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CONTENT OF SELECTED TRACE ELEMENTS IN SOILS ALONG STATE ROAD 51 (NORTH-EASTERN POLAND)

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K e y w o r d s: traffic flow, contamination, trace elements, soils.

A b s t r a c t

The aim of the study was to determine the impact of traffic flow on the content of selected trace elements, i.e. manganese, zinc, copper and iron, in the surface layers of soils along the section of State Road 51 (north-eastern Poland). The areas most exposed to contamination surrounded the road and urban zone while, with an increasing distance off the road, the content of tested elements decreased significantly. Traffic flow thus exerted a significant impact on the concentration of trace elements in soil which directly surrounded the road. It was most evident for zinc, whose content in soil at a distance of 100 m, in comparison with the roadside, decreased the most of the tested elements. All types of soil had a natural content of the examined elements.

ZAWARTOŚĆ WYBRANYCH PIERWIASTKÓW ŚLADOWYCH W GLEBACH WZDŁUŻ DROGI KRAJOWEJ 51 (PŁN.-WSCH. POLSKA)

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S ł o w a k l u c z o w e: ruch samochodowy, zanieczyszczenie, pierwiastki śladowe, gleby.

A b s t r a k t

Celem pracy było określenie wpływu ruchu samochodowego na zawartość wybranych pierwiastków śladowych: manganu, cynku, miedzi i żelaza w wierzchnich warstwach gleby wzdłuż odcinka drogi krajowej numer 51 (płn.-wsch. Polska). Najbardziej narażone na zanieczyszczenia były obszary bezpośrednio przylegające do drogi i obszarów miejskich, zaś wraz z oddalaniem się od nich

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zawartości badanych pierwiastków ulegały znacznemu zmniejszeniu. Ruch samochodowy wpływał zatem istotnie na zawartość pierwiastków śladowych w glebach przylegających bezpośrednio do drogi. Było to najbardziej widoczne w przypadku cynku, którego zawartość w glebach w odległości 100 m, w porównaniu do skraju drogi, uległa największemu ograniczeniu spośród badanych pierwiastków. Wszystkie gleby charakteryzowały się zawartością naturalną badanych pierwiastków.

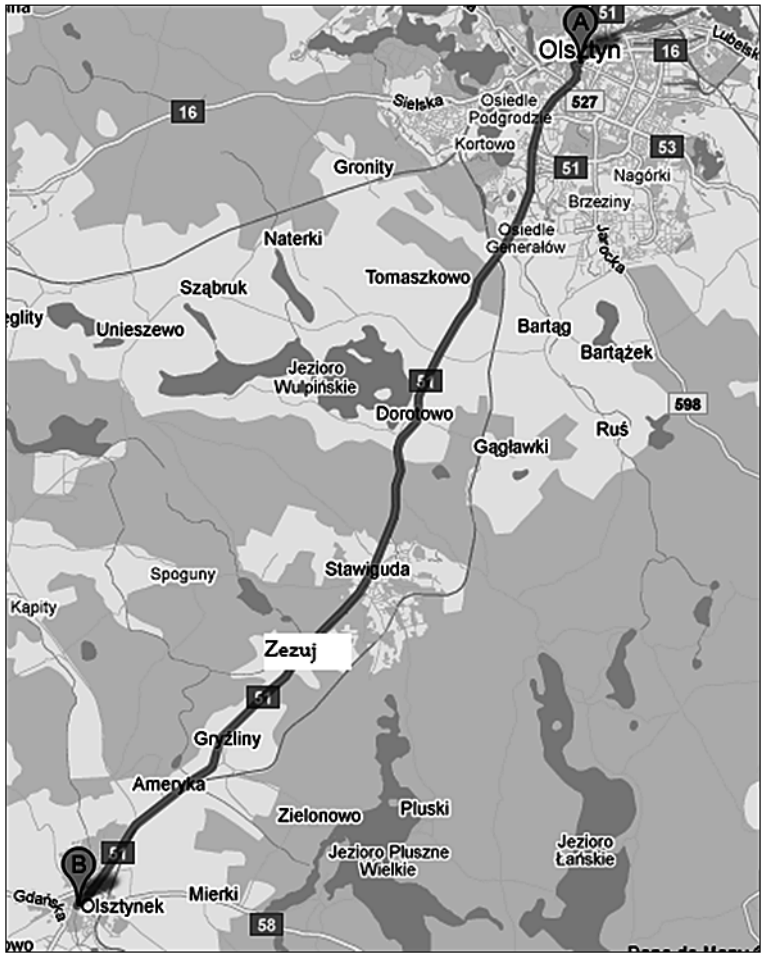
Introduction

Soil, together with water and air, belongs to the most precious resources in the natural environment which encompasses, apart from solids, gases and liquids, living faunal and floral organisms as well as pollution generated by human activities (COSKUN et al. 2006, FAIZ et al. 2009). All components of the environment are exposed to degradation due to pollution originating from industrial plants, communal industry, waste disposal sites, agriculture, ore mining and processing as well as traffic flow (FACIU et al. 2012, TAKÁČ 2009, WYSZKOWSKI and ZIÓŁKOWSKA 2013). As reported by FAIZ et al. (2009), over the last century the pollution of the natural environment has risen dramatically. The reasons are rapid development, urbanization and industrialization. Among soil pollutants, there are also trace elements that are difficult to degrade and accumulate in the environment. Their excessive concentration poses a significant risk to the natural environment (FAIZ et al. 2009, HLIHOR et al. 2009, TAKÁČ 2009). They may also be toxic to living organisms (CIEĆKO et al. 2001, COSKUN et al. 2006, HLIHOR et al. 2009, WYSZKOWSKA and WYSZKOWSKI 2002, 2003, WYSZKOWSKI and WYSZKOWSKA 2009). Apart from anthropogenic sources, soil also contains elements that are a natural background of the total content and are released during soil-formation processes (COSKUN et al. 2006, TAKÁČ 2009). Recently, the number of cars on roads has risen dramatically and, as a result, the content of heavy metals in soil has also increased (AYDINALP 2010, FACIU et al. 2012). According to CHRISTOFORIDIS and STAMATIS (2009), DUONG and LEE (2011), FAIZ et al. (2009), JOHANSSON et al. (2009), they originate from exhaust gases, tyre and brake shoe wearing, oil leakage, road surface wearing and corrosion of metal vehicle construction elements. The majority of metals have a common source of origin (QIAO et al. 2011), for instance, copper, zinc and lead (FAIZ et al. 2009).

The objective of the studies was to determine the concentration of selected heavy metals (manganese, zinc, copper and iron) in the soils along the section of State Road 51 between Olsztyn and Olsztynek (north-eastern Poland).

Material and Methods

Collection of samples. The tests for the content of trace elements were conducted on the soil located along the State Road 51 between Olsztyn and Olsztynek (north-eastern Poland). The average intensity of traffic flow was 12,581 vehicles per 24 h on the section Olsztyn-Stawiguda, (2,019 trucks and



Olsztyn (53°44'06.3''N 20°26'56.1''E), Dorotowo (53°42'22.3''N 20°25'11.6''E), Stawiguda (53°40'01.9''N 20°24'10.7''E), Zezuj (53°38'25.2''N 20°22'08.9''E), Ameryka (53°36'42.1''N 20°19'36.5''E) and Olsztynek (53°35'01.9''N 20°16'29.5''E)

Fig. 1. Map presenting the distribution of sampling sites (base map data from Č2013 Google)

105 buses), and 10,019 vehicles (2,051 trucks and 105 buses) per 24 h, respectively, on the section Stawiguda-Olsztynek (GDDKIA 2010). The recordings of traffic flow were taken by trained observers manually and with automated techniques: video recording and constant flow measuring station. The soil samples were collected from the surface layer in six towns/villages situated along this communication pathway: Olsztyn (53°44'06.3''N 20°26'56.1''E), Dorotowo (53°42'22.3''N 20°25'11.6''E), Stawiguda (53°40'01.9''N 20°24'10.7''E), Zezuj (53°38'25.2''N 20°22'08.9''E), Ameryka (53°36'42.1''N 20°19'36.5''E) and Olsztynek (53°35'01.9''N 20°16'29.5''E) (Fig. 1). In each town/village, the samples were taken at four locations: along the roadside and at distances of 25, 50 and 100 m from the analysed route. The soil samples originated from coniferous forest with the predominance of pine (Olsztyn) and from the locatFions covered with grasses (the other towns/villages), although in Zezuj and Ameryka the vegetation was scarce.

Analysis of samples. The collected soil material was dried at room temperature, comminuted and sieved through a 0.1 mm mesh. The material was then „wet" mineralized in concentrated HNO_3 (V) in a microwave furnace type MARS (CEM Corporation, USA) in Teflon dishes HP500 according to the method US-EPA3051 (1994). The total content of trace elements, i.e. manganese, zinc, copper and iron, was determined with flame atomic absorption spectrometry (FAAS) in an air-acetylene flame. The results were statistically processed with Statistica software using a two-way analysis of variance (ANOVA). The coefficients of a simple Pearson's correlation (r) were calculated between the tested factors.

Results

The content of the tested trace elements in the soil depended on the distance from the road and the town/village where the samples were collected (Tables 1–2).

Manganese. The average content of manganese in the soil near the road between Olsztyn and Olsztynek ranged from 119.6 to 240.6 mg kg^{-1} of soil (Table 1). In all towns/villages, the soil collected from the areas located on the roadside had the highest content of manganese, which gradually decreased with an increasing distance off the road. The highest concentrations of zinc on the roadside were recorded in Olsztynek and Ameryka – 284.1 mg kg^{-1} and 278.1 mg kg^{-1}. By comparing the individual towns/villages, it was found that the highest content of manganese on the roadside was recorded in Olsztyn and Zezuj where, in a zone located 100 m off the road, the concentrations of this element were 2.5 and 2.3 times lower, respectively, than near the roadside.

Table 1
Content of manganese and zinc in the surface soil layer near the State Road 51 from Olsztyn to Olsztynek (mg kg^{-1} of soil)

Towns/villages	Distances from route in m				Average	*r*
	along the roadside	25	50	100		
Manganese (Mn)						
Olsztyn	226.1	104.5	97.5	89.0	129.3	-0.745**
Dorotowo	171.3	155.1	148.7	126.3	150.4	-0.991**
Stawiguda	231.4	209.9	188.7	179.2	202.3	-0.937**
Zezuj	165.7	141.2	99.5	71.7	119.6	-0.974**
Ameryka	278.1	254.5	221.6	208.1	240.6	-0.946**
Olsztynek	284.1	229.0	217.9	199.8	232.7	-0.882**
Average	226.1	182.4	162.3	145.7	179.1	-0.919 **
LSD	town/village – 7.87, distance from route – 6.43, interaction – 15.75					
Zinc (Zn)						
Olsztyn	74.4	42.1	32.8	25.4	43.7	-0.867**
Dorotowo	106.9	83.1	59.6	36.2	71.5	-0.981**
Stawiguda	126.6	23.3	22.1	18.7	47.7	-0.703*
Zezuj	183.9	38.1	24.8	16.3	65.8	-0.752**
Ameryka	104.4	57.7	41.9	32.7	59.2	-0.865**
Olsztynek	193.1	168.9	124.6	64.5	137.8	-0.997**
Average	131.5	68.9	50.9	32.3	70.9	-0.886**
LSD	town/village – 3.25, distance from route – 2.65, interaction – 6.50					

r – correlation coefficient; significant for: * $p = 0.05$, ** $p = 0.01$

In the other towns/villages, these differences were smaller and ranged from 23% to 30%. This was probably due to anthropogenic contamination of the tested soil, caused mainly by traffic flow.

Zinc. The average content of zinc in the soil located near the road between Olsztyn and Olsztynek ranged from 43.7 to 137.8 mg kg^{-1} (Table 1). The highest concentrations of zinc on the roadside, i.e. 193.1 mg kg^{-1} and 183.9 mg kg^{-1}, were recorded in Olsztynek and Zezuj, respectively, whereas the lowest content (below 75.0 mg kg^{-1} of soil) was measured in Olsztyn. The farther from the road, the lower the concentration of this element was. The highest differentiations of zinc content were found in Stawiguda and Zezuj, where it decreased substantially 25 m and 100 m from the road. At 100 m, it was seven times (Stawiguda) and eleven times (Zezuj) lower in comparison with the roadside. This indicates that the contamination of soil with this element originates from traffic flow. In the other towns/villages, these differences were smaller (66–69%) yet still significant. This relation is well-depicted by the high significance of the correlation coefficients recorded in all towns/villages.

Copper. The average content of copper in the soil located near the road between Olsztyn and Olsztynek ranged from 11.8 mg kg^{-1} in Stawiguda

Table 2
Content of copper and iron in the surface soil layer near the State Road 51 from Olsztyn to Olsztynek (mg kg^{-1} of soil)

Towns/villages	Distances from route in m				Average	*r*
	along the roadside	25	50	100		
Copper (Cu)						
Olsztyn	19.8	19.4	12.0	11.2	15.6	-0.885**
Dorotowo	14.4	13.5	12.0	10.9	12.7	-0.978**
Stawiguda	25.0	7.8	7.3	7.0	11.8	-0.702*
Zezuj	19.5	11.0	9.9	8.3	12.2	-0.822**
Ameryka	22.8	15.7	13.2	12.1	16.0	-0.854**
Olsztynek	38.5	30.6	25.5	16.2	27.7	-0.991**
Average	23.3	16.3	13.3	10.9	16.0	-0.909 **
LSD	town/village – 0.90, distance from route – 0.70, interaction – 1.70					
Iron (Fe)						
Olsztyn	8051	4248	4111	2968	4845	-0.839**
Dorotowo	7039	5965	5667	5203	5969	-0.915**
Stawiguda	6092	4937	4041	3754	4706	-0.907**
Zezuj	6104	5175	4889	3211	4845	-0.989**
Ameryka	8624	7454	6285	5882	7061	-0.925**
Olsztynek	9442	8714	6877	6298	7833	-0.940**
Average	7559	6082	5311	4553	5876	-0.943**
LSD	town/village – 242.0, distance from route – 241.1, interaction – 543.9					

r – correlation coefficient; significant for: * $p = 0.05$, ** $p = 0.01$

to 27.7 mg kg^{-1} in Olsztynek (Table 2). The highest content of copper (38.5 mg kg^{-1}) was determined in the soil along the roadside in Olsztynek whilst the lowest was in Dorotowo (14.4 mg kg^{-1}). There was a declining tendency in the content of this metal with an increasing distance from the road. The highest differentiation in the content of copper was recorded in Stawiguda, where it was almost four times higher along the roadside than 100 m from the road. In the other towns/villages, the reduction in the content of copper was lower, yet still significant, and ranged from 24% (Dorotowo) to 57–58% (Zezuj and Olsztynek).

Iron. The average content of iron in the soil along the road from Olsztyn to Olsztynek ranged between 4706 and 7833 mg kg^{-1} (Table 2). The content of this element decreased with an increasing distance from the roadside. The highest concentration of iron was found in the soil near the road in Olsztynek whilst the lowest levels were in Stawiguda and Zezuj, whereas at 100 m from the road and, depending on the town/village, the content of iron averaged from 2968 to 6298 mg kg^{-1} of soil. The largest differences in the content of iron were demonstrated in Olsztyn, where between the roadside and 100 m from the road

its concentration decreased almost three times. In the other towns/villages, these differences were smaller (26–47%) although still significant.

In none of the towns/villages was the permissible limit for trace elements exceeded (Regulation by the Polish Minister of the Environment 2002).

The content of selected trace elements in soils along State Road No. 51 was published in other paper (MODRZEWSKA and WYSZKOWSKI 2014). The traffic flow had a significant effect on the content of other heavy metals in soils lying along the road. Further away from the road, and under a lower traffic flow intensity, the amounts of contaminants originating from the motor traffic decreased. The statistical analysis demonstrated that there was a strong negative correlation between the concentrations of nickel, lead, chromium and cadmium in soils and the distance from the road. The biggest differences in the content of an individual element were determined for lead and the smallest ones – for cadmium.

The calculated correlation coefficients (r) confirmed significant relations between manganese, zinc, copper and iron and the contents of other trace elements in the tested soil (Table 3) except for chromium and other microelements, for which no significant correlations were found.

Table 3

Correlation coefficients (r) between manganese, zinc, copper and iron and the content of other trace elements in the analysed soil

	Pb	Cd	Cr	Ni	Mn	Zn	Cu
Mn	0.69**	0.68**	0.28	0.77**			
Zn	0.85**	0.57**	0.22	0.82**	0.54**		
Cu	0.87**	0.55**	0.04	0.85**	0.60**	0.87**	
Fe	0.75**	0.65**	0.22	0.94**	0.81**	0.75**	0.78**

** significant for $p=0.01$

Discussion

The studies showed the highest content of trace elements in the area surrounding the road which decreased along with an increasing distance from the road. Similar correlations were demonstrated by KLUGE and WESSOLEK (2012). Assuming the spatial development next to the analysed route, it may be concluded that soil contamination with metals originates mainly from traffic flow and agriculture as there are no longer any large plants situated along this road. The number of vehicles has increased in recent years, which was also shown in the studies by DUONG and LEE (2011) and FAIZ et al. (2009). In addition, a higher intensity and speed of traffic flow and associated tyres and road surface wearing have caused a significant increase in soil contamination with heavy metals (AYDINALP 2010, DUONG and LEE 2011).

According to DUONG and LEE (2011), FAIZ et al. (2009), JOHANSSON et al. (2009), soil contamination with copper and zinc is a typical anthropogenic pollution along roads with the main source in traffic flow. It has been observed that copper and zinc originate from common sources (KOBZA 2005), which include mechanical brake and tyre wearing and exhaust gas emissions (DUONG and LEE 2011, JOHANSSON et al. 2009, QIAO et al. 2011). Copper is also emitted when pesticides containing copper sulphate are used (QIAO et al. 2011) and zinc is released during tyre production and originates from refineries, leakage of oil derivatives from vehicles and lubricants (CHRISTOFORIDIS and STAMATIS 2009).

Manganese are found in metal alloys which are released into the environment during corrosion. Therefore, the threshold limits for this element is not included in the Regulation issued by the Polish Minister of the Environment (2002), which indicates their natural content in soil.

Heavy metals have varied mobility and availability to plants which depend on physical and chemical properties of soil: pH, content of organic matter, type of source material, soil texture, concentration of iron and manganese oxides, sorption, cation exchange and type of metal (BARANČÍKOVÁ and MAKOVNÍKOVÁ 2003, PAVEL et al. 2011, RODRÍGUEZ-OROZ et al. 2012, TAKÁČ 2009, TOSELLI et al. 2009,). A low content of humic acids, found in the organic matter, forming complexes with trace elements causes their higher bioavailability to plants (BARANČÍKOVÁ and MAKOVNÍKOVÁ 2003, BORŮVKA and DRÁBEK 2004). In addition, pH value has a considerable impact on bioavailability since, together with the increase in acidity, heavy metals become more soluble and absorbable by plants (CIEĆKO et al. 2001, FINŽGAR 2007, PAVEL et al. 2011, TAKÁČ 2009, WYSZKOWSKI and WYSZKOWSKA 2009). For instance, TAKÁČ (2009) reported that copper and zinc bioavailability decreased together with the increase in pH. KLUGE and WESSOLEK (2012) demonstrated that the content of trace elements in soil decreased with the increase in the distance from roadside. Their observations indicate that the highest concentration of metals is found near the roadside and on the surface of soil. A similar correlation was demonstrated in the present study: the farther the distance off the roadside, the smaller the content of tested elements was.

Conclusions

The highest concentration of all analysed trace elements (manganese, zinc, copper and iron) was detected in Olsztynek followed by Ameryka (manganese) and Zezuj (zinc). This finding may be explained by the location of this town, which is situated between the national express road No.7 from Rabka to Gdańsk and the State road No. 51 from Olsztyn to Olsztynek.

The substantial number of vehicles passing on these routes causes extensive emission of heavy metals into the environment, polluting the nearby areas situated along these roads. Together with the increasing distance off the route, soil contamination was significantly reduced, which indicates that traffic flow has a considerable impact on the content of trace elements on the areas surrounding the roads. It was particularly evident for zinc in Stawiguda and Zezuj, for copper in Stawiguda and for iron in Olsztyn, where their varied content between the roadside and 100 m away where it was 11-, 7-, 4- and 3-fold lower, respectively.

Significant correlations between manganese, zinc, copper and iron and the content of other trace elements in the analysed soil were demonstrated.

The results support the thesis that traffic flow is one of the major causes of contamination of roadside soil with trace elements because there are no longer any larger plants or concentrated urban infrastructure situated along this route.

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METHODS FOR PREDICTING CARCASS LEAN CONTENT IN LIVE BIRDS

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A b s t r a c t

The aim of this study was to review recent research, conducted over the last two decades, into prediction of carcass leanness and fatness in live domestic birds. Numerous traits were measured in live birds, and relatively high correlations were found between the breast muscle content and lean meat content of the whole carcass vs. breast muscle thickness, breastbone crest length and body weight of birds. Breast muscle thickness was measured using needle catheters and, more recently, ultrasonic devices. The above three traits were used as independent variables in multiple regression equations for estimating breast muscle content and lean weight in the whole carcass. The breast muscle content of poultry carcasses can be determined *in vivo* with high accuracy by computed tomography (CT) and magnetic resonance imaging (MRI). However, both techniques are relatively expensive, for which reason they are not widely used in animal breeding.

PRZYŻYCIOWE METODY OCENY UMIĘŚNIENIA TUSZEK DROBIOWYCH

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S ł o w a k l u c z o w e: drób, tuszki, mięśnie piersiowe, przyżyciowa ocena, równania regresji.

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Abstrakt

Niniejsza praca obejmuje przegląd wyników badań dotyczących przyżyciowych metod oceny umięśnienia i otłuszczenia poszczególnych gatunków ptaków domowych, opublikowanych głównie w ostatnich dwóch dekadach. Spośród przebadanych licznych cech mierzonych u żywych ptaków, stosunkowo duże skorelowanie z zawartością mięśni piersiowych lub zawartością mięsa w całej tuszce wykazały następujące: grubość mięśni piersiowych, długość grzebienia mostka i masa ciała ptaka. Do pomiaru grubości mięśni piersiowych stosowano różnej konstrukcji zgłębniki igłowe, a ostatnio aparaty ultradźwiękowe. Bardzo dużą przydatność do określania wydajności mięśni piersiowych wykazują tomografia komputerowa i obraz rezonansu magnetycznego. Jednak obecnie techniki te ze względu na duży koszt aparatury nie mają większego znaczenia dla praktycznej hodowli zwierząt domowych. W oparciu głównie o grubość mięśni piersiowych, długość grzebienia mostka i masę ciała, jako zmienne niezależne, wyprowadzono szereg równań regresji wielokrotnej do szacowania masy mięśni piersiowych lub masy mięsa w całej tuszce.

Introduction

Livestock production has to be improved with respect to the quantity and quality of final products and raw materials intended for further processing. The adopted strategies include animal recording schemes whose results are used to improve breeding programs and production technologies. Performance testing and recording in meat-type animals is most difficult. The performance of individual animals raised for eggs, milk and wool is based on the quantity and quality of their products. In animals kept for meat production, their slaughter quality is determined by carcass dressing percentage, the content of tissue components in the carcass and meat quality, which can be accurately measured post mortem. In addition, carcass dissection is both time- and labor-consuming. The chemical composition of the whole carcass is also difficult to determine (LATSHAW and BISHOP 2001). Another disadvantage of the above methods is the fact that they can only be applied after slaughter, which makes them unsuitable for selection in pedigree flocks. Thus, accurate, simple and rapid methods for predicting carcass meatiness and fatness in live animals are continuously searched for. In poultry, such research efforts were undertaken in the 1960s. At first the researchers focused on the suitability of selected traits, measured *in vivo*, for estimating the lean meat and fat content of poultry carcasses. The results of research studies in this area, published until the 1990s, have been summarized and presented by GRASHORN (1994) in a review article.

The objective of this study was to review recent research, conducted over the last two decades, into prediction of carcass leanness and fatness in live domestic birds.

Use of selected traits determined *in vivo* for predicting carcass leanness and fatness

As previously mentioned, the slaughter quality of domestic bird species is largely determined by meat and fat percentages in their carcasses, which cannot be directly measured in the bodies of live birds. Therefore, numerous studies have been conducted to date to select reliable indicators of leanness and fatness in live birds.

Particular attention has been paid to breastbone crest length, as this trait is correlated with lean weight in the whole carcass and with breast muscle weight in Pekin ducks (MICHALIK and BOCHNO 1986, RYMKIEWICZ and BOCHNO 1998a), geese (CANOPE et al. 1997, BOCHNO et al. 1999), chickens (RYMKIEWICZ and BOCHNO 1998b, MICHALIK et al. 1999, LARIVIERE et al. 2007) and Muscovy ducks (KLECZEK et al. 2006). In chickens, relatively high correlations were also found between breast muscle weight and chest width, chest height and chest circumference (LARIVIER et al. 2007).

PINGEL et al. (1969) noted a relatively high correlation ($r > 0.62$) between the thickness and weight of breast muscles in ducks. Their findings were confirmed by later studies of ducks (SØRENSEN and JENSEN 1992, KSIĄŻKIEWICZ et al. 1993, LAVALLEE at al. 1998) and geese (GRUNDER et al. 1989, KOMENDER and GRASHORN 1990, CANOPE et al. 1997, BOCHNO et al. 1999). Breast muscle thickness is also correlated with the meat content in whole carcasses of ducks (BOCHNO et al. 1988, BRZOZOWSKI and BOCHNO 1998, BOCHNO et al. 2000b), geese (BOCHNO et al. 2001), chickens (MICHALIK et al. 1999) and turkeys (MICHALIK et al. 2002, Table 1).

Needle catheters, once widely used to measure breast muscle thickness in birds, have been replaced with commercially available ultrasonic devices. It should be stressed that the results of measurements performed by both techniques are similarly correlated with breast muscle weight (CANOPE et al. 1997, BOCHNO et al. 1999) and the meat content of the whole carcass (MICHALIK et al. 1999). Measurements taken with needle catheters and ultrasonic devices had no adverse effects on animal health and performance (OVIEDO-RONDON et al. 2007). However, in view of safeguarding animal welfare and due to the introduction of animal protection laws, needle catheters have been replaced with non-invasive techniques.

Breast muscle thickness is measured at different sites in different bird species and genotypes (REMIGNON et al. 1997, KÖNING and GRASHORN 1997). X-rays can also be used to precisely measure breast muscle thickness (HORST 1961), but this technique is rarely applied to birds due to the high cost and size of X-ray equipment.

Table 1
Coefficients of simple correlation (r) between breast muscle weight (Y_1) and selected traits (X_i)

Bird species/reference	Sex	Body weight (g) X_1	Breast muscle thickness (cm) X_2	Breastbone crest length (cm) X_3
Chickens				
Michalik et. al., 2001	♂ ♀	0.845	0.638	0.533
Rymkiewicz and Bochno, 1998b	♂ ♀	0.917	0.574	0.553
Kleczek et. al., 2009	♂	0.47	0.47	–
	♀	0.46	0.67	–
Lariviere et. al., 2007	♂ ♀	0.84	0.79	0.84
Ducks				
Rymkiewicz and Bochno, 1998a	♂ ♀	0.634	0.905	0.842
Lavallee et. al., 1998	♂ ♀	–	0.62	–
	♂ ♀	–	0.69	–
Geese				
Bochno et. al., 2000a	♂ ♀	0.751	0.629	0.740
Bochno et. al., 1999	♂ ♀	0.861	0.630	0.760
Turkeys				
Bochno et. al., 2002	♂	0.906	0.778	0.497
	♀	0.883	0.804	0.421

Real-time ultrasound (RTU) imaging is also used to determine the cross-sectional area of breast muscles. The area of transversal images of breast muscles captured by RTU is highly correlated with breast muscle weight ($r > 0.96$; Oviedo-Rondon et al. 2007). A total of 50 to 60 birds per hour can be measured with RTU.

The described methods and devices are valuable tools that can be used by geneticists in selection programs aimed at increasing the growth rate of breast muscle and the meat content of the whole carcass. They can also be deployed in experiments investigating the effects of nutritional and environmental factors on muscle development in birds. In ducks, the response to selection for increased thickness of breast muscles was observed already in the F_1 generation (Lavallee et al. 1998).

