total content of this element. Phytates are antinutritional factors which bind minerals (P, Zn, Ca) and amino acids, thus making them unavailable to animals. In order to solve this problem, pig diets can be supplemented with the enzyme phytase (LARSEN and SANDSTORM 1993, SMULIKOWSKA and VAN NGUYEN 2003, SMULIKOWSKA 2006).The content of most minerals in rapeseed cake is determined by their concentrations in whole and husked seeds (BANASZKIEWICZ 1998). In comparison with soybean meal, rapeseed meal has a higher content of calcium, phosphorus, magnesium and manganese, but approximately 40–60% of phosphorus is phytate-bound (LIPIŃSKI et al. 1998a, MICHALIK et al. 2008).

Fat content and fatty acid composition

Rapeseed cake has a relatively high fat content (10–14%), which considerably increases its energy value that has been determined at 12.6–13.5 MJ ME. The fat content of rapeseed cake may reach even 20%. The energy value of rapeseed cake increases by 0.2% per percent of crude fat (SMULIKOWSKA and VAN NGUYEN 2003, BRZÓSKA et al. 2010a, WĘGLARZY et al. 2013). Rapeseed cake and rapeseed oil are characterized by similar fatty acid composition. Rapeseed cake is rich in the following unsaturated fatty acids: oleic acid, linoleic acid and

linolenic acid. The n-6/n-3 PUFA ratio oscillates around 2, and it is more desirable than in other feeds for monogastric animals. The quality of feed fat affects the fatty acid profile of animal products. Rapeseed cake is a valuable source of both energy and essential polyunsaturated fatty acids (linoleic acid and linolenic acid). Due to its high crude fiber content, rapeseed meal has lower energy value of 10.5 MJ ME. The average crude fat content of rapeseed meal is 2–4% (SMULIKOWSKA 2006, BRZÓSKA et al. 2010a).

Rapeseed meal and cake in pig nutrition

The rapeseed and the by-products of the oil extraction process, including rapeseed meal and press/oil cake, could be highly valuable sources of protein and energy in complete diets fed to pigs, poultry and ruminants (KINAL et al. 1990, SOBOTKA 2004, RAJ 2003, PARTANEN et al. 2001, KAPUSTA 2015). Their applicability as feed components is, however, limited due to the presence of antinutritional factors and crude fiber, the amino acid composition of protein, the fatty acid composition of oil, and palatability (CHOI et al. 2015).

In the first and second phase of fattening, rapeseed meal can be included in pig diets at 5–8% and 8–12%, respectively, with no adverse effects on growth performance, provided that the feed materials are low in glucosinolates and have not been overheated during the production process. The recommended dietary inclusion levels of rapeseed cake are similar, but the amount of cold-pressed cake should not exceed 10% due to its higher content of antinutritional factors.

Diets for weaners can be supplemented with rapeseed meal at 5% and rapeseed cake at 3%. Rapeseed meal has high crude fiber content which contributes to its low energy value and low palatability. Therefore, flavor enhancers can be added to diets to increase feed intake.

Similarly to growing-finishing pigs in the first phase of fattening, dry sows and sows in early gestation can also be fed rapeseed-based diets, but the inclusion levels of rapeseed products should not exceed 10%, whereas the diets of sows in late gestation and lactating sows should contain up to 5% of rapeseed products. Such inclusion rates of rapeseed have no negative influence on litter size or piglet birth weight (LIPIŃSKI 1996, HANCZAKOWSKA 2006, HANCZAKOWSKA 2009, BRZÓSKA et al 2010b).

The effect of thermal processing on the quality of rapeseed products

The content of antinutritional factors in rapeseeds intended for animal feed and in the by-products of oil extraction (rapeseed meal and cake) can be reduced by thermal and hydrothermal processing.

Commercial seed processing involves pressing, toasting with steam and extraction with a solvent, usually hexane. The technological process, particularly temperature conditions, affects the quality of the final product, but flaking, pressing and oil extraction are non-invasive techniques. The greatest changes take place during meal toasting (PASTUSZEWSKA and RAJ 2003). Toasting reduces glucosinolate content and deactivates not only myrosinase but also other beneficial enzymes present in seeds, such as phytase. It is very important to maintain appropriate conditions during the process (temperature of 100°C for around 20 minutes). Increased temperature leads to the formation of carbohydrate-amino acid (lysine) complexes during the Maillard reaction, which are unavailable to animals. They decrease the nutritional value of rapeseed meal and reduce the digestibility of nutrients, in particular protein (GRALA et al. 1994, SŁOMIŃSKI 1997, SMULIKOWSKA et al. 2008).

Rapeseed cake is the by-product of oil extraction carried out with hydraulic presses (cold pressing). The process does not ensure complete elimination of antinutritional factors, which is why low-glucosinolate rapeseed varieties should be used (HANCZAKOWSKA and WĘGLARZY 2012). Extrusion (under high pressure and high temperature) increases oil extraction yield, and the pressed cake in lower in energy but higher in easily digestible protein (SMULIKOWSKA 2006).

In a study by OSEK and MILCZAREK (2002), rapeseed extrusion did not lead to changes in the concentrations of phytates (9.3–9.2%) or tannins (1.24–1.18%), whereas the glucosinolate content of seeds was significantly reduced (8.8–7.8 μM g^{-1}). Total glucosinolate content decreased by 11%, and the concentrations of alkenylglucosinolates were reduced by 25%.

KUŚNIEREK et al. (2005) investigated the effect of rapeseed meal extrusion on the digestibility of protein and amino acids, and glucosinolate levels. Extrusion conducted at a temperature of 140°C and 160°C and 20% humidity for 40 seconds lowered glucosinolate levels from 12.7 μM g^{-1} to 9.7 and 7.7 μM g^{-1}, respectively.

In a study by WEBER et al. (2006), hydrothermally processed rapeseed cake was used as a protein source in complete diets for growing-finishing pigs. Hydrothermal processing reduced the glucosinolate content of cake from 20.4 μM g^{-1} to 10.5 μM g^{-1}.

BANASZKIEWICZ (2000) heated the seeds of three varieties of rapeseed at a temperature of 121°C for 20, 25 and 60 minutes to evaluate the effects of thermal treatment on the amino acid composition of protein. The content of most amino acids in rapeseed protein was somewhat reduced after heat treatment. Total lysine concentrations decreased in the seeds of all rapeseed varieties after 60 minutes of thermal processing, but no such changes were reported after 20 minutes of heating. Thermal processing induced a significant decrease in lysine absorption from 94.37% after 20 minutes to 88.32% after 60 minutes of heating. Available lysine content decreased by 12% relative to unprocessed seeds.

According to PASTUSZEWSKA and RAJ (2003) and BURACZEWSKA et al. (1998), protein digestibility is largely affected by technological processes which promote the formation of complexes that are not digested by enzymes in the gastrointestinal tract of monogastric animals, in particular proteins bound to the neutral detergent fiber (NDF) fraction. A reduction in toasting temperature from 120°C to 111°C increased the content of available lysine from 3.5 to 4.7 g/16 g N.

The increase in toasting temperature and longer toasting time not only lower the content of available lysine, but also reduce the digestibility of protein and other amino acids in the gastrointestinal tract of pigs (BURACZEWSKA et al. 1998).

PASTUSZEWSKA et al. (2001) determined the effect of heating temperature applied to defatted rapeseed cake on selected protein solubility parameters *in vitro* and on the nutritional value of protein fed to rats. Cake heating at 90°C increased protein solubility in KOH from 85% to 93% and enhanced enzymatic digestibility. A temperature increase to 130°C led to a gradual decrease in all parameters analyzed *in vitro* (excluding enzymatic digestibility) and *in vivo*. A further temperature increase from 130°C to 140°C promoted a significant drop in the values of all indicators analyzed *in vitro* and *in vivo*. Protein solubility in KOH decreased from 67% to 38%, and protein utilization was reduced from 71.2% to 67.8%.

RZEDZICKI (1996) reported that rapeseed extrusion at a temperature of 140/165°C did not lead to significant changes in the amino acid composition of protein, including digestible lysine content.

JAŚKIEWICZ (2001) evaluated the efficacy of a protein concentrate made from husked faba bean seeds and whole rapeseeds which had undergone extrusion. The concentrate was used to replace soybean meal in broiler chicken diets. Extruded concentrate improved nutrient digestibility. A significant 30% increase in the assimilability of crude fat points to higher availability of oil released from extruded rapeseed. Total protein digestibility was also found to increase by more than 8%.

In an experiment conducted by BURACZEWSKA et al. (1998), rapeseed cake and meal were heated at 130°C for 20, 40, 60 and 80 minutes. The content of NDF, protein and essential amino acids was determined. The content of NDF and bound protein increased with prolonged heating. The experiment demonstrated that the NDF fraction in commercial meal increases during oil extraction and toasting, processes that involve heat. The results of the study indicate that feeds containing cold-pressed seeds are characterized by lower NDF content.

Conclusions

The development of double low varieties of oilseed rape and the growth of the biofuel sector have contributed to the launching of attractive high-protein feeds into the local market. Rapeseed meal and cake, which have a high content of protein (up to 35%) with a desirable amino acid profile (high methionine and cystine concentrations), can partially replace soybean meal in pig diets. The use of rapeseed-based feeds in pig nutrition is limited by the presence of antinutritional components:glucosinolates and their breakdown products. Double low varieties of oilseed rape are low in glucosinolates, and the content of antinutritional factors in rapeseed meal and cake can be further reduced by thermal processing. However, the levels of those compounds in rapeseed products should be regularly monitored. Increased crude fiber content, which reduces nutrient digestibility and energy value, is yet another antinutritional factor in rapeseed meal. Due to its high fat content, rapeseed cake is characterized by higher energy value of 12.6–13.5 MJ ME, and can effectively compete with other feed materials in this respect. A better knowledge of the chemical composition of rapeseed products, including the limitations resulting from the presence of antinutritional factors, will contribute to their wider use in pig nutrition.

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HISTOMORPHOMETRIC CHARACTERISTICS OF THE INTEGUMENTARY SYSTEM OF THE POLISH POPULATION OF FARMED AND WILD FOXES*

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K e y w o r d s: integumentary system, farmed fox, wild fox, hair coat quality, skin histology.

A b s t r a c t

The aim of the study was to determine the degree of differentiation between selected histomorphometric characteristics of common fox (*Vulpes vulpes*) skins with regard to the origin of animals (farmed vs wild population). Skin size parameters with evaluation of hair coat quality, trace element composition of hair, and histomorphometric characteristics of cutaneous tissue were studied. The domestic population of wild foxes was characterized by low body weight and poor hair coat quality, which showed considerable felting (10–35% of skin area), absence of down, and deficiency of elements needed for proper hair development. Histological analysis of cutaneous tissue in wild foxes showed a lower number of bundles per tuft ($P \leq 0.05$), a lower number of down hair per tuft and bundle ($P \leq 0.01$) and a greater number of sebaceous glands, which had greater length ($P \leq 0.01$) and area ($P \leq 0.05$). Analysis of the level of trace and major elements in the hair of wild and farmed foxes revealed highly significant differences in the amounts of iodine, lead, selenium and sulfur. All of these elements were more abundant in farmed foxes except for lead, which was higher in wild foxes.

CECHY HISTOMORFOMETRYCZNE UKŁADU POWŁOKOWEGO POPULACJI KRAJOWYCH LISÓW HODOWLANYCH I DZIKO ŻYJĄCYCH

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S ł o w a k l u c z o w e: układ powłokowy, lis fermowy, lis dziki, jakość okrywy włosowej, histologia skóry.

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A b s t r a k t

Celem badań było określenie stopnia zróżnicowania wybranych cech histologiczno-morfometrycznych skór lisów pospolitych (*Vulpes vulpes*) z uwzględnieniem pochodzenia zwierząt (hodowla fermowa w stosunku do populacji dziko żyjącej). Badano parametry wielkości skór wraz z oceną jakości okrywy włosowej, składem mikropierwiastków we włosach i oceną histologiczno-morfometryczną tkanki skórnej.

Krajowa populacja lisów dziko żyjących odznaczała się niższą masą ciała i jakością okrywy włosowej, która charakteryzowała się znacznym sfilcowaniem (10–35% powierzchni skóry), niewykształceniem puchu i brakami pierwiastków sprzyjających jej właściwemu rozwojowi. W ocenie histologicznej ich tkanki skórnej wykazano niższą liczbę pęczków w kępce ($P \leq 0{,}05$), liczbę włosów puchowych w kępce i pęczku ($P \leq 0{,}01$) oraz większą liczbę gruczołów łojowych odznaczających się większą długością ($P \leq 0{,}01$) i powierzchnią ($P \leq 0{,}05$). W ocenie poziomu mikro- i makroelementów we włosach lisów dzikich i hodowlanych wykazano wysoko istotne zróżnicowanie w ilości jodu, ołowiu, selenu i siarki. Z wymienionych pierwiastków wyższe wartości stwierdzono u lisów hodowlanych z wyjątkiem poziomu ołowiu, który był wyższy u lisów dzikich.

Introduction

Out of the many species of foxes living in the world, only two are raised in cages: the arctic fox (*Vulpes lagopus*) and common fox (*Vulpes vulpes).* The common fox is a carnivorous mammal (*Carnivora*) of the family *Canidae*. In its natural state, it inhabits the Northern Hemisphere, from the Arctic Circle to North America, Europe, Asia and North Africa. It easily adapts to different environments. Foxes are on the IUCN's (International Union for Conservation of Nature) list of the world's 100 worst invasive species (STATHAM et al. 2011, *100 of the word's...* 2016).

The history of fur animal domestication is relatively short. Originally, common foxes were only farmed in North America, where semi-feral reproduction with partial human intervention was used in the 18th century. The first fox farm was established on Prince Edward Island (Canada) in 1894. Fur-bearing carnivores came to European farms as breeding animals in the 1920s. The interest in cage farming increased as a result of pelts from farmed foxes receiving four times the price of best pelts from caught foxes at a large fur auction held in London in the years 1904–1910. In Poland, the origins of fur farming date back to the interwar period (1918–1939) when the first farms of silver foxes were set up in Silesia and later near Gdańsk (PIÓRKOWSKA 2015). JEŻEWSKA-WITKOWSKA et al. (2012) demonstrated that Polish farmed foxes originate from North America (Canadian), *Vulpes vulpes* subspecies.

Human domestication of the wild fur animals was aimed to modify morphological, physiological, developmental and mental characteristics so as to obtain desirable traits (GUGOŁEK et al. 2013, GUGOŁEK et al. 2014). After many generations of breeding work on fox farms, the productive traits of farmed foxes came to differ considerably from those of wild animals. Clear differences

are observed in the productive traits being improved, notably hair coat quality, coat colour, body weight, measurable traits of the organs of the skeletal and digestive systems, and even animal temperament (KULAWIK et al. 2013, GUGOŁEK et al. 2014).

The aim of the study was to determine the degree of differentiation between selected histomorphometric characteristics of fox skins with regard to the origin of animals (farmed vs wild population).

Materials and Methods

The experiment used 40 raw skins of foxes. Twenty skins originated from a breeding farm belonging to the Experimental Station of the National Research Institute of Animal Production Chorzelów Ltd., and another 20 from wild animals harvested in north-eastern Poland during late autumn. An equal sex ratio was maintained in each group.

Pretreatment, fleshing, drying and preservation of the skins were performed in accordance with relevant standards for this animal species.

Physical parameters of the raw skins and quality traits of the hair coat were evaluated based on the methods described by KASZOWSKI and KAWIŃSKA (1960) and PIÓRKOWSKA (2001, 2002). The tests included body weight at slaughter, measurement of skin size parameters (weight, length of skin and tail, width of skin, planimetric area). Based on skin weight and area, the weight of 1 dm^2 skin was calculated to determine its lightness. All skins were graded for size according to auction sale requirements (SKINPOLEX 1994, *Sagafurs*® 2016). The following skin measurements and corresponding sizes were accounted for [cm]:

Auction size	Size [cm]
30 (000)	> 115.1
20 (00)	106.1–115.0
0	97.1–106.0
1	88.1–97.0
2	79.1–88.0

The experimental skins were evaluated organoleptically for defects and damage to the integumentary system (cutaneous tissue and hair coat). The defects and their extent were defined, and measurable traits were measured.

Histological and morphometric tests were performed on cutaneous tissue samples fixed in 6% buffered formalin. Samples were dehydrated in a graded ethanol series and cleared in xylene. Sections made from paraffin blocks were

cut on a microtome into 6–8 μm thick slices and stained differentially with Delafield's hematoxylin and eosin or with Mallory's stain based on three stain types: acid fuchsin, phosphomolybdic or phosphotungstic acid, and Orange G with aniline blue and oxalic acid (ZAWISTOWSKI 1970). The preparations were analysed and their microphotographs were taken using a Nikon Eclipse E-400 microscope and MultiScan program with the ScanBase image and text database (computer image analysis system MultiScanBase v.18.03).

Chemical analysis of hair was performed as a method of evaluating the body's mineral status. Concentration of bioelements (iron, zinc, copper, manganese, cobalt, selenium, sulfur, iodine, silicon, calcium, magnesium, lead, mercury) in hair was determined using an X-ray fluorescence (XRF) spectrometer. This method analyses the amount of X-ray radiation reaching the detector after being reflected from the sample.

The results were analysed by one-way ANOVA F-test in an orthogonal design. The calculations were made with Statistica 7.1 PL package using the following linear model:

$$y_{ij} = \mu + a_i + e_{ij}$$

where:
y_{ij} – observed value of a trait
μ – mean value of a trait in the population
a_i – effect of experimental group (1, 2)
e_{ij} – random error.

Results

The mean body weight of farmed foxes was highly significantly higher than that of wild foxes (6.74 and 5.42 kg, respectively; Table 1). The low body weight of wild foxes translated into the size and area of their skins, which differed considerably from those in the skins of farmed animals. Highly significant differences in favour of farmed foxes concerned all of the skin size parameters. In relation to the wild skins, skins from farmed foxes were about 34% heavier, 12% longer and 5% wider, with surface area greater by 25%. Comparison of the furs for lightness showed that the skins of wild foxes were lighter than those of farmed foxes by 1.24 g per dm^2 of skin.

The largest proportion of wild fox skins (85%) were auction size 2 and 1 (Figure 1). Two skins (10% of all wild fox skins) were too small for auction sale. The skins of farmed foxes were classified into four auction sizes, 55% of which were size 20 and 0, corresponding to skin sizes ranging between 97.1 and 115 cm.

Table 1

Measurements of raw skins from foxes

Item	Foxes			
	farmed	SD	wild	SD
Weight body [kg]	6.74^{A}	0.659	5.42^{B}	0.793
Weight of skin [g]	454.49^{A}	57.31	301.54^{B}	63.12
Total length of skin [cm]	134.82^{A}	6.249	118.70^{B}	9.608
Auction length of skin [cm]	99.37^{A}	4.874	86.32^{B}	6.736
Tail length [cm]	35.45^{a}	2.145	32.32^{b}	4.774
Width of skin [cm]	30.85^{A}	0.875	29.30^{B}	1.218
Area of skin [dm^2]	44.503^{A}	3.975	33.516^{B}	3.898
Weight of 1 dm^2 skin [g]	10.194^{A}	0.649	8.958^{B}	1.264

Means in rows with different letters differ significantly ($^{a, b}$ – $P \leq 0.05$; $^{A, B}$ – $P \leq 0.01$)

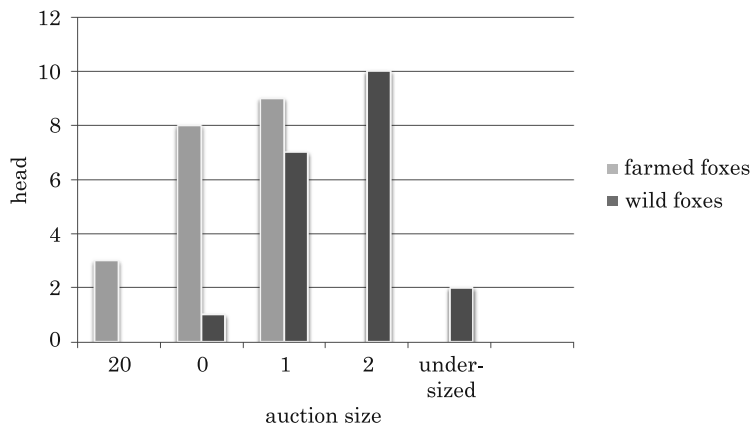

Fig. 1. Proportion of analysed skins according to auction size

Hair coat was felted in both farmed and wild foxes, but in the former this only concerned the pelvic girdle to a shallow/low extent (3 skins) and to a deep/high extent (1 skin), where hair tangling exceeded 1 cm in the cut sample. In wild foxes, felting spread from the sacrum to tail head and side, and the degree of felting was medium (2 skins) and deep/high (9 skins) – Table 2. Hair felting in wild foxes affected up to 35% of the skin area.

The histological examination of the wild foxes revealed a significantly lower number of bundles per tuft and a highly significantly lower number of down hair per tuft and bundle. The area, length and diameter of a hair bundle was highly significantly lower in these animals. No statistically significant differences were found in the number of down and awn hair. The skins of wild foxes had a greater number of sebaceous glands, which were highly significantly longer and had a significantly greater area (Table 3, Figure 2 and Figure 3).

Table 2

Degree of hair cover felting in foxes*

Foxes	Hair coat felting*						
	Site of occurrence	No. of skins acc. to degree of felting**:			No. of skins with defects	% of skin with defects	% of skin area felted, min – max
		shallow/ low	medium	deep/ high			
Farmed	pelvic girdle	3	–	1	4 (20)	20	1–5
Wild	from sacrum to tail head, side	–	2	7	9 (20)	45	10–35

* skins were grouped according to the highest degree of coat felting
** deep/high felting 1 cm and greater on a cut sample, counting from cutaneous tissue, medium felting from 0.5 to 1 cm, shallow/low felting up to 0.5 cm

Table 3

Results of histological examination of fox skins

Item	Foxes			
	farmed	SD	wild	SD
Thickness of skin layers [μm]:				
– epidermis	5.15	1.120	4.36	2.012
– dermis	219.30	46.25	222.75	45.11
No. of bundles per tuft	2.8^{a}	1.033	2.3^{b}	0.852
No. of down hair per tuft	41.3^{A}	14.46	30.2^{B}	17.41
No. of down hair per bundle	14.8^{A}	3.542	13.4^{B}	4.076
Hair bundle:				
– area [μm^2]	25064.9^{A}	5974	14695.5^{B}	4319
– length [μm]	276.63^{A}	47.15	216.67^{B}	32.16
– diameter [μm]	125.10^{A}	22.45	93.44^{B}	17.88
Guard hair:				
– area [μm^2]	5786.4	4558	7232.8	2549
– length [μm]	100.46	38.51	112.00	20.20
– diameter [μm]	73.39	24.48	82.89	14.93
Awn hair:				
– area [μm^2]	1355.9	290.5	1445.1	624.2
– length [μm]	50.37	8.256	48.97	11.04
– diameter [μm]	37.53	3.539	38.34	7.675
Sebaceous gland:				
– area [μm^2]	3823.4^{a}	1715	5633.1^{b}	3969
– length [μm]	121.07^{A}	29.22	174.51^{B}	84.19
– width [μm]	46.76	13.03	51.77	22.38

Explanations as in Table 1

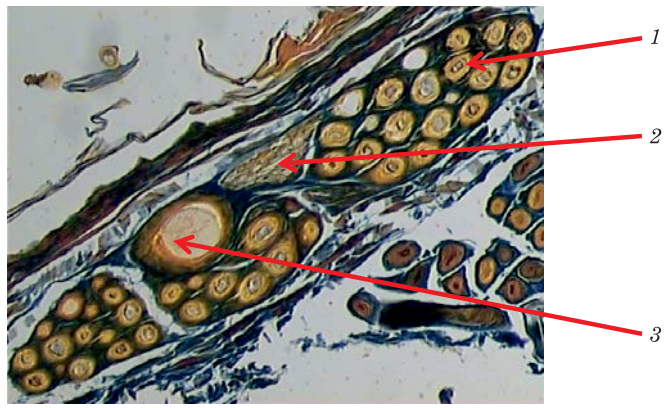

Fig. 2. Microphotograph of a tuft with sebaceous gland from farmed fox (100x magnification, Mallory's staining): *1* – down hair; *2* – sebaceous gland; *3* – guard hair

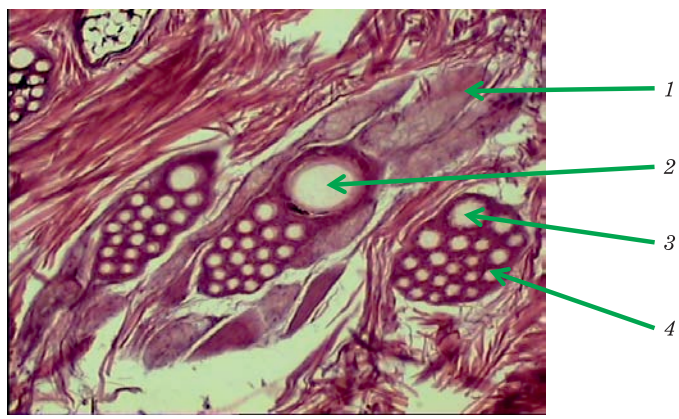

Fig. 3. Microphotograph of a tuft with sebaceous gland from wild fox (100x magnification, hematoxylin and eosin staining): *1* – sebaceous gland; *2* – guard hair; *3* – awn hair; *4* – down hair

Analysis of the level of trace and major elements in the hair of wild and farmed foxes revealed highly significant differences in the amount of iodine, lead, selenium and sulfur (Table 4). All of these elements were more abundant in farmed foxes except for lead, which was higher in wild foxes.

Table 4

Chemical analysis of hair [mg/kg]

Element	Foxes			
	farmed	SD	wild	SD
Ca (calcium)	348.25	154.1	301.94	137.8
Co (cobalt)	0.012	0.027	0.019	0.027
Cu (copper)	10.402	2.517	9.805	4.323
Fe (iron)	136.71	63.33	115.97	33.18
Hg (mercury)	0.241	0.113	0.284	0.107
I (iodine)	0.223^{A}	0.077	0.121^{B}	0.045
Mg (magnesium)	57.49	59.89	43.77	17.16
Mn (manganese)	0.782	0.446	0.958	0.363
Pb (lead)	0.996^{A}	0.452	1.528^{B}	0.334
Se (selenium)	0.429^{A}	0.109	0.304^{B}	0.124
S (sulfur)	245.30^{A}	54.85	132.48^{B}	65.42
Si (silicon)	372.93	173.6	289.81	67.78
Zn (zinc)	35.193	36.58	22.846	6.833

Explanations as in Table 1

Discussion

Fur-bearing carnivores have been farmed in Europe for about 95 years. According to authors (BRZOZOWSKI 2002, GUGOŁEK et al. 2008), differences between the skins of farmed and wild animals were small only during the initial period of farming. Today the differences in the size and quality of fur material are so large that over 86.5% of the skins offered by the auction houses originate from farmed animals (GUGOŁEK et al. 2008, GUGOŁEK 2015).

The considerably greater body weight and thus the skin size of the farmed foxes compared to the wild foxes, found in the present experiment, is the consequence of long-term breeding work and the use of properly balanced diets, which meet strict quality standards. Skin size may be indicated by its length, which reflects the animal's size. Similar results were obtained by JANISZEWSKI at al. (2010), LOREK et al. (2001), KULAWIK et al. (2013), PIÓRKOWSKA (2015), and PRZYSIECKI et al. (2006). Changes in the conformation of fur-bearing carnivores as a result of breeding work were investigated by BRZOZOWSKI (2002). The analysis covering the years 1975–2002 demonstrated that during this period the skin size of farmed foxes increased by two auction sizes, i.e. by around 18 cm. According to the information provided by the auction houses, after the year 2000 fox skin sizes 4, 3 and 2 were not included in the auctions (*Sagafurs* 2016).

The fur faults that considerably reduce the fur value of fox skins include felting, cutouts, greasiness, hair whorls, differences in the thickness and length of individual hairs and their ratio. In long-haired skins, the ratio of down hair to guard hair should be 70:30 to provide the coat with proper softness and thermal insulation. Adult foxes molt once a year – during the spring. During the summer hair growth, many hair roots remain dormant and winter hair begins to grow as late as the end of August (NATANEK et al. 2001). The lack of proper nutrients during this period may contribute to abnormal winter coat development, which we observed in foxes living in the wild. The number of down hair, in both tufts and bundles, was much smaller than in the farmed animals. The significantly larger area (thickness) of guard hair in the skins of wild foxes is evidence that their hair coat was rough and less noble (primitive).

Because hair felting, which is found in both farmed and wild animals, is a latent defect, it can be easily overlooked during preliminary visual assessment. This fault can be identified by the presence of soft, flabby and sticky hair that is devoid of elasticity. Organoleptic evaluation shows granularity at the base of hair and tufts of matted hair mass (BLOMSTEDT 2000). Many studies have shown that this defect is heritable (heritability of 0.26–0.38), similarly to hair coat quality, which means that it can be reduced through selection and deliberate selective breeding (BLOMSTEDT et al. 2001). The felting of skins from farmed foxes is probably a relic of the period when selection was for skin size as a factor having the largest effect on its price.

Histological examination is useful in evaluating the skins because it reveals changes occurring in different disease conditions or abnormalities in cutaneous tissue and hair structure. Histological analysis also allows for an accurate determination of hair coat density, making it useful for determining the hair-forming potential of skin.

Anatomically, the skin consists of three distinct layers – the epidermis, dermis and subcutaneous layer, which differ in structure, chemical composition and function – and hair coat, which is a product of the epidermis. Individual hairs penetrate deep into the dermis, to which they are attached through hair roots. Sebaceous gland secretion, which consists of fatty acids and cholesterol, protects the epidermis from drying out and the hair from being penetrated by water. Unlike the size of sebaceous glands, their number in animals does not change throughout their lifetime. In our study, the area, length and width of sebaceous glands (Figure 1 and Figure 2) were smaller in farmed foxes than in wild foxes, which can be seen as the result of domestication.

Farmed foxes, which are kept in covered cages, are not as exposed to the changing environmental conditions as animals living in the wild. Sebum

production is a natural process that creates a protective mantle for the skin and epidermis, and shields them from mechanical and chemical agents, microorganisms, changing environmental conditions, and UV radiation.

The hair coat of common foxes is classified as long due to the length of guard hair (45–110 mm). Hairs in the coat are arranged in tufts, and their number is about 10,000 per cm^2 (DUDA 1992). Hair coat characteristics have been analysed by many authors (SOCHA 1999, NOWICKI et al. 2010, 2012, PIÓRKOWSKA 2015), because these traits are continually changing under the influence of selection and environmental conditions.

Improper diet, ongoing pathological changes and the adverse environmental impact are reflected, among others, in the mineral composition of hair. The elemental concentration in hair largely depends on the diet currently in use (SKIBNIEWSKA et al., 2011). Organic trace elements are absorbed by the body and later deposited in the hair coat. Many studies suggest that there is a relationship between elemental levels in hair and internal organs (BIAŁKOWSKI and SABA 1987, ŁUCZAK-ZIELKIEWICZ and SZUTOWSKI 2013, SABA et al. 1982, *Life Line*... 2016). Thus, hair reflects the body's health status (KARCZEWSKI 1998, RADOMSKA et al. 1991). From blood tests it is impossible to conclude the accumulation of elements in different tissues in which they are stored, because their concentration varies with emotional changes or with the type of food consumed. No such variation is observed in hair. In a study on the mineral content of silver fox hair, SABA et al. (1982) determined that the mineral quantities were in the order of Na > Ca > K > Mg > P > Zn > Fe > Cu > Mn > Co, whereas the level of elements fell into the following ranges depending on the season: Na, 900–1150 ppm; Ca, 760–1170 ppm; K, 320–680 ppm; Mg, 200–300 ppm; P, 200–300 ppm; Zn, 49–64 ppm; Fe, 38–58 ppm; Cu, 10–14 ppm; Mn, 1–4 ppm; Co, 0.5–0.6 ppm.

The hair coat of wild foxes was found to contain a relatively high level of toxic element lead, which indicates that their ecological niche covers areas close to urban roads, where in fields and meadows adjacent to the roads the foxes forage for small rodents, which are the staple of their diet.

The element iodine, the content of which was almost twice as high in the hair of farmed foxes, plays a major role in hair growth and prevention of hair loss. It has an effect on the general appearance of the hair coat, and protects hair from becoming brittle and decoloured. Sulfur improves the appearance of hair and plays an essential role in its growth, whereas selenium, considered as one of the most important antioxidants, can alleviate the symptoms of hair loss. Low or zero levels of individual elements contributes to inhibited growth and loss of hair (the defect of absence of down).

The use of properly balanced diets and long-term breeding work on farms caused differences between the populations of farmed and wild foxes in many

metric traits of the gastrointestinal and integumentary systems. Compared to the population of farmed foxes, the wild foxes were characterized by poorer hair coat quality, as reflected in considerable felting, absence of down, and deficiency of elements needed for proper hair development.

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PREDICTING HOT CARCASS WEIGHT AND INSTANTANEOUS BODY WEIGHT IN YOUNG CROSSBRED BULLS AND STEERS*

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K e y w o r d s: prediction, hot carcass weight, slaughter value, beef cattle, SVMs.

A b s t r a c t

The aim of this study was to estimate hot carcass weight (HCW) and to determine the accuracy of predicting body weight in young bulls and steers, based on live animal measurements performed at 6 and 12 months of age and before slaughter with the use of Support Vector Machines (SVMs). Among the four analyzed kernel functions, a radial basis function (RBF) in the following form: $K(u,v) = \exp(-\gamma \cdot \| u - v \|^2)$, $\gamma > 0$, where γ is the kernel's parameter, provided the best fit. The most accurate and the least accurate prediction of the body weights was achieved for live animal measurements performed before slaughter and at 6 months of age, respectively. The highest and lowest values of the coefficient of correlation (Pearson's r) between experimental and model HCW values were noted for measurements taken on the day of slaughter and at 12 months of age, respectively. Early (6 months of age) prediction of HCW could contribute to optimizing the length of the fattening period in beef cattle, thus helping the animals realize their full production potential.

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PRZEWIDYWANIE MASY TUSZY (WBC) BUHAJKÓW I WOLCÓW MIESZAŃCÓW MIĘSNYCH ORAZ MASY CIAŁA W MOMENCIE WYKONYWANIA POMIARÓW (CHWILOWEJ MASY CIAŁA)

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S ł o w a k l u c z o w e: przewidywanie, waga bita ciepła, wartość rzeźna, bydło mięsne, metoda SVMs.

A b s t r a k t

Celem badań było oszacowanie masy tuszy (wbc) i dokładności przewidywania masy ciała buhajków i wolców mieszańców mięsnych, pochodzących z krzyżowania krów rasy polskiej holsztyńsko-fryzyjskiej (PHF) i buhajów ras mięsnych (HH, LM, CH), na podstawie pomiarów przyżyciowych zwierząt wykonanych w 6. i 12. miesiącu życia oraz przed ubojem, z wykorzystaniem metody Support Vector Machines (SVMs). Z czterech analizowanych funkcji jądrowych najlepsze dopasowanie uzyskano dla funkcji RBF (radial basis function) w postaci: $K(u,v) = \exp(-\gamma \cdot \| u - v \|^2)$, $\gamma > 0$, gdzie: γ, jest parametrem jądra. Najlepszą możliwość przewidywania masy ciała dają przyżyciowe pomiary wykonane przed ubojem, najgorszą w wieku 6 miesięcy. Najwyższe wartości współczynnika korelacji r Pearsona między wbc „eksperymentalną" a wbc „modelową" zaobserwowano dla pomiarów wykonanych w dniu uboju, a najniższe po ukończeniu 12. miesiąca życia. Wczesne (w wieku 6 miesięcy) oszacowanie wbc bydła mogłoby pomóc w zaplanowaniu optymalnej długości opasania zgodnie z predyspozycjami produkcyjnymi zwierząt.

Introduction

Beef carcass evaluation provides a basis for transactions between livestock producers and meat processing plants. Beef carcasses are assessed based on their weight and conformation/fat cover scores in the EUROP classification system. Therefore, reliable methods supporting early and accurate prediction of hot carcass weight (HCW) are constantly being searched for. Information on HCW and carcass composition can be used to optimize the production process, conduct food research and develop breeding programs. The development of objective carcass appraisal methods, including ultrasound (WILSON 1992, REALINI et al. 2001, LAMBE et al. 2010, SAKAMOTO et al. 2014) and zoometric measurements, has been a priority for decades. Ultrasound evaluations of live animals have attracted the attention of the meat processing industry (NASH et al. 2000, CREWS and KEMP 2001, GREINER et al. 2003, NAVAJAS et al. 2010a, NAVAJAS et al. 2010b), and a strong relationship between body size and slaughter traits has been documented, among others, by TATUM et al. (1982),

PAPSTEIN et al. (1992), NOGALSKI et al. (2000), and in our previous studies (POGORZELSKA-PRZYBYŁEK et al. 2014, POGORZELSKA-PRZYBYŁEK et al. 2015), which confirms that zoometric measurements can be used as predictors of body weight and composition in cattle (LAWRENCE and FOWLER 2002, FERNANDES et al. 2010). Visual assessment of muscling in live animals is an easy, cheap and rapid method for evaluating meat performance traits, and it is significantly correlated with carcass dressing percentage and quality ($r = 0.82$ and $r = 0.72$, respectively; CONROY et al. 2010).

The objective of this study was to estimate the hot carcass weight (HCW) of young crossbred bulls and steers, the offspring of Polish Holstein-Friesian (PHF) cows and beef bulls (Limousin, Hereford and Charolaise), based on live animal measurements performed at 6 and 12 months of age and before slaughter with the use of Support Vector Machines (SVMs). An attempt was also made to determine the accuracy of predicting the instantaneous body weight of animals based on live measurements.

Materials and Methods

Animals

The experimental materials comprised young crossbred bulls and steers, the offspring of Polish Holstein-Friesian (PHF) cows and beef bulls of the following breeds: Hereford (HH), Limousin (LM) and Charolaise (CH) – Table 1. Calves of known origin, at 2–3 weeks of age, were purchased in north-eastern Poland. One half of the calves were castrated at purchase. Bloodless castration was carried out using a rubber elastrator. The animals were fed milk replacer from automatic feeders. At 2 weeks of age, the calves were transferred to a calf shed with straw bedding, and their milk-based liquid diet was supplemented with solid feeds (concentrate, haylage and hay). The fattening period began at 6 months of age, and the calves were fed a Total Mixed Ration (TMR) provided *ad libitum*. The TMR was composed of wilted grass (first-cut) silage and concentrate (rapeseed meal, ground triticale and mineral supplements). Live body measurements were taken three times: I – at the beginning of fattening (at 6 months of age), II – at 12 months of age, III – on the day of slaughter. The body weights of animals were determined, zoometric measurements (height at sacrum, chest width between shoulder joints, pelvic width, rump length, trunk length, chest girth) and ultrasound measurements (thickness of *m. longissimus dorsi* – MLD, at the level of the 12^{th} – 13^{th} thoracic vertebrae, cross-sectional area of MLD) were carried out, and muscling was assessed. Zoometric measurements were performed by two

qualified technicians, and ultrasound measurements were performed by one person with the use of the Mysono 201 scanner (Medison Co., Seoul, Korea), equipped with a 170 mm linear probe (PB-MYL2-5/170 CD), operating in the 2–5 MHz frequency range. A visual appraisal of muscle scoring was performed on a scale of 1 (low lean content) to 10 (very high lean content). "The muscle score describes the shape of cattle independent of the influence of fatness. Muscling is the degree of thickness or convexity of an animal relative to its frame size" (Mc KIERNAN 2007). A similar method for evaluating the conformation of animals was applied by CHOROSZY et al. (2010), but it was not identical to that used in our study.

Table 1

Experimental material

Gender category	Bulls			Steers		
Number of heads	96			96		
Breed	PHF x HH	PHF x LM	PHF x CH	PHF x HH	PHF x LM	PHF x CH
Number of heads	32	32	32	32	32	32
Number of heads	192					

Statistical Analysis

Support Vector Machines (SVMs) are learning models used for regression analysis, which were originally developed for discriminating between sets. The recent algorithms associated with SVMs allow to construct regression models in the form of a linear function. Nonlinearity can be achieved by mapping sets of elements into a new larger space with the use of nonlinear transformation ϕ. A formal representation of a set assumes the following form: $\{(x_1, y_N), ..., (x_N, y_N)\}$ where $x_i \in \Re^d$ and $y_i \in \Re$ for $i = 1, ..., N$ and nonlinear transformation ϕ: $\Re^d \rightarrow Z$, where Z is a feature space. In regression and classification tasks, SVMs construct an optimal hyperplane without the need to separate feature classes for discrimination, but based on the assumption that the points of a newly-created (by transformation) set located at distance $\varepsilon > 0$ form the hyperplane. VAPNIK (1998) proposed the following function as a measure of fit:

$$L^{\varepsilon}(y, f(x, \beta)) = \begin{cases} 0, & \text{if } |y - f(x,\beta)| \leq \varepsilon \\ |y - f(x,\beta)| - \varepsilon, & \text{if } |y - f(x,\beta)| > \varepsilon \end{cases} \quad (1)$$

where:
$f(x,\beta) = \beta \cdot \phi(x) + \beta_0$.

