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IMPACT OF CLIMATE CHANGE ON CROP WATER AND IRRIGATION REQUIREMENT (CASE STUDY: EASTERN DEZ PLAIN, IRAN)

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K e y w o r d s: climate change, crops water requirement, crop irrigation requirement, Dez Plain, Lars-WG.

A b s t r a c t

This study has been conducted to assess the possible impacts of climate change on crop water and irrigation requirement of the eastern Dez Plain in south-west of Iran, according to the current crop pattern, and for the 2020s, 2050s and 2080s periods. The climate change data was generated for A_2 scenario through the global circulation model HADCM3, using the stochastic weather generator Lars-WG model. The results showed that crop water requirement was predicted to increase from the mean value of 653.5 [mm year^{-1}] in the baseline period to 663.15, 682.3 and 715.0 [mm year^{-1}] in the 2020s, 2050s and 2080s, respectively. Irrigation requirement was estimated to decrease by 2% in the 2020s and increase by 2.7% and 15% in the 2050s and 2080s periods, respectively.

WPŁYW ZMIAN KLIMATU NA ZAPOTRZEBOWANIE ROŚLIN NA WODĘ I NAWADNIANIE (STUDIUM WSCHODNIEJ RÓWNINY DEZ, IRAN)

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S ł o w a k l u c z o w e: zmiany klimatu, zapotrzebowanie roślin na wodę, potrzeby nawadniania roślin, równina Dez, Lars-WG.

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Abstrakt

Badania przeprowadzono w celu oceny ewentualnego wpływu zmian klimatu na zapotrzebowanie roślin na wodę i potrzeb nawadniania we wschodniej równinie Dez w południowo-zachodniej części Iranu, zgodnie z aktualnym układem roślin i dla okresów: 2020, 2050 i 2080. Dane dotyczące zmian klimatu wygenerowano dla scenariusza A_2 przez globalny model cyrkulacji HADCM3, za pomocą modelu stochastycznego generatora pogody Lars-WG.

Wykazano, że zapotrzebowanie roślin na wodę wzrośnie ze średniej wartości 653,5 [mm · rok^{-1}] w okresie początkowym do 663,15, 682,3 i 715,0 mm [mm · rok^{-1}] w okresach odpowiednio 2020, 2050 i 2080. Potrzeby nawadniania zmniejszą się o 2% w 2020 i zwiększą się o 2,7% i 15% odpowiednio w okresach 2050 i 2080.

Introduction

Despite the fact that agriculture is a sensitive operation which supplies food and economy of many regions all around the world, but it is the largest consumer of water among human activities which withdrawals almost 70 percent of the total anthropogenic use of renewable water resources (FISCHER et al. 2006) and it is directly affected by climate which is often defined as the general patterns of temperature, precipitation, radiation, humidity and wind over a sufficient log period of time (approximately 30 years) (CHEN et al. 2013). Any changes in climatic components over a significant period of time is defined as climate change which may affect crop yield, water requirement, irrigation requirement and consequently fresh water resources either positively or negatively (WHEELER, VON BRAUN 2013). Nowadays, agricultural production is potentially endangered by climate change in many countries (PIAO et al. 2010, WANG et al. 2009, WEI et al. 2014) and this has become an important issue worldwide. Such phenomena to a large extant is caused by human activities such as civilization, industry and agriculture, and population growth which not only has increased the rate of green-house gases emission but also has limited fresh water resources (SCHULZE 2000). The international panel on climate change has announced that, climate change will have major impacts in the near-term and beyond on water availability and supply in developing countries which will absolutely influence food security and agricultural incomes (*Climate change...* IPCC 2013). Al-Zawad predicted that 5.1°C increase in temperature in the Saudi Arabia for the period of 2070–2100 (CHOWDHURY, AL-ZAHRANI 2013). GILABERTE-BURDALO et al. (2007) figured out that under B_2 scenario the ski season length in North America will decrease by 0–2% in 2020s and by 4–7% in 2050s. Results of DOLL et al. (2002) also showed that, crop irrigation requirement in two third of the world's farm-lands will increase in 2020 and 2007. PARRY et al. (1999) estimated that, increase in temperature by 1°C may increase crop production by 5–25% in arid regions. In order to deal with food and water security problems it is important to understand the possible impacts

of climate change on crop production and water requirement. Such Varieties in temperature and precipitation which are of the major characteristics of a climate may lead to water and food security hazards. Thus in order to deal with food and water security problems it is important to understand the possible impacts of climate change on the water consumption of the agriculture sector. This study investigates the possible impacts of climate change on crop production and water requirement in the eastern section of Dez Plain.

Material and Methods

Study area. Dez Plain is known as one of the largest plains of the Dez watershed which is located to the south west of Iran and is considered as a semi-arid region. It is also of the largest plains of the Khuzestan province, with an area of about 2487 km^2 which covers northern mountain regions of the province to the central smooth regions of it. Figure 1 shows geographical location of the Dez Plain. Eastern section of the Dez Plain has been studied in this practice. Eastern section of the Dez Plain is illustrated in Figure 2 (AZARI 2014).

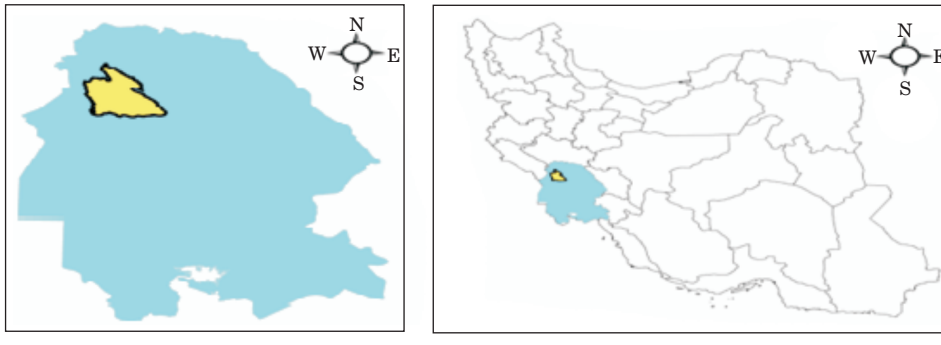

Fig. 1. Geographical location of the Dez Plain

Baseline data. The baseline data used in this study was a 24-year data-set representing 1985-2009 which was collected from three stations in the study area. Geographical characteristics of the climatology stations are illustrated in Table 1, and Figure 2. The information regarding to the crop pattern and the percentage of the cultivated area of the study area is shown in Table 2 (HOSSEINIZADEH 2014, AZARI 2014).

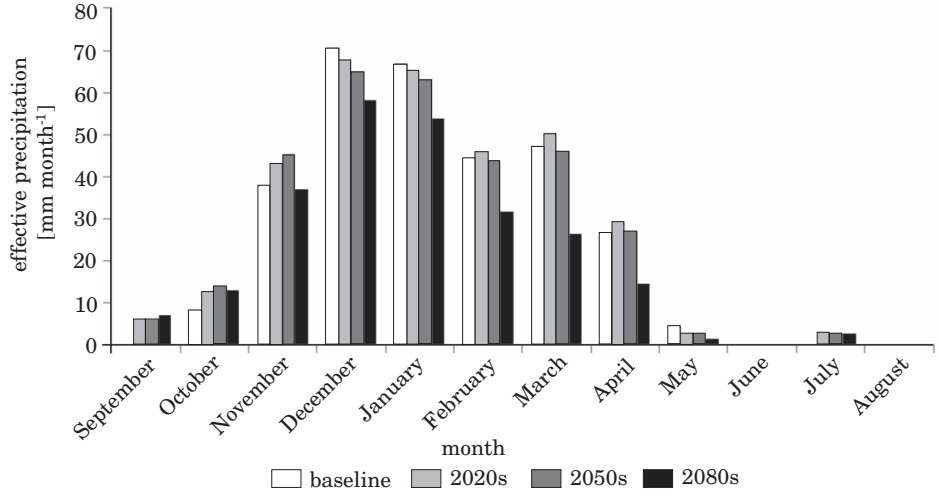

Fig. 2. Mean amount of monthly effective precipitation [mm month^{-1}] in the baseline, 2020s, 2050s and 2080s

Table 1

Geographical characteristics of the climatology stations

Station name	X [m]	Y [m]	Z [m]
Dez Regulator Dam	260 201	3 589 485	142
Harmale	268 428	3 537 516	38
Gotvand	294 312	3 570 240	100

Table 2

Crop pattern and the percentage of cultivated area of each crop type [%]

Crop name	Cul. [%]	Crop name	Cul. [%]
Winter vegetables	3.53	summer carrot	1.64
Summer lettuce	1.5	broad bean	1
Winter lettuce	1.7	summer bean	0.66
Summer cucumber	1	winter bean	1.1
Summer tomato	2	mung	2.7
Winter tomato	1	sugarcane	8.1
Potato	2.3	corn	20
Onion	3.3	wheat	39.3
Garlic	0.7	others	7.47
Summer eggplant	1		

Climate change data. Future projections were generated under A_2 scenario through the global circulation model HADCM3 for three periods, representing 2000–2020 (2020s), 2020–2050 (2050s) and 2050–2080 (2080s). The climate change data was both generated and downscaled using the

statistical down-scaling model Lars-WG developed by SEMENOV et al. (1998). Lars-WG is a stochastic weather generator which has the ability to be used for the simulation of weather data at a single site (SEMENOV et al. 1998) under current and future climate conditions. The generated data, consists of daily time series of minimum and maximum temperature [°C], solar radiation [MJm^{-2} day^{-1}] and precipitation [mm] (ETEMADI et al. 2012).

Reference evapotranspiration. The reference evapotranspiration was estimated through the FAO-Penman-Monteith approach (1) which contributes various factors such as climatic conditions, cultivation area and type, soil type and moisture, growing seasons and crop production frequencies in its estimation (ALLEN et al. 1998).

$$ET_0 = (0.408\Delta(R_n - G) + \gamma\,(900/(T + 273))\,u_2\,(e_s - e_a)) / (\Delta + \gamma\,(1 + 0.34u_2)) \quad (1)$$

where:
ET_0 – reference evapotranspiration [mm day^{-1}];
R_n – net radiation at the crop surface [MJm^{-2} day^{-1}];
G – soil heat flux density [MJm^{-2} day^{-1}];
T – mean daily air temperature at 2 m height [°C];
u_2 – wind speed at 2 m height [m s^{-1}];
e_s – saturation vapor pressure [KPa];
e_a – actual vapor pressure [KPa];
Δ – slope of vapor pressure curve [KPa $(°C)^{-1}$];
γ – psychometric constant [KPa $(°C)^{-1}$].

The FAO-Penman Monteith method was developed by the Food and Agricultural Organization (FAO). In this procedure, height of the reference grass, surface resistance and albedo coefficient are assumed to be 0.12 m, 70 m s^{-1} and 23 percent, respectively (FAO 2009).

Crop water requirement. Once the reference evapotranspiration was determined, crop water requirement for each crop was estimated using (2) and assuming crop water requirement is equal to the actual evapotranspiration by the crop.

$$CWR = ET_c = ET_0 \cdot Kc \quad (2)$$

Where:
CWR – crop water requirement;
ET_c – actual evapotranspiration by the crop [mm day^{-1}];

ET_0 – reference evapotranspiration [mm day^{-1}];
K_c – crop coefficient at a specific growth stage which mostly depends on the type of crop and climatic conditions.

The monthly values of K_c for each crop type was obtained from the agriculture organization of Khuzestan and the crop water requirement of each month was determined by multiplying the crop in mean monthly reference evapotranspiration relating to that month (SMITH et al. 2002).

Effective precipitation. For assessing irrigation requirement for a crop it is necessary to estimate the amount of occurred effective precipitation over the cultivated area. The effective rain-fall was calculated using USDA soil conservation service method which is shown in (3).

$$\begin{cases} P_{\text{eff}} = P \cdot \left(\dfrac{125 - 0.6 \cdot P}{125}\right) & \text{for} \quad P \le \dfrac{125}{3} \text{ [mm]} \\ P_{\text{eff}} = \left(\dfrac{125}{3}\right) + 0.1 \cdot P & \text{for} \quad P > \dfrac{125}{3} \text{ [mm]} \end{cases} \tag{3}$$

Where and are the total precipitation and effective rainfall [mm], respectively. The values of the estimated effective rainfall of each station were then averaged using the Thiessen polygon network coefficients. Thiessen network coefficient of each station is elaborated in Table 3.

Table 3

Thiessen network coefficient of each station

Station	Thiessen network coefficient
Dez Regulator Dam	0.31
Harmale	0.299
Gotvand	0.392

Crop irrigation requirement. The amount of crop irrigation requirement was then determined by subtracting crop water requirement from the estimated effective rainfall (4).

$$\text{CIR} = \text{CWR} - P_{\text{eff}} \tag{4}$$

where:
CIR – crop irrigation requirement [mm];
CWR – crop water requirement [mm];
P_{eff} – effective precipitation [mm].

Results

Temperature. Table 4 provides information about the variations in minimum, maximum and mean air temperatures during the baseline and simulated periods. According to this table, minimum, mean and maximum temperatures follow the same pattern during the studied periods. The predictions also indicate no temporal changes in temperature over the 2020s, 2050s and 2080s. Annual mean air temperature is expected to increase by 0.7°C, 1.98°C and 4.16°C in 2020s, 2050s and 2080s periods in comparison to baseline, respectively. Monthly mean air temperature, however, is expected not to rise evenly in all months of the year and the rate of temperature increase may be the highest in the worm months of the year. Future projections also show that, the most intensive temperature increase will occur in March, July and May in 2020s, 2050s and 2080s, respectively. The estimations were similar to the results of VOLOUDAKIS et al. (2015) who predicted an increase of about 1.8° in mean annual temperature for the 2050s in Greece.

Table 4
Monthly and annual amount of minimum, maximum and mean temperature (°C) in the 2020s, 2050s, 2080s and baseline

Period	Month												Mean Annual
	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	
T-min [°C]													
Baseline	25	21	14	9.8	8	9.2	12	17	23	26	29	29	18
2020s	25	21	15	11	8.4	9.6	13	18	23	27	29	29	19
2050s	27	23	16	12	9.3	10	14	19	25	28	31	31	20
2080s	29	25	18	13	11	12	16	22	28	31	34	33	23
T-mean [°C]													
Baseline	33.2	28	22.4	14.8	12.7	14.4	18	24.2	31	35.1	37.5	37.1	25.5
2020s	33.9	28.6	21.3	15.4	13.1	14.9	19	25	32	35.8	38.3	37.7	26.2
2050s	35.4	30	22.4	16.4	14	15.7	20	26.4	33	37.5	40.1	39.4	27.48
2080s	37.5	32	24.5	18.1	15.5	17.3	22	28.9	36	40.2	42.6	41.7	29.66
T-max [°C]													
Baseline	42	35	26	20	17	20	24	31	39	44	46	46	33
2020s	42	36	27	20	18	20	25	32	40	45	47	46	33
2050s	44	37	28	21	19	21	26	33	41	47	49	48	35
2080s	46	39	31	23	20	23	28	36	44	49	51	50	37

Rainfall. A comparison between the average effective precipitation during the projected future and the baseline periods is elaborated in Figure 2. Figure 2 shows that, effective rainfall in the projected future in the study area is expected to follow a similar temporal distribution pattern in comparison to baseline and the rate of precipitation is expected to be the highest in December

and lowest in June in the simulated periods. According to Figure 2 the monthly amount of precipitation in the study area varies in the range of 0 [mm month^{-1}] in August and June, to 70.7 [mm month^{-1}] in December. The maximum value of precipitation is predicted to decline to roughly 67.7 (2020s) [mm month^{-1}], 65 (2050s) and 58.2 (2080s), whereas the rate of precipitation in August is expected to rise to about 0.1 [mm month^{-1}] in the 2020s, 2050s and 2080s. The rate of precipitation in September is expected to increase from 0.2 [mm month^{-1}] to 6.2, 6.1 and 7.2 [mm month^{-1}] in the 2020s, 2050s and 2080s, respectively. Furthermore, an increase of about 2.6 (2020s), 2.5 (2050s) and 2.4 (2080s) [mm month^{-1}] has been predicted for July. According to the information reported in Figure 2 the rate of precipitation is expected to rise in almost eight months of year in the 2020s, six months in the 2050s and just two months of year in the 2080s. As a result, total annual amount of precipitation, is expected to increase by 6.25% and 2.73% during the 2020s and 2050s periods but, decrease by 19 percent in the 2080s. The estimations also corroborate with the results of LOHMME et al. (2009) who predicted that, precipitation will rise in the 2050s in Tunisia, whereas, GOHARI et al. (2013) estimated a 11 to 31 percent decrease in precipitation in Zayande-rud river basin.

Reference evapotranspiration. The reference evapotranspiration data relating to the baseline and future projections is presented in Figure 3. According to these results, evapotranspiration during the baseline decreases from the average cumulative amount of 159 [mm month^{-1}] in September to the minimum value of approximately 39.9 [mm month^{-1}] in January, then jumps to roughly 204.6 [mm month^{-1}] in May and reaches the peak of about 235.91 [mm month^{-1}] in July. Future projections, however, show that, the maximum quantities of are expected to increase by 1.31%, 2.72% and 3.79% in the 2020s, 2050s and 2080s, respectively. And the mean annual values of are predicted to rise by approximately 1.37% (2020s), 4.3% (2050s) and 9.1% (2080s). A comparison between Figure 2 and Figure 3 shows that, there is such a vast difference between the rate of reference evapotanspiration and rainfall in the study area and the maximum rates of reference belongs to May, June, July and August, in which the rate of rainfall is minimal.

Crop water requirement. Dez Plain is one of the most important and strategic farm-land regions of the Khuzestan province with a wide range of cultivation variety. Majority of the crops planted in this area consists of vegetables, cereal and been. The name and cultivation percentage of the most frequent cultivated crops in the study area is summarized in Table 2. The amount of total water requirement of the most frequent cultivated crops during their growth period for the baseline and projected future periods is presented in Figure 4 and Figure 5. From these charts, it can be seen that, water requirement for all crops will increase in the future, though this increase

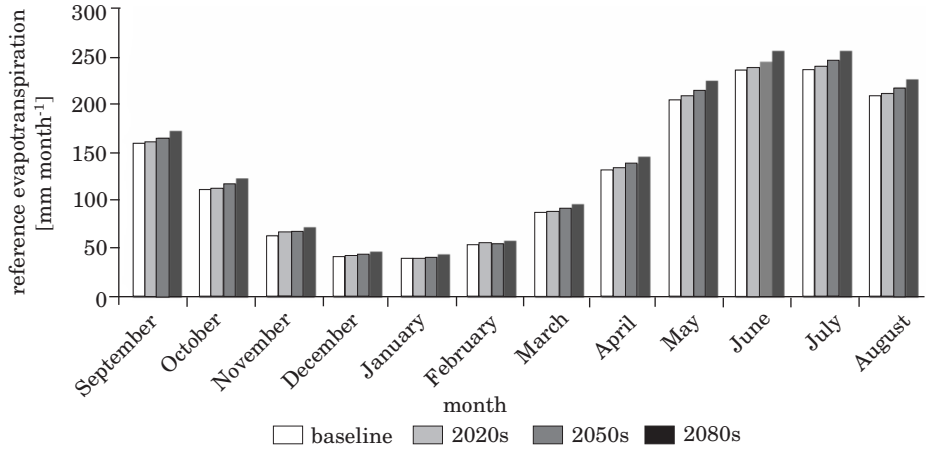

Fig. 3. Mean monthly amount of the reference evapotranspiration [mm month^{-1}] in the baseline, 2020s, 2050s and 2080s

is expected to be more marked for the crops which cover such a larger cultivated area and consequently consume more water, i.e. wheat, corn and sugarcane (The most dominant crops in the study area). Monthly crop water requirements for the study area as a whole during the baseline and the projected future periods are illustrated in Figure 6. According to this data, the average amount of crop water requirement in the study area during the baseline decreases from roughly 80.8 [mm month^{-1}] in September to 40.73 [mm month^{-1}] in October, halves in December, being around 21.02 [mm month^{-1}], but gradually increases, standing at about 23.4, 35.3 and 60.58 [mm month^{-1}] in January, February and March, respectively. This value then, falls dramatically to 43.01[mm month^{-1}] in June after reaching a peak of 81.29[mm month^{-1}] in May, but again begins to rise, standing at 69.3 [mm month^{-1}] in July and the maximum of 86.03 [mm month^{-1}] in August. However, as a result of climate change, the total amount of crop water requirement is expected to increase by 1.48%, 4.41% and 9.3% during the 2020s, 2050s and 2080s, respectively. TANASIJEVIC et al. (2014) in a study on olive crop evapotranspiration and irrigation requirements in the Mediterranean region also predicted that, crop water requirement will increase in the 2050s. According to the Table 2, sugarcane, corn and wheat are the major most frequent cultivated crops in the eastern Dez Plain which encompass approximately 67 percent of the crop area and also consume roughly 72 percent of the water in the study area. The monthly values of crop water requirement of these three most cultivated crops are illustrated in Figure 7. Figure 7 shows that, in the baseline, the total amount of monthly crop water requirement for sugarcane with an average crop area percentage of about 8.1 varies between 1.4 [mm month^{-1}] in January and

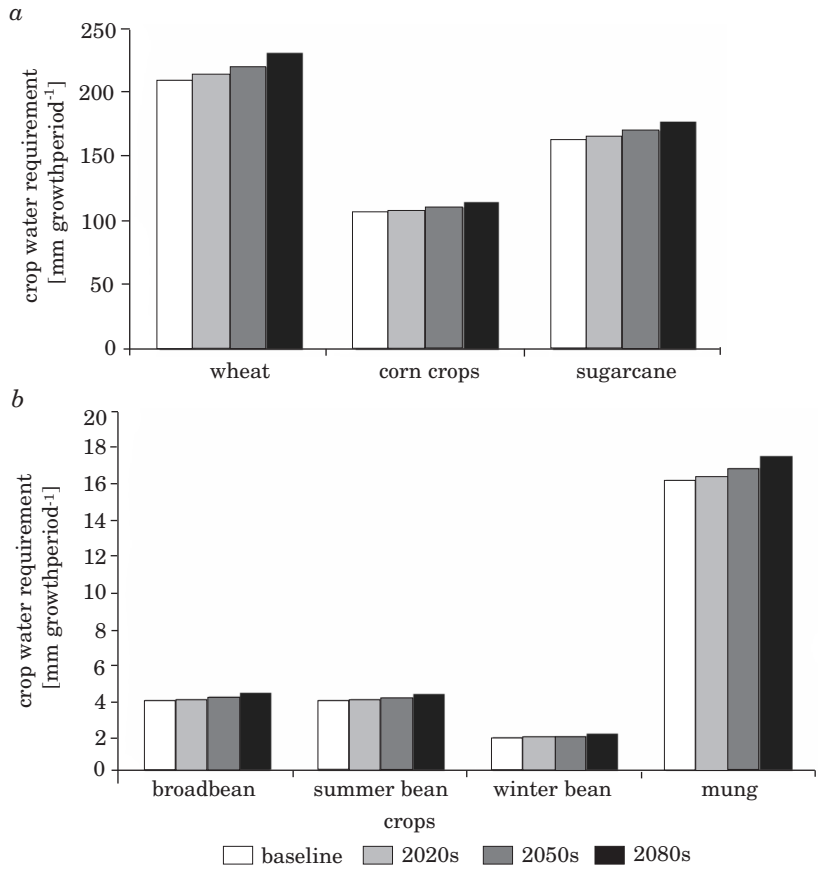

Fig. 4. Total amount of estimated water requirement [mm growthperiod^{-1}] for the crops cultivated: *a* – wheat, corn crops, sugarcane; *b* – broadbean, summer bean, winter bean, mung in the study area in the Baseline, 2020s, 2050s and 2080s