In recent years, attempts have been made to use new measurement techniques, other than ultrasounds, to estimate carcass leanness and fatness in live birds. The most promising of them is CT (Bensten and Sehested 1989, Remignon et al. 1997, Andrassy-Baka et al. 1999, Brenoe and Kolstad 2000), followed by MRI (Wiederhold et al. 1995, Wiederhold 1996, Wiederhold and Pingel 1997, Scolan et al. 1998, Mitchell et al. 1997, Davenel et al. 2000) and sonography (Grashorn 1994). Compared with the ultrasound technique, MRI and X-ray CT enable more accurate estimation of breast muscle yield, and they do not require direct contact between the sensor and bird's skin (Davenel et al. 2000). The disadvantages of MRI and X-ray CT

include high cost and size of the equipment, which makes it difficult to use them in poultry houses.

To date, MRI has been used, among others, to determine changes in the volume of breast and leg muscles in growing ducks and geese (WIEDERHOLD and PINGEL 1997).

Since the above techniques are very expensive, they probably will not be widely used in practice in the nearest future (SCOLAN et al. 1998). A viable alternative is the ultrasound technique that supports easy and relatively accurate measurement of the thickness and cross-sectional area of breast muscles.

In all bird species, the meat content of carcass is highly correlated with body weight. This results primarily from autocorrelation of the above traits, since body weight includes muscle weight.

Despite considerable research efforts, reliable *in vivo* indicators of carcass fatness in poultry species have not been developed to date.

Regression equations for estimating breast muscle weight and meat content in whole carcasses of birds

For comparative purposes, carcass lean content can be evaluated based on the values of the indicators described above. However, data regarding the meat content of the whole carcass and the weight of breast muscles (*Pectoralis major* and *Pectoralis minor*) are more meaningful while estimating the slaughter quality of birds. An increase in carcass lean content and breast muscle weight should be one of the goals of selection in pedigree flocks (Table 2).

Table 2

Table 2. Coefficients simple correlation (r) between the some traits and meat (Y_2) content (g) of the carcass

Bird species/reference	Sex	Body weight (g) X_1	Breast muscle thickness (cm) X_2	Breastbone crest length (cm) X_3
Chickens				
MICHALIK et. al., 2001	♂ ♀	0.950	0.574	0.600
RYMKIEWICZ and BOCHNO, 1998b	♂ ♀	0.956	0.401	0.586
Ducks				
BOCHNO, 2000b	♂ ♀	0.822	0.815	0.851
BRZOZOWSKA et. al., 1999	♂ ♀	0.902	0.676	0.756
Geese				
BOCHNO et. al., 2001	♂ ♀	0.923	0.490	0.780
Turkeys				
MICHALIK et. al., 2002	♂	0.952	0.750	0.440
	♀	0.920	0.620	0.417

Table 3
Regression equations for predicting breast muscle content ($\hat{Y}_1$; g) and lean weight in the whole carcass ($\hat{Y}_2$; g), standard errors of the estimate (S_y) and coefficients of multiple correlation (R)

Bird species/reference	Sex	Equation	S_y	R
Chickens				
Rymkiewicz and Bochno, 1998b	♂ ♀	$\hat{Y}_1 = 0.139X_1 + 78.32X_2 - 106.3$	13.4	0.972
Michalik et. al., 2001	♂ ♀	$\hat{Y}_1 = 0.115X_1 + 7.501X_2 + 7.064X_3 - 299.8$	24.2	0.902
Michalik et. al., 1999	♂ ♀	$\hat{Y}_2 = 0.409X_1 + 11.179X_2 - 279.6$	38.2	0.956
Ducks				
Bochno et. al., 2000b	♂ ♀	$\hat{Y}_2 = 0.184X_1 + 125.4X_2 + 25.12X_3 - 255.8$	39.0	0.942
Brzozowski et. al., 1999	♂ ♀	$\hat{Y}_2 = 0.225X_1 + 64.85X_2 + 16.08X_3 - 185.3$	37.22	0.919
Rymkiewicz and Bochno, 1998a	♂ ♀	$\hat{Y}_1 = 0.039X_1 + 139.5X_2 + 17.16X_3 - 313.1$	24.0	0.944
Muscovy ducks				
Kleczek et. al., 2006	♂	$\hat{Y}_2 = 0.235X_1 + 58.503X_2 + 35.120X_3 - 796.6$	81.2	0.843
	♀	$\hat{Y}_2 = 0.232X_1 + 27.622X_3 + 10.503X_5 - 439.9$	37.7	0.842
Goose				
Bochno et. al., 2000a	♂ ♀	$\hat{Y}_1 = 0.066X1 + 7.800X_2 + 21.602X_3 - 341.4$	30.6	0.907
Bochno et. al., 1999	♂ ♀	$\hat{Y}_1 = 0.0492X_1 + 9.645X_2 + 16.48X_3 - 205.8$	30.8	0.906
Turkeys				
Bochno et. al., 2002	♂	$\hat{Y}_1 = 0.201X_1 + 174.1X_2 + 53.0X_3 - 1647.8$	134.8	0.932
	♀	$\hat{Y}_1 = 0.159X_1 + 127.9X_2 + 5.04X_3 - 168.8$	64.3	0.939
Michalik et. al., 2002	♂	$\hat{Y}_2 = 0.551X_1 + 195.23X_2 + 25.82X_4 - 1193.4$	228.3	0.956
	♀	$\hat{Y}_2 = 0.451X_1 + 94.27X_2 + 52.65X_4 - 966.4$	145.2	0.938

Independent variables: X_1 – body weight (g)
X_2 – breast muscle thickness (cm)
X_3 – breastbone crest length (cm)
X_4 – drumstick circumference (cm)
X_5 – chest girth (cm)

The most appropriate multiple regression equations developed to predict muscle weight in live birds are given in Table 3. In the derived equations, the following three traits were used as independent variables: body weight, breast muscle thickness and breastbone crest length. Different traits were used as independent variables by other authors. In the multiple linear regression equations developed by OVIEDO-RONDON et al. (2007) to estimate breast muscle weight, two independent variables were body weight and the area of transversal images of breast muscles captured by RTU. DAVENEL et al. (2000) demonstrated that the correlation between the transverse cross-section area of breast muscles determined by MRI and the weight of breast muscles improved when body weight was used as the second independent variable.

The proposed multiple regression equations are a valuable tool for predicting carcass leanness in birds. Similar results were reported when lean meat content in broilers, ducks and geese was estimated using equations developed for both sexes and for males and females separately. This suggests that in the above species carcass meatiness and fatness can be calculated using equations based on data for both sexes. In turkeys, which show high sexual dimorphism, the content of lean meat and fat in the carcass should be estimated using different equations for males and females (BOCHNO et al. 2002).

It should be noted that *in vivo* measurement of breast muscle content has already been used in poultry selection (POPOVIC and PYM 1995, PYM 1996, PYM et al. 1998). Selection for increased growth rate of breast muscles has also been based on breast muscle thickness (WILLIAM 2005).

It can be concluded that the analyzed traits, i.e. body weight (g), breast muscle thickness (cm) and breastbone crest (cm), are good indicators of lean meat content in the carcasses of geese, ducks, turkey and chickens. The derived multiple regression equations for predicting carcass lean content, with the above traits as independent variables, are characterized by very small standard errors of the estimate.

The authors recommend the equations given in Table 3 for selection programs aimed at increasing the content of carcass lean meat in poultry.

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EFFECT OF HEAT TREATMENT OF RAPESEED AND METHODS OF OIL EXTRACTION ON THE CONTENT OF PHOSPHORUS AND PROFILE OF PHOSPHOLIPIDS

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K e y w o r d s: cold- and hot-pressing, extraction, heat treatment, rapeseed oil, phosphorus and phospholipids content

A b s t r a c t

The aim of research was to determine the effect of heat treatment of whole and milled seeds and methods of extraction on the content of phosphorus and profile of phospholipids. The experimental material consisted of 15 laboratory samples of rapeseed oil, and 1 sample of industrial hot-pressed oil. The phosphorus content, the share of phospholipids with column chromatography and the profile of phospholipids with thin layer chromatography were determined. The methods of oil extraction had a significant impact on the content of phosphorus and the profile of phospholipids, while the most significant to the share of PA. It was found that the heat treatment of seeds increased the content of phosphorus, share of total PL and PA in the samples of cold-pressed and extracted with petroleum ether oils.

Abbreviation:
HPL – hydratable phospholipids,
NHPL – nonhydratable phospholipids,
NPL – non-polar lipids,
GL – glycolipids,
PC – phosphatidylcholine
PI – phosphatidylinositol,
lyso-PL – lysophospholipids,
PE – phosphatidylethanolamin
PA – phosphatidic acid,
pp – percentage point

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WPŁYW CIEPLNEJ OBRÓBKI NASION RZEPAKU I METODY WYDOBYWANIA OLEJU RZEPAKOWEGO NA UDZIAŁ I PROFIL FOSFOLIPIDÓW

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S ł o w a k l u c z o w e: tłoczenie na zimno i gorąco, ekstrakcja, obróbka termiczna, olej rzepakowy, zawartość fosforu i fosfolipidów.

A b s t r a k t

Celem badań było określenie wpływu obróbki termicznej całych i rozdrobnionych nasion rzepaku, jak również metody wydobywania oleju na udział i profil fosfolipidów. Materiał badań stanowiło 15 laboratoryjnych próbek oleju rzepakowego i jedna przemysłowa próbka oleju tłoczonego na gorąco. W olejach określono skład lipidowy metodą chromatografii kolumnowej oraz profile fosfolipidowe metodą chromatografii cienkowarstwowej. Metoda wydobywania oleju miała istotny wpływ na zawartość fosforu oraz udział i profil fosfolipidów, z czego w największym stopniu na udział PA. Stwierdzono, iż ogrzewanie nasion rzepaku wpłynęło na zwiększenie zawartości fosforu oraz ogólny udział PL i udział PA w próbkach tłoczonych na zimno i ekstrahowanych eterem naftowym.

Introduction

The major compounds present in crude rapeseed oil are triacylglycerols (> 90%), while the minor amounts of non-triacylglycerol compounds are phospholipids, free fatty acids, sterols, tocopherols, pigments, flavonoids and glycolipids. SZYDŁOWSKA-CZERNIAK (2007) said that since most problems are caused by phospholipids, they should be removed to the maximum extent. From the technological point of view, those compounds can be divided into hydratable (HPL) and non-hydratable (NHPL). Such phospholipids as PC, PI and lyso-PL are easily hydratable, PE is partially hydratable, while PA is a non-hydratable compound (SUBRAMANIAN et al. 1999, ZUFAROV et al. 2009). Although free PA is formed as a result of phospholipase D activity, due to the presence of free metal ions (calcium and magnesium) in oil, it forms non-hydratable salts with them. In industrial conditions, they are removed from oil with the use of mineral acid (mainly phosphoric acid) and organic acid (citric acid). The added acid separates metal ions from phosphatidic acid salts, restoring their easily-hydratable acid form (ROTKIEWICZ and KONOPKA 2001).

During the treatment of rape seeds and oil pressing, degradation of membranes leads to the release of phospholipids, which freely migrate to the extracted oil (PRIOR et al. 1991). Oils obtained by a cold pressing method,

preserving a temperature < 45°C are characterized by a low content of phosphorus < 20 mg kg^{-1} (PRIOR et al. 1991, ROTKIEWICZ et al. 1995), while those obtained at a higher pressing temperature and from seeds conditioning in different temperatures of about 125–476 mg kg^{-1} (UNGER 1990, SZYDŁOWSKA-CZERNIAK and SZŁYK 2003, AMBROSEWICZ et al. 2012, TAŃSKA et al. 2013a, TAŃSKA et al. 2013b). Extracted oils are characterized by a much higher content of this compound (300–1,190 mg kg^{-1}) (UNGER 1990, SZYDŁOWSKA-CZERNIAK and SZŁYK 2003, PRZYBYLSKI and ESKIN 1991, CVENGROŠ et al. 1999). Most phospholipid studies have focused on clarifying the technological conditions of their content in vegetable oils. Research in this subject area has mainly concerned the impact of extraction technologies on the phosphorus and/or total phospholipid content in oils (PRZYBYLSKI and ESKIN 1991, SOSULSKI et al. 1981, BREVEDAN et al. 2000, CARELLI et al. 1997, 2002, TAŃSKA and ROTKIEWICZ 2003), while little attention is paid to explain the impact of extraction method or heat treatment of seeds on the profile of phospholipids in rapeseed oils. The objective of this paper was thus to evaluate the effect of heat treatment of whole and milled seeds and extraction methods on the content of phosphorus and profile of phospholipids in rapeseed oils.

Material and methods

Rapeseed oil samples

The experimental material consists of 15 laboratory samples of oil, including: 4 samples of cold- pressed oil extracted from unheated (CP_1 and CP_1) and heated in different temperatures seeds (CP_3 and CP_4), 4 samples of oil extracted with petroleum ether from unheated (EPN_1) and heated milled seeds (EPN_2, EPN_3 and EPN_4), 1 sample of oil extracted with hexane from unheated milled seeds (EH_{uh}), 1 sample of oil extracted with ethanol (EET_{uh}), 2 samples of oil extracted with chloroform and methanol ($EChM_1$ and $EChM_2$) and 3 samples of oil extracted from cold-pressed meal (EPE_1, EPE_2 and EPE_3). Moreover, 1 sample of industrial hot-pressed oil (HP) was used (Table 1).

Rapeseed oil sample extraction methods

The cold-pressed oils obtained from un-heated and heated seeds (130°C during 60 and 90 min) were extracted by using a screw oil expeller featuring a cylindrical perforated strainer basket Komet laboratory CA 59 G (IBG Monforts & Reiners, Germany), with temperatures ≤ 40°C. Mechanical impuri-

Table 1

Characteristics of the experimental material

Sample	Cultivar of rapeseed	Parameters of heat treatment of whole seeds	Parameters of heat treatment of milled seeds	Extraction method	Temperature of extraction	Solvent
CP_1	Californium	–	–	cold-pressing	≤ 40°C	–
CP_2	Industrial mixture of seeds	–	–	cold-pressing	≤ 40°C	–
CP_3	Californium	130°C/60 min	–	cold-pressing	≤ 40°C	–
CP_4	Californium	130°C/90 min	–	cold-pressing	≤ 40°C	–
HP	Industrial mixture of seeds	–	110°C/30 min	hot-pressing	110–120°C	–
EPN_1	Californium	–		extraction in Soxhlet apparatus	>60°C	petroleum ether
EPN_2	Industrial mixture of seeds	–	130°C/45 min, in an aluminum foil	extraction in Soxhlet apparatus	>60°C	petroleum ether
EPN_3	Californium	–	115°C/60 min, in an aluminum foil	extraction in Soxhlet apparatus	>60°C	petroleum ether
EPN_4	Californium	–	115°C/60 min, in an airtight container	extraction in Soxhlet apparatus	>60°C	petroleum ether
EH_{uh}	Californium	–	–	extraction in Soxhlet apparatus	>60°C	hexane
EET_{uh}	Californium	–	–	extracted directly under a reflux condenser	>60°C	ethanol
$EChM_1$	Californium	–	–	Folch's extraction	18±3°C	chloroform : methanol (2:1)
$EChM_2$	Industrial mixture of seeds	–	–	Folch's extraction	18±3°C	chloroform : methanol (2:1)
EPE_1	Californium	cold-pressed pulp		extraction in Soxhlet apparatus	>60°C	petroleum ether
EPE_2	Californium	cold-pressed pulp of seeds heated in 130°C/60 min		extraction in Soxhlet apparatus	>60°C	petroleum ether
EPE_3	Californium	cold-pressed pulp of seeds heated in 130°C/90 min		extraction in Soxhlet apparatus	>60°C	petroleum ether

ties were removed from the pressed oil by centrifugation in a centrifuge type C 5810 R (12 000 rpm, 10 min) (Eppendorf, Germany). The sample of industrial hot-pressed rapeseed oil was extracted from crushed seeds conditioned at 110 ± 5°C for 30 minutes and then pressed at 110-120°C.

Extraction of oils from un-heated seeds with petroleum ether and hexane was conducted in Soxhlet's apparatus type FoodAlyt R 60 (Omnilab, Germany).

Extraction of oils with petroleum ether from seeds heated in 115°C during 60 and in 130°C during 45 min. First, the samples of rapeseed were comminuted in a laboratory mill (WZ-1 type, Spomasz, Poland) and then placed in a drier (KC-65M type, PREMED, Poland) and heated in 115°C/60 min and 130°C/45 min in an aluminium foil and in 115°C/60 min in an airtight container. Fat was extracted from the samples over petroleum ether in Soxhlet's apparatus. The extract was distilled on a vacuum evaporator (type R-210, Büchi Labortechnik, Switzerland).

Extraction under a reflux condenser. First, the sample of rapeseed was comminuted in a laboratory mill (WZ-1 type, Spomasz) and weighted into a round-bottomed flask. Then the ethanol was added. In next step the round-bottomed flask with sample was connected to the reflux condenser and set at a heating bath order to heat the solvent to boiling point (approx. 60°C). After extraction the sample was filtered and extract was distilled on a vacuum evaporator type R-210 (Buchi, Switzerland).

Extraction of oil with chloroform and methanol was conducted according to Folch's method (FOLCH et al. 1957), while extraction of oils from cold-pressed meal (of unheated and heated seeds) was conducted in Soxhlet's apparatus (type FoodAlyt R 60, Omnilab).

Determination of the content of phosphorus

The content of phosphorus was determined according to Polish standard method PN-88/A-86930 (Polish standard PN-88/A-86930). Five millilitres of HNO_3 (6 mol/L), 10 mL of ammonium molybdate (4.05×10^{-2} mol/L), 10 mL of ammonium metavanadate were added to ashed samples, and the absorbance was measured at 460 nm against a reagent blank (5mL of HNO_3 + 10 mL of ammonium molybdate + 10 mL of ammonium metavanadate) with a UNICAM UV/Vis UV2 spectrophotometer (ATI Unicam, UK). The phosphate content was measured as a function of absorbance. A standard curve of absorbance versus known phosphate concentrations was prepared by making KH_2PO_4 (Sigma-Aldrich) solutions in different concentration and analysing them by the same method.

Determination of the share of phospholipids in oils

The share of phospholipids in oils was determined according to Ohm and Chung's method (OHM and CHUNG 1999). The measurement was carried out in MEGA BOND SI 1GM 6ML columns (Sigma-Aldrich, Poland). The flow of solvent through the columns was forced at a negative pressure of 18 kPa in a BAKER SPE-12 G chamber (J.T. Baker, USA). At the first stage, the column was conditioned with 5 mL chloroform. The sample was prepared by dissolving the extract of lipids in 25 mL of chloroform and then, depending on the sample, 10 mL of such solution was placed on a column. Lipids were fractionated by gradual washing with proper solvents. Fraction of non-polar lipids (NPL) was washed away with 10 mL chloroform: acetone mixture (4:1), glycolipids (GL) with 15 mL acetone: methanol mixture (9:1) and phospholipids (PL) with 10 mL methanol. Following fractioning, the solvents were evaporated in a vacuum evaporator (Buchi type R-210) below 50°C and lower pressure and the samples were then weighed. The resulting weight of the phospholipids was converted to the percentage on the weight of total lipid fraction.

Determination of the phospholipid profiles

The profile of phospholipids was determined according to Nzai and Proctor's method (NZAI and PROCTOR 1998). The measurements were carried out on 20 x 20 cm chromatographic plates with silica gel (MERCK, Poland). The plates were conditioned in a drier at 105°C for 1 h (KC-65M type). The chromatographic chamber was saturated with a mixture of solvents (chloroform: methanol: water = 75: 25: 3). The previously-extracted phospholipid fractions and phospholipid standards (Sigma-Aldrich) (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, lysophosphatidylcholine, phosphatidic acid) were placed (approx. 25 fg) on the plates. The plates were then put into a chromatographic chamber and the chromatograms were stained in iodine atmosphere and then scanned in order to process the results. Treatment of the results was carried out using a densitometry method, according to Nzai and Proctor (1998).

Statistical analysis

Obtained results of researches were statistically analyzed using the Statistica 10.0 PL (StatSoft, Kraków, Poland) program. In order to indicate significance of differences between oil samples unvaried analysis of variance

(ANOVA) with Tukey's test of $p \leq 0.05$ significance level was used. Moreover, the Pearson correlation coefficients (r) between individual discriminates were determined.

Results

Comparison of the content of phosphorus in rapessed oils obtained from seeds heated in different temperatures and pressed by different extraction methods

The method of oil extraction significantly differentiated the phosphorus content in the rapeseed oils (Table 2). Generally, it was found that the cold- and hot-pressed oils were characterized by a lower content of phosphorus, ranging from 6.2 to 209.9 mg kg^{-1}. The content of this compound in the samples obtained from the heated seeds (CP_3 and CP_4) and in the hot-pressed oil (HP) was higher than in the samples pressed from the unheated seeds (CP_1 and CP_2). It was found that the low content of phosphorus in the pressed oils resulted from a higher content of this compound in cold-pressed meal, what was evidenced by the high content of phosphorus in the samples of EPE_1, EPE_2 and EPE_3 (1136.6–1445.2 mg kg^{-1}). The oils extracted with a mixture of

Table 2

Share of phospholipids in oils extracted by different methods

Oil samples	Content of phosphorus mg kg^{-1}	Share of phospholipids %
CP_1	6.2^{a}	0.045^{a}
CP_2	8.9^{a}	0.088^{b}
CP_3	152.0^{ab}	0.517^{e}
CP_4	195.4^{ab}	0.564^{f}
HP	209.9^{ab}	0.730^{h}
EPN_1	352.3^{bc}	0.361^{c}
EPN_2	677.3^{d}	0.890^{i}
EPN_3	540.6^{cd}	0.522^{e}
EPN_4	608.2^{d}	0.654^{g}
EH_{uh}	582.5^{d}	0.441^{d}
EET_{uh}	2237.9^{g}	1.749^{j}
$EChM_1$	2508.8^{g}	2.455^{k}
$EChM_2$	3987.1^{h}	2.780^{k}
EPE_1	1136.6^{e}	0.379^{c}
EPE_2	1303.1^{ef}	0.593^{f}
EPE_3	1445.2^{f}	0.633^{g}
Correlation coefficients (r)		
Content of phosphorus mg kg^{-1}	1.0	0.91
Share of phospholipids %	0.91	1.0

a, b... – mean values in columns marked with the same letter are not significantly different ($p \leq 0.05$)

chloroform and metanol, and with ethanol were characterised by much higher content of this compound (smaple $EChM_2$ – 3987.1 mg kg^{-1}, sample $EChM_1$ – 2508.8 mg kg^{-1} and sample EET_{uh} – 2237.9 mg kg^{-1}). In the case of oils extracted with petroleum ether from seeds heated in different conditions (EPN_2, EPN_3 and EPN_4) the content of phosphorus was not significantly diversified and identical with the content of this compound in the oil extracted with hexane (EH_{uh}) (Table 2).

Comparison of the share of phospholipids in rapessed oils obtained from seeds heated in different temperatures and pressed by different extraction methods

The share of phospholipids in the oils extracted by different methods was significantly diversified and ranged from 0.045 to 2.780% (Table 2). As well as in the case of phosphorus content, the cold-pressed oils obtained from the unheated seeds (CP_1 and CP_2) were characterized by a lower share of phospholipids (respectively 0.045 and 0.088%), while a higher share of these compounds were showed in the samples of oil extracted with a mixture of chloroform and methanol ($EChM_1$ i $EChM_2$ samples) and ethanol (EET_{uh}) (from 1.749 to 2.780%). The share of phospholipids in the hot-pressed was significantly higher than in the samples of cold-pressed oils obtained from the heated seed (CP_3 and CP_4) and extracted with ether from the unheated and heated seeds (115°C/60 min). In turn the sample of oil extracted with petroleum ether from the seeds heated at 130°C for 45 min was characterized by a higher share of those compounds (0.890%) (Table 2). In the case of oils extracted from the cold-pressed meal after cold-pressing of the heated and unheated seeds it was found that the temperature and time of the seeds treatment had a significant effect on the release of phospholipids from cellular structures, as indicated by increased share of these compounds in the samples of EPE_2 and EPE_3.

Comparison of the profile of phospholipids in rapessed oils obtained from seeds heated in different temperatures and pressed by different extraction methods

The main phospholipids indentified in the oils extracted by different methods were PC, PI, PE and PA (Table 3). Exceptions were samples of the cold-pressed oils obtained from the unheated seeds (CP_1 and CP_2) with the lowest share of phospholipids, for which the profile of phospholipids was not identified. In turn, the profile of phospholipids of the samples of oil extracted

with petroleum ether from the seeds heated at 130°C was presented by PC and PE, while the share of PC was lower and PE higher by approximately 13.98 pp in the oil pressed seed from the seeds heated for a longer time (CP_4). Also, the profile of phospholipid of the oil extracted with petroleum ether from the unheated seeds was represented mainly by PE and PC, while PE accounted for the dominant share (66.65%). In the case of oils extracted with petroleum ether from the seeds heated at 115°C (EPN_3 and EPN_4) the presence of PC, PE and PI was showed, while the share of PI was the highest and PC the lowest (Table 3). The profile of phospholipid of the hot-pressed oil was in the PC>PI>PE>PA system, whereas for the oil extracted with petroleum ether from the seeds heated at 130°C during 45 min in the PC>PA>PI>PE system. In turn, the predominant phospholipid in the sample of oil extracted with ethanol (EET_{uh}) was PA (32.94%), the share of PI and PE were almost at the same level, and the share of PC accounted for the lowest share of the identified phospholipids (14.49%). It was also observed that the profile of phospholipids of the samples of oil extracted with hexane (EH_{uh}) and a mixture of chloroform and methanol ($EChM_1$) were not statistically different. On the other hand,

Table 3

Profile of phospholipid of oils extracted by different methods

Oil samples	Profile of phospholipids [%]			
	PC	PI	PE	PA
CP_1	N.D.	N.D.	N.D.	N.D.
CP_2	N.D.	N.D.	N.D.	N.D.
CP_3	$62{,}47^{h}$	N.D.	37.53^{e}	N.D.
CP_4	$48{,}49^{g}$	N.D.	51.51^{f}	N.D.
HP	38.82^{f}	29.59^{b}	21.91^{d}	9.68^{b}
EPN_1	33.35^{de}	N.D.	66.65^{g}	N.D.
EPN_2	33.10^{d}	22.11^{a}	21.91^{c}	22.89^{ef}
EPN_3	22.08^{b}	51.14^{ef}	26.79^{d}	N.D.
EPN_4	22.90^{bc}	40.65^{d}	36.45^{f}	N.D.
EH_{uh}	14.33^{a}	49.52^{e}	21.35^{c}	13.81^{bc}
EET_{uh}	14.49^{a}	27.05^{b}	27.98^{d}	32.94^{g}
$EChM_1$	14.28^{a}	47.38^{e}	20.51^{c}	17.84^{cde}
$EChM_2$	39.10^{g}	21.81^{a}	21.80^{c}	17.30^{cd}
EPE_1	37.04^{ef}	54.93^{f}	4.91^{a}	3.13^{a}
EPE_2	19.86^{b}	36.83^{d}	21.29^{c}	22.04^{def}
EPE_3	26.75^{c}	30.47^{c}	16.05^{b}	26.73^{f}
Correlation coefficients (r)				
Content of phosphorus mg kg^{-1}	–	–	–	–
Share of phospholipids %	–	–	–	0.52
Share of PC %	1.00	–	–	–
Share of PI %	–	1.00	–	–
Share of PE %	–	–	1.00	–
Share of PA %	–	–	–	1.00

N.D. not detected

a, b... – mean values in columns marked with the same letter are not significantly different ($p \leq 0.05$)

comparing the share of individual phospholipids in the samples $EChM_1$ and $EChM_2$ it was found that the $EChM_1$ sample was characterised by the high share of PI, while $EChM_2$ sample PC (Table 3). Reported differences between these samples resulted from the use of different samples of seeds for oil extraction (sample $EChM_1$ – winter variety of Californium, sample $EChM_2$ – industrial mass of seeds) (Table1). Moreover, it was found that the temperature and time of heat treatment of seeds used in the cold-pressing method contributed to the significant increase of the share of PA (form 3.13% for the EPE_1 sample to 26.73% for the EPE_3 sample) and decrease of the share of PI (from 54.93 to 30.47%) in the oils extracted with petroleum ether from cold-pressed meal (Table 3).