Sets of measurement data are burdened with various errors, and outliers that lie beyond (ε) the optimal hyperplane are often encountered. Therefore, additional variables $\xi_1,..., \xi_N, \xi_1^*,..., \xi_N^* \geq 0$ were introduced in SVMs, which enabled to rewrite the optimization problem, aimed at finding the optimal hyperplane, in the following way:

$$\begin{cases} \min_{\beta,\beta_0,\xi} \frac{1}{2} \|\beta\|^2 + C \sum_{i=1}^{N} (\xi_i + \xi_i^*), \\ y_i - (\beta \cdot \phi(x_i) \leq \varepsilon + \xi_i, \qquad i = 1, ..., N, \\ -y_i + (\beta \cdot \phi(x_i) \leq \varepsilon + \xi_i^* \qquad i, = 1, ..., N, \\ \xi_i, \xi_i^* \geq 0. \end{cases} \tag{2}$$

In SVMs, there is no need to directly define the transformation (ϕ). It is enough to calculate the dot products of certain functions in a larger space, with the use of a kernel function in the following form: $K(u,v) = \phi(u) \cdot \phi(v)$. The accuracy of a regression model developed using SVMs is primarily determined by nonlinear transformation ϕ and the value of parameter C which gives a tradeoff between model complexity and training error. The *libsvm* library (CHANG and LIN 2011) cooperating with Matlab 2014a environment (Statistics Toolbox 2014) was used in the present study *libsvm* algorithms enabled to test many forms of a kernel function, including:

– linear function: $K(u,v) = u^T \cdot v$,
– polynomial function: $K(u,v) = (\gamma \cdot u^T \cdot v + t)^d$, $\gamma > 0$,
– sigmoid function: $K(u,v) = \tanh(\gamma \cdot u^T \cdot v + t)$,
– radial basis function (RBF): $K(u,v) = \exp(-\gamma \cdot \| u - v \|^2)$, $\gamma > 0$,

where γ, d, t are kernel parameters.

Parameters for the above four forms of the kernel function were determined based on optimization procedures using genetic algorithms. The criterion adopted in the study was the maximum value of the linear correlation coefficient (Pearson's r) between the model and experimental values of a validation set (HCW values). The training set and the validation set were generated by bootstrapping. This method relies on random sampling with replacement, and it creates a training set where the number of elements is equal to the number of elements in the existing set of empirical data. The validation set represents the difference between the empirical set and the set of elements that were never sampled for the training set. The training set was used to determine the parameters of the kernel function. The values of zoometric variables from the validation set were input into the created model to determine the model values of HCW. The bootstrapping procedure was repeated 50 times, and the goodness-of-fit was the mean value from all iterations.

Results and Discussion

The analyzed population of crossbred bulls and steers was characterized by satisfactory body dimensions (height at sacrum, chest width between shoulder joints and pelvic width) and muscle score (visual assessment of muscling, thickness of MLD, cross-sectional area of MLD) – Table 2. The live animals measurements performed in this study were selected in view of the fact that trunk length, pelvic length and the thickness of MLD are highly significantly correlated with carcass weight (BLANCO ROA et al. 2003, CONROY et al. 2009). Height at sacrum, included in the prediction model, increases the accuracy of muscle yield prediction (BERGEN et al. 2005). Regardless of the height at sacrum, the width between the processes of the hip bones is correlated with higher live body weight at slaughter and higher values of carcass quality traits (NOGALSKI et al. 2012). TRELA and CHOROSZY (2011) noted highly significant positive correlations between the thickness of MLD, measured behind the 12^{th} rib, and lean meat yield (r = 0.73). Correlations between live ultrasound measurements of subcutaneous adipose tissue and the cross-sectional area of MLD vs. the actual values of those parameters were reported by SMITH et al. (1992), BRETHOUR (2000), MAY et al. (2000) and GREINER et al. (2003).

Table 2

Descriptive statistics

Variables	Measurement I		Measurement II		Measurement III	
	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd
Age on the day of measurement [days]	183.26	3.14	364.15	4.44	557.09	69.03
Independent variables:						
Height at sacrum [cm]	106.58	3.92	124.60	5.51	136.19	5.53
Forechest width [cm]	31.73	3.52	40.75	3.64	49.21	4.05
Pelvic width [cm]	29.60	2.75	40.00	2.97	47.26	3.25
Pelvic length [cm]	35.70	2.14	44.65	2.66	51.13	3.28
Trunk length [cm]	67.68	4.83	83.48	6.13	95.92	8.04
Chest girth [cm]	129.27	7.65	167.03	8.76	194.73	10.51
Thickness of *M. longissimus dorsi* [mm]	43.98	6.26	55.71	6.57	67.26	8.09
Cross-sectional area of *M. longissimus dorsi* [cm]	39.80	7.25	57.41	8.01	84.57	11.89
Intravital muscle score [pts]	5.95	1.41	6.88	1.30	7.75	1.10
Dependent variables:						
Body weight [kg]	193.2	22.0	358.5	46.4	521.9	74.1
Hot carcass weight [kg]	$\bar{x}$		sd			
	288.0		43.4			

All analyses were performed for a normalized dataset in the <0, 1> range. Among the four analyzed kernel functions, a radial basis function (RBF) provided the best fit. The value of parameter C (system of equations (2)) represented the difference between the maximum and the minimum value of variable y for the training set. Due to the size and randomness of the sample, $C \cong 1$. The remaining parameters and the model's goodness-of-fit are presented in Table 3. The highest values of the coefficient of correlation (Pearson's r) between experimental and model HCW values were noted for measurements taken on the day of slaughter. A higher value of the coefficient of correlation between live weight at slaughter and carcass weight ($r = 0.94$) was reported by MŁYNEK and LITWIŃCZUK (1999). In our previous study, HTC was estimated by stepwise regression based on backward elimination. Live body measurements were performed immediately before slaughter, disregarding weighing results, which confirms the usefulness of HCW estimation on the farm, before the animals are transported to the meat processing plant, assuring a just system of payment for slaughtered animals. The derived equation ($\hat{Y} = 1.507x_1 + 1.103x_2 + 4.043x_3 + 5.53x_4 + 0.379x_5 + 8.076x_6 - 678.93$,
where:
x_1 – height at sacrum [cm]
x_2 – chest girth [cm]
x_3 – pelvic width [cm]
x_4 – pelvic length [cm]
x_5 – thickness of *M. gluteo-biceps* [mm]
x_6 – thickness of MLD (points) overestimated the predicted value by 1.25% (3.9 kg) on average.

Table 3
Values of model parameters and the coefficient of correlation (Pearson's r) for the hot carcass weight (HCW) and body weights of animals at different ages

Measurement	HCW			Body weight at the moment of performing measurements		
	γ	ε	r	γ	ε	r
I	0.1687	0.2597	0.5162	0.2091	0.2768	0.7907
II	0.0002	0.2513	0.3919	0.0142	0.0570	0.8961
III	0.0832	0.0260	0.9231	0.0672	0.2503	0.9185

The coefficient of determination and the standard error of estimation reached $R^2 = 0.892$ and Sy = 16.28, respectively (POGORZELSKA-PRZYBYŁEK et al. 2014). The model developed in this study enables to predict, with lower but sufficient accuracy, the HCW of six-month-old bulls and steers slaughtered

at 18.5 months of age. Figure 1 shows regression lines for experimental and model HCW values determined based on zoometric measurements performed on three dates. The relationships were obtained for one of the 50 iterations selected randomly from the validation set. The slopes of regression lines indicate that the most and least accurate prediction was achieved based on the measurements taken on date III and date II, respectively. The lower goodness-of-fit noted for HCW estimated based on the measurements performed at 12 months of age can be attributed to the lower weight gains of bulls and steers, resulting from changes in their feeding regime and housing conditions (transfer to a free-stall barn).

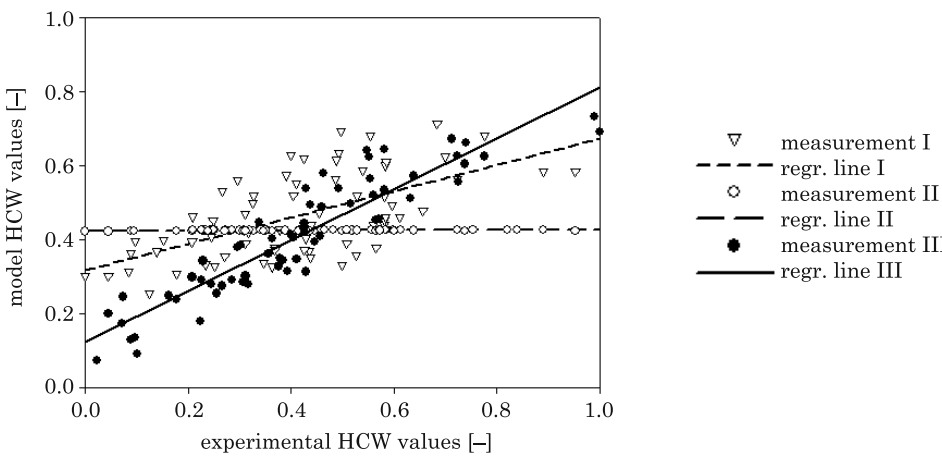

Fig. 1. Regression (RG) lines, with the corresponding points, for experimental and model values of hot carcass weight (HCW), determined based on live measurements performed on three dates; normalized data from the validation set

Selected live measurements allow to predict, with high accuracy, the body weights of animals at the moment of performing zoometric and ultrasound evaluations. The coefficients of correlation between the model-derived and actual body weights increase proportionally to the age of animals subjected to measurements. Figure 2 presents regression lines for experimental and model body weight values determined based on live measurements performed on three dates. The relationships were obtained for one of the 50 iterations selected randomly from the validation set. The slopes of regression lines show that the most and least accurate prediction was achieved based on the measurements taken on date III and date I, respectively. Unlike in Figure 1, the slopes of regression lines in Figure 2 differ slightly. The lower accuracy of estimation observed in younger animals could result from greater variation in

their body dimensions. At successive stages of somatic maturation, the analyzed population was characterized by more uniform values of body measurements, and the body parts of individual animals developed more proportionally.

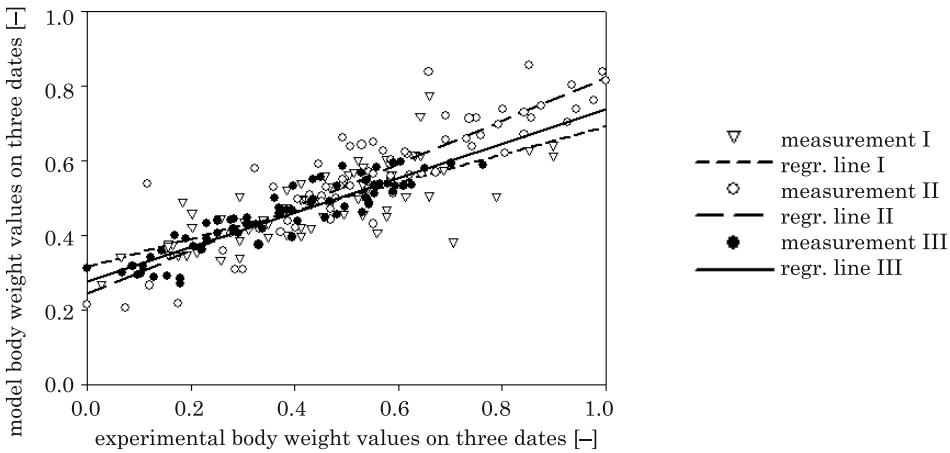

Fig. 2. Regression (RG) lines, with the corresponding points, for experimental and model values of body weight, determined based on live measurements; normalized data (from the validation set) are presented for all three dates when the measurements were performed

The regression model developed with the use of SVMs enables to estimate the body weights of young crossbred bulls and steers at a selected age, and the accuracy of estimation tends to increase with the animal's age. The model also supports early (at 6 months of age) prediction of the HCW of animals slaughtered at 18.5 months of age on average. The estimation of HCW before or during fattening could contribute to optimizing the length of the fattening period in beef cattle, thus helping the animals realize their full production potential.

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A NEW STAND AND THE CURRENT STATUS OF THE *NUPHAR PUMILA* POPULATION IN WARMIŃSKO-MAZURSKIE PROVINCE

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K e y w o r d s: endangered species, glacial epoch survivor, protected areas, forest lake.

A b s t r a c t

During a floristic study conducted in 2003 in the "Beaver Refuge on the Pasłęka River" reserve (Olsztyn Lakeland, north-eastern Poland), a new stand was found of *Nuphar pumila* (Timm) DC (a rare species in Poland, one of the glacial epoch survivors) in a humic and forested closed water body within Warmińsko-Mazurskie Province. The aim of the study, conducted in 2013 and presented in this paper, was to confirm the presence of the stand, and to determine the current conservation status of the *N. pumila* population. In 2003, the occurrence of *N. pumila* in the southern bay of the lake was found to be only one stand. The study conducted in 2013 confirmed the presence of the species in question in a lake (regionally known as Jeziorko Leśne). In addition, a distinct extension of the range of *N. pumila* compared to the status in 2003 was observed; currently, the species is found in several dozen stands. As in previous years, *N. pumila* grew on the bottom of the water body to a depth of approx. 70–90 cm, and developed mainly submerged leaves. It covered the largest areas in the north-eastern part of the lake.

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NOWE STANOWISKO I AKTUALNY STAN ZACHOWANIA POPULACJI *NUPHAR PUMILA* W WOJEWÓDZTWIE WARMIŃSKO-MAZURSKIM

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S ł o w a k l u c z o w e: gatunek zagrożony wyginięciem, relikt glacjalny, obszary chronione, śródleśne jezioro.

A b s t r a k t

Podczas badań florystycznych prowadzonych w 2003 r. w Rezerwacie przyrody „Ostoja bobrów na rzece Pasłęce" (Pojezierze Olsztyńskie, północno-wschodnia Polska) w humusowym, śródleśnym, bezodpływowym zbiorniku stwierdzono nowe stanowisko *Nuphar pumila* (Timm) DC (rzadkiego w Polsce gatunku należącego do reliktów glacjalnych) w granicach województwa warmińsko-mazurskiego. Celem badań przedstawionych w pracy (przeprowadzonych w roku 2013) było potwierdzenie tego stanowiska oraz określenie aktualnego stanu zachowania populacji *N. pumila*. W 2003 r. stwierdzono występowanie *N. pumila* w południowej zatoce opisywanego zbiornika tylko na jednym stanowisku. W badaniach z 2013 r. potwierdzono obecność tego gatunku w jeziorze (regionalna nazwa Jeziorko Leśne) na południe od Gamerek Wielkich. Stwierdzono również wyraźne rozprzestrzenianie się zasięgu *N. pumila*, w porównaniu ze stanem z roku 2003 – w 2013 r. występował on na kilkudziesięciu stanowiskach. Podobnie jak w latach ubiegłych grążel drobny porastał dno zbiornika do głębokości około 70–90 cm, wytwarzając głównie liście zanurzone. Największe powierzchnie zajmował w północno-wschodniej części jeziora.

Introduction

Nuphar pumila (Timm) DC, syn. *N. pumilum* (Timm) DC, is a member of the *Nymphaeaceae* family. *N. pumila* is found in northern, central and western Europe (where it is endangered), in Siberia, in eastern Asia, and in the central-eastern part of North America. Since 1983, the species has been under strict protection in Poland (Journal of Laws 2014, item 1409), and, according to the Polish Red Data Book of Plants, is classified as an endangered species. Given that the species is critically endangered in Germany and Czech Republic, endangered in Belarus, rare in Lithuania, and no longer found in Kaliningrad Oblast, it is also listed in the European Red List of Vascular Plants (KŁOSOWSKI 2014). *N. pumila* is a perennial plant with a rather long (20–70 cm) rhizome and ovate floating leaves that are morphologically similar to the leaves of *N. lutea* (L.) Sibith. & Sm., but much smaller, as well as submerged leaves,

which are cordate or orbicular in shape. The characteristic feature of *N. pumila* is a pistil topped with a flat stigma disc, with 8–12 radiated lines reaching the clearly dentate edge, and a fruit that is usually curved in the upper part (KŁOSOWSKI and KŁOSOWSKI 2012, KŁOSOWSKI 2014). It grows in meso- to oligotrophic and dystrophic ponds and lakes with cool water of low hardness, on a peaty and slimy organic substratum (KRASKA et al. 2006, KŁOSOWSKI and KŁOSOWSKI 2012, KŁOSOWSKI 2014). *N. pumila* is a component of aquatic plant communities classified as the alliance *Nymphaeion* Oberd. 1957. Most often, it forms phytocoenoses of the association *Nupharetum pumili* Oberd. 1957, in combination with the following species: *Potamogeton natans* L., *Nymphaea alba* L. and *Myriophyllum spicatum* L., in which it is a dominant species. In Poland, *N. pumila* primarily grows within the Lakeland Belt, mainly in the Suwałki Lakeland and Pomorskie Lakeland (ZAJĄC and ZAJĄC 2001, KŁOSOWSKI and KŁOSOWSKI 2012, MATUSZKIEWICZ 2014, KŁOSOWSKI 2014).

During the floristic study conducted in 2003 in the "Beaver Refuge on the Pasłęka River" reserve, the collaborators found a new stand of *Nuphar pumila* (Timm) DC within Warmińsko-Mazurskie Province.

The aim of the study, conducted in 2013 and presented in this paper, was to confirm the presence of the stand and to determine the current conservation status of the *N. pumila* population.

Material end Methods

Study area

The floristic study was focused on a small, forested, closed water body (regionally known as Jeziorko Leśne), which is situated in north-eastern Poland, in the Olsztyn Lakeland, (PUWG 1992: 575127, 664519) – Figure 1. The lake is located at a height of 82.4 m.a.s.l., ith an actual area of 2.34 ha, length of 260 m, and width of 150 m. The deepest spot found in the lake was a depth of 2.5 m. In the south-western part, a periodic outlet is situated, allowing outflow to the Pasłęka River. The immediate surroundings of the lake are forests. The southern shores of the lake are covered with a 90-year-old mixed marshy coniferous forest, parts of the western shore by a more than 100-year-old mixed fresh coniferous forest, and the north-eastern shore by a 110-year-old mixed fresh forest.

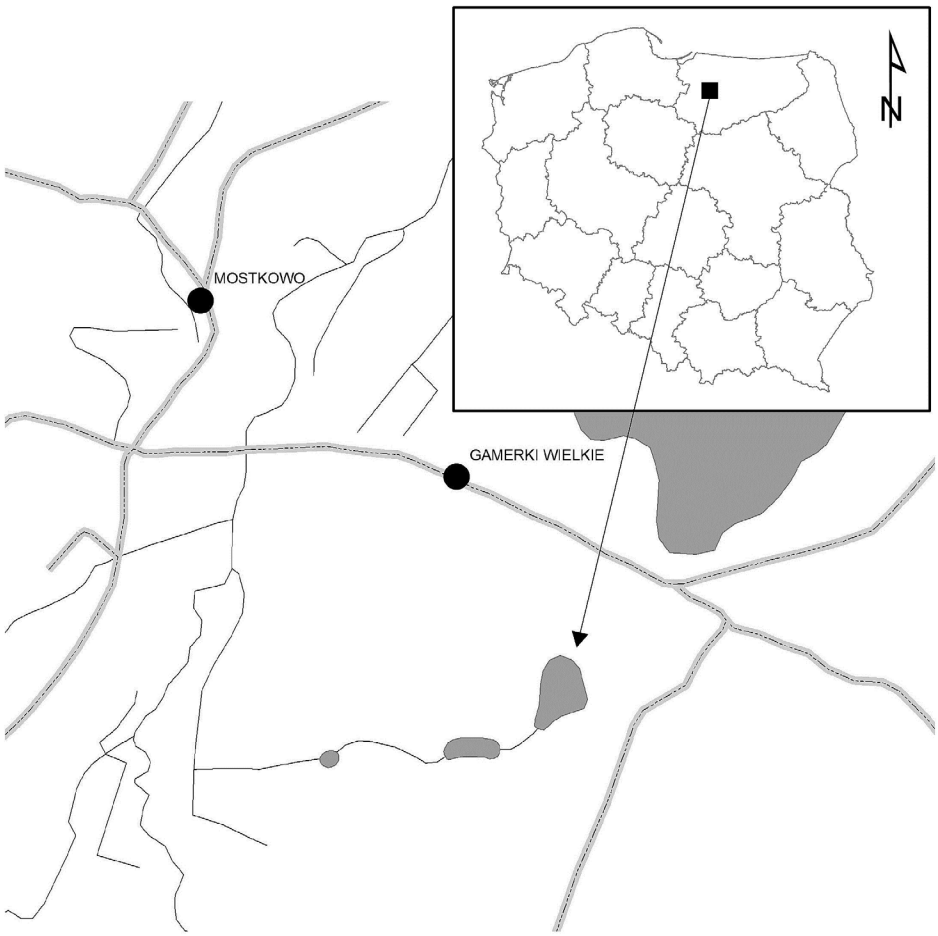

Fig. 1. The location of study site

Sampling and data collection

The field study was conducted with the use of a boat, an anchor for sampling macrophytes, and a Garmin eTrex Vista GPS satellite receiver. Geographical positions were determined for the stands of individual plant communities and stands of the small yellow pond-lily. The range of occurrence of individual species and communities was determined using ArcMAP 9.3.1. software. The performed floristic analyses were based on the commonly used method of phytosociological releves (BRAUN-BLANQUET 1951). Based on the characteristic species, individual plant communities ranked as an association were identified (HERBICH 2004, MATUSZKIEWICZ 2014). The paper presents

protected species in accordance with the classification of the Regulation of the Minister of the Environment (Journal of Laws 2014, item 1409). The nomenclature of vascular plant species is presented in accordance with MIREK et al. (2002). Using a Combo pH & EC tester (Hanna Instruments), the pH, temperature and conductivity of water were measured. Water transparency was determined using a Secchi disc.

Results and Discussion

According to literature data, in north-eastern Poland, *N. pumila* is currently present in only five known stands found in the Suwałki Lakeland (Okliny Lake near Okliny, Pobłędzie Lake near Skajzgiry, Wersele Lake near Wersele, Dziadówek Lake near Dzierwany, and Jegliniszki Lake near Soliny), and in Serwent Lake in the Olsztyn Lakeland (ZAJĄC and ZAJĄC 2001, SZYMKIEWICZ 2011, DZIEDZIC et al. 2012, KŁOSOWSKI 2014). The stands of *N. pumila*, unconfirmed after 1990, include, *inter alia*: Prosno in the commune of Morąg, Morąskie Łąki, Gamerki Wielkie in the commune of Jonkowo, Małdyty near Morąg, Dywity near Olsztyn, Jonkowo near Olsztyn, Kiemno swamp near Purda, Smolajny in the commune of Dobre Miasto, Rudzienickie Lasy near Iława, Czarne Lake near Ostróda, Kierzlińskie Lake, and Lisunie Lake near Mikołajki (DZIEDZIC 2001, KŁOSOWSKI 2014). Jeziorko Leśne is therefore the second stand of *N. pumila* confirmed after 1990 and situated in Olsztyn Lakeland.

In 2003, the occurrence of *N. pumila* was found in the southern bay of the presented lake in only one stand. The study conducted in 2013 have confirmed the presence of this species in Jeziorko Leśne. In addition, a visible spread of the range of *N. pumila* compared to the status as of 2003 was observed, as this species was found in several dozen stands. Its largest coverage is in the north-eastern part of the lake (Figure 2, Table 1 – relevé no 6). As in previous years, *N. pumila* grew on the bottom of the water body to a depth of approx. 80 cm. Some 90% of individuals developed only submerged leaves. The floating leaves (intensely bitten by insects), flowers and fruits were only found occasionally (Figure 3). In Serwent Lake, the situation was opposite. *N. pumila* developed mostly floating leaves, while submerged leaves accounted for approx. 20% of the total. *N. pumila* individuals growing in Serwent Lake developed more flowers and fruits compared to those growing in Jeziorko Leśne. In addition, reduced insect pressure was observed (own data, unpublished).

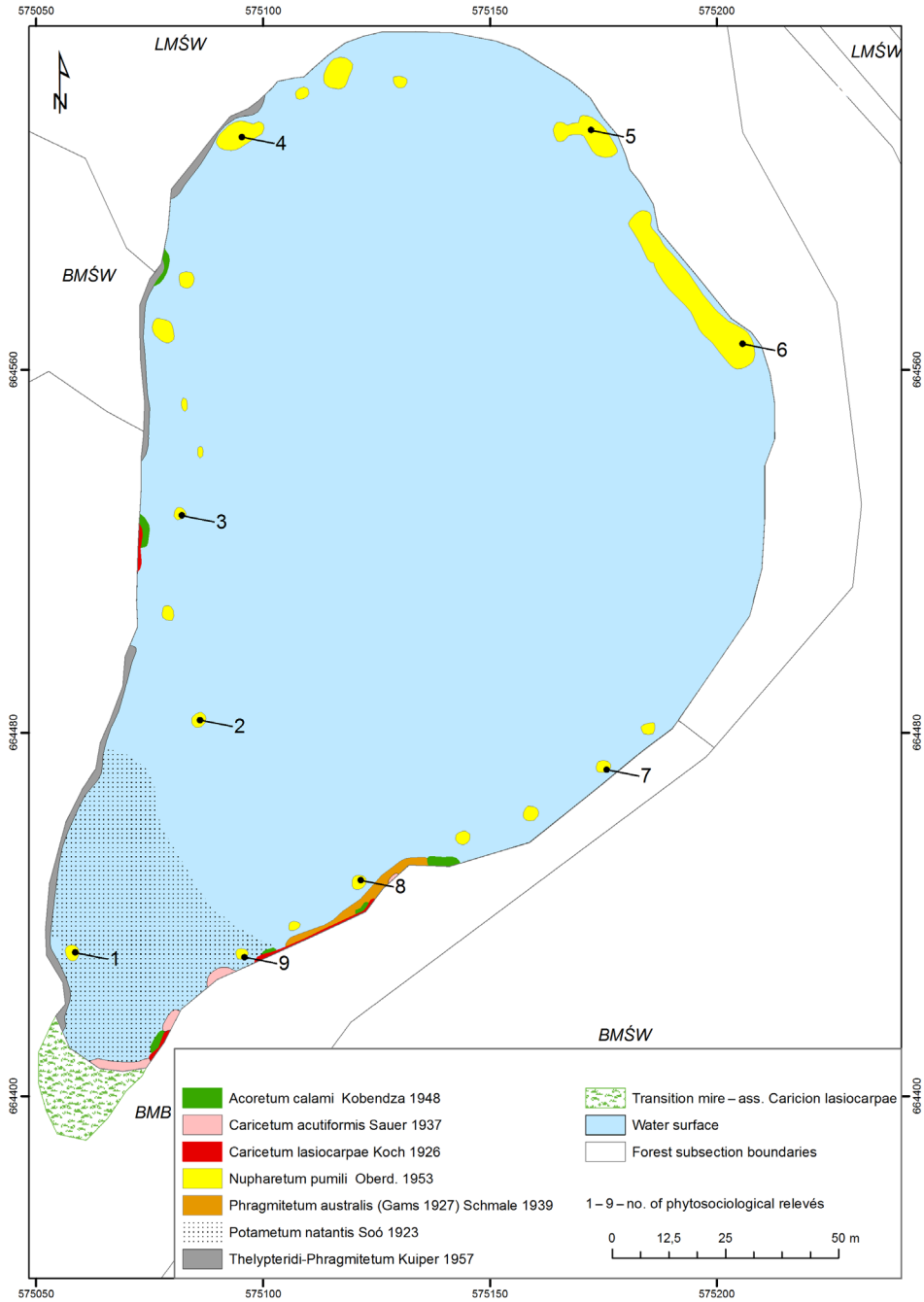

Fig. 2. Map of actual vegetation of Jeziorko Leśne

Table 1

Association *Nupharetum pumili* Oberd. 1953

No. of relevé	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Date	20.08.2013									6.08.2003
Forest division	Kudypy									
Forest district	Bobry									
Forest section	350i									
Cover of herb layer [%]	90	20	40	70	20	20	30	30	100	20
The area of record [m^2]	20	40	20	20	20	20	20	20	20	20
The number of species	3	2	1	1	1	1	1	1	2	2
Nuphar pumila	2	2	3	4	2	2	3	3	2	2
Potamogeton natans	3	+	·	·	·	·	·	·	4	1
Nymphaea alba	1	·	·	·	·	·	·	·	·	·

Source: Herbich et al. 2003 (unpublished) for data of the 2003, own study for data of the 2013

Fig. 3. *Nuphar pumila* – leaves, flower and fruit (Jeziorko Leśne, 20 August, 2013; photo by P. Dynowski)

The characteristics of individual plant associations found in the lake under study in 2013 are presented in detail below.

The aquatic plant communities

1. Association *Nupharetum pumili* Oberd. 1953. On the national level, this is a rather rare community, poor from a floristic point of view, with a boreal type of range and probable relict nature (KŁOSOWSKI et al. 1996, MATUSZ-

KIEWICZ 2014). *N. pumila*, which is a characteristic species for the association, grows along the south-eastern, western, and northern shores to a depth of 1.6 m. Patches of this association were scattered, and their area was approx. 10–20 m^2. Near the eastern shore, over a distance of approx. 100 m, no phytocoenoses of the association concerned were found (near a small beach with a pier) – Figure 2). *N. pumila* mostly forms single-species phytocoenoses with different areas. In only the southern bay were found two small patches of the association *Nupharetum pumili* adjacent to a dense patch of the broad-leaved pondweed association *Potametum natantis* (Table 1).

2. Association *Potametum natansis* Soó 1923. A characteristic species for this association is *Potamogeton natans*, which forms a single patch of the association in the southern bay (Figure 2). Within the phytocoenosis with the dominant *P. natans*, *Nymphaea alba* and *N. pumila* grew in separate stands, and in shallower spots, near the shore, moss *Fontinalis antipyretica* grew.

The rush communities

1. Association *Phragmitetum australis* (Gams 1927) Schmale 1939. A poorly developed, narrow, discontinuous strip of reed bed is formed of *Phragmites australis* and *Acorus calamus*. Phytocoenoses of the association *Phragmitetum australis* were recorded at a 40 m-long section of the south-eastern shore (Figure 2). As regards the species forming the reed bed, in addition to the dominant *Phragmites australis*, also *Carex acutiformis*, *Acorus calamus* and *Carex lasiocarpa* were recorded.

2. Association *Acoretum calami* Kobendza 1948. Small phytocoenoses with the dominant *Acorus calamus* were found in several scattered stands near the south-eastern and western shores (Figure 2). In the patches of calamus rushes, in addition to the dominant species, the occurrence of *Phragmites australis*, *Carex pseudocyperus*, *Carex lasiocarpa*, *Carex acutiformis*, *Cicuta virosa*, *Bidens connata* and *Phalaris arundinacea* was recorded.

3. Association *Thelypteridi-Phragmitetum* Kuiper 1957. The phytocoenosis is determined by the presence of *Thelypteris palustris* and the rush species. Along the entire western shore (with several metre-long gaps), patches of this association with a width of 3–5 m had developed (Figure 2). In the formation of the community, in addition to *Thelypteris palustris*, *Typha latifolia* was playing a significant role as well. As regards rush species, the occurrence of *Equisetum fluviatile*, *Carex rostrata*, *Carex pseudocyperus*, *Peucedanum palustre* and *Lycopus europaeus* was observed. They were accompanied by *Comarum palustre*, *Solanum dulcamara* and *Juncus conglomeratus*. Near the western shore, phytocoenoses of this association develop in the form of a strip with a width of 1.5–3 m, which are significantly poorer in species. In addition to

Thelypteris palustris and *Typha latifolia, Acorus calamus*, *Carex pseudocyperus* and *Scutellaria galericulata* were recorded. In certain locations, they were accompanied by *Juncus conglomeratus*.

4. Association *Caricetum acutiformis* Sauer 1937. A characteristic species for the association is *Carex acutiformis*. Along the shores of the lake, the occurrence of four small patches of the association, with a width of 1–3 m and a length of 2–5 m, were found (Figure 2). Phytocoenoses of this association develop near the south-eastern shore, and are accompanied by reed and calamus rushes. In addition to *Carex acutiformis*, patches of this association are formed by *Acorus calamus*, *Cicuta virosa*, *Peucedanum palustre* and *Phalaris arundinacea*.

The marsh-sedge peat bogs and morasses communities

1. Association *Caricetum lasiocarpae* Koch 1926. A characteristic species for this association is *Carex lasiocarpa*. Phytocoenoses of this association were found in three stands (Figure 2). The largest patch, with a length of 30 m and a width of 0.5 m, develops along the outer limits of the high rushes, near the south-eastern shore. Patches of the association, with the dominant *Carex lasiocarpa*, develop with the participation of species penetrating from adjacent communities. In certain locations, it was accompanied by *Phragmites australis*, *Acorus calamus* and *Carex limosa*. The other two small patches with lengths of 2 m and 5 m developed along the calamus rushes.

Community with *Eriophorum angustifolium*, a characteristic species of the *Scheuchzerio-Caricetea* nigrae class

Near the shore of the southern bay, at the outer limits of a patch of the association *Thelypteridi-Phragmitetum*, a community developed with the dominant *Eriophorum angustifolium*, which covers part of the shore to a length of 60 m and width of 10–12 m. With its species composition, it is similar to phytocoenoses of transitional peat bogs. *Eriophorum angustifolium* was accompanied by *Equisetum fluviatile*, *Peucedanum palustre*, *Thelypteris palustris*, *Typha latifolia*, *Cicuta virosa*, *Carex rostrata*, *Carex pseudocyperus*, *Alisma plantago-aquatica* and *Lycopus europaeus*. In addition, *Comarum palustre*, *Bidens cernua*, *Salix cinerea*, *Salix aurita* and *Vaccinium myurtillus* also occurred there. The community concerned develops near a mixed coniferous forest, in a highly hydrated habitat with *Sphagnum spp*.

In the tree stand that forms the immediate surroundings of the water body, *Pinus sylvestris* is dominant. In the layer of trees, *Betula pendula*, *Carpinus betulus*, *Fagus sylvatica*, *Quercus robur*, *Quercus rubra*, *Alnus glutinosa*, *Populus tremula*, *Sorbus aucuparia*, *Picea abies* and a sapling *Acer platanoides* were found. As regards shrubs and dwarf shrubs, *Frangula alnus*, *Vaccinium myrtillus* and *Vaccinium vitis-idaea* were recorded. In the group of herbaceous plant species, the occurrence of *Lysimachia thyrsiflora*, *Scutellaria galericulata*, *Peucedanum palustre*, *Carex nigra*, *Carex lasiocarpa*, *Trientalis europaea*, *Juncus conglomeratus* and *Maianthemum bifolium* was found.

As mentioned earlier, *N. pumila* grows in lakes, ponds and oxbow lakes. This species is characterised by wide ecological amplitude as regards the fertility of the habitat. It grows in oligotrophic, humotrophic (dystrophic) and eutrophic waters, and *N. pumila* has the biggest chance for survival in water bodies with humotrophic (dystrophic) characteristics, not being subject to rapid succession changes (KŁOSOWSKI 2014).

Jeziorko Leśne is characterised by the following parameters: temperature of 17.3°C; pH of 6.5; conductivity of 180 μS/cm (75 ppm); water transparency of 65 cm. In addition, the lack of submerged vegetation other than *N. pumila, N. alba, F. antipyretica* and *P. natans* was recorded (despite the average depth of approx. 1 m). Therefore, presented lake may be preliminarily classified as humic, forest, small lake.

The data obtained by the authors concerning the phytocoenoses of *Nupharetum pumili* in the new stand coincide with data presented for the phytocoenoses of *Nupharetum pumili* from north-eastern Poland (KŁOSOWSKI et al. 1996, 2011, JABŁOŃSKA and KŁOSOWSKI 2012). However, a detailed study of the physicochemical properties of the water and sediments must be conducted in the future.

According to the information included in the Polish Red Data Book of Plants, in Poland, the most serious risk to the presented species is an increase in water hardness, which contributes to the more intense development of other plants forming communities of the *Potametea* class, e.g., *Potamogeton natans*, and in particular *Nuphar lutea*, which is one of the strongest competitors to *N. pumila* (KŁOSOWSKI 2014). At all stands where both of those species occurred, the formation of an increasing number of hybrids (*N. x intermediata*), and the gradual domination of *N. lutea* were recorded. This process has been observed in such places as Serwent Lake (SZYMKIEWICZ 2011, DZIEDZIC et al. 2012), Okilny Lake, or an astatic water body near Nożyk (KŁOSOWSKI 2014). This issue has also been noticed in other parts of the world, e.g., in the lakes Kämmoosteich and Lac de Lussy in Switzerland (KOZŁOWSKI and EGGENBERG 2005), and in the Podilskyi Reserve in Ukraine (DIDUKH et al. 2010). On the other hand, in Japan, natural hybridisation occurring between *N. pumila* and *N. japonica* has been observed (SHIGA and KADONO 2007).

The literature suggests that all lakes with stands of *N. pumila* should be turned into reserves (as a form of protection actions). In other stands of this species, maintaining low water hardness must be pursued (KŁOSOWSKI 2014). The lake described in this paper is situated within the "Beaver Refuge on the Pasłęka River" reserve, Natura 2000 site PLB 280002 Dolina Pasłęki, the Special Protection Area Dolina Pasłęki (PL.ZIPOP.1393.OCHK.370). Given its natural values, during the preparation of the plan of protective measures it was proposed that the lake, along with the immediate surroundings, should be incorporated into the Natura 2000 SITE PLH 280006 Rzeka Pasłęka.

In 2003, only a single phytocoenosis of the small yellow pond-lily was found (Table 1, relevé no 10). In 2013, it turned out that the small yellow pond-lily spread along the shores. Therefore, if the habitat conditions in the lake do not change, the conservation status of the *N. pumila* population will not be at risk. A potential threat to this site is a possible decrease in the water level (which can be controlled by making the valve downstream), forestry clearance and increased supply of humus compounds.

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LONG-TERM CHANGES IN THE FLORA AND VEGETATION OF OLECKO WIELKIE LAKE, EŁK LAKE DISTRICT, POLAND

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K e y w o r d s: urban lake; anthropogenic pressure; macrophytes; protected, threatened and rare plant species.

Abstract

The paper presents detailed results of research into floristic and phytosociological studies on the vegetation of Olecko Wielkie Lake, carried out in 2009. During the study 28 hydrophytic taxa, 27 helophytic taxa and 14 species classified as co-existing were identified, including of those forming the structure of particular plant communities. In total 69 plant taxa were found, without trees and shrubs growing on the lake shoreline. The paper presents the occurrence of protected, endangered, and rare species in Poland. The paper also presents a comparison of recent studies with does carried out in the period in 1983–1986, allowing for the identification of changes in the flora and vegetation of Olecko Wielkie Lake between 1983 and 2009.

DŁUGOTERMINOWE ZMIANY FLORY I ROŚLINNOŚCI JEZIORA OLECKO WIELKIE NA POJEZIERZU EŁCKIM

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S ł o w a k l u c z o w e: jezioro miejskie; antropopresja; makrofity; rośliny chronione, zagrożone i rzadkie.

A b s t r a k t

W pracy przedstawiono wyniki badań florystyczno-fitosocjologicznych szaty roślinnej jeziora Olecko Wielkie, które prowadzono w roku 2009. W trakcie badań stwierdzono występowanie 28 taksonów hydrofitów, 27 gatunków szuwarowych i bagiennych oraz 14 gatunków określonych jako gatunki towarzyszące, z podaniem które z nich uczestniczą w budowie określonych zbiorowisk roślinnych. Łącznie odnotowano występowanie 69 taksonów roślin z wyłączeniem drzew i krzewów,

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które rosną na brzegu jeziora. W pracy przedstawiono występowanie gatunków chronionych, zagrożonych i rzadkich. Porównano także otrzymane wyniki badań z wynikami z lat 1983–1986. Na tej podstawie podsumowano zmiany, które zaszły w obrębie flory i roślinności jeziora Olecko Wielkie na przestrzeni lat 1983–2009.

Introduction

Lakes are habitats for specific aquatic plant species known as macrophytes, which are a group of plants with highly diversified morphology (e.g. FLEMING et al. 2012). Macrophytes include all *Charophyta* species native to Poland, selected *Bryophyta,* very few ferns (*Pteridophyta*), and a small group of seed plants (*Spermatophyta*). Macrophytes have a number of important ecological functions in reservoirs, e.g. by affecting the water's trophic state and the composition of plant and animal biocenosis. They are places of feeding and breeding, create refugia for many aquatic invertebrate and fish species, and are also favourable habitats for birdlife (SUTELA et al. 2013, FLEMING and DIBBLE 2015, LAURIDSENET al. 2015). The presence of macrophytes also increases the biodiversity of ecosystems (FLEMING et al. 2012, ALAHUHTA et al. 2013). Macrophytes are relatively stable groups of aquatic plants. Nevertheless, they undergo rapid degradation under extreme conditions (BAKKER and NOLET 2014, JUSIK and MACIOŁ 2014, FLEMING and DIBBLE 2015).