34.4 [mm month^{-1}] in July. The crop water requirement for sugarcane in the baseline, falls dramatically from nearly 19 [mm month^{-1}] in September to 6.15 [mm month^{-1}] in October, and then decreases gradually to the minimum value of about 1.4 [mm month^{-1}] in January. After that, it continuously rises, reaching the peak of about 34.4 [mm month^{-1}] in July, but then decreases to about 27.6 [mm month^{-1}] in August. By the same token, future projections show that, in the 2020s, 2050s and 2080s, this value follows the same pattern, though the rates are on average 1.26, 4.14 and 8.72 percent higher in the 2020s, 2050s and 2080s, respectively (Table 5). The higher rates of crop water requirement in June, July and August may be explained by hot summer. Information regarding to the monthly amount of crop water requirement in the simulated periods for wheat which is the most dominant crop in the study

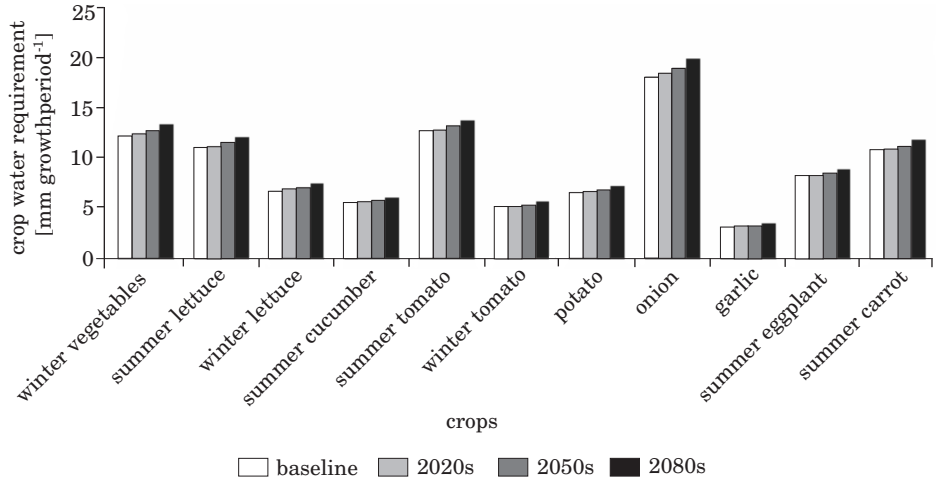

Fig. 5. Cumulative amount of water requirement [mm growthperiod^{-1}] for the crops cultivated in the study area in the baseline, 2020s, 2050s and 2080s

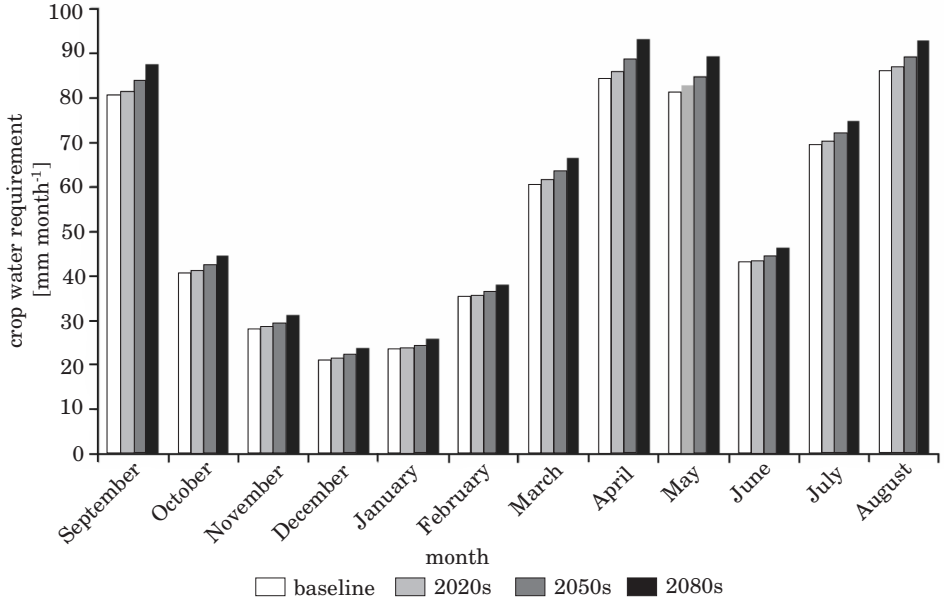

Fig. 6. Mean monthly crop water requirement [mm month^{-1}] in the eastern Dez Plain in the baseline, 2020s, 2050s and 2080s

area is described in Figure 7. According to these charts, the crop water requirement for wheat during the baseline initially decreases from the monthly rate of about 17.8 [mm month^{-1}] in November to the minimum value of 12.7 [mm month^{-1}] in December. After that, it increases to the peak

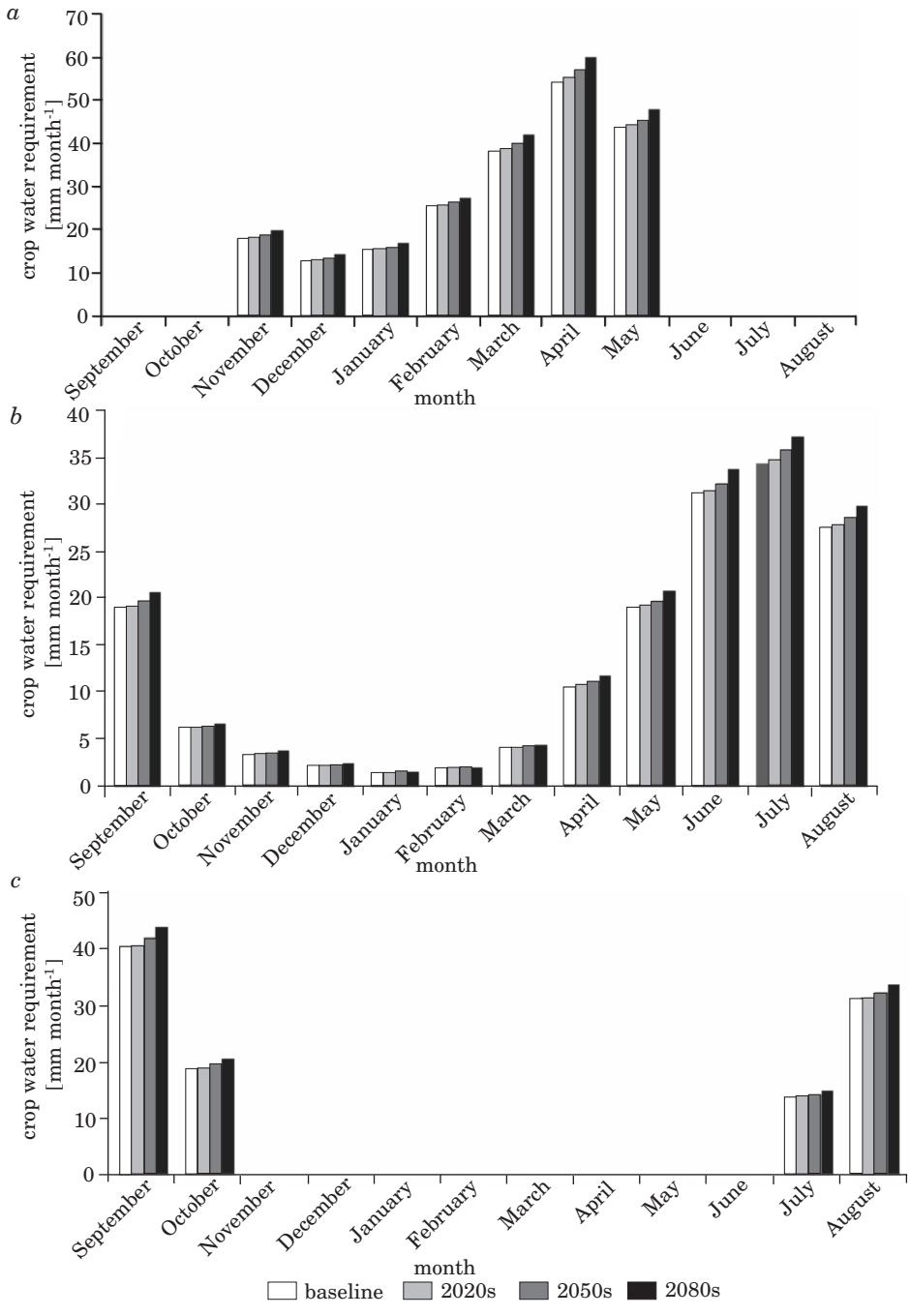

Fig. 7. Monthly amount of water requirement [mm month^{-1}] estimated for: *a* – wheat; *b* – sugarcane; *c* – corn in the baseline, 2020s, 2050s and 2080s

of approximately 54.3 [mm month^{-1}] in April and then tails off. The estimations also indicate that, the monthly values of water requirement for wheat follow the same pattern in the 2020s, 2050s and 2080s, though the rates are on average 1.9% (2020s), 4.8% (2050s) and 10.22% (2080s) higher than baseline (Table 5). Figure 7 also shows fluctuations in the rate of crop water requirement for corn with a percentage of cultivated area of about 20 percent. According to this Figure there is sharp increase in the rate of corn water requirement rises from the minimal amount of about 13.7 [mm month^{-1}] in July to the peak of approximately 40.1 [mm month^{-1}] in September, and then falls to 18.7mm/month in October. These values however, as a result of climate change will increase by 1, 4.05 and 8.4 percent in the 2020s, 2050s and 2080s, with respect to baseline, respectively (Table 5).

Table 5

Changes in crop water requirement in the 2020s, 2050s and 2080s according to the baseline [%]

Period	Crop name		
	sugarcane	wheat	corn
2020s	1.26	1.91	1
2050s	4.14	4.8	4.05
2080s	8.72	10.22	8.4

Crop irrigation requirement. Information regarding to the crop irrigation requirement of the study area as a whole is provided in Figure 8. The total amount of irrigation requirement for the study area during the baseline was estimated to be 458 mm year^{-1}. This value, however, is expected to decrease by 2% (2020s), but increase by 2.7% (2050s) and 15% (2080s), due to climate change. From Figure 8 it can also be seen that, irrigation requirements for February, January, December and November have estimated to be zero in all studied periods, since, the amount of precipitation is more than crop water requirement during the mentioned months. What is more, a comparison between Figure 6 and Figure 8 shows that, almost all of crop water requirement is supplied by irrigation in six months of year, namely; September, October, May, June, July and August during baseline. Once the crop water requirement of sugarcane, corn and wheat was analyzed, the monthly values of irrigation requirement for these crops were calculated for the study periods. Figure 9 provides information on the irrigation requirement of these crops in the baseline, 2020s, 2050s and 2080s. According to this data, sugarcane irrigation requirement in the baseline decreases dramatically from the monthly rate of about 19 [mm month^{-1}] in September to roughly 5 [mm month^{-1}] in October and then hits a four month plateau, between November and February with the smallest rate of irrigation requirement. After that,

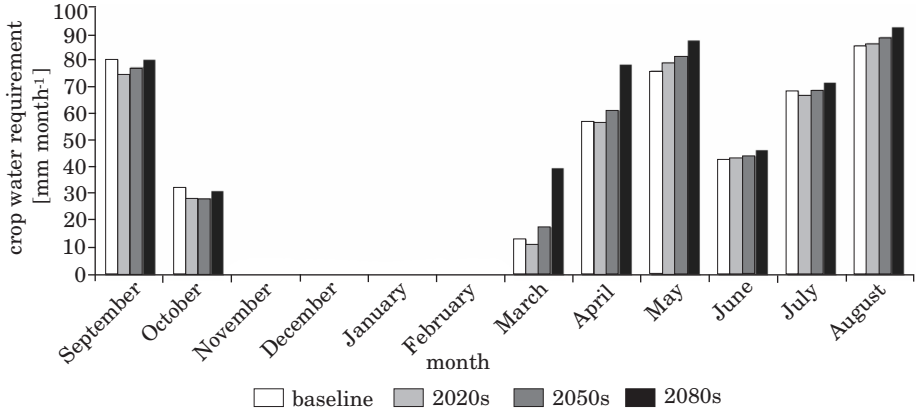

Fig. 8. Mean monthly crop irrigation requirement [mm month^{-1}] in the Eastern Dez Plain in the baseline, 2020s, 2050s and 2080s

there is a continuous sharp increase in the rate of monthly crop irrigation requirement which tails off after hitting the peak of 34.2 [mm month^{-1}] in July. In the 2020s and 2050s, however, despite the fact that monthly amount of sugarcane irrigation requirement is expected to decline as result of a 6.25% and 2.73% increase in the annual rate of rainfall but, predictions show an increase of about 0.24% (2020s) and 3.49% (2050s) in monthly irrigation requirement of sugarcane and this may be explained by an average increase of about 0.7 and 1.98 in the mean annual temperature in the 2020s and 2050s, respectively (Table 6; Table 4). In 2080s, irrigation requirement of sugarcane increases by 10.23 percent as a result of a 19% decrease in the rate of total annual rainfall and a 4.16 increase in the rate of mean annual temperature. According to the data shown in Figure 9 the monthly rate of crop irrigation requirement for wheat in the baseline, declines from approximately 3 mm/month in November to zero in December and remains steady at this rate until the next month, then shows an upward trend, which ends after reaching the maximum value of about 43.8 [mm month^{-1}] in April. Future projections show that, this value is expected not to change significantly in the 2020s but grow by roughly 6.19% and 29.6% in the 2050s and 2080s, respectively (Table 6). Figure 9 also shows that, there is a sharp increase in the rate of crop water requirement for corn from July and September, with the minimum rate of around 13.6 mm month^{-1} in July and maximum rate of about 40.3 [mm month^{-1}] in September. Estimations show that, as a result of climate change, the monthly amount of crop irrigation requirement for corn in expected to decrease by 1.56% in 2020s but increase1.34% and 5.74% in the 2050s and 2080s, respectively (Table 6).

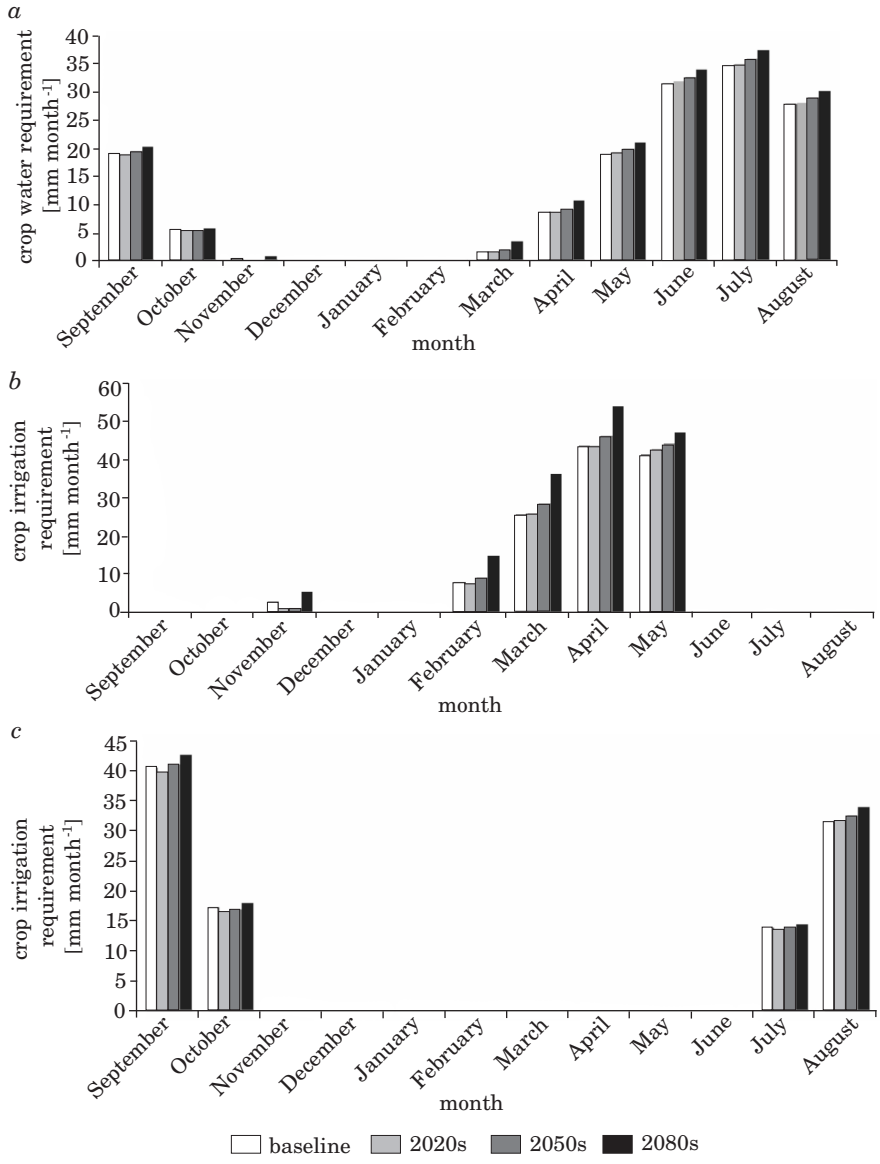

Fig. 9. Monthly amount of irrigation requirement [mm month^{-1}] estimated for: *a* – sugarcane, *b* – wheat, *c* – corn in the baseline, 2020s, 2050s and 2080s

Table 6
Changes in crop irrigation requirement in the 2020s, 2050s and 2080s according to the baseline [%]

Period	Crop name		
	sugarcane	wheat	corn
2020s	0.23696	0.00094	–1.56053
2050s	3.48579	6.1862	1.33951
2080s	10.2311	29.6147	5.73604

Conclusions

According to the data recorded during the baseline, long term mean annual air temperature in the Dez Plain stands at roughly 25.5°C and the monthly mean air temperature varies between 12.7°C in January and 37.5°C in July. However, estimations show that, these values are expected to change in the future. Mean annual air temperature in the study area under A2 scenario, apart from temporal changes is expected to rise by approximately 2.6%, 7.6% and 15.1% in the 2020s, 2050s and 2080s, respectively. Such an increase in temperature may cause changes in the crop water requirement in the eastern Dez Plain, rising from the annual amount of about 653.5 [mm year^{-1}] in the baseline to about 663.15 [mm year^{-1}] in the 2020s, 682.3 [mm year^{-1}] in the 2050s and 714.13 [mm year^{-1}] in the 2080s. Crop irrigation requirement, in contrast, is expected to decrease from around 458.4 [mm year^{-1}] by two percent in the 2020s, standing at roughly 449.2 [mm year^{-1}] and such a decline may be explained by the higher rate of precipitation in the 2020s which is predicted to be 6.25% greater than the long term mean annual rainfall in the baseline which is about 307.4 [mm year^{-1}]. In the 2050s, however crop irrigation requirement is expected to increase by 2.7% with respect to baseline, though the predictions show an increase of about 2.73% in the rate of long term mean annual rainfall in the 2050s. Furthermore, as a result of higher rate of air temperature and a decrease of about 19% in mean rainfall, crop irrigation requirement is estimated to be roughly 15 percent greater than baseline in the 2080s.

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CARCASS QUALITY OF EUROPEAN ROE DEER (*CAPREOLUS CAPREOLUS*) FROM FOREST AND FIELD HUNTING GROUNDS

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K e y w o r d s: European roe deer, carcass quality, measurements.

A b s t r a c t

The experimental materials comprised 180 carcasses of European roe deer (bucks, does and kids) hunter-harvested in forest (90 carcasses) and field (90 carcasses) habitats. Zoometric measurements of carcasses were carried out and the percentage shares of edible and non-edible components were determined.

Significant differences were found in carcass size and weight between roe deer from different sex and age groups representing forest and field populations.

Fawns were characterized by the highest percentage share of non-edible parts in the carcass. Legs constituted the largest portion of roe deer carcasses, accounting from 40.53% in bucks from afforested areas to 41.39% in fawns from field habitats. No significant differences were observed in the proportions of the analyzed cuts in the carcasses of roe deer harvested in forests and fields hunting grounds.

JAKOŚĆ TUSZY SARNY EUROPEJSKIEJ *CAPREOLUS CAPREOLUS* Z ŁOWISK LEŚNYCH I POLNYCH

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S ł o w a k l u c z o w e: sarna europejska, jakość tuszy, wymiary.

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Abstract

Badaniom poddano 180 tusz sarny europejskiej (samców, samic i osobników w 1 roku życia) pochodzących z łowisk leśnych (90 tusz) oraz polnych (90 tusz). Wykonano pomiary zoometryczne tusz oraz analizowano udział elementów jadalnych i niejadalnych.

Stwierdzono zróżnicowanie w wymiarach i masie tuszy osobników z poszczególnych grup płciowo-wiekowych bytujących w obwodach łowieckich polnych oraz leśnych. Największy procentowy udział elementów niejadalnych stwierdzono w tuszach osobników w pierwszym roku życia. Największym wyrębem w tuszach saren były udźce, które stanowiły od 40,53% (kozły pochodzące z terenów leśnych) do 41,39% (koźlęta pozyskane na terenach polnych). Nie stwierdzono jednak statystycznego zróżnicowania w procentowym udziale poszczególnych wyrębów między sarnami pozyskanymi na terenach obwodów łowieckich leśnych oraz polnych.