Correlations

Calculated on the basis of statistical analysis, the Pearson correlation coefficients (r) revealed that the share of phospholipids in the analyzed oils was dependent on the phosphorus content ($r = 0.91$) (Table 2), while the share of PA was correlated with the share of phospholipids ($r = 0.52$) (Table 3).

Discussion

Phosphorus contained in the oil is almost completely phospholipid phosphorus. This compound migrates to oil due to the degradation of biological membranes, which may occur during commodity turnover, storing and processing (ROTKIEWICZ and KONOPKA 2000). During the processing of seeds, due to mechanical and/or thermal degradation of membranes of oil bodies, especially at the beginning of roasting, phospholipid are released and dissolve in the oil (ROTKIEWICZ and ROTKIEWICZ 2000, ROTKIEWICZ et al. 2002). ROTKIEWICZ and KONOPKA (2000), examining the effect of selected technological factors on the content of phosphorus in rapeseed oil, found that all of the factors which maximize the degree of extraction of oil such as the increased temperature of roasting and extending period of this process, the increased humidity of roasted milled seeds, and the small size of seeds contributed to increase of content of this compound. PRZYBYLSKI et al. (2005), after ČMOLIK, reported that the conditioning of milled seed of rape contributed to increase of the share of phospholipids from about 0.5% to about 15%. In turn, Unger found that the extraction of pressed meal caused the migration of the phospholipids to oil. What is more, the quoted author reported that the rate of phospholipid migration to oil is in direct proportion to the dilution degree of miscella used

for extraction and the depth of oil extraction. SOSULSKI et al. (1981), SHAHIDI (2001) and TAŃSKA and ROTKIEWICZ (2003) found that the significant impact on the content of phospholipids in oil had the temperature of pressing and the polarity of the solvent using during extraction.

Phospholipids as polar compounds (BOUKHCHINA et al. 2004) may be easily extracted with the more polar solvents, which was reported in presented work. The high share of these compounds was showed in the sample extracted with ethanol (index of polarity 5.2), as well as in the oil obtained with using a mixture of chloroform and methanol (index of polarity 4.1 and 5.1, respectively). In turn, a lower share of phospholipids was shown in the oils obtained by using petroleum ether and hexane (index of polarity 0.1 and 0.0, respectively). SZYDŁOWSKA-CZERNIAK and SZŁYK (2003) analysing the content of phosphorus and the share of phospholipids in the rapeseed oils found that the sample of extracted oil after last evaporator was characterized by the highest amount of these compounds (500 mg kg^{-1} and 1.30%, respectively), while the lowest the sample of refined rapeseed oil (2.021 mg kg^{-1} and 0.0053%, respectively). Moreover, PRZYBYLSKI and ESKIN (1991) examining, for example, the composition of lipids and phospholipids of the oils extracted and pressed from the roasted seed meal found that the oil obtained by extraction was characterized by a higher share of phospholipids than the pressed oil. The quoted study also noted that the pressed oil was characterized by about 3-fold higher share of PA and about 3.5-fold lower share of PC and PI than the extracted oil (PRZYBYLSKI and ESKIN 1991). The significant diversification of the content of phospholipids in the oils obtained from different stages of technological process was also showed by SZYDŁOWSKA-CZERNIAK (2007). The quoted author found that the highest amount of these compounds was in the extracted oil after last evaporation (22710 mg kg^{-1}) and the lowest in the bleached pressed oil (224 mg kg^{-1}). It is also worth mentioning that analyzed by SZYDŁOWSKA-CZERNIAK (2007) the crude pressed oil was characterized by a high content of these compounds (5656 mg kg^{-1}). In turn, in our previous studies it was showed that the share of phospholipids in the oils extracted with a mixture of chloroform and methanol ranged from 1.91 to 3.07%, while the profile of phospholipids was represented by PC, PI and PE, of which PC has the highest share (TAŃSKA et al. 2009, AMBROSEWICZ et al. 2010).

SIMPSON (1991), after HELLER et al., reported that phospholipase D, responsible for PA production, was subject to inactivation in increased temperatures in a „water" environment. The quoted author found in own research that in a „non-water" environment (in hexane) a temperature of 65°C for 60 min did not have any effect on inhibition of the phospholipase D action. On the other hand, the activity of this enzyme in hydrated hexane decreased by about eight times, both at 65°C, and at 100°C for 10 min. MOREOVER, SIMPSON (1991)

also found that a further increase in the PA content in the raw material previously subject to thermal treatment in order to inactivate the phospholipase D, might be an evidence of both the reactivation of this enzyme and the presence of more hydrophobic phospholipase D, resistant to high temperature. TOSI et al. (2002), after KOCK, reported that the phospholipase D reached the highest activity at 85°C, whereas in temperatures close to 110°C this activity was inhibited.

Conclusions

1. The method of oil extraction had a significant impact on the content of phosphorus and the profile of phospholipids, of which the most significant to the share of phosphatidic acid.

2. It was found that the heat treatment of whole and milled seeds increased the content of phosphorus and the share of phospholipids (including phosphatidic acid) in the cold-pressed and extracted with petroleum ether oils.

3. The samples of cold-pressed oil obtained from the unheated and heated seeds were characterized by a lower content of phosphorus and a lower share of phospholipids. It was resulted from the fact that the majority of these compounds remained at the cold-pressed meal. The profile of phospholipids of the samples obtained from the heated seeds consisted of PC and PE.

4. The oils extracted with chloroform-methanol and etanol were showed a higher content of phosphorus and share of phospholipids. Moreover, these samples were characterized by a high share of nonhydratable phospholipids (PA + PE).

5. The profile of phospholipid of the analyzed oils was significantly diversified and consisted mainly of PC and PE for the samples pressed from the heated seeds and extracted with petroleum ether from the unheated seeds; PC, PE and PI for the sample extracted from the seeds heated at 115°C and of PC, PE, PI and PA for others sample.

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THE LEVEL OF CHOSEN POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN MEAT PRODUCTS SMOKED BY USING AN INDUSTRIAL AND A TRADITIONAL METHOD*

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K e y w o r d s: smoked meat products, industrial smoking, pollutants, PAHs, B[*a*]P.

A b s t r a c t

Constantly changing consumer market manifests itself with greater competitiveness of cured meat products of particular sensory qualities produced both industrially and traditionally. Improper conditions of curing may cause excessive accumulation of polycyclic aromatic hydrocarbons (PAHs), which in turn casts doubt on whether cured products are healthy for consumer.

The aim of this research was to estimate the sum content of the four PAH compounds benzo[*a*]pirene – B[*a*]P, chrysene – CHR, benz[*a*]anthracene – B[*a*]A and benzo[*b*]fluoranthene – B[*b*]F (Σ 4PAH) contamination in selected meat products which had been industrially and traditional smoked. The experimental material were smoked meat products from the meat processing plant operates on the local market (West – Pomeranian region). The research was conducted on selected cured three assortment groups: smoked meat products-bacon, smoked sausages – „jałowcowa" and others: smoked poultry sausages.

Samples were collected from the surface (1 cm deep) and from the core of each product and then were ground and lyophilized. Qualitative and quantitative analysis of PAHs compounds were performed by adopting liquid chromatography coupled with the selective detector (HPLC/FLD). For the isolation of PAHs from the solid matrix, accelerated solvent extraction (ASE) and purification, followed by enrichment with solid phase extraction (SPE) on silica gel were conducted. Analytical procedure was validated.

The results constitute a fragmentary part of qualitative and quantitative analyses which have been assumed in one of the points of the grant project and will enable preliminary evaluation of the

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actual level of B[*a*]P, B[*a*]A, CHR i B[*b*]F (ΣPAH4) in industrially method and traditionally cured products.

It should be noted that the smoking industry in a controlled environment allows for the possibility of change, so you can reduce the content of compounds of PAHs in smoked products. In the case of smoking traditional method based on natural air flow or convection, the critical point in reducing the presence of PAHs in the finished product is the experience and ability to control the reaction conditions of pyrolysis.

POZIOM WYBRANYCH WIELOPIERŚCIENIOWYCH WĘGLOWODORÓW AROMATYCZNYCH (WWA) W MIĘSNYCH PRZETWORACH WĘDZONYCH METODĄ PRZEMYSŁOWĄ I TRADYCYJNĄ*

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S ł o w a k l u c z o w e: przetwory mięsne wędzone, wędzenie przemysłowe, zanieczyszczenia, WWA, B[*a*]P.

A b s t r a k t

Stale zmieniający się rynek konsumentów przejawia większą konkurencyjność wędlin o szczególnych walorach sensorycznych produkowanych zarówno przemysłowo, jak i tradycyjnie. Niewłaściwe warunki wędzenia mogą powodować nadmierne gromadzenie się wielopierścieniowych węglowodorów aromatycznych (WWA), których obecność narzuca wątpliwość, czy wędliny gwarantują zdrowie konsumenta.

Celem badań było określenie poziomu zanieczyszczenia 4 związkami z grupy WWA: benzo[*a*]pirenem – B[*a*]P, chryzenem – CHR, benz[*a*]antracenem – B[*a*]A oraz benz[*b*]fluorantenem – B[*b*]F w wybranych produktach mięsnych, które podlegały tradycyjnemu procesowi wędzenia oraz w warunkach przemysłowych.

Materiałem do badań były mięsne przetwory wędzone pochodzące z zakładu mięsnego funkcjonującego na rynku lokalnym (region zachodniopomorski). Badania przeprowadzono dla 3 wybranych produktów wędzonych: wędzonka-boczek; kiełbasa wędzona: jałowcowa; oraz kiełbasa drobiowa. Próbki do analizy pobierano z powierzchni (1 cm głębokości) oraz ze środkowej części każdego produktu, a następnie mielono i liofilizowano. Jakościową i ilościową analizę związków PAHs wykonano techniką chromatografii cieczowej z selektywnym detektorem (HPLC/FLD). Do izolowania WWA z matrycy stałej wykorzystano przyspieszoną ekstrakcję rozpuszczalnikiem (ASE) oraz oczyszczanie i wzbogacanie za pomocą ekstrakcji do fazy stałej (SPE) na żelu krzemionkowym. Procedurę analityczną poddano walidacji.

Wyniki stanowią fragmentaryczną część analiz ilościowych i jakościowych, które zostały zaplanowane w jednym z punktów projektu i umożliwiły wstępną ocenę rzeczywistego poziomu B[*a*]P, B[*a*]A, CHR i B[*b*]F w przetworach wędzonych metodą przemysłową i tradycyjną.

Należy stwierdzić, że wędzenie przemysłowe w kontrolowanych warunkach pozwala na możliwość wprowadzenia zmian, dzięki którym można obniżyć zawartość związków WWA w produktach wędzonych. W przypadku wędzenia metodą tradycyjną opartą na naturalnym przepływie powietrza lub konwekcji, punktem krytycznym w ograniczaniu obecności WWA w gotowych produktach jest doświadczenie i umiejętność panowania nad warunkami reakcji pirolizy.

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Introduction

Our global food supply chain recently raised concerns about food security. Products are grown and processed in widely differing environments under a variety regulatory frameworks, travels thousands of miles, are kept in various storage conditions, experience temperature fluctuations that may affect shelf life, and are handled by many different people. At any points in this process, products can be contaminated or may become unfit for consumption (CIECIERSKA and OBIEDZIŃSKI 2007, JIRA et al. 2006, KUBIAK 2012, KUBIAK 2013, MEADOR et al. 2006).

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds that consist of two or more condensed aromatic rings in a molecule. Thermal processing of food not only gives food desirable sensory quality, but it may also enhance the formation of undesirable chemical compounds (JIRA et al. 2006, KLIMASZEWSKA 1999). Smoking, drying, roasting on fire (grilling/barbecuing) are well-known methods of preserving food of plant and animal origin which might also pollute food products with considerable amounts of aforementioned PAHs (CIECIERSKA and OBIEDZIŃSKI 2007, Codex Committee on Food Additives and Contaminants 2005, Environmental Protection Agency 1981, KUBIAK et al. 2011, KUBIAK 2012, KUBIAK and POLAK-ŚLIWIŃSKA 2012a, TKACZ et al. 2012]. Smoking is a technological process which aims at extending the food product's life (meat, fish) through its dehydration and antibacterial action of generated smoke constituents, especially phenols. The changes in European law concerning food pollution repeatedly force the necessity to introduce modifications in the production technology of smoked food (GARCIA FALCON et al. 1996, JANKOWSKI 2004, KLIMASZEWSKA 1999, KUBIAK and JAKUBOWSKI 2013). PAHs are classified as carcinogenic compounds and consequently are monitored worldwide in a wide range of environmental matrices, including drinking water, waste water, furnace emission, soil, and hazardous waste extracts and smoked food (KUBIAK 2013). Polish smoked meat products, due to the smoking process, constitute a group of products which are distinguished by their specific sensory qualities resulting from the specific character of the process. It is extremely important from the customer's point of view as it applies to food in commercial traffic that has been thermally processed and thus at risk of cumulation of these compounds during industrial technological processes (KUBIAK and JAKUBOWSKI 2013, KUBIAK and POLAK-ŚLIWIŃSKA 2012a, KUBIAK 2012b). The compounds resulting from the pyrolysis create clearly defined sensory and product discriminants of smoked products, however, besides the highly desirable compounds, undesirable constituents questionable in terms of health are also created in the smoke, including polycyclic aromatic hydrocarbons (PAHs). Polycyclic aromatic hydrocarbons are a very

diverse and ubiquitous group of chemical contaminants present in the human environment. It has been estimated that nearly 70% of PAHs are consumed with food, including the consumption of smoked meat. B[*a*]P for a long time has been a marker of total PAHs content in food and environmental analysis. However, in many cases, B[*a*]P constitutes only 1–20% of the total PAHs content in the examined matrix. In 2011, changes in the European law on food contaminants (European Food Safety Authority – EFSA) led to the publication of the legislative act on the control of impurity content in food products. One of such documents is the Commission Regulation (EU) No 835/2011 from August 19, 2011 amending regulation (EC) No 1881/2006 regarding maximum levels of polycyclic aromatic hydrocarbons (PAHs) in foods, including smoked meat products [Commission Regulation (EU) No 835/2011]. *EFSA* concluded that B[*a*]P is not a suitable indicator for the occurrence of PAH in food and assessed that the sum content of the four PAH compounds B[*a*]P, CHR, B[*a*]A and B[*b*]F („Σ4PAH") is the most suitable indicator of PAHs in food (EFSA 2008, 2012). On the basis of the EFSA opinion (2008), modifications on numerical values regarding acceptable levels of PAHs for particular food groups, were also carried out. In 2011, changes in the European law on food contaminants made by the European Food Safety Authority (*EFSA*) has resulted in the publication of legal acts regarding the control of impurities in food products – Commission Regulation (EU) No 835/2011 (SANCO 2011).

In the EU, including also the Polish legislation, amendments included in the Commission Regulation (EU) No 835/2011 of 19 August 2011, regarding modification of maximum levels accepted for polycyclic aromatic hydrocarbons (PAHs) in food products have been adopted. In this document, a change related to the existing marker, indicating the presence of PAHs in food, namely B[*a*]P has been established with the highest acceptable level (5.00 μg kg-1). From 1 September 2014, the maximum level for B[*a*]P will be established at 2.00 μg kg-1 and for the sum of four PAHs, at 12.00 μg kg-1 (Commission Regulation (EC) No 1881/2006; Commission Regulation (EU) No 835/2011). What is more, Commission Regulation (EU) No 1327/2014 from December 12, 2014 provides for exceptions (quote): However, recent evidence shows that in a number of member countries – in some cases including traditionally smoked meat and smoked meat products, as well as traditionally smoked fish and fishery products – it is not possible to achieve lower levels of PAHs in spite of the widest possible use of good practices of smoking, because in those cases there is no possibility to change the practice of smoking without also causing a significant change in the organoleptic properties of food. The consequence of such action would be the disappearance of this kind of traditionally smoked products from the market, which would result in the closure of many small and medium-sized enterprises.

The aim of this research was to estimate the content of the four PAH compounds: B[*a*]P, CHR, B[*a*]A and B[*b*]F (Σ4PAH) in selected meat products which had been industrially and traditional smoked.

Materials and Methods

In Poland, the most popular meat products, the products have undergone the process of smoking. Traditional method of smoking included drying of meats surface and smoking in traditional smoking chamber with an internal smoke generation (PEK-MONT). Terms of the smoking process were not fully controlled by the nature of the treatment preservation. The next step after the smoking process was cooling of the storage temperature – were chilled in temperature of 4°C for 24 hours. The smoking process was carried out under industrial conditions in medium-sized *Grabowscy* general partnership meat processing enterprise (Ościęcin near Gryfice). Material for studies consisted of meat processing facility located in the northern Polish region. The collected samples were divided into three assortment groups: smoked meat products: bacon, smoked sausages: „jałowcowa" and poultry smoked sausages.

Experimental

The following methods were used to determine ΣPAH4 (B[*a*]P, CHR, B[*a*]A and B[*b*]F) in meat products: extraction of lipid fraction, SEC (Size Exclusion Chromatography) technique to separate hydrocarbons from fat. Quality and quantity analysis of the PAH was carried out using liquid chromatography with a selective detector – HPLC-FLD (LAGE YUSTY, CORTIZO DAVIÑA 2005).

Reagents, standards and equipments

Methanol, acetonitrile (J.T. Baker) for gradient elution, cyclohexane, hexane, dichloromethane, HPLC grade (POCH, Gliwice), double-distilled water. Stock solution of PAHs was prepared by diluting the standard solution (10 mg L-1) of each of the PAHs (PAH-MIX 9 in acetonitrile; Dr. Ehrenstorfer Reference Materials). 250 μl of standard PAHs stock solutions were sampled and transferred to a 25 ml flask and filled to the mark with acetonitrile *gradient grade* (J.T. Baker). Working solutions were prepared by diluting 100 ng l^{-1} solution. Chromatography was performed with an 1100 system (Agilent Technologies, Waldbronn, Germany) equipped with quaternary pump, mobile

phase degasser, autosampler, thermostat for autosampler and columns, and fluorescence detector (FLD). For data acquisition and processing ChemStation B.04.03 software was used. The separation of the analytes was performed with the use of Bakerbond PAH (J.T. Baker) (250 x 3 mm, 5 um particle diameter) with a precolumn. Mobile phase flow was 0.5 ml min^{-1}.

Methodology of isolation, purification and enrichment of PAHs

For the isolation of PAHs from the solid matrix, accelerated solvent extraction (ASE) together with purification and enrichment using solid phase extraction (SPE) on silica gel was used. The detailed conditions for accelerated solvent extraction (Dionex ASE300).

Analytical procedure

Freeze-dried samples were weighed with 0.05 mg precision. They were subjected to homogenization from 2 g of Florisil, and 1 g of diatomaceous earth, and then placed in an extraction flash completing the free space. The resulting extract was enriched with the use of the vacuum rotary evaporator to a volume of about 2 ml, which was then subjected to purification on silica gel SPE cartridge (12 ml; 2 g). The sorbent was conditioned with 12 ml of dichloromethane and 12 ml of hexane. For such a prepared bed, the extract was applied. Adsorbed PAHs were eluted with 3 x 3 ml of hexane/dichloromethane (70/30% v/v) mixture. Acetonitrile was added to the sample and the sample was subjected to evaporation to 500 μl in a nitrogen stream. The resulting extract was subjected to chromatographic analysis.

Determination of PAHs was performed using gradient elution of water/acetonitrile mobile phase. The sample was applied with a composition of the mobile phase (50/50% v/v). The concentration of acetonitrile at 40 min increased to 100%, and was maintained for 10 min, and then returned to the initial conditions (50/50 v/v). Based on the retention time, optimal working conditions for fluorescence detector. Applicability of HPLC/FLD method for the determination of PAHs in the prepared samples was determined, and the stability of the system was checked by injecting each standard solutions five times, and selected samples containing standards as well.

Analytical procedure was validated by establishing such parameters as accuracy, injection precision and methods; precision, linearity, limit of detection (LOD) and limit of quantification (LOQ).

Results and Discussion

A multitude of reports related to the monitoring of PAHs pollution coming from smoking treatment in smoked meat products, indicates the need to deal with this issue. The use of different methods and smoking techniques creates the possibility of placing meat products with varying degrees of PAH compounds contamination on the market. Changes of parameters and stages of the smoking process do not constitute a sufficient method to reduce PAH compounds contamination of meat products because they significantly depend on many other factors, for example: physical parameters of wood or structural determinants of smoking chambers, as well as meat itself.

On the basis of own research results concerning pollution levels of PAH compounds coming from smoke of smoking-treated meat, it can be concluded that in selected product groups, Σ4PAH levels exceed the standards regulated by the new European Union regulations Different levels of PAHs in smoked products, presented in this article, are contained in a wide range, what indicates a significant influence of a smoke-treated raw material. The reason is also the difference resulting from the technology used for conducting the smoking process, as well as the input parameters and the distribution of the mixture of smoke in the smoking chamber.

The results of the analyzes conducted in order to indicate PAHs levels in meat products subject to industrial and traditional smoking are shown in Table 1.

The greatest content of B[*a*]P was found in samples taken from the outer portion of bacon (8.49 μg kg^{-1}), „jałowcowa" sausage (8.41 μg kg^{-1}) and chicken sausage (7.68 μg kg^{-1}), subject to traditional smoking. The values obtained for B[*a*]P in the analyzed products smoked by using the traditional method were above the maximum permissible limit (2.00 μg kg^{-1}) established for the smoked meat products group in the EU Commission Regulation No. 835/2011, of 19 August 2011. The results obtained for the content of B[*a*]P in bacon and „jałowcowa" sausage smoked in an industrial environment controlled by a monitoring system for the bacon and „jałowcowa" sausage, were also above the maximum level of 2.00 μg kg^{-1}. Only in the chicken sausage the content of B[*a*]P did not exceed the permissible limit and was 1.60 μg kg^{-1}. Meat products smoked in two methods were also analyzed in terms of penetration of (into the inner part) PAH compounds contained in the smoke. The content of B[*a*]P in the inner part of all meat products smoked in a traditional method was exceeded and ranged between 3.04-3.44 μg kg^{-1}. The content of B[*a*]P in the inner part of meat products smoked in an industrial method (controlled process conditions) was at a level of 1.02 μg kg^{-1} to 1.10 μg kg^{-1} (below the values specified in the Regulation – Table 1). This is due to, inter alia, the

Table 1

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PAHs [μg kg^{-1}]	Smoked meat products, industrially smoked					
	bacon		sausage „jałowcowa"		poultry sausage	
	external part	internal part	external part	internal part	external part	internal part
Σ4PAHs (B[*a*]P, B[*a*]A, CHR and B[*b*]F)	$20.06^{X*}\pm2.13$	$3.38^{x*}\pm0.51$	$16.61^{Y*}\pm2.07$	$3.25^{x*}\pm0.43$	$8.62^{Z*}\pm0.33$	$4.20^{x*}\pm0.61$
B[*a*]P	$6.30^{X*}\pm0.55$	$1.10^{x*}\pm0.14$	$3.40^{X*}\pm0.68$	$1.02^{x*}\pm0.23$	$1.60^{Y*}\pm0.15$	$1.09^{x*}\pm0.16$
PAHs	Smoked meat products, traditional technique					
	bacon		sausage „jałowcowa"		poultry sausage	
	external part	internal part	external part	internal part	external part	internal part
Σ4PAHs (B[*a*]P, B[*a*]A, CHR and B[*b*]F)	$24.27^{1**}\pm2.29$	$6.83^{3**}\pm1.28$	$24.13^{1**}\pm2.07$	$6.72^{3**}\pm1.25$	$19.68^{2**}\pm1.52$	$5.82^{3**}\pm0.14$
B[*a*]P	$8.49^{1**}\pm0.33$	$3.13^{a**}\pm0.44$	$8.41^{1**}\pm0.32$	$3.04^{a**}\pm0.38$	$7.68^{1**}\pm0.30$	$3.44^{a**}\pm0.52$

X-x, X,Y-y, Z-z, valuesmarked withdifferentlettersonthe internalandexternal part of smoked products-industrially smoked (the line in this product group) statistically significant difference between means at $p \leq 0{,}05$

1-a, 2-b, 3-c, valuesmarked withdifferentlettersonthe internalandexternal part of smoked products-traditional technique (the line in this product group) statistically significant difference between means at $p \leq 0{,}05$

*, ** values marked with different letters of smoked products, industrial smoke and traditional technique (the line in this product group) statistically significant difference between means at $p \leq 0{,}05$

Source: own study.

influence of temperature on the cover and denaturation of proteins on the surface, which constitute a barrier for the penetration of PAH compounds into the products.

According to EFSA, benzo[*a*]pyrene is not a sufficient, suitable marker indicating the presence of PAHs in food and the most appropriate arrangement is the one of four specific PAHs (B[*a*]P, B[*a*]A, B[*b*]F and CHR). In 2011, based on the opinion of EFSA, modifications of the numerical limits of permissible PAH levels for individual food groups were carried out. Since September 1, 2014, the maximum level for the sum of four PAHs has been 12.00 μg kg^{-1} of a product (Commission Regulation (EU) No 835/2011). A total of 4 PAHs indicated in the research in the outer part in all three of meat products smoked using a traditional and an industrial method, exceeded the level of these aryls. Only in the chicken sausage smoked in the industrial method, Σ4PAH value was at the level of 8.62 μg kg^{-1}, and was below the value specified in the Regulation No. 835/2011 of the EU Commission. It should be noted that in all

products, regardless of the way of smoking treatment, undergoing analyses of the content of the sum of 4 PAHs in the inner part, the sum was below the value of 12.00 μg kg^{-1}. Similar relationships resulting from the industrial and traditional smoking, though for other smoked meat products, are presented in the work by CIECIERSKA and OBIEDZIŃSKI (2007). One should keep in mind many factors which may change the value of individual compounds from the PAH group. However, most authors recall those which are the most important, concerning variable parameters regulation: temperature, time, and smoke density. Another example of the variation of PAH compounds concentration are the studies presented by PÖHLMANN et al. (2012), in which PAH content depending on the variable factors and conditions of smoking (chip kind, the temperature of embers, smoke density and air access, as well as the time of the very process) was researched. Thus, both the parameters of the process, the type chips used and their physical parameters (humidity and granulation), repeatability of meat products undergoing the process of smoking and, of course, the very smoking method, have a significant impact on the aryls accumulation in smoked meat products.

According to KILJANEK et al. (2013) in studies conducted on smoked meat products by the Ministry of Agriculture and Rural Development, and the Ministry of Health, both for B[*a*]P and Σ4PAH were not significantly exceeded. In the reports on the 1400 samples that were tested for the content of 15 PAHs, the authors show that the contents of B[*a*]P were exceeded only in 5.3%. In contrast, the average concentration in relation to Σ4PAH, was at the level of 1.20 μg kg^{-1}. Dominating, approximately 40%, share in the indicated total of 4 PAHs came from chrysene, while the share of benzo[*a*]pyrene was about 15%. The authors mention that in the industrial method of smoking, the heat-treatment was an important part of the process, thanks to which meat products do not show signs of an increased accumulation of PAH compounds.

One should also keep in mind the speed and the distribution of the smoke-air mixture in the smoking chamber with a forced circulation, which cause the exchange of ingredients and provide fresh smoke. This allows for avoidance of exceeding permissible limits for PAH compounds content in meat products. Smoke held in a chamber without the exchange and the access of air, subjected to a high temperature, causes undesirable changes to take place between compounds.

All of these factors, mentioned both in the literature and in this study, can certainly contribute to obtaining a discrepancy of results. Thus, it seems important to present as many reports related to the level of PAH compounds content in meat products smoked in a variety of technological systems, as possible. This applies mainly to the industrial and traditional smoking process, taking into account the parametrics, the type of wood and the design of the

smoking chamber, as well as adjusting the speed of the distributed mixture of smoke. This allows for the continuous monitoring of smoked foods of animal source and thus, will eliminate errors made in industrial and traditional smoking technology.

Conclusions

Meat products smoked by using the traditional method were characterized by a higher concentration of B[a]P, both in the inner and outer parts of the products, which exceeds the permissible limit. The content of B[*a*]P was exceeded in meat products smoked in controlled conditions. In the industrial conditions, it was exceeded only in the outer part of the bacon (6.30 μg kg^{-1}) and „jałowcowa" sausage (3.40 μg kg^{-1}). In the inner part of all meat products smoked in controlled industry conditions, the content of Σ4PAH and B[*a*]P was not exceeded.The overall conclusion is that the industrial smoking in controlled conditions allows for the possibility of introducing changes which will enable reducing the excessive accumulation of PAH compounds in smoked products.