Lakes are exposed to endogenic and exogenic factors, and respond by the transformation of their ecological structure, including that of macrophytes. Very important is also the manner of management in lake's catchment area. Changes in lakes trophy may lead to decline of valuable and rare species in the lakes, as well as decline of valuable habitats, including Nature 2000 habitats (SUTELA et al. 2013, HANSEN and SNICKARS 2014, JUSIK and MACIOŁ 2014, KOLADA et al. 2014, SOANA and BARTOLI 2014, LAURIDSEN et al. 2015).

The aim of this study was to determine the changes in the flora of the urban lake Olecko Wielkie Lake during 25 years and making an inventory of natural valuable and protected species. The main reason for the implementation of the research was observed in recent years, more and more anthropopressure (extension Olecko city, tourism and recreation) on featured lake (own study – unpublished).

Material and Methods

Study area

Olecko Wielkie Lake (*jezioro Olecko Wielkie*) is located in an urban area in north-east part of Poland (54°03'27"N/ 22°29'42"E). The town of Olecko, with

a population of about 22,038 (data for 31 December 2014), spreads out on the western shoreline of the lake. According to CHMIELEWSKI et al. (2007), the lake covers an area of 227.3 ha and has a maximum depth of 45.2 m. The lake basin is ribbon-shaped, with a maximum length of 4,860 m and a maximum width of 1,110 m, stretching from the north-west towards the south-east (Figure 1). Olecko Wielkie Lake is located near the eastern border of Ełk Lake District, a mesoregion within the macroregion of the Masurian Lake District (KONDRACKI 2011).

Fig. 1. The location of the study site

Olecko Wielkie Lake is a flow-through reservoir. The mouth of the Lega river is in the lake's northern bay, and the river's outflow is located in the central part of the western shoreline. The Lega, through subsequent rivers (Małkinia and Jegrznia), and further through the rivers Ełk, Biebrza and

Narew, discharges water into the Vistula river (CHMIELEWSKI et al. 2007). Since 2003 Olecko Wielkie Lake has been a part of the Olecko Wielkie Lake Protected Landscape and the Lega Valley Protected Landscape (O.J. of Warmia and Mazury 2003, No. 52, item. 725). In 2007 and the "Długi Mostek" Ecological Site, located in the bay of Olecko Wielkie Lake near the outflow of the Lega river, were established (O.J. of Warmia and Mazury 2007, No. 1, item 1).

Sampling and data collection

In 1989 research was conducted using classic method of profiles made from the shore to a depth of occurrence of plant communities. Phytocoenoses were documented by phytosociological records performed according to generally used method of Braun-Blanquet. On the basis of documented positions 59 phytosociological records were made.

In June and July 2009 field research were carried out using a GPS eTrex Vista satellite receiver (Garmin). 179 geographical locations corresponded with the sites of protected, rare or declining plant taxa (classification is made in accordance with RUTKOWSKI 2011) and endangered or vulnerable *Charophyte* species (classification is made in accordance with SIEMIŃSKA et al. 2006). Protected species in this paper are presented according to the classification published in the Regulations of the Minister of the Environment. The survey was carried out using grappling hooks and dragging rakes down to the depth of plant occurrence. The distribution range for individual species was established using the ArcMAP 9.3.1 application.

Nomenclature of vascular plant species was adopted after MIREK et al. (2002), and for charophyte species after DĄMBSKA (1964), PEŁECHATY, PUKACZ (2008) and URBANIAK, GĄBKA (2014).

Results and Discussion

Studies investigating the status of flora in Olecko Wielkie Lake were carried out in June and July 2009, in parallel with research aimed at the identification and distribution of plant communities. Results demonstrated that the vegetation of Olecko Wielkie Lake included phytocenoses of 13 hydrophytic associations and 11 helophytic associations (DZIEDZIC, DYNOWSKI 2009).

Floristic studies carried out in 2009 revealed the presence of 23 hydrophytic taxa (Table 1), 26 helophytic taxa, and 14 other species classified as co-existing

Table 1

Systematic classification of hydrophytes in Olecko Wielkie Lake in 2009

No. of hydroph.	*R* – rooted *S* – semi-rooted *F* – floating	Taxa in systematic order
		Division: *Chlorophyta*
		Class: *Charophyceae*
		(1.) Family: *Characeae*
1.	*S*	*Ch. fragilis* Desvaux = *Chara globularis* Thuillier
2.	*S*	*Chara contraria* A. Braun ex Kutzing
3.	*S*	*Nitella flexilis* (L.) Agardh
4.	*S*	*Chara tomentosa* L.
5.	*S*	*Nitellopsis obtusa* J. Groves
		Division: *Telomophyta*
		Subdivision: *Bryophytina*
		(2.) Family: *Fontinalaceae*
1.	*F*	Fontinalis antipyretica Hedw
		Subdivision: *Magnoliophyta (Angiospermae)*
		Class: *Magnoliopsida (Dicotyledones)*
		(4.) Family: *Polygonaceae*
1.	*R*	*Polygonum amphibium* L. f. *natans* Moench
		(5.) Family: *Nymphaeaceae*
2.	*R*	*Nuphar lutea* (L.) Sibth.
3.	*R*	*Nuphar* x *intermedia* Leder. (= *N* x *spenneriana* Gaudin)
4.	*R*	*Nymphaea alba* L.
		(6.) Family: *Ceratophyllaceae*
5.	*F*	*Ceratophyllum submersum* L.
6.	*F*	*Ceratophyllum demersum* L.
		(7.) Family: *Ranunculaceae*
7.	*R*	*Batrachium circinatum* (Sibth.) Fr.
		(8.) Family: *Holoragaceae (Holorrhagidaceae)*
8.	*R*	*Myriophyllum spicatum* L.
		(9.) Family: *Lentibulariaceae*
9.	*F*	*Utricularia vulgaris* L.
		Class: *Liliopsida (Monocotyledones)*
		(10.) Family: *Lemnaceae*
10.	*F*	*Lemna minor* L.
		(11.) Family: *Alismataceae*
11.	*R*	*Alisma gramineum* Lej.
		(12.) Family: *Hydrocharitaceae*
12.	*S*	*Stratiotes aloides* L.
		(13.) Family: *Potamogetonaceae*
13.	*R*	*Potamogeton pectinatus* L.
14.	*R*	*Potamogeton crispus* L.
15	*R*	*Potamogeton lucens* L.
16.	*R*	*Potamogeton perfoliatus* L.
17.	*R*	*Potamogeton praelongus* Wulfen

plants (Table 2). In total 63 plant taxa were recorded, without trees and shrubs growing on the lake shore.

Sites of protected, rare and declining plants in Poland are presented in Figures 2–5.

Table 2

Systematic classification of helophytes in Olecko Wielkie Lake in 2009

	Division: *Telomophyta*
	Subdivision: *Sphenophytina*
	Class: *Sphenopsida*
	(1.) Family: *Equisetaceae*
1.	*Equisetum fluviatile* L. (*E. limosum* L.)
	Subdivision: *Magnoliophytina (Angiospermae)*
	Class: *Magnoliopsida* (*Dicotyledones*)
	(2.) Family: *Polygonaceae*
2.	*Rumex hydrolapathum* Hudson
	(3.) Family: *Brassicaceae* (*Cruciferae*)
3.	*Rorippa amphibia* (L.) Besser
	(4.) Family: *Apiaceae* (*Umbelliferae*)
4.	*Berula erecta* (Huds.) Coville
5.	*Cicuta virosa* L.
6.	Sium latifolium L.
	(5.) Family: *Primulaceae*
7.	*Lysimachia thyrsiflora* L.
	(6.) Family: *Rubiaceae*
8.	*Galium palustre* L.
	(7.) Family: *Lamiaceae* (*Labiatae*)
9.	*Lycopus europaeus* L.
10.	*Scutellaria galericulata* L.
	Subdivision: *Magnoliophytina* (*Angiospermae*)
	Class: *Liliopsida* (*Monocotyledones*)
	(8.) Family: *Alismataceae*
11.	*Alisma plantago-aquatica* L.
	(9.) Family: *Iridaceae*
12.	*Iris pseudacorus* L.
	(10.) Family: *Poaceae* (*Gramineae*)
13.	*Glyceria maxima* (Hartman) Holmb. (*G. aquatica* (L.) R. Br.
14.	*Phalaris arundinacea* L.
15.	*Phragmites australis* (Cav.) Trin. ex Steud. (*P. communis* Trin.)
	(11.) Family: *Araceae*
16.	*Acorus calamus* L.
	(12.) Family: *Sparganiaceae*
17.	*Sparganium erectum* L. em. Rchb.
18.	*Sparganium emersum* Rehmann (*S. Simplex* Hudson)
	(13.) Family: *Typhaceae*
19.	*Typha angustifolia* L.
20.	*Typha latifolia* L.
	(14.) Family: *Cyperaceae*
21.	*Carex acutiformis* Ehrh.
22.	*Carex gracilis* Curtis (*C. acuta* L.)
23.	*Carex pseudocyperus* L.
24.	*Eleocharis acicularis* (L.) Roem. et Sch.
25.	*Eleocharis palustris* (L.) Roem. et Sch.
26.	*Schoenoplectus lacustris* (L.) Palla (*Scirpus lacustris* L.)

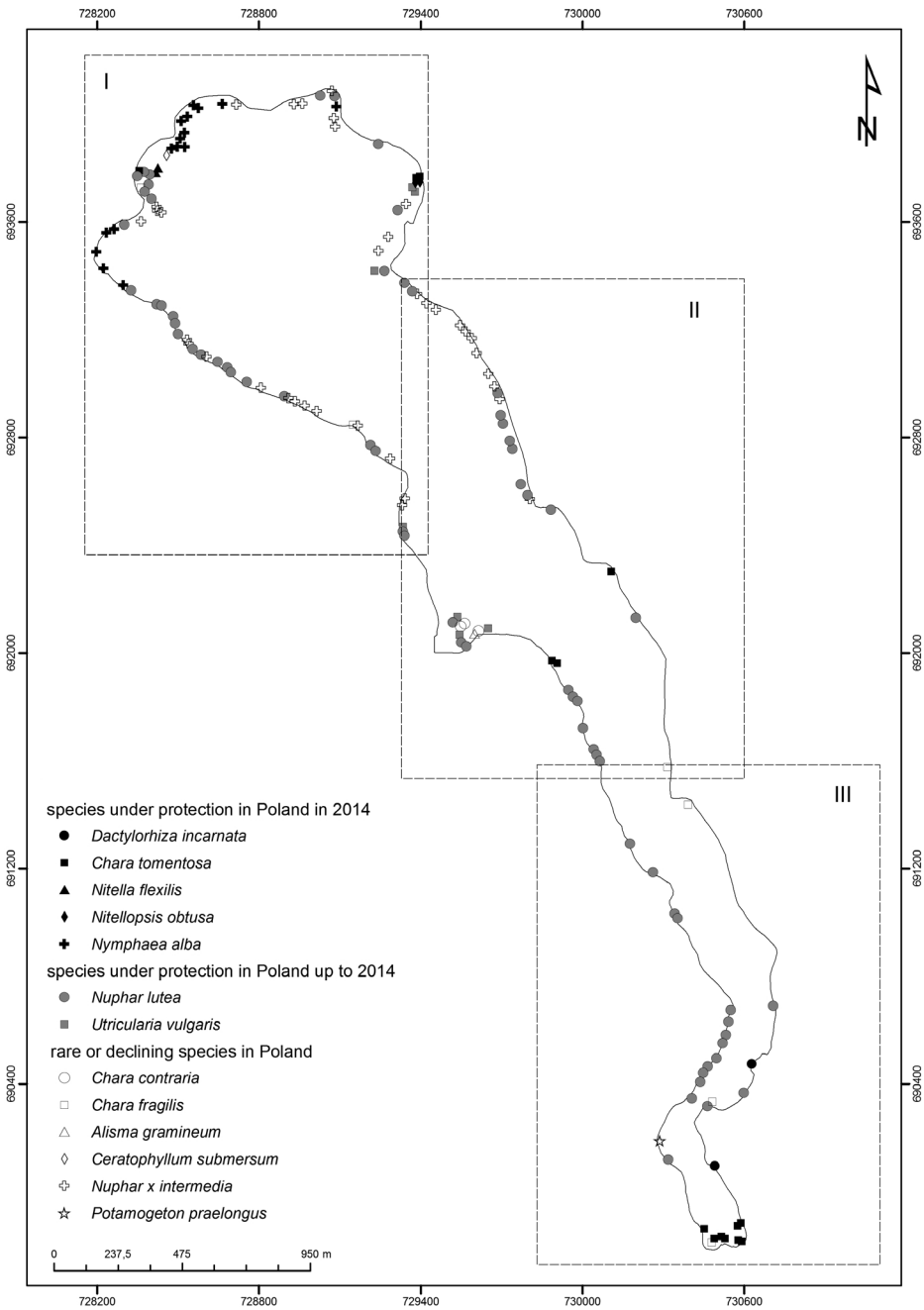

Fig. 2. Olecko Wielkie Lake divided into sheets individual sheets. Sites of protected, rare and declining taxa in Poland

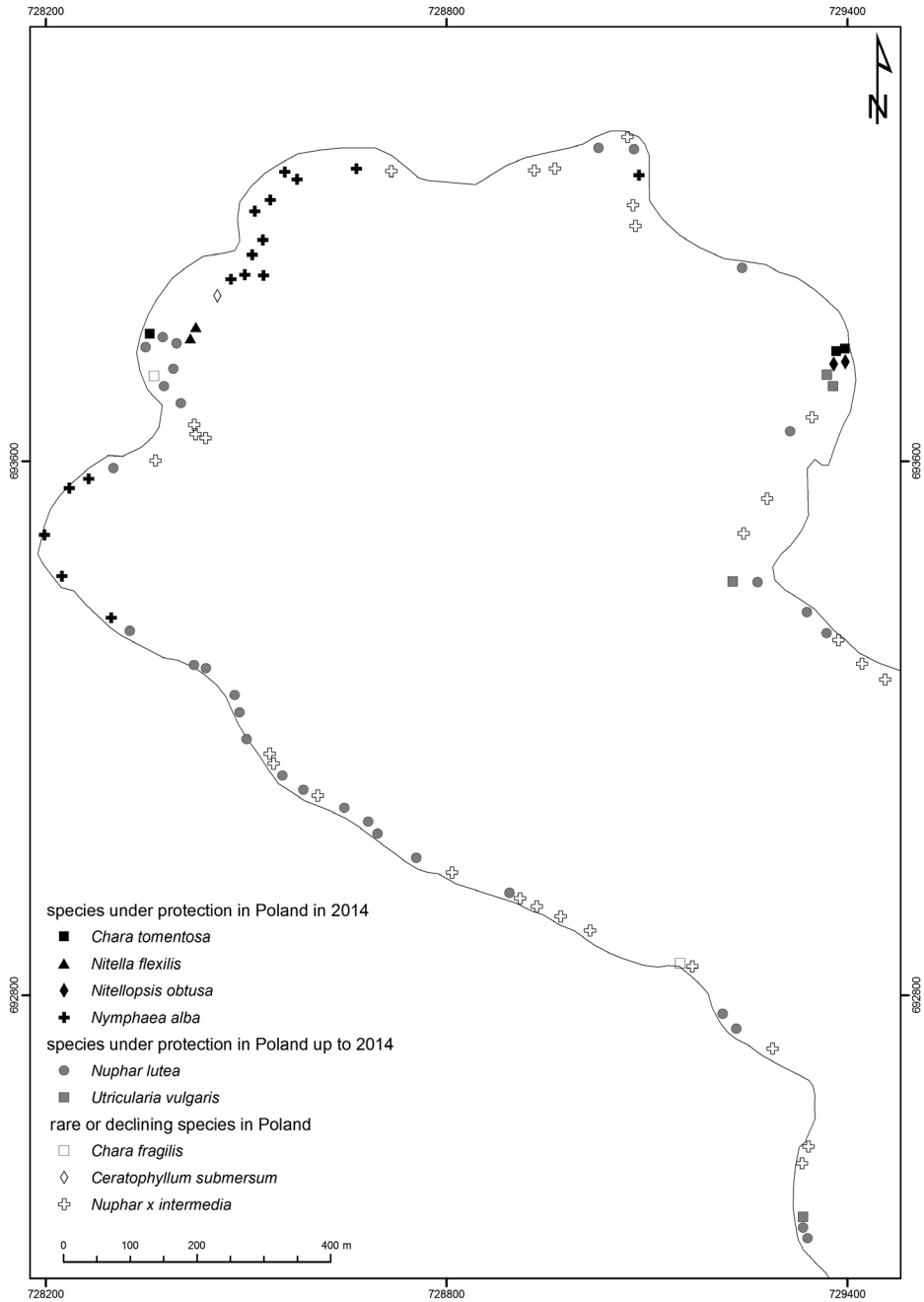

Fig. 3. Olecko Wielkie Lake, Sheet I. Map of sites of protected, rare and declining taxa in Poland

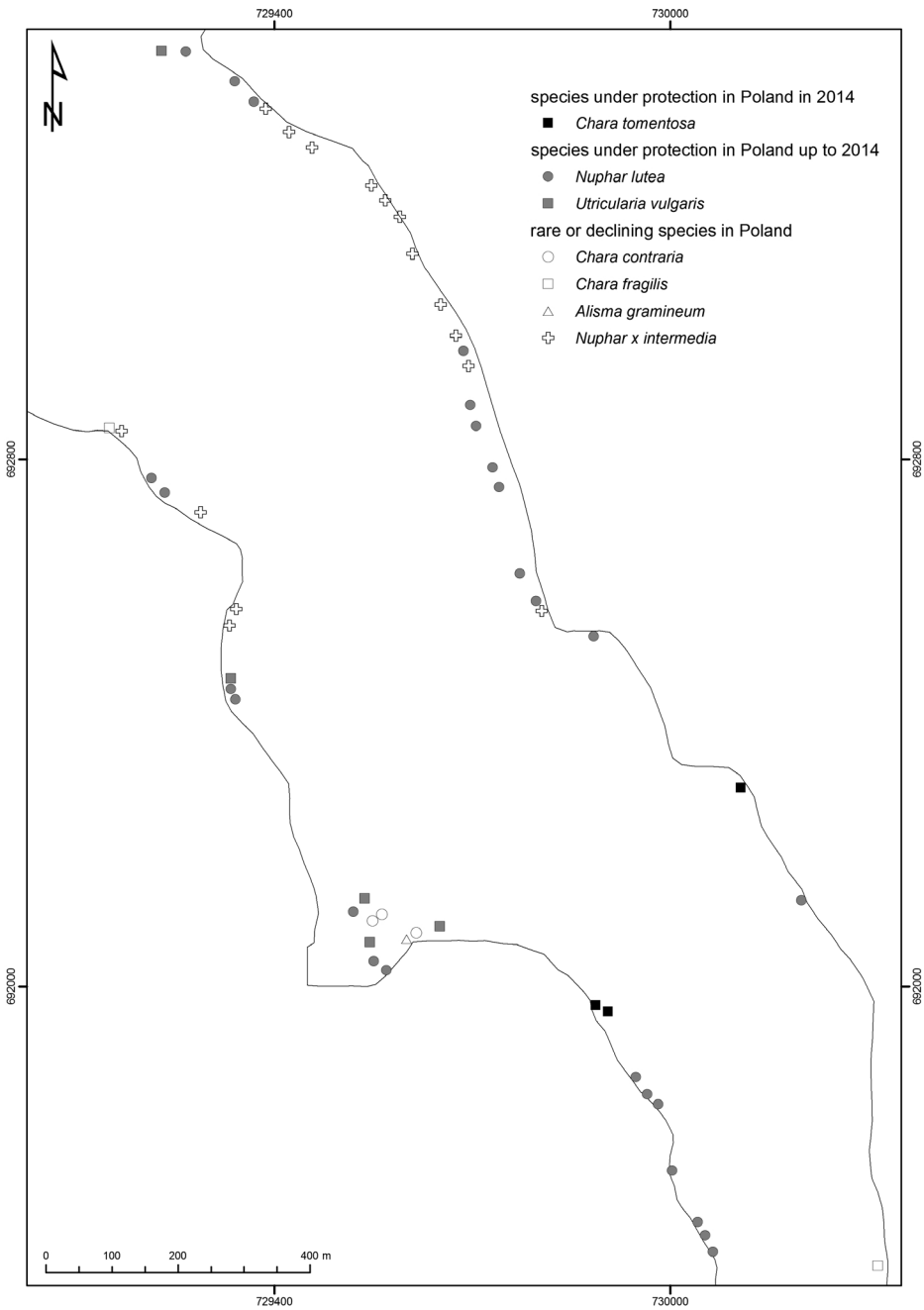

Fig. 4. Olecko Wielkie Lake, Sheet II. Map of sites of protected, rare and declining taxa in Poland

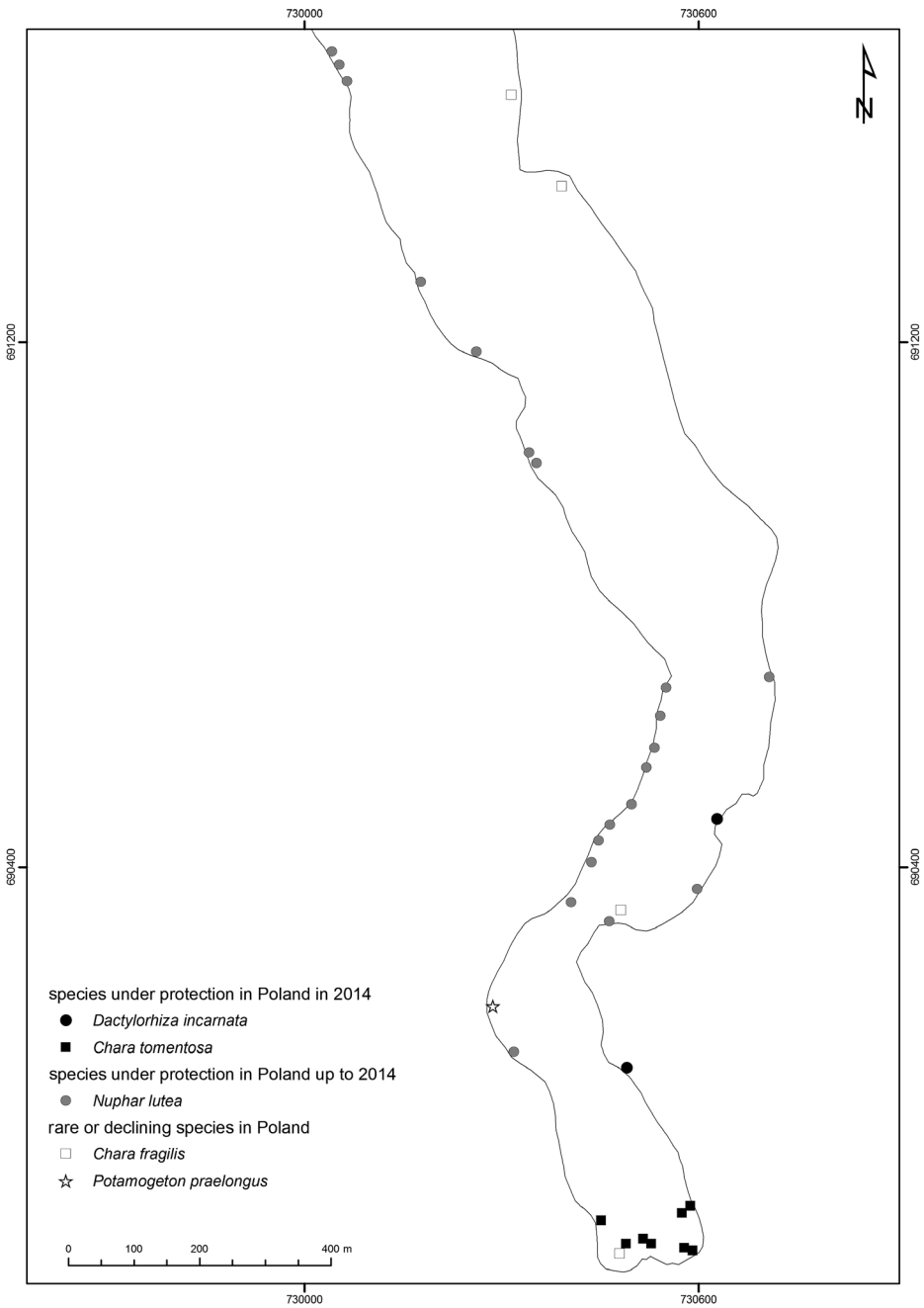

Fig. 5. Olecko Wielkie Lake, Sheet III. Map of sites of protected, rare and declining taxa in Poland

In the 1980s the recorded communities were formed by 4 charophyta species: *Chara globularis* (Thuiller), *Chara rudis* [(Braun) von Leonhardi], *Chara contraria* (Braun ex Kutzing), *Nitellopsis obtusa* [(Desvaux) Groves] (DZIEDZIC 2004a). In 2009 four charophyta species were also recorded: *Chara globularis, Chara contraria, Nitellopsis obtusa, Chara tomentosa* (L.). In 2009, the decline of communities formed by *Chara rudis* is being observed, while those formed by *Chara tomentosa* are emerging. Most likely, *Chara tomentosa* also occurred in the 1980s, but was not found due to the use of low-precision research methods. The emergence of this macroalgae may also be associated with the fact that it has a very wide ecological spectrum and can grow, unlike other *Charophyte* species found in the lake, in waters from highly eutrophic to polytrophic. Studies carried out in the 1980s revealed that all recorded *Charophyte* species formed their own communities (DZIEDZIC 2004 a). However, research form 2009 demonstrated that *Chara globularis* and *Chara contraria* no longer form their own phytocenoses but occur only as co-existing species within other plant communities. This is most likely associated with the increase in the trophic state of the lake caused by the inflow of large amounts of biogenic compounds to the catchment area and intense exploitation of the lake for recreational purposes (including power boating), but also the succession on native plant species such as *Ceratophyllum demersum* (L.) or *Myriophyllum spicatum* (L.), characteristic of eutrophic lakes (eg. ALAHUHTA et al. 2012, KOLADA et al. 2014). The wastewater treatment plant for the town of Olecko was put into operation relatively late, in 1995 (RÓŻAŃSKI et al. 2004). The decline of *Charophyte* species may also be associated with specific fishery management, e.g. the use of bottom dragging equipment (own study – unpublished).

With reference to aquatic mosses, the decline of *Eurhynchium riparioides* [(Hedw.) Richards]sites was observed. In recent years, sites of this species have not been recorded, butthedistribution range of *Fontinalis antipyretica* (L.) has clearly increased. The decline of sites was also observed for *Elodea canadensis* (Michx.), *Polygonum amphibium* f. *natans* (Michx.), *Potamogeton friesii* (Rupr.) and *Potamogeton trichoides* (Cham. et Schltdl.).

In the 1980s no sites of *Nuphar* x *intermedia* (Ledeb.) or *Nymphaea alba*(L.) were recorded, but in 2009 they form their own communities. *Ceratophyllum submersum* (L.), a species indicating improved water quality, was recorded on several sites. This ephemeral species occurs in lakes where trophic conditions have improved. However, its regression occurs after some time, until complete decline (TOMASZEWICZ 1979).

In 2009 communities formed by *Potamogeton crispus* (L.), *Potamogeton praelongus* (Wulfen), *Stratiotes aloides* (L.) and *Eleocharis acicularis* (L.) were found (not recorded previously). However, sites of *Potamogeton friesii* and *Potamogeton trichoides* have declined.

Characteristics of individual plant formations found in Olecko Wielkie Lake in 2009 are presented below.

Aquatic plants – hydrophytes

Most of the 23 aquatic taxa found in Olecko Wielkie Lake in 2009 were represented by plants rooted in the lake bottom (Table 1 – *R*), followed by semi-rooted plant species (Table 1 – *S*) and plants submerged in water or floating on its surface (Table 1 – *F*).

Helophytes

The shoreline of Olecko Wielkie Lake was a habitat for 26 helophytic species (Table 2), and 14 co-existing terrestrial plant species encroaching on the helophytic communities (Table 3).

Table 3

Plants co-existing with helophytic communities in Olecko Wielkie Lake in 2009

	Division: *Telomophyta*
	Subdivision: *Magnoliophytina* (*Angiospermae*)
	Class: *Magnoliopsida* (*Dicotyledones*)
	(1.) Family: *Cannabaceae*
1.	*Humulus lupulus* L
	(2.) Family: *Urticaceae*
2.	*Urtica dioica* L.
	(3.) Family: *Polygonaceae*
3.	*Rumex obtusifolius* L.
4.	*Polygonum amphibium* L. f. *terrestre* Leyss
	(4.) Family: *Rosaceae*
5.	*Filipendula ulmaria* (L.) Maxim.
	(5.) Family: *Lythraceae*
6.	*Lythrum salicaria* L.
	(6.) Family: *Onargaceae* (*Oenotheraceae*)
7.	*Epilobium hirsutum* L.
	(7.) Family: *Primulaceae*
8.	*Lysimachia vulgaris* L.
	(8.) Family: *Convolvulaceae*
9.	*Calystegia sepium* (L.) R. Br.
	(9.) Family: *Boraginaceae*
10.	*Symphytum officinale* L.
	(10.) Family: *Solanaceae*
11.	*Solanum dulcamara* L.
	(11.) Family: *Asteroideae* (*Tubiflorae*)
12.	*Bidens tripartita* L.
13.	*Eupatorium cannabinum* L.
	Division: *Magnoliophytina* (*Angiospermae*)
	Class: *Liliopsida* (*Monocotyledones*)
	(12.) Family: *Orchidaceae*
14.	*Dactylorhiza incarnata* (L.) Soósubsp. *incarnata*

Sites of protected species in Poland

Species under legal protection up to 2014 (under legal protection in 2009, currently not protected)

Species: *Utricularia vulgaris* (L.)
Family: *Lentibulariaceae*
Class: *Magnoliopsida* (*Dicotyledones*)

Utricularia vulgaris had been under strict protection in Poland since 2004, together with other species from the genus *Utricularia* (O.J. 2004, No. 168, item 1764). However, it is not listed in the recent Regulation of the Minister of the Environment (O.J. 2014, item 1409) on protected plant species in Poland. *U. vulgaris* is common in Poland and found mainly in nutrient-rich stagnating waters (RUTKOWSKI 2011, KŁOSOWSKI and KŁOSOWSKI 2012). The plant is carnivorous (insectivorous), like all representatives of the genus *Utricularia*. Small aquatic animals are captured by leaves transformed into bladder-like traps, closed with a trapdoor which allows entry but cannot be open from within the bladder. The bladderwort is an aquatic and marshland plant, rootless, with creeping or free floating stems. The leaves are finely innately divided, and some are transformed into bladder-like traps. The major threat to this species results from the decline of natural habitats.

In Olecko Wielkie Lake it was recorded infrequently near reeds at a depth of 1.3 m, and in *Fontinalis antipyretica* communities at a depth of 1.9 m, in phytocenoses with dominant *Potamogeton pectinatus* at a depth of 0.5 m, in patches with dominant *Potamogeton lucens* at a depth of 0.5 to 1.2 m and in communities with dominant *Batrachium circinatum* (Sibth.) at a depth of 0.7 to 1.9 m. Single specimens were found rarely at depths of 1.4 to 2.8 m. This species was found in littoral zone in the central and northern parts of the lake (Figures 2–4)

Species: *Nuphar lutea* [(L.) Sibth.]
Family: *Nymphaeaceae*
Class: *Magnoliopsida (Dicotyledones)*

Nuphar lutea had been under strict protection in Poland since 1983 (O.J. 1983, No. 27, item 134). In the Regulation of the Minister of the Environment of 2001 it was still listed as a strictly protected species (O.J. 2001, No. 106, item 1167). Since 2004 it had been under partial protection (O.J. 2004, No. 168, item 1764). However, it is not listed in the recent Regulation of the Minister of the Environment (O.J. 2014, item 1409).

Nuphar lutea is a common hydrophyte with floating leaves. It is found in natural and man-made reservoirs, and in slowly flowing rivers. It forms phytocenoses of the *Nupharo-Nymphaeetum albae* association. In the vegeta-

tion of Olecko Wielkie Lake phytocenoses dominated by *Nuphar lutea* covered 1.178 ha, versus 3.859 ha covered by all identified hydrophytic communities (DZIEDZIC and DYNOWSKI 2009). Patches of the *Nupharo-Nymphaeetum albae* association with dominant *Nuphar lutea* were found along the whole lake shoreline (Figures 2–5).

Species under legal protection according to the recent Regulation of the Minister of the Environment (O.J. 2014, item 1409)

Species: *Dactylorhiza incarnata* [(L.) Soó] subsp. *incarnata*

Family: *Orchidaceae*

Class: *Liliopsida* (*Monocotyledones*)

The family *Orchidaceae* is represented in the flora of Poland by 46 species (SZLACHETKO 2014). Plants with colourful flowers are at risk of being collected or dug out. Generally, threats to these species are associated with the transformation of natural habitats caused by human activity.

All orchid species in Poland were covered by protection relatively early, in 1983 (O.J. 1983, no. 27, item 134). Currently, *Dactylorhiza incarnata*is under partial protection (O.J. 2014, item 1409). This orchid was found on two sites near the eastern shoreline of Oleckie Wielkie Lake, in the southern part (Figure 2 and Figure 5). In Poland *Dactylorhiza incarnata* occurs in a number of varieties and subspecies. Specimens found near the lake had characteristics of a typical subspecies, *Dactylorhiza incarnata* subsp. *incarnata*, most common in the southern part of Poland (SZLACHETKO 2014).

Species: *Nymphaea alba* (L.)

Family: *Nymphaeaceae*

Class: *Magnoliopsida (Dicotyledones)*

Nymphaea alba was covered for the first time by partial protection in 1957 (O.J. 1957, No. 15, item 78). In 1995, this status was changed to strict protection, which was reflected in the regulation of 2001 (O.J. 2001, no. 106, item 1167). Since 2004 *Nymphaea alba* has been under partial protection (O.J. 2004, no. 168, item 1764, O.J. 2014, item 1409).

Nymphaea alba and *Nuphar lutea* are species characteristic of the *Nupharo-Nymphaeetum albae* association (MATUSZKIEWICZ 2012). In Olecko Wielkie Lake one large patch (0.148 ha) of the association formed by *Nymphaea alba* was found in the northern part of the lake, on the western side of the Lega river mouth (Figure 3). *Nymphaea alba* was recorded at a depth of 0.9 to 2.4 m (DZIEDZIC and DYNOWSKI 2009). Other rare and single sites of the white water lily were recorded only in the northern part of the lake, in the north-western bay, and one separate site in the north-eastern bay.

Occurrence of threatened, rare and protected charophyte species in Poland

Charophytes, known as sensitive indicators of water quality, were found in the studied lake. These plants are also attributed with habitat- and environment-forming functions, associated with the intake of biogenic compounds and water decalcification, as well as effects on other aquatic organisms (CIECIERSKA et al. 2003, GĄBKA et al. 2007, URBANIAK and GĄBKA 2014).

Species: *Chara fragilis* (Desvaux) = *Chara globularis* (Thuillier)
Family: *Characeae*
Class: *Charophyceae*

According to the Red List of Algae in Poland (SIEMIŃSKA et al. 2006) *Chara fragilis* is classified as a vulnerable species. During the carried out study no phytocenoses of *Charetum fragilis* association were found (DZIEDZIC and DYNOWSKI 2009). A few sites were recorded in the northern and southern parts of Olecko Wielkie Lake (Figures 2, 3 and 5). In the northern part of the lake *Chara fragilis* was found in a phytocenosis with dominant *Nuphar lutea* near a tributary of the Lega river, at a depth of 1.2 m, and near the western shoreline, northwards from the public beach, near a patch with dominant *Nuphar* x *intermedia* at a depth of 0.6 m. In the southern part of the lake (southern bay), *Chara fragilis* was a component of a phytocenosis with dominant *Chara tomentosa* at a depth of 1.4 m. Separate single sites were recorded near the eastern shoreline at depths of 0.7 to 0.9 m.

Species: *Chara contraria* (Braun ex Kützing)
Family: *Characeae*
Class: *Charophyceae*

Chara contraria is classified as a vulnerable species (SIEMIŃSKA et al. 2006). In Olecko Wielkie Lake it was found only in the bay near the outflow of the Lega river by the western shoreline, in the central part of the lake (Figure 2 and Figure 4). In the central part of the bay, at a depth of 2.5 m, *Chara contraria*formed a patch with the dominant *Fontinalis antipyretica*, and at a depth of 0.9 m it was found in a phytocenosis with the dominant *Potamogeton lucens*. It also grew near the southern tip of the bay, at a depth of 0.6 m, in a community with the dominant *Fontinalis antipyretica*.

Species: *Nitella flexilis* [(L.) Agardh]
Family: *Characeae*
Class: *Charophyceae*

Nitella flexilis is classified as a vulnerable species (SIEMIŃSKA et al. 2006). In Olecko Wielkie Lake it was recorded only on one site, in the northern part of the lake near a tributary of the Lega river, at depths of 1.0 and 1.2 m, in a community with the dominant *Nuphar lutea* (Figure 3). In a recent Regula-

tion of the Minister of the Environment (O.J. 2014, item 1409) *Nitella flexilis* is listed as a partly protected species.

Species: *Chara tomentosa* (L.)
Family: *Characeae*
Class: *Charophyceae*

According to the Red List of Algae in Poland (SIEMIŃSKA et al. 2006) *Chara tomentosa* is a rare species. Two patches of the *Charetum tomentosae* association were found in Olecko Wielkie Lake (DZIEDZIC and DYNOWSKI 2009). The first patch was identified in the northern part of the lake, in the eastern bay within a swimming sector (Figure 2 and Figure 3). *Chara tomentosa* formed a phytocenosis of the *Charetum tomentosae* association, 180 m^2 of surface area at depths of 0.7 to 1.4 m. In this part of the lake, near the north-western shoreline, it was found in a patch of the *Nupharo-Nymphaeetum albae* association, at a depth of 0.7 m.

Chara tomentosa also grew in the southern part of the lake (Figure 2 and Figure 5), in the southern bay. It dominated in an area of 228 m^2, at depths of 0.7 to 1.9 m. Currently, *Chara tomentosa* is under partial protection (O.J. 2014, item 1409).

Species: *Nitellopsis obtusa* (Groves)
Family: *Characeae*
Class: *Charophyceae*

Nitellopsis obtusa is a rare species (SIEMIŃSKA et al. 2006). In the northern part of the surveyed lake it formed a patch of the *Nitellopsidetum obtusae* association at a single site (Figure 2 and Figure 3). This phytocenosis was formed in the eastern bay within the swimming sector, next to a patch of the *Charetum tomentosae* association. *Nitellopsis obtusa* dominated in an area of 100 m^2 and penetrated the littoral zone at depths of 0.4 to 0.9 m. The recent Regulation of the Minister of the Environment (O.J. 2014, item 1409) established partial protection of this species.

Rare and declining species in Poland

Taxon: *Nuphar* x *intermedia* [Ledeb.] (= *N* x *spenneriana* [Gaudin])
Family: *Nymphaeaceae*
Class: *Magnoliopsida (Dicotyledones)*

Nuphar x *intermedia* is a hybrid of *Nuphar pumila* and *Nuphar lutea*, with distinct morphological features (RUTKOWSKI 2011, KŁOSOWSKI 2014). *Nuphar pumila* is a glacial relic, a rare and vulnerable species, listed in the Polish Red Data Book of Vascular Plants (KŁOSOWSKI 2014). *Nuphar lutea* is classified as

a frequent (RUTKOWSKI 2011) or common species (KŁOSOWSKI and KŁOSOWSKI 2012). The most serious threats to the sites of *Nuphar pumila* are posed by the increase in water hardness, but also *Nuphar lutea*, which as a result of cross-breeding produces hybrids and gradually becomes the dominant species (KŁOSOWSKI 2014).

In Olecko Wielkie Lake *Nuphar lutea* and *Nuphar* x *intermedia* formed distinct plant communities (DZIEDZIC and DYNOWSKI 2009). The presence of this hybrid indicates that *Nuphar pumila* occurred initially in the lake flora. *Nuphar* x *intermedia* was recorded in the northern and central parts of the lake (Figure 2–4). It formed its own community or separate single sites at depths of 0.4 to 1.8 m. In the northern part of the lake phytocenoses with dominant *Nuphar* x *intermedia* occupied mainly the western shoreline, and in the central part of the lake the northern part of the eastern shoreline.

Species: *Ceratophyllum submersum* (L.)
Family: *Ceratophyllaceae*
Class: *Magnoliopsida (Dicotyledones)*

Ceratophyllum submersum, with leaves branched dichotomously three times, is considered a rare species, unlike the common *Ceratophyllum demersum*, with leaves branched once or twice or classified as quite frequent in some regions (RUTKOWSKI 2011).

In Olecko Wielkie Lake it was recorded only at a single site, in the northern part of the lake (Figure 2 and Figure 3). *Ceratophyllum submersum*, at a depth of 1.2 m, formed a phytocenosis of the *Nupharo-Nymphaeetum albae* association with the dominant white water lily, in a patch near the mouth of the Lega river.