Introduction

The European roe deer *Capreolus capreolus* is widespread in Europe. The geographical range of the species covers an area of approximately 7.2 mln km^2, and the total number of animals has been estimated at 15 mln (BURBAITE, CSÁNYI 2009).

The body weight and size of European roe deer may vary widely depending on geographical, climatic and environmental conditions. The carcass weight and measurements of roe deer are also determined by other factors such as the age and growth rate of individual animals (BOBEK et al. 1984, PETTORELLI et al. 2002).

Roe deer meat is much appreciated by consumers for its specific physicochemical properties (DZIERŻYŃSKA-CYBULKO, FRUZIŃSKI 1997). The roe deer is the most common representative of the deer family in Poland. According to the Central Statistical Office, the total estimated number of roe deer in Polish habitats is 873 500, and 186 667 animals were harvested in the 2013/2014 hunting season.

KULAK and WAJDZIK (2009) demonstrated that the carcass weight and skull measurements of roe deer are useful parameters in ecotype classification. According to the cited authors, different ecotypes of male roe deer can be distinguished based on selected measurements.

In view of the above, the objective of this study was to perform a detailed analysis of carcass quality in roe deer bucks, does and kids from forest and field hunting grounds.

Materials and Methods

The experimental materials comprised the carcasses of European roe deer (*Capreolus capreolus*) hunter-harvested in North-Eastern and Central Poland during two successive hunting seasons of 2009/2010 and 2010/2011.

Roe deer carcasses with the following characteristics were analyzed in the study:

– no extensive bullet damage or contamination due to bullet,
– correct evisceration procedure,
– correct chilling and transportation procedures.

Based on the data provided by a game processing plant, roe deer were divided into those harvested in hunting districts where woodland covers less than 40% and more than 60% of the total area, referred to as the field group and the forest group, respectively. A total of 180 roe deer carcasses were analyzed (Table 1).

Table 1

Number of roe deer carcasses analyzed in the study (combined data for two hunting seasons)

Specification	Forest roe deer			Field roe deer		
	bucks	does	fawns	bucks	does	fawns
Number of analyzed carcasses	30	30	30	30	30	30

The carcasses of bucks, does and yearlings were eviscerated prior to analysis. The heads and antlers of bucks were removed and prepared for reporting the harvest to a Game Commission responsible for evaluating selective culling.

The following zoometric measurements were performed on each carcass (JANISZEWSKI et al. 2007):

a) carcass length (± 1 cm)
   – does and fawns – from the tip of the nose to the base of the tail, along the spine,
   – bucks (following removal of the head and antlers) – from the atlas to the base of the tail, along the spine;
b) height at withers (± 1 cm) – from the highest point of the withers to the tip of the hoof, along the leg, measured in midline;
c) height at sacrum (±1 cm) – from the highest point of the back to the tip of the hoof, along the straight leg;
d) chest girth (± 1 cm) – behind the withers and shoulders;
e) chest width (± 0.5 cm) – at the widest point, behind the shoulders;
f) chest depth (± 0.5cm) – at the deepest point, behind the shoulders.

The carcasses were dressed at the game processing plant. After skinning, non-edible carcass parts such as the head and lower legs were removed and weighed on an electronic scale accurate to 0.01 kg.

The following cuts were separated from the carcass according to the procedure proposed by JANISZEWSKI (2009):

– loin – by cutting in the front along the ribs, perpendicular to the spine between the second and the third rib, and in the back along the line separating the leg;

– ribs – by separating the legs, the saddle and the shoulders;

– neck – by cutting between the first cervical vertebra and the base of the skull in the cranial direction and along the line separating the saddle in the caudal direction;

– shoulders – by performing a semi-circular cut through the muscles connecting the forelimbs and the chest cavity;

– rumps – by cutting between the second to last and the last lumbar vertebrae, along the spine line at the top; the upper section of the hindlimb is classified as part of the leg.

A statistical analysis was performed to determine:

– arithmetic means ($\bar{x}$) and standard deviations (SD),

– the weights and percentage shares of cuts and non-edible parts in the carcass,

– significance of differences between carcass measurements and the percentage shares of cuts and non-edible parts in the carcasses of roe deer from different sex and age groups representing forest and field populations – by one-way ANOVA at $\alpha = 0.01$ and $\alpha = 0.05$.

Results and Discussion

Table 2 presents the carcass measurements of roe deer from different sex and age groups representing forest and field populations.

Table 2

Carcass measurements of roe deer from forest and field populations [cm; $\bar{x} \pm$ SD]

Specification	Forest roe deer			Field roe deer		
	bucks	does	fawns	bucks	does	fawns
Carcass length	$90.10^{a} \pm 4.54$	$114.02^{a} \pm 5.92$	100.45 ± 7.80	$92.13^{b} \pm 4.61$	$116.11^{b} \pm 6.01$	100.98 ± 7.81
Chest girth	64.05 ± 3.40	62.11 ± 4.31	53.66 ± 6.32	64.71 ± 3.51	62.69 ± 4.42	53.87 ± 6.28
Height at withers	$74.89^{A} \pm 4.25$	$71.06^{a} \pm 2.82$	57.98 ± 2.39	$77.09^{B} \pm 4.33$	$72.25^{b} \pm 2.86$	58.12 ± 2.41
Height at sacrum	86.70 ± 3.91	$82.36^{a} \pm 2.76$	64.55 ± 2.69	87.16 ± 3.92	$84.43^{b} \pm 2.81$	64.86 ± 2.66

$^{A, B}$ – $P \leq 0.01$

$^{a, b}$ – $P \leq 0.05$

Bucks, does and fawns from the forest population were characterized by smaller carcass measurements than their counterparts from the field

population. Table 2 data show that the carcasses of does from the field population were significantly (by 1.91 cm) longer than the carcasses of does from the forest population. The carcasses of females were longer than the carcasses of males, but the latter were measured after head removal. The carcasses of bucks from the field population were also significantly (by 2.03 cm) longer than the carcasses of bucks from the forest population. The carcass length of fawns from field and forest populations was similar at 100.98 cm and 100.45 cm, respectively. No significant differences in chest girth were observed between bucks, does and fawns representing forest and field populations.

A comparison of our findings with those of DROZD et al. (2000) revealed that the chest girth of bucks from the field population (64.70 cm) and from the forest population (64.05 cm) was greater compared with bucks from south-eastern and north-eastern regions of Poland (61.30 cm and 63.30 cm, respectively). The chest girth of bucks from the forest population was similar to that reported for bucks from Central and Eastern Poland (63.9 cm). Does from field and forest populations differed significantly in chest girth (63.69 cm and 61.11 cm, respectively) from does inhabiting different regions of Poland. The highest value of chest girth was noted in does from Central and Eastern Poland (67.50 cm), followed by those from south-eastern (66.90 cm) and north-eastern (64.80 cm) regions.

The average height at withers was significantly (by 2.2 cm) greater in bucks from the field population (77.09 cm) than in bucks from the forest population (74.89 cm). Smaller differences in height at withers were observed in does and fawns, but field roe deer were generally taller than forest roe deer.

Bucks and fawns from field and forest populations were characterized by similar average height at sacrum, but bucks from field habitats were somewhat taller. The most significant difference in height at sacrum was noted between females – does from the field population were 2.07 cm taller than their counterparts from forest habitats.

In a study by VACH (1993), the average height at withers of roe deer bucks hunter-harvested in the Czech Republic was 72.30 cm; the values noted in our experiment were slightly higher. In the cited study, the heights at sacrum and carcass length of young males were 68.6 cm and 99.2 cm, respectively. Fawns analyzed in our experiment had longer carcasses, but were shorter. VACH (1993) demonstrated that the average carcass length of does harvested in the Czech Republic reached 107.40 cm, which indicates that they were considerably smaller than does analyzed in our study. The average height at sacrum of female roe deer from Czech habitats (69.64 cm) was also lower than the values noted in our study for does from field and forest populations.

Table 3 shows carcass weight, and the weights and percentage shares of non-edible parts in the carcasses of roe deer representing forest and field

populations. Field roe deer had higher carcass weight than forest roe deer. The average carcass weight of bucks from field and forest habitats was 19.87 kg and 17.56 kg, respectively. The average carcass weight of does from forest and field populations reached 16.32 kg and 16.98 kg, respectively. Fawns from forest habitats were also characterized by lower carcass weight than their field counterparts – 10.12 kg vs. 11.03 kg.

Table 3

Carcass weight and the weights [kg] and percentage shares [%] of non-edible parts in the carcasses of roe deer from forest and field populations [$\bar{x}$ ± SD]

Specification	Forest roe deer			Field roe deer		
	bucks	does	fawns	bucks	does	fawns
Carcass weight						
[kg]	$17.56^{A} \pm 2.85$	16.32 ± 3.23	$10.12^{a} \pm 2.65$	$19.87^{B} \pm 2.34$	16.98 ± 3.05	$11.03^{b} \pm 2.42$
[%]	100	100	100	100	100	100
Skin weight						
[kg]	2.11 ± 0.36	1.85 ± 0.32	$1.03^{a} \pm 0.18$	2.11 ± 0.34	1.83 ± 0.30	$1.14^{b} \pm 0.19$
[%]	12.01 ± 1.88	11.33 ± 1.38	10.18 ± 1.30	10.62 ± 1.91	10.78 ± 1.58	10.33 ± 1.29
Lower leg weight						
[kg]	0.71 ± 0.09	0.61 ± 0.08	0.42 ± 0.07	0.70 ± 0.09	0.59 ± 0.09	0.47 ± 0.09
[%]	4.04 ± 0.72	3.74 ± 0.70	4.15 ± 0.65	3.52 ± 0.71	3.47 ± 0.70	4.26 ± 0.69
Head weight						
[kg]	–	1.12 ± 0.10	1.01 ± 0.08	–	1.10 ± 0.11	0.99 ± 0.11
[%]	–	6.86 ± 0.81	$9.98^{a} \pm 0.69$	–	6.48 ± 0.80	$8.97^{b} \pm 0.78$
Total non-edible parts						
[kg]	2.82 ± 0.45	3.58 ± 0.50	$2.46^{a} \pm 0.33$	2.81 ± 0.43	3.52 ± 0.50	$2.60^{b} \pm 0.39$
[%]	$16.05^{A} \pm 2.60$	$21.93^{a} \pm 2.89$	24.31 ± 2.64	$14.14^{B} \pm 2.62$	$20.73^{b} \pm 3.08$	23.56 ± 2.76

A, B – $P \leq 0.01$
a, b – $P \leq 0.05$

Highly significant differences in carcass weight were observed between male roe deer from field and forest populations – the former were 2.31 kg heavier. Significant differences in carcass weight were also noted between young roe deer – fawns from field habitats were 0.91 kg heavier. The difference in carcass weight between does from field and forest populations was statistically not significant (0.66 kg). Our findings point to considerable differences in body size between roe deer from various habitats, which was also reported by PIELOWSKI (1988).

JANISZEWSKI and KOLASA (2007) also demonstrated that the habitat had a significant effect on the carcass weight of male roe deer. In the above study, the average carcass weight of bucks from field and forest habitats

in north-eastern Poland was 17.15 kg and 15.65 kg, respectively (a difference of 1.5 kg).

In a study by PETELIS and BRAZAITIS (2003) conducted in Lithuania, field roe deer were heavier than forest roe deer. The average carcass weight of adult bucks from field habitats reached 21.2 kg, and it was 1.2 kg higher in comparison with bucks from forest habitats. Significant differences in carcass weight were also noted between does and fawns – animals from the field population were heavier than those from the forest population.

Such a relationship was also reported by KLEIN and STRANDGAARD (1972) who analyzed roe deer in Denmark. The cited authors found that roe deer from hunting grounds with a high farmland to woodland ratio had significantly higher carcass weight (17 kg), whereas animals from areas with high forest cover were lighter.

WAJDZIK and JAMROZY (2001) compared the average carcass weight of forest and field roe deer from various regions of Poland. The average carcass weight of bucks aged 3 and 5 years varied in the range of 20% to 40%. The average carcass weight of bucks from forest habitats reached 13.4 kg, whereas bucks from field habitats were 3.6 kg heavier (17.0 kg).

DROZD et al. (2000) observed differences in carcass weight between roe deer bucks from various forest complexes in Central and Eastern Poland. The heaviest bucks were harvested in areas of woodland and woodland/farmland mosaic, i.e. in the eastern part of the Lublin Upland. The carcass weight of male yearlings was 15.2 kg, and the carcass weight of three-year-old bucks reached 19.2 kg and it was lower than the carcass weigh of bucks from a field population. The most significant differences in carcass weight were noted between bucks harvested in the western part of the Lublin Upland and Solska Wilderness – in both cases, field roe deer were heavier.

According to BRZUSKI et al. (1997), bucks from non-forested areas were characterized by higher carcass weight (ranging from 16.39 kg to 16.48 kg) than bucks from afforested areas (15.26 kg to 15.49 kg). The bucks analyzed in our study were heavier. RAESFELD (1987) reported that the average carcass weight of roe deer from different regions of Germany ranged from 13.78 kg to 15.10 kg, which indicates that they were significantly lighter than those examined in our experiment.

Table 3 presents also the weights and percentage shares of non-edible parts separated from roe deer carcasses. The heads of bucks were removed by hunters to prepare a trophy, which is why they were not included in our analysis. The highest weight of non-edible parts in the carcass was noted in does from forest and field populations, at 3.58 kg and 3.52 kg, respectively. Fawns from forest habitats were characterized by the lowest weight of non-edible parts in the carcass (2.46 kg). Head weight was comparable in does

from forest and field populations, ranging from 1.12 kg to 1.10 kg. Similar values of head weight were noted in fawns from forest and field habitats, at 1.01 kg and 0.99 kg, respectively.

Table 3 data show that fawns from the forest population were characterized by the highest total percentage share of non-edible parts in the carcass (24.31%). The lowest skin weight was noted in fawns from forest habitats (1.03 kg), and the highest skin weight was observed in bucks from forest and field populations (2.11 kg). The highest percentage share of skin in the carcass was found in bucks from the forest population (12.01%) and the lowest – in fawns from forest habitats (10.18%).

Lower leg weight was also highest in the carcasses of bucks from afforested areas and lowest – in the carcasses of fawns from the forest population. The percentage share of this non-edible carcass part was lowest in does from field habitats (3.47%) and highest – in fawns from the field population (4.26%).

In a study by TRZISZKA (1975), non-edible parts had the following percentage shares of roe deer carcasses: head – 6.6%, lower legs – 4.4%, skin – 9.2%. The above percentage shares of the head and skin in the analyzed carcasses were lower than those noted in our study, whereas small differences were observed in the percentage share of lower legs.

A comparison of the proportions of non-edible parts in the carcasses of different game species revealed that they had the highest total percentage share of wild boar carcasses – 20.91% (ŻMIJEWSKI, KORZENIEWSKI 2000).

Table 4 shows the weights and percentage shares of cuts in the carcasses of forest and field roe deer.

The percentage share of legs, which constitute the largest portion of roe deer carcasses, varied from 40.53% in bucks from afforested areas to 41.39% in fawns from field habitats. Significant differences in leg weight were noted between bucks representing forest and field populations. The remaining cuts had the following percentage shares of roe deer carcasses: saddle – approx. 17%, neck – approx. 10%, shoulder – approx. 17%, ribs – approx. 13%. No significant differences were observed in the proportions of the analyzed cuts in the carcasses of males, females and yearlings hunter-harvested in forests and fields.

ŻMIEJEWSKI et al. (2007) reported that the percentage share of legs in roe deer carcasses (combined data for sex and age groups) reached 40.41%. A similar value was noted in our study in the carcasses of male roe deer. The proportions of the other carcass cuts noted by the cited authors (shoulder – 17.62%, saddle – 17.04%, neck – 11.8%, ribs – 13.13%) were also comparable with those determined in the present experiment. Based on a review of previous findings, DZIERŻYŃSKA-CYBULKO and FRUZIŃSKI (1997) presented the following percentage shares of cuts in roe deer carcasses: legs 41,2%, saddle 16,9%, shoulder 18,1% and neck 9,4%.

Table 4

The weights [kg] and percentage shares [%] of non-edible parts in the carcasses of roe deer from forest and field populations [$\bar{x}$ ± SD]

Specification	Forest roe deer			Field roe deer		
	bucks	does	fawns	bucks	does	fawns
Carcass weight without non-edible parts [kg] [%]	14.74 ± 1.95 100	12.74 ± 1.84 100	7.66 ± 1.44 100	17.06 ± 2.13 100	13.46 ± 1.97 100	8.43 ± 1.27 100
Saddle: [kg] [%]	2.57 ± 0.34 17.46 ± 2.31	2.20 ± 0.32 17.31 ± 2.52	1.34 ± 0.25 17.39 ± 3.24	2.97 ± 0.35 17.44 ± 2.05	2.33 ± 0.33 17.32 ± 2.45	1.46 ± 0.26 17.31 ± 3.08
Neck: [kg] [%]	1.56 ± 0.28 10.61 ± 1.90	1.28 ± 0.26 10.03 ± 2.04	0.76^{a} ± 0.15 10.01 ± 1.97	1.76 ± 0.29 10.26 ± 1.69	1.39 ± 0.27 10.35 ± 2.01	0.84^{b} ± 0.16 10.01 ± 1.91
Shoulder: [kg] [%]	2.64^{a} ± 0.29 17.90 ± 1.96	2.24 ± 0.27 17.62 ± 2.12	1.36 ± 0.17 17.77 ± 2.22	3.08^{b} ± 0.31 18.04 ± 1.81	2.37 ± 0.27 17.64 ± 2.01	1.49 ± 0.18 17.69 ± 2.14
Ribs: [kg] [%]	1.99^{a} ± 0.30 13.50 ± 2.03	1.74 ± 0.29 13.63 ± 2.27	1.05 ± 0.20 13.68 ± 2.61	2.30^{b} ± 0.37 13.51 ± 2.17	1.85 ± 0.29 13.65 ± 2.14	1.15 ± 0.22 13.60 ± 2.60
Leg: [kg] [%]	5.97^{a} ± 0.74 40.53 ± 5.02	5.28 ± 0.70 41.41 ± 5.49	3.15^{a} ± 0.42 41.15 ± 5.49	6.95^{b} ± 0.81 40.75 ± 4.75	5.52 ± 0.71 41.04 ± 5.28	3.49^{b} ± 0.45 41.39 ± 5.34

$^{a, b}$ – $P \leq 0.05$

Conclusions

The following conclusions can be drawn from the present study, which investigated carcass quality in roe deer bucks, does and fawns representing forest and field roe deer:

1. Roe deer from different sex and age groups, hunter-harvested in forest and field habitats, differed in carcass weight and measurements – field roe deer were larger and heavier.

2. The average carcass weights of animals from the forest population were as follows: bucks – 17.56 kg, does – 16.32 kg, fawns – 10.12 kg. The average carcass weights of animals from the field population were 19.87 kg, 16.98 kg and 11.03 kg, respectively.

3. The carcasses of yearlings had the highest percentage share of non-edible parts.

4. The percentage share of legs, which constitute the largest portion of roe deer carcasses, varied from 40.53% in bucks from afforested areas to 41.39% in fawns from field habitats.

5. No significant differences were observed in the proportions of the analyzed cuts in the carcasses of roe deer hunter-harvested in forests and fields.

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THE EFFECT OF ENVIRONMENTAL ENRICHMENT AND SEASON ON THE FATTENING PERFORMANCE, SLAUGHTER VALUE AND SERUM BIOCHEMICAL PARAMETERS OF PIGS

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K e y w o r d s: growing-finishing pigs, management system, season, serum biochemical parameters, carcass quality.

A b s t r a c t

Two pig fattening trials were conducted, one in summer and one in winter. A total of 96 crossbred pigs were analyzed, including 48 animals in each experiment. The pigs were randomly assigned to 4 groups: group 1 – pigs kept in litter-less pens and fed a complete diet *ad libitum*, group 2 – pigs kept in litter-less pens and fed a complete diet supplemented with fresh alfalfa (experiment 1) or alfalfa hay (experiment 2), group 3 – pigs kept in pens with straw bedding and fed a complete diet *ad libitum*, group 4 – pigs kept in pens with straw bedding and fed a complete diet supplemented with fresh alfalfa or alfalfa hay. The body weights of pigs, average daily gains, feed intake, feed conversion ratio, water intake, serum urea nitrogen concentrations and carcass quality were evaluated. Both management systems provided animals with optimal welfare and contributed to highly satisfactory fattening performance and carcass quality. The evaluated systems had no significant effect on average daily gains or slaughter value. However, average daily gains were significantly higher in summer than in winter. Water intake was reduced in pigs fed a complete diet supplemented with fresh alfalfa. Pigs slaughtered in summer were characterized by lower triacyloglicerol level in the blood serum than the animals reared in winter.

WPŁYW WZBOGACANIA ŚRODOWISKA CHOWU I SEZONU NA WARTOŚĆ TUCZNĄ, RZEŹNĄ ORAZ WSKAŹNIKI BIOCHEMICZNE SUROWICY KRWI ŚWIŃ

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Abstrakt

Przeprowadzono dwa doświadczenia, pierwsze w sezonie letnim, a drugie w zimowym. Do badań użyto łącznie 96 świń mieszańcowych, po 48 w każdym eksperymencie. Świnie podzielono na 4 grupy doświadczalne i umieszczono w kojcach (4,2 x 3,6 m), zgodnie z układem: gr. 1 – kojce bez ściółki, mieszanka pełnoporcjowa podawana do woli; gr. 2 – kojce bez ściółki, mieszanka pełnoporcjowa i zielonka z lucerny (doświadczenie 1) lub siano z lucerny (doświadczenie 2); gr. 3 – kojce ze ściółką ze słomy, mieszanka pełnoporcjowa podawana do woli; gr. 4 – kojce ze ściółką ze słomy, mieszanka pełnoporcjowa i zielonka lub siano z lucerny. Oceniano masę ciała, średnie przyrosty dobowe, spożycie paszy, zużycie paszy, ilość pobranej wody pitnej, poziom mocznika w surowicy krwi oraz jakość tusz. Zastosowane systemy żywienia i utrzymania zapewniły świniom doświadczalnym wysoki poziom dobrostanu i w związku z tym cechowała je wysoka wartość tuczna i rzeźna, jednak średnie przyrosty dobowe masy ciała tuczników w okresie letnim były wyższe niż w okresie zimowym. Obserwowano mniejsze pobranie wody przez tuczniki otrzymujące zielonkę. Tuczniki ubijane latem charakteryzowały się niższym poziomem triacylogliceroli w surowicy krwi w porównaniu z tucznikami utrzymywanych w sezonie zimowym.