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EFFECT OF THE HOMOGENIZATION PROCESS ON THE RHEOLOGICAL PROPERTIES OF FOOD EMULSIONS

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K e y w o r d s: rheology of emulsions, pressure homogenization.

A b s t r a c t

The aim of this study was to assess changes in rheological characteristics and droplet size distribution in emulsions studied under the influence of the one- and two-stage homogenization processes, carried out at a pressure higher than in standard industry practice. The material consisted of food emulsions of the o/w type, with an oil phase content of 9%, 12%, and 18% of mass, in a 20% solution of milk protein concentrate. The one- and two-stage homogenization processes were carried out using a „Panda" laboratory homogenizer from GEA Niro-Soavi at the pressure of 30 MPa and 30/6 MPa at 45,0 ± 0,5°C. The degree of fat despersion was determined with the microscopic method, by using computer image analysis. The flow curves of the emulsions tested were determined using the rheometer of the Rheostress1 type with a concentric cylinder system Rotor 234 DIN 53019, at an increasing shear rate ($\dot{\gamma}$) ranging from 0,04 to 700 s^{-1}. Changes in shear stress versus shear rate value $\sigma = f(\dot{\gamma})$were described using the Ostwald de Waele power-law model. It was found that subjecting food emulsions to one- and two-stage homogenization pressure resulted in changes in the rheological parameters of flow curves. Droplet size depended on the percentage of oil in the emulsion and homogenization conditions. The homogenization process was followed by a significant decline of the consistency coefficient and a slight increase in the flow rate. These changes prove the effect of the homogenization process on the structure of emulsions.

WPŁYW PROCESU HOMOGENIZACJI NA WŁAŚCIWOŚCI REOLOGICZNE EMULSJI SPOŻYWCZYCH

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S ł o w a k l u c z o w e: reologia emulsji, homogenizacja ciśnieniowa.

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Abstrakt

Celem niniejszej pracy było dokonanie oceny zmian charakterystyk reologicznych oraz rozkładu wielkości kropel w badanych emulsjach pod wpływem procesu jedno- i dwustopniowej homogenizacji prowadzonej przy ciśnieniu wyższym niż w praktyce przemysłowej. Materiał do badań stanowiły emulsje spożywcze typu o/w o zawartości fazy olejowej 9%, 12% oraz 18% mas, w 20% roztworze koncentratu białek mleka. Proces homogenizacji jedno- i dwustopniowej prowadzono za pomocą laboratoryjnego homogenizatora „Panda" firmy GEA Niro-Soavi przy ciśnieniu odpowiednio 30 MPa i 30/6 MPa w temperaturze 45.0 ± 0.5°C. Stopień dyspersji tłuszczu określono metodą mikroskopową z wykorzystaniem komputerowej analizy obrazu. Krzywe płynięcia badanych emulsji wyznaczano za pomocą reometru typu Rheostress1 z układem cylindrów koncentrycznych Rotor 234 DIN 53019 przy rosnących szybkościach ścinania $\dot{\gamma}$ w zakresie od 0,04 do 700 s^{-1}. Zmiany naprężenia stycznego w funkcji szybkości ścinania $\sigma = f(\dot{\gamma})$ przybliżono modelem potęgowym Ostwalda de Waele. Stwierdzono, że poddanie emulsji spożywczych jedno- i dwustopniowej homogenizacji ciśnieniowej prowadziło do zmian parametrów reologicznych krzywych płynięcia. Wielkości kropel zależały od udziału oleju w emulsji i warunków homogenizacji. Po procesie homogenizacji następował wyraźny spadek wartości współczynnika konsystencji oraz nieznaczny wzrost wskaźnika płynięcia. Zmiany te dowodzą wpływu procesu homogenizacji na strukturę emulsji.

Introduction

The pressure homogenization process is an effective method used in many industries in the production of highly dispersed biological emulsions and suspensions (THIEBAUN et al 2003). The final result of this operation depends on the properties of the emulsion's components, type and amount of an emulsifier introduced and the manner of conducting the process. An appropriate selection of these parameters leads to a high quality product of homogeneous structure, better consistency and high stability (THIEBAUN et al. 2003, STEFFE 1996.). Perception of consistency and taste sensation during the consumption of food emulsions and homogenized juices are closely related to the particles size distribution of the dispersed phase (STEFFE 1996, PERRECHIL and CUNHA 2010, BIASUTTI et al. 2010, YONG WANG DONG et al 2011). The degree of dispersion of the emulsion droplets and the resulting changes in viscosity depend primarily on the participation of the dispersed phase and the homogenization pressure (KIEŁCZEWSKA et al. 2006, MASSON et al 2011, RÓŻAŃSKA et al. 2012). Flow characteristics of emulsions containing a relatively high proportion of the dispersed phase may be of a non-Newtonian character, as indicated by publications (DŁUŻEWSKA and LESZCZYŃSKI 2005, PERRECHIL and CUNHA 2010).

The aim of the study was to assess changes in the parameters and rheology characteristics and droplet size distribution of the food emulsions tested under the influence of one- and two-stage homogenization processes carried out at a pressure higher than that of standard industry practice.

Material and Methods

The material consisted of food emulsions of the o/w type, with an oil content of 9, 12 and 18% of mass, in a 20% solution of milk protein concentrate, prepared in a laboratory. The emulsion was prepared at 45 ± 0,2°C by adding up to 20% of the milk protein solution (MPC 75), comprising a continuous phase and olive oil in an amount shown in Table 1. In order to obtain the primary emulsion, a seven-minute dispersion was applied with the use of a laboratory mixer. Then, maintaining the temperature at 45 ± 0,2°C, emulsions were deaerated under a vacuum.

Table 1

Composition of food emulsions of o/w type

Product name	Concentration of the oil phase in the emulsion [%] w/w	20% solution of milk proteins [g]	Olive oil [g]
Food emulsions of o/w type	9.0 12.0 18.0	910 880 820	90 120 180

The one-and two-stage homogenization processes were carried out using a „Panda" laboratory homogenizer from GEA Niro Soavi at a pressure of 30MPa and 30/6 MPa respectively, at the same temperature as of when preparing the emulsion. Microscopy was used to assess the structures of the studied emulsions. Oil droplet size wasa determined by computer image analysis using the Lucia G software (Laboratory Imaging Ltd. LUCIA, Czech Republic). The diameter of oil droplets visible in the microscopic field were measured at 40x magnification. The flow curves of the emulsion before and after homogenization were determined at an increasing shear rate ranging from 0.04 to 700 s^{-1} using a Haake Rheo Stress® 1 rotating rheometer with a cylinder concentric system (234 DIN 53019). Shear stress changes as a function of shear rate was presented by the power law equation

$$\sigma = K\dot{\gamma}^n \tag{1}$$

where:
σ – shear stress [Pa],
K – consistency coefficient [Pas^n],
n – flow rate,
$\dot{\gamma}$ – shear rate.

Calculations of flow curves' parameters and a statistic analysis were performed with the use of *Statistica* v. 8.0 (StatSoft Inc. 2008), assuming the level of significance $p \leq 0.05$ to verify statistical hypotheses.

Conclusions and Discussion

Figure 1 shows the flow characteristics of food emulsions, while Table 2 summarizes the results of calculating the characteristics equation of flow curves, consisting of the power functions of the media tested. As a measure of the rheological model adjustment to the empirical flow curve assumed there were assumed high values of the coefficient of determination R^2 (Tab.2). The non-linear process based on the shear stress versus shear rate $\sigma = f(\dot{\gamma})$ relation, shows a non-Newtonian flow of emulsions that pseudoplastic liquids exhibit. Similar tendencies were found by BIASUTTI et al. 2010. The compiled data shows that the concentration of the oil phase has a very strong effect on the rheological properties of the emulsion. A doubled concentration of oil causes an increase in the apparent viscosity (Fig. 2.) The process of pressure homogenization in each test case led to changes in droplet size in the dispersed phase, which also contributed to changes in the value of consistency coefficients K and a slight increase in flow rates n (SAN MARTIN – GONZALEZ et al. 2009). The highest coefficient of consistency in the processes of one- and two-stage homogenization (K = 0.128 Pas^n and K = 0.095 Pas^n respectively) was characteristic to the emulsion containing an oil phase of 18%, whereas the smallest value of the consistency coefficient K was attributed to the emulsion with the 9% oil phase concentration (K = 0.037 Pas^n).

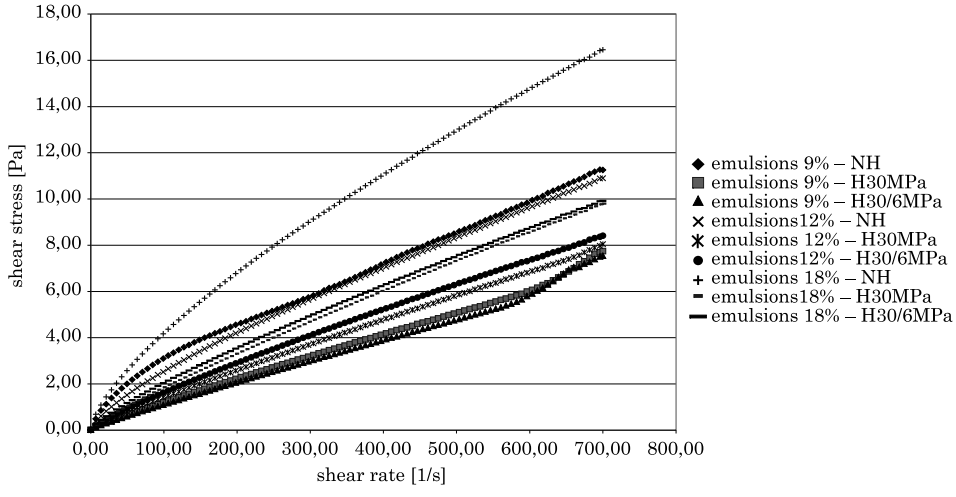

Fig. 1. Flow characteristics of the tested food emulsions

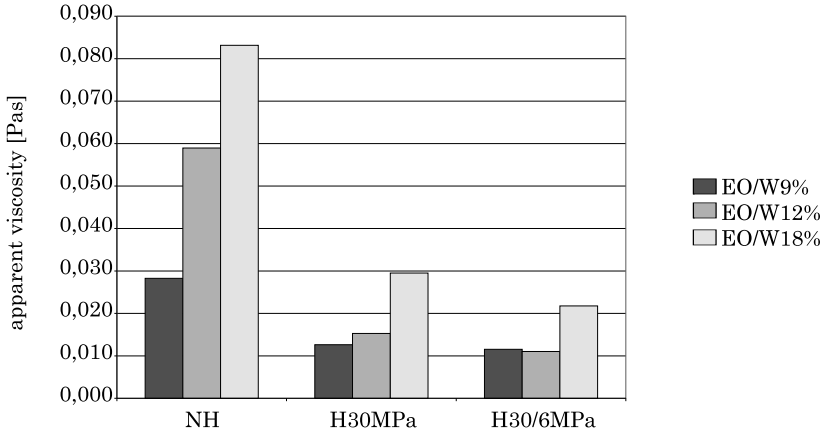

Fig. 2. Effect of the content of the oil phase to the apparent viscosity of the emulsion tested

Table 2

Parameters of the power low equation

Food emulsion of oil phase content [%] mas.	Process type	Ostwald – de Waele Model		Współczynnik determinacji
		K [Pas^n]	n [-]	R^2
9.0	Non-homogenized NH	0.163	0.635	0.997
	Homogenized I^o 30 MPa H30MPa	0.066	0.682	0.994
	Homogenized II^o 30/6MPa H30/6MPa	0.037	0.757	0.993
12.0	Non-homogenized NH	0.110	0.683	0.991
	Homogenized I^o 30 MPa H30MPa	0.058	0.721	0.991
	Homogenized II^o 30/6MPa H30/6MPa	0.082	0.695	0.996
18.0	Non-homogenized NH	0.190	0.678	0.998
	Homogenized I^o 30 MPa H30MPa	0.098	0.689	0.997
	Homogenized II^o 30/6MPa H30/6MPa	0.095	0.693	0.998

The figures 3–5 present sample images of the distribution of drops in the emulsions tested that show clear differences in the dispersed phase. These pictures prove that prior to homogenization droplets size in emulsions is the biggest and the value of Sauter diameter mean size D_{32} ranges from 2.792 to 3.482 μm (Fig. 3). Submitting the emulsion to single-stage homogenization process at a pressure of 30MPa resulted in almost double reduction in the size of droplets, the average value D_{32} of which ranged from 1.262 to 1.673 μm (Fig. 4), whereas the process of two-stage homogenization at a pressure of 30/6 MPa led to further reduction of the droplets diameter D_{32}, ranging from 1.097 to 1.265 μm. This caused the decrease of the apparent viscosity, which is also confirmed in the works of FLOURY et al. and KUHN et al. The dependence of the average value of Sauter diameter D_{32} on the pressure homogenization process is shown in Figure 6.

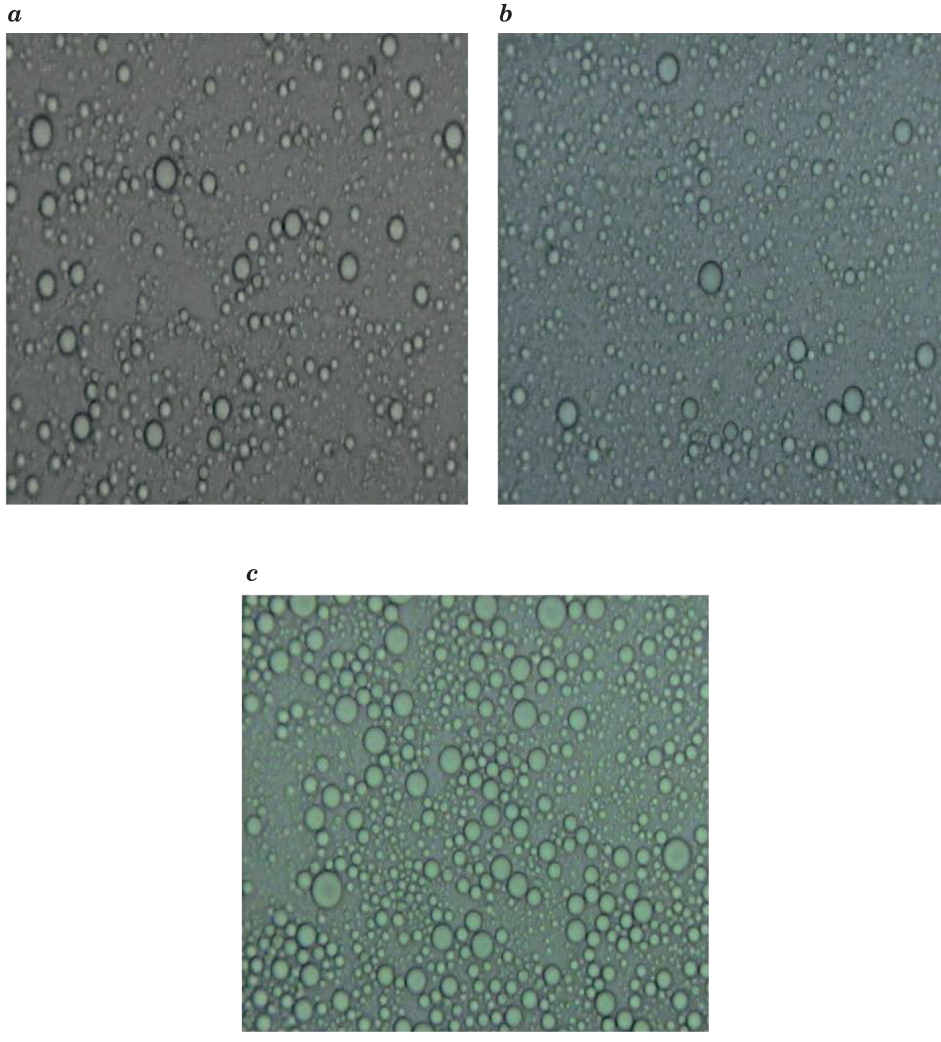

Fig. 3. Microscopic images of food emulsion before pressure homogenization process of the concentration of oil phase: *a* – 9%, *b* – 12%, *c* – 18%

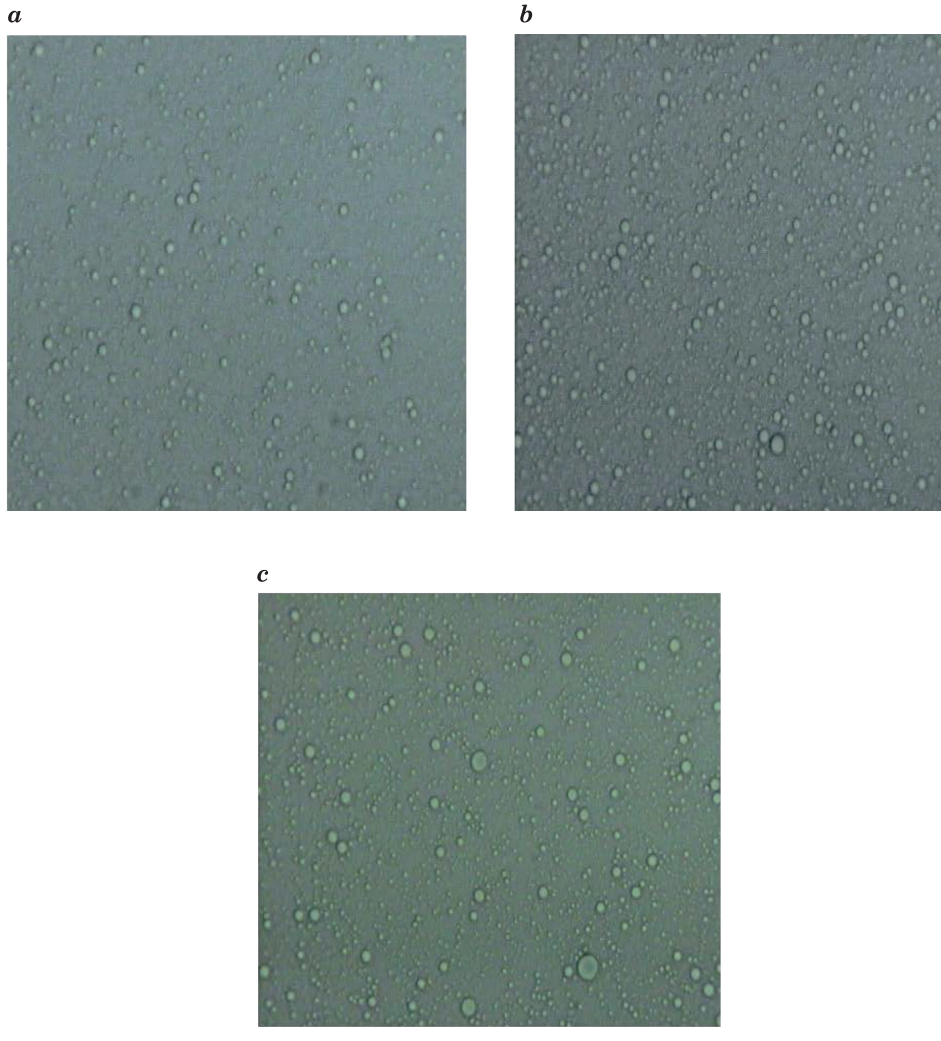

Fig. 4. Microscopic images of food emulsion after the single-stage homogenization process at a pressure of30 MPa of the concentration of oil phase: *a* – 9%, *b* – 12%, *c* – 18%

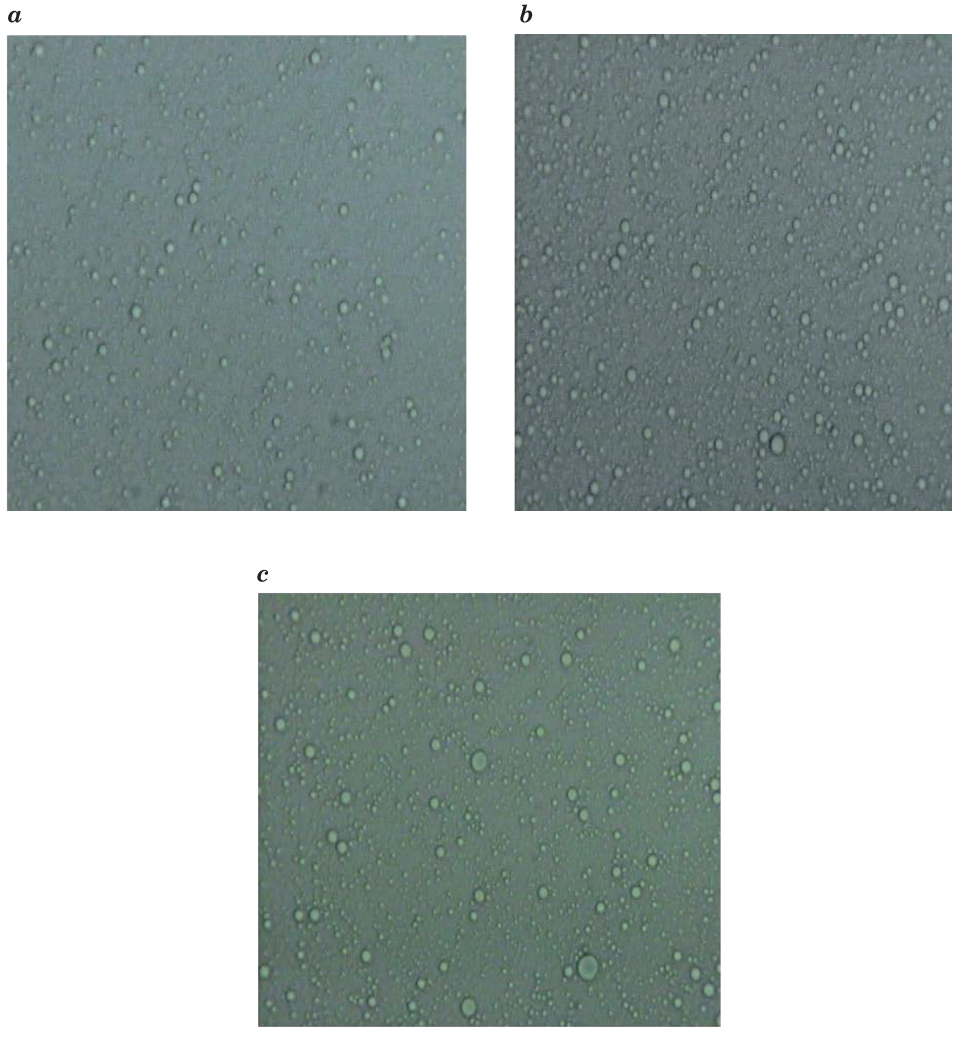

Fig. 5. Microscopic images of food emulsion after the two-stage homogenization process at a pressure of 30.6 MPa of concentration of oil phase: *a* – 9%, *b* – 12%, *c* – 18%

The influence of homogenization and oil phase concentration type on the droplet's distribution in food emulsions was evaluated by the one-way analysis of variance using an NIR test. Sources of variation under consideration were the type of process (non-homogenized NH, one-stage homogenized H30MPa, two-stage homogenized H30/6MPa) and the type of oil phase concentration (9%, 12%, 18%). The obtained results of the analysis of variance indicate that on the size of droplets in the emulsions tested 12 and 18% of the concentration

of the oil phase had no effect. In each tested case no significant differences between the single and two-stage homogenization were stated.

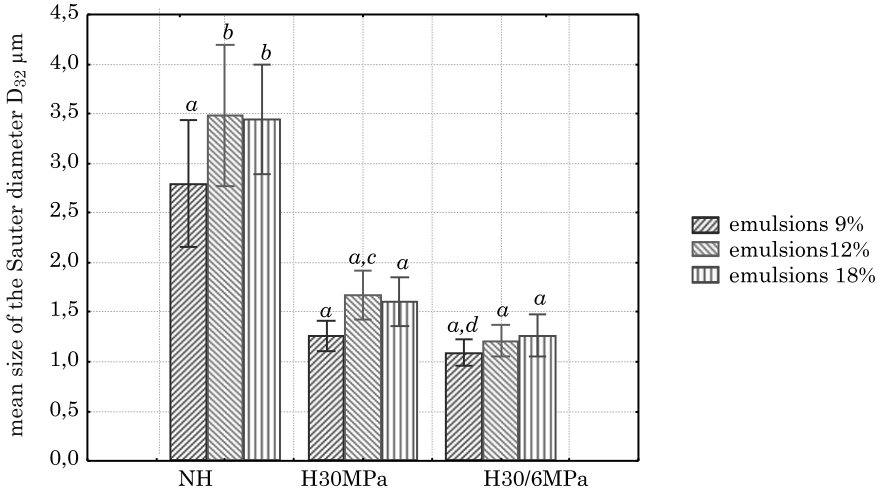

Fig. 6. Effect of the pressure homogenization process on the mean size of the Sauter diameter D_{32} µm– means marked with the same letter are not significantly different ($p \leq 0.05$)

Conclusions

The tests carried out and the results obtained show that the concentration of the oil phase had a significant effect on the change of coefficients of consistency and the emulsions' apparent viscosity. Submission of food emulsions of one- and two-stage homogenization pressure led to a similar degree of dispersion of droplets, which had an impact on the decrease in apparent viscosity. The homogenization process was followed by a decrease in value of the coefficient consistency *K* and a slight increase in the flow index, which reflects the effect of the homogenization process on the structure of the emulsion and its rheological properties.

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EFFECT OF THE EXPANDING PROCESS ON THE CONTENT OF PHENOLIC COMPOUNDS IN EXPANDED GRAINS

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K e y w o r d: millet grains, expanded grains, bioactive compounds, phenolic compounds.

A b s t r a c t

The aim of the study was to determine to what extent the process of expansion affects the interaction of phenolic compounds with other dry matter components such as starch, cellulose, fiber and protein.

As a research material millet grains and popping were used. The content of total phenolic compounds and contents of free and liberated from the ester and glycosidic bonds phenolic acids were determined. The content of phenolic compounds was analysed spectrophotometrically. To induce a characteristic color reaction a Folin-Ciocalteu reagent was used. The measurement was performed at a wavelength of 720 nm to the blank sample. Results were expressed as D-catechin.

The content of phenolic compounds, extracted with 80% methanol was 157.97 mg/100g dry matter for millet grain and 129.45 mg/100 g dry matter for popping.

WPŁYW PROCESU EKSPANDOWANIA NA ZAWARTOŚĆ ZWIĄZKÓW FENOLOWYCH W POPPINGU

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S ł o w a k l u c z o w e: ziarna prosa, popping, substancje biologicznie aktywne, związki fenolowe.

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Abstrakt

Celem pracy było ustalenie, w jakim stopniu proces ekspandowania wpływa na interakcję związków fenolowych z innymi składnikami suchej masy np. skrobią, celulozą, błonnikiem i białkiem.

Materiałem badawczym było ziarno prosa oraz popping. Oznaczono zawartość związków fenolowych ogółem, zawartość wolnych i uwolnionych z połączeń estrowych i glikozydowych fenolokwasów. Zawartość związków fenolowych oznaczono spektrofotometrycznie. Do wywołania charakterystycznej reakcji barwnej stosowano odczynnik Folina-Ciocalteu. Pomiar wykonano przy długości fali 720 nm wobec próby odczynnikowej. Wyniki podano w przeliczeniu na D-katechinę.

Zawartość związków fenolowych, wyodrębnionych 80% metanolem, wynosiła w ziarnie prosa 157,97 mg/100 g s.m., a w poppingu 129,45 mg/100g s.m.

Introduction

The terms „natural phytocompounds" or „bioactive plant compounds" apply to chemical substances which occur in plants and are potentially bioactive, for example, as antioxidants (TROSZYŃSKA, CISKA 2002, ZIELIŃSKIET al. 2012, IZADI et al. 2012). Bioactive compounds differ in terms of their physical and chemical properties and can be classified into hydrophilic ones (phenols, vitamin C, glucosinolates), which protect the aqueous environment of cytosol, and hydrophobic ones (tocopherols, carotenoids, phytosterols), which protect cellular membranes and lipoproteins from the destructive action of free radicals (KOPCEWICZ, LEWAKA 2002).