Species: *Alisma gramineum*(Lej.)
Family: *Alismataceae*
Class: *Liliopsida (Monocotyledones)*

Alisma gramineum, in the Polish Red List of Vascular Plants (ZARZYCKI and SZELĄG 2006), is classified as a vulnerable species.

In Olecko Wielkie Lake it was recorded only on a single site, in the central part of the lake at a depth of 0.6 m, near the southern tip closing the bay near the outflow of the Lega river (Figure 2 and Figure 4).

Species: *Potamogeton praelongus* (Wulfen)
Family: *Potamogetonaceae*
Class: *Liliopsida (Monocotyledones)*

Potamogeton praelongus is found most frequently in north-western Poland, but much less in the Masurian Lake District (ZALEWSKA-GAŁOSZ 2008). It has also been reported as a rare and declining species (RUTKOWSKI 2011).

In Olecko Wielkie Lake it was found only on a single site, in the southern part of the lake, in the bay near the western shoreline (Figure 2 and Figure 5), at a depth of 1.4 m next to a *Phragmitetum australis* association.

Changes in the flora of Olecko Wielkie Lake in 1983–2009

The first preliminary floristic surveys of Olecko Wielkie Lake were carried out in 1983 and 1986 (DZIEDZIC 2004 a, b). After over 25 years, changes in the lake flora can be identified in the case of 11 species, of which at 9 species (43% of all species) *in plus* and in 3 (11% of all species) *in minus*, with the specification of declined and vulnerable species (Table 4).

Table 4
Registry of hydrophytes species found in the years 1983–1986 and in 2009 in Olecko Wielkie Lake

Species		1983–1986		2009	
		occurence	creating community	occurence	creating community
Charophyta	*Chara globularis*	+	+	+	–
	Chara rudis	+	+	–	–
	Chara contraria	+	+	+	–
	Nitellopsis obtusa	+	+	+	+
	Chara tomentosa	–	–	+	+
Bryophyta	*Fontinalis antipyretica*	+	+	+	+
	Eurhynchium riparioides	+	+	–	–
Magnoliopsida	*Batrachium circinatum*	+	+	+	+
	Nuphar lutea	+	+	+	+
	Nuphar x *intermedia*	–	–	+	+
	Nymphaea alba	–	–	+	+
	Elodea canadensis	+	+	+	–
	Ceratophyllum demersum	+	+	+	+
	Ceratophyllum submersum	–	–	+	–
	Myriophyllum spicatum	+	+	+	+
	Polygonum amphibium f. *natans*	+	+	+	–
	Utricularia vulgaris	–	–	+	–
Liliopsida	*Potamogeton pectinatus*	+	+	+	+
	Potamogeton lucens	+	+	+	+
	Potamogeton crispus	–	–	+	+
	Potamogeton perfoliatus	+	+	+	+
	Potamogeton friesii	+	+	–	–
	Potamogeton trichoides	+	–	–	–
	Potamogeton praelongus	–	–	+	+
	Stratiotes aloides	–	–	+	+
	Lemna minor	+	–	+	–
	Lemnatrisulca	+	–	+	–
	Eleocharis acicularis	–	–	+	+
	Alisma gramineum	+	–	+	–

+ the occurrence of a species or communities, – no occurrence of a species or communities

Declined species are represented by:
Species: *Chara rudis* [(Braun) von Leonhardi]
Family: *Characeae*
Class: *Charophyceae*

Chara rudis is classified as a vulnerable species (SIEMIŃSKA et al. 2006). In the past, a single patch of plant association formed by *Charetum rudis* was found in the southern part of the lake, near the eastern shoreline, in its southern part, northwards from the southern tip. The community with the dominant *Chara rudis* covered an area of 75 m^2 at depths of 0.5 to 0.8 m (DZIEDZIC 2004 a, b). In 2009, this species has not been found. The recent Regulation of the Minister of the Environment (O.J. 2014, item 1409) established strict protection of this species.

Species: *Eurhynchium riparioides* [(Hedw.) Richards] [= *Platyhypnidium rusciforme* (Fleisch.)]
Family: *Brachytheciaceae*
Class: Bryopsida
Subdivision: *Bryophytina*

Eurhynchium riparioides is an aquatic moss that used to form a small 10 m^2 patch of the *Platyhypnidietum rusciformis* association at depths of 3.0 to 3.5 m in the northern part of the lake, near the northern shoreline, in the south-western bay (DZIEDZIC 2004 a, b). This species was not identified during our study.

Species: *Potamogeton trichoides* (Cham. et Schltdl.)
Family*: Potamogetonaceae*
Class: *Liliopsida (Monocotyledones)*

The hairlike pondweed used to form phytocenoses of three associations: *Potametum pectinati* with the dominant *Potamogeton pectinatus*; *Ranunculetum circinati* with the dominant *Batrachium circinatum,* and *Potametum perfoliati* with the dominant *Potamogeton perfoliatus*. In 2009, the patches of these associations do not include *Potamogeton trichoides* (DZIEDZIC and DYNOWSKI 2009), which is considered a rare and declining species (RUTKOWSKI 2011), with very few sites in the Masurian Lake District reported (ZALEWSKA-GAŁOSZ 2008).

Plants for which single sites were identified in Olecko Wielkie Lake are considered vulnerable species. These include *Nitella flexilis*, *Ceratophyllum submersum*, *Alisma gramineum* and *Potamogeton praelongus*.

Studies carried out in 2009 revealed the occurrence of several taxa which had not been previously reported from Olecko Wielkie Lake. These include *Chara tomentosa, Nuphar x intermediata, Nymphaea alba, Potamogeton crispus, Potamogeton praelongus, Stratiotes aloides* and *Eleocharis acicularis*.

The reason of decline of some plant species could be a change in the trophic state of the lake. It is known, after the 1950s degradation occurred in many urban lakes. This mainly resulted from the direct or indirect discharge of communal and industrial wastewater (CIECIERSKA 2000, RÓŻAŃSKI et al. 2004, LOSSOW et al. 2005, SUTELA et al. 2013, JUSIK and MACIOŁ 2014). The increasing trophic state and decreasing transparency of lake water resulted in transformation of plant communities, mainly reflected in the shrinking distribution range of hydrophytes. Within this group of plants, the share of *Potamogeton* spp. was increasing, with a decreasing share of *Characeae* species (CIECIERSKA 2000).

In the early 1950s Olecko Wielkie Lake was classified as an α-mesotrophic reservoir, with about a 20% oxygen level measured above the bottom. Water transparency, measured on 16 September 1951, was 3.5 m (OLSZEWSKI and PASCHALSKI 1959). In 1987 the water purity was between the second and third class. Water tests in 1996 revealed that the lake had water purity of the third class. In 1996, a slight deterioration in the quality of the lake water probably resulted from unfavourable weather conditions (IMIELSKI and KOZARKIEWICZ 1997). A wastewater treatment plant, put into operation in 1995, contributed to an improvement in the quality of the lake water, but further actions of public authorities with a focus on environmental policy within the catchment area of Olecko Wielkie Lake are still needed (RÓŻAŃSKI et al. 2004).

The results allow us to conclude that over the last 50 years the Olecko Wielkie Lake was converted from α-mesotrophic type to strongly eutrophic type. Increasing human pressure on the reservoir through the development of a residential and tourist infrastructure directly on the banks undoubtedly contributed to this fact. Changes in the scope of occurrence, appearance and disappearance of macrophyte species presented in this article indicates intensively occurring processes of change of trophic status of the lake water. For this reason, it is advisable to increase the frequency of the surveys the ecological status of the lake for the purposes of monitoring, using all elements of the assessment, in accordance with the Water Framework Directive. This activity will enable the identification of a number of existing threats to the lake and will allow take appropriate measures to protect this reservoir.

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IMPROVING THE STABILITY OF COLD-PRESSED OILS BY THEIR ENRICHMENT IN SEA-BUCKTHORN OIL*

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K e y w o r d s: cold pressed oils, sea-buckthorn oil terpenoids, oil stabilisation, amaranthus seed oil, pumpkin seed oil.

A b s t r a c t

Cold pressed oils from pumpkin and amaranthus seed are valued because of their health--promoting effect. Of particular importance are the contents of sterols in pumpkin seed oil and squalene in amaranthus oil, among others. Because of their high susceptibility to oxidation, methods of prolonging their shelf life are needed. One of such methods is to enrich them in antioxidants naturally occurring in plant oils.

This study analysed the opportunities to use of rich in antioxidants sea-buckthorn oil, including terpenoids, to increase the oxidation stability of cold pressed amaranthus and pumpkin seed oils.

The experiment involved blends of amaranthus and pumpkin seed oils with 0.5–12.0% of sea-buckthorn oil. In the oils and in the obtained blends, the fatty acid composition, the contents of selected terpenoid derivatives (carotenoids, tocopherols, sterols, squalene) and the oxidation stability as the induction time using a Rancimat apparatus were determined.

Sea-buckthorn oil rich in selected terpenoid derivatives proved to be effective in prolonging the shelf life of pumpkin and amaranthus seed oils, wherein the strongest relationship of oil stability indicators were observed in connection with carotenoid contents.

POPRAWA STABILNOŚCI OLEJÓW TŁOCZONYCH NA ZIMNO POPRZEZ ICH WZBOGACANIE OLEJEM ROKITNIKOWYM

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S ł o w a k l u c z o w e: oleje tłoczone na zimno, terpenoidy oleju rokitnikowego, stabilizacja oleju, olej amarantusowy, olej dyniowy.

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Abstrakt

Tłoczone na zimno oleje dyniowy i amarantusowy cenione są ze względu na ich oddziaływanie prozdrowotne. Szczególne znaczenie ma zawartość steroli w oleju dyniowym oraz skwalenu w oleju amarantusowym. Z uwagi na ich wysoką podatność na utlenianie poszukiwane są metody przedłużania ich trwałości. Jednym z takich sposobów jest wzbogacenie ich w przeciwutleniacze naturalnie występujące w olejach roślinnych.

Za cel pracy postawiono określenie możliwości wykorzystania oleju rokitnikowego bogatego w antyoksydanty, m.in. terpenoidowe, do zwiększenia stabilności oksydacyjnej tłoczonych na zimno olejów amarantusowego i dyniowego.

W doświadczeniu sporządzono blendy olejów amarantusowego i dyniowego z olejem rokitnikowym w ilości 0,5–12,0%. W olejach i otrzymanych blendach określono skład kwasów tłuszczowych, zawartość wybranych pochodnych terpenoidowych (karotenoidów, tokoferoli, steroli, skwalenu) oraz stabilność oksydacyjną wyznaczaną jako czas indukcji w aparacie Rancimat.

Olej rokitnikowy bogaty w wybrane pochodne terpenoidowe okazał się skuteczny w przedłużaniu trwałości olejów dyniowego i amarantusowego, przy czym najsilniejszą zależność wskaźników stabilności olejów obserwowano w powiązaniu z zawartością karotenoidów.

Introduction

Plant oils in the human diet constitute a source of essential unsaturated fatty acids. The property distinguishing them from other fats is their high content of polyunsaturated fatty acids. In the contemporary diet of highly developed countries, improper diet balancing is often observed in the proportion of omega-3 and omega-6 fatty acids, which is an identified risk factor for the occurrence of so-called "diseases of affluence" (SIMOPOULOS 2001, SIMOPOULOS 2002, MOZAFFARIAN et al. 2005). Supplementation of the diet using appropriate plant oils is recommended in the prevention of these diseases. In the oils, non-glycerol components are also present, including terpene derivatives characterised by biological activity in the body, as well as by antioxidant activity (KELLY 1999, QUILES et al. 1999, BERG et al. 2000, BERGER et al. 2004, TANG et al. 2005, GUPTA et al. 2011). Antioxidant properties of tocopherols have long been used for the preservation of fat products. Squalene and carotenoids, among others, are also known for their ability to inhibit the oxidation of lipids (MAŁECKA 1994, MUELLER and BOEHM 2011). The biological function of the natural components is also significant, and introducing plant terpenoids (carotenoids, phytosterols, squalene, tocopherols) into fat products could maintain the health safety of food. Cold pressed amaranthus seed oil is a rich source of tocopherols, squalene and phytosterols. However, despite the fact that some of them have potential antioxidant activity, it does not protect the oil from oxidation changes. Similarly, pumpkin seed oil, in spite of certain amounts of squalene, sterols and tocopherols, undergoes the action of antioxidant factors. Plant oils with a high percentage of polyunsaturated fatty acids are particularly susceptible to

oxidation changes. Using synthetically obtained antioxidants for preservation of the plant oils helps to prevent and inhibit on-going oxidation changes. However, there are doubts raised by contradictory studies indicating both the harmful effect of these compounds and a lack of such effect on human health. Both opinions seem to be correct, since the activity of these compounds depends on the ingested dose. This dose, in turn, may be too high considering the multitude of products preserved with synthetic antioxidants (WILLIAMS et al. 1999, SARAFIAN et al. 2002, SOUBRA et al. 2007, GULTEKIN and DOGUC 2013). Introducing natural antioxidants into the products would increase their shelf life while maintaining their natural character. Methods of preventing oxidation of lipids in exactly such a way have long been sought. In many studies, the source of antioxidants are often, for example, plant extracts rich in natural antioxidants (ECONOMOU et al. 1991). Sea-buckthorn oil used in the work is valued because of its broad spectrum of biological activity (SURYAKUMAR and GUPTA 2011). It is particularly rich source of α-tocopherol and β-carotene which have documented antioxidant properties (SIES and STAHL 1995, GOULSON and WARTHESEN 1999).

For this reason, this study sought to assess the opportunities to use rich in terpenoid derivatives sea-buckthorn oil as a factor increasing the oxidation stability of cold pressed amaranthus and pumpkin seed oils.

Material and Methods

The pumpkin seed oil, amaranthus seed oil and sea-buckthorn fruit oil were used in this study. The seeds (cleaned, without foreign odour, moisture content not more than 8%) and fruits (harvested at the stage of full ripeness, without foreign odour) were purchased from "Szarłat" company (Łomża, Poland). Oils from seeds were obtained by cold pressing (temperature < 45°C) the raw material on a IBG Monforts & Reiners, Komet CA59G (Germany) laboratory expeller equipped with a 4 mm diameter nozzle and purified by centrifugation at 8000 x g on a Eppendorf centrifuge (type 5810R, Eppendorf AG, Hamburg, Germany). Sea-buckthorn fruit oil were obtained from lyophilised oleosomes (isolated from fruit juice) by hexane extraction.

Blends used in experiment were prepared triplicate by 0.5, 1.0, 2.0, 4.0, 8.0, 12.0% of sea-buckthorn oil addition to analysed pumpkin and amaranthus seeds oils.

In stabilised oils initial state of their rancidity were analysed. The acid (AV), peroxide (PV), and *p*-anisidine (*p*-AV) values were determined in accordance with procedures of EN ISO 660:2009 (CEN 2009), EN ISO 3960:2012 (CEN 2012), and EN ISO 6885:2008 (CEN 2008), respectively.

Fatty acids derivatization was done according to method described by Zadernowski and Sosulski (1978). Methylated fatty acids were analysed by gas chromatography with a GC-MS QP2010 PLUS (Shimadzu, Japan) system. Separationı was performed on a BPX70 (25 m x 0.22 mm x 0.25 μm) capillary column (SGE Analytical Science, Victoria, Australia) with helium as the carrier gas at a flow rate of 0.9 mL/min. The column temperature was programmed as follows: a subsequent increase from 150°C to 180°C at the rate of 10°C/min, to 185°C at the rate of 1.5°C/min, to 250°C at the rate of 30°C/min, and then 10 min hold. The interface temperature of GC-MS was set at 240°C. The temperature of the ion source was 240°C and the electron energy 70 eV. The total ion current (TIC) mode was used in 50–500 m/z range. Obtained results of fatty acids composition were used to oxidation index (U) calculation according to formula given by COSGROVE et al. (1987): $U = (0.02 \cdot (C_{16:1} + C_{18:1}) + 1 \cdot C_{18:2})/100$.

Carotenoids in oils were analysed with a reversed phase high performance liquid chromatography (RP-HPLC) technique according to method previously described by CZAPLICKI et al. (2016). Carotenoids separation was performed at 30°C on a YMC-C_{30} 150 x 4.6 mm, 5 μm column (YMC-Europe GmbH, Germany) with the use a 1200 series liquid chromatograph manufactured by Agilent Technologies (Palo Alto, CA, USA), equipped with a diode array detector (DAD). Gradient of methanol – methyl tert-butyl ether (MTBE) was used as a mobile phase. Carotenoids were identified based on retention times and by comparing the UV–Visible absorption spectra of available standards (Sigma-Aldrich, USA). For quantitative analysis of carotenoids to the oil samples internal standard of β-Apo-8'-carotenal was added.

The content of sterols in oils was determined by gas chromatography coupled with mass spectrometry (GC-MS QP2010 PLUS, Shimadzu, Japan) according to the method previously described by CZAPLICKI et. al (2011). The sample was saponified by adding a 0.5 mL 2M NaOH methanolic solution at ambient temperature for 2 hours. Unsaponifiables were extracted with diethyl ether which was evaporated under nitrogen conditions. The dry residues were re-dissolved in 1.5 mL of n-hexane and a 0.2 mL 5α-cholestane internal standard solution was added (0.4 mg/g). After evaporation, the residues were re-dissolved in 100 μL of pyridine and 100 μL BSTFA (N,O-bis (trimethylsilyl) trifluoroacetamide) with 1% TMCS (trimethylchlorosilane) and left in 60°C for 60 minutes to complete derivatization. One mL of hexane was then added to the sample and 1 μL of the obtained mixture was analysed. After silylation sterols were separated on ZB-5MSi (Phenomenex Inc., Torrance, CA, USA) capillary column. The quantifications using the internal standard method was done with the use of total ion current (TIC) mode at 100–600 m/z range.

The tocopherols analysis was carried out by high performance liquid chromatography (HPLC), according to the method described by CZAPLICKI et

al. (2011). The analysis was performed using a 1200 series liquid chromatograph manufactured by Agilent Technologies (Palo Alto, CA, USA), equipped with a fluorescence detector. The separation was done on a Merck LiChrospher Si 60 column, 250 mm x 4 mm, 5 μm. A 0.7% isopropanol solution in hexane at a 1 mL/min flow rate was used as the mobile phase. The fluorescence detector was set at 296 nm for excitation and 330 nm for emission. Peaks were identified on the basis of retention times determined for α-, β-, γ- and δ-tocopherol standards (Merck, Darmstadt, Germany) separately, and their content was calculated using external calibration curves.

Induction time of oils was measured on a Rancimat apparatus 743 (Metrohm, Herisau, Switzerland). The analysis was performed according to method described by FARHOOSH (2007). Determination of the induction time was based on the conductometric detection of volatile oxidation products. The time that elapsed until these oxidation products appeared was saved as the induction time.

Statistical analysis

The results of all analysis performed in triplicate were statistically analysed using Statistica 12.0 PL software (StatSoft Inc., Kraków, Poland). In order to indicate the significance of differences between oil samples, unvaried analysis of variance (ANOVA) with a Duncan test at $p \leq 0.05$ significance level was used. In order to develop the prediction model for oxidative stability of oils the linear regression and coefficient of determination (R^2) were estimated for each bioactive compound.

Results and Discussion

This study assessed the opportunities to use of sea-buckthorn oil, which is rich in bioactive terpenoid derivatives (α-tocopherol, carotenoids), to preserve cold pressed oils. In the experiment, freshly-pressed oils from amaranthus and pumpkin seeds were used. The oils had low acid values (AV) defining the degree of hydrolysis of oils, which for amaranthus and pumpkin seed oils were 2.33 and 1.88 mg KOH $\cdot$ g^{-1} of oil, respectively. High acid value (6.06 mg KOH $\cdot$ g^{-1}) in case of sea-buckthorn fruit oil was connected with very high organic acids concentration in fruits used to oil production. All used oils were characterised by a low degree of oxidation, indicated by low peroxide (PV) and anisidine values (p-AV). In this case, lower values were observed for amaranthus seed oil (PV = 0.17 mEq $O_2 \cdot kg^{-1}$ of oil; p-AV = 0.25). Pumpkin seed oil, despite having

slightly higher oxidation values, met the requirements of the Codex Alimentarius Commission standard for cold-pressed and virgin oils, determined as 4 mg KOH g^{-1}, and 15 mEq O_2 kg^{-1} of oil, respectively (Codex Alimentarius Commission 2001).

Table 1
Rancidity indices of amaranthus and pumpkin seed oils before enrichment with sea-buckthorn oil

Specification	Acid value [mg KOH g^{-1}]		Peroxide value [mEq O_2 kg^{-1}]		Anisidine value [–]	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Amaranthus seed oil	2.33	0.04	0.20	0.06	0.86	0.08
Pumpkin seed oil	1.88	0.02	1.96	0.27	2.95	0.68
Sea-buckthorn fruit oil	6.06	0.01	0.17	0.01	0.25	0.02

$\bar{x}$ – mean value, SD – standard deviation, $n = 3$

In oils, the most valuable are their unsaponifiable fraction components (carotenoids, squalene, sterols and tocopherols). There is many reports recommended their consumption in prevention of many diseases (TUCKER and TOWNSEND 2005, DEVARAJ and JIALAL 2006, FARVIN et al. 2006, GRATTAN 2013). Due to the oxidation of oils ingredients their value is lost during the storage. The fatty acid composition has a great effect on the susceptibility of oils to oxidation. In the discussed oils, a large proportion of fatty acids were unsaturated acids (about 70%). The acid composition of pumpkin seed oil suggests its greater susceptibility to oxidation. Its fatty acids are about 55% linoleic acid, which is about twice as susceptible to oxidation as monounsaturated oleic acid (COSGROVE et al. 1987), whose proportion in this oil constituted nearly 18%. Amaranthus seed oil had a slightly lower proportion of unsaturated acids, but the proportions of oleic acid (27%) and of linoleic acid (40%) were observed to be more favourable in these terms. The proportion of unsaturated acids in oil from sea-buckthorn fruit reached over 60%, but linoleic acid constituted only 12%. Such a proportion of fatty acids has an effect on the susceptibility of oils to oxidation changes. The oxidation index computed based on the relation formulated by COSGROVE et al. (1987) for amaranthus seed oil and pumpkin seed oil reached the values of 0.41 and 0.56, while the value computed for sea-buckthorn oil was only 0.16 (Table 2). This indicates that using sea-buckthorn oil as a source of natural antioxidants will also have a preserving effect on oils, by decreasing the value of the oxidation index.

Table 2

Fatty acids composition, oxidation indices and main bioactive compounds content in oils

Compound/discriminant	Amaranthus seed oil		Pumpkin seed oil		* Sea buckthorn fruit oil	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Fatty acids [%]						
palmitic	27.08^{a}	0.57	19.65^{b}	3.13	36.31^{c}	0.01
palmitoleic	nd^{a}		nd^{a}		40.97^{b}	0.04
stearic	5.01^{a}	0.07	7.43^{b}	1.01	0.55^{c}	0.01
oleic	27.27^{a}	0.21	17.73^{b}	0.15	8.77^{c}	0.11
linoleic	40.65^{a}	0.71	55.20^{b}	1.41	12.12^{c}	0.13
Oxidation index [–]	0.41^{a}	0.01	0.56^{b}	0.04	0.16^{c}	0.00
Carotenoids [mg · 100 g^{-1}]						
lutein	0.13^{a}	0.04	0.50^{b}	0.09	3.24^{c}	0.49
all-trans β-carotene	0.07^{a}	0.09	0.40^{b}	0.03	118.36^{c}	9.58
other carotenoids	0.04^{a}	0.01	0.26^{b}	0.02	74.82^{c}	6.25
total carotenoids	0.24^{a}	0.07	1.16^{a}	0.11	206.04^{b}	15.63
Tocopherols [mg · 100 g^{-1}]						
α-tocopherol	26.80^{a}	2.04	11.40^{b}	0.38	144.14^{c}	4.10
β-tocopherol	25.18^{a}	1.90	4.00^{b}	0.00	3.98^{c}	0.23
γ-tocopherol	9.20^{a}	0.83	49.69^{b}	0.21	4.63^{c}	0.32
δ-tocopherol	9.67^{a}	0.82	nd^{b}		0.75^{c}	0.00
total tocopherols	70.86^{a}	2.50	65.09^{b}	1.87	153.50^{c}	4.10
Sterols [mg · 100 g^{-1}]						
campesterol	18.41^{a}	0.52	nd^{b}		7.87^{c}	0.45
Δ5-avenasterol	251.14^{a}	1.68	nd^{b}		20.46^{c}	1.54
β-sitosterol	377.17^{a}	9.46	71.22^{b}	3.47	536.30^{c}	1.25
Δ7-stigmastenol	319.93^{a}	3.84	9.40^{b}	0.56	nd^{c}	
Δ7-stigmasterol	210.62^{a}	13.29	nd^{b}		nd^{b}	
Δ7-avenasterol	49.76^{a}	3.57	3.39^{b}	0.16	nd^{c}	
other sterols	71.93^{a}	2.02	53.42^{b}	8.26	262.81^{c}	16.61
total sterols	1299.0^{a}	0.12	137.4^{b}	0.68	855.9^{d}	8.95
Squalene [mg · 100 g^{-1}]	2560.8^{a}	358.65	310.6^{b}	12.5	nd^{c}	
Induction time [h]	4.46^{a}	0.06	7.50^{b}	0.32	>48^{c}	

* Sea buckthorn fruit oil characteristic data was published in work of CZAPLICKI et al. (2016)

$\bar{x}$ – mean value, SD – standard deviation, $n = 9$

Means in the same line with difierent letters are significantly difierent (P=0.05).

nd – not detected

The chemical characteristics of the oils proved that the preserved oils were poor in carotenoids (Table 2), with the dominant carotenoid being lutein – whose content was 0.13 and 0.50 mg · 100 g^{-1} in amaranthus and in pumpkin seed oils, respectively. Sea-buckthorn oil is an unusually valuable source of these components. The total carotenoid content of this oil was 206 mg · 100 g^{-1}, of which over 50% was β-carotene.

For tocopherols, the observed differences were not significant (Table 2). The content of these components in amaranthus and pumpkin seed oils was about 71 and 65 mg · 100 g^{-1}, respectively, and in amaranthus seed oil α- and

β-tocopherol dominated and their contents were comparable (about 25–27 mg · 100 g^{-1}). Tocopherols of pumpkin seed oil are 76% γ homologue, and the remaining 17.5% and about 6% are α- and β-tocopherol, respectively. Taking into account the antioxidant activity of tocopherols (MADHAVI et al. 1995), their favourable proportions can be observed in sea-buckthorn oil, in which nearly 94% constitutes α-tocopherol. The content of this homologue reached 144 mg · 100 g^{-1} in sea-buckthorn oil, which is nearly 13-fold higher than in pumpkin seed oil.

In terms of phytosterol contents, sea-buckthorn oil also exceeded pumpkin seed oil (Table 2). In both oils, the dominant compound was β-sitosterol, but in pumpkin seed oil its content was about 7.5-fold lower (71.22 mg · 100 g^{-1}). It was different for amaranthus seed oil, whose dominant sterol was β-sitosterol (about 320 mg · 100 g^{-1}), but its content was close to the contents of Δ7-stigmastenol and Δ5-avenasterol, and somewhat higher than of Δ7-stigmasterol. Although the content of the dominant β-sitosterol in sea-buckthorn oil was about 1.4-fold higher, in terms of the total sterol content, amaranthus seed oil was 1.5 times richer. Analysing the effect of an addition of sea-buckthorn oil on the phytosterols content in the obtained blend, it was found that it resulted in an increase in the total sterol content and in the β-sitosterol content in the composition with pumpkin seed oil, but it had a negative effect on the sterol content in the enriched amaranthus seed oil.

The situation was similar for squalene, which was not found in sea-buckthorn fruit oil (Table 2). Pumpkin seed oil was characterised by a squalene content in the amount of 310.6 mg · 100 g^{-1}, which is a high content among plant oils. Amaranthus seed oil, in turn, regardless of the method it is obtained with, is the richest plant source of squalene (CZAPLICKI et al. 2012). The squalene content in amaranthus seed oil is about 2,560 mg · 100 g^{-1} and, as in the case of pumpkin seed oil, an addition of sea-buckthorn oil lowered the squalene content in the product.

Taking into account the contents of the analysed bioactive substances and fatty acids composition, it is not surprising that the oxidation stability of sea-buckthorn oil was the highest (Table 2). The induction time for this oil exceeded 48 hours. The measurement results of the induction time of the other oils indicated amaranthus seed oil to be more susceptible to oxidation (4.46 h). The induction time determined for pumpkin seed oil was close to the times observed for cold pressed rapeseed oil (ROSZKOWSKA et al. 2015). Both of the studied oils proved to be far less stable than sea-buckthorn oil. BHATNAGAR et al. (2009) and HAMED and ABO-ELWAFA (2012) described the preservation of plant oils by mixing them with other, oxidatively-stable. In these studies, the authors preserved oils by introducing, e.g. the natural antioxidant of sesame oil (sesamin). However, they also considered the importance of the oxidative

stabilisation of oil by changing its fatty acid composition. Increasing the proportion of saturated fatty acids also resulted in an increase in the oxidation stability of the blend.

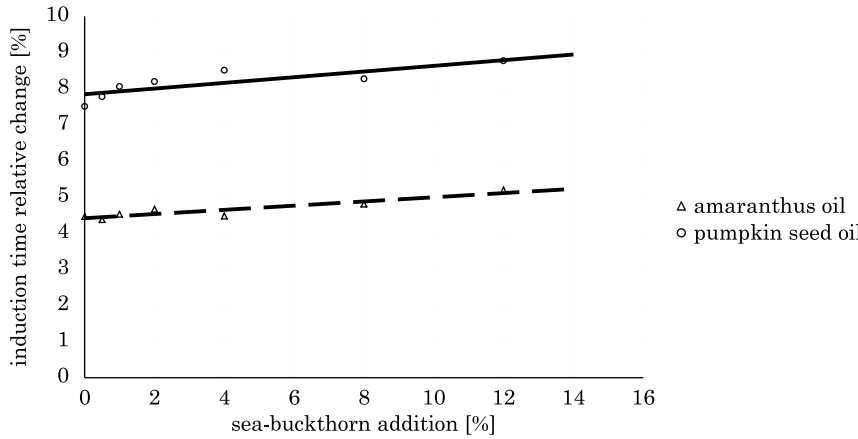

Fig. 1. Relation observed for induction time relative changes and percentage of sea-buckthorn oil addition in enriched oils

Table 3
The relation between the relative change in the induction time and the amount of the sea-buckthorn oil addition

	Linear regression equation	R^2	Induction time increase [min · 1%$^{-1}$ of sea-buckthorn fruit oil]
Amaranthus seed oil	$y = 1.2171x + 0.4102$	0.94	3.65
Pumpkin seed oil	$y = 1.0448x + 4.2568$	0.74	6.30

R^2 – regression coefficient, $n = 9$

Figure 1. presents the relation which was observed following analysis of enriched amaranthus and pumpkin seed oils. An addition of sea-buckthorn oil resulted in positive changes in the induction time of the studied oils. In the case of amaranthus seed oil, a slightly lower slope of the curve was observed compared to that obtained for pumpkin seed oil. In order to objectively compare the preservation efficiency of both oils, regression equations were determined for the relation between the relative change in the induction time and the percentage of the addition of sea-buckthorn oil. For pumpkin seed oil, the equation was characterised by a 10-fold higher value of the shift coefficient (Table 3). This suggests that the efficiency of the use of an addition of sea-buckthorn oil as a source of antioxidants is much higher for pumpkin seed

oil than for amaranthus seed oil, even for the lowest of the applied concentrations of sea-buckthorn oil. The induction times for the oils with enrichment levels from 0.5 to 12% increase gradually, and at the maximal addition they already assume similar values of about 16.5%. This represents a 50% increase in the stability obtained by GÁMEZ-MEZA et al. (1999) who, in order to preserve soy oil, used a 0.02% addition of butylated hydroxyanisole (BHA). The same concentration of tertiary butyl hydroquinone (TBHQ) used in their experiment resulted in a nearly three-and-a-half-fold increase in the induction time value. Numerous reports emphasise the efficiency of synthetic antioxidants in the preservation of plant oils (KHAN and SHAHIDI 2001, AZEEZ et al. 2013).

An increase in the induction time for the enriched oils, which was determined as an increase in the number of minutes as a result of the addition of each percent of the added sea-buckthorn oil is presented in Table 3. For amaranthus seed oil, this value reached 3.65 min · $1\%^{-1}$ and it was almost twice lower than for pumpkin seed oil (6.3 min · $1\%^{-1}$). These observations indicated that the addition of sea-buckthorn oil results in a change in the fatty acids composition and the contents of substances dissolved therein. In order to determine the effect of the amount of an addition of particular bioactive components of the studied oils on the stability of the obtained blends, the relations were determined between the contents of the components and the induction times determined for the mixtures.

Figure 2 presents the relations observed between the determined induction times of the studied oils enriched to a different degree with α-tocopherol, β-carotene and β-sitosterol, with a decrease in the squalene content. The β-carotene content in amaranthus seed oil with a 12% addition of sea-buckthorn oil increased to almost 13 mg · 100 g^{-1}, which is a value nearly 66-fold higher than the initial value. At the same time, a 66% increase was observed in the α-tocopherol content and a 12% increase in the β-sitosterol content. The content of squalene of which this oil is a rich source decreased by about 103 mg · 100 g^{-1} of oil. It is only a four percent decrease in the squalene content.

The obtained data were analysed by determination of the relation between the contents of particular antioxidants and the induction times of the mixtures (Table 4). It was found in amaranthus seed oil that lengthening the induction time by 0.73 hours had the greatest effect on the β-carotene content. This can be explained by the fact that the percent change in the β-carotene content was greatest. The literature emphasises the antioxidant properties of both β-carotene and of its metabolites (MUELLER and BOEHM 2011).

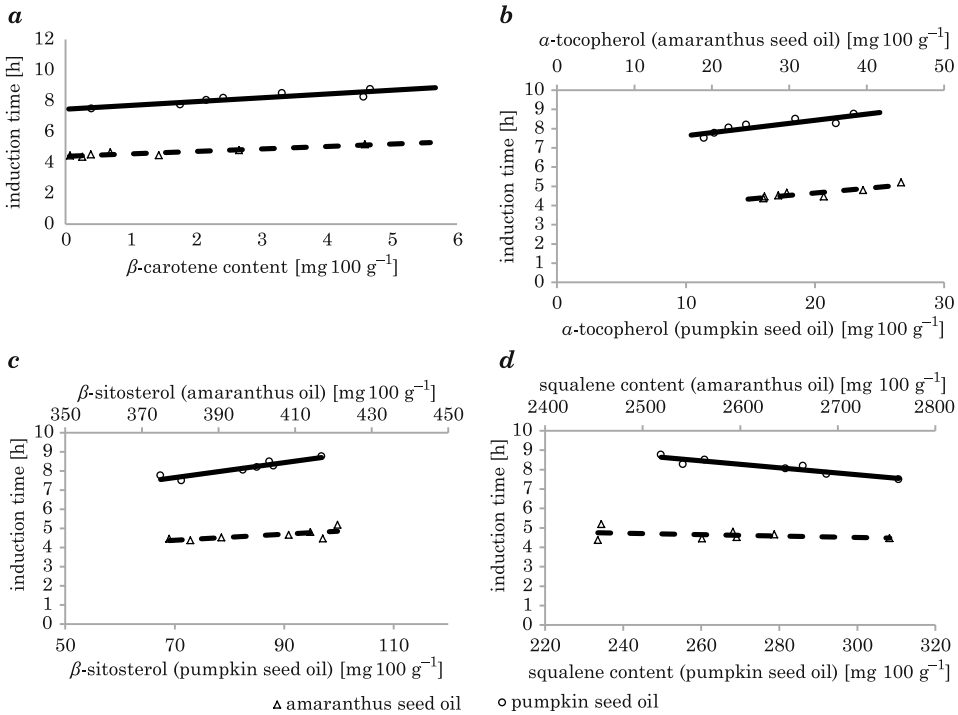

Fig. 2. Relations observed for induction time and bioactive compounds content in enriched oils

Table 4

Linear regression equations and determination coefficients (R^2) for the relation between the bioactive compound concentration and enriched oils induction time

Bioactive compound	Amaranthus seed oil		Pumpkin seed oil	
	linear regression equation	R^2	linear regression equation	R^2
β-carotene	$y = 0.2478x + 7.4655$	0.81	$y = 0.1594x + 4.4088$	0.87
α-tocopherol	$y = 0.0364x + 3.4403$	0.79	$y = 0.0802x + 6.8343$	0.77
β-sitosterol	$y = 0.0111x + 0.1944$	0.49	$y = 0.0391x + 4.9181$	0.87
Squalene	$y = -0.0179x + 13.105$	0.87	$y = -0.0009x + 6.9575$	0.11

R^2 – regression coefficient, $n = 9$

In pumpkin seed oil, together with an over 11-fold increase in the β-carotene content, an increase in the induction time by 1.26 hours was observed. This change was also connected with relatively smaller changes in the contents of other antioxidants. The β-sitosterol content increased by about 36% and the α-tocopherol content increased as much as 2-fold. At the same time, the content of squalene, as a result of its lack in sea-buckthorn oil,

decreased by 20%. Analysing the information presented in Table 4, it is possible to find that the change in the squalene content had the least effect on the oxidation stability of pumpkin seed oil.

As presented in Figure 3, preservation of amaranthus seed oil with sea-buckthorn oil results in a change in its natural colour. The high carotenoid content in sea-buckthorn oil is the reason that its 4% addition is noticeable and its 8–12% proportion may be a reason for its lack of acceptance among consumers. However, this colouration does not have to be perceived as a flaw. Cold pressed pumpkin seed oil also has intense colouration, which is not an obstacle in its wide use in gastronomy and as a health-promoting dietary supplement. What is important is the benefit resulting from increasing the shelf life of oils thanks to the use of natural antioxidants of sea-buckthorn oil. In both preserved oils, supplementation with sea-buckthorn oil at the level of 12% resulted in a 16% increase in the stability of oils measured by the induction time. The literature also describes the use of natural antioxidants of oils for stabilisation during frying. Lavender and thyme herbs also have a positive effect on the stabilisation of sunflower oil. In sunflower oil, by reacting with free radicals created under the effect of heating, antioxidants of the herbs may prevent degradation of tocopherols (BENSMIRA et al. 2007). A similar antioxidant effect was found by the use of cassia essential oil used as cooking oil and its optimum content was 0.012% (DU and LI 2008).

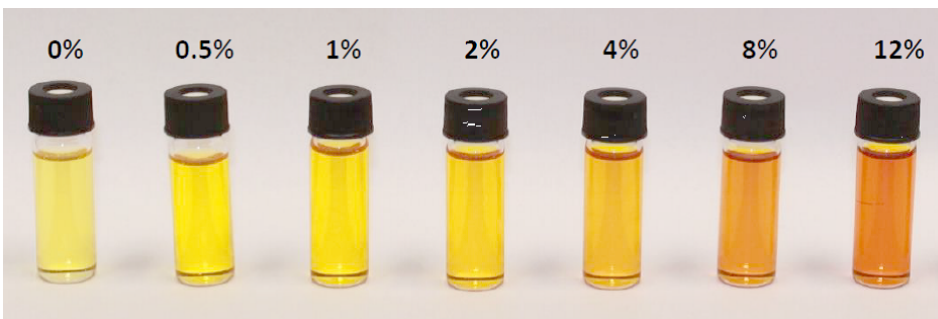

Fig. 3. The amaranthus oil with varying degree of sea-buckthorn oil enrichment

Conclusions

The results of the presented studies confirm the effectiveness of an addition of sea-buckthorn oil as a source of natural antioxidants, especially α-tocopherol and carotenoids, to improve the oxidation stability of cold pressed amaranthus and pumpkin seed oils. Supplementation with sea-buckthorn oil

has the greatest effect on changes in the contents of carotenoids. The data indicate greater dependence between these changes and the oxidation stability of the preserved oils than for tocopherols.

The results indicate that on the obtained blended stability has a greater influence natural antioxidants concentration than fatty acid composition. This thesis is confirmed by the enriched amaranthus and pumpkin seed oil "oxidation index" and "induction time" values relation. Pumpkin seed oil significantly exceeded amaranthus seed oil in its carotenoid content, and was richer in polyunsaturated fatty acids.

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CRITICAL TRACEABILITY POINTS IN A MASS CATERING – A PRACTICAL APPROACH

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K e y w o r d s: food traceability, CTP, critical traceability points, catering, mapping of traceability.

A b s t r a c t

In view of the very broad scope of purchased raw materials and produced meals, mass catering facilities belong to those food chain links, in which product traceability is very limited, and the available literature does not provide information concerning the traceability of materials used for producing meals. The study involved development of maps showing the material and information flow for catering processes carried out in a mass catering facility of a closed type, thus making it possible to identify critical traceability points (CTP). The results obtained proved that the catering process was characterised by numerous CTPs and an analysis provided a basis to establish corrective actions, enabling improvements of the traceability system under analysis. As a result of those measures, a system of labelling material and meal batches was introduced, together with ID cards and registers, which led to the elimination of the majority of CTPs identified. The result of the research can significantly improve the area of traceability in food safety management systems used in mass catering facilities.