Introduction

In contemporary animal production, intensive pig farming methods are introduced to boost growth and fattening performance. Modern pig farms are characterized by high stocking density, the use of separate buildings for rearing specific production groups, slatted-floor pens without bedding and complete diets (KALLABIS, KAUFMANN 2012, MARSCHANT-FORDE 2009), which can significantly compromise animal welfare.

The 1970s witnessed a growing interest in environmental protection, animal rights and welfare, which have become important issues also in studies investigating the growth performance of pigs (WHITTEMOR 1987). The concept of environmental enrichment has been introduced to animal research, and experiments evaluating the impact of different management systems on pigs from various production groups, in particular sows and piglets, have been carried out (BEATTIE et al. 1995, MILLET et al. 2005, WHITTEMOR 1987). However, studies of the type are still rarely conducted in Poland (GRELA 2008, KARPIESIUK, FALKOWSKI 2009, KARPIESIUK et al. 2013, KOZERA 2007), although environmental enrichment aimed at improving animal welfare is expected to play an increasingly important role in livestock research (KOZERA 2007, LEBRET et al. 2014, ZAPOTOCZNY et al. 2014). The production of large quantities of high-quality pork is determined by the use of high-quality feed, a balanced diet and adequate environmental and management conditions (KARPIESIUK, FALKOWSKI 2008A, WASILEWSKI et al. 2014).

The objective of this study was to analyze the fattening performance, serum biochemical parameters, including blood urea concentrations as an indicator of protein metabolism, and carcass quality of pigs kept in pens with and without bedding, fed a complete diet with or without the addition of roughage.

Materials and Methods

The study comprised two experiments conducted between June and September (summer), and between December and March (winter). The experiments were carried out in a commercial pig farm in a closed production cycle. A total of 96 weaners raised on the farm were used, including 48 animals in each experiment. The animals were produced by simple four-breed crossing that involved F_1 sows (Polish Landrace x Polish Large White) and F_1 boars (Pietrain x Duroc). They were reared in a fattening unit in pens with an area of approximately 15 m^2, with a solid concrete floor, with or without straw bedding. In each trial, the pigs were randomly allocated to 4 treatment groups of 12 animals each:

– group 1 – pigs kept in litter-less pens and fed a complete diet offered *ad libitum*;

– group 2 – pigs kept in litter-less pens and fed a complete diet offered *ad libitum,* which was supplemented with fresh alfalfa in summer and alfalfa hay in winter;

– group 3 – pigs kept in pens with shallow cereal straw bedding and fed a complete diet offered *ad libitum*;

– group 4 – pigs kept in pens with shallow cereal straw bedding and fed a complete diet offered *ad libitum,* which was supplemented with fresh alfalfa in summer and alfalfa hay in winter.

The animals were tagged and divided into treatment groups by the analogue method, based on age, initial body weight and sex. Growing-finishing pigs were fed two complete diets. The diets were formulated to contain 17% crude protein in the first phase of fattening (PT-1 for pigs weighing 30 to 70 kg) and 15% crude protein in the second phase (PT-2 for pigs weighing 70 to 110 kg), according to the Nutrient Requirements of Swine (1993). Feed ingredients were obtained locally. The experimental diets had the following composition: ground wheat (60% in PT-1, 40% in PT-2), ground triticale (13% in PT-1, 28% in PT-2), ground barley (12% in PT-2) and ground oats (7% in PT-1, 5% in PT-2). Protein sources in the diets were protein concentrate and soybean meal. Pigs had free access to feed in automatic dispensers and water in nipple drinkers. In groups 2 and 4, diets were additionally supplemented with fresh alfalfa (1.2 kg per day per pig) and alfalfa hay (0.13 kg per day per pig), provided once daily in metal feeding racks.

The pigs were weighed individually at the beginning of the experiment, during transition from PT-1 to PT-2 diets, and at the end of the fattening period. Concentrate, fresh alfalfa and alfalfa hay intakes were monitored. Water intake was recorded based on readings from water meters. During both experiments, temperature and relative humidity in the fattening unit were measured continuously with the LAB-EL LB-520 hytherograph.

Seven days before slaughter, blood samples for biochemical analyses were collected from the *vena cava cranialis* from each animal. The serum concentrations of protein, urea, total cholesterol, cholesterol fractions and triacylglycerols were determined. The samples were examined in the COBAS INTEGRA 800 chemistry analyzer. Protein content was determined by the biuret method, and urea concentrations were determined in a kinetic test with urease and glutamate dehydrogenase. Total cholesterol and triacylglycerol levels were determined by the enzymatic-colorimetric method, and HDL cholesterol was measured in an enzymatic colorimetric assay. The LDL fraction was calculated based on the following formula: LDL = total cholesterol – LDL – triacylglycerols/5.

Slaughter and carcass quality assessment were carried out in accordance with industrial standards. Lean meat content was determined in hanging hot right half-carcasses with the use of the ultrasound SYDEL SGM apparatus. Half-carcasses are classified according to the EUROP (Commission Regulation (EC) No. 1249/2008) system using a CGM (Capteur Gras/Maigre) apparatus by Sydel, operated by authorized and trained personnel. The CGM is a hand-held device equipped with an optical probe that determines the thickness of the loin muscle and the fat layer by measuring the light reflected in the probe. The device determines the lean meat content of the carcass, i.e. the ratio of the total mass of the striated muscles to the mass of the carcass ($LMC_{CGM} = 59{,}42 + 0{,}1322M_2 - 0{,}6275T_2$ (1); T_2 – the thickness of the backfat between the 3rd and 4th ribs, 6 cm from the line of carcass partition; M_2 – the thickness of the longissimus dorsi muscle, 6 cm from the line of carcass partition), which is weighed no later than 45 minutes after the animal is stunned.

The pH of the LD muscle (*musculus longissimus dorsi)* was measured 45 minutes (pH_{45}) post-mortem and after 24 hours of chilling at 0°C (pH_{24}). pH_{45} and pH_{24} were determined with the use of the WTW 3310 pH meter and combination electrode (WTW-Wissenschaftlich-Technische Werkstaetten GmbH, Weilheim, Germany) and calibrated with the same standard solutions of pH 4.01 and 7.00 at 20°C. Additionally, their accordance was tested with meat samples at the beginning and regularly during the measuring period. Back fat thickness was determined at five points in chilled half-carcasses: at the thickest point above the shoulder, on the back behind the last rib, above the cranial edge of the GM muscle (*musculus gluteus medius*) (first sacral vertebra – sacrum point I), in the midline of the GM muscle (second sacral vertebra – sacrum point II), above the caudal edge of the GM muscle (third sacral vertebra – sacrum point III). The length of the carcass (from the anterior end of the connection between the first rib and the sternum to the anterior end of the symphysis pubis) and loin eye area were measured.

The analyzed animals were housed in groups, therefore, only mean values in groups were determined for the daily intake of feed, alfalfa and water, and the feed conversion ratio. The remaining results were analyzed statistically, and significant differences between means in groups were determined by two-way ANOVA with an orthogonal design and Duncan's test. Analysis was performed using the general lineal model (GLM) procedure of the StatSoft software package (version Statistica PL 12.5). The model of analysis was:

$$Yijk = \mu + FTi + RSj + (FT \times RS)\,ij + \varepsilon ijk$$

where
FTi is the feeding type (i = 1,2), RSj is the rearing systems (j = 1,2),
(FT x RS)ij is the interaction between the treatment and rearing effects, 4ijk is the residual error.

Data were processed with the use of Statistica PL 12.5 software (2015).

Results

The mean temperature inside the fattening unit was determined at 20.9°C in summer and 14.7°C in winter. Mean relative humidity reached 67.8% in summer and 77.1% in winter.

Disease symptoms and animal deaths were not reported during the study.

The fattening period lasted 96 days in summer and 104 days in winter. The average initial body weight of pigs was similar in both experiments, and no statistically significant differences were observed (Table 1). Significant differences in initial body weight were not noted between groups in summer or winter, and average final body weight was determined at 113.8 kg in summer and 108.8 kg in winter (Table 2). The differences between the mean values of this trait were statistically significant ($p \leq 0.05$). The animals were characterized by a high growth rate, and highly significant differences were observed between seasons – average daily gain was 125 g higher in summer than in winter. The average daily gains of all animals ranged from 794 g in winter in pigs kept in litter-less pens and fed a complete diet to 968 g in summer in pigs housed in pens with bedding and fed a complete diet supplemented with fresh alfalfa. No significant differences in average daily gains were noted between groups in each season. However, highly significant differences were observed between seasons, and average daily gains were considerably higher in summer.

Average feed consumption per kg of body weight gain reached 2.70 kg in summer and 2.85 kg in winter (Table 1).

Table 1
The effect of environmental enrichment, season and dietary alfalfa supplementation on the fattening performance of pigs

Specification	Season*	Pens without bedding		Pens with bedding		Mean**
		complete diet	complete diet + alfalfa	complete diet	complete diet + alfalfa	
Initial body weight [kg]	*S*	23.0	22.9	23.1	22.9	22.9
	W	23.7	23.2	23.4	23.1	23.3
Final body weight at slaughter [kg]	*S*	114.1	114.6	110.5	115.9	113.8^{a}
	W	106.3	107.6	109.7	111.5	108.8^{b}
Average daily gain [g]	*S*	948	955	911	968	946^{A}
	W	794	811	830	849	821^{B}
Daily feed intake [kg]	*S*	2.58	2.58	2.48	2.60	2.56
	W	2.23	2.32	2.40	2.43	2.18
Daily intake of fresh alfalfa [kg]	*S*	–	1.20	–	1.20	1.2
Daily intake of alfalfa hay [kg]	*W*	–	0.14	–	0.12	0.13
Daily water intake per pig [L]	*S*	5.74	4.57	5.41	4.09	4.95
	W	6.06	5.52	5.79	5.57	5.73

* Season: *S* – summer; *W* – winter
** Means within a column without a common superscript differ significantly ($^{a, b}$ – $P < 0.05$; ^{A,B} – $P < 0.01$)

In the experiments conducted in summer and winter, the applied housing systems (with and without bedding) and feeding regimes (complete diets with or without the addition of roughage) had no significant influence on the fattening performance, growth rates or slaughter value of pigs. All of the evaluated management systems supported the production of carcasses characterized by a high lean meat content, high quality and processing suitability.

The lowest average daily water intake of 4.57 L and 4.09 L was noted in the summer experiment in groups whose diets were supplemented with fresh alfalfa (groups 2 and 4, respectively) – Table 1. Reduced water intake in summer can probably be attributed to the high water content of fresh alfalfa.

The average values of serum biochemical parameters were generally within the reference range, and the only parameter that exceeded the norm was total serum cholesterol measured in summer (Table 2). It should be noted, however, that reference values (WINNICKA 2015) do not account for differences between the age groups or production groups of animals. Pigs slaughtered in summer were characterized by lower triacylglycerol levels in the blood serum than the animals reared in winter.

Table 2

The effect of environmental enrichment, season and dietary alfalfa supplementation on the serum concentrations of blood urea nitrogen and lipids [mmol/L] in pigs

Specification	Season*	Pens without bedding		Pens with bedding		Mean***
		complete diet	complete diet + alfalfa	complete diet	complete diet + alfalfa	
Blood urea nitrogen	*S*	4.98**	5.87^{A}	4.50^{B}	5.29	5.16^{a}***
	W	5.89	5.45	5.58	5.72	5.66^{b}
Total cholesterol	*S*	2.38	2.26	2.38	2.34	2.34^{A}
	W	1.90	1.86	1.89	1.76	1.86^{B}
HDL cholesterol	*S*	1.18	1.09	1.09	1.23	1.15^{A}
	W	1.09^{a}	1.06^{a}	1.03	0.88^{b}	1.02^{B}
LDL cholesterol	*S*	1.09	1.05	1.14	1.05	1.08^{A}
	W	0.67	0.65	0.73	0.78	0.71^{B}
Triacylglycerols	*S*	0.28	0.29	0.30	0.24	0.28
	W	0.35	0.37	0.29	0.25	0.32

* Season: *S* – summer; *W* – winter

** Means within a row without a common superscript differ significantly (^{a,b} – $P < 0.05$; ^{A,B} – $P < 0.01$)

*** Means within a column without a common superscript differ significantly (^{a,b} – $P < 0.05$; ^{A,B} – $P < 0.01$)

In winter, significant differences were observed in HDL-C concentrations between group 1 (1.09 mmol/L) and group 2 (1.06 mmol/L) vs. group 4 (0.88 mmol/L). Total cholesterol (2.34 mml/L), HDL-C (1.15 mml/L) and LDL-C (1.08 mml/L) levels were highly significantly higher ($p \leq 0.01$) in summer than in winter when the respective values were determined at 1.86, 1.08 and 0.71 mmol/L. Serum triacylglycerol levels were below the average reference values reported by WINNICKA (2015), and they reached 0.28 and 0.32 mmol/L in summer and winter, respectively

In summer, serum urea concentrations differed significantly between group 2 (5.87 mmol/L) and group 3 (4.50 mmol/L). The increase in urea levels in group 2 can be attributed to higher feed and green forage intake and, consequently, higher protein intake (Table 1).

The dietary treatments had no significant influence on slaughter traits or meat quality in either experiment (Table 3). Lean meat percentage, loin eye area, carcass length and pH_{45} were not significantly affected by the season, feeding regime or housing system. Carcasses from the summer experiment were characterized by significantly higher back fat thickness in all measurement points and higher pH_{24} values (Table 3) than carcasses from the winter experiment. PSE or partially PSE meat was not observed, and active acidity (pH_{45}) was high in all analyzed carcasses in both seasons, ranging from 6.02 in summer to 6.26 in winter. pH is measured 24 hours (pH_{24}) after slaughter to determine the presence of DFD (dark, firm, dry) meat with pH higher than 6.2.

In the present study, DFD meat was not detected, and the average value of pH_{24} ranged from 5.43 in carcasses produced in summer to 5.42 in carcasses produced in winter.

Table 3
The effect of environmental enrichment, season and dietary alfalfa supplementation on the carcass characteristics of pigs

Specification	Season*	Pens without bedding		Pens with bedding		Mean**
		complete diet	complete diet + alfalfa	complete diet	complete diet + alfalfa	
Lean meat percentage	*S*	55.61	55.55	55.04	55.15	55.33**
	W	56.09	56.41	55.52	55.42	55.86
Back fat thickness*** [mm]	*S*	22.80	22.70	23.18	24.90	23.39^A
	W	15.90	18.20	14.50	17.60	16.59^B
Loin eye area [cm^2]	*S*	55.50	54.70	54.20	52.20	54.19
	W	52.60	55.20	53.30	54.30	53.18
Carcass length [cm]	*S*	83.83	83.50	83.33	83.91	83.64
	W	81.60	83.20	82.20	83.80	82.73
pH_{45min}	*S*	6.53	6.64	6.58	6.67	6.61
	W	6.52	6.59	6.54	6.64	6.57
$pH_{24\ h}$	*S*	5.56	5.54	5.53	5.57	5.55^A
	W	5.46	5.49	5.49	5.48	5.48^B

* Season: *S* – summer; *W* – winter
** Means within a column without a common superscript differ significantly ($P < 0.01$)
*** Mean values of five measurements.

Discussion

In this study, temperature and humidity in the fattening unit were generally consistent with industrial standards for growing-finishing pigs (KOŁACZ, DOBRZAŃSKI 2006). CHMIELOWIEC-KORZENIOWSKA et al. (2012) reported greater variations in temperature and relative humidity in pens with deep litter in summer and winter seasons.

Average daily gains noted in our study were similar to those reported by JORDAN et al. (2008) and MILLET et al. (2005). In other experiments conducted in recent years, both lower (JORDAN et al. 2008, KARPIESIUK, FALKOWSKI 2008a, KOZERA 2007, LEBRET et al. 2014) and higher (CHMIELOWIEC-KORZENIOWSKA et al. 2012) daily weight gains were noted in growing-finishing pigs. In a study by LEBRET et al. (2006), season had a significant ($P < 0.001$) influence on pig growth rates. In the cited study, the highest weight gains were reported in winter, the lowest weight gains were noted in summer, whereas medium weight gains that did not differ significantly from the reported values were observed in spring. In the work of BRZOZOWSKI et al. (2013) who analyzed the

influence of effective microorganisms on fattening performance, average daily gains in both experimental groups were similar to those noted in our winter experiment, but lower by 100 g and 133 g than in our summer experiment.

KORNIEWICZ et al. (2001), who studied growing-finishing pigs from commercial four-breed crossing, reported slightly lower average feed consumption per kg of body weight gain (2.67 kg). In the previously cited French study (LEBRET et al. 2006), the management system had no significant effect on the feed conversion ratio which was determined at 3.07, 3.27 and 3.30 kg/kg in winter, spring and summer, respectively.

In the Nutrient Requirements of Swine published by the US National Research Council (2012), the minimum water demand of growing-finishing pigs was set at 2 L per kg of a complete diet. Pigs fed *ad libitum* usually drink approximately 2.5 L of water per kg of a complete diet. In growing pigs, water intake can be influenced by environmental factors, diet composition, the health status of animals and stress exposure (SHAW et al. 2006). The relationship between fresh alfalfa intake and reduced water intake had been established in earlier studies (KARPIESIUK, FALKOWSKI 2008b, KARPIESIUK, FALKOWSKI 2009).

In a study into the compensatory growth of growing-finishing pigs, WIĘCEK et al. (2008) demonstrated lower concentrations of total cholesterol and LDL-C in animals whose diets were restricted in the first phase of fattening, compared with pigs fed semi *ad libitum* diets, regardless of the type of administered diets.

Similar or slightly higher triacylglycerol concentrations were reported in previous studies of growing-finishing pigs of the same genotype (EGGUM 1970, KARPIESIUK, FALKOWSKI 2008b). GRELA et al. (2012) supplemented pig diets with inulin and garlic and observed a drop in the serum concentrations of total cholesterol, LDL-C and triacylglycerols as well as an increase in HDL-C levels. In another study, GRELA et al. (2013) reported an increase in total cholesterol and LDL-C levels in pigs fed an organic diet supplemented with fish meal and premix. A reduction in triacylglycerol, total cholesterol and LDL-C concentrations in the blood and tissues of pigs could improve the nutritional value of pork.

According to the literature, excessive protein intake increases urea synthesis and secretion, which is manifested by higher urea concentrations in the blood serum (EGGUM 1970, WIĘCEK et al. 2008). SEMENIUK, GRELA (2011) reported (throughout the fattening period) lower values of protein metabolism parameters, including the plasma levels of total protein, uric acid, blood urea nitrogen (BUN) and ammonia, in growing-finishing pigs fed diets with 10% reduced protein content in comparison with animals whose protein intake was consistent with the Polish edition of the Nutrient Requirements of Swine (1993)

The pH_{45} values noted in our study are indicative of normal meat acidity, according to KORTZ (2001). In a study investigating the influence of different pig management systems on meat quality and the fatty acid profile of the LD muscle, KARPIESIUK et al. (2013) reported much greater variations in pH45 values (5.80 – 6.70) in comparison with the present findings. In the above study, pH_{24} values were determined in the range of 5.35 to 5.61 and were similar to our results. In the work of KARPIESIUK, FALKOWSKI (2009), pH_{45} and pH_{24} values were lower than those noted in the present study.

Conclusions

Fattening performance and carcass quality values were satisfactory and remained within the reference ranges in all experimental groups of growing--finishing pigs raised in summer and winter.

Dietary supplementation with fresh alfalfa reduced water intake by pigs.

The average daily gains of pigs were determined at 946 g in summer (first experiment) and 821 g in winter (second experiment).

The evaluated carcasses were characterized by a satisfactory or highly satisfactory lean meat content. The average lean meat percentage was determined at 55.33% in the first experiment and 55.86% in the second experiment, and the difference between the noted values was not statistically significant.

It can be concluded that environmental enrichment did not improve the fattening performance of pigs.

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COMPARATIVE ANALYSIS OF VARIATION OF CONFORMATION TRAITS IN DIFFERENT-COLOUR MINKS

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K e y w o r d s: minks, colour variety, quality of fur, auction value, performance evaluation.

A b s t r a c t

The aim of the study was to characterize the variability of performance of the colour varieties of pastel, silver blue, and pearl American mink. Observations were made on 996 minks, whose conformation was assessed in accordance with the standard of evaluation. The analysis includes auction results. Among the investigated colour varieties, the most preferred performance features were exhibited by the pearl mink, and the worst were attributed to the pastel mink pelt. The results indicate poorer fur quality of males compared with females. It is evident that there is an association between evaluation of the conformation class obtained by the tested animals and the value of mink pelts in the auction sale.

ANALIZA PORÓWNAWCZA ZMIENNOŚCI CECH POKROJU RÓŻNYCH ODMIAN BARWNYCH NOREK

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S ł o w a k l u c z o w e: norki, odmiana barwna, jakość okrywy włosowej, wartość rynkowa skór, wzorzec oceny.

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Abstrakt

Celem badań była charakterystyka zmienności cech pokroju norek amerykańskich (*Neovison vison*) odmiany pastel, silver blue oraz pearl. Obserwacje prowadzono na 996 norkach urodzonych w 2011 r., z których 404 zwierzęta miały oceniony pokrój przez licencjonowanego sędziego i zostały zakwalifikowane do klas A, B+, B oraz C zgodnie z obowiązującym wzorcem oceny. W analizie uwzględniono wyniki aukcji z trzech domów aukcyjnych (SAGA, Kopenhagen Fur, NAFA) w sezonie 2014 i 2015. Na podstawie zaprezentowanych wyników można zauważyć, że istnieje zależność między uzyskaną przez zwierzęta klasą oceny pokroju a wartością aukcyjną skór norek. Wśród badanych odmian barwnych najkorzystniej wyrażonymi cechami pokroju charakteryzowały się norki odmiany pearl, natomiast najgorzej oceniono okrywę włosową norek pastel.

Introduction

In breeding of fur-bearing animals, including mink, the most important features from an economic point of view are the high quality of fur and the size of the skin. However, the effectiveness of mink farming strongly depends on the reproductive performance of the herd. It depends largely on genetic and a number of environmental factors that must be considered throughout the production cycle. The quality of the fur is an inherited trait in mink breeding; therefore, this factor should be an important element of selection ($h = 0.613$) (SOCHA et al. 2008).