Cereal grain contains considerable amounts of bioactive compounds (polyphenols, tocopherols and phytosterols), which are strong antioxidants (PETERSON et al. 2001, WOLOCH et al. 2007, CZAPSKI, GORECKA 2014). Moreover, they protect grain from internal and external threats. They stimulate the growth of microflora, the feeding of insects and rodents and heal damaged parts of plants (KOPCEWICZ, LEWAKA 2002). The properties of bioactive substances depend mainly on their chemical structure. Bioactive compounds of plant origin include phenols, terpenoids, carotenoids, glucosinolates, alkaloids, capsaicinoids, betalains, allyl compounds, polyacetylenes and polysaccharides. Phenolic compounds are among the most common bioactive substances. Consumed with other ingredients of food, these compounds contribute to improvement of human health and play an important role in disease prevention. For example, they reduce the risk of a number of diseases, such as cancers, heart diseases or diet-related diseases (GRAJEK 2007, CZAPSKI, GORECKA 2014). Cereals and farinaceous products are among the most frequently consumed foods. They contain a range of phenolic compounds, which are a good source of natural antioxidants.

Millet is a cereal, which is rich in phenolic compounds. It is not very popular and often overlooked, but is a valuable material for the production of functional foods. It is one of the oldest crops and it used to be one of the main

cereals (CZERWIŃSKA 2010). Millet grain is mainly used to produce millet groats, but recently it has also been used to produce flakes and expanded grain (popping). Grain changes its physical and chemical properties during the process of expanding, for example, its volume increases many times (TYBURCY 2000). ZIELIŃSKI et al. (2012) report that processing grain can result in changes in polyphenol content, which include an increase in the content of phenolic acids.

The available literature contains a number of reports, which indicate the possibility of the formation of strong chemical bonds between phenolic compounds and components of dry matter, mainly proteins and polysaccharides (CHANDRA, SEKARA, SHAHIDI 2010).

Therefore, the hypothetical question arises: to what extent can the thermal energy of the expanding process cause interaction between the components mentioned above? In order to answer this question, the aim of this study was to evaluate the changes, which take place in phenolic compounds of millet grain during the production process of expanded grain. It involved examination of how the process of expanding affects interactions of phenolic compounds with other components of dry matter, such as starch and protein.

Material and methodology

Millet grains and expanded millet grains (popping) were used as the study material. The expanding process was conducted on a prototype device at Przedsiębiorstwo Produkcyjno-Handlowo-Usługowe „Szarłat" s.c. in Łomża. Grains containing 9–12% of moisture were heated at 280°C for a few seconds. The experimental part was conducted in three independent replications and the samples of the expanded grains were further analysed.

In order to verify the hypothesis put forward in this study and to achieve the study objective, its scope was restricted to determination of the total content of phenolic compounds, occurring both in free forms and as bound by ester and glycoside bonds in millet grain and expanded millet grain.

The material was prepared for the experiment by grinding and degreasing by the Soxhlet method, as per the Polish Standard (PN-ISO 6492:2005).

Phenols were isolated from the prepared material and were subsequently analysed by the method described by ZADERNOWSKI (1987).

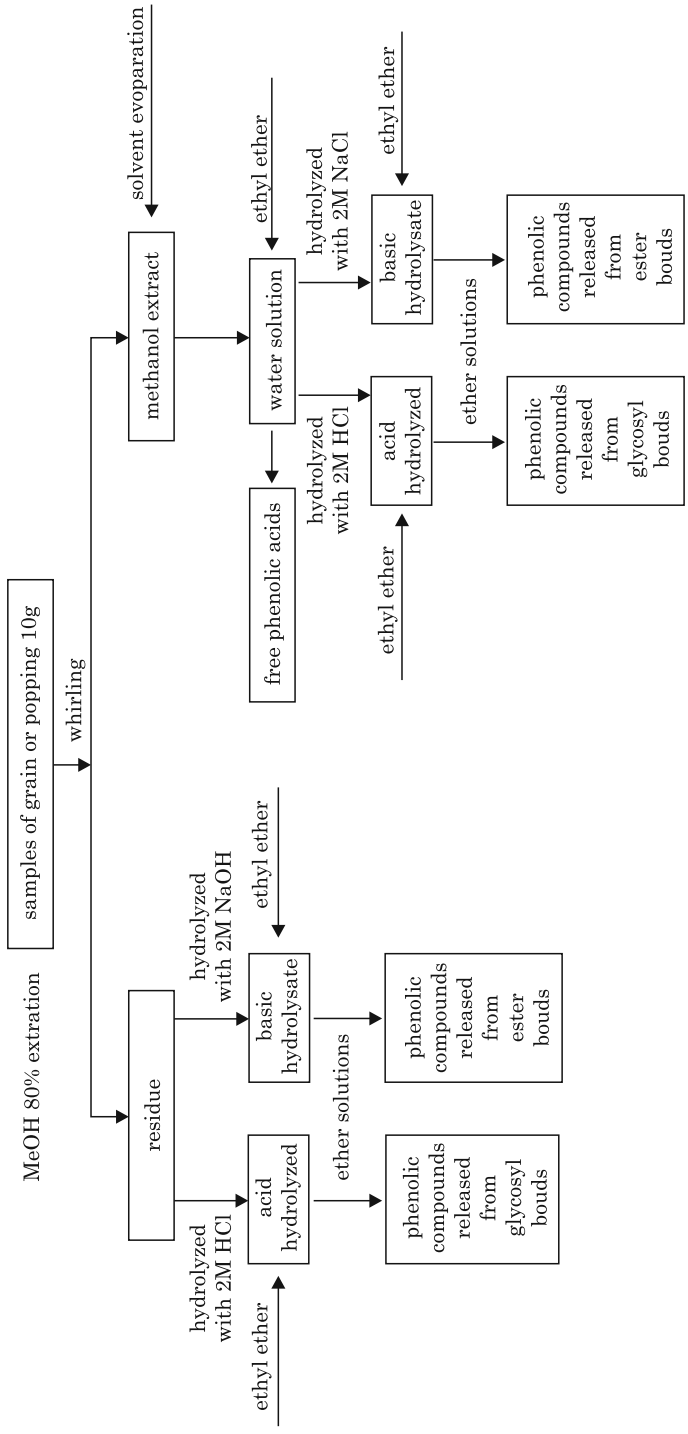

Fig. 1. A diagram of the extraction and isolation of phenolic compounds

Isolation of low-molecular phenolic compounds from grains and expanded grains

A 10 g sample was taken from the ground study material, to which 50 cm^3 of 1% solution of HCl in 80% MeOH was added and the whole sample was shaken in a shaker for 20 minutes.

The extraction was conducted twice more; each time 50 cm^3 of a 1% solution of HCl in 80% methanol was used and the whole sample was shaken in a shaker for 15 minutes. The extracts were filtered and then condensed on an R210 rotary vacuum evaporator (Buchi Labortechnik AG, Postfach, Switzerland) until the solvent had evaporated completely. The condensed extract obtained in this manner was dissolved in methanol or water at pH 2, transferred to a 50 cm volumetric flask and made up to volume. The extract dissolved in methanol was used to assay the total amount of phenolic compounds. The extraction residue was dried and kept for further analyses.

Isolation of free low-molecular phenolic compounds (FLPC) from a mixture of the organic compounds extracted with methanol from millet grains and expanded millet grains

The extract isolated from ground millet grains and expanded grains, which is a mixture of low-molecular organic compounds, was dissolved in water at pH 2. Phenolic compounds were extracted from the prepared mixture with ethyl ether by continuous liquid-liquid extraction for 24 hours. Subsequently, ether was evaporated and the condensed sample was transferred to a 10 mL volumetric flask and rinsed out with 80% methanol. Free low-molecular phenolic compounds were assayed in the prepared samples by the colorimetric method. The FLPC extraction residue was subjected to alkaline and acidic hydrolysis in order to isolate phenolic compounds bound by ester and glycoside bonds with other low-molecular organic compounds soluble in 80% methanol.

Isolation of phenolic compounds bound by the ester bond with other low-molecular organic compounds, isolated with methanol from millet grains and expanded grains

The ether extraction residue was evaporated to dryness in a stream of nitrogen and the sample was then dissolved in 50 cm^3 of 2M NaOH and stirred with an electromagnetic agitator for 4 hours. After that time, the sample was acidified to pH 2 and the phenolic compounds released from esters were

extracted with ethyl ether by continuous liquid-liquid extraction. Subsequently, ether was evaporated and the condensed sample was transferred to a 10 mL volumetric flask and rinsed out with 80% methanol. The amount of released compounds was determined by the spectrophotometric method using the Folin-Ciocalteu reagent.

Isolation of phenolic compounds bound by the glycosidic bond with other low-molecular organic compounds, isolated with methanol from millet grains and expanded grains

The ether extraction residue was evaporated to dryness in a stream of nitrogen and subsequently dissolved in 50 cm^3 of 2M HCl and hydrolyzed for 1 hour by heating it up on a boiling water bath under a reflux condenser. After that time, the sample was acidified to pH 2 and free phenolic compounds were extracted with ethyl ether by continuous liquid-liquid extraction. Subsequently, ether was evaporated and the condensed sample was transferred to a 10 mL volumetric flask and rinsed out with 80% methanol.

The amount of the phenolic compounds released from glycosides was determined by the spectrophotometric method using the Folin-Ciocalteu reagent.

Isolation of phenolic compounds released from esters which are present in extraction residue of millet grain and expanded grain

2 g portions of the methanol extraction residue were weighed after the residue was dried and homogenized. Subsequently, 50 cm^3 of 2M NaOH was added and the whole sample was stirred with an electromagnetic agitator for 4 hours. After that time, the sample was acidified to pH 2 and free phenolic compounds were extracted with ethyl ether by continuous liquid-liquid extraction. Subsequently, ether was evaporated and the condensed sample was transferred to a 10 mL volumetric flask and rinsed out with 80% methanol.

The amount of phenolic compounds released from esters was determined by the spectrophotometric method using the Folin-Ciocalteu reagent.

Isolation of phenolic compounds released from glycosides which are present in extraction residue of millet grain and expanded grain

The extraction residue was dried and 2 g portions of it were weighed. Subsequently, 50 cm^3 of 2M HCl was added and the whole sample was

hydrolyzed for 1 hour, heating it up in a boiling water bath under a reflux condenser. After that time, the sample was acidified to pH 2 and free phenolic compounds were isolated with ethyl ether by continuous liquid-liquid extraction. Subsequently, ether was evaporated, then condensed sample was transferred to a 10 mL volumetric flask and rinsed out with 80% methanol. The amount of phenolic compounds released from the glycosides was determined by the spectrophotometric method using the Folin-Ciocalteau reagent.

Plotting a calibration curve 100 mg of D-catechin was dissolved in methanol and the volume was made up to 100 cm^3. The stock solution was used to make dilutions from 1 to 10, containing 0.01 to 1.0 mg of D-catechin, respectively. 0.25 cm^3 of the solution was sampled from each diluted solution and the following were added in sequence: 0.25 cm^3 of the Folin-Ciocalteu reagent and 0.5 cm^3 of sodium carbonate solution; the whole sample was made up with distilled water to the volume of 5 cm^3. After 30 minutes, the absorbance was read out at the wavelength of 720 nm against a blank sample.

Phenolic compounds assay in the sample before and after hydrolysis

The amount of phenolic compounds released from glycosides was determined by spectrophotometry, using the Folin-Ciocalteu reagent, by the method described in AOAC (Association of the Official Analytical Chemists 1974).

0.5 mL of the extract, 0.25 mL of Folin-Ciocalteu reagent (diluted at 1:1 v/v with distilled water, prepared several minutes before), 0.5 mL of sodium carbonate and 3.75 mL of distilled water were sampled to a centrifuge tube. After all the components were added, the samples were mixed, capped with a stopper and left for 25 minutes. After that time, they were centrifuged at 12,000 rpm in an Eppendorf centrifuge. Subsequently, absorbance was measured at the wavelength of 720 nm against a blank sample (0.25 mL of Folin-Ciocalteu reagent, 0.5 mL of sodium carbonate and 4.25 mL of distilled water were sampled to a centrifuge tube). The content of phenolic compounds was converted to the content of D-catechin and is given as the number of mg per 100 g dry matter of millet grain.

Discussion of results

An analysis of increased reactivity of phenolic compounds compared to other components of dry matter, caused by increased energy potential during industrial processing, should take into account the chemical diversity of this

group of organic compounds. Phenolic compounds are a very large group of organic substances which perform diverse, non-nutritional, functions in the body. After they are consumed with food, the majority of phenolic compounds retain their bioactive properties and positively affect the function of the human body.

Literature reports have described the anti-oxidative properties of phenolic compounds which occur in grain, grains (IZADI et al. 2012, ZIELIŃSKI et al. 2012, KIM et al. 2010, TROSZYŃSKA, CISKA 2002), fruit and vegetables (GRAJEK 2007, CZAPSKI, GÓRECKA 2014,) and their inhibitory effect on enzyme activity (KUBICKA, JĘDRYCHOWSKI 2001, KOPCEWICZ, LEWAKA 2002). CHOI et al. (2007) showed methanol extracts of sorghum, rice, millet and barley contain high concentrations of polyphenolic compounds and, consequently, high anti-oxidative activity. KOPCEWICZ, LEWAKA (2002) reported that phenolic compounds in plants and crops perform the function of enzymatic activity inhibitors. On the other hand, KUBICKA, JĘDRYCHOWSKI (2001) claim that phenolic compounds extracted from pumpkin with an enzyme do not have an inhibitory effect on the enzyme activity. In their opinion, the lipoxygenase activity depends on the chemical composition of extraction solutions.

Phenolic acids, derivatives of cinnamic and benzoic acids, are the main group of phenolic compounds. Free phenolic acids are present in small amounts in plants and their presence usually results from the fact that they have not been transformed into bound forms during physiological processes. Phenolic acids usually occur in a bound form in different parts of plants, including grain and grain, as components of low- or high-molecular weight polyphenolic compounds. For example, various forms of depsides are low-molecular polyphenols. High-molecular polyphenols include lignin procyanidins, hydrolyzing tannins, flavonoids, with which phenolic acids form conjugates bound by ester or glycoside bonds. GAWLIK-DZIKI (2004) reports that some derivatives of cinnamic acid are commonly present in esters with carboxylic acids or glucose, while derivatives of benzoic acid usually occur as glycosides. Derivatives of cinnamic acid occur as free compounds, as a depside or as glycosides. Chlorogenic acid, which occurs in grain, in oily seeds and in coffee, is an example of a depside. It is formed by binding caffeic acid with quinic acid. Another example is ellagic acid, a dimer of gallic acid, whose molecules are bound at the second position by a C-C bond and by two symmetric ester bonds between the carboxylic group of one acid and the hydroxyl group of the other, forming a system of four condensed rings (BOCK et al. 1981). Esters of synapinic acid and choline, or sinapine, is a compound which typically occurs in the *Brassicaceae* family plants (ZADERNOWSKI 1987). Most depsides occur in bound forms, for example, they are parts of hydrolyzing tannins and form complexes with proteins and polysaccharides.

Apart from the structures described above, phenolic acids bind with lipids, sterols, polysaccharides, peptides.

Due to the high diversity of their chemical structure and mutual relationships, as well as their ability to interact with other organic compounds, such as sugars, proteins and lipids, phenolic compounds are difficult to isolate and assess physicochemically, for example, in terms of their antioxidative or inhibitory properties.

Therefore, an analytical procedure was applied to extract phenolic compounds in this study whose detailed description is provided in the methodology part of the paper. Phenolic compounds are usually extracted with methanol or acetone (SHAHIDI, NACZK 2006). Methanol is an organic polar solvent, which is effective in the extraction of low-molecular polyphenols, such as phenolic acids, depsides, flavonoids, glycosides and esters of phenolic acids with monosaccharides, fatty acids and sterols. Acetone is used to extract procyanines, catechins, tannins and polyphenols, typical of grain shells. In this study, phenolic acids were extracted from millet grain and expanded grain with 80% methanol acidified with 1% HCl. A similar extraction solvent has been used by other researchers. It must be borne in mind that extraction of phenolic compounds is frequently incomplete, with only free phenolic acids and their conjugates as low-molecular esters and glycosides being isolated. This is because some phenolic acids occur in plants as cellular walls polymers, e.g. pectin, protein, fiber, polysaccharides.

One such example is ferrulic acid, which forms esters and glycosides present in the cellular walls of cereals. Most of the ferrulic acid and p-coumaric acid present in cereals is bound with arabinoxylans (SHAHIDI, NACZK 2006). Therefore, it seems justified to conduct pre-hydrolysis and to cleave the bonds between phenolic compounds and the protein or polysaccharide matrix. ZADERNOWSKI (1987), ROSS et al. (2009), GARCIA-SALAS et al. (2010), WATSON (2014) propose conducting alkaline hydrolysis with 2 M NaOH, followed by acidic hydrolysis with 2 M HCl. Both of these methods of hydrolysis have been applied in this study.

Fig. 2 shows the total amount of phenolic compounds found in four samples of millet grain and in two samples of expanded grain. The amount comprises low-molecular forms of phenolic compounds soluble in 80% methanol; these include mainly free phenolic acids and their conjugates as well as free forms of other polyphenols. It does not include phenolic acids bound with cellular wall polymers because these are compounds with high reactivity and affinity, bound by strong chemical bonds, which cannot be broken by methanol. Easy formation of complexes of phenolic acids with flavonoids, structural molecules of plant cells (proteins, lignins, cellulose) and other acids (e.g. maleic and tartaric acid) determines the diversity of compounds in the group and their solubility in methanol and acetone.

The mean total content of phenolic compounds is listed in table 1; the values were converted to the content of catechin and expressed in mg/100 g of sample. The total content of phenolic compounds in the samples of millet grains under analysis ranged from 134.68 to 178.83 mg/100 g (mean 157.97 ± 19.08 mg/100 g) and from 122.08 to 136.82 mg/100 g (mean 129.45 ± 10.42 mg/100 g) in samples of expanded grains. Similar amounts of phenolic compounds were found in millet grains by ZHANG et al. (2014)

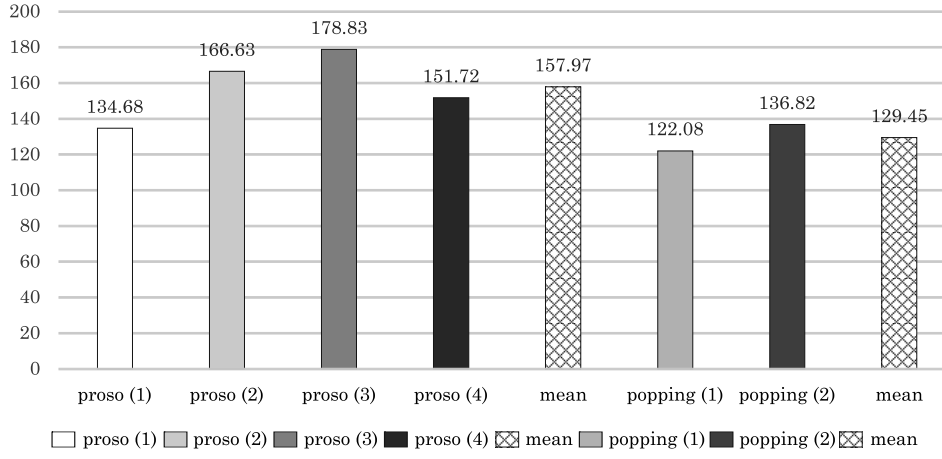

Fig. 2. The total content of free phenolic compounds (soluble in 80% methanol), expressed in mg per 100 g of millet grains and expanded grains

Table 1
Mean content of phenolic compounds, expressed in mg per 100 g of millet grains and expanded grains

	Total phenolic compounds [mg/100 g]	Free phenolic acids [mg/100 g]	Free phenolic acids and other forms of phenolic compounds released by hydrolysis of methanol extract and breaking:		Phenolic acids and other forms of phenolic compounds released by hydrolysis of millet grains extract residue and breaking:	
			ester bonds	glycoside bonds	ester bonds	glycoside bonds
Millet grains	157.97±19.08	110.98±10.15	13.25±1.15	25.74±0.89	90.27±8.31	78.94±8.01
Expanded grains	129.45±10.42	98.99±5.19	11.83±1.06	17.62±1.36	92.87±1.51	116.68±12.64

ZHANG et al. (2014) report that the content of free polyphenols ranged from 27.48 to 151.14 mg of gallic acid equivalent / 100 g of dry weight of a sample.

An analysis of the total content of phenolic compounds showed that expansion resulted in an 18% reduction of the content of free low-molecular

phenolic compounds in samples of expanded grains. Some phenolic compounds were probably not isolated from expanded grains by extraction with methanol because of their secondary, strong bonding with components of dry matter of expanded grains. Such bonds are too strong to be broken by extraction of grains and expanded grains with methanol. Bound phenolic compounds were also present in millet grains, but their content was much smaller than in expanded grains.

According to ZHANG et al. (2014) the amount of phenolic compounds which occur in a bound form in millet grain ranged from 55.95 to 305.81 mg of gallic acid equivalent per 100 g of dry matter. The contents of bound phenolic compounds and of total phenolic compounds were 62.08% and 67.05%, respectively (ZHANG et al. 2014). The interaction of free polyphenols, mainly phenolic acids, with other substances, may result from supplying large amounts of energy to the system during the expanding process, but it may also be caused by changes in the ring structure in aromatic polyphenol rings.

Low-molecular phenolic compounds isolated from millet grain or expanded grain with methanol include mainly free phenolic acids, their depsides and conjugates with flavonoids or glycosides, which are formed by binding phenolic acids with other polyphenols or mono- and disaccharides, inositol. The content of free phenolic acids in millet grain was 110.98 ± 10.15 mg/100 g of sample and it was lower by 11.99 mg/100 g in expanded grain. This demonstrates that during the expanding process, part of the free phenolic acids or their conjugates can form stable complexes with polymers present in dry matter, for example, with proteins, lignins and cellulose.

The solution obtained after free phenolic acids were isolated was hydrolyzed in both alkaline and acidic environments. Both the methods of hydrolysis are not fully selective and both ester and glycoside bonds can be broken simultaneously. For example, both glycoside bonds and weak ester bonds can be broken in an acidic environment. This increases the amount of polyphenols released and disturbs the total balance of phenolic compounds.

Alkaline hydrolysis produced 13.25 ± 1.15 mg of phenolic compounds released from esters/100 g of millet grains and 11.83 ± 1.06 mg/100 g (Fig. 3.) of expanded grain. Many more phenolic acids were isolated by acidic hydrolysis: 25.74 ± 0.89 mg/100 g of millet grain and 17.62 ± 1.36 mg/100 g of expanded grain. The phenolic compounds isolated from millet grain and expanded grain by extraction with methanol were a mixture of free phenolic acids and other species into which phenolic acids were incorporated. More phenolic acids in these compounds were bound in the form of glycosides than as esters.

Very interesting findings were obtained in an assay of phenolic acids in the residue after extraction of low-molecular phenolic compounds with methanol. Phenolic acids were isolated from the extraction residue during alkaline and

acidic hydrolysis by breaking ester and glycoside bonds. Mainly complexes of phenolic acids with cellular wall polymers (proteins, polysaccharides) were broken. The amount of phenolic acids released from esters in the samples under analysis varied slightly and was 90.27 ± 8.31 mg/100 g of millet grain and 92.87 ± 1.51 mg/100 g (Fig. 4.) of expanded grain. Significant differences were observed in terms of the amount of phenolic acids released from glycoside compounds. Expanded grain contained 116.68 mg of free phenolic acids/100 g, which was considerably more than in millet grain (78.94 mg/100 g). This shows that the content of bound phenolic compounds was higher than that of free forms. This also indicates that the process of expanding may result in some phenolic compounds forming stable complexes with other components of dry matter, e.g. cellular wall polymers. MAILLARD and BERSET (1995) claim that these compounds perform various functions, e.g. they act as antioxidants.

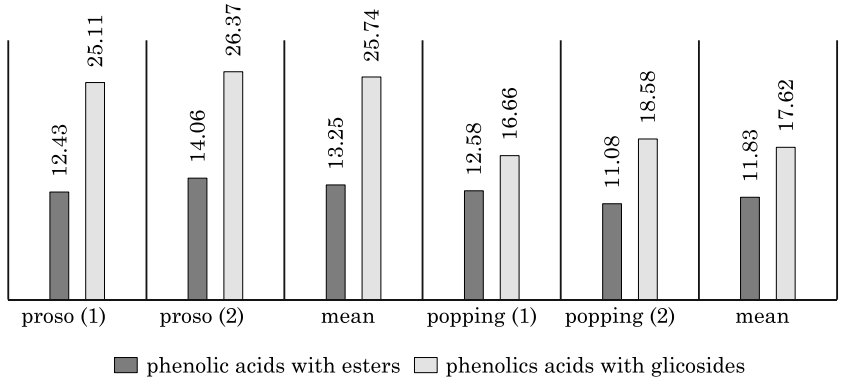

Fig. 3. Content of phenolic acids released from esters and glycosides, expressed in mg per 100 g of millet grains and expanded grains

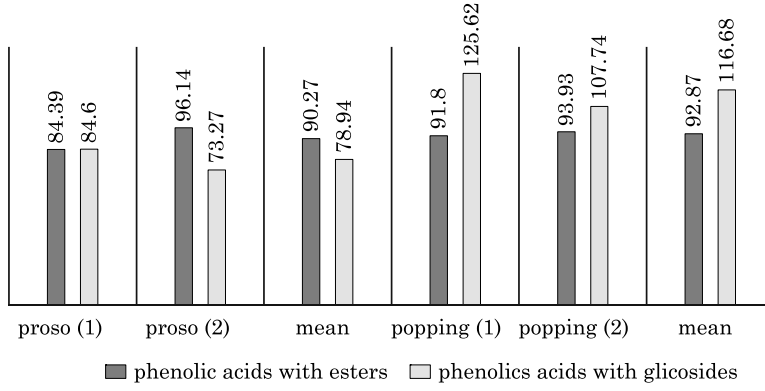

Fig. 4. Content of phenolic acids bound with components of extraction residue, released from esters and glycosides, expressed in mg per 100 g of millet grains and expanded grains

Summary and conclusions

The total phenolic compounds content was higher in millet grains than in expanded millet grains. This means that some of the phenolic compounds were bound during the expanding process. The polyphenol content was probably affected by the high temperature of the expanding process.

Phenolic acids bound in esters were isolated in alkaline hydrolysis. The content of free phenolic acids was higher in millet grains than in expanded millet grains.

Phenolic acids bound in glycosides were released in acidic hydrolysis. The amount of free phenolic acids was higher in millet grains than in expanded millet grains.

It may be claimed that more phenolic acids forms conjugate with carbohydrates (glycosides) than with proteins (esters).

The general conclusion can be drawn on the basis of the study that the thermal energy supplied during the millet grain expanding process increases the amount of phenolic compounds bound with other components of dry matter of expanded grain. This may make protein in expanded grain less digestible.

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EFFECT OF NITROGEN FERTILIZATION METHOD ON THE YIELD AND QUALITY OF MILEWO VARIETY SPRING TRITICALE GRAIN

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K e y w o r d s: nitrogen fertilization, grain and protein yield, nutrients.

A b s t r a c t

In this paper the effect of soil fetilization (NH_4NO_3 and $CO(NH_2)_2$) foliar fertilization ($CO(NH_2)_2$) with and without Ekolistem on selected grain quality parameters and the yield of Milewo variety spring triticale was determined. It was found based on the obtained results that the nitrogen fertilization level and the years of research affected the grain, protein yield and the concentrations of (selected) minerals in spring triticale grain. The highest grain and protein yield of Milewo variety spring triticale was obtained after the application of nitrogen fertilization at the rate of 120 kg ha^{-1}. Protein yield increased with the increasing grain yield and the coefficient of determination was close to the linear correlation coefficient (R^2 = 0.936). The applied nitrogen fertilization 120 kg ha^{-1} affected grain phosphorus content. Lack of basic mineral fertilization (120 kg N ha^{-1}) supplementation with the mixed fertilizer with additional micronutrients resulted in an increase in grain potassium content. Joint application of urea plus Ekolist with 80 kg N ha^{-1} caused an increased amount of magnesium compared to the higher fertilization level.