KRYTYCZNE PUNKTY IDENTYFIKOWALNOŚCI W ŻYWIENIU ZBIOROWYM – PODJEŚCIE PRAKTYCZNE

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S ł o w a k l u c z o w e: identyfikowalność żywności, CTP, krytyczne punkty identyfikowalności, żywienie zbiorowe, mapowanie identyfikowalności.

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Abstrakt

Ze względu na szeroki zakres zakupowanych surowców oraz wytwarzanych potraw zakłady żywienia zbiorowego należą do tych ogniw łańcucha żywnościowego, w których identyfikowalność wyrobów jest bardzo ograniczona, a w dostępnej literaturze brakuje informacji dotyczących identyfikowalności materiałów stosowanych do wytwarzania potraw. W badaniach opracowano mapy przepływu materiałów i informacji dla procesów gastronomicznych realizowanych w zakładzie żywienia zbiorowego typu zamkniętego, które umożliwiły zidentyfikowanie krytycznych punktów identyfikowalności (CTP). W badaniach ujawniono, że proces gastronomiczny charakteryzuje się licznymi CTP, których analiza stanowiła podstawę ustanowienia działań korygujących umożliwiających udoskonalenie analizowanego systemu identyfikowalności. W wyniku tych działań wprowadzono system znakowania partii materiałów i potraw, karty ID oraz rejestry, które umożliwiły eliminację większości zidentyfikowanych CTP. Wyniki pracy mogą znacząco usprawnić obszar identyfikowalności w systemach zarządzania bezpieczeństwem żywności funkcjonujących w zakładach żywienia zbiorowego.

Introduction

Traceability, understood as the ability to track the history, use or location of the analysed subject (*Quality management systems*... ISO 9000), with regard to food covers activities directed towards identification of the origin and location of all components of the food product, as well as activities aimed at identification of all recipients of the product under examination (DZWOLAK 2008, DZWOLAK 2008a, *Food safety management*... ISO 22000)). Tracking (MOUSAVI et al. 2002), also referred to as "traceability forward" (KELEPOURIS 2007) or "tracing forward" (ANON 2007), with reference to food products means the ability to trace the path taken by any specific food product unit between individual links of the food chain (ANON 2007). Tracing (DUPUY et al. 2005, CAC/GL 2006), also referred to as backward traceability (JANSEN-VULLERS et al. 2003) or tracking back (ANON 2007) denotes the ability to establish the origin of a specific unit and/or batch of a food product located within the food chain on the basis of available records (ANON 2007).

Traceability assurance is required by the EU Food Law, under Art. 18. of Regulation (EC) No. 178/2002 (Regulation 178/2002). Additionally, a traceability system is a key element of all food safety and quality management systems and is one of the basic requirements of such standards as *Food safety management*... ISO 22000, ISO 9001, BRC, IFS and GlobalGAP (CZARNIECKA-SKUBINA and NOWAK 2012, DZWOLAK 2009, MAI et al. 2010).

Implementation of the traceability system makes it possible to gain several advantages, the most important of which include improvement of production management and product distribution management, as well as improved management of nonconforming products, particularly in the phase of withdrawing the nonconforming product from the market, which can be reflected

in the reduction of costs resulting from complaint procedures or a food crisis (DUPUY et al. 2005, ANON 2007, FOLINAS et al. 2006, KIJOWSKI and FABISZ-KIJOWSKA 2008, PINTO et al. 2006). In the context of improving food safety or quality management systems, the most important advantage of the effective traceability system is the possibility to quickly identify and eliminate the cause of producing a nonconforming/unsafe product (DZWOLAK 2008a, ANON 2007, SALTINI and AKKERMAN 2012).

The literature on the subject includes mostly publications dealing with traceability in the food processing industry (RUIZ-GARCIA et al. 2010, SALTINI and AKKERMAN 2012), as well as at the stage of obtaining raw materials of plant (CANAVARI et al. 2010, HU et al. 2013, MANOS and MANIKAS 2010) and animal origin (BYKOWSKI and LOREK 2005, DONNELY et al. 2009, GÓRNA 2012, MOUSAVI et al. 2002, RANDRUP et al. 2008, SMITH et al. 2008). In view of the importance of quick identification of the products in the traceability system, particularly with the application of Global Solution One (GS1) and RFID systems (DZWOLAK 2009), the subject of many publications concerns applications of those types of IT systems for supporting the traceability of raw materials and food products (CZARNIECKA-SKUBINA and NOWAK 2012, KELEPOURIS 2007, PAPETTI et al. 2012, ZHANG et al. 2010). However, there are no available publications taking up the issues of traceability in catering processes. In this area of the food chain, operations carried out within the traceability system aim at reconstructing the meal production history, including determination of the type and the origin of the materials used (raw materials, additives, etc.), as well as identification of persons and conditions related to meal production (DZWOLAK 2008).

Objectives

The aim of the research was to improve the system of internal traceability in a mass catering facility by mapping the flow of materials and information, identification of critical traceability points (CTP), and defining corrective actions in order to enhance the traceability system in the facility. The scope of the research covered the system of internal traceability in the catering process, from purchase of food raw materials to serving of ready meals. The subject of the research was the internal traceability system in a closed-type catering facility.

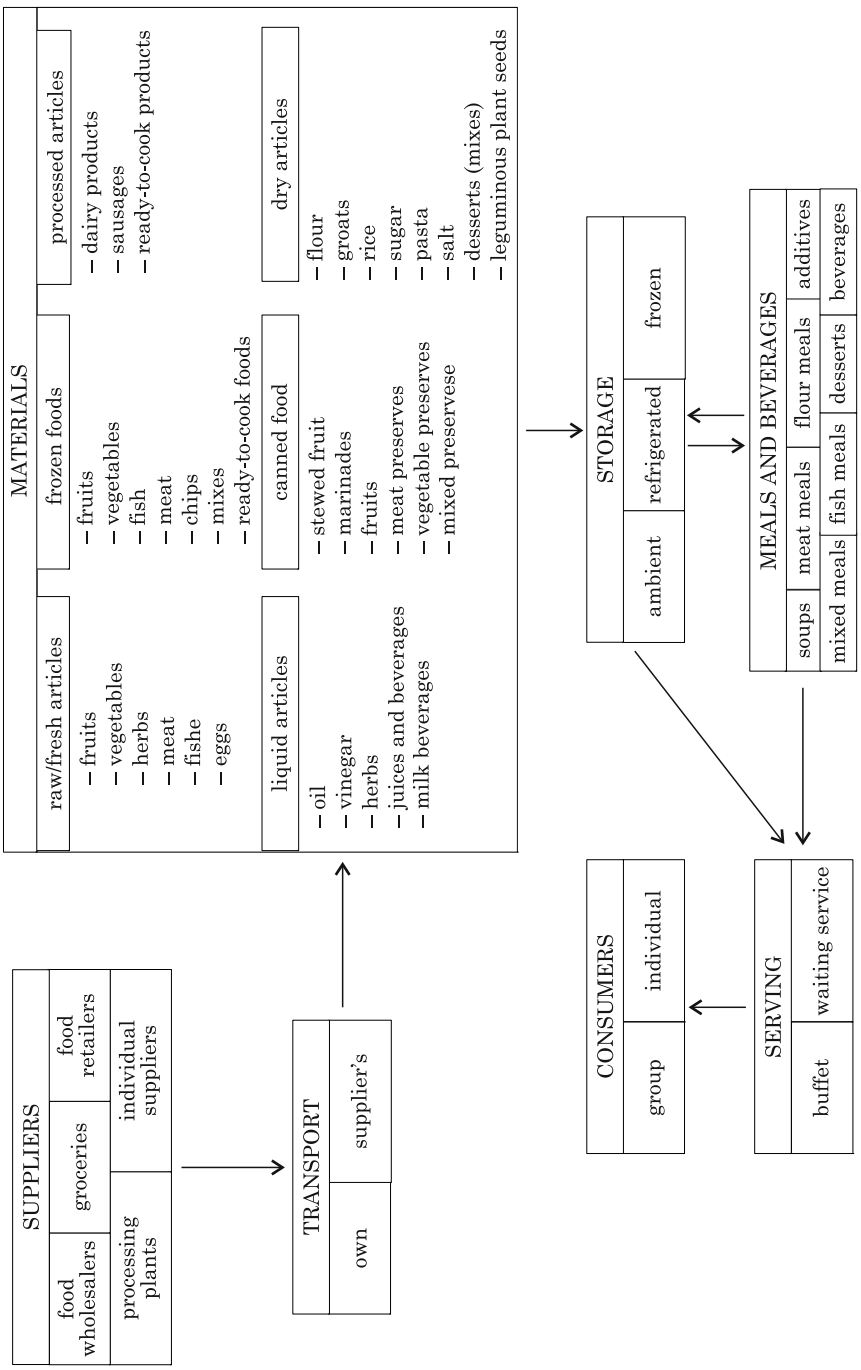

Fig. 1. Map of material and information flow

Materials and Methods

Internal audit and mapping of the flow of materials and information

The research methods included an internal audit of the system, mapping of the material and information flow, CTP identification and establishing corrective actions necessary to improve the traceability system in the facility.

On the basis of direct observations and the analysis of the available documentation of the facility, the audit criterion was developed in the form of the list of control questions, which provided a basis for the internal audit concerning the traceability system in the analysed mass catering facility (MCF). The internal audit was performed according to the audit methodology (DZWOLAK 2009a, DZWOLAK 2010), and its aim was to determine the flow of materials and information related to product traceability. The scope of the audit covered all purchased raw materials, additives, seasonings and packages, as well as products for direct consumption, semi-finished products and products intended for refrigerated and frozen storage.

On the basis of the internal audit, a map of material and information flow was prepared, including the stage of receiving deliveries, storage of purchased materials, production and storage of meals, as well as their serving (Figure 1).

CTP identification

Critical Traceability Points (CTP) mean those spots in which the continuity of the information chain, necessary to establish the origin or location of the food product, is broken (KARLSEN and OLSEN 2011). A decision concerning determination of the piece of information under analysis as a CTP was taken on the basis of a developed decision tree, referring to the location of materials/meals, stopping the process of taking out/withdrawing meals, links between materials (raw materials, additives, seasonings) and meals or between meals and materials, as well as to usefulness of information for establishing the cause for producing nonconforming/unsafe meals (Figure 2).

Corrective actions

After analysing the maps of material and information flow and the CTPs identified, corrective actions related to improving identification of material batches and product batches were formulated.

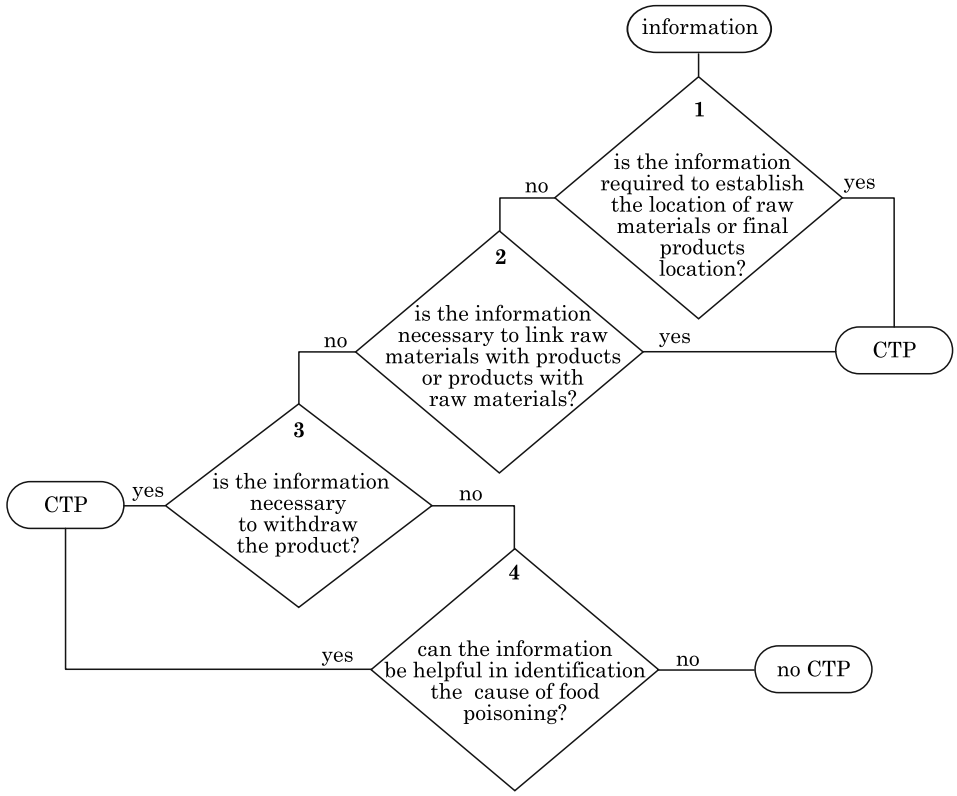

Fig. 2. Decision tree for CTP identification

Verification of corrective actions

After eight weeks following implementation of the corrective actions, the improved traceability system was verified. Three meals selected at random – one from the freezer, one from the refrigerator and one directly from the kitchen – were the subject of verification. The aim of this stage of the research was to evaluate the efficiency of the corrective actions applied by checking the continuity of two-way flow of information concerning the above mentioned meals, and the final determination of the scope of the internal traceability system in the facility.

Results and Discussion

Internal audit and mapping the flow of materials and information

The results of the audit concerning the internal traceability system of the MCF under analysis revealed that the system had various points in which continuity of information concerning identification (labelling) and/or location of materials was not assured. As results from the map of material and information flow (Figure 1), the continuity of information flow was ensured at the stage of receiving deliveries of materials only with reference to transport means. At this stage, the continuity of information broke when materials were transferred to their storage locations, except for eggs which were always stored in the designated and labelled refrigerator. A loss of traceability chain continuity between receiving deliveries and storage of materials resulted from the lack of labelling for the batch of materials received by MCF, as well as from the fact that materials were stored in several unmarked refrigerators, freezers and rooms (Figure 3). Also, at the storage stage, some dry goods (e.g. groats, beans and peas), vegetables and fruit, bread as well as unpacked articles (e.g. cured meat and raw meat) were placed in collective containers. This resulted also in mixing various batches of materials, which brought about a loss of information flow continuity. Those observations correspond to previous reports by other authors, who indicated mixing of batches as one of main difficulties emerging in traceability systems (DUPUY et al. 2005, SALTINI and AKKERMAN 2012).

The lack of possibility to identify batches of materials and their origin, except for eggs, was also noticed for meal production. No methods of recording the name, the batch or other forms of identifying materials were used at this stage, in spite of the fact that identification of the batch of material/ product is a crucial element of the recall and product withdrawal procedures as well as searching for the cause of the nonconforming product (DZWOLAK 2009, ANON 2007). Although this situation is typical for many catering facilities, it is unacceptable in the aspect of food safety assurance and public health, since it enables undertaking effective corrective actions in case of food poisoning.

CTP identification

Critical traceability points are determinants of the traceability system completeness. CTPs identified in this research (Figure 2) were a consequence, first of all, of the lack of labelling batches of received materials at their storage stage (CTP1-CTP10) and while making the meals (CTO11-CTP20). Other

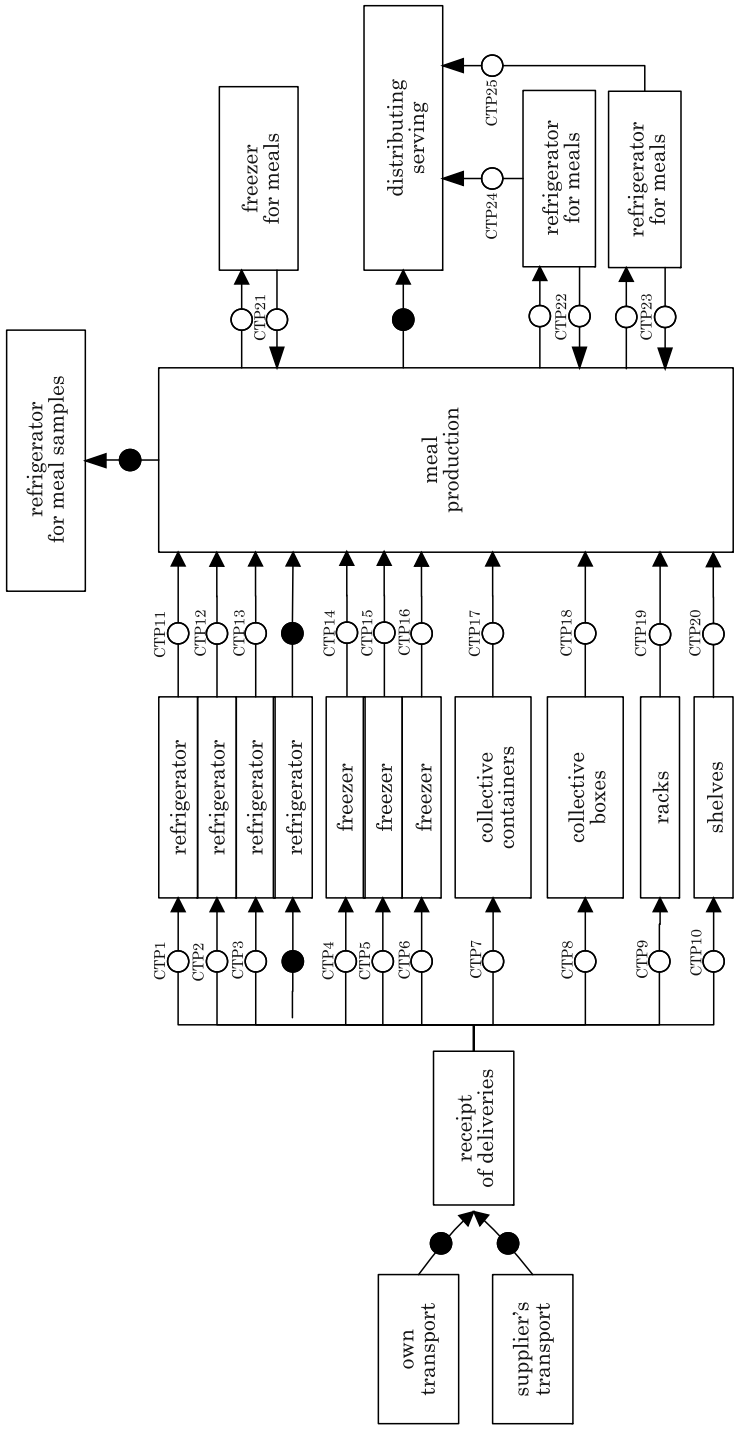

Fig. 3. CTPs in the catering process under analysis: • – information continuity, o – information discontinuity

CTPs (CTP21-CTP25) resulted from the lack of identification labels on semi-finished products and meals at their frozen storage (CTP21) and refrigerating storage stage (CTP22-CTP25) – Figure 3. Those results correspond to the findings by KARLSEN and OLSEN (2011) and KARLSEN et al. (2011), who explained CTPs identified in fish farming by mixing the batches of fodder components and the lack of explicit labels on traceable units.

Meal production in mass catering facilities is not of the serial production type, except from mass events and the so-called system gastronomy (CZARNIECKA-SKUBINA et al. 2009), which to a great extent hinders assurance of information flow continuity and generates numerous CTPs. Additional difficulties in this regard result from the fact that catering facilities are characterized by significant diversity of suppliers and their high rotation, particularly in summer and autumn seasons. The emergence of CTPs in the analysed MCF was also influenced by specific conditions typical for catering facilities, resulting first of all from a wide range of meals produced, as well as from numerous batches of materials (raw materials, additives, seasonings, etc.) and varied sources of their origin (DZWOLAK 2013). Numerous batches of raw materials and manufactured products result in increasing the so-called dispersion of batches of raw materials and products, which is indicated in the literature as one of the reasons for lowering the efficiency of the traceability system in a food chain (DUPUY et al. 2005, SALTINI and AKKERMAN 2012).

Corrective actions

In view of the fact that the primary problem reducing the effectiveness of recalls and withdrawals of products and identification of causes for the emergence of nonconforming products is the impossibility to determine the batch of materials (ANON 2007), the first step was to define a batch of materials (raw materials, additives and packages)[1] supplied to the MCF, as well as a batch of products (semi-finished products and final meals)[2]. This operation was necessary for clear identification of materials originating from various sources, and also provided the basis for applying an appropriate system of labelling material batches (ANON 2007, SMITH et al. 2008).

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[1] Production/distribution unit (net weight, number of pieces, package, multi-pack, box container, etc.) of materials originating from one supplier, produced in uniform production conditions and delivered to MCF at the same time.

[2] Production unit of MCF products (pieces of net weight) produced in uniform conditions, at the same time, by the same persons, using the same equipment and kitchen appliances.

In case of catering facilities, introduction of sophisticated information technologies, such as GS1 bar codes or RFID transponders (CANAVARI et al. 2010, CZARNIECKA-SKUBINA and NOWAK 2012, HU et al. 2013, PAPETTI et al. 2012) is not economically viable. Taking into account the fact that simple traceability schemas based on manually prepared records and documents have been used in production and food trade facilities for many years (ANON 2007, FOLINAS et al. 2006, GÓRNA 2012), the chosen solution was well-suited to the technical and economic possibilities of the MCF under analysis. For the labelling of material batches, the system of stickers/notes/tags with written code of the material batch was used, of the following general pattern:

$$000/0/00/A \tag{1}$$

where:
000 – subsequent day of the year
0 – subsequent number of batch on a given day
00 – subsequent number of material batch in the same delivery,
A – place code.

In order to ensure full identification of materials received, the given batch code was entered together with the name of the material and the number of invoice or another proof of purchase in the "Delivery register" book created. Due to difficulties in identifying some supplies of vegetables and fruit, the group of accidental suppliers from marketplaces was excluded from the group of suppliers.

To precisely designate the storage place (location) of a given batch of materials, stickers/notes showing the code were used for labelling freezers (Z_1, Z_2, Z_3), refrigerators (C_1, C_2, C_3, C_4), racks and other places where the above-mentioned materials were stored (Figure 4). Those labels also provided the code of the storage place, which was recorded together with the batch code (pattern 1).

At the meal production stage, the use of the so-called identification cards (ID cards) between the storeroom and the proper kitchen was introduced. While collecting the material from the storeroom, a cook or a cook helper copied the batch number from the label onto an ID card and entered the proper place code (of the freezer, the refrigerator, etc.). The ID card was brought together with the collected material into the kitchen and was then placed in the labelled container (Figure 5). ID cards were kept in the container until the end of the shift and were then transferred to the envelope marked with a date and stored for 72 h (in case of directly served meals) or by the end of their use-by date (frozen products).

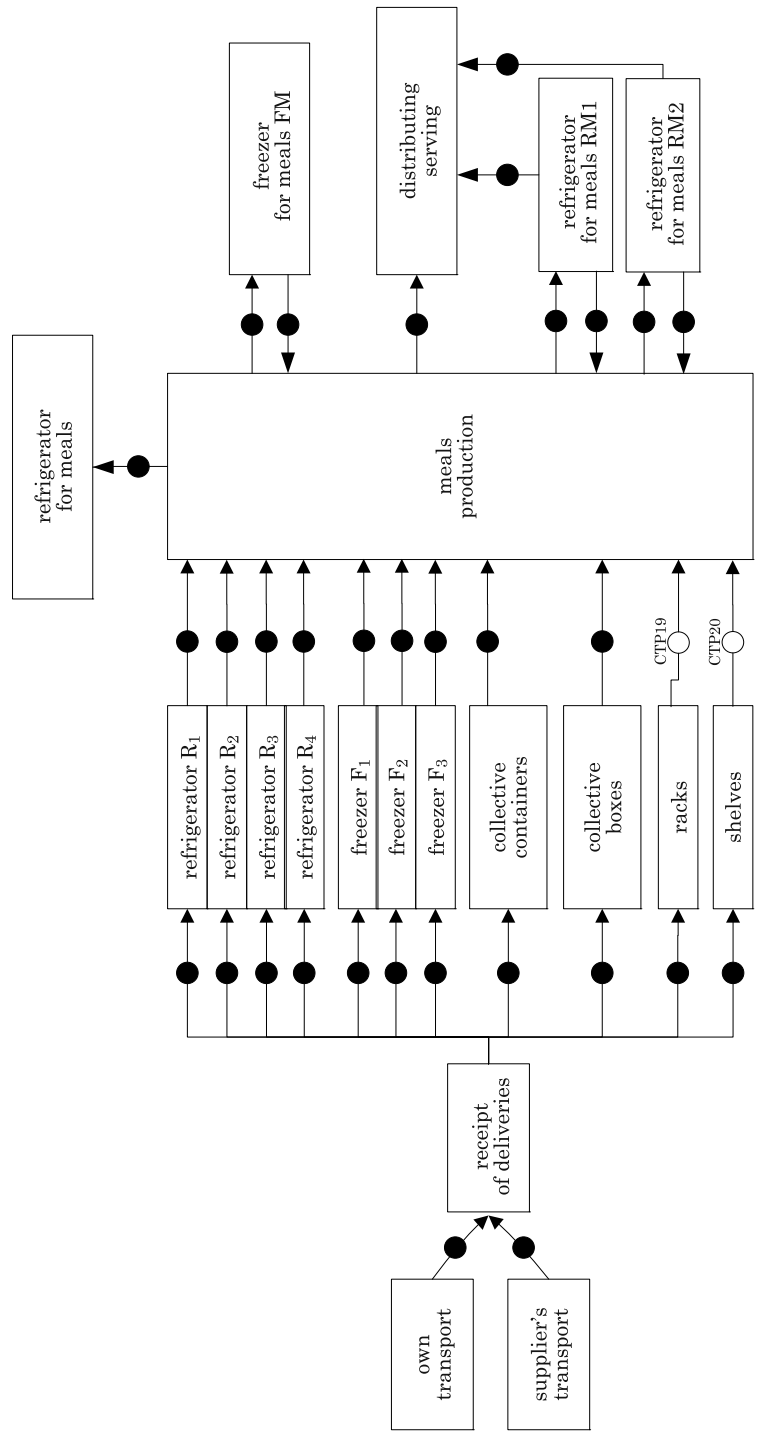

Fig. 4. CTPs after introduction of corrective actions: • – information continuity, o – information discontinuity

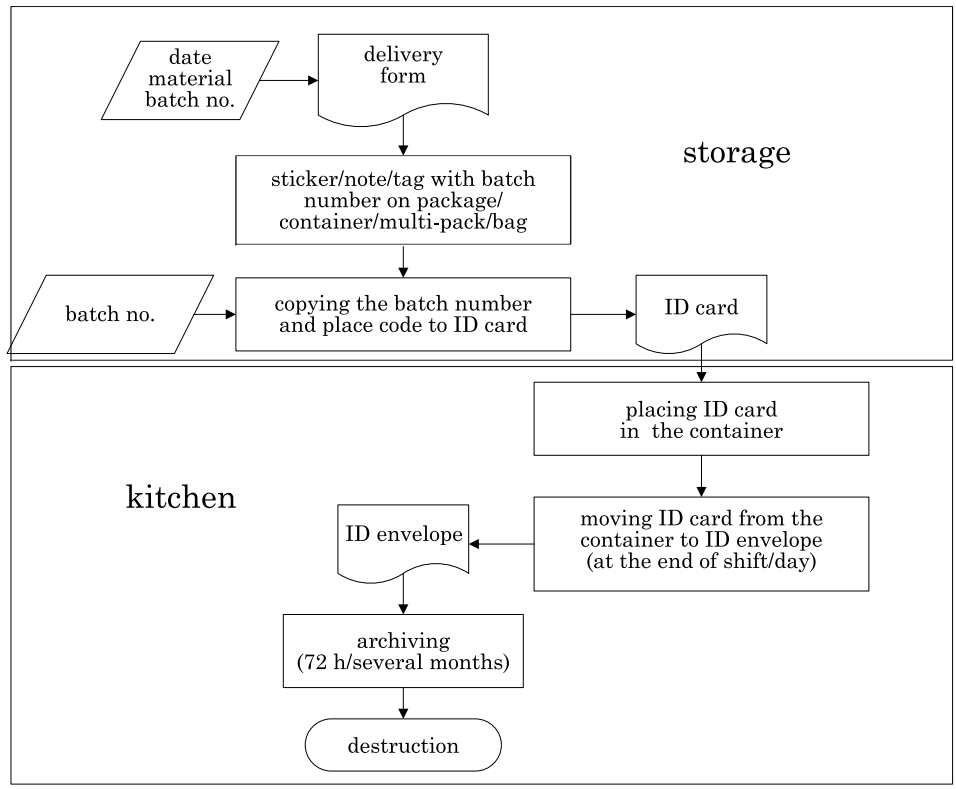

Fig. 5. Corrective actions – flow of information with regard to the origin of materials used for meal production

In this way, the ability to identify all material batches used for producing meals on a given day was ensured. In combination with the above-described corrective actions at the stage of receiving and storing the material, it was also possible to establish the origin (suppliers) of the materials of which the meals were produced on a given day.

The last group of corrective actions was related to labelling batches of ready meals and semi-finished products stored in refrigerators and freezers. In this case, labelling was used in the form of stickers or notes specifying the day of production, the date and time of packing, as well as the date and time by which the meal should be consumed or the semi-finished product should be used. To identify samples of meals collected during mass events, a description of samples was used, specifying the name of the meal, the date of its production and the time when the sample was collected.

While implementing corrective actions in the MCF analysed, a fundamental problem was the additional load for employees related to new duties of labelling the batches and use of ID cards. For the first three weeks of using a new batch identification and traceability system, employees also had problems with the proper performance of activities related to the new system. Those observations correspond to information provided in the literature, according to which simple systems based on manual records are laborious and time-consuming (PINTO et al. 2006, ANON 2007). Unquestionably, the application of modern IT technologies (e.g. RFID or GS1) significantly improve the rate of operation and improve the efficiency of the entire traceability system (GÓRNA 2012, MANOS and MANIKAS 2009), but solutions of this type exceed the financial possibilities of most MCFs and are applied only by large restaurants and catering businesses. In the context of improving the rate of information flow, at moderate financial cost, the introduction of barcode printers and readers could be a good solution.

Verification of corrective actions

The scheme of the identification procedure with marked traceability elements and proper documents and records is presented in Figure 5. For all three samples, it was possible to reconstruct the history of the meal production, establishing technological parameters, health condition of employees participating in the production of the analysed meals, registered technological parameters, materials used for the production of meals, conditions for their receipt and storage, as well as the origin of those materials (identification of suppliers). Additionally, for the meal selected directly from the kitchen, it was possible to identify and locate the sample of this meal (Figure 6). The obtained scope of the analysed traceability system from the meal to the material suppliers confirmed the effectiveness of corrective actions applied and it was compliant with the minimum requirements defined in the Regulation of the European Parliament and the Council No. 178/2002 (Regulation 178/2002).

The applied methodology of material and information flow mapping, CTP identification and implemented solutions improving the traceability system in the facility can be also used in other mass catering facilities. Nevertheless, a basic limitation of this established traceability system is employee turnover. Newly recruited employees must, at least for a few weeks, adjust to the required procedures, and if the employee turnover rate is too high, the system may be destabilised. However, this constraint does not result from the specific character of the system described, but it is conditioned by the specificity of the catering industry, in which some closed-type facilities (e.g. resort hotels) are closely related to the seasonal character of the tourist activity.

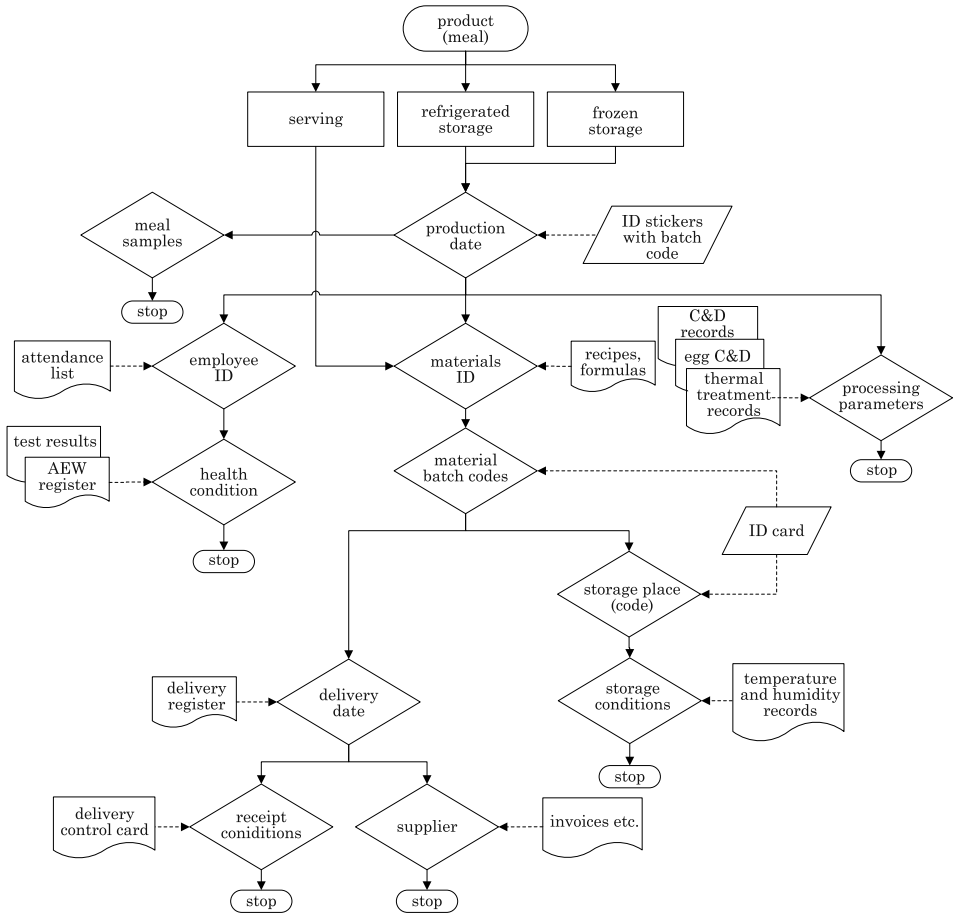

Fig. 6. Scheme of internal traceability verification after implementing corrective actions: AEW – records concerning admission of employees to the workplace, ID – identification, CandD – cleaning and disinfection. A dashed line is used to mark the flow of information available in the existing records

Conclusions

1. Numerous critical traceability points (CTP) established in the analysed catering process were a consequence of a partial or total loss of information flow (concerning location of materials or meals) as a result of mixing various batches at the storage stage or during refrigerated and frozen storage of semi-finished products or ready meals.

2. In order to improve the traceability system through reduction of CTPs in the analysed mass catering facility, it was necessary to introduce numerous

corrective actions. Those measures consisted, first of all, in introducing a labelling system for material batches at the stage of their delivery, and the batches of materials stored under refrigerated and frozen conditions. The system of material purchases also required some corrections, in which occasional suppliers were eliminated and the register of suppliers was established, with reference to internal codes of material batches and place codes.

3. As proven during the practical verification of the corrective actions introduced, the possibility to trace the product from serving the meal to material suppliers as well as from the material suppliers to serving ready meals was able to achieve.

4. The mapping of material and information flow applied in this study, combined with CTP identification and establishment of corrective actions, which included coding and labelling material batches and meals as well as the introduction of identification cards, made it possible to improve the internal traceability system in the facility. These results may be useful for designing traceability systems in mass catering facilities.

Translated by Biuro tłumaczeń „Oscar" w Olsztynie

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PROTEOLYTIC CHANGES IN RIPENED COW, SHEEP AND GOAT CHEESES MADE BY LOCAL PRODUCERS*

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K e y w o r d s: proteolysis, water-soluble nitrogen compounds, peptide nitrogen, amino acid nitrogen, cheese ripening.

A b s t r a c t

The range of proteolytic changes that take place in ripened cheeses produced by local and mass manufacturers from cow, goat and sheep milk was assessed by determination of nitrogen compounds in the products, such as: total nitrogen, water-soluble nitrogen compounds at pH 4.6, peptide nitrogen content and amino acid nitrogen content. The results indicate that cheese made from sheep milk by local manufacturers contained the largest amounts of all the nitrogen forms under study. The total nitrogen content in them was 5.75%, the content of water-soluble nitrogen compounds at pH 4.6 was 18.27% N_{total} on average, peptide nitrogen was 2.63% N_{total} and amino acid nitrogen was 12.75% N_{total}. Moreover, the study showed that the concentration of individual forms of nitrogen compounds was higher in products made by local manufacturers compared to the same products made by mass manufacturers. Other parameters determined in cheese samples included pH, water, NaCl and fat content, fat content.

ZMIANY PROTEOLITYCZNE W SERACH DOJRZEWAJĄCYCH KROWICH, KOZICH I OWCZYCH POCHODZĄCYCH OD LOKALNYCH PRODUCENTÓW

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S ł o w a k l u c z o w e: proteoliza, rozpuszczalne związki azotowe, azot peptydowy, azot aminokwasowy, sery dojrzewające.

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Abstrakt

Oznaczono zmiany proteolityczne w serach dojrzewających pochodzących od lokalnych i masowych producentów, wytworzonych z mleka krowiego, koziego oraz owczego. Ocenę zakresu zmian proteolitycznych przeprowadzono, oznaczając zawartość poszczególnych form związków azotowych w badanych produktach. Oznaczono zawartość: azotu ogółem, azotu rozpuszczalnego w pH 4,6, azotu peptydowego oraz azotu aminokwasowego. Wykazano, że najwyższą zawartością wszystkich badanych form azotu charakteryzowały się sery owcze pochodzące od lokalnych producentów. W produktach tych zawartość azotu ogółem kształtowała się na poziomie średnio 5,75%, azotu rozpuszczalnego w pH 4,6 średnio 18,27% N_{total}, azotu peptydowego 2,63% N_{total}, a azotu aminokwasowego – 12,75% N_{total}. Na podstawie przeprowadzonych badań wykazano ponadto, że stężenie poszczególnych form związków azotowych jest wyższe w produktach pochodzenia lokalnego niż w tego samego rodzaju produktach wytwarzanych przez masowych producentów. W badanych próbkach serów dojrzewających oznaczono również kwasowość czynną (pH), zawartość wody, zawartość tłuszczu w suchej masie oraz zawartość NaCl. W badaniach wykazano, iż badane wyróżniki jakości mieściły się w granicach wyznaczonych przez polskie standardy dla tego rodzaju produktów.

Introduction

Nowadays, consumers are searching for safe food of known origin. They want the food they buy to be free of any risk to their health and to have above-standard quality (ANGULO and GIL 2007, VERBEKE et al. 2007). Product origin is playing an increasingly important role. Products from rural areas are regarded as being of high quality. The EU legal regulations (EC No 509/2006, EC No 510/2006, EC No 2081/92, EC No 2082/92) provide the possibility of protecting original agricultural and food products which are characteristic in terms of their origin and traditional method of production. These include: Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Speciality Guaranteed (TSG). Product quality, which is a result of the method of production and processing, as well as the raw materials used, should be the most important food characteristic, providing the basis for building its positive image. Therefore, the important issues of registration and standardization of traditional foods arise in order for these products to be protected against imitations, to be of high quality and to conform to contemporary rules of appropriate and safe production. A way to ensure authenticity and high quality of traditional food products is to establish criteria for their registration that will thereafter determine standards for their commercial production (TRICHOPOULOU et al. 2007).

Ripened rennet cheeses are among the most important components of the human diet in the temperate zones and are one of the best sources of calcium in food (BROOME and HICKEY 1991, LANE and FOX 1996, MISTRY 2001). The degree of their ripeness is one of the major factors which determine their quality and sensory characteristics. One of the most significant biochemical

processes which determine the taste and texture of cheese is proteolysis. The process comprises a whole range of microbiological, enzymatic and physicochemical phenomena. The processes can be divided into three phases: proteolysis of casein before starter bacteria and coagulating enzyme are added, enzymatic coagulation of milk and proteolysis during the cheese ripening process (Fox 1989, GRAPPIN and BEUVIER 1997, TRUJILLO et al. 2002). An important role in developing the taste and flavour of cheese is played by bacterial enzymes through releasing low-molecular products of degraded para-casein (BROOME and HICKEY 1991, SKEIE et al. 1995). The most important native enzyme, responsible for proteolysis of milk proteins is plasmin. Plasmin's proteolytic effect on α_{s1}-casein leads to the formation of peptides and λ-casein. The products of β-casein hydrolysis induced by plasmin are γ-caseins (γ_1, γ_2, γ_3, γ_4) and proteose-peptones (KUNCEWICZ et al. 2009). Proteinases of starter bacteria effect hydrolysis of β-casein, whereas peptidases are active after cell autolysis (BROOME and HICKEY 1991, LANE and FOX 1996). Producers, whose main goal is to maximise profits, frequently reduce cheese ripening time to the minimum, which considerably lowers the sensory quality of the finished product. It has been observed in recent years that products obtained from cow milk, e.g. rennet cheese, increasingly often are becoming a cause of food allergies. Consumers who want to have a good source of calcium in ripened cheese seek alternative products, made from a different type of raw material. Products made from sheep or goat milk are a perfect alternative for people who are allergic to dairy products made from cow milk. The literature does not provide exact data on the quality of ripened cheese made from sheep or goat milk from local producers. There are no specific requirements for the physico-chemical quality for products produced by local manufacturers. Under current law the legal conditions of production of dairy products produced from milk obtained at the farm, where the business is involved in the production of products intended for direct sale, apply only to the veterinary requirements that should be met in carrying out activities of local, organic and limited (Regulation of The Minister of Agriculture And Rural Development 2010). Therefore, the aim of this study was to evaluate the range of proteolytic transformations (by determining the content of different nitrogen compounds), which are an indicator of the maturity of rennet cheeses and basic determinants of quality, such as active acidity, salt and fat content in cow, sheep and goat ripened cheese made by local producers and to compare selected parameters with those of cheeses made by larger manufacturers.