The most important indicator of the profitability of mink farming is the litter size expressed in the number of pups born and reared. However, litters with more than 10 young ones as well as with less than 3 pups can be born, which is not uncommon on the farm. Therefore, minks with a low number of pups should not be mated in the next breeding season. A high herd reproduction rate will ensure profitability of the farm. Additionally, considerable attention should be paid to the differences in the price of mink pelts depending on the variety of the coat colour. The colour varieties of mink skin obtained through breeding work reach higher prices than skins in the standard version (BIELAŃSKI et al, 2005). The aim of the study was to characterize the variability of performance of the colour varieties of pastel, silver blue, and pearl American mink (*Neovison vison*).

Material and Methods

Data for the study were obtained from breeding records of a farm in the South-East of Poland. Observations were made on 996 American minks (*Neovison vison*) born in 2011 (473 males, 523 females). The animals represented by 3 colour types: 306 silver blue (159 males, 147 females), 321 pearl (158 males, 163 females), and 369 pastel (156 males, 213 females).

The characteristics of the tested herd are shown in Table 1. Evaluation of the fur quality was carried out on 404 minks in November, according to accepted assessment procedures (A, B+, B, C) by a licensed judge (*Wzorzec oceny fenotypu*... 2010). The housing and feeding conditions on the farm remained unchanged. The herd was fed in accordance with standards for carnivorous fur animals. The animals were also subjected to necessary preventive and veterinary treatments.

Table 1

Characteristics of the tested herd

Specification	$\bar{x}$	SD	Min.	Max
Date of birth	130.97	2.79	123.00	139.00
Born	6.58	1.80	1.00	11.00
Weaned	6.24	1.76	1.00	11.00

The analyses included the number of pups born and the number of pups weaned per litter and date of birth (in days since the beginning of the year). The probability of assignment of a specific conformation class was analysed by multivariate analysis of variance using the least squares method, taking into account the fixed effects of assessment procedures, sex, colour type, and the colour type x sex interaction. The calculations were made using SAS statistical package (SAS Institute, Cary, NC, USA). The values of the analysed traits are shown as least square means (lsm), providing standard errors (se) that determine the reliability of estimation. Statistical significance was established as $P \leq 0.05$.

Results and Discussion

According to the evaluation standard of the American mink phenotype (2010), the animals are classified into one of four groups (A, B +, B, C), depending on the features of conformation. Minks with best-expressed characteristics receive a rating of ''A' and the worst – ''C''. During evaluating the phenotype of minks, the overall appearance of the animal is assessed, i.e. its physique and size, colour type, colour and purity of the fur, and fur quality, i.e. the length, density, silkiness, and elasticity.

The key aspects of profitability in mink breeding are the size of animals and quality of fur, which have a high share in the price of the skin. FILISTOWICZ et al. (1999) found that the economic value of the size and quality of the skin was 0.64 and 0.34, respectively. The research by WIERZBICKI (2005) confirmed that

the size of the skin accounted for about 60%, and the quality for 28% of the overall volatility of its price. This means that the value of the skins is dependent largely on the above-mentioned characteristics. It should be emphasized that the price of mink pelts also depends on the colour variety (Table 2), which is related to, among others, fashion trends.

Table 2
Average prices for mink (EUR) in two seasons at auctions: Saga®, Kopenhagen Fur and NAFA

Colour type	Sex	2015			2014			$\bar{x}$
		SAGA	Kopenhagen	NAFA	SAGA	Kopenhagen	NAFA	
Pastel	♂	41.20	26.26	nd*	nd*	39.95	54.81	40.55
	♀	28.20	17.03	nd*	nd*	25.44	35.24	26.48
Silver blue	♂	56.65	58.49	nd*	44.47	45.95	51.06	51.32
	♀	36.05	37.48	nd*	28.44	28.41	33.83	32.84
Pearl	♂	50.74	54.61	nd*	45.11	52.81	67.25	54.10
	♀	36.62	39.71	nd*	29.94	35.66	49.68	38.32

* no action results published yet

Due to selective breeding carried out on farms, farmed minks are relatively heavier than wild minks and on average have by 30% larger body sizes and by 50% larger skins (PIÓRKOWSKA, KOWALSKA 2014). The size and quality of the fur is also dependent on the sex. Due to the sexual dimorphism in the minks, the male skins are larger and have a better quality of fur reflected in longer and thicker hair. As indicated by the results of sales at three auction houses: SAGA® (www.sagafurs.com), Kopenhagen Fur (www.kopenhagen-fur.com), and NAFA (www.nafa.ca), regardless of the colour variety, male skins achieve higher prices, which is associated with the higher body weight of males as well as the length and quality of their skins, compared with females. Based on sales reports in the period 2014–2015, it can be concluded that the pearl minks had the best-rated skins. They reached a price by ca. 27.46% and ca. 8.94% higher than that of the pastel minks and the silver blue minks, respectively.

Table 3 shows the probability of assignment of a particular conformation class for the three tested mink colour varieties. The pearl minks were characterized by the greatest likelihood of achieving class A among the three studied colour varieties. Among the pearl minks, it was found that the B + skin class was most likely to occur. The highest probability of obtaining the weakest quality of the fur was recorded in the pastel mink group (class C). The auction sales may explain the results obtained – the highest prices were reached by the pearl minks and the lowest by the pastel-colour animals (Table 2).

Table 3
Probability (lsm) of occurrence of individual classes of mink conformation ratings depending on the variety of colour

Colour	A		B+		B		C	
	lsm	se	lsm	se	lsm	se	lsm	se
Silver blue	0.051^{b}	0.020	0.136	0.03	0.110	0.028	0.071^{b}	0.029
Pearl	0.123^{a}	0.019	0.175^{a}	0.028	0.115	0.027	0.069^{b}	0.027
Pastel	0.009^{b}	0.021	0.095^{b}	0.03	0.094	0.028	0.241^{a}	0.029

^{a,b} – The values in the columns marked with various letters differ significantly at $p \leq 0.05$

The results of evaluations of the particular classes of mink conformation depending on the sex are shown in Table 4. Class B + was noted most likely in females, whereas males were assigned to the weakest class C. The size of the animal is taken into account in the assessment of conformation due to the pronounced sexual dimorphism. Therefore, the results indicate poorer fur quality of males compared with females. The lowest lsm value was recorded for class A, most desirable by breeders, in both females and males (Table 4). This may be due to both the very small size of the animals and the unsatisfactory quality of the coat, which demonstrates the possibilities of further breeding work to improve the quality of the coat in the tested mink colour varieties. KOŁODZIEJCZYK, SOCHA (2006, 2008) have shown that the type of colour, age of the animal, and sex of the examined mink have a significant impact on the characteristics of conformation. Results of other authors evaluating the effect of sex on the conformation traits of different species of fur-bearing animals do not indicate a direct correlation between these characteristics. A study conducted by ŚLASKA (2002) showed that males were characterized by a significantly higher average conformation rating compared with females, but there was no difference in the average overall scores between the sexes. For a majority of mink conformation traits, except for the body size, SOCHA et al. (2001) obtained higher scores in the group of males although statistically significant differences between the sexes were only noted for the quality of the coat and the size of the body. The differences in the skin size depending on the sex, due to the sexual dimorphism of mink, as well as the quality of hides are related to the average of the value pelts in auction sales (Table 2).

Table 4
Probability (lsm) of occurrence of individual classes of mink conformation ratings based on the sex

Sex	A		B+		B		C	
	lsm	se	lsm	se	lsm	se	lsm	se
♂	0.038^{b}	0.018	0.085^{b}	0.026	0.049^{b}	0.025	0.096^{b}	0.026
♀	0.084^{a}	0.017	0.186^{a}	0.024	0.164^{a}	0.023	0.158^{a}	0.024

^{a,b} – The values in the columns marked with various letters differ significantly at $p \leq 0.05$

Based on the results of the research and the price of skins from the auction data, a relationship between the evaluation of the conformation class obtained in our study and the auction value of mink can be noticed. The largest number of animals with high coat quality was found in the evaluation of the pearl minks. Females with this colour variety were characterized by a significantly better coat quality than males (Table 5). The intermediate quality classes (B + and B) were most often obtained by pearl and silver blue mink females. The greatest probability of being assigned to class C was found in both male and female pastel minks.

Table 5

Probability (lsm) of occurrence of individual classes of mink conformation ratings depending on the variety of colour and sex

Colour	Sex	A		B+		B		C	
		lsm	se	lsm	se	lsm	se	lsm	se
Silver blue	♂	0.047^{b}	0.024	0.043^{b}	0.035	0.067^{bc}	0.034	0.063^{bc}	0.035
	♀	0.055^{b}	0.023	0.230^{a}	0.034	0.152^{ac}	0.032	0.080^{bc}	0.033
Pearl	♂	0.065^{b}	0.023	0.108^{b}	0.034	0.026^{b}	0.032	0.044^{bc}	0.033
	♀	0.182^{a}	0.023	0.242^{a}	0.033	0.205^{ac}	0.031	0.094^{bc}	0.032
Pastel	♂	0.003^{c}	0.025	0.104^{b}	0.037	0.053^{b}	0.035	0.180^{c}	0.036
	♀	0.015^{b}	0.022	0.087^{b}	0.033	0.136^{ac}	0.031	0.301^{a}	0.032

^{a,b} – The values in the columns marked with various letters differ significantly at $p \leq 0.05$

PIÓRKOWSKA et al. (2014) evaluated the conformation in a population of pastel minks kept in different numbers per cage using the dual standards of evaluation: the first one in force until 2009 (2000) and the one that is currently being used (2010). For minks kept in pairs in cages, the average overall rating according to the first evaluation standard (2000) amounted to 16.97, while the following results for the different classes were obtained according to the new method of evaluation (2010): A (12.12%), B + (54.55%), B (21.21%), and C (12.12%). The results obtained are not consistent with the herd of pastel minks tested in our study, where 52.60% of the animals were assigned to class C. In accordance with the new evaluation system, such minks are eliminated from further breeding. Similar results were obtained in the group of silver blue minks where 11.96% of the animals were assigned to class A and 41.30% class B+. Pearl minks achieved the best assessment class, where as many as 64.75% of the animals were classified to the most desirable Class A and B+. While assessing the conformation traits for standard and palomino minks, KOŁODZIEJCZYK, SOCHA (2008) reported an average overall rating of 17.93–19.47 points, which can be classified to class B+ and A in the new assessment system.

Based on these results, it can be concluded that the pearl colour variety was the most preferred mink conformation trait, while the pastel colour minks were characterized by the worst rating. The differences in the probability of assignment of each conformation class indicate high variability of the coat quality between males and females, as well as between the different mink colour varieties. The differences in the size and quality of skins depending on sex and colour variations are related to the average auction value of pastel, silver blue, and pearl pelts.

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SEASONAL VARIABILITY OF CARBON FORMS IN WATER AND BOTTOM SEDIMENT IN LAKES WITH A DIFFERENT TYPE OF MIXING*

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K e y w o r d s: TOC, DOC, IC, bottom sediments, urban lakes.

A b s t r a c t

The aim of this study was to determine seasonal changes in the occurrence of carbon forms in water and bottom sediment in lakes with a different type of mixing. The study was conducted in five urban lakes located in Olsztyn: Track, Sukiel, Podkówka, Redykajny and Tyrsko. The research was carried out in March, April, August and November. We analyzed TOC, DOC and IC in samples of water collected near the surface and near the bottom, as well as in the overlying and interstitial layer of 0–5 cm and 5–10 cm of sediment. The dominant form of carbon in urban lakes is organic carbon, whose share in the surface water layer in the lakes analyzed in the present study was from 29.3 to 58.4% of the total pool of carbon, and the near-bottom layer was characterized by values ranging from 28.2 to 66%, while the layer of overlying water contained from 34.2 to 63.6% of the total amount of carbon. In the interstitial water, the percentage of organic carbon in the total pool was from 40.1 to 94% within the 0–5 cm layer of sediment and from 56.4 to 88.1% in the layer of sediment at the depth of 5–10 cm. The percentage of inorganic carbon forms ranged from 2.94% to 71.79% of the total amount of carbon. The carbon cycle in lake water depends not only on the inflow of this element from the catchment, but also on its release from the bottom sediment, which can be a large reservoir of organic carbon in lakes.

SEZONOWA ZMIENNOŚĆ FORM WĘGLA W WODZIE I OSADACH DENNYCH JEZIOR O ZRÓŻNICOWANYM TYPIE MIESZANIA

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S ł o w a k l u c z o w e: TOC, DOC, IC, jeziora miejskie, osady denne.

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Abstrakt

Celem pracy było określenie sezonowych zmian zawartości węgla w wodzie i osadach dennych w jeziorach o zróżnicowanym typie mieszania. Badania przeprowadzono w pięciu jeziorach miejskich Olsztyna: Track, Sukiel, Podkówka, Redykajny, Tyrsko w marcu, kwietniu, sierpniu i w listopadzie. W pobranych próbkach wody zbadano zawartość TOC, DOC oraz IC. Wodę do badań pobierano z warstwy powierzchniowej, naddennej, nadosadowej oraz interstycjalnej (0–5 i 5–10 cm). Dominującą formą węgla w jeziorach miejskich był węgiel organiczny, którego udział w całkowitej puli węgla wynosił od 29,3% do 58,4% w wodzie powierzchniowej, a w wodzie naddennej od 28,2% do 66%, natomiast w wodzie nadosadowej od 34,2% do 63,6%. W wodzie interstycjalnej procentowa zawartość węgla organicznego wynosiła od 40,1% do 94% w warstwie 0–5 cm osadów, a w warstwie 5–10 cm od 56,4% do 88,1%. Zawartość procentowa węgla nieorganicznego wynosiła od 2,94% do 71,29% całkowitej puli węgla. Uzyskane wyniki wskazują, że obieg węgla w wodzie jeziorowej będzie zależał nie tylko od jego dopływu ze zlewni, ale również od uwalniania tego składnika z osadów dennych, które mogą być dużym rezerwuarem węgla organicznego w jeziorze.

Introduction

In surface waters, carbon appears in the form of total organic carbon (TOC) and inorganic carbon (IC). Total organic carbon consists of dissolved organic carbon (DOC), particulate organic carbon (POC) and volatile organic compounds (VOCs). Inorganic carbon, which dominates in water, comes in three forms: CO_3^{-2}, HCO^{-3}, CO_2 (DUNALSKA 2009). Dissolved organic carbon (DOC) is a key component of all aquatic ecosystems. The pool of DOC in a lake consists of both autochthonous organic matter, produced in the lake, and allochtonous matter, delivered from the catchment. Dissolved organic carbon is an important production regulator in aquatic ecosystems. With its absorption properties, DOC may inhibit the process of photosynthesis (JONES 1998); on the other hand, it provides substrate for heterotrophic bacteria (TRANVIK and DOWNING 2009). DOC originating from the catchment is less readily transformed than that derived from the production in the lake, because the former, before entering the lake, already undergoes a series of changes in soil (SCHIFF et al. 1997). Total DOC contained in the water of a lake is subject to numerous processes which modify its concentration. The impact on these processes depends primarily on the catchment characteristics, such as the nature of the catchment, its runoff coefficient and land use. An external supply of organic carbon in lakes often dominates the amount of carbon produced within these water bodies (CARACO, COLE 2004). In addition, DOC can affect the fate of other dissolved substances such as heavy metals and other organic pollutants (HAITZER et al. 1998). DOC may also perform a protective function towards ultraviolet for flora and fauna inhabiting the ecosystem of a lake (MOLOT et al. 2004).

Inorganic carbon (IC) is an important constituent of lake systems. The dominant form of inorganic carbon is largely dependent on the water pH.

In typically hard water lakes, pH values may be above 8 and HCO_3^- is usually a dominant species in solution (WETZEL 2001). The amount of IC in lake water depends on a variety of processes, including the equilibrium state with atmospheric CO_2, bicarbonate-carbonate balance, external loadings, contributions from metabolic respiration, consumption via photosynthesis, temperature and redox reactions including microbial methane fermentation, nitrification, sulfide oxidation, sulfate reduction, Fe and Mn oxide reduction and denitrification (LAMPERT, SOMMER 2007).

The aim of this study was to determine seasonal changes in the occurrence of carbons in different components of a lake ecosystem, including surface water, bottom water, overlying water and interstitial water in lakes with a different type of mixing.

Material and Methods

Study site

The study was conducted in five urban lakes located in Olsztyn: Track, Sukiel, Podkówka, Redykajny and Tyrsko (Figure 1), which belong to the Masurian Lake District. The morphometric, mictic characteristics and present trophic status of studied lakes are shown in Table 1.

Table 1

Morphometric, mictic characteristics and present trophic status of studied lakes

Lake	Area [ha]	Catchment area [ha]	Depth max [m]	Type of mixing	TSI (Chl)	TSI (TP)	TSI (TOC)	TSI (SD)
Tyrsko	**18.6**	**68.2**	**30.4**	**di-**	**45.74**	**65.23**	**54.11**	**41.92**
Redykajny	29.9	187.4	20.6	di-	56.04	82.22	62.16	44.15
Podkowka	**6.9**	**25.6**	**6.0**	**poly-**	**66.34**	**92.60**	**59.20**	**46.22**
Sukiel	20.8	26.1	25	di-	42.44	55.44	61.38	48.62
Track	**52.8**	**387.1**	**3.8**	**poly-**	**77.65**	**80.07**	**66.33**	**61.52**

Track Lake is located on the north-eastern outskirts of Olsztyn. The lakeshores are generally flat or slightly sloping.

Sukiel Lake is located in the western part of Olsztyn. The lake has no outlet. The shores of the lake are gently elevated.

Podkówka Lake is located in the north-western part of Olsztyn. The shores of the lake are distinctly raised and mostly overgrown with trees.

Redykajny Lake is located in the northern part of Olsztyn. The basin is physiographically varied and the shores comprise long elevated sections.

Tyrsko Lake is located in the north-western part of Olsztyn.

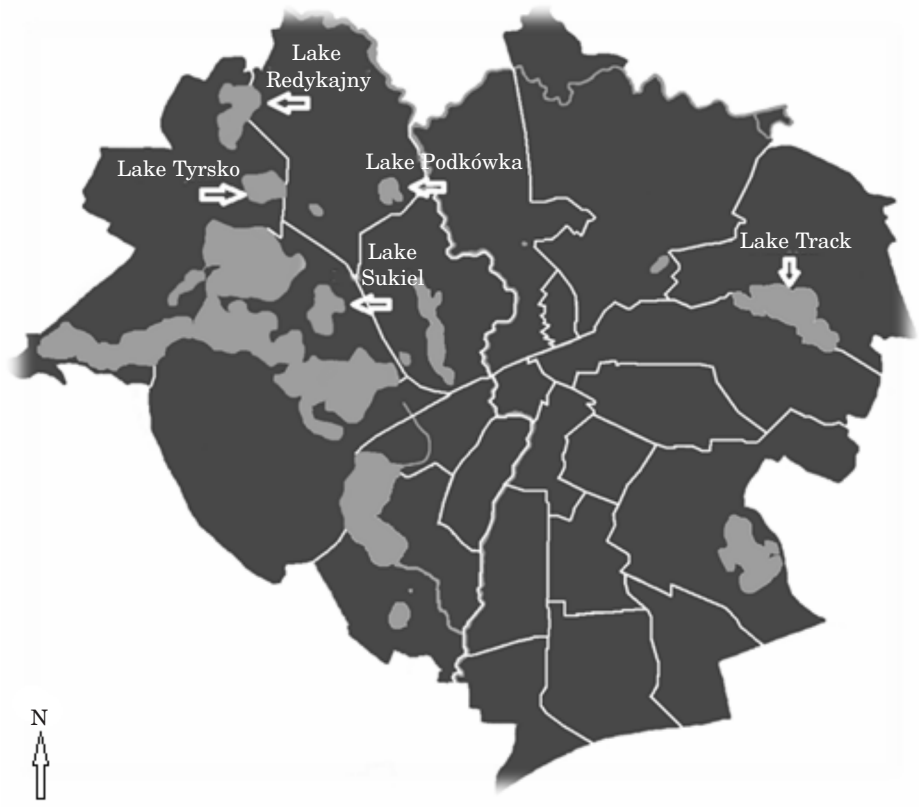

Fig. 1. Location of the lakes in Olsztyn

Water sample collection

The research was carried out in March, April, August and November. We analyzed samples of water collected near the surface and near the bottom, as well as in the overlying and interstitial layer of 0–5 cm and 5–10 cm of sediment. Bottom sediments was collected in the deepest place in each lakes. Water and bottom sediment samples were collected using the Ruttner water sampler and Kajak sediment sampler, respectively. Interstitial water was collected by centrifugation in a centrifuge at a speed 2500 rpm.

Carbon determination and chemical analyze

Each water sample was divided into two parts. One was filtered through a Millipore filter with a pore diameter of 0.45 μm, in order to isolate the

fraction of dissolved organic carbon (DOC) (DUNALSKA et al. 2012). The other one was not filtered and used for the determination of total carbon (TC) and inorganic carbon (IC) via high-temperature combustion (HTC) using a IL 550 TOC-TN HACH analyzer. TOC was calculated from the results of TC and IC.

Total phosphorus was determined according to the Standard Methods (1999). Chlorophyll was determined by the spectrophotometric method with the correction for phaeopigments (PN-86/C-05560/02). Water transparency was assayed with the Secchi disc.

Statistical analysis

In order to verify whether the content of carbons depends on the season, the results were submitted to statistical analysis, including tests of normality of the distribution and homogeneity of variance. Normality was checked with the Shapiro-Wilk's test, while homogeneity was assessed with the Levene's test. When both conditions were met, an analysis of variance was performed by ANOVA. ANOVA procedure was used to compare TOC, DOC and IC between different components of a lake ecosystem. In case of given factor, *post hoc* tests were performed by Tukey test.

Results

Total Organic Carbon

The content of TOC in the surface water of the lakes ranged from 7.33 mg C dm^{-3} (Tyrsko Lake in summer) to 18.39 mg C dm^{-3} (Track Lake in spring). In the near-bottom water layer, the highest amount of TOC was observed in Podkówka Lake, and the lowest one in Tyrsko Lake (23.51 mg C dm^{-3} and 6.77 mg C dm^{-3}, respectively). The highest and lowest values were obtained in spring. The overlying water in the lakes was characterized by much higher organic carbon content (7.32 mg C dm^{-3} – 522.80 mg C dm^{-3}) than in surface and bottom layers (Figure 2). The smallest value was recorded in Tyrsko in summer, and the highest one occurred in Redykajny in spring. The amount of TOC in the interstitial water within the 0–5 cm layer of sediments was of similar magnitude as in overlying water and ranged from 14.41 mg C dm^{-3} in Podkówka Lake in summer to 516.50 mg C dm^{-3} in Redykajny Lake in spring. The concentration of organic carbon in the interstitial water within the 6–10 cm layer of sediments was in the range of 36.82 mg C dm^{-3} in Podkówka Lake in summer to 320.30 mg C dm^{-3} in Sukiel Lake in winter.