WPŁYW SPOSOBU NAWOŻENIA AZOTEM NA PLONOWANIE I JAKOŚĆ ZIARNA PSZENŻYTA JAREGO ODMIANY MILEWO

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S ł o w a k l u c z o w e: nawożenie azotem, plon ziarna i białka, składniki pokarmowe.

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Abstrakt

Celem pracy było określenie wpływu zróżnicowanego nawożenia doglebowo (NH_4NO_3 and $CO(NH_2)_2$) oraz dolistnie ($CO(NH_2)_2$) bez i z Ekolistem na wybrane parametry jakościowe ziarna i plonowanie pszenżyta jarego odmiany Milewo. Na podstawie uzyskanych wyników stwierdzono, że poziom nawożenia azotem i lata badań wpływały na plon ziarna, białka i koncentracje (wybranych) składników mineralnych w ziarnie pszenżyta jarego. Najwyższy plon ziarna i białka pszenżyta jarego odmiany Milewo uzyskano po zastosowaniu nawożenia azotem w dawce 120 kg ha^{-1}. Wraz ze wzrostem plonu ziarna wzrastał plon białka, a współczynnik determinacji był bliski współczynnikowi korelacji liniowej (R^2=0,936). Nawożenie azotem w dawce 120 kg ha^{-1} wpłynęło na zawartość fosforu w ziarnie. Brak uzupełnienia podstawowego nawożenia mineralnego (120 kg N ha^{-1}) nawozem wieloskładnikowym z dodatkiem mikroelementów zwiększało zawartość potasu w ziarnie. Łączne stosowanie mocznika i Ekolistu w dawce 80 kg N ha^{-1} w porównaniu z wyższym poziomem nawożenia spowodowało wzrost ilości magnezu.

Introduction

Modern plant production technology aimed at obtaining a high grain yield of proper quality should include balanced fertilization with all necessary nutrients (KOCOŃ 2009).

In intensive agricultural production, the fertilization level and the time of application is the decisive measure in achieving a high crop output with good quality properties (KNAPOWSKI et al. 2009, JANUŠAUSKAITĖ 2013, WARECHOWSKA et al. 2013). Nitrogen is undoubtedly the key factor determining cereal yield to the greatest degree. This nutrient modifies both the yield of its chemical composition and the grain feed value (WRÓBEL 2005, KNAPOWSKI et al. 2010). An important element of crop cultivation technology is foliar nitrogen fertilization combined with mixed fertilizers containing a set of properly selected micronutrients (DOMSKA et al. 2009, WOJTKOWIAK et al. 2014). This fertilization method enables the quick provision of minerals in short supply for the plants, both if they are deficient in soil and if the uptake is difficult. These nutrients are responsible for the synthesis and metabolism of many compounds and thus affect the quality of the obtained yield and the possibilities of its use in human and animal nutrition. Research by MALAKOUTI (2008) shows that the application of combined mineral and micronutrient fertilization increases grain yield and also improves the nutritional value of cereal grain.

Spring triticale cultivation is becoming increasingly important, especially in areas with unfavourable conditions in winter. According to SANTIVERI et al. (2002), spring triticale grain is characterized by a higher grain yield (from 896 to 1758 kg ha^{-1}) than the winter form of triticale. According to ROYO and PARES (1996) winter triticale yielded about 43% more forage than spring types, but after forage removal the spring types yielded about 36% more grain than winter triticale. The area under triticale in the world amounts to 3.7 million

ha, with 1/3 in Poland (FAO 2013), but this is mainly winter cultivation. Compared to winter triticale, the number of varieties is small, yet they differ considerably in terms of many agricultural and quality traits. Nine spring triticale varieties are currently registered in the national variety register (COBORU 2013). The Milewo variety was developed in the Strzelce Breeding Center (IHAR group) and entered in the register in 2008. It is the highest yielding spring triticale variety in Poland in the years 2011 and 2012.

In this paper the effect of soil fetilization (NH_4NO_3 and $CO(NH_2)_2$) foliar fertilization ($CO(NH_2)_2$) with and without Ekolistem on selected grain quality parameters and the yield of Milewo variety spring triticale was determined.

Materials and Methods

A field experiment was conducted in the years 2010-2011 at the Didactic and Experimental Station in Tomaszkowo belonging to the University of Warmia and Mazury in Olsztyn (53°72'N; 20°42'E). The experiment was carried out using the random block method in triplicate on surface brown soil with the granulometric composition of light loam classified as Haplic Cambisol according to FAO-WRB (2006). The soil was characterized by an average abundance of phosphorus, potassium, zinc and manganese and a low abundance of copper and a slightly acid reaction (Table 1).

Table 1

Soil properties (average 2010–2011)

Parameter, unit		Value
Type of soil (FAO-WRB 2006)		Haplic Cambisol
pH in KCl		5.05
$C_{org.}$ [g kg^{-1}]		7.71
$N_{tot.}$ [g kg^{-1}]		0.97
Content of mineral [mg kg^{-1}]	P	108.8
	K	207.5
	Mg	50.0
	Zn	14.5
	Mn	182.0
	Cu	2.10

Triple superphosphate at a rate corresponding to 30.2 kg P ha^{-1} was used for fertilization to enrich the soil with phosphorus, and potassium was applied in the form of 56% potash salt at a rate equal to 83.1 kg K.

The factor differentiating the fertilized objects was the nitrogen rate and application method and the fertilizer form (Table 2). Nitrogen fertilization was applied in the amount of 80 and 120 kg N ha^{-1}. For soil fertilization ammonium

nitrate (NH_4NO_3) and urea ($CO(NH_2)_2$) was used and foliar fertilization was done with application of urea ($CO(NH_2)_2$) with and without Ekolist. A mixed fertilizer (Ekolist) with a specially-developed chelating complex – chelacid was applied in the experiment. The following were introduced with Ekolist at a rate of 2.0 dm^{-3} ha^{-1} (g dm-3): N – 240.0; K – 130.0; Mg – 40.0; S – 10.0; Cu – 10.0; Zn – 5.0; Mn – 1.0; Fe – 2.0; Mo – 0.04 and B – 10.0.

Table 2

Fertilization diagram

Object	Sum of N [kg ha^{-1}]	Applying time, and type N fertilizer (dose N kg ha^{-1})		
		before sowing	BBCH 23–29	BBCH 31–32
		soil fertilization	foliar fertilization	
1	80	–	(40) $CO(NH_2)_2$	(40) $CO(NH_2)_2$
2	80	–	(40) $CO(NH_2)_2$	(39.76) $CO(NH_2)_2$ + (0.24) Ekolist*
3	120	(40) NH_4NO_3	(40) $CO(NH_2)_2$	(40) $CO(NH_2)_2$
4	120	(40) NH_4NO_3	(40) $CO(NH_2)_2$	(39.76) $CO(NH_2)_2$ + (0.24) Ekolist*

$CO(NH_2)_2$ – urea; NH_4NO_3 – ammonium nitrate;
BBCH – Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie
* mixed fertilizer

Cultivation measures for spring triticale were conducted according to the cultivation requirements for this species. After the forecrop was harvested, ridge plowing for winter was performed to improve the soil structure and store post-winter water. Spring measures were limited to shallow tillage starting with harrowing. The next spring measure was phosphorus and potassium fertilization, fertilization with pre-sowing nitrogen rate, cultivating, harrowing, cereal sowing and post-sowing harrowing. Depending on the used variants, the fertilizers were broadcast on the field surface and during the crop's growing season the fertilizers were broadcast at the BBCH 23–29 stage and applied to foliage at the BBCH 31–32 stage. The harvest was carried out within the first ten days of August with a plot harvester.

Temperature and precipitation measurements were conducted during the experiment. The average temperature was similar in both years of research and the monthly temperature distribution did not diverge from the averages from the multi-annual period (Table 3). The low precipitation in April deserves special attention. In May 2011, the precipitation (51.1 mm) was close to the averages from the multi-annual period and in 2010 (131.9 mm) it exceeded the average precipitation totals for the multi-annual period by more than twice. A higher precipitation total was recorded from July to August compared to the multi-annual period. The month of July 2011, in which the precipitation was very high (202.0 mm), deserves special attention.

Table 3

Weather conditions during the study

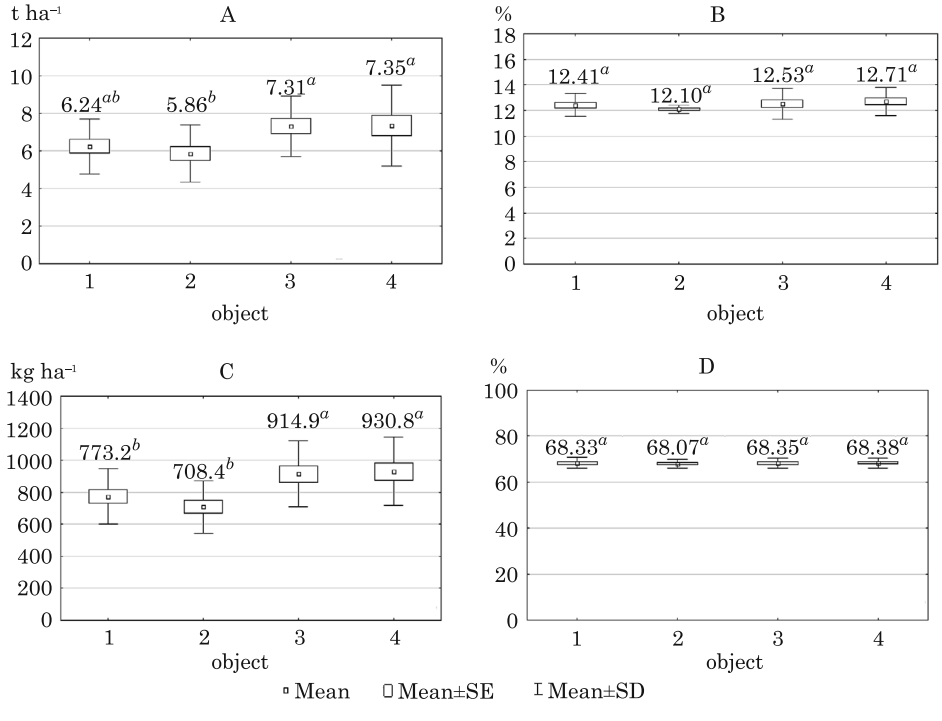
Year	March	April	May	June	July	August	Average March--August
	Temperature (°C)						
2010	2.1	8.1	12.0	16.4	21.1	19.3	13.2
2011	1.6	9.1	13.1	17.1	17.9	17.6	12.7
Reference period*	1.2	6.9	12.8	15.9	17.8	17.7	12.1
Precipitation (mm)							
2010	36.7	18.2	131.9	84.8	80.4	95.3	74.6
2011	16.3	22.5	51.1	81.7	202.0	82.1	75.9
Reference period*	27.6	35.7	51.9	78.5	75.1	66.1	55.8

* Monthly temperature and precipitation average in the period 1961–2005

Grain was harvested, dried and cleaned annually during the experiment. Yields were determined and samples were collected and chemically analyzed for macronutrient content by methods used in the agricultural chemistry. Grain samples were wet-mineralized to determine the macronutrients in H_2SO_4 with an addition of H_2O_2 as the oxidant. Nitrogen was determined by the hypochlorite method (BAETHEN and ALLEY 1989) and phosphorus was determined by the vanadium-molybdenum method. Potassium and calcium were determined by the AES atomic spectrometry method and magnesium was determined by AAS (PANAK 1997) in an AA-6800 Shimadzu apparatus. An Infratec™ 1241 grain analyzer, which uses near infrared analysis in the wavelength range of 570–1100 nm, was used to measure grain starch content. Protein yield was determined by multiplying the grain nitrogen content by a factor of 5.7 and then by grain yield. Analysis of variance, which was consistent with the mathematical model of the experiment arrangement (random blocks) was applied for statistical computations. Average values were determined for individual experimental objects. In addition to the basic statistical parameters, statistically homogeneous groups (groups without differences between average values) were identified using Duncan's test, at the significance level $\alpha = 0.05$. The relationships between grain protein content and grain and protein yield were also analyzed. Excel software and the Statistica statistical package were used to perform the statistical computations and analyses.

Results and Discussion

Milewo variety spring triticale grain yield was from 5.86 to 7.35 t ha^{-1} in own research (Fig. 1A). The applied nitrogen fertilization 120 kg ha^{-1} in the solid form of NH_4NO_3 and $CO(NH_2)_2$ and as foliar application of $CO(NH_2)_2$, without or together with a mixed fertilizer (Ekolist) at the BBCH 31-32 stage, contributed to an increased grain yield. Compared to the standard adopted by COBORU (2013) for spring triticale at an average fertilization level (90 kg ha^{-1}), grain yield was higher by 17.2 to 47.0% in the years 2010–2011. According to REHM (2006) and NADIM et al. (2012), micronutrients needed in trace quantities have a positive effect on the physiological processes of crops, which is reflected in an improved output. The effects of combining nitrogen fertilization with the provision of micronutrients (singly or in the form of mixed fertilizers) have been presented in many papers (KNAPOWSKI et al. 2009, NOGALSKA et al. 2012, WOJTKOWIAK et al. 2014,). In the conducted own research, fertilization with the higher nitrogen rate (120 kg ha^{-1}) together with



1,2... – explanation in the Materials and Methods chapter, averages followed by the same letter are insignificant ($\alpha < 0.05$)

Fig. 1. Grain yield (*A*), protein content (*B*), protein yield (*C*), starch content (*D*)

Ekolist at the BBCH 31–32 stage and without Ekolist contributed to obtaining the highest yield of spring triticale (7.35 t ha^{-1} and 7.31 t ha^{-1}, respectively). Increasing the fertilization level from 80 to 120 kg N ha^{-1} affected an increase in yield spring of triticale by 25.43% after the application of $CO(NH_2)_2$ together with Ekolist and by 17.33% after spraying with a 10% $CO(NH_2)_2$ solution.

Statistical analysis confirmed the effect of nitrogen rates on grain and protein yield (Table 4). Research by LESTINGI et al. (2010) proved that the nitrogen rate for ensuring good triticale grain quality is ca. 50 kg ha^{-1}. Under this rate, grain and protein yield increased by 14.2% and 25.0%, respectively. According to KNAPOWSKI et al. (2009), increasing the fertilization level from 80 to 120 kg N ha^{-1} increased grain yield spring of triticale by 9.6%. According to JANUŠAUSKAITĖ (2013), nitrogen rate of 90–120 kg ha^{-1}, under which grain yield increased by 33.9 and 37.0%, respectively, compared to the object without fertilization, proved optimally economical for achieving a high spring triticale grain yield. The positive effect of higher nitrogen rates, 160–180 kg ha^{-1}, was confirmed in research by CIMRIN et al. (2004) and MUT et al. (2005).

Table 4

Protein, starch content and grain and protein yield of Milewo variety spring triticale

Average for		Grain yield [t ha^{-1}]	Protein content [%]	Protein yield [kg ha^{-1}]	Starch content [%]
Year	2010	$7.12^a \pm 0.85$	$12.21^b \pm 0.37$	$870.0^a \pm 118.5$	$68.08^a \pm 0.95$
	2011	$6.26^b \pm 1.04$	$12.67^a \pm 0.48$	$793.7^a \pm 135.8$	$68.50^a \pm 1.04$
Nitrogen dose [kg ha^{-1}]	80	$6.05^b \pm 0.72$	$12.26^a \pm 0.35$	$740.8^b \pm 85.3$	$68.20^a \pm 1.02$
	120	$7.33^a \pm 0.88$	$12.62^a \pm 0.55$	$922.9^a \pm 97.9$	$68.36^a \pm 1.02$

Averages in columns (separately for years and nitrogen rates) followed by the same letter are insignificant ($\alpha < 0.05$), ±SD

According to KALBARCZYK (2008), a precipitation shortage at the earing-dough stage can cause a decrease in triticale grain yield by 10–20% and at the sowing-dough and sowing-harvest stages by 12–16% and 10–14%, respectively. In the first year of own research, 2.6 times more precipitation was recorded in the period of highest water needs of spring triticale (BBCH 30–39) compared to the 2nd year of research. This affected an increased grain yield by 0.86 t ha^{-1} and decreased protein content by 0.46%.

Depending on the fertilization variants, the average Milewo variety triticale grain protein content was from 12.10 to 12.71% (Fig. 1B) and varied in the years of research (Tab. 4). Protein yield was from 708.4 to 930.8 kg ha^{-1} (Fig. 1C) and depended mainly on grain yield and not on protein content (Fig. 2A, B). Increasing the nitrogen rate from 80 to 120 kg ha^{-1} by soil NH_4NO_3 application contributed to a significant increase in protein yield by 31.4% in

the variants with Ekolist and 18.3% in the variants without Ekolist. According to FAGERIA et al. (2009), foliar fertilization does not affect an increased output, but can raise cereal protein content if used during flowering or after flowering. ALARU et al. (2003) claim that the main factor affecting protein content and grain yield is the variety and the least affecting factor is nitrogen fertilization. In that research, nitrogen fertilization used in the tillering phase caused an average increase in triticale grain protein content by 1.57%. In addition, according to WRÓBEL (2005), the application of the second nitrogen rate in a solution form did not have a significant effect on the percentage protein yield content. According to DOMSKA et al. (2009), the action of micronutrients was more efficient in triticale cultivation compared to fertilization with nitrogen alone. In their research, supplementation of basic fertilization with copper, zinc, manganese and Ekolist increased protein yield by 17.9%, 26.8%, 35.3% and 28.1%, respectively. A high grain yield and protein concentration of nitrogen-fertilized spring triticale in the rate range of 90–120 kg ha^{-1} was confirmed by the research of SPYCHAJ-FABISIAK et al. (2005).

Starch is the main reserve material stored in caryopses. Its content in triticale grain is from 62.4–70.9% (BURESOWA 2010). According to LABUSCHAGNE et al. (2007), OBUCHOWSKI et al. (2010) end BECKLES and THITISAKSAKUL (2014), the variety, nitrogen fertilization, location and years of research as well as the internal interaction between these factors have a considerable determining effect on the starch level in triticale grain. In conducted own research, the years of research (Tab. 4) and the fertilizer variants (Tab. 4 and Fig. 1D) did not affect significant changes in grain starch content. On average, irrespective of the years of research and the fertilized objects, Milewo variety spring triticale grain contained from 68.07–68.38% starch (Fig. 1D). NOWOTNA et al. (2007) also did not confirm an effect of nitrogen fertilization on grain starch content. SPYCHAJ et al. (2013) even found a decreased starch content after nitrogen fertilization. A drop in starch content by 2.0 and 3.1% was recorded with increasing nitrogen rates (60–90–120 kg N ha^{-1}). OBUCHOWSKI et al. (2010) also confirm the effect of a lower fertilization level (90 kg nitrogen compared to 140 kg ha^{-1}) on increasing starch content.

It was found, based on regression analysis that an increase in spring triticale grain protein content was not accompanied by a yield increase (Fig. 2). Analysis of the dependence between protein content and grain yield showed a linear dependence. Protein yield increased with the increasing grain yield and the coefficient of determination was close to the linear correlation coefficient ($R^2 = 0.936$).

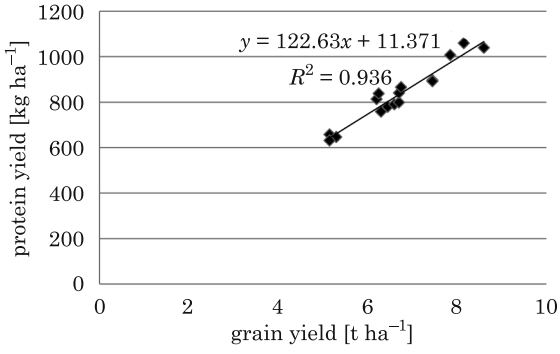


Fig. 2. Dependence of protein yield on grain yield

The content of minerals, among others, such as P, K, Mg, Ca, is crucial for cereal species selection in functional food production (SIDHU et al. 2007). According to MAKARSKA et al. (2010), nutritional value can be improved by the proper selection of parents and selection during breeding work on X *Triticosecale* Wittmack. According to KOWIESKA et al. (2011), spring triticale grain fertilized with nitrogen in the amount from 60 to 100 kg N ha^{-1} differed from wheat in a higher magnesium, potassium and phosphorus content. In own research, statistical analysis confirmed the effect of the years of research and increasing nitrogen rate, from 80 kg to 120 kg on average, on grain phosphorus content (Tab. 5). Nitrogen, potassium content varied in the years of research. Increasing nitrogen rate affected a decrease in magnesium content by 0.18 g kg^{-1} of d.m.

Table 5

Mineral content in Milewo variety spring triticale grain

Average for		Content of minerals [g kg^{-1} of d.m.]				
		nitrogen	phosphor	potassium	calcium	magnesium
Year	2010	$21.4^{b} \pm 0.65$	$4.21^{a} \pm 0.87$	$4.83^{b} \pm 0.19$	$1.66^{a} \pm 0.18$	$1.35^{a} \pm 0.21$
	2011	$22.2^{a} \pm 0.86$	$3.28^{b} \pm 0.63$	$5.12^{a} \pm 0.22$	$1.61^{a} \pm 0.18$	$1.22^{a} \pm 0.02$
Object	1*	$21.8^{a} \pm 0.78$	$3.12^{b} \pm 0.45$	$4.98^{ab} \pm 0.05$	$1.54^{a} \pm 0.16$	$1.33^{ab} \pm 0.13$
	2	$21.2^{a} \pm 0.28$	$3.13^{b} \pm 0.34$	$4.99^{ab} \pm 0.40$	$1.66^{a} \pm 0.17$	$1.41^{a} \pm 0.22$
	3	$22.0^{a} \pm 1.07$	$4.23^{a} \pm 0.93$	$5.14^{a} \pm 0.20$	$1.75^{a} \pm 0.16$	$1.18^{ab} \pm 0.07$
	4	$22.3^{a} \pm 0.96$	$4.50^{a} \pm 0.73$	$4.79^{b} \pm 0.16$	$1.60^{a} \pm 0.20$	$1.22^{b} \pm 0.10$
Nitrogen dose [kg ha^{-1}]	80	21.5a ± 0.62	$3.12^{b} \pm 0.37$	$4.98^{a} \pm 0.26$	$1.60^{a} \pm 0.17$	$1.38^{a} \pm 0.17$
	120	$22.1^{a} \pm 0.96$	$4.36^{a} \pm 0.79$	$4.97^{a} \pm 0.25$	$1.68^{a} \pm 0.19$	$1.20^{b} \pm 0.08$

* explanation in the Materials and Methods chapter

Averages in columns (separately for years, objects and nitrogen rates) followed by the same letter are insignificant ($\alpha < 0.05$), ±SD

Spring triticale grain, on average for the fertilizer variants, contained 21.2–22.3 g kg^{-1} nitrogen, 3.12–4.50 g kg^{-1} phosphorus, 4.79–5.14 g kg^{-1} potassium, 1.54–1.75 g kg^{-1} calcium and 1.18–1.41 g kg^{-1} magnesium.

Increasing the fertilization level from 80 to 120 kg N ha^{-1} contributed to increasing phosphorus by 43.8% after foliar application of $CO(NH_2)_2$ together with Ekolist (object 2 compared to 4) and 35.6% after spraying only with $CO(NH_2)_2$ at the BBCH 31–32 stage (object 1 compared to 3). Lack of basic nitrogen fertilization (120 kg N ha^{-1}) and supplementation with Ekolist raised grain potassium content by 7.3% (object 3 compared to 4). Joint application of Ekolist with 80 kg N ha^{-1} (object 2) caused an increased amount of magnesium by 15.6% compared to the higher fertilization level (object 4). According to WOJTKOWIAK et al. (2014), foliar application of $CO(NH_2)_2$ raised phosphorus, calcium and magnesium content in the grain of the Andrus spring triticale variety. According to KNAPOWSKI et al. (2010), increasing the fertilization level from 80 to 120 kg N ha^{-1} caused a significant increase in the amount of nitrogen, phosphorus and potassium in spring triticale grain (by 5.8%, 5.8% and 17.5%, respectively). BOBRECKA-JAMRO et al. (2013) found the highest calcium and magnesium content after the application of 120 kg N ha^{-1}, but without the effect of phosphorus or potassium accumulation in grain as a result of increasing nitrogen rates. According to NOGALSKA et al. (2012), the applied mixed fertilizers showed equal action and variation in chemical composition occurred only between individual years of research.

Conclusions

Summing up, the highest grain and protein yield of Milewo variety spring triticale was obtained after the application of nitrogen fertilization at the rate of 120 kg ha^{-1}. The protein yield increased with the increasing grain yield and the coefficient of determination was close to the linear correlation coefficient. On average, nitrogen rate affected the protein percentage and did not change grain starch content. The years of research and increasing nitrogen rate, from 80 kg to 120 kg on average, affected grain phosphorus content. Nitrogen and potassium content varied only in the years of research. On average, an increasing nitrogen rate affected a decrease in magnesium content. Basic mineral fertilization supplementation with Ekolist in Milewo variety spring triticale cultivation does not indicate the appropriateness of using this mixed fertilizer in practice.

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ASSESSMENT OF HUMAN THERMAL SENSATIONS BASED ON BIOCLIMATIC INDICES IN A SUBURBAN POPULATION, WROCŁAW (SW POLAND)

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K e y w o r d s: thermal perceptibility scale, temporal distribution, effective temperature, radiative-effective temperature.

A b s t r a c t

The aim of this study was to characterize thermal sensations and distribution across the years 2006–2011 on the basis of two bioclimatic indicators. The study was based on hourly meteorological data for the period April-October collected from the station, located on the eastern outskirts of Wrocław. TE and TRE were calculated with the use of BioKlima 2.6. software, while the evaluation of thermal sensations by the local population was performed according to the scale developed by Mikhailov. The average TE in the period April-October was 8.3°C and on average about 1.1°C lower than TRE. The highest TE and TRE were recorded in the summer months. In July, their above-average levels were most frequently reported between 9:00 and 17:00. During the 7-month-long study period, all kinds of thermal sensations were reported; from „very cold" to „hot". In suburban Wrocław, sensations of „cold" dominated. „Hot" temperatures were indicated only by TRE in four months.

OCENA ODCZUĆ CIEPLNYCH CZŁOWIEKA W WARUNKACH PODMIEJSKICH WROCŁAWIA NA PODSTAWIE WSKAŹNIKÓW BIOKLIMATYCZNYCH, POLSKA POŁUDNIOWO-ZACHODNIA

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S ł o w a k l u c z o w e: skala odczuwalności cieplnej, rozkład czasowy, temperatura efektywna, temperatura radiacyjno-efektywna.

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Abstrakt

Celem pracy była charakterystyka rozkładu czasowego w latach 2006–2011 i ocena odczuć cieplnych człowieka na obszarze podmiejskim Wrocławia. W pracy wykorzystano godzinne dane meteorologiczne w okresie kwiecień–październik, zebrane ze stacji Państwowego Monitoringu Środowiska, położonej na wschodnich peryferiach Wrocławia. Wartości wskaźników TE i TRE obliczono w programie BioKlima 2.6, natomiast ocenę odczuć cieplnych człowieka przeprowadzono wg skali opracowanej przez Michajłowa. Średnia temperatura TE w okresie kwiecień-październik wynosiła 8.3°C i była przeciętnie niższa, o 1.1°C, od temperatury TRE. Najwyższe wartości obu rozpatrywanych wskaźników notowano w miesiącach letnich – czerwiec-sierpień. W lipcu ponadprzeciętne, dodatnie wartości rozpatrywanych wskaźników notowano najczęściej w godzinach 9:00–17:00. W rozpatrywanym okresie wystąpiły wszystkie rodzaje odczuwalności cieplnej człowieka od „bardzo zimno" do „gorąco". W warunkach podmiejskich Wrocławia dominują odczucia zimna o różnym nasileniu w każdym z badanych miesięcy przez większą część doby. Na wystąpienie odczucia gorąca wskazał tylko wskaźnik TRE w czterech miesiącach w badanym okresie, od maja do sierpnia.