Materials and Methods

Samples

Stock was taken of ripened cheeses made by agritourist farms and dairy micro-companies operating in the region of Warmia and Mazury in Poland. Selected from the indicated products were those with above-standard quality, which results from traditional production methods and the raw materials (of local origin) used in the process. Of the selected rennet cheeses, samples of 15 hard ($n = 4$) and semi-hard ($n = 11$) cow (hard $n = 4$, semi-hard $n = 5$) goat ($n = 3$) and sheep ($n = 3$) cheeses were taken for analysis. Moreover, hard (cow $n = 3$) and semi-hard (cow $n = 3$, goat $n = 2$ and sheep $n = 2$) cheeses from 10 large producers were purchased on the local market (these samples were labelled as conventional). Research was conducted in 2011.

Proteolytic changes

The proteolytic changes was evaluated by the reference method (Kjeldahl's method), taking into account changes in the content of different forms of nitrogen compounds: total nitrogen (HELDRICH 1990), water-soluble nitrogen at pH 4.6 according to Sode-Mogensen (HELDRICH 1990), peptide nitrogen according to SCHOBER et al. (HELDRICH 1990) and amino acid nitrogen according to Sirks (HELDRICH 1990). The samples were mineralized and distilled using FOSS apparatus.

NaCl, fat, water, pH

NaCl, fat, water content and active acidity (pH) were determined by the method contained in the Polish Standard (*Mleko i przetwory*... PN-73/A-86232). NaCl content were determined using Volhard's method. To estimate fat content has been used Soxhlet extraction.

Statistical analysis

The data obtained were statistically analysed with the use of basic statistics. The results were statistically analysed using Statistica 10 software. Basic statistics (average and standard deviation) were calculated. A one-factor variation analysis ANOVA and Tukey's post-hoc tests were used to test differences between products and origin (local and conventional). The significance of differences was tested at the significance level of 0.05. The results are shown in Tables 1–3 and in Figures 1–4.

Results and Discussion

NaCl, fat, water, pH

The determination results have shown that active acidity (pH) in mass-produced cheeses was higher than those of local origin (Tables 1–3). The relationship was observed in all the three types of ripened cheeses (made from cow, sheep and goat milk). A statistical evaluation of experimental results has shown that acidity of all local products was significantly higher from the conventional products. According to the requirements of the standard PN-68-A-86230, salt content in ripened cheese should not be higher than 2.5%.

Table 1

Chemical composition and acidity (pH) of cow cheeses (mean ± SD)

Specification		Cow cheese		ANOVA
		local	conventional	
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	
Acidity (pH)		6.09 ± 0.15	6.49 ± 0.15	$F = 5.29, p = 0.016$
NaCl	%	1.85 ± 0.22	1.63 ± 0.45	$F = 3.15, p = 0.187$
Water content	%	40.05 ± 1.24	40.97 ± 0.95	$F = 1.70, p = 0.101$
Fat content in d.m.	% d.m.	46.14 ± 2.14	45.01 ± 2.89	$F = 1.82, p = 0.615$

Table 2

Chemical composition and acidity (pH) of goat cheeses (mean ± SD)

Specification		Goat cheese		ANOVA
		local	conventional	
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	
Acidity (pH)		6.01 ± 0.19	6.23 ± 0.17	$F = 1.18, p = 0.049$
NaCl	%	2.15 ± 0.18	1.86 ± 0.06	$F = 8.94, p = 0.001$
Water content	%	39.95 ± 1.04	41.47 ± 1.75	$F = 8.83, p = 0.003$
Fat content in d.m.	% d.m.	49.06 ± 1.84	41.65 ± 1.34	$F = 1.18, p = 0.003$

Table 3

Chemical composition and acidity (pH) of sheep cheeses (mean ± SD)

Specification		Sheep cheese		ANOVA
		local	conventional	
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	
Acidity (pH)		5.78 ± 0.04	6.23 ± 0.19	$F = 21.09, p = 0.001$
NaCl	%	1.52 ± 0.03	1.48 ± 0.02	$F = 2.71, p = 0.052$
Water content	%	40.05 ± 0.85	41.52 ± 0.78	$F = 1.17, p = 0.043$
Fat content in d.m.	% d.m.	48.36 ± 2.01	44.83 ± 1.74	$F = 1.32, p = 0.082$

The study results have shown that salt content in cheese from local producers was higher than in cheese produced by larger dairy plants, but in none of the samples did it exceed the highest acceptable value for the product (Tables 1–3).

The differences between the average content of NaCl are statistically significant only in goat cheeses (Table 2). The water content in all the cheese samples ranged from 39.95% (in goat cheese from a local manufacturer) to 41.52% (in mass-produced sheep cheese) – Tables 1–3, and in none of the samples did it exceed the highest value allowed by the Polish Standard, which is equal to 45% for this type of product.

The highest fat content per dry weight was found in the locally produced goat cheeses (49.06% on average) – Table 2, and the lowest was in the same type of cheese produced on a large scale (41.65%) – Table 2. A statistical evaluation has shown that water content was significantly differed in goat and sheep (Table 3) cheeses and fat content – in goat cheeses (Table 2).

Range of proteolytic changes

The range of proteolytic transformations in the cheese samples was compared by determination of the different forms of nitrogen compounds (total nitrogen, water-soluble nitrogen at pH 4.6, peptide nitrogen and amino acid nitrogen). The content of different forms of nitrogen is shown in Figures 1–4. The results show that the highest content of total nitrogen is found in locally produced sheep cheeses (5.75% on average) – Figure 1. Average total nitrogen content in conventional sheep cheeses was 4.82% (Figure 1). The values found in the other types of ripened cheeses were similar with no differences observed between local and conventional products (Figure 1). The total nitrogen content in cow cheeses was 4.56% in local products and 4.54% in conventional ones, whereas the values for goat cheeses were 4.62% and 4.63%, respectively (Figure 1). The differences between the average content of total nitrogen are statistically significant only in sheep cheeses ($F = 4676$; $p = 0.00$).

The range of proteolytic transformations in ripened cheeses is indicated by the presence of water-soluble nitrogen, amino acid nitrogen and peptide nitrogen contained in products of para-casein hydrolysis. The study results show that ripened cheeses produced by local manufacturers contain the highest amounts of nitrogen compound in all forms.

The content of soluble nitrogen in local products made from sheep milk was equal to 18.33% N_{total} (Figure 2), the content of peptide nitrogen was 2.63% N_{total} (Figure 3), and the content of amino acid nitrogen was 12.75% N_{total} (Figure 4). Those values for the products made from goat milk were: 17.84%

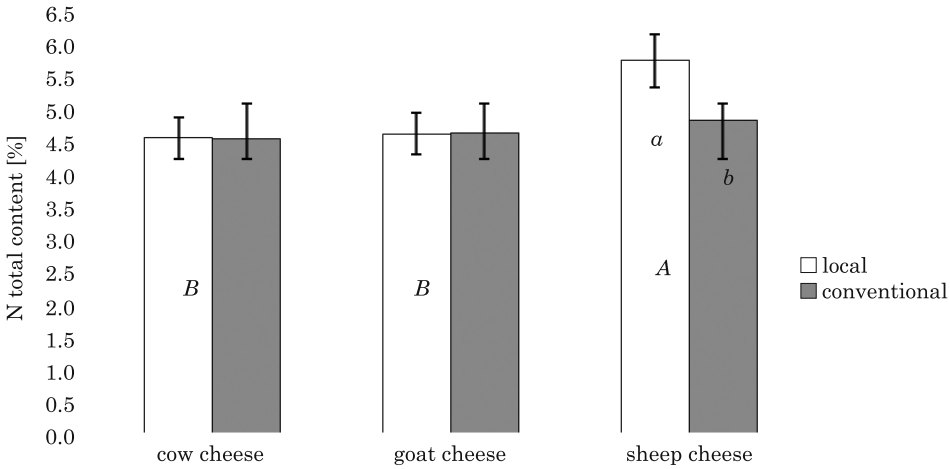

Fig. 1. Content of total nitrogen [%] in local and conventional cow, goat and sheep cheeses: statistically high significant differences between averages marked with the same letters in series *A*, *B*, *C* ($p \leq 0.05$) and origin of product – local and conventional – *a*, *b* ($p \leq 0.05$)

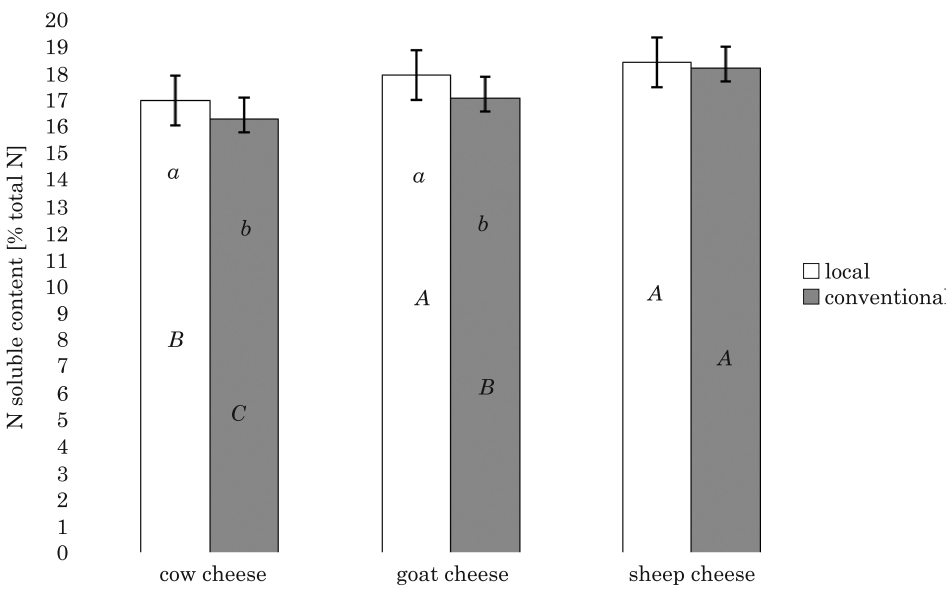

Fig. 2. Content of nitrogen soluble at pH 4.6 in local and conventional cow, goat and sheep cheeses [% N total]: statistically high significant differences between averages marked with the same letters in series *A*, *B*, *C* (cow $F = 5.64$; $p = 0.02$; goat $F = 7.68$; $p = 0.01$) and origin of product – local and conventional – *a* (F = 7.61; $p = 0.00$), *b* ($F = 12.34$; $p = 0.00$)

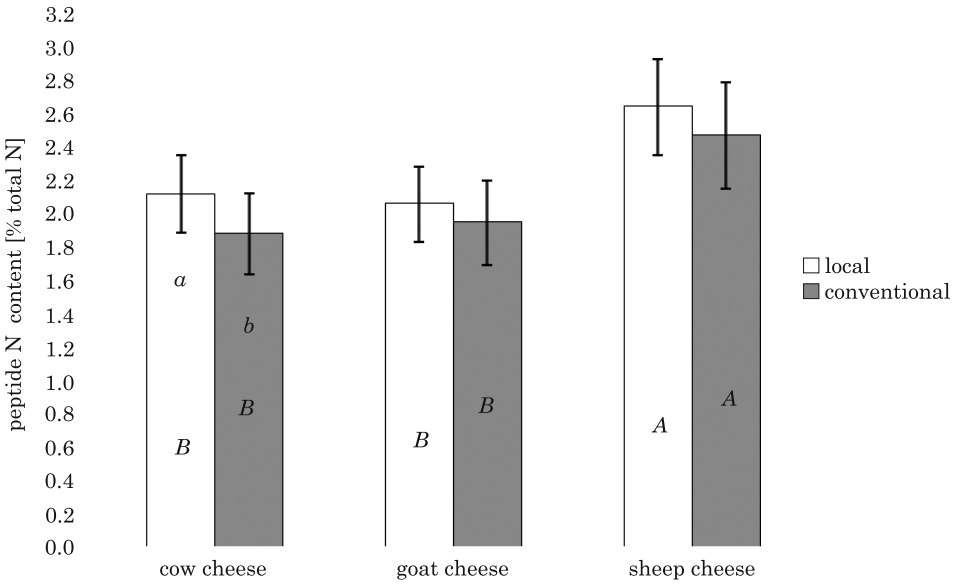

Fig. 3. Content of peptide nitrogen in local and conventional cow, goat and sheep cheeses [% N total] statistically high significant differences between averages marked with the same letters in series *A*, *B*, *C* (cow $F = 8.29$; $p = 0.08$) and origin of product – local and conventional – *a* ($F = 20.15$; $p = 0.00$), *b* ($F = 18.64$; $p = 0.00$)

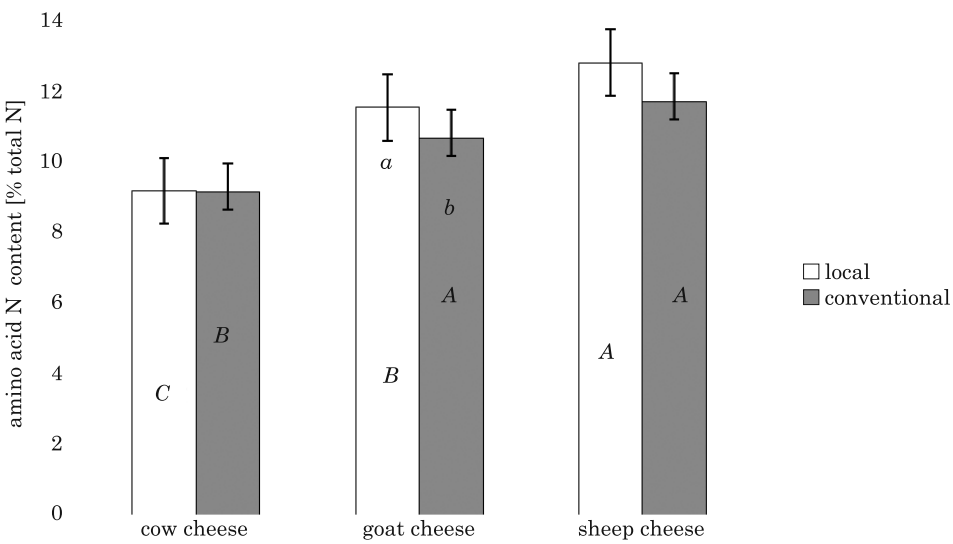

Fig. 4. Content of amino acids nitrogen in local and conventional cow, goat and sheep cheeses [% N total] statistically high significant differences between averages marked with the same letters in series *A*, *B*, *C* (goat $F = 6.04$; $p = 0.01$) and origin of product – local and conventional – *a* ($F = 136$; $p = 0.00$), *b* ($F = 19.03$; $p = 0.00$)

N_{total} (Figure 2), 2.05% N_{total} (Figure 3) and 11.49% N_{total} (Figure 4), respectively, while for cow cheeses it was 16.91% N_{total} (Figure 2), 2.11% N_{total} (Figure 3) and 9.15% N_{total} (Figure 4), respectively. The content of nitrogen compounds in conventional products made from sheep milk was: soluble nitrogen – 18.12% N_{total} (Figure 2), peptide nitrogen – 2.46% N_{total} (Figure 3), amino acid nitrogen – 11.64% N_{total} (Figure 4). Those values for cow cheeses were: 16.22% N_{total} (Figure 2), 1.87% N_{total} (Figure 3), 9.12% N_{total} (Figure 4), respectively, and for goat cheese: 17.01% N_{total} (Figure 2), 1.94% N_{total} (Figure 3) and 10.63% N_{total} (Figure 4), respectively. Statistical evaluation has shown that nitrogen soluble content in cow and goat local cheeses ($p \leq 0.05$) was significantly differed from the levels determined in conventional products (Figure 2). Peptide nitrogen content was significantly differed only in cow cheeses ($p \leq 0.05$) (Figure 3), and amino acid nitrogen was significantly differed in goat cheeses ($p \leq 0.05$) (Figure 4). The statistically significant differences in total nitrogen content, soluble nitrogen content peptide nitrogen content and amino acid nitrogen content were also found between sheep, cow and goat cheeses ($p \leq 0.05$) produced by local producers (Figure 1–4). In cheeses cow goat and sheep produced conventionally statistical evaluation has shown significantly differences in soluble nitrogen content, peptide nitrogen content and amino acid nitrogen content ($p \leq 0.05$) (Figure 2–4). The higher content of nitrogen forms determined in local products (which is a sign of cheese maturity) may indicate that the ripening process lasts longer compared to mass-produced cheeses. The business activities of mass producers are profit-oriented and shortening the ripening process helps to reduce production costs. The study conducted by other authors showed higher content for approx. 5% of water-soluble nitrogen in ripened cheese (PARK 2001, AWAD 2006, CICHOSZ et al. 2006) examined changes in the content of different forms of nitrogen compounds in the process of ripening of Gouda cheese. The authors showed that after 6 weeks of ripening, Gouda cheese contained 13.44% N_{total} of water-soluble nitrogen compounds, 4.99% N_{total} of amino acid nitrogen and 1.5% N_{total} of peptide nitrogen (CICHOSZ et al. 2006). According to earlier studies conducted by CICHOSZ et al. (2005) on Gouda cheese, the content of water-soluble nitrogen after 6 weeks of storage was 17.27% N_{total}, amino acid nitrogen was 10.66% N_{total} and peptide nitrogen was 1.29% N_{total}.

The content of different forms of nitrogen is depending on many factors such as: time and conditions of ripening product, protein content in raw material, amount and quality of starter culture. The highest content of different forms of nitrogen compounds was found in sheep cheeses, both those produced locally and by large dairy farms, whereas the lowest content was found in cheeses produced from cow milk (Figures 1–4). Sheep milk contains the largest amounts of protein (4.90 – 6.80%, including 4.40 – 5.90% of casein)

(PANDYA and GHODKE 2007, DANKÓW and PIKUL 2011). Hence, the total nitrogen content and that of individual forms of nitrogen compounds in ripened cheese is higher compared to products made from cow or goat milk. Ripened cheeses produced by local manufacturers contain the highest amounts of all forms nitrogen compound.

Conclusions

1. The basic chemical composition and active acidity (pH) of the ripened cheese under study, made from different types of raw material, complied with the requirements set by the Polish Standards for such products.

2. The content of different forms of nitrogen compounds, which is a sign of cheese maturity, was higher in products made by local manufacturers. This may indicate that the process of cheese ripening is longer, which results in better taste and flavour characteristics, largely affecting the quality of the final product.

3. The content of each form of nitrogen compounds, both for local and conventional products, was the highest in cheese produced from sheep milk, which may be attributed to the composition of the raw material and the cheese ripening time.

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THE STUDY ON OIL DROPLET SIZE DISTRIBUTION IN O/W EMULSIONS PREPARED BY THE USE OF THE ASYMMETRIC MEMBRANE*

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K e y w o r d s: membrane emulsification, oil droplet size distribution, milk protein concentrate, whey protein concentrate.

A b s t r a c t

This paper analyses the impact of two types of emulsifiers originating from milk: milk protein concentrate (MPC) and whey protein concentrate (WPC), on droplet size distribution using an asymmetric membrane process. The results indicated that the size, span and uniformity of oil droplets in emulsions depend on the velocity of shear stress on the internal surface of a membrane channel and on the physical and chemical parameters of the medium used as an emulsifier. The use of WPC produced an emulsion with optimum (the lowest) parameters of oil droplet size distribution. Switching from WPC to MPC resulted in an increase in the average characteristic diameter of the emulsion droplets and simultaneously caused widening of the distribution and a reduction in the uniformity index.

STUDIA NAD ROZKŁADEM WIELKOŚCI KULECZEK TŁUSZCZOWYCH EMULSJI TYPU O/W OTRZYMANYCH Z WYKORZYSTANIEM MEMBRAN ASYMETRYCZNYCH

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S ł o w a k l u c z o w e: emulgowanie membranowe, rozkład wielkości kuleczek tłuszczowych, koncentrat białek mleka, koncentrat białek serwatkowych.

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Abstrakt

W publikacji analizowano wpływ dwóch typów emulgatorów: koncentratu białka mleka (MPC) i koncentratu białek serwatkowych (WPC) na rozkład wielkości kuleczek tłuszczowych w emulsjach uzyskanych z wykorzystaniem membran asymetrycznych. Wielkość kuleczek tłuszczowych, równomierność i ich rozstęp w emulsjach uzyskanych metodą membranową najbardziej zależą od prędkości zmian naprężeń na powierzchni wewnętrznej kanałów membrany oraz właściwości fizycznych i chemicznych medium wykorzystywanego jako emulgator. Zastosowanie WPC pozwoliło na otrzymanie emulsji charakteryzującej się optymalnymi (najmniejszymi) parametrami rozkładu wielkości kuleczek tłuszczowych. Zmiana WPC na MPC spowodowała wzrost wielkości średnich średnic charakteryzujących daną emulsję oraz zwiększenie zakresu zmienności rozmiaru kuleczek tłuszczowych emulsji oraz redukcję indeksu równomierności.

Introduction

Emulsions are heterogeneous dispersive systems that are composed of several phases: a continuous phase and one or several phases that are dispersed in it. They are widely used in different industrial branches, such as the pharmaceutical, cosmetic, petrochemical, agricultural and food industries. In the majority of applications it is attempted to obtain an average size of droplets in the dispersed phase of below 1 μm and to achieve the highest uniformity of the dispersed phase possible (URBAN et al. 2006). A smaller size of droplets in the dispersed phase has an impact on the optical properties of a produced emulsion such as clarity and colour (LEE et al. 2013). In the case of emulsions in which the dispersed phase contains biologically active compounds, the average size of particles impacts their bioavailability (SCHUCHMANN and SCHUBERT 2003). The degree of dispersion of the dispersed phase (defined as the ratio of the surface of a dispersed phase to its volume) and droplet size distribution in the dispersed phase depend on the method and conditions of emulsification. Providing the size of droplets and their distribution that are proper for each emulsion depends on such factors as the volume of supplied mechanic energy, the type and concentration of an emulsifier, physical properties of the dispersed phase and continuous phase and physical parameters of the process itself (e.g. pressure and temperature) (MCCLEMENTS 1999). The average droplet size, the difference between the maximum and minimum diameter of droplets of the dispersed phase and the degree of their dispersion are considered as the significant parameters characterizing a given emulsion.

Physical stability is an important descriptor of an emulsion quality and it is defined as the capacity of the emulsion to maintain the same properties for a long period of time. This feature is associated with rheological properties such as viscosity, texture and lubricity. In the case of food emulsions, import-

ant features include sensory properties like colour, taste and smell. Interfacial tension at the border between the continuous and dispersed phases is one of the factors that impact the stability of an emulsion. Its reduction prevents such faults of an emulsion as coalescence, flocculation or inversion of the phases. In an emulsion, superficially active substances of an amphiphilic nature selected based on the substrates forming an emulsion are responsible for the interfacial tension at the border between the phases (BEROT et al. 2003). In the food industry, this role is most often taken by amphiphilic macromolecules such as proteins. Proteins, as emulsifiers, allow for a reduction of the surface tension and constitute a component of the macromolecular layer which is formed during emulsification process, which determines the stability of the entire arrangement (FLOURY et al. 2000). The studies on ultra-high pressure homogenization (UHPH) carried out by HEBISHY et al. (HEBISHY et al. 2013) are an example of the increase in the stability of an emulsion due to a reduction of droplet size. The reduction of droplet size during the UHPH process (over 100 MPa) produced a vegetable oil emulsion with similar viscosity to an emulsion produced with a colloidal mill. The droplet size was, however, smaller, which resulted in an increase in protein concentration on the surface of dispersed molecules and effectively prevented coalescence of lipid droplets (HEBISHY et al. 2013).

The properties of an emulsifier have an impact on the average size of the dispersed phase. Petersen and Ulrich (2013) compared the size of droplets in an emulsion produced with maize oil with low-molecular emulsifiers: Polysorbat 20 and sodium caseinate. The use of Polysorbat 20 contributed towards a smaller size of droplets and higher stability of the produced emulsion.

During production of emulsions in emulsifying machines, droplets of the dispersed phase are formed as a result of supplying mechanical energy to the system. In food emulsions, it is most often attempted to produce a dispersed oil phase in water (o/w type emulsions) or vice-versa (w/o type emulsions). Deformation and consequent fragmentation of oil droplets in the aqueous phase also occur during such processes as mixing, pumping, spraying or extruding under conditions of both laminar and stormy flow (WINDHAB et al. 2005).

In order to produce an emulsion with a proper size of droplets in the dispersed phase, high-pressure homogenizers, rotor-stator machines, membrane techniques and ultrasound homogenizers are used (SCHUCHMANN and SCHUBERT 2003, SCHULTZ et al. 2004, URBAN et al. 2006). The correlation between the size of droplets in the dispersed phase and the method of emulsification was demonstrated by SIDDIQUI (2011). The author compared the average size of droplets of sunflower oil in an emulsion produced with soy lecithin as an emulsifier that was dispersed in a high-pressure homogenizer,

a silverson rotor-stator device and a silverson impinging jet and an ultrasonic device (SIDDIQUI 2011). The smallest average size of droplets in the dispersed phase (3 mm) was detected in the emulsions produced with a high-pressure homogenizer. Emulsifying in an ultrasonic device generated the emulsion with the size of droplets between 3 and 10 μm. A similar range of variability (from 4 to 15 μm) was produced with an impinging jet system, whereas the largest droplets (15 to 45 μm) were detected in the emulsions homogenized in a silverson rotor-stator device (SIDDIQUI 2011). In their publication, PERRIER--CORNET and GERVAIS (2005) also demonstrated that the size of droplets in an emulsion and its homogeneity depended on the method of emulsification. The use of a high-pressure jet generates smaller particles, yet with lower uniformity than the emulsion produced with a micro-fluidizer. According to those authors, a nature of the flow generated at the spot of jet decompression could have a strong impact on the shape of a curve depicting the volume distribution of the tested droplets (PERRIER-CORNET and GERVAIS 2005).

Membrane techniques are gaining increasing popularity as emulsifying modalities (VAN DER GRAAF 2005, CHARCOSSET 2009, HEBISHY et al. 2013). During emulsification with membranes, different mechanisms of droplet formation in the dispersed phase are involved than with the above methods. The phase that is dispersed is pushed through the pores in a membrane to the continuous phase flowing along the surface of a membrane. At the ends of the pore tubules, droplets of the dispersed phase are formed and thrown by shear stress into the liquid flowing along a membrane (CHARCOSSET et al. 2004). According to many authors, there is a close relationship between the size of pores in a membrane and the size of droplets in the dispersed phase (JOSCELYNE and TRÄGÅRDH 2000, BEROT et al. 2003, NAZIR et al. 2010). The use of a membrane with a larger pore size roduces larger droplets in the dispersed phase in both oil/water (O/W) and water/oil (W/O) emulsions (NAKASHIMA et al. 2000). The distribution of droplet size in the emulsions produced with membrane techniques also depends on the speed of adsorption of an emulsifier on the surface of droplets. Berot et al. demonstrated that O/W emulsions generated with membrane techniques using protein as an emulsifier had a higher size of droplets than those produced with SDS as an emulsifier which has a higher rate of the changes in surface tension at the border of the phases (BEROT et al. 2003). Shear stresses found in the continuous phase near the surface of a membrane are an important factor determining the size distribution of the dispersed phase. According to VLADISAVLEVIC and SCHUBERT (2003), the size of a droplet generated with membrane emulsification decreases with an increase in shear stresses below 30 Pa. A further increase in the velocity of flow and, consequently, in shear stresses caused an increase in their magnitude. Further studies into optimizing the process of membrane emulsification are thus warranted.

The objective of the studies was to investigate the impact of the protein type and concentration, flow efficiency of the continuous phase and transmembrane pressure on the distribution of droplet size in the dispersed phase in rapeseed oil emulsions generated with the membrane emulsification technique.

Material and Methods

The O/W emulsions with 30% dry matter content were prepared and they contained the following components of the continuous phase: demineralized water, milk protein concentrate MPC 75 (Z.P.M. MLECZ Wolsztyn, Poland) or whey protein concentrate WPC 80 as emulsifying agents and maltodextrin N (dextrose equivalent – DE 7–13) – MLT from PEPEES JSC Starchworks Lomza. Milk protein concentrate MPC 75 and whey protein concentrate WPC 80 were chosen base on the preliminary experiments during which different types of MPC and WPC concentrate were tested. The dispersed phase was composed of refined rapeseed oil from EOL Poland. The mass fraction of oil in the dry matter of emulsion was $x = 0.3$ w/w. The other part constituted a mixture of protein concentrates and maltodextrin in the proportion of 0.3 proteins + 0.4 MLT or 0.1 proteins + 0.6 MLT depending on the experiment design (Table 1).

Table 1

Independent variables considered in the experiment design

Independent variable	Lower level "–1"	Upper level "+1"
X_1 – protein concentration in emulsion solids [w/w]	0.10	0.30
X_2 – kind of protein concentrate	MPC 75	WPC 80
X_3 – circulation flow-rate of the continuous phase [$dm^3\ s^{-1}$]	0.111	0.222
X_4 – transmembrane pressure [kPa]	300	500

The experimental emulsions were produced with a membrane emulsification technique on a post whose design is presented in Figure 1. An INSIDE CéRAM™ asymmetric, one-channel ceramic membrane with an internal diameter of 0.006 m and length 0.3 m manufactured by TAMI Industries (France), is the main component of the system. According to the specification provided by the manufacturer, the nominal diameter of pores in the membrane was 0.8 μm. The schematic diagram of the experimental setup is presented in Figure 1. Dispergation of rapeseed oil in the aqueous phase was run in the membrane module (*D*). During emulsification, the aqueous phase was pumped into the internal membrane channel (*D*) from the feed container (*A*) with

a two-stage circulatory pump system (*B*). The regulating valve (*H*) was used to regulate the intensity of flow of the continuous phase. Oil was pumped out of the pressure container (*E*) into the outer part of membrane module. The samples of emulsion for PSD analyses were collected through the drainage valve (*I*).

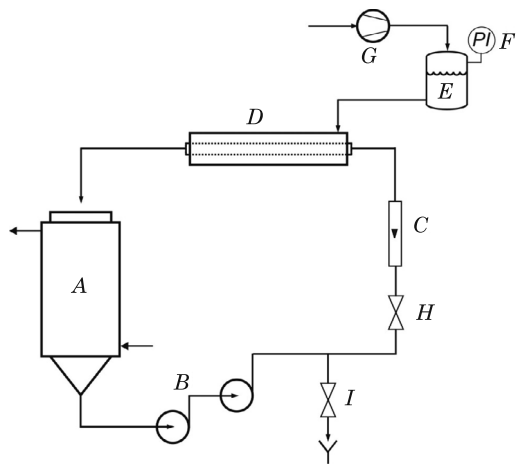

Fig. 1. Schematic diagram of the experimental setup: *A* – continuous phase supply tank with a heating/cooling coat, *B* – pump system, *C* – flowmeter, *D* – membrane module, *E* – pressurized oil tank, *F* – pressure gauge, *G* – air compressor, *H* – control valve, *I* – drainage valve

The process of emulsification was run at 30°C ± 0.5°C (a measurement with a HI 935005 one-channel thermometer with a *K*-type probe) at the input pressures of oil pumping and jets of flow volume of the continuous phase (Table 1). The volume jet corresponded to the shearing velocity of the internal surface of membrane channel in the range of $\gamma = 3800$ to 11000 s^{-1}, which was calculated from the following equation:

$$\gamma = \frac{8u}{d} \tag{1}$$

where:
γ – shear rate [s^{-1}]
u – flow velocity [m s^{-1}],
d – diameter of the membrane channel [m].

Particle size distribution of oil droplets in the emulsions was assessed by laser diffraction analysis using a particle size analyzer *Mastersizer 2000 (Malvern Instruments Ltd Great Britain.)*. The measurements resulted in a data set comprising: percentile readings of equivalent diameters: $d_{0.1}$, $d_{0.5}$, $d_{0.9}$ – i.e. diameters of the droplets at which 10, 50 or 90% of the sample is smaller

than the size measured, d_{32} – volume-surface mean diameter (so-called Sauter diameter) and d_{43} – weight-volume mean diameter. The distribution width was expressed as span value calculated as (JOSCELYNE and TRÄGÅRDH 1999):

$$Span = \frac{d_{0.9} - d_{0.1}}{d_{0.5}} \tag{2}$$

and its uniformity as the ratio:

$$\frac{\Sigma v_i \, |d(v,0.5) - d_i|}{d(v,0.5)\Sigma v_i} \tag{3}$$

where:
$d(v,0.5)$ is the median size of the distribution and d_i and v_i are respectively the mean diameter of, and result in, size class i (MALVERN MANUAL 2005).

These magnitudes have been analyzed as dependent variables in the full factorial experiment type 2^4 according to (MAŃCZAK 1976). The independent variables considered in the experiments are summarized in Table 2. The experiment design matrix (Table 2) was generated and analysed by the DOE module of *StatSoft, Inc.* [2011]. *STATISTICA (data analysis software system), version 10* software.

Table 2

Full factorial experiment design matrix type 2^4

Standard No. of experiment	Independent variables				Dependent variable vector
	X_1	X_2	X_3	X_4	y_i
1	–1	–1	–1	–1	y_1
2	–1	–1	–1	+1	y_2
3	–1	–1	+1	–1	y_3
4	–1	–1	+1	+1	y_4
5	–1	+1	–1	–1	y_5
6	–1	+1	–1	+1	y_6
7	–1	+1	+1	–1	y_7
8	–1	+1	+1	+1	y_8
9	+1	–1	–1	–1	y_9
10	+1	–1	–1	+1	y_{10}
11	+1	–1	+1	–1	y_{11}
12	+1	–1	+1	+1	y_{12}
13	+1	+1	–1	–1	y_{13}
14	+1	+1	–1	+1	y_{14}
15	+1	+1	+1	–1	y_{15}
16	+1	+1	+1	+1	y_{16}

Results and Discussion

The emulsions produced during the experiments contained oil droplets with diameters significantly higher than the nominal diameter of pores in the membrane (Figure 2).

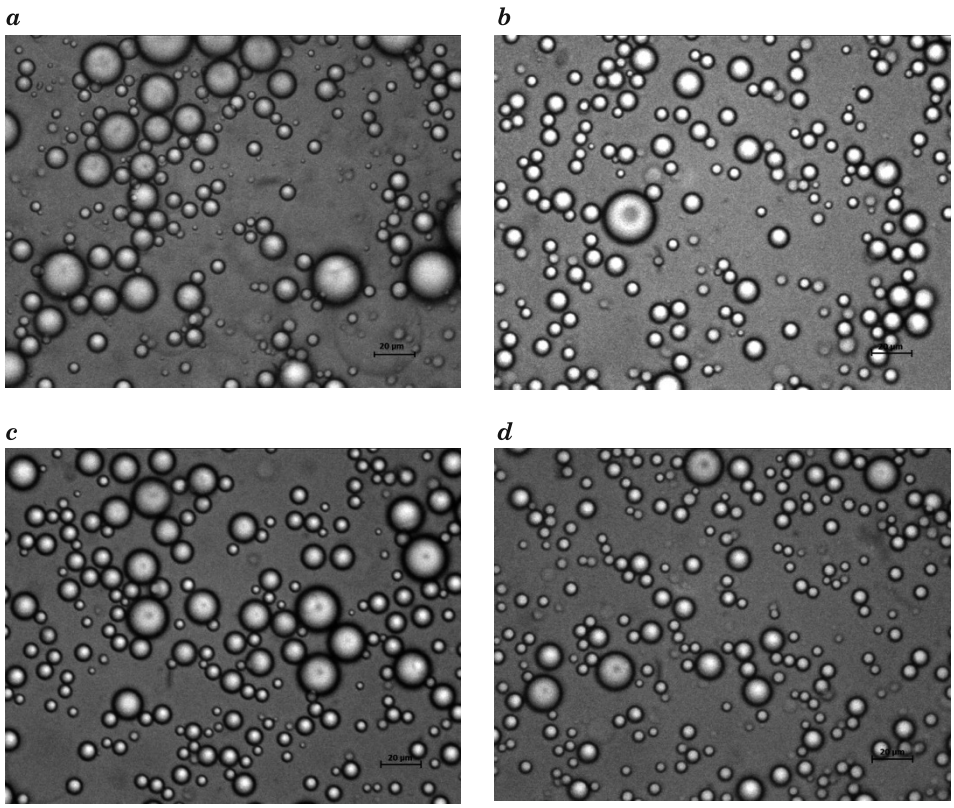

Fig. 2. Examples of emulsions; structure from experiments: *a* – no. 2; *b* – no. 4; *c* – no. 8; *d* – no. 11 (photomicrographs from light microscope taken at lens 5 × 40)

Although the smallest droplet size detected by laser during PSD measurements in the individual experiments ranged from 0.68 to 5.37 mm, their number in the sample was small and thus the diameter $d_{0.1}$, below which droplets constituted 10% of the volume, was 4.50–12.13 µm. Droplets of 9.87–30.04 µm represented by the size $d_{0.5}$ constituted the main fraction.

In the majority of cases, a monomodal distribution was generated (Figure 3).

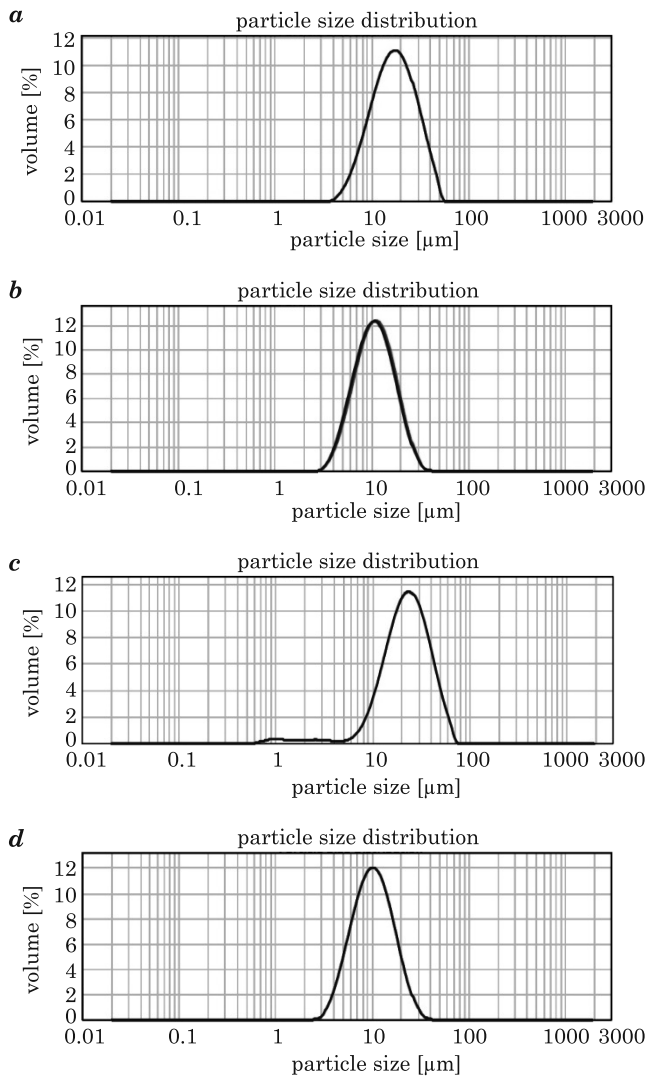

Fig. 3. Examples of particle size distribution from experiments: *a* – no. 2; *b* – no. 4; *c* – no. 8; *d* – no. 11

Only in experiments 3, 5 and 6, apart from the main fraction of droplets with a diameter of 3÷50 μm, was the second weak peak observed and it corresponded to the presence of droplets with diameters over 50 μm. The general characteristics of variability in the results of droplet size distribution measurements in the produced emulsions are presented in Table 3.