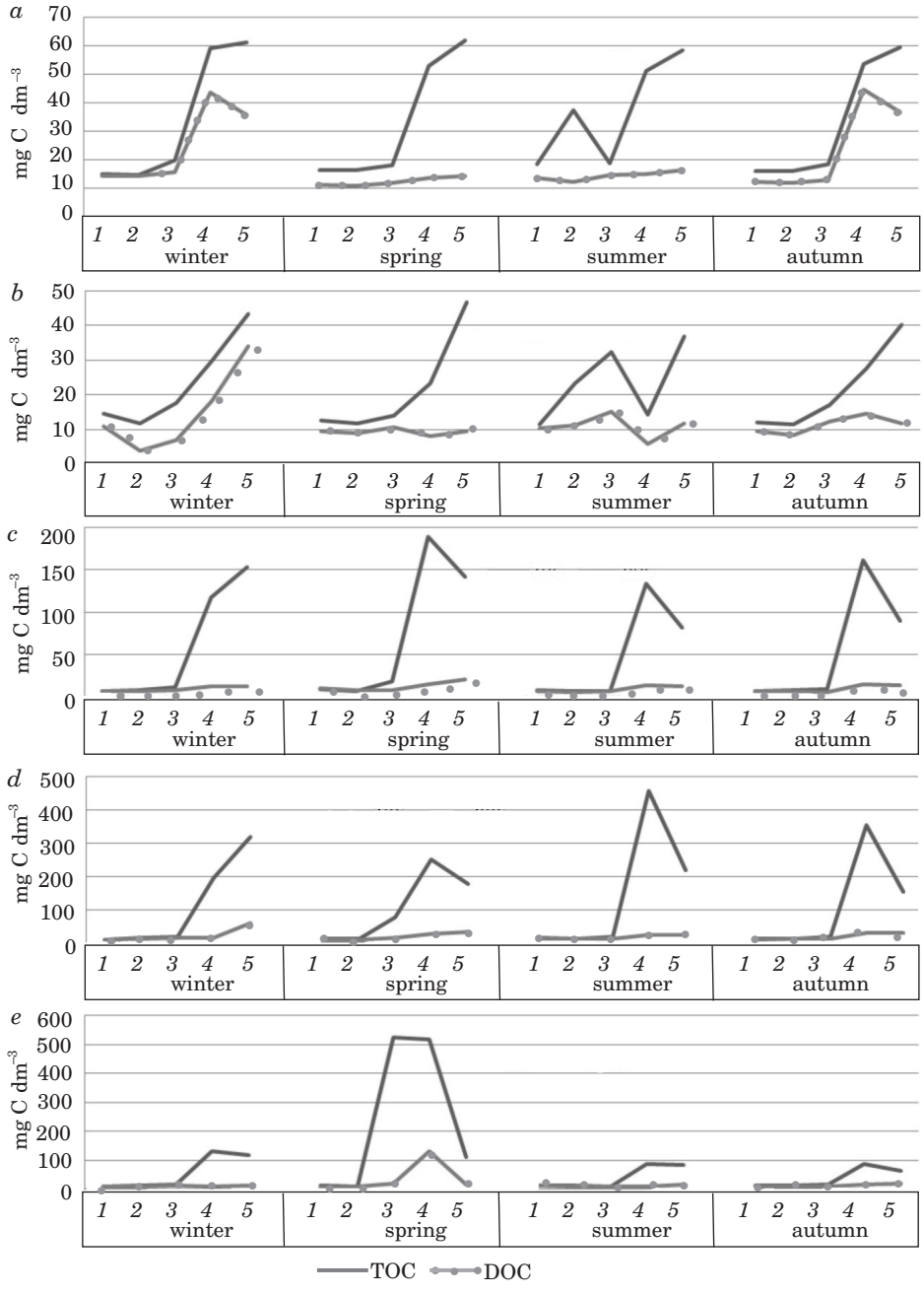

Fig. 2. Organic carbon content and dissolved organic carbon content in water of the analyzed lakes: *a* – Lake Track, *b* – Lake Podkówka, *c* – Lake Tyrsko, *d* – Lake Sukiel, *e* – Lake Redykajny; (*1* – surface water, *2* – bottom water, *3* – overlying water, *4* – interstitial water, 0–5 cm, *5* – interstitial water, 5–10 cm)

Dissolved Organic Carbon

The concentration of DOC in the surface water of the lakes ranged from 6.35 mg C dm^{-3} in Tyrsko Lake in autumn to 14.26 mg C dm^{-3} in Track Lake in winter. The content of DOC in the near-bottom water was on the level of 3.82 mg C dm^{-3} in Podkówka Lake to 14.50 mg C dm^{-3} Track Lake, both in winter. The highest content of DOC in the overlying lake water appeared in Track Lake in winter (15.76 mg C dm^{-3}), and the lowest one – in Tyrsko Lake in autumn (5.61 mg C dm^{-3}). The DOC in the interstitial water within the 0–5 cm layer of sediment ranged from 5.82 mg C dm^{-3} in Podkówka Lake in summer to 131.00 mg C dm^{-3} in Redykajny Lake in spring. The 6–10 cm layer of sediment was characterized by the content of DOC in the interstitial water ranging from 9.64 mg C dm^{-3} in Podkówka Lake in spring to 59.27 mg C dm^{-3} in Track Lake in spring (Figure 2).

Inorganic Carbon

The surface water of the examined lakes were characterized by the inorganic carbon content ranging from 7.90 mg C dm^{-3} to 36.33 mg C dm^{-3}. In the near-bottom water, the IC content was within the range of 8.14 mg C dm^{-3} to 37.16 mg C dm^{-3}, while in the overlying waters it varied from 6.57 mg C dm^{-3} to 37.91 mg C dm^{-3} (Figure 3). In the surface, near bottom and overlying water, the minimum and maximum values were found at the same sampling sites: in Tyrsko Lake in spring and in Track Lake in winter, respectively. The concentration of IC in the interstitial waters of the 0–5 cm layer of bottom sediment ranged from 11.57 mg C dm^{-3} to 48.21 mg C dm^{-3}, in Tyrsko Lake in winter (the lowest value) and in Sukiel Lake in autumn (the highest value). The lowest content of IC in the interstitial water of the 6–10 cm layer of sediment was determined in Podkówka Lake in spring (13.74 mg C dm^{-3}), and the highest one was in Sukiel Lake in autumn (and 54.98 mg C dm^{-3}).

Discussion

Seasonal changes of carbon content in lakes ecosystem

Many studies focus on examining the content of organic carbon in lake sediment (MOLOT, DILLON 1996, STALLARD 1998, EINSELE et al. 2001, KORTELAINEN et al. 2004, ANDERSON et al. 2009, KASTOWSKI et al. 2011), and the results provide information on the carbon budget in lakes and its role in the

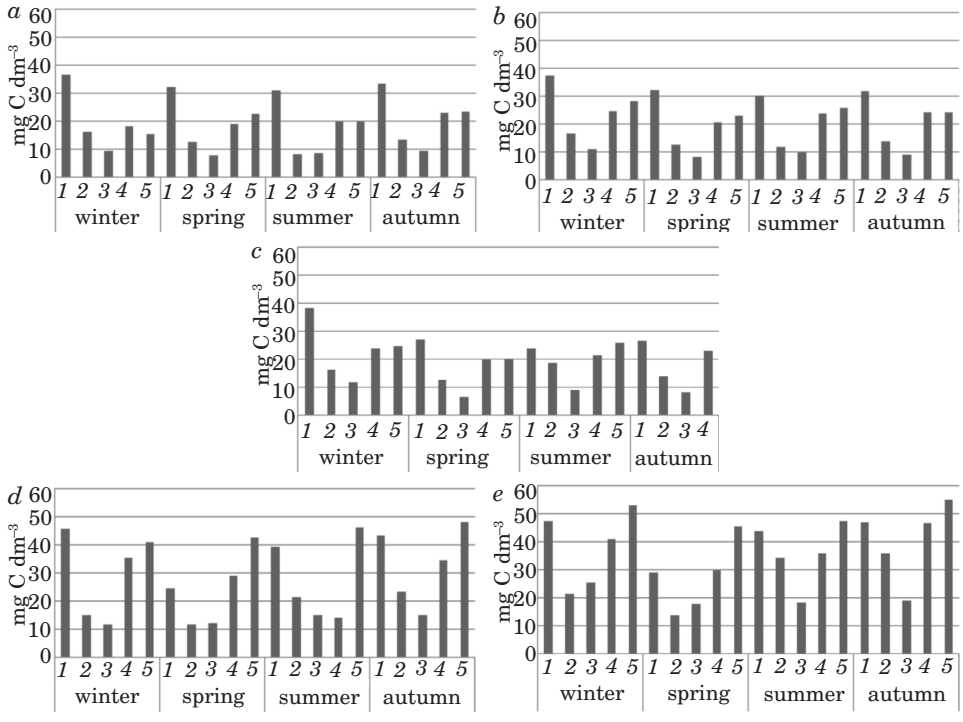

Fig. 3. Inorganic carbon content in water of the analyzed lakes: *a* – surface water, *b* – bottom water, *c* – overlying water, *d* – interstitial water (0–5), *e* – interstitial water (5–10); *1* – Track Lake, *2* – Podkówka Lake, *3* – Tyrsko Lake, *4* – Sukiel Lake, *5* – Redykajny Lake

global cycling. Climate change, hydrology and geochemical processes are different in different parts of the world, hence the carbon cycle will differ in particular regions.

The dominant form of carbon in studied urban lakes is organic carbon, whose share in the surface water was from 29.3 to 58.4% of the total pool of carbon, and the near-bottom layer was characterized by values ranging from 28.2 to 66%, and in the layer of overlying water contained from 34.2 to 63.6% of the total amount of carbon. The lowest values of concentration were recorded in polymictic Track Lake, which was due to the ongoing process of intensive mineralization of organic carbon. The highest values of concentration were observed in Podkówka Lake. The high percentage of TOC in the waters of this lake results probably from the nature of the catchment, dominated by forests. In spring, rainfall imports hardly decomposable organic matter to the lakes. In the interstitial water, the percentage of organic carbon in the total pool was from 40.1 to 94% within the 0–5 cm layer of sediment and from 56.4 to 88.1% in the layer of sediment at the depth of 5–10 cm. Such a significant share

of organic carbon in interstitial water can be associated with the sedimentation of dead phytoplankton (CARMOUZE et al. 1998).

Sediment can play a very important role in the eutrophication process by retaining nutrients, including carbon, and undergoing possible evolution. Until now, many authors have drawn attention to the release of nitrogen and phosphorus from sediments, but too little interest has been paid to the internal loading in carbon, whose availability may significantly affect the ecological status of water bodies.

The current study has demonstrated seasonal changes in the forms of carbon in different parts of the analyzed lake ecosystems. The largest seasonal variation was observed in interstitial water, both in the 0–5 cm and 5–10 cm layer. This can be attributed to sedimentation of dying phytoplankton and organic matter decomposition in sediment, as demonstrated by numerous authors (YACOBI, OSTROVSKY 2012).

The abundance of the particular carbon forms was rather stable ($F_{(4,13)}$ = = 9.72; $p < 0{,}0001$). The increase in TOC and DOC in spring was most likely related to the inflow of organic matter along with the spring thaw washed out from soils and forest litter. In summer, the increase in TOC and DOC corresponds to the primary production in a lake basin, which is stimulated by more insolation, higher temperatures as well as an ample supply of nutrients (unpublished data), which all contribute to the enhanced primary production (COLE et al. 2000, GIORGIO del, PETERS 1993).

Reducing the impact of bottom sediment on water is of great importance, especially in urban lakes, which used to receive polluted discharge for many years but now are often used for recreational purposes. An additional supply of nutrients from the bottom sediment, which in some cases may exceed the supply of nutrients from allochthonous sources, can lead to an avalanche of degradation events in a lake and prohibit its use by people.

Carbon cycling and the mixing type

Interstitial water and the overlying are a rich reservoir of organic carbon in the analyzed lakes, so that under favorable conditions during periods of circulation they can supply the water column with organic carbon, thus stimulating metabolic processes in a lake. Carbon trapped in the solid phase of sediments is first released to the interstitial water and then migrates to the overlying water, depending on the concentration gradient. It can be assumed that the actual dynamics of organic carbon in sediment of polymictic lakes (e.g. Track, Podkówka) is higher due to the frequent mixing of water masses and accelerated mineralization, organic carbon is released to the atmosphere as

CO_2. Mineralization of organic matter also promotes the release of phosphorus into the water column and thus contributes to the growth of lake trophy. Regarding the lakes discussed herein, the highest trophic index based on the content of phosphorus was achieved by the two polymictic lakes: Track and Podkówka (TSI (TP) = 80 and 92.6, respectively, Table 1). In the dimictic lakes, the bottom sediment can play a positive role, as they accumulate organic matter and pollutants inflowing from the catchment. This way, sediment may reduce the adverse impact of the catchment by removing a pool of pollutants deposits. This assumption was confirmed by our results, according to which a deep, dimictic lake is characterized by lower rates of the trophy based on a phosphorus content – TSI (TP). In polymictic lakes, organic carbon trapped in bottom sediment, via mineralization, supplies the water column with CO_2 and contributes to the degradation of the lake.

Based on the TOC transfer through all the elements of a lake ecosystem, it can be concluded to depend on the mictic type of a lake. Polymictic lakes (Track, Podkowka) (Figure 4) are characterized by the highest TOC content in the interstitial water within the 5–10 cm layer of sediments. However, in dimictic lakes (Tyrsko, Redykajny, Sukiel) – Figure 5, the interstitial water of shallower sediment (0–5 cm) was the richest in TOC.

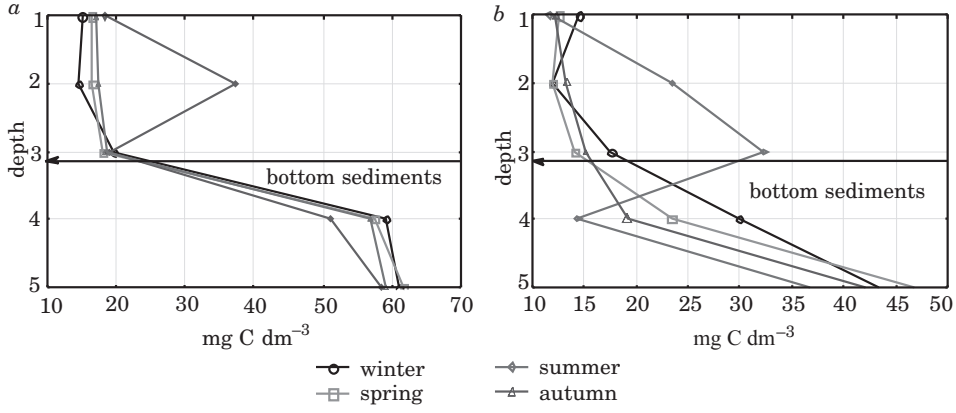

Fig. 4. TOC distribution in particular lake ecosystem elements in polymictic lakes: *a* – Lake Track, *b* – Lake Podkówka; depth: *1* – surface water, *2* – bottom water, *3* – overlying water, *4* – interstitial water (0–5), 5– interstitial water (5–10)

The shallower, polymictic lakes with higher water temperature in the bottom layer, and additionally frequent mixing water masses accelerate the decomposition of organic matter, then large amounts of sediment-trapped pollutants can be liberated, thus stimulating metabolic processes and deteriorating the ecological status of the water body. In dimictic lakes, an increase

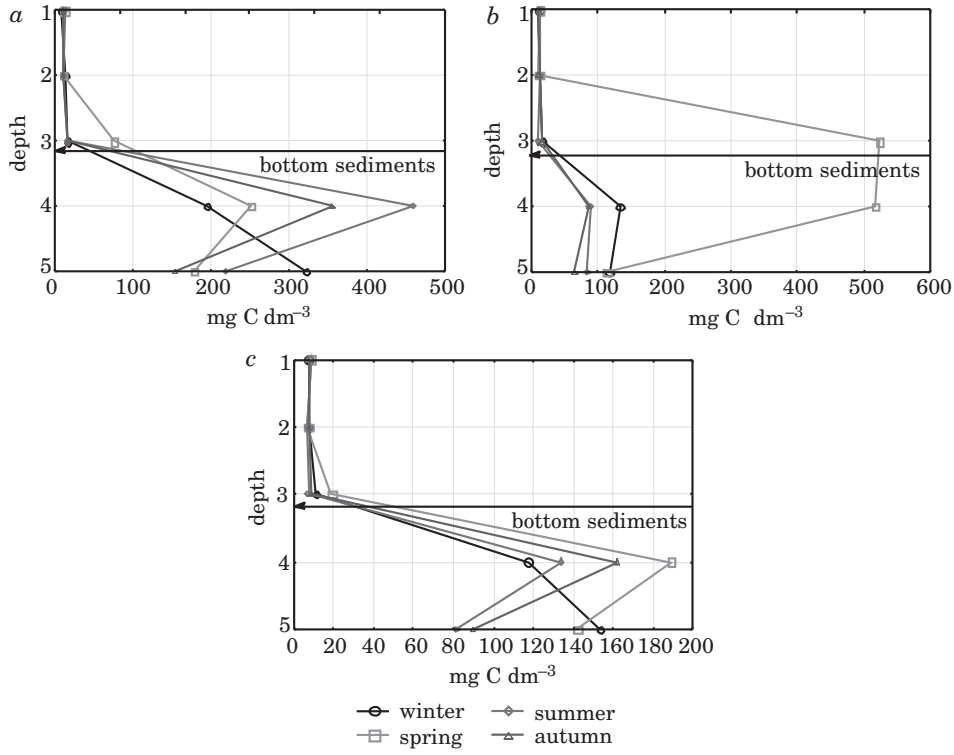

Fig. 5. TOC distribution in particular lake ecosystem elements in dimictic lakes: *a* – Lake Sukiel, *b* – Lake Redykajny, *c* – Lake Tyrsko; depth: *1* – surface water, *2* – bottom water, *3* – overlying water, *4* – interstitial water (0–5), *5* – interstitial water (5–10)

in the carbon content will be different than in polymictic lakes. Organic carbon is accumulated in sediment and therefore sedimentation will limit its impact on the processes of degradation of lakes (Xu et al. 2012). In polymictic lakes studied, the amount of organic carbon in sediment was 3- to 4-fold higher than in water, but the analogous difference in dimictic lakes can be up to 45-fold. The analytical results confirm that a dimictic lake may accumulate more carbon in sediment than polymictic one.

The buffering properties of water are closely related to its alkalinity, which is affected by bicarbonates, carbonates and hydroxides. These compounds enable the maintenance of the pH of water by neutralizing pollutants. The percentage of inorganic carbon ranged from 2.94% to 71.79% of the total amount of carbon. The results also indicate a greater share of this form of carbon in the interstitial water of polymictic lakes, probably due to more intensive organic matter mineralization in such lakes.

The content of IC in water varies with depth, which is a consequence of an increased CO_2 solubility in cooler water. In the hard-water eutrophic lakes,

CO_2 becomes especially depleted in the epilimnion due to photosynthesis and precipitation of calcite. An increase in IC found in the hypolimnion corresponds mainly to the dissolution of calcite and decomposition of organic matter (Xu et al. 2012). This assumption is supported by the results showing that the content of IC increased with the depth, reaching the highest value in the lower layer of interstitial water. In the analyzed lakes, the highest IC values were recorded in winter, resulting from the prevalent meteorological conditions during that season and IC content was less varied than the TOC content.

Among all the lakes, the highest IC content was found in Track Lake. This was reflected by the trophic index based on chlorophyll *a*. The inorganic form of carbon alongside the high availability of phosphorus in water (a high trophic index based on total phosphorus) promote excessive growth of phytoplankton, adding to the degradation processes of the lake. However, the smallest amount of IC throughout the whole research was determined in Tyrsko Lake. A small amount of IC co-occurring with a high amount of available phosphorus (TSI (TP) = 65.23, Table 1) may indicate limitation of the primary production by IC. However, there is a risk that if larger quantities of IC are delivered from the basin or atmosphere, Tyrsko Lake can undergo rapid eutrophication.

Conclusions

The carbon cycle in lake water depends not only on the influx of this element from the catchment, but also on its release from the bottom sediment, which can be a large reservoir of organic carbon in lakes. Bottom sediment have a dual role. On the one hand, they may lock nutrients from a water body; on the other hand, they can release large amounts of nutrients into water, contributing to the degradation of a lake. The release of carbon into water will depend on the mictic status of a lake. In a polymictic lake, the highest content of organic carbon was in the surface (0–5 cm) layer of bottom sediment, while in dimictic lakes the 5–10 cm layer of sediment contained most organic carbon. Polymictic lakes are shallower and mineralization processes in such lakes are accelerated by more frequent mixing of water masses than in dimictic lakes, so that more organic carbon is released from sediment of polymictic lakes.

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EFFECT OF RAPESEED SIZE AND STORAGE CONDITIONS ON THE CONTENT OF PHOSPHORUS AND PHOSPHOLIPIDS IN SEEDS AND OILS

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K e y w o r d s: rapeseed, oil, seed fractions, storage temperature, phosphorus, phospholipids.

A b s t r a c t

This paper analysed the impact of rapeseed size and storage conditions on content of phosphorus and phospholipids in seeds and oils. The study was carried out with variably-sized seed fractions of „Bios" rapeseed cultivar and the extracted oils. The seed and oil samples were tested for phosphorus content and the phospholipid profiles. Seed samples were stored for one year at ambient temperature (20 ± 4°C) and at refrigerating temperature (7 ± 1°C). The fine seed fraction (2.0–1.6 mm) and extracted oils from this sample had the highest total phosphorus content, phospholipid proportion and total share of their non-hydratable form, such as phosphatidic acid, and phosphatidylethanolamine. The assumed method of storing seeds at ambient temperature and refrigerating temperature practically did not affect the phosphorus content or the phospholipid proportions in the majority of the seed and oil samples. On the other hand, the temperature of storage had different impact of the proportion of individual phospholipids in phospholipids profile of seeds and oils.

WPŁYW WIELKOŚCI NASION RZEPAKU I WARUNKÓW PRZECHOWYWANIA NA ZAWARTOŚĆ FOSFORU I FOSFOLIPIDÓW W NASIONACH I OLEJACH

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S ł o w a k l u c z o w e: rzepak, olej, frakcje nasienne, temperatura przechowywania, zawartość fosforu i fosfolipidów.