Introduction

The inhabitants of large cities are exposed to thermal stress that is usually greater than in adjacent rural areas (UNGER 1999, FORTUNIAK et al. 2006, NDETTO and MATZARAKIS 2013, MAJEWSKI et al. 2014). This adverse effect is additionally exacerbated by air pollution, noise, and the intense pace of life in the city, which increases susceptibility to various diseases, especially among the elderly (DUBICKI et al. 2002, SARRAT et al. 2006, KALBARCZYK and KALBARCZYK 2007, JOHNSON and WILSON 2009, BURKART et al. 2011, RASZKA et al. 2014). This is one of the reasons behind the decision to move out of the city in search of better living conditions (BASU and SAMET 2002, GABRIEL and ENDLICHER 2012, LAAIDI et al. 2012). Polish cities are no exception; in the 2000s they saw a construction boom in the suburbs and adjacent rural areas (with a peak in 2008) and a rapid outflow of residents from the city centers. However, the increased intensity of settlements surrounding the cities may contribute to the deterioration of inner thermal conditions. This hypothesis can be partially verified by the determination and comparison of thermal perceptibility in suburban areas with the previously known conditions in the city center (SZYMANOWSKI 2005, SIKORA 2008). The specific aim of this research was to analyze the temporal distribution of thermal sensations by the population in suburban areas of the city of Wrocław. Our analysis were based on effective temperature (TE) and radiative-effective temperature (TRE), the basic indicators of thermal perceptibility commonly used in bioclimatic research (MAKOKHA 1998, TEJEDA-MARTINEZ and GARCIA-CUETO 2002, WERESKI and WERESKI 2009, PÓŁROLNICZAK 2011, CZERNECKI and PÓŁROLNICZAK 2013).

Materials and Methods

The study used hourly meteorological data for the period from April to October, from the National Environmental Monitoring Station (PMS) in Wrocław belonging to the Lower Silesian Inspectorate for Environmental Protection, located in Wrocław, in Bartnicza Street (λ=17°08'28"E, φ=51°06'58"N, hs=120 m above sea level, code – PL0193A), across subsequent years 2006–2012 (Fig. 1). The data included air temperature (t, °C), relative humidity (f, %) at about 2 m agl and wind speed (v, m s^{-1}) at about 10 m agl. The station is located on the eastern outskirts of the city, the Psie Pole district, at the premises of the Swojec Agricultural Experimental Station, approx. 8 km from the center of Wrocław. The measuring point is surrounded by single-family houses and farmland, with no industrial areas nearby. Any missing values of global solar radiation (Kglob, W m^{-2}) were supplemented by measurements from the station located in Wrocław-Karłowice (λ=17°01'46"E, φ=51°07'46"N, hs=121 m above sea level) located in Korzeniowskiego Street.

Station of the NEM	Longitude	Latitude	Elevation [height above sea level]	Type of area
Wrocław – Bartnicza	17°08'28" E	51°06'58" N	120	suburban

Fig. 1. Location of the national environmental monitoring station (NEM) in Wrocław

Meteorological data collected for consecutive years across the multiannual period 2006–2012 were used to assess human thermal sensations based on two indicators: effective temperature (TE) and radiative-effective temperature (TRE). TE is known as quasi temperature, describing the combined effect of three elements: t, f and v on the human thermal comfort of a man dressed in simple summer clothes and performing light work (eg. walking) under shade. The TRE index, in addition to the three meteorological elements (t, f and v), also takes into account an additional element – Kglob. The TRE index defines human thermal sensations in the open air in direct sunlight. Before determining TE and TRE, the wind speed was reduced to a level of 2 m above the ground level by the following formula (KRAWCZYK 1991, PÓŁROLNICZAK 2011):

$$v_z = v_w(h_z/h_w)^{0.2}$$

where
v_z – wind velocity at a height of 2 m (m s^{-1}),
v_w – wind velocity at the height of measurement (m s^{-1}),
h_z – target height (m),
h_w – measurement height (m).

TE ratios and TRE were calculated based on positive air temperatures using the procedure implemented in the BioKlima 2.6 software, developed and then made available for research by BŁAŻEJCZYK (2004):

$$TE = t - 0.4 \cdot (t - 10.0) \cdot (1 - 0.01 \cdot f), \text{ when } v \leq 0.2 \text{ m s}^{-1} \text{ or}$$

$$TE = 37 - \frac{37 - t}{0.68 - 0.0014 \cdot f + \frac{1}{1.76 + 1.4 \cdot v^{o}\ 75}} - 0.29 \cdot t \cdot (1 - 0.01 \cdot f)$$

$$\text{when } v > 0.2 \text{ m s}^{-1}.$$

The lower wind velocity limit in the formulas used in the BioKlima 2.6. software is based on the assumption that the movement of air below 0.2 m s^{-1} is experienced as no air movement.

TRE was determined according to the formula developed by BŁAŻEJCZYK (2004):

$$TRE = TE + (1 - 0.01 \cdot ac) \cdot Kglob \cdot [(0.0155 - 0.00025 \cdot TE) - (0.0043 - 0.00011 \cdot TE)]$$

where
t – air temperature (°C),
f – relative humidity (%),
v – wind speed (m s^{-1}),
Kglob – global solar radiation (W m^{-2}),
ac – albedo of the human skin or clothing (assumed to be 31% in this paper).

The evaluation of thermal sensations on the basis of TE and TRE was conducted according to the scale developed by Mikhailov (cited by BŁAŻEJCZYK 2004), in which temperature intervals correspond to thermal sensations (Table 1).

Temporal distribution of TE and TRE was characterized on the basis of basic statistics; arithmetic mean ($\bar{x}$) and standard deviation (Sd) by month for the period April-October in both the entire multiannual period and in individual years. The study also involved the incidence of individual human thermal sensations based on the Mikhailov scale (cited by BŁAŻEJCZYK 2004) in different time steps (day, week and month).

Table 1

The scale of human heat perceptibility by Mikhailov (cited by BŁAŻEJCZYK 2004)

Class of temperature (°C)	Impact on the human body
<1	very cold
1–8.9	cold
9–16.9	cool
17–20.9	fresh
21–22.9	comfortable
23-26.9	warm
≥27	hot

Results and Discussions

Across the period 2006–2011, in suburban Wrocław the average daily effective temperature (TE) for the period April-October was 8.3°C (Table 2). Radiative-effective temperature (TRE) in the examined seven-month period was higher than TE, on average by 1.1°C. The largest differences between the TRE and TE were recorded in June (1.4°C) and then in July and April (1.3°C). Much greater differences between the temperatures were found for measurements at 12:00. Moreover, in the studied years in the period from April to October, the average TE at 12:00 was about 1.5°C lower than the daily average in the same period for Wrocław in the multiannual period 1981–2000 (SIKORA 2008). This difference may be due to the considerable distance of the study area from the center of Wrocław (approx. 8 km). The studies by DUBICKI et al. (2002) confirm the presence of an urban heat island in Wrocław – its average annual intensity in the hottest central area of the city ranges from 0.5°C (in the day) to 1.6°C (at night). The influence of the urban heat island in Wrocław can be felt even at a distance of 5.5 km from the center, even though its average intensity is low, from 0.1°C (in winter) to 0.6°C (in summer, at night).

In all the examined months TRE at noon was higher than TE, in April by as much as 3.5°C. Of course the clearly higher noon TRE than TE can be explained by the intense inflow of solar radiation. In turn, TE at 12: 00, with the exception of April, was lower in the following months than in the multiannual period 1981–2000 (SIKORA 2008), especially at the end of the analyzed period, ie. in September and October, by as much as 2.5–2.7°C.

Table 2

Mean ($\bar{x}$) and standard deviations (Sd) of TE and TRE by month. Years 2006–2011

Month	TE index				TRE index			
	$\bar{x}$		Sd		$\bar{x}$		Sd	
	24 hours	12:00	24 hours	12:00	24 hours	12:00	24 hours	12:00
April	3.2	6.5	6.0	5.2	4.5	10.0	6.9	5.8
May	7.3	10.1	5.9	6.0	8.4	13.4	6.7	6.8
June	11.4	14.2	5.4	5.2	12.8	17.4	6.1	5.8
July	13.7	16.4	5.5	5.5	15.0	19.7	6.3	6.1
August	12.5	15.2	4.7	4.7	13.6	18.1	5.4	5.3
September	7.7	10.0	5.4	5.4	8.6	13.2	6.0	6.1
October	2.3	5.0	5.6	5.9	2.9	6.7	5.9	6.3

24 hours – the average of 24 measurements, 12:00 – measurement at noon

The standard deviation of TRE compared to TE was greater in all the analyzed months.

Temporal distributions of TE and TRE – by month in consecutive years – were similar to the distribution of air temperature; the maximum recorded in the summer months, from June to August (Fig. 2). Similarly, the presence of the highest average TE and TRE in July is confirmed by other studies, including those conducted in Wroclaw, Wielkopolska and north-eastern Poland (CHABIOR and MICHALSKA 2007, SIKORA 2008, SZYGA-PLUTA 2011). The standard deviation determined for the individual months and years, similar to the multiannual period (Table 2), was about 0.3–1.1°C higher for TRE than TE.

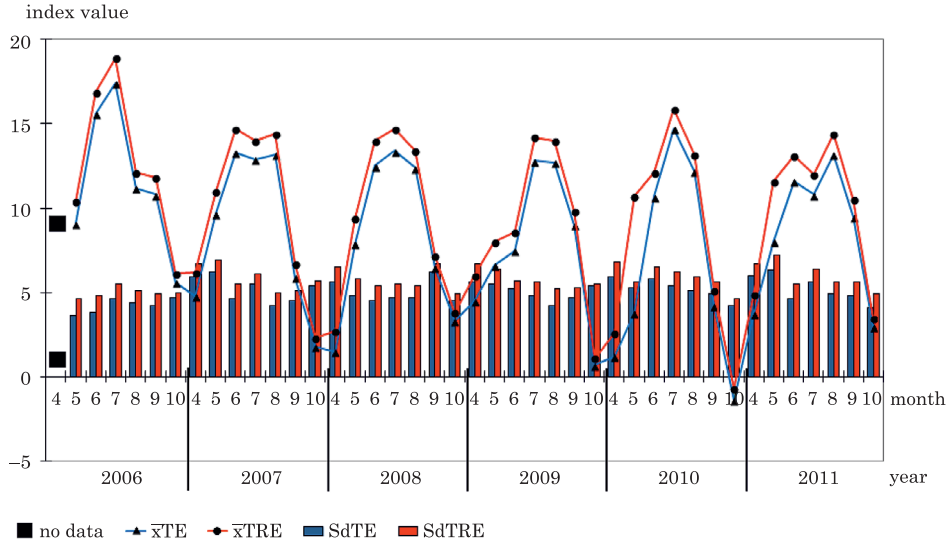


Fig. 2. Mean ($\bar{x}$) and standard deviations (Sd) of TE and TRE by month and year

The values of the aforementioned indices varied depending on the month and time of day (Fig. 3). In the summer months across the years 2006–2011, the highest TE was primarily recorded in the hours 10:00–18:00 in the period from 1st decade of July to 2nd decade of August, and in the hours of 8:00–20:00 in the 2nd decade of July. Higher ranges, were observed for longer time for TRE between 9:00 and 17:00 in the period June-August, and from 7:00 to 20:00 in the 2nd decade of July. The values in the range of 20–25°C of TRE were observed at 10:00–15:00 in the 2nd decade of July. In the night, in the time between sunset and sunrise, in the absence of solar radiation, TRE had the same values as the TE in the range 5–10°C, both ratios occurring mostly from 0:00–5:00.

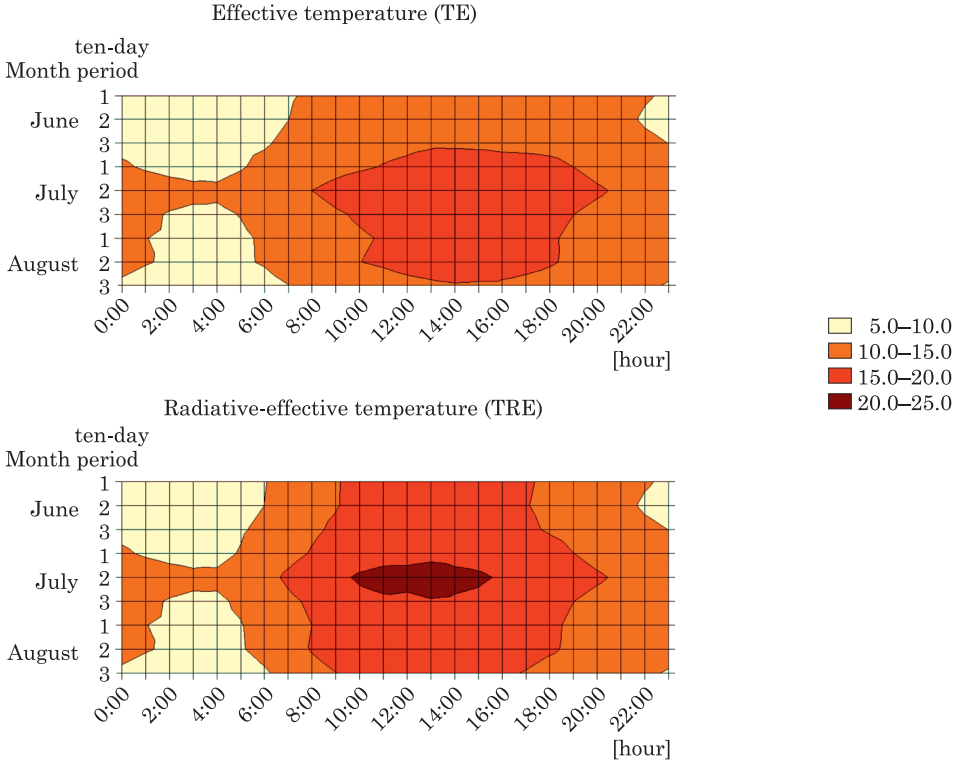


Fig. 3. Temporal distribution of average decade TE and TRE in the period June-August by hour in the analyzed multiannual period (2006-2011)

An analysis of the distribution of mean daily TE and TRE, by summer month and by year, shows that the number of days with TE≥15°C averaged about 9 and 13 days, respectively for two indicators, the highest number in July, the lowest in June (Fig. 4). The greatest number of days in July with the

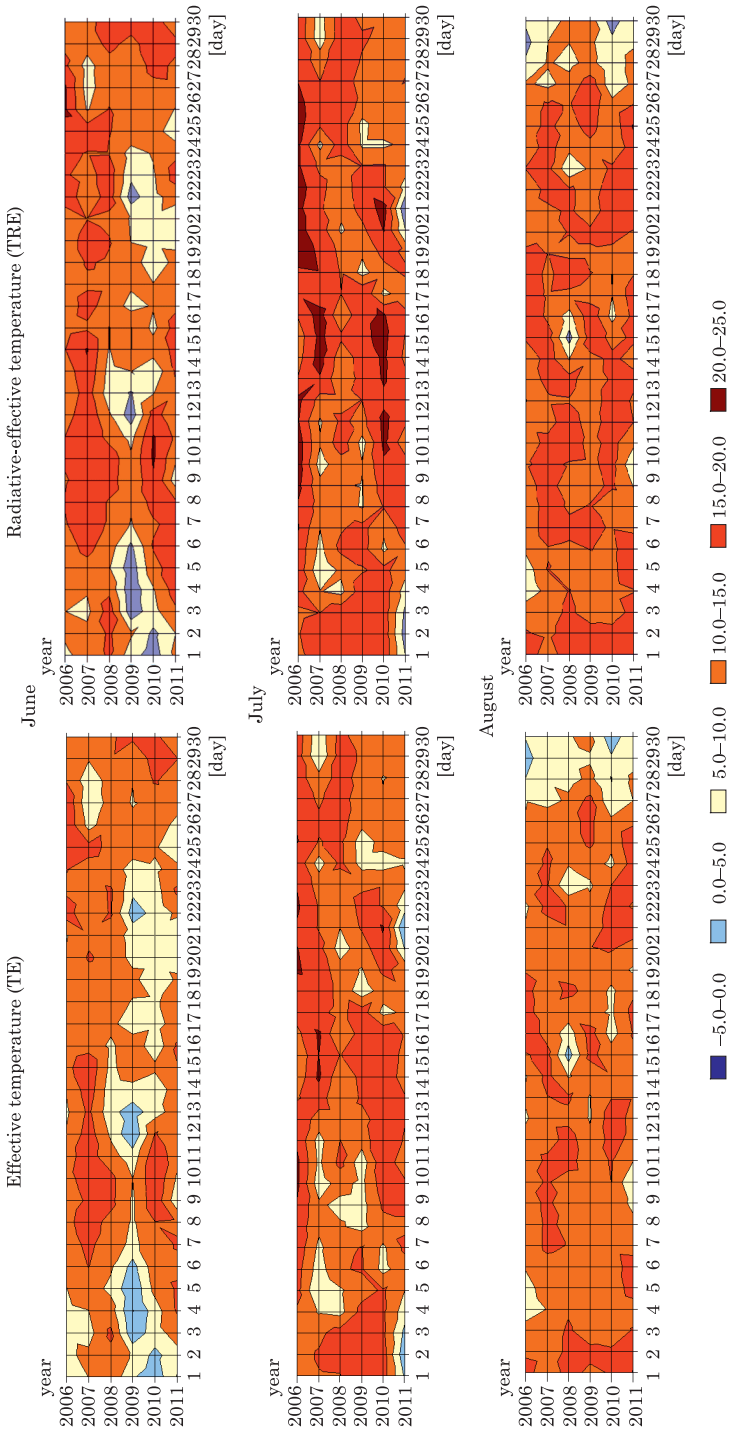


Fig. 4. Distribution of daily average TE and TRE by months and years

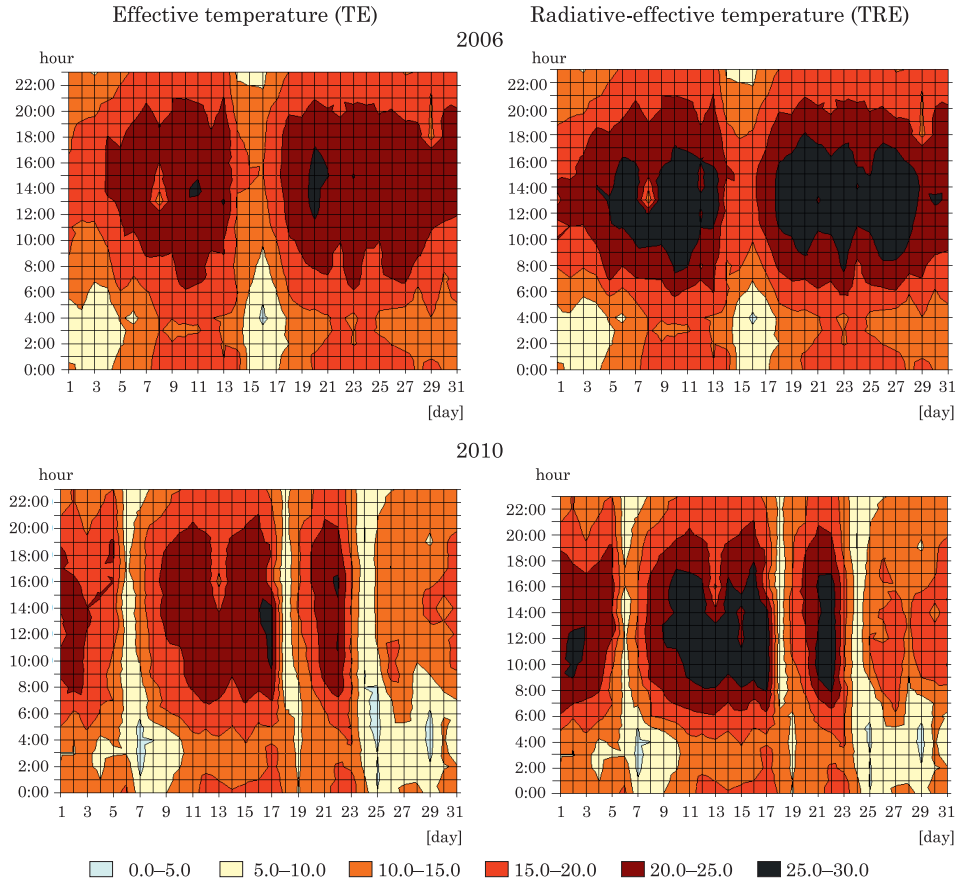


Fig. 5. Distribution of hourly average values of TE and TRE in July

mean daily temperature ≥15°C were recorded in 2006 and 2010 – 23 and 17 days for TE with 26 and 20 days for TRE, respectively. Temperatures ≥20°C occurred on average on 1 day for TE and 2 days for TRE, and on 6 and 14 days, respectively, in July 2006. TE and TRE <0°C occurred occasionally, on 1 day of each summer month.

In the months with the highest TE and TRE, i.e. in July 2006 and July 2010, the temporal distribution was similar (Fig. 5). In July 2006, from 11:00–18:00, the TE≥20°C generally occurred for 20–24 consecutive days broken by a 4-day period. The TRE≥20°C and the TRE≥25°C in July 2006 occurred for longer periods than TE during the day and for a greater number of days in the month. Lower temperatures of TE≤10°C and TRE≤10°C were recorded primarily at 0:00–5:00. In July 2010, the TE≥20°C and TRE≥20°C were reported primarily from 7:00–19:00 for about 10 days, the TE≥25°C was recorded for 2 days and TRE≥25°C for 12 days. In contrast to 2006, TE≤10°C in

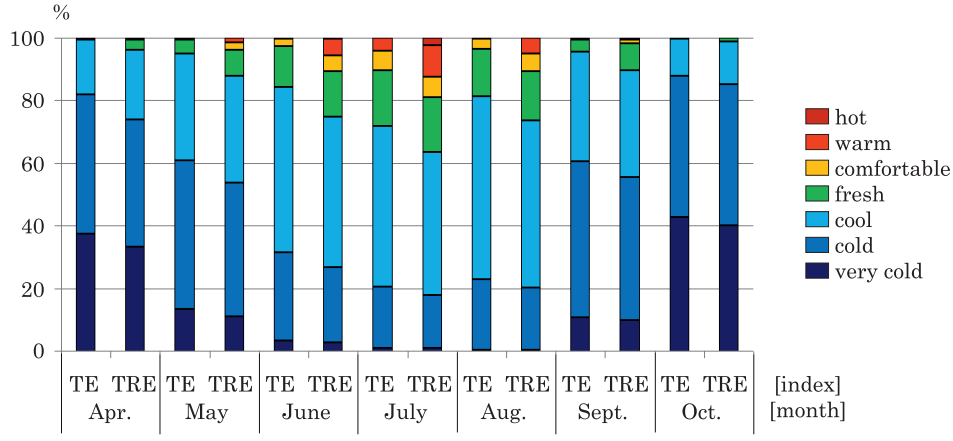


Fig. 6. The frequency of human thermal sensations determined on the basis of TE and TRE in the analyzed multiannual period (2006–2011), by month

2010 was recorded not only at night and early morning, but also in the daytime.

During the period from April to October, and in the analyzed multi-annual period, all types of heat sensations on the Mikhailov scale had been reported, i.e. from „very cold" to „hot" (Fig. 6). TE had been reported as „comfortable" in only the three months, and for TRE this period was two months longer. A „comfortable" TE and TRE was reported in July, then in August and June, similar to other studies in Wrocław and Wielkopolska (SIKORA 2008, SZYGA-PLUTA 2011). A „comfortable" TRE was also recorded occasionally in May and – even less frequently – in September. The observed low incidence of comfortable conditions is consistent with the opinion on the higher frequency of climatic stimulation in suburban areas than in the city center (DUBICKI et al. 2002).

The coldest TRE conditions, „very cold", were reported in April-July and September-October, most frequently in October, then in April. Cool and cold sensations were reported with a similar frequency for TE and TRE. The differences between the TE and TRE gained in significance for the warmest conditions. Sensations described as „hot" were reported only for TRE and in only one month. The sensation of „warm" was reported in five months (TRE) or in one month (TE). The period April-October, which constitutes the warm season in Poland, was therefore dominated by sensations of cold. The most common among these were the sensations of cool, cold and then very cold temperatures. The next – in terms of occurrence – was the sensation of fresh temperatures, which ranged from 1% (April for TE and October for TRE) to 18% (July for TE and TRE). Definitely less frequent were the feelings of warm

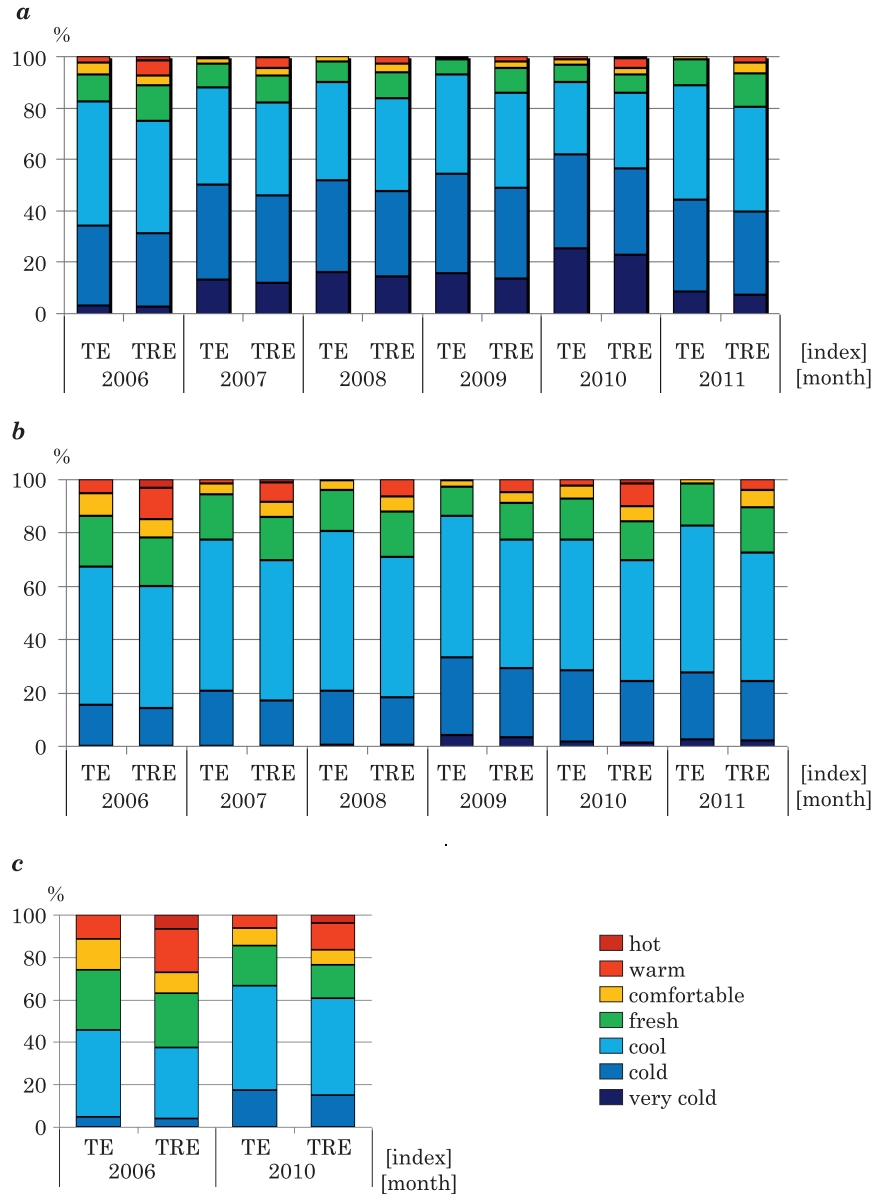


Fig. 7. The frequency of human thermal sensations determined on the basis of TE and TRE, by period and year; *a* – in the period April-October; *b* – in the period June-August; *c* – in July

temperatures (comfortable, warm and hot). Comfortable temperatures occurred a maximum of 6% of days in the month (July). In total, the various sensations of warm were reported for a maximum of 18% days (for TRE) of the hottest month of the year, i.e. July. A twice lower incidence of sensations of warm and hot temperatures on the outskirts of the city compared with the city center confirms the results of DUBICKI et al. (2002).