Table 3

Overall variability characteristics of oil droplet size distribution in the emulsions

Dependent variable	Droplet diameter d [μm]				Variability coefficient v(y)%
	min	max	average	standard deviation s(y)	
$d_{0.1}$	4.50	12.13	7.71	2.21	28.7
$d_{0.5}$	9.87	30.04	16.97	6.06	35.7
$d_{0.9}$	18.53	125.84	40.32	26.36	65.4
d_{32}	8.49	25.13	13.36	4.27	31.9
d_{43}	11.33	51.55	21.81	10.43	47.8
Span	1.033	3.785	1.783	0.674	37.8
Uniformity	0.321	1.120	0.601	0.223	37.1

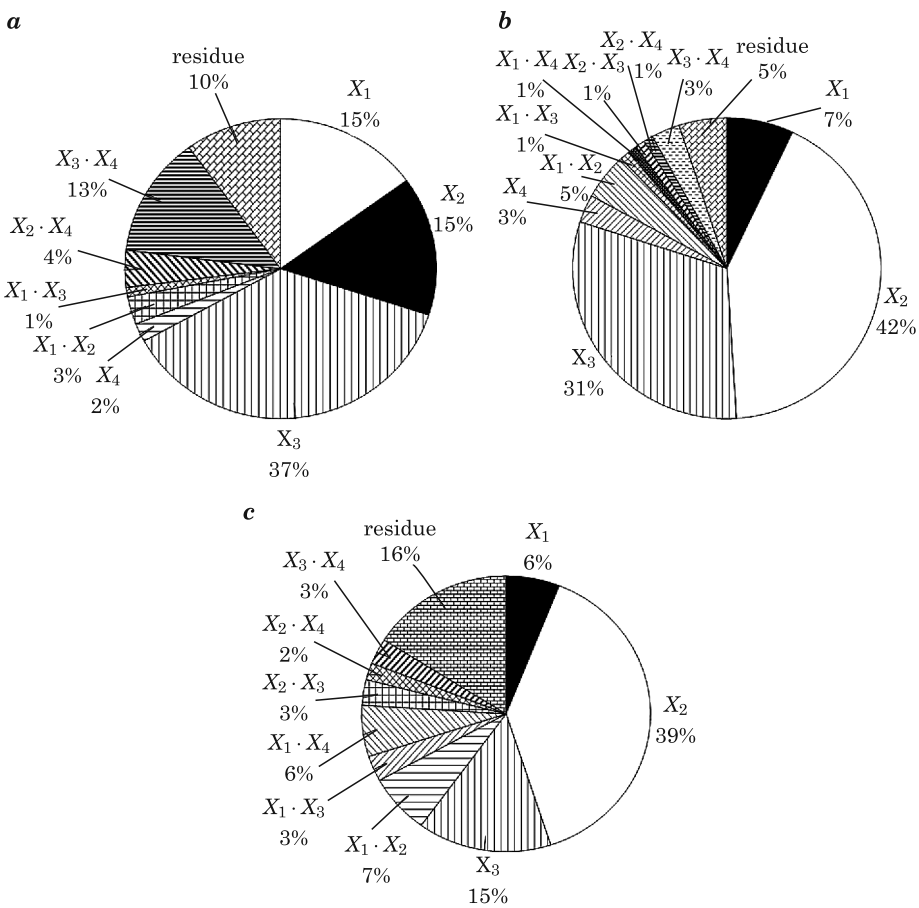

Fig. 4. The results of the variance analysis of "percentile" diameters of oil droplets expressed as percentage of total sum of squares explained by the particular effects: a – $d_{0.1}$; b – $d_{0.5}$, c – $d_{0.9}$

Both the presence of small diameter droplets and large droplets ($d_{0.9}$) in the emulsions resulted in varied parameters describing the span and uniformity of size distribution (Table 3). This variability could have been caused by the changes of initial sizes in a two-factor experiment (Tables 1 and Table 2), which is indicated by the analysis of variance involving all initial values in the experiment.

A graphic depiction of the results of analysis of variance carried out for the variability of percentile diameter of oil droplets is presented on Figure 4. The following factors had the greatest impact on the droplet size, defined as the percentile readings of equivalent diameters $d_{0.1}$, $d_{0.5}$, and $d_{0.9}$: protein concentration in the emulsion solids, kind of protein concentrate, and circulation flow-rate of the continuous phase. The role of these three factors in elucidating the variability of particle size distribution of the values $d_{0.1}$, $d_{0.5}$, and $d_{0.9}$ was 67%, 80% and 60%, respectively. The impact of individual process factors $X_1 \div X_4$ and their interactions on the size of $d_{0.1}$, $d_{0.5}$, $d_{0.9}$ descriptors (Figure 4) in the tested emulsions was 90% explained (at 10% share of the residual sum of squares (SS)), 95% (at 5% residual (SS)) and 84% (at 16% residual (SS)), respectively. The interactions of independent variables did not have any significant impact on the value of individual descriptors of droplet size distribution parameters in the produced emulsions. Interaction of the independent variables had no significant effect on the value of the droplet size distribution descriptors $d_{0.1}$, $d_{0.5}$, $d_{0.9}$. For the interaction of X_1X_4 factors, it was within 0–6%, for X_1X_2 1–7%, for X_3X_4: from 3 to 13% only for $d_{0.1}$ (Figure 4a). The impact of interactions of X_1X_3, X_2X_3, X_2X_4 factors on the analyzed descriptors of droplet size distribution was minor and below 5%.

A graphic depiction of the results of analysis of variance for the surface-based diameter (Sauter) d_{32} and volume-based diameter d_{43} is presented in Figure 5.

The following factors exerted the greatest impact on these values: protein concentration in the emulsion solids, the kind of protein concentrate and the circulation flow-rate of the continuous phase. The role of linear effects of all investigated process factors and their interactions on the size of Sauter's diameter (Figure 5a) and the volume-based diameter d_{43} (Figure 5b) of the tested emulsions was 87% and 90% explained, respectively. The total share of all variable conjugations that were important for d_{32} and d_{43} was 20% and 18%, respectively.

The results of a variance analysis for the variability in span and uniformity of droplet size distributions in the emulsions (generated with variable concentrations of WPC and MPC proteins), different shearing forces (resulting from varied efficiency in the flow of a liquid through the membrane channel) and variable pressure of oil supply (Figure 6), unambiguously indicate that these

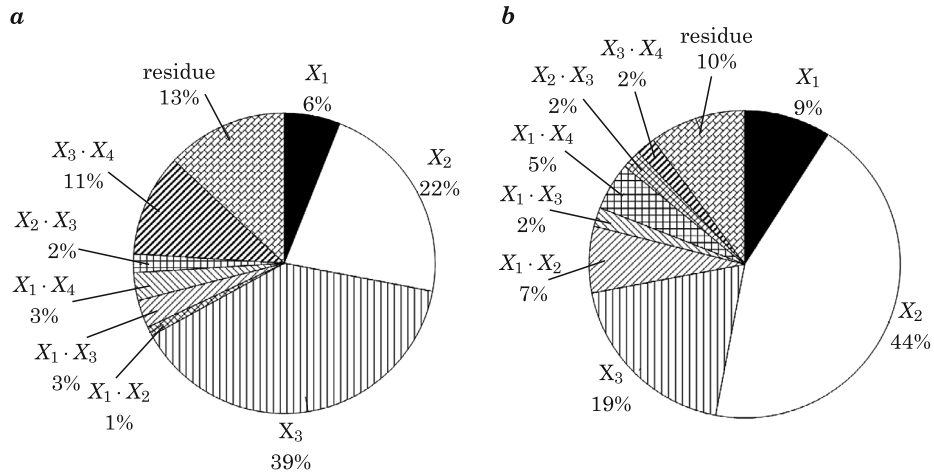

Fig. 5. The results of the variance analysis of: *a* – surface based (Sauter) diameter d_{32}; and *b* – volume based diameter d_{43}; explained by the particular effects under consideration

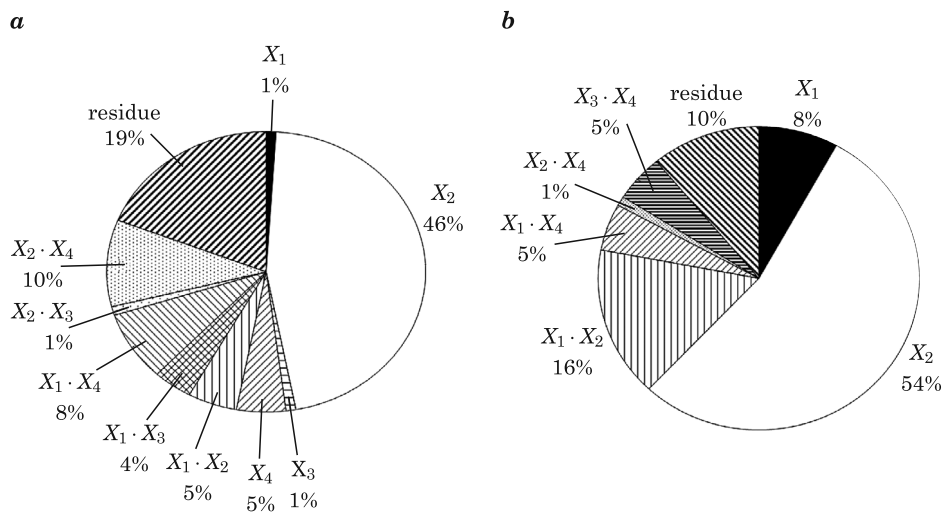

Fig. 6. The results of the variance analysis of: *a* – span of droplets size distribution; *b* – uniformity of the droplets size distribution; expressed as percentage of sum of squares explained by the particular effects under consideration

distribution features mainly depend on the type of protein used as an emulsifier (X_2). This observation is consistent with the research results of CHARON et al. (2011) and YE (2011). They used a different biopolymers as emulsifiers and received differing size distributions of droplets in emulsions produced.

The relations depicted on Figure 5 and Figure 6 are presented in more detail with the following regression equation:

$$\hat{y}_j = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_4X_4 + B_{12}X_{12} + B_{13}X_1X_3 + B_{14}X_1X_4 + B_{23}X_2X_3 + B_{24}X_2X_3 + B_{34}X_3X_4 \quad (4)$$

where:

$\hat{y}_j$ – denotes the estimated dependent variable
X_i – standardized independent variable
B_0 – is the average y_j value in the experiment
$B_{1–4}$ – are the linear regression coefficients for independent variables
$B_{12–34}$ – are the regression coefficients for the interactions of independent variables.

Numerical values of regression coefficients are collected in Table 4.

Table 4

Regression coefficients in the equation (4)

Variable		Droplet diameter d [μm]					Span	Uniformity
		$d_{0.1}$	$d_{0.5}$	$d_{0.9}$	d_{32}	d_{43}		
Coefficients	X_0	7.712*	16.967*	40.316*	13.357*	21.814*	1.783*	0.600*
	X_1	-0.842*	-1.591*	-6.390	-0.998	-3.028	-0.129	-0.038
	X_2	0.833*	3.787*	15.728*	1.959*	6.702*	0.880*	0.142*
	X_3	-1.293*	-3.263*	-9.845	-2.565*	-4.366*	-0.155	0.001
	X_4	0.322	1.093	-1.741	0.109	-0.009	-0.290	-0.060
	X_1X_2	-0.358	-1.315	-6.675	-0.307	-2.745	-0.286	-0.048
	X_1X_3	0.232	0.420	4.714	0.757	1.443	0.245	0.018
	X_1X_4	0.074	0.581	6.129	0.659	2.159	0.376	0.075
	X_2X_3	0.011	-0.494	-4.768	-0.509	-1.584	-0.144	-0.023
	X_2X_4	0.439	0.623	-3.297	0.177	-0.231	-0.422	-0.045
	X_3X_4	0.768	1.059	4.536	1.400	1.522	0.031	-0.023
	R^2	0.902	0.949	0.839	0.866	0.904	0.812	0.789

* Asterisk denotes effects significant at $p = 0.05$

They correspond with the variability of independent variables within limits <-1, +1> according to Table 1 as follows:

$$X_1 = \frac{C - 0.20}{0.10} \quad (5)$$

$$X_2 = -1 = \text{WPC}$$
$$X_2 = +1 = \text{MPC} \quad (6)$$

$$X_3 = \frac{U - 0.167}{0.10} \tag{7}$$

$$X_4 = \frac{p - 0.40}{0.10} \tag{8}$$

The results presented in (Table 4) clearly show that the most important factors influencing droplet size distributions in emulsions obtained using membrane as the dispersing system are: kind of protein used as the surface active agent and the flow-rate of the liquid in the membrane channel. Switching from WPC to MPC results in an increase of every characteristic diameter of the droplets in the emulsions and simultaneously causes widening of the distribution and a reduction in the uniformity index. It is thus concluded that the use of whey proteins in producing and stabilizing emulsions with a membrane technique is more beneficial than using total milk proteins (MPC), which is probably associated with the size of molecules or micelles in the aqueous phase and the kinetics of their adsorption on the interfacial surface (TCHOLAKOVA et al. 2004, RAYENER et al. 2005, YE 2011). Under the conditions of the conducted experiment, the investigated change of proteins in the dry matter of an emulsion had a significant impact only on diameters $d_{0.1}$ and $d_{0.5}$.

MCCARTHY et al. (2012) used whey protein concentrate (WPC-75) to stablizie sunflower o/w emulsions. They have demonstrated the relationship between the concentration of this emulsifier in the dry matter and the diameters of the emulsion formed during the emulsification of the dispersed phase. Based on results of this, researchers can be concluded that increasing the protein concentration favors the formation of small droplets of the dispersed phase.

The factor X_3 had a comparably significant impact on the size of oil droplets in the produced emulsions. This value determines the shearing velocity on the surface of a membrane, eqn. (1) and, consequently, the magnitude of shearing stresses that make oil droplets tear away from the margin of pores in a membrane (RAYNER et al. 2005).

For all analyzed dependent variables, the impact pressure at which oil was fed into an emulsion was statistically insignificant at $p = 0.05$.

The analysis of relations between all investigated diameters of oil droplets in the emulsions and the conditions of emulsification indicates that the median d_{32} and the Sauter's d_{32} and Herdan's d_{43} diameters react most strongly to the changes introduced to the parameters of the process.

Conclusions

1. The possibility of manufacturing of the o/w emulsions stabilized by milk-originated proteins using regular asymmetric ceramic membranes has been demonstrated.

2. Oil droplet diameters in the emulsions are more than 10-times greater than nominal pore size in the membrane and are influenced by the process variables.ą

3. The greater is the velocity of continuous phase / emulsion the smaller oil droplets are produced by this technique.

4. Whey proteins as the stabilizing agent enable better oil droplet size distribution than may be obtained with milk protein concentrate.

Translated by AUTHORS

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THE GERMINATION OF *ALICYCLOBACILLUS ACIDOTERRESTRIS* SPORES AND THE RELEASE OF DIPICOLINIC ACID UNDER SUPERCRITICAL CARBON DIOXIDE

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K e y w o r d s: *Alicyclobacillus acidoterrestris*, spore germination, supercritical carbon dioxide, dipicolinic acid.

A b s t r a c t

Alicyclobacillus acidoterrestris (AAT) is an acidothermophilic spore forming bacterium that causes the contamination of pasteurized fruit and vegetable juices. Since it survives typical heat treatment, the use of more effective techniques, such as supercritical carbon dioxide (SCCD), are considered for preserving juices.

Dipicolinic acid (DPA) is a universal component of bacterial spores and its release can serve as an indicator of spore germination.

The aim of this study was to determine the relationship between the release of DPA and the germination of AAT spores, initiated by SCCD. ąSamples of the spores of two AAT strains suspended in apple juice and pH 4.0 and pH 7.0 McIlvain buffers were treated with pressure of 10–60 MPa, at a temperature of 35–75°C for 30 min. The results showed that some of the process parameters, mainly the temperature and pH, strongly affected spore germination. The amount of released DPA correlated strongly (R = 0.928) to the number of germinated AAT spores.

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KIEŁKOWANIE PRZETRWALNIKÓW *ALICYCLOBACILLUS ACIDOTERRESTRIS* I UWALNIANIE KWASU DIPIKOLINOWEGO POD WPŁYWEM NADKRYTYCZNEGO DITLENKLU WĘGLA

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S ł o w a k l u c z o w e: *Alicyclobacillus acidoterrestris,* kiełkowanie przetrwalników, nadkrytyczny ditlenek węgla, kwas dipikolinowy.

A b s t r a k t

Alicyclobacillus acidoterrestris (AAT) należy do bakterii acidotermofilnych, tworzących przetrwalniki, które powodują psucie się pasteryzowanych soków owocowych i warzywnych. Ze względu na zdolność do przetrwania procesów pasteryzacji poszukuje się alternatywnych metod do ich inaktywacji, m.in. ditlenku węgla w stanie nadkrytycznym (SCCD). Kwas dipikolinowy (DPA) jest związkiem swoistym tylko dla przetrwalników, a jego uwalnianie do środowiska jest wskaźnikiem procesu kiełkowania przetrwalników. Celem badań było określenie korelacji między ilością uwalnianego DPA a procesem kiełkowania przetrwalników AAT, zainicjowanego przez SCCD. Przetrwalniki zawieszone w soku jabłkowym oraz w buforach McIlvain o pH 4,0 i pH 7,0 poddawano działaniu SCCD o ciśnieniu 10–60 MPa, w temperaturze 35–75°C, w czasie 30 min. Zaobserwowano, że niektóre parametry procesu, m.in. temperatura i pH, miały znaczący wpływ na proces kiełkowania przetrwalników. Ilość uwolnionego DPA była silnie (R = 0,928) skorelowana z liczbą kiełkujących przetrwalników AAT.

Introduction

The presence of *Alicyclobacillus acidotersestris* (AAT), a thermoacidophilic and spore-forming bacterium, in pasteurized acidic juices poses a serious problem for the processing industry. The typical sign of spoilage in contaminated juices, mostly apple and orange-is a characteristic phenolic off-flavour associated with the production of guaiacol (GOCMEN et al. 2005, JENSEN et al. 2003, SPLITTSTOESSER et al. 1994).

AAT spores demonstrate extremely high thermal resistance. The values of D95 in apple juice, which can be found in literature, vary from 1.85 to 15.1 min. The standard pasteurization process, which uses temperatures of 85–95°C, is therefore not effective against these bacteria (SPLITTSTOESSER et al. 1994, BAUMGART et al. 2000, STEYN et al. 2011 KOMITOPOULOU et al. 1999, SOKOŁOWSKA et al. 2008).

Generally spores are a unique dormant form of many types of bacteria, which develop through a remarkable series of stages to render the vegetative cells into forms that are naturally resistant to environmental conditions. Their resistance is clearly due to the cumulative effect of structural, chemical and biochemical features. The most important part of the spore is its numerous layers, which constitute up to 50% of the dry weight of the whole spore. These layers are composed of proteins containing large amounts of cysteine. The structure which is particularly important to the spore's resistance is the spore cortex. The spores are also highly dehydrated; water constitutes only 15% of the cells. Compared with vegetative cell spores, they contain more protein and 75% less carbohydrates. The structure of the spore contains a lot of dipicolinic acid, associated primarily with calcium ions, as well as other bivalent elements. Complex Ca^{2+} – DPA (calcium dipicolinate) may constitute up to 10% by dry weight of the spore. The resistance of the spores is also connected to the family of proteins known as SASP (small amide-soluble proteins). They alter the structure of the DNA, stiffening and straightening it by saturating the biomolecules on the outer side of the DNA helix (LEGETT et al. 2012, SETLOW et al. 2006).

To effectively kill spores, a temperature of 121°C is commonly used in a steam autoclave, whereas many other vegetative bacteria are killed at temperatures of between 60 and 100°C. To enhance the effectiveness of decontamination processes, it is recommended that spore germination be induced and that the spores be transformed into a vegetative form which greatly increases their susceptibility to inactivation with the use of physical or chemical agents, while their metabolic activity remains unchanged. It is a commonly accepted and well-documented theory that pressure triggers spore germination and during this process, the spores progressively lose their typical resistance and more readily become inactivated (SETLOW 2003, NGUYEN et al. 2010). Therefore, at present, the hope of a final solution to the *Alicylobacillus* problem is seen to be in unconventional preservation methods, based on elevated pressure, such as high hydrostatic pressure (HHP) or SCCD (supercritical carbon dioxide).

SCCD is considered a promising technique for food preservation because it requires much lower pressures than those used in HHP. The mechanism of inactivating vegetative bacteria with the use of SCCD has been widely investigated. The lethal action of highly pressurized CO_2 on bacteria can be explained by cell-membrane modification, reduction in the internal pH of the bacterial cell, the effects of CO_2 and HCO_3^- on metabolism, alteration of the intracellular electrolyte balance, and the extraction of vital constituents from cells and cell membranes. CO_2 is physiologically safe, inexpensive and easily available in high purity and in large quantities (BAE et al. 2009).

The deactivation effect of SCCD has been evaluated on spores of numerous species (SPILIMBERGO et al. 2003, WHITE et al. 2006, ZHANG et al. 2006A, 2007). Only a few researchers have found this technique effective for killing AAT spores in combination with heat. So far there has only been one approach to applying this technique for the deactivation of AAT spores which has been quite successful. A reduction of above 5 log was obtained after 20-min treatment at 70°C and 10 or 12 MPa (BAE et al. 2009).

The mechanism of destroying bacterial spores using supercritical carbon dioxide has not been elucidated and it is not clear whether the germination step is involved. ZHANG et al. (2006b,c) investigated the effect of this process on *B. atrophaeus* and after treatment at 40°C, 27.5 MPa detected no significant release of dipicolinic acid, thus indicating that germination was not triggered. However, in this experiment the spores were lyophilized and inoculated into paper, so the conclusions might not be applicable to spore suspensions. Moreover (FURUKAWA et al. 2004) found that high-pressure gaseous carbon dioxide treatment at 35°C, 65 bar for 120 min initiated the germination of B. *coagulans* and *B. licheniformis* spores. The effect was confirmed by phase-contrast microscopy.

The aim of this study was to analyse the process of the germination of the spores of two strains of AAT, initiated by an innovative food preservation technique-supercritical carbon dioxide (SCCD)-and to estimate the relationship between the release of DPA and the germination of AAT spores after SCCD treatment.

Material and Methods

The International Federation of Fruit Juice Producers' method (2004/2007) was used to isolate the AAT strains TO-169/06 and TO-117/02 from Polish concentrated apple juice. These strains were chosen from among eight wild strains tested in our previous study (SKĄPSKA et al. 2012, SOKOŁOWSKA et al. 2008, SOKOŁOWSKA et al. 2012A). The TO-117/02 was highly resistant to external factors whereas the TO-169/06 strain was the sensitive one.

Spores were produced based on a method described by SOKOŁOWSKA et al. (2012B) and were then suspended in apple juice (11.2°Bx, pH 3.4) or in McIlvain buffer solutions of pH 4.0 and pH 7.0. The number of spores in the suspensions was approximately 6 log cfu/mL for determining spore germination and approximately 9 log cfu/mL for determining the release of dipicolinic acid.

Samples of AAT spores were treated with supercritical carbon dioxide using apparatus for the extraction with supercritical fluids Speed SFE®, Applied

Separations, USA. The volume of the treatment chamber was 10 mL, with working pressure of up to 69 MPa and temperatures of up to 120°C.

Seven-millilitre samples tubes were exposed to supercritical carbon dioxide at pressures of 10, 30, 60 MPa at temperatures of 35, 50, 75°C for 30 min. The temperature was measured in the chamber. The assays were performed using two independent samples.

The spread plate method on BAT-agar (Merck) with incubation for 5 days at 45°C was used. Pressure-induced germination was the difference between the plate count before and after SCCD treatment, followed by heat treatment at 80°C for 10 min (VERCAMMEN et al. 2012, SOKOŁOWSKA et al. 2015). The results were expressed as log (cfu/mL).

The quantification of the DPA concentration in the samples was performed using the HPLC method (WARTH 1979). A Waters 2695 Separations Module with Waters 2996 Photodiode Array Detector system and SunFire C8 Column, (5 μm, 4.6 mm x 250 mm) with SunFire C8 Guard Pre-column, (5 μm, 4.6 mm x 20 mm) were used. Elution was with 1.5% *tert*-amyl alcohol in 0.2 M potassium phosphate, pH 1.8, at 25°C. To determine the total amount of DPA in the spore suspensions, 3 mL of each individual batch (in 0.05 M PBS buffer pH 7), was sterilized at 121°C for 20 min and then analysed (REINEKE et al. 2013).

An analysis of the variance and Duncan's multiple-range test, using StatSoft® Statistica 7.1, was used to test the significance of the differences ($p<0.05$). The assays were performed using two independent samples. The bars on the figures indicate the mean standard deviation for the data points. Microsoft Office Excel 2007 was used for linear regression and to calculate the coefficient of determination *(R^2)* and coefficient of correlation (r).

Results and Discussion

The effect of pressure and temperature on the release of DPA and germination of the spores are presented in Figures 1–2. Two strains of *A. acidoterrestris*, treated with pressures of 10, 30 and 60 MPa at temperatures of 35, 50 and 75°C were used in this study.

The results indicate that the germination of AAT TO-169/06 spores in apple juice depended on the pressure and temperature. It was observed that pressure of 10 MPa applied at 50°C was not efficient for spore germination, which was 0.56 log under these conditions. Better results were achieved at 75°C when 1.5 log of germinated spores was observed. When the apple juice was treated with pressure of 30 MPa at 50°C and 75°C, the germination of the spores was 0.75 log and 1.8 log respectively. At 35°C germination was significantly less than at 50°C and after 30 min at 60 MPa achieved only 0.42 log in apple juice

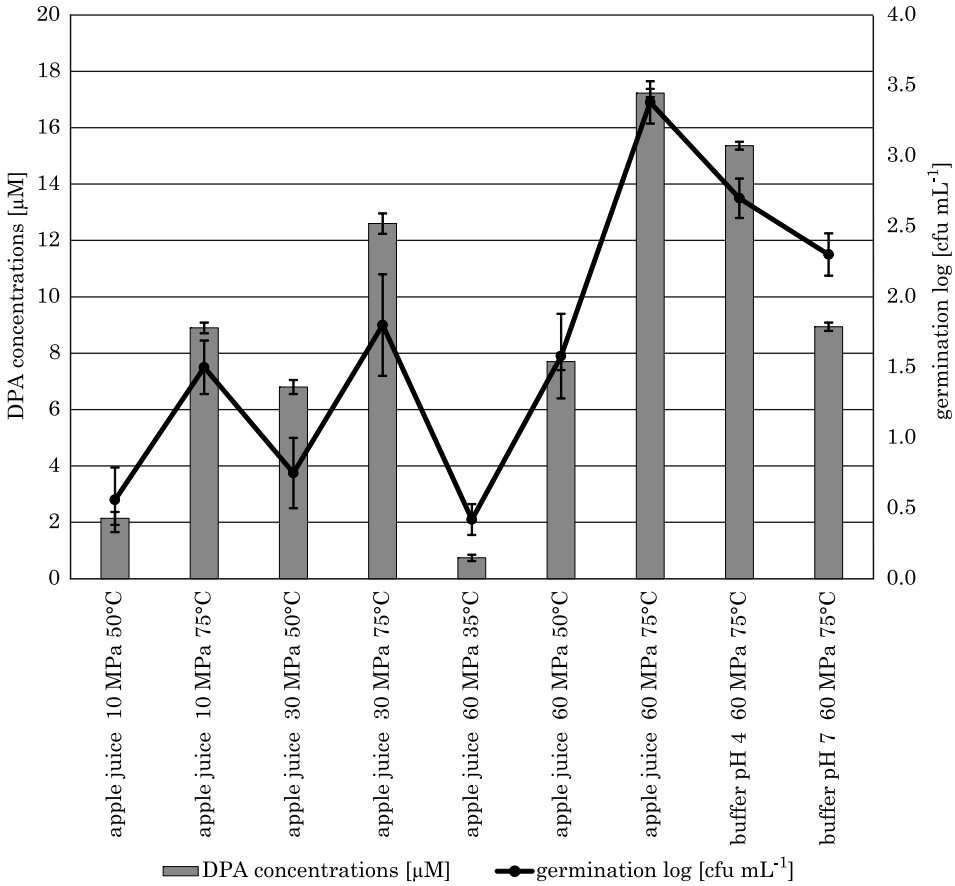

Fig. 1. Germination and DPA released from AAT TO-169/06 spores after 30 min of SCCD treatment

($p < 0.05$). When the temperature was increased to 50°C germination achieved 1.58 log ($p < 0.05$). The largest number of germinated spores-3.38 log-was observed during the experiment carried out using the highest pressure and temperature values, 60 MPa and 75°C ($p < 0.05$) – Figure 1.

To study the effect of pH on the germination of AAT TO-169/06 spores, a temperature of 75°C and pressure of 60 MPa were selected. The results of the process conducted in low (4.0) and neutral (7.0) pH buffer and real food-apple juice (pH 3.4)-were compared (Figure 1). Germination in the pH 4.0 buffer achieved 2.7 log and under the same conditions, however in the pH 7.0 buffer only 2.3 log of spores germinated ($p < 0.05$).

The results achieved in this part of our study show that low pH and the nutrients present in commercial apple juice can promote the germination of AAT spores during SCCD treatment. The same phenomenon was observed in

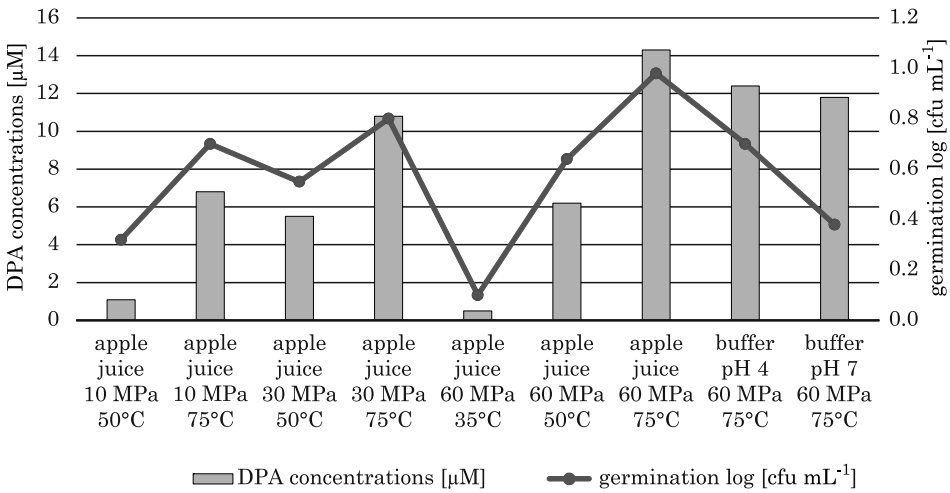

Fig. 2. Germination and DPA released from AAT TO-117/02 spores after 30 min of SCCD treatment

apple juice during treatment of these spores using high hydrostatic pressure (PORĘBSKA et al. 2015a,b, SOKOŁOWSKA et al. 2013, 2015) and in tomato juice (VERCAMMEN et al. 2012).

The total amount of DPA present in AAT TO-169/06 spores (released during sterilization) was 50.3 μM and 42.7 μM for the TO-117/02 strain (data not shown). The amount of DPA released from the spores after SCCD processing was strongly affected by the pressure and temperature and corresponded with the degree of germination of the spore population (Figures 1–2). When the processes were carried out at 50°C in apple juice, the highest amount of released DPA – 7.7 fM (15.30% of total DPA) was observed at 60 MPa for spores of the AAT TO-169/06 strain (Figure 1). When lower pressures, 10 and 30 MPa were used, the amounts of DPA released were slightly lower and reached 2.14 μM and 6.8 μM respectively. Temperature strongly stimulated DPA release, and it achieved 0.74 μM at 35°C and increased to 17.23 μM at 75°C (34.25% of the total DPA) when the process was conducted at 60 MPa. The effect of pH on the release of DPA was also observed. An acidic environment stimulated the release of DPA as well as germination. The DPA released at pH 4.0 achieved 15.36 μM, but only 8.94 μM at pH 7.0.

The same experiments were conducted with the second strain of AAT TO-117/02, giving similar results with regard to the spore germination and DPA release trends, however this strain turned out to be far more resistant to SCCD treatment (Figure 2). At 35°C only 0.1 log spores germinated in apple juice after 30 min at 60 MPa. Treatment at 50°C slightly supported germination in apple juice, and resulted in 0.32, 0.55 and 0.64 log of germinated spores after

processing at 10, 30 and 60 MPa ($p < 0.05$). The highest germination – 0.98 log-was achieved in apple juice when 60 MPa was used at 75°C. The effect of pH on germination was also observed for the TO-117/02 strain spores. In pH 4.0 buffer, germination achieved 0.8 log when 60 MPa at 75°C was used. A neutral pH inhibited germination, and only 0.38 log of spores germinated under the same conditions (Figure 2). Similar results were observed by SOKOŁOWSKA et al. (2015) in apple juice.

Similarly to the observations made for the previous strain, the amount of DPA released from TO-117/02 was proportional to the number of germinated spores. During 30 min of SCCD treatment with 60 MPa at 50°C, the amount of DPA released was 6.2 μM (14.52% of the total DPA). The temperature affected the DPA release process. The amount of DPA released in apple juice at 35°C after treatment at 60 MPa was 0.49 μM DPA and increased to 14.3 μM at 75°C (33.49% of the total DPA). The acidic environments also stimulated the release of DPA from the TO-117/02 spores, as well as germination. At pH 4.0 the amount of DPA released achieved 12.4 μM, and 6.2 μM at pH 7.0 (Figure 2).

The data obtained on the release of DPA corresponded with the level of both AAT strain TO169/06 and TO-117/02 spore germination. Similar results were obtained by other authors for *Bacillus subtilis* spores (REINEKE et al. 2013).

The relationship between the release of DPA after SCCD treatment and the pressure-induced germination of *A. acidoterrestris* spores is presented in Figure 3. A strong positive correlation ($R^2 = 0.863$, $r = 0.928$) was achieved.

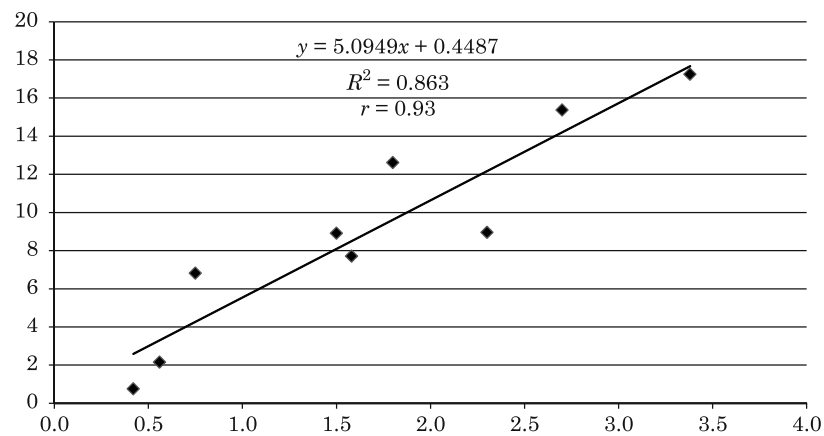

Fig. 3. DPA released from the spore suspensions vs the number of germinated spores of *A. acidoterrestris* after SCCD treatment

The significant variations in the DPA released from the population observed in the present study for different AAT strains are consistent with the results of a study by MARGOSCH et al. (2004) who also reported significant differences in the DPA levels between the populations of spores of different *Bacillus* species and *Clostridium* species (FRANCIS et al. 2015). These levels could also vary between the individual spores in a spore population, perhaps due to cellular heterogeneity (HUANG et al. 2007, LUU et al. 2014).

To summarize, the results obtained once again confirm that the resistance of AAT to pressure and elevated temperatures is strongly strain-dependent. The phenomenon of differentiation of the strains within the AAT species was also shown by BEVILACQUA et al. (2007). These processes may be associated with the complex spore structure and the related existence of a number of mechanisms of gene expression that govern the germination of spores. This is the first study which confirms the release of DPA during SCCD induced germination of AAT spores.

Conclusions

SCCD can induce the germination of AAT spores. Some of the process parameters, mainly temperature and low pH, strongly affected spore germination. The ability of spores to germinate under SCCD depended on the strain. The nutrients present in apple juice probably promoted the germination of AAT spores after pressurization using SCCD. The process of DPA release from the spores depended on the strain, pressure and temperature used. The amount of DPA released had a strong positive correlation to the amount of germinated AAT spores.

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WAKE PARKS IN POLAND – CURRENT STATE, CONDITIONS AND PROSPECTS FOR DEVELOPMENT

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K e y w o r d s: lift for water sports, wakeboard, constructional solutions, formal requirements, environment protection.

A b s t r a c t

Wake parks are structures for recreational and professional wakeboarding, a sport where a person rides on a towed board. The cradle of wakeboarding started in the USA, where the beginnings of this sport can be traced back to the mid-1980s. The first Polish wake part was opened in Augustów in 1999. Our aim was to collate information about wake parks in Poland, and to analyse the environmental as well as legal grounds for their development. The history of Polish wakeboarding is short but dynamic. Most wake parks with cable systems were created in 2013–2014. Today, there are over 40 wake parks across the country. This growth is stimulated by newer and less expensive technical solutions. Most constructions are based on mobile, two-tower installations, which are easy to assemble and maintain. Wake parks attract visitors to regions which until now have not been perceived as destinations for water tourism and recreation. However, building a wake park on a lake or other water body must comply with legal regulations pertaining to nature conservation. Two-tower wakeboarding installation is eco-friendly and can be installed in silence zones as well as nature protected areas.

WAKEPARKI W POLSCE – UWARUNKOWANIA I PERSPEKTYWY ROZWOJU

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Abstrakt

Wakeparki są to konstrukcje przeznaczone do rekreacyjno-sportowego uprawiania wakeboardingu, czyli pływania na holowanej desce. Kolebką światowego wakebordu są Stany Zjednoczone, a jego początki datowane na połowę lat 80. W Polsce pierwszy wakepark powstał w 1999 r. w Augustowie.

Celem pracy była charakterystyka wakeparków w Polsce oraz analiza środowiskowych i formalno-prawnych uwarunkowań ich rozwoju. Historia polskiego wakeboardingu jest krótka, ale rozwój bardzo dynamiczny. Większość wakeparków z wyciągami linowymi do sportów wodnych powstała w latach 2013–2014. W Polsce funkcjonuje obecnie ponad 40 takich obiektów. Sprzyja temu wprowadzanie nowych i tańszych rozwiązań konstrukcyjnych. Większość instalacji oparta jest na mobilnym i prostym w obsłudze systemie dwusłupowym. Wakeparki stają się atrakcją w regionach, które dotąd nie były kojarzone z turystyką i rekreacją wodną. Czynnikami warunkującymi ich rozwój i lokalizację w obrębie naturalnych zbiorników wodnych są m.in. regulacje związane z ochroną środowiska. System dwuwieżowy jest przyjazny dla środowiska. Można go instalować w strefach ciszy i na obszarach chronionych.

Introduction

Wake parks are installations and constructions created for recreational and professional wakeboarding. The name "wakeboarding"coins two words: "a wake" (a track left by a ship in water) and "a board;. Wakeboarding can be defined as "riding on a board behind a motorboat". The sport was created by combining water skiing, snowboarding and surfing. It is not easy to think of a good word in Polish for a wake park, but the name can be understood as a cable water park. What all wake parks have in common is a system for pulling or lifting. Other elements are various water features, such as jumps, kicks, platforms, etc., located on both sides of the cableway.

The cradle of wakeboarding is the United States of America, and the beginnings of this sport date back to the mid-1980s. In 1985, Tony Finn, a surfer from San Diego, invented "a skurfer", which was a combination of a surfing board and water skis. The prototype lacked foot straps, but once they were added, various maneuvers began to be developed. First, skiboarding appeared. In 1990, the first US championships in surfer riding were aired on television. In the years to come, new materials and construction solutions have been tried in board making. With time, "wakeboarding" was adopted as the official name of the new sport (E-WAKEBOARD 2014).

Wakeboard as a sports discipline was officially recognised in 1992. In 1993, the World Publications issued the first "Wakeboarding" magazine. Soon, other magazines appeared (E-WAKEBOARD 2014). A turning point for wakeboarding was in 2000, when various obstacles were created, and these added the so-called "street-like atmosphere" to the sport. Work continues on constructions which will allow wakeboard riders to perform new and more advanced tricks. In 2005, wakeboarding became part of the World Championships. For

many young people, this sport is a lifestyle and therefore can be seen as part of a youth culture (NAORNIAKOWSKI 2011).

Wakeboarding came to Poland in the 1990s. Prior to that, water skiing, another form of water sport, had been practised in our country. Waterskiing as a recreational activity appeared in the 1950s, although the first public show of riding on two water skis took place back in the 1920s (NAORNIAKOWSKI 2011). The first sports clubs associating water skiers were established in the 1960s, and the following developed most vigorously: KSW „Hutnik" Pogoria, AKS "Sparta" Augustów, LOK Szczecin and LOK Bytom. Precursors of waterskiing in Poland were Zbigniew Naorniakowski, Bolesław Talago, Zygmunt Kowalik, Jurand Jarecki and Krystyna Jarecka. Owing to their effort, the first Polish Waterskiing Competition was held in Augustów in 1965. At that time, waterskiing lifts were very rare, so riders were towed by motorboats (NAORNIAKOWSKI 2011). The first waterskiing lift was constructed in Augustów, in 1999. Today, it is also used for wakeboarding. The last years of the 20th century witnessed a dynamic growth of cable water sports across Europe. Waterskiing and wakeboarding were no longer an elite form of recreation and amateur sport. Once the equipment became more easily available, wakeboarding gained popularity. For several years, the European Championships and – more recently – the Polish Chamionships have been held regularly (NAORNIAKOWSKI 2011).