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Abstrakt

Celem pracy było określenie wpływu wymiarów nasion rzepaku oraz warunków ich przechowywania na zawartość fosforu i fosfolipidów w nasionach i olejach. Materiałem badań były różnowymiarowe frakcje nasienne odmiany rzepaku „Bios", jak również wyekstrahowane z nich oleje. W próbkach nasion i olejach oznaczono zawartość fosforu oraz profil fosfolipidowy. Po przeprowadzeniu analizy wstępnej frakcji nasiennej próbki nasion przechowywano przez 1 rok w temperaturze otoczenia (20 ± 4°C) oraz w temperaturze chłodniczej (7 ± 1°C). Stwierdzono, że udział fosfolipidów w lipidach nasion i olejów oraz zawartość fosforu były istotnie zależne od wymiarów nasion. Najwyższą zawartością fosforu, udziałem ogólnym fosfolipidów i sumarycznym udziałem fosfolipidów niehydratowalnych, tj. kwasu fosfatydowego i fosfatydyloeatnoloaminy, cechowała się frakcja nasion drobnych (2,0–1,6 mm) oraz olej z nich wydobyty. Wykazano ponadto, że przyjęty w badaniach sposób przechowywania nasion w temperaturze otoczenia i chłodniczej nie miał statystycznie istotnego wpływu na zawartość fosforu i udział fosfolipidów w lipidach większości próbek nasion i olejów. Z drugiej jednak strony, wykazano, że temperatura przechowywania nasion miała zróżnicowany wpływ na zmiany profilu fosfolipidowego nasion i olejów.

Introduction

High phospholipid content is a major problem associated with oils produced with the pressing-extracting method used in the seed processing industry. Crude oils, especially extraction oil, have particularly high phospholipid content and are hydrated in an extraction facility. Hydrated extraction oil, combined with pressed oil, with a total amount of phosphorus < 200 mg kg^{-1} (Polish Standard – *Tłuszcze roślinne*... PN-87/A-86906), is subjected to acid degumming, which reduces the content of phosphorus to ≤ 10 mg kg^{-1} and, under optimal conditions, to less than 5 mg kg^{-1} of oil (YANG et al. 2006, DIJKSTRA 2013). Higher phosphorus content hinders the correct course of further refining processes and/or oil modification (UNGER 1990, SUBRAMANIAN and NAKAJIMA 1997, VAN GERPEN 2005, DIJKSTRA 2013).

From a technological point of view, phospholipids are divided into hydratable and non-hydratable compounds. Phospholipids such as phosphatidylcholine (PC), phosphatidylinositol (PI) and lyso-phospholipids (lysoPL) are easily hydratable, phosphatidylethanolamine (PE) is partially hydratable, whereas phosphatidic acid (PA) is classified as non-hydratable (SUBRAMANIAN et al. 1999, ZUFAROV et al. 2009).

During initial rapeseed processing and pressing, due to physical, thermal or enzymatic degradation of the membranes, phospholipids are released and freely diffuse to the extracted oil (PRIOR et al. 1991). Phospholipids in oils involve several issues. Since these compounds have a hydrophobic-hydrophilic structure, they are capable of binding with water found in oil and forming colloids which are deposited as sediments. Moreover, they deteriorate oil taste, smell and colour (SIMPSON 1991, SUBRAMANIAN et al. 1999, HAFIDI et al. 2005, KORIS and MARKI 2006, YANG et al. 2006, PŁATEK 2009).

During oil refining, phospholipids are removed by degumming. Ineffective degumming causes increased losses, reduces mechanical efficiency and inactivates hydration catalysts (SUBRAMANIAN and NAKAJIMA 1997, SZYDŁOWSKA-CZERNIAK 2007, SINGH and VIRENDRAKUMAR 2008, AMBROSEWICZ et al. 2012).

In biodiesel production, phospholipids in oil contribute towards catalyst inactivation (e.g. KOH), among others, and strongly emulsify the reactive system – reducing processing efficiency and hindering the separation of ester-phase from glycerine-phase. According to WALISIEWICZ-NIEDBALSKA (2004), transesterification rapeseed oil should have phosphorus content of less than 50 mg kg^{-1}, while American Society for Testing and Materials (*Standard specification*... ASTM D6751) and British Standard (*Liguid petroleum*... BS EN 14214) have stipulated the maximum total phosphorus content in biodiesel products to be 10 mg kg^{-1}. VAN GERPEN (2005) reported that the content of phosphorus in oil > 50 mg kg^{-1} causes a reduction in biodiesel efficiency by 3–5%. Moreover, SIDIBÉ et al. (2010) and HALDAR et al. (2009) observed that high phosphorus concentrations in the oil cause clogging in diesel engines.

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 phospholipid content in oils (SOSULSKI et al. 1981, PRZYBYLSKI and ESKIN 1991, CARELLI et al. 1997, BREVEDAN et al. 2000, CARELLI et al. 2002, SHAHIDI 2001, TAŃSKA and ROTKIEWICZ 2003, CHEN et al. 2013, TAŃSKA et al. 2013a,b, GHAZANI et al. 2014, AMBROSEWICZ-WALACIK et al. 2015). Few publications have attempted to explain the impact of other factors, such as seed dimensions (ROTKIEWICZ et al. 2002, TAŃSKA and ROTKIEWICZ 2003) or storage conditions (SOSADA 1996, TAŃSKA and ROTKIEWICZ 2003), on the phospholipid share in seed and oil lipids. However, so far there are no studies on the effect of seed size on the profile of phospholipids in seeds and extracted oils. Accordingly, the objective of this study was to evaluate the impact of rapeseed sizes and storage conditions on content of phosphorus, as well as and profile of phospholipids in seeds and oils. This knowledge may be useful in prediction costs of edible oil or biodiesel production, because amount and type of phospholipids determine degumming parameters, especially amount chemicals.

Materials and Methods

The study was carried out with variably-sized seed fractions of the „Bios" spring rapeseed cultivar as well as oils extracted from the seeds. The seed fractions were produced with a sieve separation method using sieves with squared holes (sides of 2.2 mm, 2.0 mm and 1.6 mm). Three seed fractions were

separated: a large seed fraction > 2.2 mm (F_1) (26.26% share of seeds weight), a medium seed fraction 2.2–2.0 mm (F_2) (67.38% share of seeds weight) and a fine seed fraction 2.0–1.6 mm (F_3) (6.36% share of seeds weight). Next, the seed samples were analysed. The rapeseeds were stored for one year at ambient temperature (20 ± 4°C) and refrigerating temperature (7 ± 1°C). Samples of equal weight (10 kg) were stored in containers in a dimmed room (variant I) and in a refrigerator (variant II). The seed samples were collected for the analyses after 3, 6, 9 and 12 months of the storage.

The seed samples were characterized by determining: seed moisture (Polish Standard *Oznaczanie zawartości...* PN-EN ISO 662:2001P), fat content (Polish Standard *Nasiona oleiste...* PN-EN ISO 659:2010P), mass of 1000 seeds (by weight), equivalent diameter (TAŃSKA et al. 2005), phosphorus content (Polish standard *Tłuszcze roślinne...* PN-ISO 10540-1:2005P), phospholipid proportions (OHM and CHUNG 1999) and phospholipids profile (AMBROSEWICZ-WALACIK et al. 2015).

Determination of equivalent diameter. The equivalent diameter measurements for the rapeseed samples were carried out with a set for digital image analysis composed of a Nikon DXM 1200 digital camera, a Kaiser RB 5004 HF – High Frequency Daylight Copy Light set with 4 x 36 W fluorescent light tubes (colour temperature about 5400 K), a computer with an image acquisition card for a DXM 1200 Digital Camera, LUCIA G ver. 4.80 software, a screen and a printer. Three hundred seeds without visible mechanical damage were randomly selected for measurements from each sample. The individual seeds were placed onto a measuring plate at a distance of 13 cm from the objective-lens of the camera. The equivalent diameter was determined automatically in LUCIA G software.

Determination of proportion of phospholipids. The share of phospholipids in lipid fraction of seeds was determined with column chromatography using MEGA BOND SI 1GM 6ML columns. The lipid fraction was extracted with Folch's method (FOLCH 1957), assuming that this method extracts all seed lipids (SOSULSKI et al. 1981, SHAHIDI 2001).

Determination of phosphorus content. Samples of milled rapeseed were digested as follows: MgO and samples (in the range 0.1000–10.000 g) were weighed in quartz crucible, placed in a furnace and ashed at 800–900°C. Then HNO3 solution (6 mol L^{-1}), ammonium molybdate solution (4.05 x 10^{-2} mol L^{-1}) and ammonium metavanadate solution were added to ashed samples, and the absorbance was measured at 460 nm against a reagent blank 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.

The oils used in the experiment were hot-extracted. First, the samples of rapeseed were comminuted in a laboratory mill (WZ-1 type) and then heated under conditions simulating industrial roasting. To this end, milled seed samples were tightly packed in aluminium foil and placed in a drier (KC-65M type) at 130°C for 45 minutes. Fat was extracted from the samples with petroleum ether with the Soxhlet's method. The extract was distilled on a vacuum evaporator (VIPAR, 350 type). The extracted oils were tested for content of total phosphorus and non-hydratable phosphorus (Polish standard *Tłuszcze roślinne...* PN-ISO 10540-1:2005P), phospholipid proportions (OHM and CHUNG 1999) and phospholipids profile (AMBROSEWICZ-WALACIK et al. 2015).

Determination of non-hydratable phosphorus. The preparation of oils for hydration included heating up to 80°C and then adding re-distilled water at 0.5% in relation to the weighed oil portion. During hydration (15 minutes), the oils were continuously mixed with an electromagnetic mixer (rotating velocity = 250 rpm) and a constant temperature of the sample was maintained (80°C). Hydratable phospholipids were removed from oil with centrifugation (time = 10 minutes, rotating velocity = 10,000 rpm). In such oil, the non-hydratable phosphorus content was determined using the ammonium vanadium method according to the Polish Standard *Tłuszcze roślinne...* PN-ISO 10540-1:2005P.

Statistical analysis

Obtained results of researches were presented as the arithmetic mean ± standard deviation (from three replicates) and statistically analysed using the Statistica 9.0 PL (StatSoft Poland) program. In order to indicate significance of differences between seeds and oils analysis of variance (ANOVA) with Tukey's test of $p \leq 0.05$ significance level was used.

Results and Discussion

Relation between the seed dimensions, phosphorus contents and phospholipid proportions

Seed fraction samples F_1, F_2 and F_3 were significantly diversified in their total phosphorus content (Table 1). Fine seeds (fraction F_3) with low fat content had the highest concentration of phosphorus (6123 mg kg^{-1} DM). Together with an increase in equivalent diameter, the phosphorus content

decreased in the following arrangement: $F_3 > F_2 > F_1$. In comparison with the finest seeds (fraction F_3), larger seeds in the samples F_1 and F_2 had a lower (by 15.1% and 10.9%, respectively) content of this compound. One-way variance analysis showed that the seed dimensions had a significant impact on the total phosphorus content in the examined seed fractions (Table 1).

Table 1
The phosphorus content and phospholipids proportions in the seeds with diverse size and in oils

Discriminants		Seed fractions		
		F_1	F_2	F_3
Phosphorus content				
total in seeds [mg kg^{-1} DM]		5199^{a}* ± 13.1	5456^{b} ± 15.0	6123^{c} ± 9.3
total in oil [mg kg^{-1}]		351.7^{a} ± 6.3	360.4^{b} ± 3.6	387.8^{c} ± 2.3
nonhydratable in oil [mg kg^{-1}]		103.6^{a} ± 0.5	111.3^{b} ± 1.6	127.5^{c} ± 0.2
proportion of nonhydratable [%]		29.46^{a} ± 0.38	30.88^{b} ± 0.14	32.88^{c} ± 0.14
Phospholipid proportion	in seed lipids [%]	2.78^{a} ± 0.02	3.09^{b} ± 0.02	3.54^{c} ± 0.08
	in oil [%]	1.11^{a} ± 0.00	1.13^{b} ± 0.01	1.24^{c} ± 0.04

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

The total phosphorus content in the oils that were hot-extracted from the individual seed fractions was significantly varied and ranged from 351.7 mg kg^{-1} for fraction F_1 to 378.8 mg kg^{-1} for fraction F_3. It was found that the oils extracted from fine seeds with a lower fat content and higher total phosphorus content had a higher concentration of phosphorus. The oils extracted from larger seeds (fractions F_1 and F_2) had a lower content of this compound. The non-hydratable phosphorus content in the oil extracted from variably-sized seeds was significantly diversified. The content of this compound in the tested samples was arranged in the following order: $F_3 > F_2 > F_1$. The oil extracted from fine seeds (fraction F_3) which had the highest content of total phosphorus also had the highest concentration of non-hydratable phosphorus. The oil extracted from the largest seeds (fraction F_1) had the lowest content of phosphorus (by 18.7%) than the oil extracted from seed fraction F_3 (127.5 mg kg^{-1}). Based on a one-way analysis of variance, it was shown that the seed dimensions exerted an impact on the non-hydratable phosphorus content (Table 1).

The non-hydratable phosphorus proportion in total phosphorus in the tested samples was markedly varied and ranged from 29.46% (the oil from the fraction F_1) to 32.88% (the oil from the fraction F_3). Sample F_2 had an intermediate content of this compound, amounting to 30.88% (Table 1).

The recorded results are consistent with the findings reported by ROTKIEWICZ et al. (2002) and with our previous studies (TAŃSKA et al. 2009) in which it was found that the cultivars with fine seeds had a higher content of phosphorus.

Non-polar lipids were predominant in the lipid composition of variably-sized seeds, ranging from 95.0% (for the fraction F_3) to 95.9% (for the fraction F_1). The group of these compounds was also predominant in the lipid composition of oils. Phospholipids predominated among the polar lipids of seeds and oils, with the highest content detected in the finest seed fraction and the oil extracted from this fraction (3.54 and 1.24%, respectively). The lowest proportion of these compounds was detected in the largest seeds (2.78%) and in the oil extracted from these seeds (1.11%) (Table 1). These results, indicating a higher proportion of phospholipids in lipids in finer seeds, are consistent with the findings reported by ROTKIEWICZ et al. (2002).

Generally, phospholipids of oilseeds are located in spherosomes consisting of with triacylglycerols surrounded by a membrane of phospholipids forming complexes with oleosin and occurring in small amount caleosins (BEISSON et al. 2001, FRANDSEN et al. 2001, JOLIVET et al. 2004, KATAVIC et al. 2006, CHAPMAN et al. 2012). MURPHY et al. (1989) and TZEN et al. (1993) indicated that a varied proportion of phospholipids in seed lipids is associated with the size of spherosomes, because – as these authors explain – in fine seeds, the spherosomes are smaller and have a higher phospholipid-protein-surface-to-triglycerides ratio that fills their interior, which results in a higher content of phospholipids. Spherosomes of rapeseeds are smaller than other oilseeds. The diameter of organelles in the cells of mature rapeseeds is in the range of 0.5–3 μm (MURPHY et al. 1989, TZEN and HUANG 1992, TZEN et al. 1993, KÜHNEL et al. 1996, FRANDSEN i in. 2001, KATAVIC et al. 2006). Size of the sferosomes is dependent on the nutritional status of the plants and environmental factors (KÜHNEL et al. 1996, FRANDSEN et al. 2001). Moreover, TING et al. (1996) reported that shperosomes' size is conditioned by the content of fat and oleosin synthesized during maturation of maize seed, and more specifically the ratio between them. The embryos of maize seeds varieties with high fat content (48%), that were characterized by approx. 6-fold higher proportion of fat to oleosin, have bigger spherosomes (diameter approx. 2.16 μm) than embryos with low fat (8%) (diameter up to 0.79 μm).

Impact of seed fraction storage on phosphorus content, phospholipid proportion in seeds and oils

The seed fractions F_1, F_2 and F_3 stored both at ambient temperature and at refrigerating temperature and the extracted oils were not markedly varied in the content of total phosphorus (Tables 2, 3). The non-hydratable phosphorus contents and proportions in the oils were not significantly different after storage for 12 months (Table 3).

Table 2
Total content of phosphorus in F_1, F_2 and F_3 seed fractions storage in different temperatures [mg kg^{-1} DM]

Storage period [month]	Seed fractions		
	F_1	F_2	F_3
seeds stored 12 months in 20°C			
0	5199^a* ± 13.1	5456^a ± 15.0	6123^a ± 9.3
3	5203^a ± 23.2	5454^a ± 9.7	6122^a ± 9.3
6	5200^a ± 23.9	5454^a ± 11.5	6119^a ± 12.3
9	5196^a ± 20.5	5452^a ± 6.8	6121^a ± 4.5
12	5197^a ± 17.7	5450^a ± 12.4	6117^a ± 9.7
seeds stored 12 months in 7°C			
0	5199^a ± 13.1	5456^a ± 15.0	6123^a ± 9.3
3	5198^a ± 6.2	5455^a ± 9.8	6122^a ± 9.9
6	5195^a ± 14.1	5453^a ± 19.3	6120^a ± 9.0
9	5198^a ± 18.5	5454^a ± 14.1	6122^a ± 14.6
12	5193^a ± 17.3	5451^a ± 10.6	6121^a ± 10.0

*$^{a, b}$ ... – mean values in column marked with the same letter are not significantly different ($p \leq 0.05$) for values indicated in 3, 6, 9 and 12 month of storage variant

The recorded results are also consistent with the findings presented by ROTKIEWICZ et al. (2002). In addition, TAŃSKA et al. (2009), while investigating the technological value of selected national rapeseed cultivars, found that fine seeds and extracted oils had higher phosphorus contents.

The storage conditions had a minor impact on the changes in phospholipid proportions in seeds and extracted oils. It was found that at ambient temperature the proportion of phospholipids in seed lipids and oils of F_1 and F_2 fractions significantly decreased by 0.04 p.p. after 12 months of storage (Table 4). A similar tendency was shown for those samples stored at 7°C. No significant variations in the phospholipid proportions were detected for fraction F_3 stored at ambient temperature or the oils extracted from these seeds and the samples stored at refrigerating temperature.

These results differ from TAŃSKA and ROTKIEWICZ (2003) who found that the phospholipid proportion in seed fractions stored for one year at 4–16°C was significantly lower. A possible reason for the phospholipid reduction in seed lipids may be phospholipid peroxidation, which caused a decrease in their content (MAWATARI and MURAKAMI 1998).

The profile of phospholipids in seed lipids of F_1, F_2 and F_3 fractions consisted of PC, PI, PE and PA. The proportion of these compounds in seed lipids significantly changed during the 12-month storage period (Table 5). The share of PC in seed lipids of all fractions, that were stored at ambient and refrigerating temperature significantly increased, but more in seed lipids

Table 3

Total content and nonhydratable phosphorus [mg kg^{-1}] in oils extracted from seeds storage in different temperatures

Storage period (month)	Phosphorus content [mg kg^{-1}]								
	F_1			F_2			F_3		
	total	nonhydratable	proportion of nonhydratable [%]	total	nonhydratable	proportion of nonhydratable [%]	total	nonhydratable	proportion of nonhydratable [%]
seeds stored 12 months in 20°C									
0	351.7^{a*} ±6.3	103.6^{a} ±0.5	29.46^{a} ±0.38	360.4^{a} ±3.6	111.3^{a} ±1.6	30.88^{a} ±0.14	387.8^{a} ±2.3	127.5^{a} ±0.2	32.88^{a} ±0.14
3	350.2^{a} ±5.6	103.1^{a} ±2.0	29.45^{a} ±0.10	359.7^{a} ±2.1	111.1^{a} ±0.6	30.89^{a} ±0.02	387.4^{a} ±2.8	127.4^{a} ±0.7	32.90^{a} ±0.04
6	349.2a±5.6	103.4a±1.4	29.62a±0.08	358.2a±2.8	110.6a±0.8	30.89a±0.01	386.1a±1.6	127.1a±0.5	32.93a±0.01
9	350.1^{a} ±4.4	104.0^{a} ±1.5	29.72^{a} ±0.06	356.7^{a} ±4.6	110.3^{a} ±1.4	30.93^{ab} ±0.01	387.2^{a} ±1.1	127.5^{a} ±0.2	32.93^{a} ±0.03
12	349.8^{a} ±6.4	105.5^{a} ±1.9	30.15^{b} ±0.01	356.9^{a} ±1.6	110.9^{a} ±0.4	31.06^{b} ±0.13	385.6^{a} ±1.6	127.1^{a} ±0.5	32.95^{a} ±0.01
seeds stored 12 months in 7°C									
0	351.7^{a} ±6.3	103.6^{a} ±0.5	29.46^{a} ±0.38	360.4^{a} ±3.6	111.3^{a} ±1.6	30.88^{a} ±0.14	387.8^{a} ±2.3	127.5^{a} ±0.2	32.88^{a} ±0.14
3	350.6^{a} ±5.2	103.3^{a} ±1.7	29.47^{a} ±0.06	359.5^{a} ±3.8	111.0^{a} ±1.1	30.89^{a} ±0.04	387.8^{a} ±1.5	127.5^{a} ±0.4	32.89^{a} ±0.02
6	350.0^{a} ±5.6	103.2^{a} ±1.5	29.48^{a} ±0.04	360.8^{a} ±5.8	111.5^{a} ±1.9	30.91^{a} ±0.01	386.7^{a} ±1.5	127.2^{a} ±0.6	32.89^{a} ±0.01
9	351.1^{a} ±7.3	103.5^{a} ±1.7	29.48^{a} ±0.13	357.7^{a} ±3.9	110.5^{a} ±1.0	30.88^{a} ±0.06	387.1^{a} ±1.3	127.4^{a} ±0.4	32.92^{a} ±0.01
12	350.4^{a} ±6.4	103.2^{a} ±0.4	29.47^{a} ±0.43	358.2^{a} ±2.6	110.6^{a} ±0.7	30.89^{a} ±0.02	386.6^{a} ±2.7	127.3^{a} ±0.9	32.93^{a} ±0.01

*$^{a, b}$ ... – mean values in column marked with the same letter are not significantly different ($p \leq 0.05$) for values indicated in 3, 6, 9 and 12 month of storage variant

Table 4
Phospholipids proportion in seed lipids and oil of F_1, F_2 and F_3 fractions storage in different temperatures [%]