In the period April-October „very cold" sensations were most often reported in 2010 (>20% days), while least often in 2006 (approx. 3%) (Fig. 7a). Cold sensations of varying severity (from very cold to cool) occurred in each of the years of the multiannual period. The most common were the sensations of „cool", which – with the exception of 2010 – occurred on average 36–44% days (TRE) or 38–48% (TE) days a year. The sensation of „fresh" temperatures occurred most frequently in 2006 and the least frequently in 2009. Thermal comfort conditions were most often reported in 2006 (approx. 4.5% days) (TE) and 2011 (TRE), while least often in 2009. Warm temperatures were reported most frequently in 2006, and least often in 2009 (TRE) and 2008 (TE). The occurrence „warm" was reported only for TRE. It was the least frequently occurring sensation in each of the years, its frequency not exceeding 2%. In total, sensations of warmth at varying severity (comfortable-warm-hot) occurred most frequently in 2006, and least often in 2009.

In the period June-August the sensation of cold with varying severity was reported on 60% of days (TRE) and 67% of days (TE) in 2006 to 86% (TRE) and 77% of days (TE) in 2009 (Fig. 7b). The sensation of „very cold" temperatures occurred sporadically. Similar to the longer period of April-October, the period of June-August was dominated by the sensation of cool temperature, with an incidence of 46–60% days. The sensation of „fresh" temperature occurred relatively frequently, almost 20% days in 2006, the least often in 2009. Sensations from „comfortable" to „hot" occurred on less than 2% of days to 22% of days. Hot temperatures (TRE) were reported only in three years (2006, 2007 and 2010). In terms of TE, a comfortable temperature was the warmest sensation in two of the years, 2008 and 2011.

In July 2006 and 2010, the sensation of „very cold" temperature did not occur (Fig. 7c). The most common was the sensation of „cool" temperature, especially in 2010. In 2010 the sensation of „cold" temperature was more frequent, even 3–4 times than in 2006. The second most common sensation was „fresh" temperature, especially in 2006. In July 2006, the sensation of „warm" temperature was relatively frequent, more than „comfortable" temperature (TRE). The occurrence of hot temperature was shown only for TRE, for 7% days in July 2006, while for 4% days in July 2010. No sensation of „hot" temperature was reported in terms of TE. „Comfortable" temperature was more frequently reported in 2006.

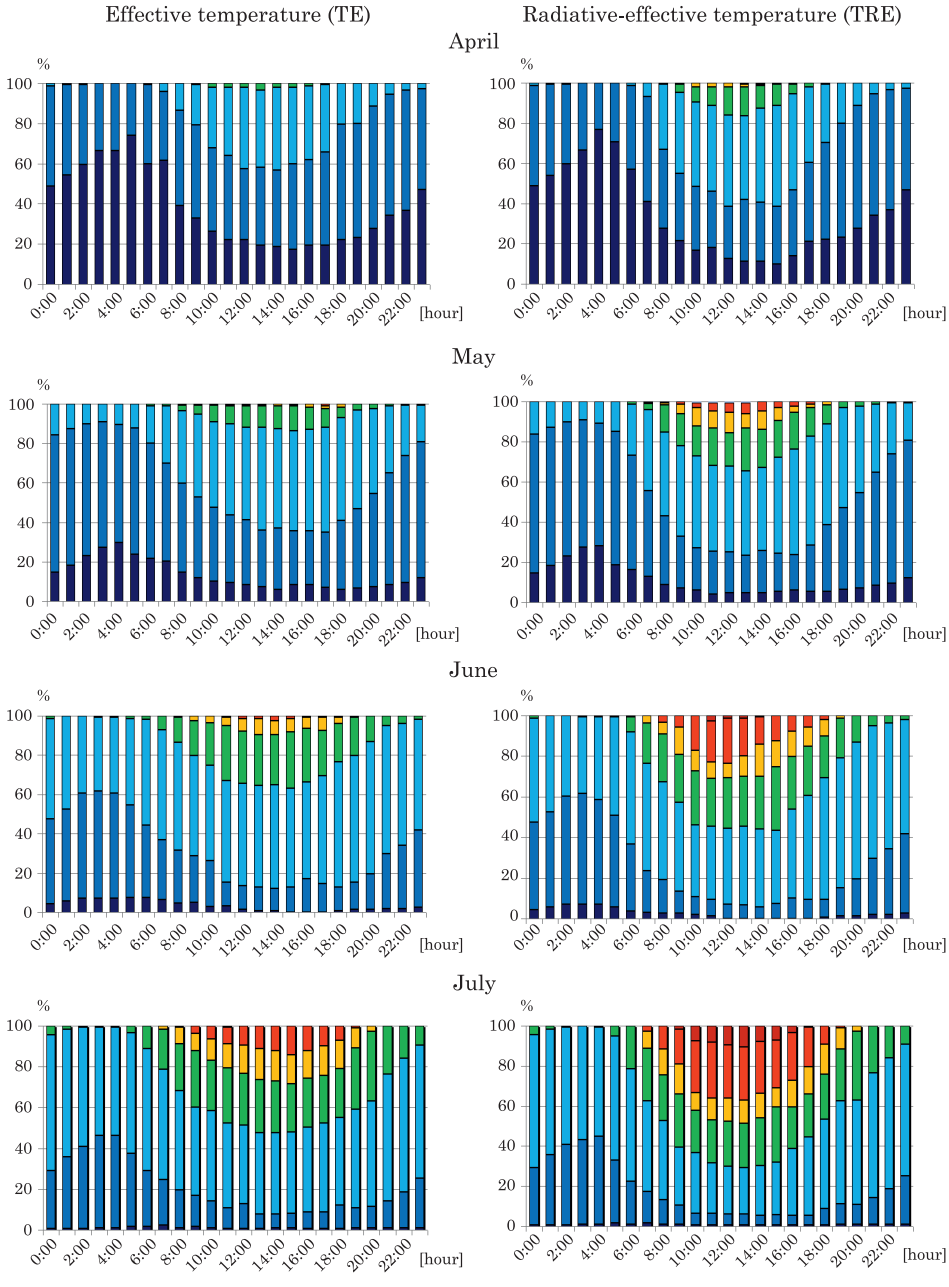
In hourly terms, it can be confirmed that the sensations of cold with varying degrees dominated in Poland, in this case it was reported in each of the studied months for most of the day (Fig. 8). The coolest hours of the day were in the morning: in the spring and early autumn (April-May) at approx. 4:00–5:00, in the summer (June-August) at approx. 3:00–4:00, in the fall (October) at approx. 5:00–6:00. The sensation of „very cold" temperature was experienced at least once in each hour of the day in the months of April, May, July and September. The sensation of „cold" occurred in each of the months, similar to the sensation of „cool" temperature, with the only exception of April in hours 3:00–5:00. The sensation of „fresh" temperature occurred on average from an entire day in July to 6–7 hours in October. „Comfortable" was rarely reported, from 13 hours a day in July to three hours a day in October. The sensation of „warmth" did not occur in October (in terms of TE it also did not occur in September), and only for one hour on average, between 13:00 and 14:00 in April, but for 11–12 hours in July. Hot temperature was only indicated by TRE in the four months during the period: May, June, July and August. The greatest frequency of hot temperature (10–12%) was reported in the hours 13:00–14:00.

The bioclimatic conditions in the suburbs are considered to be more beneficial to people than in the centers of large cities. In the summer, the urban heat island strengthens the sensation of hot temperature and is a strong burden for the human thermoregulatory system. Because of the smaller number of frosty and ground frost days in spring, autumn and winter, the city center contributes to a reduced thermoregulatory efficiency (DUBICKI et al. 2002). The positive aspect of the heat island is a lower risk of overcooling during the winter season, but this season was not covered in this research.

The used indicators of thermal conditions provide a description of thermal sensations of the inhabitants of the suburbs of Wrocław. At the same time, they represent a point of departure for further research with the use of more complex and comprehensive indicators of bioclimatic conditions (LI and CHAN 2000, BŁAŻEJCZYK et al. 2012, SZYMANOWSKI and KRYZA 2012, MAJEWSKI et al. 2014).

Conclusions

In suburban Wrocław, in the years 2006–2011, the courses of the effective temperature (TE and TRE) were characterized by a great diversity not only during the day, but also during the month and the entire analyzed period of April-October. TRE showed a greater variability in terms of the standard deviation in all months of the entire period, both on an annual and multian-


Effective temperature (TE)
Radiative-effective temperature (TRE)
April
May
June
July
%
100
80
60
40
20
0
0:00
2:00
4:00
6:00
8:00
10:00
12:00
14:00
16:00
18:00
20:00
22:00
[hour]

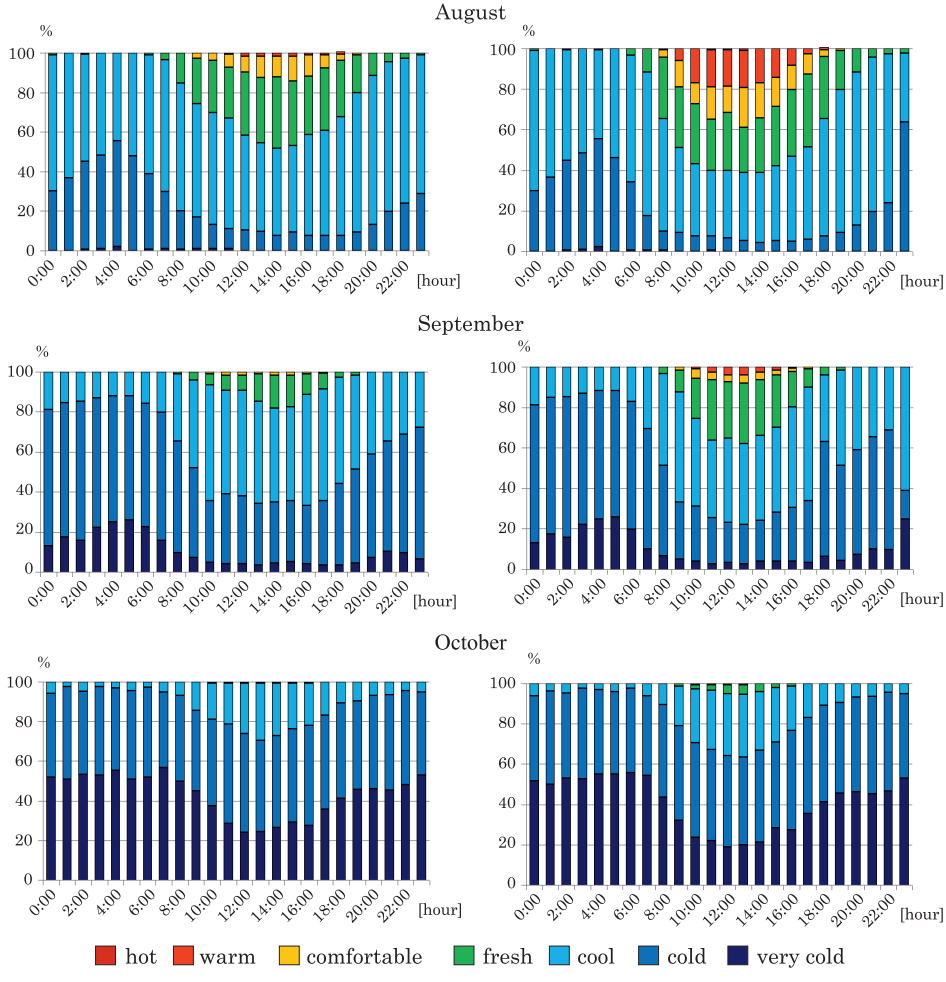


Fig. 8. The frequency of human thermal sensations determined on the basis of TE and TRE in the analyzed multiannual period (2006–2011), by month and hour

nual basis. In the period from sunrise to sunset, TRE was clearly higher than TE, which can be explained by the difference in solar radiation. During the period from April to October, all thermal sensations from the Mikhailov scale were reported (from „very cold" to „hot"), which indicates a significant climatic stimulation in the area.

The bioclimatological indicators TE and TRE used to evaluate the thermal sensations differed in indications. TE showed „very cold" to „fresh" sensations 2% more often than TRE, while TRE indicated „comfortable" to „hot" sensations 1.5% more often. Extremely adverse human thermal sensations in

the period were recorded in October, mostly at night with „very cold", and in July around noon for „hot".

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CRYPTOSPORIDIUM CANIS AND *C. FELIS* AS A POTENTIAL RISK TO HUMANS

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K e y w o r d s: *Cryptosporidium* spp., dogs, cats, infectivity, diagnostics, treatment.

A b s t r a c t

Cryptosporidium spp. are protozoan parasites found in the respiratory and gastrointestinal tracts of many vertebrates. This paper aims to present information about *Cryptosporidium* spp. related to their biology, life cycle, pathogenesis, infectivity, diagnostic methods and the treatment of diseases caused by them. *C. canis* and *C. felis* are common parasites of dogs and cats, therefore contact with ill or asymptomatically infected animals could pose a risk of infection especially for children and people with immunodeficiency disorders. The diagnostic difficulties and inadequately developed methods to treat pets make an infection with *Cryptosporidium* worse, and this has been proven by many confirmed cases of the disease.

CRYPTOSPORIDIUM CANIS I *C. FELIS* JAKO POTENCJALNE ZAGROŻENIE DLA CZŁOWIEKA

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S ł o w a k l u c z o w e: *Cryptosporidium* spp., psy, koty, zarażenie, diagnozowanie, zwalczanie.

A b s t r a k t

Cryptosporidium spp. są to pierwotniaki znajdowane w układzie pokarmowym i oddechowym wielu kręgowców. Praca ta ma na celu przedstawienie wiadomości na temat biologii, rozwoju, patogenezy, inwazjologii, metod diagnostycznych oraz leczenia *Cryptosporidium* spp. z uwzględnieniem tych gatunków, które mogą być zagrożeniem dla człowieka. U psów i kotów często występują *C. canis* i *C. felis*, a skażenie środowiska oocystami lub kontakt ze chorym zwierzęciem może być szczególnie groźne dla dzieci i osób z immunosupresją. Trudności w diagnozowaniu i nie w pełni opracowane leczenie zwierząt towarzyszących pogłębiają problem zakażeń ludzi kryptosporydiozą, czego dowodem jest wiele potwierdzonych przypadków zachorowań.

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Cryptosporidium spp. is a genus of apicomplexan parasitic protozoans belonging to Eimeria suborder of the Cryptosporidiae family. They commonly exist in 280 species of vertebrates, reptiles and fish (UPTON et al. 1985, MORGAN et al. 1999, CAVALIER-SMITH 2003). The parasite was first described in 1907 by E. E. TYZZER, who found it in the gastric glands of mice and named it as *Cryptosporidium muris* (TYZZER 1910). These protozoans live within host epithelial cells inhabiting the surface of the brush border, usually of the distal intestine. They may attack the epithelium of the respiratory track and stomach of humans especially individuals with immunosuppression. So far, 26 species and 73 genotypes have been described, including 8 that are pathogenic to human: *C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, *C. canis*, *C. suis*, *C. muris, C. cuniculus*, *C. ubiquitum*, *C. viatorum*, *C. fayeri*, *C. andersoni*, *C. bovis*, *C. scrofarum*, *C. tyzzeri*, *C. erinace* and *C. cervine* (RYAN and HIJJAWI 2015). Weakened, young and old organisms or those with immunosuppression are susceptible to invasion. The protozoa is transmitted through faeces, contaminated food, water and air. 1 g of human faeces may contain about 50,000 oocysts, which can enter the soil and water (WEBER et al. 1991). Theoretically, one oocyst is enough to infect an individual. The most known epidemic outbreak of cryptosporidiosis was reported in Milwaukee, USA, in 1993, when the water supply was contaminated with oocysts of *C. parvum* (MAC KENZIE et al., 1994). As a result, 100 people died, and the disease was confirmed in 403,000 cases. Pets, mainly dogs and cats, may be an important reservoir for the germs pathogenic for humans (CAUSAPE et al. 1996, THOMPSON et al. 2008, OVERGAAUW et al. 2009, PALMER et al. 2008, BOWMAN and LUCIO-FORSTER 2010). In Poland, the dog population is estimated at 11 million, and cat at 6 million. This indicates that every third Pole may be a dog owner, and every fifth may have a cat. Dogs are natural hosts to commonly occurring *C. canis* and cats to *C. felis*. Studies confirm that these animals are susceptible to infection with many other species/genotypes of *Cryptosporidium*, for example: *C. hominis, C. parvum, C. meleagridis, C. felis, C. andersoni, C. muris* (FITZ GERALD et al. 2011, XIAO and FAYER 2008).

Life cycle of *Cryptosporidium*

Cryptosporidium spp. is a homoxenic parasite, i.e. it completes its life cycle in a single host. The main place of residence for the parasite is villus epithelial cells in the small intestine or epithelial cells in the respiratory tract. Humans and animals acquire infection via the fecal-oral route and sporadically by inhalation. Following infection, each invasive oocyst releases four mobile sporozoites, which invade villus epithelial cells in the intestine (and/or in the respiratory tract). They locate extracytoplasmatically in host cells within

parasitophorus vacuoles, where they differentiate into spherical trophozoites. Trophozoites undergo asexual multiplication to form two types of meronts: type I meronts (containing 6 or 8 merozoites) leave the parasitophorus vacuole, enter villus epithelial cells next and undergo asexual multiplication or develop into type II meronts (containing 4 merozoites). The type II meronts undergo sexual multiplication to form gamonts. Following gametogony, micro- and macrogamont form a zygote, which develops into an oocyst. Two types of oocysts develop during the life cycle of *Cryptosporidium*: thin-walled oocysts, which initiate a new life cycle (autoinvasion), resulting in chronic infection of the host, and thick-walled oocysts, which are shed in faeces to the environment (BARR 1998). Oocysts are invasive immediately after excretion and very resistant to environmental conditions, so they may be rapidly transmitted to the next host. The incubation period of cryptosporidiosis varies and depends mainly on the species of the parasite and its host.

Pathogenesis of the disease – The course of the disease in humans and animals is similar. The parasites cause villous atrophy, leading to a decrease in absorption area, especially in the duodenum and distal intestine. The mechanism of diarrhoea induction has not been fully described, but it is supposed that it is a result of secretion disorder or poor absorption. These processes are based on abnormal secretion of prostaglandin (PGE), which stimulates the contraction of smooth muscles of the gastrointestinal tract, substance P, responsible for an increase in the vascular permeability of endothelial cells in the intestine and inflammations, and tumour necrosis factor (TNF), which is involved in inflammation processes as well. Disorders of the active transport of sodium and H2O dependent on glucose have been proven, too. It is supposed that toxins produced during the life cycle of the parasite negatively influence the absorption of chloride ions. Disorders of secretion of the abovementioned components and a negative influence on the absorption mechanism lead to villous atrophy caused by inflammation and to diarrhoea caused by poor absorption. Inflammations in particularly chronic cases result in severe damage of villus and their enterocytes. Diarrhoea is caused by disorders of the balance of water and electrolytes. Depending on the condition of the organism, the disease caused by *Cryptosporidium* spp. may be symptomless, acute or chronic. The symptomless disease occurs in young individuals more often than older ones. It is characterized by a lack of symptoms during pre-patent and patent periods. These individuals are a reservoir for the parasite. While there are no symptoms, the individual is theoretically considered as healthy. The disease self-cures after several cycles of parasite multiplication. The chronic disease lasts up to 21 days and is particularly dangerous for young individuals. The patent period, i.e. the stage in which parasites enter the intestine wall, lasts up to 7 days. Lack of appetite, slightly increased body temperature and nausea were observed. The patent period is characterized by profuse, self-

limited diarrhoea. Following the given period, the physical condition suddenly improves, and the disease self-cures. The chronic disease may even last several months. It occurs in cachectic organisms or those with immunosuppression. Symptoms include coughing, dyspnoea, increased body temperature, vomiting, frequent diarrhoea and, as a consequence, cachexia, body weight loss and bloody diarrhoea. It may lead to death. Factors predisposing to the development of the disease are, in particular, acquired immunodeficiency syndrome and diseases causing immunosuppression, e.g. leukaemia (CLARK and SEARS 1996, LAURENT et al. 1999, KOSEK et al. 2001, TZIPORI and WARD 2002, DOMENECH et al. 2011).

Infectivity – Animals under 90 days old are most often affected (JIAN et al., 2014). Studies carried out in many countries showed a different number of infected animals, e.g. 2.3–26.2% of dogs and 2.3–26.2% of cats in Brazil, 7.4–9.3% of dogs and 7.3% of cats in Canada, 3.9% and 3.8%, respectively, in Japan, 3.8% and 4% in the USA, 3.8% of dogs in China, 3.3% in Italy and 8.1% of cats in Great Britain (ARAI et al. 1990, EL-AHRAF et al. 1991, MTAMBO et al. 1991, GENNARI et al. 1999, SHUKLA et al. 2006, GIANGASPERO et al. 2007, MUNDIM et al. 2007, BALASSIANO et al. 2009, COELHO et al. 2009, YOSHIUCHI et al. 2010, UEHLINGER et al. 2013, JIAN et al., 2014). An animal with cryptosporidiosis excretes in faeces invasive oocysts to the environment. They constitute a reservoir for the animals using the waterhole. Isolation of the parasite from water in rivers, where their number was from 2 to 112 oocysts per litre of H2O, can cause infection at a considerable distance from the source of contamination (ONGERTH et al., 1987). Incorrect manure storage, drainage leakage and absence of hygienic precautions in farms or other animal communities promote water contamination. It was shown that wild animals, e.g. foxes, can constitute a serious hazard (SMITH et al., 2006). An important transmission route is contaminated food of plant origin and gardens in playgrounds for children. For example, it was stated in Peru that a 3-year-old boy and his siblings were infected by a pet dog (XIAO et al. 2007). It was indicated that an increased incidence rate of cryptosporidiosis in people occurs in spring and autumn during abundant rainfalls and in the summertime, when recreation and water sports are more frequent (MAJEWSKA 2003, LAL et al. 2012). Usage of water reservoirs containing oocysts promotes infection. It was specified that 20.5% of wild animals were infected with *Cryptosporidium*, so they constitute a dangerous reservoir to the environment, animals and humans. The parasites represented 21 genotypes, and 11 of them were in water (FENG et al. 2007). Dogs and cats infected with cryptosporidia are a source of infection to household members, in particular to children attending public institutions, such as nursery schools or schools, and to elderly people living in nursing homes, etc. An outbreak of cryptosporidiosis in children attending a nursery school in Georgia, USA, was described, where 49% of children were

infected and as many as 70% of them suffered from diarrhoea. It should be noted that the parasite was identified in 13% of adults who were there, too, but the course of infection implied that children were more susceptible to infection with the parasite (TANGERMANN et al. 1991). When elderly people are concerned, cryptosporidiosis has been described in 36% of patients hospitalized in the USA (NEILL et al. 1996). The occurrence of cryptosporidiosis has been documented in paediatric hospitals as well, e.g. above 20 such invasions were mentioned in 1980 in China. It has been demonstrated that 1.62% of infected patients had 6 genotypes of *C. hominis* and 4 genera of *Cryptosporidium*, including *C. canis* and *C. felis* (FENG et al. 2012). 159 children of 533 children under 4 years of age examined in Peru were infected with *Cryptosporidium* spp., and approx. 7% of cases were caused by cryptosporidiosis in dogs and cats. *C. canis* and *C. felis* are a specific threat to people with immunosuppression, in particular patients infected with HIV, which is caused by CD+ lymphocytes deficiency. There are many indications that *Cryptosporidium* is a significant pathogen that might cause death from cachexia through dehydration and diarrhoea (BLANSHARD et al. 1992, TIANGTIP and JONGWUTIWES 2002). In different countries, *C. felis* was found in 6% of people with immunosuppression (TIANGTIP et al. 2002). Studies carried out in Brazil showed that as many as 18.5% of people infected with HIV were carriers of *C. felis*, and 3.7% of *C. canis* (LUCCA et al. 2009). There is the possibility that one individual is infected with various species of *Cryptosporidium* spp., e.g. a patient of Jamaica with immunosuppression was infected with *C. felis* and *C. hominis* (GATEI et al. 2008). Another example is a Peruvian infected with three types of protozoan: *C. felis, C. hominis* and *C. meleagridis* (CAMA et al. 2006). Studies by MEINHARDT et al. (1996) showed that there were no preferences for sex of the host regardless of parasite species or genotype. Throughout history, there have been many mass invasions of *Cryptosporidium* spp. Examples given below confirm that cryptosporidiosis poses a common threat and is a serious problem that can affect highly developed countries, too. In 1987, 13,000 residents of the state of Georgia, USA, were affected by an outbreak of cryptosporidiosis. Oocysts were found in drinking water (HAYES et al. 1989). During an agricultural fair in 1993, it was stated that cider was the main source of oocysts of *Cryptosporidium*. 89% of individuals exposed to contact with the protozoan were infected. This is the first description of the spread of this parasite in the population through contaminated food (MILLARD et al. 1994). In 1997, contamination of borehole water in the area in the northern part of the River Thames in England was recorded; 746,000 inhabitants were exposed to the infection, and 345 with documented diagnosis of cryptosporidiosis were hospitalized (WILLOCKS et al. 1998). Other large outbreaks of cryptosporidiosis caused by water contaminated with oocysts were described by TAYLOR et al. 1985,

RICHARDSON et al. 1991, BRIDGMAN et al. 1995, YOKOI et al. 2005, CAUSER et al. 2006, VANDENBERG et al. 2012.

Diagnostic methods – Diagnostics of cryptosporidiosis in dogs and cats depends on the severity of symptoms and place of residence of the parasite in the organism. A stool examination should be performed if there are symptoms from the gastrointestinal tract, and sputum is the material to test if respiratory cryptosporidiosis is considered. It is hard to find oocysts in stool, so it should be collected over a few days. In exceptional circumstances, oocysts can be found in bile. Biopsy of the duodenum, distal intestine or lung is undertaken in severe cases. Microscopic examination of a stool smear stained with the use of the acid-fast procedure (Ziehl-Neelsen), i.e. the method of Kinyouna, Gram and Giemsa, is the simplest, but not always accurate, method for detection of *Cryptosporidium* spp. (O'DONOGHUE, 1995). Specialised laboratories use immunological methods, i.e. western blotting, detection of antibodies in stool or identification of oocysts by the direct immunofluorescence method (ARROWOOD et al., 1991). PCR is the most sensitive method, as it allows to identify particular species of *Cryptosporidium*, what is very useful in determining the source of infection. An autofluorescence method can be applied in diagnostics, too (VAREA et al. 1998).

Treatment – Methods to treat cryptosporidiosis in dogs and cats have not been fully developed. There is no effective medication for animals with *Cryptosporidium* spp. Therapy against the protozoan has been much better developed for humans. Macrolide antibiotics azithromycin and paramycin are applied (ARMITAGE et al. 1992, BLANSHARD et al., 1997). Their use has permitted the cure of pulmonary cryptosporidiosis in an AIDS patient (PALMIERI et al. 2005). Therapy with the use of spiramycin had no desired effects, because there was no clinical improvement (WITTENBERG et al. 1989). Nitazoxanide, applied to cure children with diarrhoea, can become a future treatment of cryptosporidiosis (SMITH and CORCORAN 2004, FOX and SARAVOLATZ 2005). Halofuginone is considered to be of some significance for the control of oocysts. It was proven in *in vitro* studies that use of monensin and halofuginone can cause a decrease in the number of the oocysts by as much as 90% (MCDONALD et al. 1990, NACIRI et al. 1993). *Cryptosporidium spp*. oocysts are resistant to external factors, and therefore the treatment of water supply or food can often be unreliable. Water chlorination is unreliable too. Filters applied to intake water for humans should have pores of a size smaller than 1 ,m to protect against oocysts.

Summation – *Cryptosporidium canis* and *felis* are important and underestimated human's pathogen. Many scientific works cited in article confirms, that this protozoan are a serious threat to young people, elders and with immunosuppression. Illness which is caused by *Cryptosporidium* spp. depends

on health status and may be symptomless, acute or chronic. *Cryptosporidium* spp. that occurs in dogs and cats are able to contaminate gardens, parks and water reservoirs. Due to high resistance of the oocysts, contamination of the environment can take several months. There is lack of information about prevalence of *C. canis* and *C. felis* in dogs and cats in Poland have a negative impact on the prevention aimed against this parasite. These protozoa can cause diarrhoea in puppies or kittens which often lead to increased mortality. Pet animals are an important vector of the parasite due to its proximity with humans. The exposed persons should examine their animals for the presence of this protozoan. Early diagnosis of cryptosporidiosis prevent the spread of *Cryptosporidium* spp. The aim of the veterinarian in the protection of public health should be to minimize environmental pollution with oocysts by rapid identification of sick animals, as well raising awareness of animal owners to ensure hygiene which will reduce the the chance of infection to humans.

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