Our aim has been to find out the current number of wake parks in Poland and to explore what technical solutions they employ. Another purpose has been to analyse the underlying conditions for wake park building and development.

Materials and Methods

Wake parks are a relatively new concept in Europe. Such issues as their location, underlying development conditions and impact on nature have not been researched thoroughly thus far. Hence, a lack of analytical descriptions or reviews concerning these problems. There was applied factual analysis method in this study. This method is commonly used in the socio-economic research, including those based on archival materials (OLENSKI 2001, KLESZCZOWA and GWOŹDZIK 2009). Factual material was collected by query of the literature sources and materials dispersed in the websites. Literature sources and materials dispersed in the websites are commonly used to determine the status of objects, processes or events (OLENSKI 2001, FRANCZAK 2014). The data resources were**:** electronic databases of libraries**,** databases of legal regulations**,** websites and web portals designed for wakeboarding enthusiasts **(**http://pl.youcanwake.com; http://wakestyle.pl; http://wakefocus.ibiss.pl;

http://iwakeboard.weebly.com; http://e-wakeboard). Information was also obtained through direct interviews with investors and representatives of companies that sell and service cable water lifts (Wakepark SA.; SesitecPolska; WakeStation Polska).

Results and Discussion

Engineering solutions in wake parks

Currently, there are two types of constructions built in wake parks: a full size cable system and a two-tower installation (2.0 system). A full size cable system typically consists of 4, 5 or 6 towers, which can be 14 meters high. The cables and anchores ensure the stability of the whole construction. Because of the size of a whole installation, the minimum surface area of a water body is 5 to 10 ha. Artificial water basins of at least 1.2 m in depth are preferred. Special constructions for anchoring the towers may be required when the depth exceeds 10 meters. Also, when water levels fluctuate within 1 meter or so, the starting platform may need special engineering solutions. A full-size cable lift is typically an immovable installation. Depending on the size of an installation and local conditions, it usually takes 3 to 4 weeks to assemble the whole structure. Six-tower systems are most popular among riders because they ensure high comfort of wakeboarding rides. The cable is usually well stretched and does not jerk a rider who is passing a turning point. While the average speed of the running cable is 30 km/h, it can be adjusted within the range of 0 to 60 km/h (SESITEC 2015a).

A two-tower system (2.0) is a light and portable construction, which does not require to install the supporting structures in water. A two-tower installation can be used alone or as an additional facility in larger water parks. System 2.0 has been specially designed for wakeboarding and allows for a continuous ride over several runs. The installation consists of two towers (pylons) standing on land or in water (on the bottom of a lake). They are immobilised with concrete anchors, each approximately 1.5 x 1.5 x 1.5 m in size. Towers up to 7 m high require two anchors, each weighing 5.5 tons. Higher towers will need heavier anchors, 8 tons each. The towers can be raised on two opposite shores of a water body or along the shoreline. It is recommended to install two-tower systems in water bodies of the minimum depth of 1 m, 9–230 meters long and 25–36 meters wide. The minimum length is 60 m. The whole system is powered by a three-phase (400 V) or two-phase (230 V) electricity current, and the latter requires a phase convertor and transformer. The installation takes up to 2 days. This is a modular construction, which makes it mobile and multi-

functional. Technical solutions also include automated maintenance and a remote control, which can be operated from a distance of around 100 meters away from the engine tower. Electronic control systems guarantee efficient operation of the wakeboarding system (YOUCANWAKE 2014a).

Wake parks in Poland – the current state

The history of wake parks in Poland goes back to the turn of the 20th and 21st century. The first wake park was created on Necko Lake in Augustów, in 1999. The second, started in 2001, was constructed on Zalew Zemborzycki, an artificial water basin in Lublin. In the late 2000s, there were five wake parks in Poland, and they were all equipped with a by 4- or 5-tower cable installation made by the German company Sesitec (Table 1). The length of riding runs varied from 680 m in Augustów to 1100 m on Trzesicko Lake in Szczecinek. The latter wake park was opened in 2008 and it is still one of the longest wakeboarding systems in Europe. In total, 12 riders can use the cable system simultaneously. The sixth full-size system was installed in 2014, in the wake park Rueda Januszkowice. This was the first, and still remains the only six-tower system in Poland. The installation is part of a large recreational and sports centre located on Zbiornik Januszkowicki (an artificial water body), which features many cafes and accommodation facilities. Next to the six-tower cable system, Rueda Januszkowice Wake Park has a 2.0 system installed, which is used for learning and improving wakeboarding skills as well as for sporting events (KOMPLEKS RUEDA 2015).

Table 1

Wake parks in Poland, equipped with Sesitec cable systems

No. of wake park	Year of installing the system	Name and location of the wake park
1.	1999	Augustów
2.	2001	Reland Lublin
3.	2008	Ostróda
4.	2008	Szczecinek
5.	2010	Margonin near Chodzież
6.	2014	Rueda Januszkowice near Opole

The vast majority of the Polish wake parks have two-tower systems (2.0). Such installations first appeared in Poland in the early 2010s (Table 2). In 2011–2012, there were 12 two-tower installations built, all employing System 2.0 developed by Sesitec. Twelve more wake parks were created in 2013,

Table 2

Wake parks in Poland with two-tower installation system

Number of wake park	Year of system installation	Name and location of the wake park
1.	2011	Port Rynia near Warszawa*
2.	2011	Żukowo near Gdańsk*
3.	2011	Wakeprojekt Sławutówko near Rumia*
4.	2011	Wierzbowe Ranczo – Wakehouse near Grodzisk Maz.*
5.	2011	Wrocław*
6.	2011	Kraków Kryspinów*
7.	2012	Bydgoszcz Myślęcinek*
8.	2012	Wakespot Poznań*
9.	2012	Szczecin – Floating Park Głębokie*
10.	2012	Wake up Silesia Świętochłowice*
11.	2012	Wakepoint Kraków Bagry*
12.	2012	Opole*
13.	2013	Wakecity Stęszew*
14.	2013	Wakefamily Trzciany near Warszawa**
15.	2013	Łomianki***
16.	2013	Solina**
17.	2013	Wake Roll near Łódź**
18.	2013	Wakeart Krubin near Legionowo**
19.	2013	Kapitan wake Rokitnica near Łódź**
20.	2013	Wake Lake Hubertus near Katowice*
21.	2013	Skorzęcin near Gniezno*
22.	2013	Wakeport Kaniów near Kielce*
23.	2013	Wakeplace Śrem*
24.	2013	Wawa Wake Konstancin Jeziorna***
25.	2014	Rueda Januszkowice near Opole*
26.	2014	Giżycko**
27.	2014	Wisła Zalesie near Piaseczno***
28.	2014	Wake up Radzymin**
29.	2014	Wake for Friends Adamów near Grodzisk Maz.*
30.	2014	Wake Skate Park Książenice near Warszawa*
31.	2014	Czeszki Gliwice*
32.	2014	Wake Zone Stawiki near Sosnowiec*
33.	2014	Koszalin**
34.	2014	Kuwaka Pobiedziska**
35.	2015	Nice Bay Olsztyn**

* – Sesitec; ** – Primus Cable; *** – Wakestation

and seven of these used installations offered by manufacturers other than Sesitec. There were five installations with system called Primus Duo, made by Polish branch of American company Primus Cable, and two of them – with two-tower straight-line cablesystem created by the Lithuanian company Wakestation.

In 2014, ten new installations with two-tower system were started, half of which were manufactured by Sesitec, while one was supplied by Wakestation. Just one more new wake park was found to open in the first half of 2015. This wake park, called Nice Bay, was created in Olsztyn and equipped with a two-tower system construction made by Primus Cable. All across Poland, new wake parks are being planned and prepared to be opened.

At the moment, there are 41 wake parks operating in Poland, of which 35 have two-tower systems. Noteworthy are wake parks with double two-tower systems, such as Wrocław, Kraków (Kryspinów), Wake City Stęszew and Wake Up.

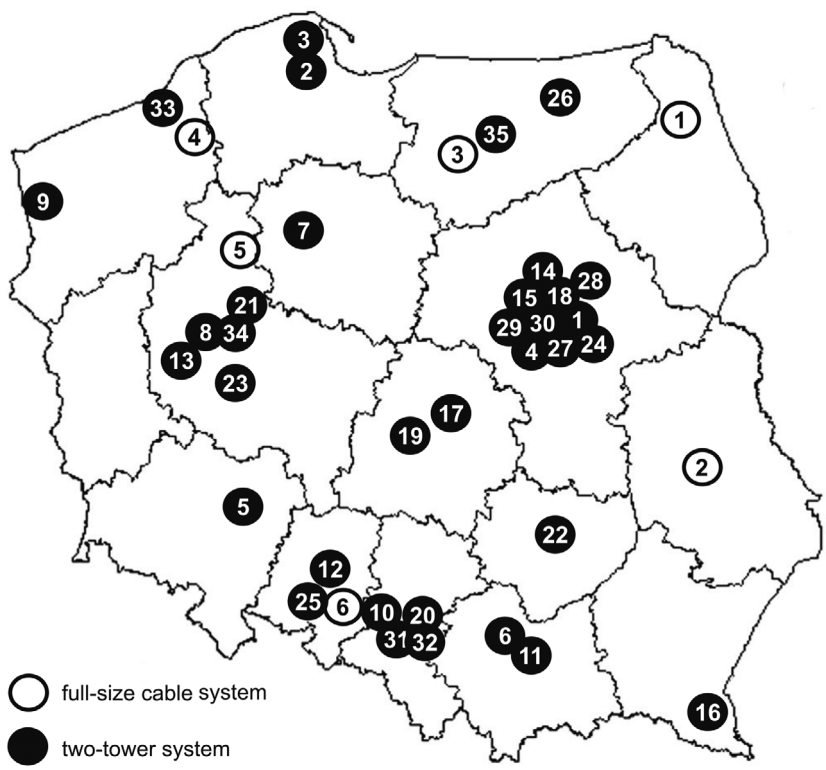

Fig. 1. Location of wake parks in Poland (numbers on the map correspond to the numbers in Table 1 and Table 2

Source: the authors, based on analysis of scattered information sources

Besides, Wakepark Wrocław plans to add a full-size cable installation. Currently, most wake parks are situated in central Poland, in Mazowsze (10 two-tower systems within less than 100 km distance from Warsaw), and in the west, in Wielkopolska (5 two-tower systems less than 100 km from Poznań, and one full-size cable system in Margonin) – Figure 1.

The environmental, formal and legal considerations in wake park development

Obviously, investors intending to build a wake park need enough capital, but equally important they must comply with all formal and legal requirements. While each project is a specific case, most procedures are standard. The choice of a location and a water body, including its surface area, depth, water quality and current tourist and recreational functions, is of strategic importance. When looking for the best location, the key question is the transport accessibility of the immediate surroundings and the quality of existing infrastructure. Equally important is to explore the possibilities of buying or leasing the land. The subsequent stage is to make an inventory of the natural and man-made features. The investment project cannot violate the provisions of the binding spatial management plans or legal regulations governing nature conservation (Ustawa z 27 marca 2003 r... Dz.U. 2015 poz. 528). The fact that water skiing installations are classified as ones which potentially produce significant impact on nature (Rozporządzenie Rady Ministrów... Dz.U. 2010 nr 213 poz. 1397) gives rise to significant legal consequences. As a result, it is mandatory to obtain a decision on environmental conditions (Ustawa z 3 października 2008 r... Dz.U. 2013 poz. 1235). In line with the so-called screening procedure, it is verified whether a Report on Environmental Impact will be in order. This procedure entails an assessment of the type and specification of an investment project (here, construction of a wake park), its location as well as the type and extend of potential impact on the environment. Such a report is one of the steps in the general environmental impact evaluation. It can also be included in the proceeding for issuing a decision about environmental conditions.

If a location chosen for a future wake park lies in a nature protected area (a national park, a nature reserve, a landscape park, a protected landscape area, Natura 2000 sites, ecological utility, a nature and landscape complex, a documentation site), it is obligatory to make an inventory and assessment of nature resources. It is also required to analyse the impact of a planned investment on the environment. Additionally, it is necessary to take into account the influence of the project on ecological corridors, especially in terms of the cohesion of Natura 2000 network.

Sometimes, the presence of protected animals or plants is discovered after works on the project have been commenced, in which case the investor must apply for a permit to perform action prohibited towards legally protected animal or plant species. The application should be submitted to the proper Regional Directorate for Environmental Protection. If a wakeboarding installation is to be anchored on the bottom of a water body, obtaining a water legal permit is also mandatory. All relevant regulations are comprised in Water Law of 18 July 2010 (Ustawa z 18 lipca 2001 r... Dz.U. 2015 poz. 469).

If the construction of a wake park necessitates the removal of some trees or shrubs, a permit for tree felling has to be sought. The legal regulations governing this issue can be found in the Act on Nature Protection, of 16 April 2004 (Ustawa z 16 kwietnia 2004 r... Dz.U. 2013 poz. 627).

The final step is to obtain a building permit. The legal regulations for submitting an application are specified in Building Law of 7 July 1994 (Ustawa z 7 lipca 1994 r... Dz.U. 2013 poz. 1409).

Having obtained all the required permits and completed all the procedures, an investor can now proceed with the installation, which usually takes a few days and consists of assembling the elements of the supporting construction (towers and anchors) and preparing its installation within the water body or in its immediate surroundings. Depending on the investor's vision, other facilities can be built, such as cafes, scenic platforms, a swimming and sunbathing beach, sports facilities, etc.

Prospects for development of wake parks

The development of wake parks depends on the determination of investors and groups of people interested in the growth of this sports discipline. The latter are often youth culture members, involved in extreme sports and recreational activities. Tourism and extreme sports are gaining popularity owing to mass media, which create the fashion for active lifestyle and extreme experiences (ADAMCZYK 2011). An investment into a wake park reflects the economic situation. New wake parks are likely to be created and existing ones developed if the general economic conditions are favourable. Any risk of decelerated economic growth, decrease in the corporate investment level, or an increase in debt affects negatively the demand (ROGOWSKI and MICHALCZEWSKI 2005). Risk investment analyses were made in 2010, when Wakepark S.A. (WPK) – the first company in Poland directly engaged in wake park development was established. On 28 September 2012, the company appeared on the Warsaw Stock Exchange. Wakepark S.A., which was created by water sports enthusiasts, now manages the WakePark Wrocław. The identified sources of risk in the company's operation are: problems in the acquisition of land for

wake parks and high fixed costs, including rent for leased land; seasonality of the business activity; external, independent factors affecting the financial results; competition on the recreation and sports market. Due to the specific character of the above risk, the company is unable to manage it (WAKEPARK S.A. 2014).

The economic efficiency of all wake parks in Poland depends on weather. The key consideration is the number of sunny days in a year, which is almost impossible to predict when making an economic efficiency analysis of wake parks. The question of weather is closely connected with other risk factors. Another constraint is the seasonal character of wakeboarding, done mostly in summer and during holidays. The maximum duration of a season in wake parks is from mid-April to the end of September.

The first wakeboarding installations that sprung in Poland were full-size cable lifts based on four or five towers. Since 2011, a rapid growth has been observed in the number of lifts based on less expensive and mobile two-tower system constructions. At around the same time, the monopolistic position of Sesitec was undermined by Wakestation, which broke into the European market in 2011. Wakestation offers constructing and servicing full-size (multi-tower) and two-tower system installations. The latter were advertised as solutions which required less red tape and had competitive prices, i.e. 30% lower than prices quoted by their competitors (WAKESTATIONPOLSKA 2015). In mid-2012, a new company entered the Polish market. It was a Polish branch of the American firm Primus Cable. The company offered making repairs and inspections of installations across whole Poland, available in no more than 24 hours, and delivery of spare parts in just a few hours. The biggest advantage was that Primus Cable used such construction solutions that enabled the user to carry out minor maintenance and service works in about an hour. Moreover, the company offered an assortment of wake park features, e.g. aluminium and polyethylene obstacles, which allow wakeboarders to make various tricks (WAKESTYLE 2015). The latest player on the market is a Polish company, Wake Focus, which makes wakeboarding cable systems (WAKEFOCUS 2015).

The total cost of creating a wake park depends on several factors. The major investment is to build a cable system. The final price will depend on specific conditions in a given location and on the investor's expectations, and is usually considered to be a trade secret. A cable system manufacturer prepares an individual price quotation with a detail specification of costs. Companies frequently offer other services, such as preparing documentation for technical inspection. Building a wake park with a full-size cable system will cost from 450,000 to 800,000 Euros on average. This cost calculation includes making an artificial water reservoir, which on average will cost from 200,000 to 600,000 Euros, depending on a site) as well as: a starting platform, a cable lift, toilets,

and other amenities (YOUCANWAKE 2014b). The cost of a cable lift itself and its installation is around 300,000 Euros. The net price for a two-tower cable system starts at 22,900 Euros. This solution has many advantages, such as a light construction and just two anchoring points. A tow-tower system can serve a few users at a time. The installation can be used all year round, that is in a wake park in summer and a snow park in winter (SESITEC 2015b). The capacity of a two-tower system is about 300 person-hours a week, operated by 1 to 3 staff members. This equates to the work of about 30 motor boats with 60 boat drivers and boat persons that would have to be engaged to achieve comparable capacity. Two-tower cable system is environmentally friendly because it is emission free. Besides, it can be installed in silence zones, nature protected areas and near swimming beaches. Unlike motor boats, it does not generate waves that damage the shores and reed plants. The manufacturers even claim that a cable system is beneficial to an aquatic environment owing to the constant oxygenation of water through waves created by riders. Two-tower cable system consumes less energy. If a rider is towed by a motor boat, the energy consumption is about 60 kW/h/person. However, a rider using a cable system will use up just 4 kW/hour (WAKEBOARD 2016).

As two-tower cable system is mobile, it is now possible to rent one for a season or for a single event, and such offers include shipment, installation and operation of a cable system. Another advantage is that it can be powered by a diesel power generator, which costs (depending on a manufacturer) from 3,000 to 7,000 Polish zloty to purchase. The total rental cost depends on time, location and installation requirements (land/water). The price is negotiable and, realistically speaking, may be down to 2,000 to 2,500 Polish zloty per day.

Wakeboarding is a relatively new sports discipline. Owing to the growing number of places where it can be trained and by being made increasingly attractive to the general public, wakeboarding is becoming a more popular form of recreational activity. This change inscribes itself in the growing interest among Poles in physical exercise. It also reflects the pro-health policy of the EU countries, development of appropriate infrastructure and promotion of active rest. In 2007 the EU has set up a High Level Group on Nutrition and Physical Activity. Representatives of the governments of all EU countries (plus Norway and Switzerland) jointly seek solutions to health problems associated with obesity (*Zrozumieć politykę...* EC 2014). In the third multiannual program for the financial years 2014–2020 the EU complements and supports national efforts in the area of promoting healthy lifestyles and disease prevention. Projects focused on creating the tourism and recreation based on natural resources are also funded. In this category there are also water parks, marinas and ski lifts (Rozporządzenie Parlamentu Europejskiego... Dz.U. UE 2014 rozdz. 21 t. 3).

Conclusions

The appearance of wake parks in Poland is most certainly a manifestation of the popularity of an active lifestyle among young people. Wakeboarding belongs to extreme forms of sports and recreational activities, and is associated with urban youth cultures. Meanwhile, wakeboarding is promoted by mass media, including websites, which effaces its image as an elite sport. The growing number of sports championships and events in wake parks is accompanied by camps and schools for wakeboarding fans. The dynamically developing network of wake parks is mostly supported by two-tower system installations, which assist beginner riders in learning the first steps.

Poland seems to be predisposed to develop water recreation and sports owing to a large number of natural water bodies. In Europe, it is second only to Scandinavia. However, with regard to the formal and legal requirements as well as the specific character of investment into wake parks and the business risk involved, the above considerations appear secondary in importance. The seasonality of wake parks (summer and holiday seasons) favours construction of wake parks on artificial water reservoirs. Moreover, wake parks now concentrate in areas which are not associated with either summer holidays or with water tourism and recreation. The key decision-making factor seems to be the proximity to large urban agglomerations, with good transport access. The conclusion finds support in the distribution of wake parks recently built in whole Poland.

A wake park can be an investment project that will generate numerous benefits for economy and will enhance the tourist appeal of a given site. It contributes to the promotion of a locality, town or region. However, for a wake park to be opened, several formal and legal requirements must be fulfilled. Most frequently, a concept of locating a wake park on a lake creates more red tape. Wakeboarding is an offer addressed to demanding customers. It is highly important to ensure high quality of service and safety. Concentration as well as further development and diversification of a whole range of services concerning leisure time and rest activities are necessary in order to reduce the investment risk and improve the chance of a new wake park to survive on the market.

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TRIPLOIDIZATION OF PERCID FISHES – A CHANCE FOR IMPROVEMENT AND DIVERSIFICATION OF EUROPEAN AQUACULTURE?*

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K e y w o r d s: triploid, percids, thermal shock, pressure (hydrostatic) shock, aquaculture.

A b s t r a c t

The paper presents review of experimental triploidization trials in percid fishes, important for European aquaculture due to improve and diversification of fish production. The triploidization lead to obtain individuals with three sets of homologous chromosomes (3n) theoretically sterile and showing a faster growth rate compare to the normal diploid fish. Triploidization in aquaculture is usually performed with the use of thermal/pressure and chemical shocks. Parameters of environmental shocks are species specific and it is extremely important to optimize the exact conditions for procedure. In percids the efficiency of the pressure and thermal shocks is varied, and the survival rate of triploids relatively low. However the production of triploid percids stocks using a pressure shock, can be adapted widespread in the future in the fishery practice.

TRIPLOIDYZACJA RYB OKONIOWATYCH – SZANSA NA UDOSKONALENIE I DYWERSYFIKACJĘ AKWAKULTURY RODZIMYCH GATUNKÓW RYB?

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S ł o w a k l u c z o w e: triploidyzacja, ryby okoniowate, szok termiczny, szok ciśnieniowy, akwakultura.

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Abstrakt

W pracy przedstawiono wyniki eksperymentalnych zabiegów triploidyzacji u przedstawicieli ryb okoniowatych, ważnych dla akwakultury europejskiej ze względu na poprawę i dywersyfikację produkcji ryb. Celem triploidyzacji, jako manipulacji genomowej, jest uzyskanie osobników o powiększonym o 50% zestawie chromosomów homologicznych ($3n$) wobec osobników rodzicielskich, teoretycznie sterylnych i wykazujących szybsze tempo wzrostu niż typowe ryby diploidalne. W akwakulturze zabieg triploidyzacji przeprowadza się najczęściej z wykorzystaniem szoków termicznych oraz ciśnieniowych i chemicznych. Parametry stosowanych szoków środowiskowych są swoiste gatunkowo, dlatego niezwykle ważna jest doświadczalna optymalizacja ich warunków. U ryb okoniowatych efektywność szoków termicznych i ciśnieniowych jest bardzo zróżnicowana, a przeżywalność triploidów stosunkowo niska. Jednak metody produkcji triploidalnych stad okoniowatych z zastosowaniem szoku ciśnieniowego mogą znaleźć w przyszłości powszechne zastosowanie w praktyce rybackiej.

Percids aquaculture

During the last two decades, in the context of inland aquaculture diversification in Europe, percid fishes, namely Eurasian perch (*Perca fluviatilis* L.) and pikeperch (*Sander lucioperca* L.), are receiving increasing attention from scientists and fish farmers. Moreover, high flesh quality and reasonable market value made culture of both percid species economically justified (KESTEMONT and MÉLARD 2000). The main consumer markets for percid fish products (mainly fillets) concerns Finland and Sweden in Scandinavia and especially four European countries in Alpine region, Switzerland, Northern Italy, Germany and France (TONER 2015). Nowadays, EU aquaculture of percids is dominated by France, Netherlands and Denmark based on intensive rearing techniques as recirculation systems (RAS). Intensive culture in RAS provides optimal conditions for fish growth, high survival rate and shorter production cycle, but still needs huge economical inputs. A large part of the European fish farms are micro-enterprises, in most cases using rather extensive production technology (NIELSEN et al. 2015). Therefore, initial larval and juvenile perch culture under pond conditions has been combined with intensive ongrowing of fish to a commercial size in RAS. Combination of pond and RAS perch culture is successfully used mainly in countries of Central Europe where large pond area is available (POLICAR et al. 2013). The success of the comprehensive implementation of any fish species on a commercial scale production using RAS, depends on many factors. The key to the sustainable development of the production of percids was optimized methods for reproduction under controlled condition in both periods, spawning and out of reproductive season. Mastering techniques for conducting the controlled reproduction of percid species, by selecting the appropriate environmental combined with the use of hormonal stimulation have a significant impact on the effectiveness

of spawning, and in turn, on the quantity of stocking material produced (KUCHARCZYK et al. 1996, KOUŘIL et al. 1997, DEMSKA-ZAKĘŚ and ZAKĘŚ 2002, ZAKĘŚ and SZCZEPKOWSKI 2004, MIGAUD et al. 2004, 2006, ZAKĘŚ and DEMSKA-ZAKĘŚ 2009). In the case of the controlled reproduction of European percids, also major problems include synchronization and prediction of the time of ovulation, as well as variable quality of eggs. This may suggest, among others, that the procedure of artificial spawning itself can affect the quality of eggs. It has already been proven that in the process of artificial spawning, the quality of eggs was affected by water temperature and the type of hormonal preparation used for induction of ovulation (ŻARSKI et al. 2011a, 2011b). Progress of domestication of animals, including fish species seems to be a key factor for the development of further cultivation, however domestication of percid species still is progressing (TELETCHEA and FONTAINE 2014). Effects of domestication process on fish growth, low stress response, and reproduction have already been observed in other fish species. Therefore domestication of percids certainly allows to develop breeding programs similar to those that operate in the salmonids, consequently affecting the aquaculture of these species (FONTAINE et al. 2015).

Implementing culture of a given species in RAS is determined not only by developing reproductive technique, but also by creating effective larvae and fry rearing methods in this production system. The obstacles encountered at this stage of perch and pikeperch production are mainly connected with the size of larvae (belonging to the smallest freshwater ichthyofauna representatives), not fully developed gastrointestinal tract after hatching, necessity of filling the swim bladder in the first days of life and intra-cohort cannibalism. Undoubtedly, the stage of optimizing rearing of larvae and juveniles is the key to further development of both percid fish aquaculture (ZAKĘŚ et al. 2008). The phenomenon of intra-cohort cannibalism, mentioned before, is one of the major problems predatory fish farmers are confronted with at this stage of production. The moment of occurrence of cannibalism depends on how well the structures of the gastrointestinal tract are developed and whether an individual has obtained the ability to start exogenous feeding. As for predatory fish, the first cannibalistic attempts appear between 7 and 12 days post hatching (DPH) (BARAS et al. 2003, KESTEMONT et al. 2003, BABIAK et al. 2004, KRÓL et al. 2015, KRÓL and ZAKĘŚ 2016). At early stages of rearing larvae of predatory fish in RAS, the farmer is forced to use the first live food, usually *Artemia* sp. nauplii, which is a source of nutrients and, additionally, enriches fish intestinal environment with exogenous enzymes facilitating digestion process. At the further stages of rearing predatory fish larvae, co-feeding procedure (*Artemia* + dry diet) is used, mainly as a cost-cutting scheme, to be later entirely replaced with composed feed. Converting into serving solely

composed feed to larvae is a critical moment in rearing of predatory fish species causing the greatest losses as a result of cannibalism. In both cultured European percid species, several authors suggested that further studies are required for an optimization of the weaning protocol at the earliest possible stage of larval rearing (KESTEMONT et al. 2007, KRÓL and ZIELIŃSKI 2015). The latter is a result of the fact that the nutritional requirements of percid fish are still unknown and commercial diet dedicated to these species is no available on the market.

Growth heterogeneity is a main problem in larviculture especially in predatory species (KESTEMONT et al. 2003). Controlling the phenotypic sex of farmed fish is potentially one of the most promising strategies for improving production and profitability in aquaculture (STRÜSSMANN and NAKAMURA 2002). Reducing growth heterogeneity of the cultured stocks was one of the reasons for which methods of production percid monosex populations were developed (MALISON et al. 1986, ROUGEOT et al. 2002, 2005). Moreover, in some species, the use of monosex can provide additional benefits such as reducing aggressive interactions between conspecifics or controlling spontaneous reproduction in captivity. In case of percid species because of the faster growth and later maturation of females, the production of all-female stocks may have potential applications in culture management of this species (KESTEMONT and MÉLARD 2000). STEJSKAL et al. (2009) found that the significance differences in body weight between all-female and mixed sex stocks of perch began at age 144 days, when fish were over 13 g. The sexual growth dimorphism in perch probably appears markedly when sexual maturation reduces somatic growth. The large-scale production of percid species would be improved by producing sterile (triploid) fish. Sterility provides opportunities for increasing economic growth to use energy on the somatic growth rather than the development of the gonads. Triploidization, may cause sterilization of fish and therefore probably induced the improvement of growth performances by the reduction of the gonad development above 20% (ROUGEOT et al. 2003, STEJSKAL et al. 2009).

Methods of triploidization

Triploidization is a genomic manipulation leading to obtain organisms with one additional chromosome sets. It is achieved by prevent the extrusion of the second polar body in the egg shortly after egg fertilization, but before the first mitotic division of the zygote (CHOURROUT 1988, MALISON et al. 1993). The fertilized egg has then three haploid nuclei derived from the egg, the sperm and the second polar body, which after the fusion constitute the nucleus of the triploid (autotriploid) zygote. Sterility of organisms created as the result of

triploidization is caused by the fact that dividing three sets of chromosomes equally under subsequent phases of meiosis becomes impossible. Although there are a few naturally occurring triploid species of fish that exist as all-female populations with unique reproductive strategies (PURDOM 1984), for most species triploidy is not a natural condition (BENFEY 2001). Triploidization of fish can be achieved by several methods include physical (thermal or pressure shocks) and chemical treatments (with colchicine or cytochalasin B). Generally physical methods are the most successful used to induce triploidy in fish (THORGAARD 1986, IHSSEN et al. 1990, MALISON et al. 1993, PIFERRER et al. 2009). Parameters of thermal shock are determined experimentally and individually for each fish species. The most important parameters are: temperature of the shock, time of its implementation – the time after fertilization of the egg – and the duration of the thermal shock itself application (PANDIAN and KOTEESWARAN 1998). The most common principle to determine the temperature of the shock is that high temperature (26–32°C) so called hot shock is used for cold-water species (ARAI and WILKINS 1987), while low temperature (4–7°C) so called cold shock is used for warm-water species (BASAVARAJU et al. 2002, DIAS DA SILVA et al. 2007). The most crucial parameter of triploidization seems to be the moment of shock initiation. As it has been already mentioned, shock has to be applied before the second polar body leaves the egg, after egg fertilization, but before the first mitotic division of the zygote (CHOURROUT 1988). In salmonids during triploidization procedures thermal shocks were used in 5 to 45 minutes (ARAI and WILKINS 1987). For cyprinids the time between 1–4 minutes after fertilization of eggs was adapted (BASAVARAJU 2002). The duration of shock is also dependent on the species and is most often performed in 5 to 25 minutes. Triploidization with use of the thermal shock itself is relatively easy to made. Usually, fertilized gametes placed on the sieves are kept for a few minutes in water bowls at significantly higher or lower temperature compared to the temperature of the water where egg fertilization was made (ROUGEOT 2005). For the use of pressure shock to induce the triploidization, two parameters, i.e. time of initiation and duration of the shock, are determined analogously to the described thermal method. It also requires the establishment of the third parameter, which is the condition of the high pressure shock to which the fertilized eggs are subjected (PANDIAN 1994). Triploidization with the use of this method requires a special device with the possibility to regulate and stabilize the condition of the pressure shock for at least several minutes, mostly between 58–85 Mpa (MALISON et al. 1993, PRESTON et al. 2013).

Verification of the triploidization effectiveness

The first one is to compare the size of the nuclei of erythrocytes in groups of fish subjected effected by shocks to the ones in the control group (unshocked group). For this purpose blood is collected from the tail vein of the fish, a drop of blood is then applied to the microscope slide and stained with Wright's dye (WOLTERS et al. 1982) or Giemsa method (FELIP et al. 1997). Then diameter of several dozens erythrocytes from each sample that has been made is measured and the results are verified by statistical analysis. This method is often used to estimate the ploidy since it easily identifies differences in the nuclear volume of erythrocytes of triploid and diploid organisms (CHERFAS et al. 1994). However it is limited by an adequately large size of the examined organisms from which we can take intravitally blood samples. Second method of verification of triploization's efficiency is analysis of the number of chromosomes (karyotyping). For this method was used fragments of fish tissues, for example spleen, gill epithelium or kidney of which we make cytological preparations. This method is not commonly used in analytics due to long time and high costs needed to perform it (JANKUN et al. 2008). The most reliable and relatively quick diagnostic test verifying the ploidy of the fish offspring is analysis by flow cytometry. This method is based on the measurement of the content of the nuclear DNA, isolated from small fragments of fish fins or muscles. The biological material is incubated with a fluorescent DNA-specific dye DAPI (4',6-diamidino-2-phenylindol), which crosses the nuclear membrane and binds to the DNA nucleotides, which therefore corresponds to the level of ploidy of the examined organisms (LECOMMANDEUR et al. 1994).The fluorescence intensity for each nucleus is proportional to the content of the DNA and which therefore corresponds to the level of ploidy of the examined organisms (LECOMMANDEUR et al. 1994).

Triploidization of percid fishes

As part of the experimental work so far were tested effects of environmental shocks on perch fish eggs. For the yellow perch *Perca flavescens* (MITCHILL 1814) the most efficient variants were these with the use of the heat shock (MALISON et al. 1993, MALISON and GARCIA-ABIADO 1996) – Table 1. REUGEOT et al. (2003), performing triploidization of the Eurasian perch *Perca fluviatilis* L. also obtained 100% of triploids with very similar shock parameters (Table 1). For the yellow perch the use of the shock resulted in the lack of any effects of the procedure (MALISON et al. 1993). However for the walleye *Stizostedion vitreum* (MITCHILL 1818) results of the experiments with thermal shocks were

not as good as for the aforementioned perch species. MALISON et al. (2001), analyzed the efficiency of the selected options, as a best variant obtained only 44% of triploid organisms (Table 1). In the triploidization of the European seabass *Dicentrarchus labrax* L., cold shocks were used, which resulted in a stock consisting entirely of triploids while maintaining a relatively high survival rate and obtaining around 80% of triploids (FELIP et al. 1997). The induction of the triploidization with the use of the short cold shocks was highly dependent on water temperature (FELIP et al. 1997). Generally it was observed, that the shorter exposure of eggs time for environmental factor caused the greater efficiency of triploidization. Similarly good results, were also obtained by PERUZZI and CHATAIN (2000) who used short cold shocks on European seabass.

Table 1

The most effective results of triploidization in percids fishes with use of thermal shock

Species	Shock conditions (initiation time/ /water temp./ /duration time)	Percentage of triploids [%]	Survival rate [%]	Source
Yellow perch *Perca flavescens* (Mitchill, 1814)	5 min AF / 30°C/25 min	100.0	16.7 ± 6.7	MALISON et al. (1993)
	5 min AF/ 31°C/25 min	100.0	3.3 ± 3.3	
	2 min AF/ 30°C/10 min	100.0	30.0 ± 15.3	
	5min AF/ 30°C/10 min	93.3 ± 6.7	43.3 ± 14.5	
Walleye *Stizostedion vitreum* (Mitchill, 1818)	2 min AF/ 31°C/25 min	25.0 ± 14.4	13.3 ± 8.8	MALISON et al. (2001)
	5 min AF/ 31°C/25 min	35.3 ± 19.2	16.7 ± 12.0	
	2 min AF/ 30°C/25 min	44.3 ± 29.4	13.3 ± 8.8	
	5 min AF/ 30°C/25 min	30.3 ± 19.2	16.7 ± 8.8	
Eurasian perch *Perca fluviatilis* L.	5 min AF/ 30°C/10min	88.0 ± 6.0	36.0 ± 3.0	REUGEOT et al. (2003)
	7 min AF/ 30°C/10 min	98.0 ± 2.0	38.0 ± 7.0	
	5 min AF/ 30°C/25 min	100.0	43.0 ± 34.0	
	7 min AF/ 30°C/25 min	93.0	27.0 ± 17.0	
Pikeperch *Sander lucioperca* L.	10 min AF/29°C/40 min	75.0	–	BLECHA et al. (2016)
	5min AF/31°C/20 min	100.0	–	
European sea bass *Dicentrarchus labrax* L.	5 min AF/ 0°C/5 min	87.0	70.0	FELIPI et al. (1997)

During experiments related to the production of percid triploids hydrostatic pressure shocks were also used (Table 2). However, the effectiveness of these procedures was strongly varied. MALISON et al. (1993) obtained 50% of triploid yellow perch, but at a very high mortality rate. For the walleye the use of the pressure shocks resulted 100% of triploids. The problem, similarly to the

situation of the Eurasian and yellow perch, was a high mortality rate of larvae (MALISON et al. 2001). However, PERUZZI and CHATAIN (2000) using pressure shocks in European seabass, obtained 100% of triploids.

Table 2
The most effective results of triploidization in percids fishes with use of hydrostatic pressure shock

Species	Shock conditions (initiation time/ /water temp./ /duration time)	Percentage of triploids [%]	Survival rate [%]	Source
Yellow perch *Perca flavescens* (Mitchill, 1814)	5 min AF /9000PSI/12 min	54.4 ± 27.2	80.0 ± 5.8	MALISON et al. (1993)
	5 min AF /11000PSI/12 min	50.0 ± 16.7	63.3 ± 6.7	
Walleye *Stizostedion vitreum* (Mitchill, 1818)	4 min AF/7000PSI/15 min	93.0 ± 3.0	88.3 ± 4.4	MALISON et al. (2001)
	4 min AF/7000PSI/30 min	73.1 ± 4.4	56.7 ± 8.8	
	4 min AF/8000PSI/15 min	72.2 ± 11.1	73.3 ± 6.7	
	4 min AF/8000PSI/30 min	100.0	63.3 ± 8.8	
European sea bass *Dicentrarchus labrax* L.	6 min AF/8500PSI/2 min	100.0	41.0–89.0	PERUZZI and CHATAIN (2000)

Conclusions and possibilities of using the triploid percids

Genetic manipulations techniques, including triploidization, are gaining more and more interest of world aquaculture. This is due to the fact, that their use creates potential possibilities of fish production with features of gigantism or sterile stocks, which can indirectly contribute to improve the economic efficiency of fish aquaculture (PURDOM 1983, PANDIAN and KOTEESWARAN 1998). An adequate scaling-up of the method from laboratory to hatchery is a key step if the triploidization is to be applied at the large scale required for mass production. It should be noted that polyploids are not considered to be genetically modified organisms (GMOs). This method has many useful applications to aquaculture. The major consequence of triploidy is gonadal sterility, which is of advantage in the aquaculture with supporting of superior growth. In fish, the induction of triploidy is mainly used to avoid problems associated with sexual maturation such as lower growth rates, higher aggressive and territorial behavior, increased incidence of diseases and deterioration of the organoleptic properties. Triploidy can also be used to increase the viability of some hybrids, and is regarded as a potential method for the genetic containment of farmed fish (PIFERRER et al. 2009).

In the initial stage of rearing diploids perch grow faster than triploid fish. After reaching a weight of about 20 g, the triploid perch start to gain weight faster than the diploids due to the beginning of sexual maturation process as well as the initiation of ovo- and spermatogenesis (MALISON et al. 1986). In this stage the diploid gonads are significantly more developed than triploid fish gonads (MALISON et al. 1993). These results confirm the thesis that the triploids use more energy for somatic growth than diploids, which formed their gonadal structures (MALISON et al. 1993). It was observed the differences in the ovaries development between the diploid and triploid yellow perch. With the total length of the fish being approx. TL = 75 mm, the oocyte diameter of the diploids was TL = 110 μm, while the triploids had the oocyte diameter of only 60 μm. After the fish achieved the total length of TL = 125 mm, the ovaries of the diploid females contained the vitellogenic oocytes while in the gonads of the triploids only few single previtellogenic oocytes were discovered. The diploid males (with the total length of 75 mm) had in testis numerous spermatogonia and several scattered spermatocytes. When the fish reached the total length of approx. TL = 100 mm, testes already contained primary and secondary spermatocytes as well as the spermatids. In total length of TL = 125 mm, all germ cells of spermatogenesis including spermatozoids were observed. In the same time triploid males (range 100–125 mm of total length) had only spermatogonia in seminiferous lobules (MALISON et al. 1993). Among the salmonids, triploid males develop much larger gonads than triploid females and often produce functional spermatozoa, but these spermatozoa are aneuploid (BENFEY et al. 1986)

Parameters of applied physical shocks used to induce triploidy are fish species dependent. For high procedure effectiveness extremely important is to determine their optimal conditions. Alternative production methods of Eurasian perch triploids with the hydrostatic pressure shock can be commonly used in the innovative aquaculture and fishery practice since, theoretically it's easier to optimize the standard conditions for such procedure.

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