Storage period [month]	Phospholipids proportion in seed lipids [%]			Phospholipids proportion in oil [%]		
	F_1	F_2	F_3	F_1	F_2	F_3
seeds stored 12 months at 20°C						
0	$2.78^{b} \pm 0.02$	$3.09^{b} \pm 0.02$	$3.54^{a} \pm 0.08$	$1.11^{b} \pm 0.00$	$1.13^{b} \pm 0.01$	$1.24^{a} \pm 0.04$
3	$2.75^{ab} \pm 0.02$	$3.09^{b} \pm 0.03$	$3.54^{a} \pm 0.04$	$1.11^{b} \pm 0.04$	$1.11^{ab} \pm 0.00$	$1.23^{a} \pm 0.03$
6	$2.77^{b} \pm 0.01$	$3.07^{ab} \pm 0.00$	$3.51^{a} \pm 0.00$	$1.10^{ab} \pm 0.00$	$1.11^{ab} \pm 0.04$	$1.23^{a} \pm 0.02$
9	$2.74^{a} \pm 0.02$	$3.06^{ab} \pm 0.00$	$3.51^{a} \pm 0.04$	$1.09^{ab} \pm 0.01$	$1.09^{a} \pm 0.03$	$1.22^{a} \pm 0.01$
12	$2.74^{a} \pm 0.02$	$3.05^{a} \pm 0.02$	$3.50^{a} \pm 0.01$	$1.08^{a} \pm 0.00$	$1.09^{a} \pm 0.04$	$1.20^{a} \pm 0.01$
seeds stored 12 months at 7°C						
0	$2.78^{b} \pm 0.02$	$3.09^{b} \pm 0.02$	$3.54^{a} \pm 0.08$	$1.11^{b} \pm 0.00$	$1.13^{b} \pm 0.01$	$1.24^{a} \pm 0.04$
3	$2.77^{a} \pm 0.01$	$3.08^{a} \pm 0.02$	$3.54^{a} \pm 0.01$	$1.11^{a} \pm 0.04$	$1.12^{a} \pm 0.03$	$1.23^{a} \pm 0.03$
6	$2.77^{a} \pm 0.02$	$3.08^{a} \pm 0.02$	$3.53^{a} \pm 0.01$	$1.10^{a} \pm 0.00$	$1.13^{a} \pm 0.00$	$1.24^{a} \pm 0.01$
9	$2.76^{a} \pm 0.02$	$3.08^{a} \pm 0.01$	$3.54^{a} \pm 0.05$	$1.10^{a} \pm 0.03$	$1.13^{a} \pm 0.02$	$1.22^{a} \pm 0.01$
12	$2.76^{a} \pm 0.01$	$3.07^{a} \pm 0.00$	$3.52^{a} \pm 0.00$	$1.10^{a} \pm 0.01$	$1.11^{a} \pm 0.01$	$1.23^{a} \pm 0.02$

*$^{a, b}$ ... – mean values in column marked with the same letter are not significantly different ($p \leq 0.05$) for values indicated in 3, 6, 9 and 12 month of storage variant

stored in 20°C (approx. 10–11 percentage points – pp.). On the other hand, share of the PI in seed lipids of fractions stored at two variants of temperature, were significantly lower. However, lower decrease was observed in seed lipids stored at refrigerating temperature. What is more, share of PE in seed lipids of F_1 and F_3 fractions stored at ambient temperature and for all samples stored at refrigerating temperature did not change significantly, while the seed lipids of F_2 fraction stored at 20°C were characterized by a significant, but slight decrease in the share of this phospholipid (< 2.4 pp.). The concentration of PA in seed lipids of F_1 and F_3 fractions stored at ambient temperature was at a similar level in subsequent periods of storage, while in sample F_2 significantly decreased by 1.31 pp. Moreover, seed fractions stored under refrigerating temperature were characterized by a small, but statistically significant decrease of PA (Table 5).

So far there are no data in the literature on the effect of storage temperature on changes in the profile of phospholipids in seeds of different dimensions. However, it was found that a factor inhibiting the conversion of phospholipids in seed samples stored at refrigerating temperature was low temperature, what, as reported MURTHY et al. (2003), could inhibit the degradation of the phospholipids. In turn, changes in seed lipids stored at ambient temperature can be related to the action of enzymes converting some of the phospholipids in another one (MCNEIL et al. 2001, RONTEIN et al. 2003). Demonstrated low share of the PA in the profile of phospholipids of stored seeds indicated that during one year of storage phospholipase D was not activated.

Table 5

Phospholipids profile in seed lipids of F_1, F_2 and F_3 fractions storage in different temperatures [%]

Storage period [month]	F_1				F_2				F_3			
	PC	PI	PE	PA	PC	PI	PE	PA	PC	PI	PE	PA
seeds stored 12 months at 20°C												
0	38.57^{a}* ±0.93	35.63^{c} ±0.37	22.52^{c} ±0.48	3.28^{a} ±0.08	38.47^{a} ±1.80	34.24^{c} ±1.35	23.94^{c} ±0.40	3.35^{b} ±0.05	40.09^{a} ±1.10	34.42^{c} ±0.93	22.26^{a} ±0.30	3.23^{a} ±0.12
3	43.15^{b} ±0.55	34.32^{c} ±0.71	$18.77^{ab/A}$ ±1.17	3.76^{ab} ±0.09	40.45^{ab} ±0.55	30.53^{b} ±1.42	24.63^{c} ±1.93	4.39^{c} ±0.04	40.34^{a} ±0.23	31.84^{b} ±1.61	23.98^{c} ±1.46	3.84^{b} ±0.08
6	42.68^{b} ±0.91	35.11^{c} ±1.09	$17.87^{a/A}$ ±1.41	4.34^{b} ±0.59	42.68^{b} ±0.91	30.17^{b} ±1.00	22.81^{ab} ±1.33	4.34^{c} ±0.59	41.86^{b} ±0.00	31.38^{b} ±0.71	22.90^{ab} ±0.69	3.86^{b} ±0.056
9	46.65^{c} ±1.49	28.92^{b} ±0.89	$20.00^{b/A}$ ±0.12	4.43^{b} ±0.72	46.84^{c} ±1.69	27.13^{a} ±1.07	23.19^{bc} ±0.42	2.84^{b} ±0.20	45.21^{c} ±0.08	27.83^{a} ±1.04	23.61^{bc} ±0.69	3.35^{a} ±0.26
12	49.41^{d} ±0.78	25.17^{a} ±1.35	$21.66^{c/A}$ ±0.42	3.76^{ab} ±0.15	48.76^{c} ±0.63	27.62^{a} ±0.91	21.58^{a} ±0.01	2.04^{a} ±0.27	48.56^{d} ±0.82	25.88^{a} ±0.52	22.45^{a} ±0.21	3.11^{a} ±0.09
seeds stored 12 months at 7°C												
0	38.57^{a} ±0.93	35.63^{b} ±0.37	22.52^{a} ±0.48	3.28^{d} ±0.08	38.47^{b} ±1.80	34.24^{b} ±1.35	23.94^{ab} ±0.40	3.35^{c} ±0.05	40.09^{a} ±1.10	34.42^{c} ±0.93	22.26^{a} ±0.30	3.23^{b} ±0.12
3	43.76^{bc} ±0.45	32.64^{a} ±0.59	21.02^{a} ±0.95	2.58^{ab} ±0.08	36.82^{a} ±0.39	34.95^{b} ±1.05	24.95^{b} ±0.62	3.28^{c} ±0.04	39.14^{a} ±0.86	34.11^{bc} ±1.11	23.62^{a} ±0.08	3.13^{b} ±0.13
6	42.70^{b} ±0.91	31.66^{a} ±0.86	22.82^{a} ±0.63	2.82^{bc} ±0.01	38.27^{b} ±0.91	33.51^{b} ±1.81	24.98^{b} ±1.36	3.24^{c} ±0.06	39.60^{a} ±0.91	34.49^{c} ±0.53	22.76^{a} ±1.83	3.15^{b} ±0.18
9	45.27^{c} ±1.32	31.41^{a} ±2.07	20.39^{a} ±0.66	2.93^{c} ±0.08	43.72^{c} ±2.06	30.82^{a} ±1.77	22.61^{a} ±0.47	2.85^{b} ±0.18	42.28^{b} ±0.06	32.05^{b} ±2.14	22.41^{a} ±1.86	3.26^{b} ±0.13
12	45.11^{c} ±0.65	30.81^{a} ±1.10	21.64^{a} ±0.75	2.44^{a} ±0.30	47.26^{d} ±1.49	28.28^{a} ±0.04	22.46^{a} ±1.32	2.00^{a} ±0.21	43.71^{b} ±0.69	29.48^{a} ±0.45	23.99^{a} ±0.18	2.82^{a} ±0.07

*$^{a, b}$ ... – mean values in column marked with the same letter are not significantly different ($p \leq 0.05$) for values indicated in 3, 6, 9 and 12 month of storage variant

Impact of seed fraction storage on phospholipid profile in oils

The profiles of phospholipids in oils extracted from seeds stored at two variants of temperature were characterized by PC, PI, PE and PA. Oils extracted from F_1 and F_2 seed fractions stored at ambient temperature were characterized by a lower share of PC (respectively 7.75, 7.05 pp.) – Table 6. One the other hand, oils obtained from F3 seed fraction and from all fractions stored at refrigerating temperature did not significantly change in turns of PC and PI share. Moreover, it was observed that share of PI in oils extracted from sample F_1 and F_2 stored at ambient temperature slightly decreased (0.98 and 2.86 pp., respectively). The concentration of PE in oils obtained from F_1, F_2 and F_3 seed fractions stored at ambient temperature and in sample of F_1 stored at 7°C was increased, while in oils extracted from samples F_2 and F_3 stored at refrigerating temperature share of this phospholipid was not significantly different. In turn, share of PA significantly increased in oils extracted from F_1 and F_2 seed fractions stored at ambient temperature (by 3.76 and 9.53 pp., respectively). On the other hand, PA concentration in oil obtained from F_3 sample and oils extracted from seeds stored in refrigerating temperature remained at the same level (Table 6).

Moreover, comparing the profile of phospholipids in seed lipids and oils, it was found that oils were characterized by a higher concentration of the PC, while share of PI was lower compared to profile of seeds, which was associated with a higher proportion of PA in these samples. This indicates on the activity of phospholipase D (TOSI et al. 1999) and non-enzymatic hydrolysis of these compounds (TOSI et al. 1999). It was also observed that the phospholipids profile of oils was characterized by approx. 4–5 times higher proportion of PA than the profile of seed lipids.

Minor changes in the contribution of different phospholipids in oils obtained from seeds stored at refrigerating temperatures are a derivative of the minor changes observed also in the seed lipids. As earlier mentioned low temperature of the storage could contribute to the inhibition of degradation of phospholipids, as was demonstrated by SOSADA (1996) and MURTHY et al. (2003).

LEE et al. (2011) analysed changes in phospholipid profile in soybean lipids (wild type and transgenic type) storage in 25°C and 17% relative humidity for 33 months, observed that total amount of phospholipids increased, respectively, from 34.37 to 34.77 nmol mg^{-1} and from 32.22 to 38.81 nmol mg^{-1}, while share of PC, PG, PI and PA in both samples increased and share of PE decreased. Moreover, cited authors observed that phospholipids of wild type soybean increased in unsaturation during storage, while in lipids of transgenic sample phospholipids did not change as much. It is believed that during the

Table 6

Phospholipids profile in oils of F_1, F_2 and F_3 fractions storage in different temperatures [%]

Storage period [month]	F_1				F_2				F_3			
	PC	PI	PE	PA	PC	PI	PE	PA	PC	PI	PE	PA
seeds stored 12 months at 20°C												
0	56.92^{b*} ±0.56	6.43^{c} ±0.16	20.87^{b} ±0.35	15.78 ±1.07	53.75^{b} ±0.16	7.45^{d} ±0.16	23.00^{b} ±0.62	15.80 ±0.62	48.05 ±0.35	10.08^{bc} ±0.29	22.10^{bc} ±0.20	19.77 ±0.13
3	57.23^{b} ±1.56	7.14^{d} ±0.37	18.45^{a} ±1.38	17.18^{a} ±0.55	53.31^{b} ±0.01	7.21^{c} ±0.11	22.91^{ab} ±0.83	16.57^{a} ±0.94	49.00^{b} ±0.95	10.82^{c} ±0.74	19.43^{a} ±0.16	20.75^{a} ±0.81
6	56.68^{b} ±0.49	4.36^{a} ±0.22	21.99^{b} ±1.05	16.97^{a} ±1.32	52.60^{b} ±0.59	3.93^{a} ±0.32	24.54^{b} ±0.28	18.93^{b} ±0.55	47.96^{a} ±0.35	8.09^{a} ±1.21	20.28^{b} ±0.71	21.67^{b} ±1.57
9	49.61^{a} ±0.69	5.30^{b} ±0.13	25.13^{c} ±0.27	19.96^{b} ±0.55	47.84^{a} ±0.43	4.78^{b} ±0.12	23.30^{ab} ±0.29	24.08^{c} ±0.84	47.76^{a} ±0.39	9.92^{bc} ±0.46	22.28^{c} ±0.23	20.04^{a} ±0.12
12	49.17^{a} ±0.36	5.45^{b} ±0.47	25.84^{c} ±1.00	19.54^{b} ±1.82	46.70^{a} ±0.71	4.59^{b} ±0.13	23.38^{ab} ±0.57	25.33^{c} ±0.28	46.85^{a} ±0.49	9.52^{b} ±0.37	23.47^{d} ±0.45	20.16^{a} ±0.33
seeds stored 12 months at 7°C												
0	56.92^{bc} ±0.56	6.43^{a} ±0.16	20.87^{a} ±0.35	15.78^{c} ±1.07	53.75^{a} ±0.16	7.45^{b} ±0.16	23.00^{a} ±0.62	15.80^{b} ±0.62	48.05^{b} ±0.35	10.08^{ab} ±0.29	22.10^{a} ±0.20	19.77^{a} ±0.13
3	57.85^{c} ±0.25	6.31^{a} ±0.11	20.33^{a} ±0.06	15.51^{bc} ±0.09	53.92^{a} ±0.40	7.39^{b} ±0.25	22.93^{a} ±0.86	15.76^{b} ±0.71	47.43^{ab} ±0.64	10.35^{b} ±0.64	22.66^{ab} ±1.24	19.56^{a} ±0.04
6	55.88^{ab} ±0.46	6.61^{c} ±0.22	22.09^{b} ±0.28	15.42^{a} ±0.40	54.18^{a} ±1.07	7.56^{b} ±1.07	23.66^{a} ±1.81	14.60^{a} ±0.36	46.90^{a} ±0.44	9.70^{ab} ±0.28	23.76^{b} ±0.78	19.64^{a} ±1.50
9	55.20^{a} ±0.32	6.87^{b} ±0.16	22.44^{b} ±0.14	15.49^{bc} ±0.02	54.17^{a} ±0.51	6.77^{a} ±0.22	23.30^{a} ±0.29	15.76^{b} ±0.00	47.76^{ab} ±0.04	9.92^{ab} ±0.05	22.28^{a} ±0.23	20.04^{a} ±0.23
12	56.06^{ab} ±0.91	6.95^{b} ±0.24	22.29^{b} ±0.93	14.70^{ab} ±0.22	53.70^{a} ±0.71	7.59^{b} ±0.13	23.38^{a} ±0.57	15.33^{ab} ±0.28	47.16^{ab} ±0.06	9.62^{a} ±0.23	23.21^{ab} ±0.09	20.01^{a} ±0.26

*$^{a, b}$ ... – mean values in column marked with the same letter are not significantly different ($p \leq 0.05$) for values indicated in 3, 6, 9 and 12 month of storage variant

natural ageing process the lipid degradation (including phospholipids) is caused by lipid degrading enzymes (DEVAIAH et al. 2007). Phospholipases can catalyse degradation of membrane phospholipids, including PC, PE and PG, generating significant amounts of PA and a free head group. KOOIJMAN et al. (2003) found that accumulation of PA in the cell may lead to the formation of hexagonal II phase lipid particles with loss of cell membrane integrity, while FAN et al. (1997) suggested that degradation of membrane lipids by phospholipases can also lead to senescence and cell death.

Conclusions

1. The phosphorus content and proportion of phospholipids in the tested seed fractions and the extracted oils depended significantly on the seed dimensions.

2. The fine seed fraction (2.0–1.6 mm) had the highest total phosphorus content, phospholipid proportion and total share of their non-hydratable form, such as phosphatidic acid, and phosphatidylethanolamine.

3. The oils extracted from the finest seeds also had the highest total phosphorus content, non-hydratable phosphorus and phospholipids.

4. The assumed method of storing seeds at ambient temperature and refrigerating temperature practically did not affect the phosphorus content or the phospholipid proportions in the majority of the seed and oil samples. On the other hand, the temperature of storage had significant impact on the shares of individual phospholipids in phospholipid fraction of seeds and oils.

5. Commonly used in the food industry mass of rapeseed, being a mixture of several winter varieties, differing in dimensions of seeds do not guarantee the stable quality of extracted oils. This is due to the fact that the quality and technologies characteristics of these samples vary depending on the composition of the obtained seed mass. It would therefore be justified to discontinue the marketing of rapeseed mass, and introduce a varietal trading, putting an emphasis on the growth of rapeseeds, that are characterized by favourable profile of phospholipid.

6. Improving the quality of extracted oils would be possible by introducing in a first stage of processing the screening of the raw material delivered to factories in order to remove the smallest fraction of seeds with unfavourable profile of phospholipids.

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CHANGES IN CREAM CHEESE RHEOLOGICAL PROPERTIES DURING MIXING WITH A FRUIT CONCENTRATE

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Key words: cottage cheese, fruit concentrate, fruit flavored cottage cheese, static mixer, rheological properties.

Abstract

A static mixer was used to mix a strawberry-vanilla concentrate with cream cheese produced with the centrifugal method and a cherry concentrate with cream cheese produced with the ultrafiltration method. Rheological properties of the components and the mixtures were examined at temperature 15°C with a rotational viscometer in a range of shear rate corresponding to processes of chewing and swallowing food products by man ranging from 1.5 to 121.5 s^{-1}. Their flow and viscosity curves were plotted. All the fluids examined were shown to display characteristics of shear thinning. Attempts were also made to describe the flow curves with Ostwald-de Waele, Herschel-Bulkley and Casson models. The values of correlation coefficients obtained in the study indicated that the Herschel-Bulkley model was the most suitable for the description of the curves, and that the curves exhibited the yield stress. In both cases an increase in the shear rate increased the internal tension difference and decreased the viscosity difference between the components and their mixture. In both cases of mixing cream cheese with a fruit concentrate, a flavored cream cheese, was obtained with a lower viscosity than its components.

ZMIANY WŁAŚCIWOŚCI REOLOGICZNYCH SERKA TWAROGOWEGO PODCZAS MIESZANIA Z KONCENTRATEM OWOCOWYM

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Słowa kluczowe: reologia serka homogenizowanego, reologia koncentratu owocowego, mieszanie serka homogenizowanego.

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Abstrakt

Za pomocą mieszadła statycznego mieszano koncentrat truskawkowo-waniliowy z serkiem homogenizowanym wyprodukowanym metodą wirówkową oraz koncentrat wiśniowy z serkiem wyprodukowanym metodą ultrafiltracyjną. Właściwości reologiczne komponentów i mieszanin zmierzono w temperaturze 15°C reometrem rotacyjnym. Badano szybkość ścinania odpowiadającą procesowi żucia i przełykania żywności przez człowieka. Stwierdzono, że komponenty i mieszaniny są płynami wykazującymi cechy rozrzedzania ścinaniem, do opisu których najlepiej pasuje model Herschela-Bulkleya. Na podstawie badań stwierdzono, że w zakresie szybkości ścinania od 1,5 do 121,5 s^{-1} lepkość uzyskanych mieszanin była mniejsza od lepkości komponentów.

Introduction

Fruit-flavored cream cheese is part of the basic human diet (GÓRSKA-WARSEWICZ 2007). According to unpublished data of the Central Office of Statistics, the market for fruit-flavored cream cheese, yoghurt and other fermented milk products in Poland in 2013 was estimated at ca. 348 thousand tons, with a value of ca. 1725 million zlotys and growing. The reason for the growing consumption of cream cheese can be seen in the increasing range of flavors, its consistency, texture and additives, as well as its pro-health benefits, such as boosting the immune system or restoring the microbiological balance of the body. The organoleptic properties of flavored cream cheese, including its consistency and flow properties, depend on such factors, as, for example, the type and content of fruit concentrate and the method of the cream cheese manufacture. One of the quantitative methods of estimating the changes of cream cheese properties during the process of mixing it with fruit concentrate are rheological tests of shear rate within the range responsible for a consumer's organoleptic sensations (MARZEC 2007, SURÓWKA 2002, SZCZEŚNIAK 2002).

The aim of this study was to determine the rheological properties of two types of fruit-flavored cream cheese, obtained by mixing white cream cheese with fruit concentrate and to compare it to the properties of the ingredients.

Materials and Experimental Methods

Cream cheese with a fat content of 5% produced by the centrifugal method at the Dairy Plant in Kalisz (PL) was mixed with strawberry-and-vanilla concentrate and cream cheese with a fat content of 5% produced by the ultrafiltration method at the Dairy Plant in Chełm (PL) was mixed with cherry concentrate. Both concentrates were produced by Zentis in Żelków near Siedlce (PL). The mixing process of components was conducted on a semi-technical scale in a vertical flow mixer using a static mixer constructed by the

author (LIMANOWSKI 2006). The mixing length was 1.76 m and the mixer diameter was 0.05 m. The ingredients were supplied separately with OnLine' lobe pumps manufactured by the Johnson Pump (UK) Ltd. company. The mass flow of the centrifugal cream cheese was 105 kg h^{-1}, the mass flow of the concentrate was 25.8 kg h^{-1} and the concentrate content in the mixture was 21.2%. The mixing process and the rheological measurements were conducted at 8°C (DRAKE et al. 2009). The mass flow of the ultrafiltration cream cheese was 1896.9 kg h^{-1}, the mass flow of the concentrate was 432 kg h^{-1} and the concentrate content in the mixture was 18.5%. The mixing process and the rheological measurements were conducted at 15°C. The rheological properties of the cream cheese were determined because of the surprising results of the tests of the centrifugal cream cheese, which had been conducted earlier. They showed that the flowability of the mixture of cream cheese and strawberry-vanilla concentrate was greater than that of the ingredients separately. Measurements conducted for the ultrafiltration cream cheese and cherry concentrate were an attempt at expanding the study scope; moreover, the tests were expected to confirm the previous findings (BRIGHENTI et al. 2008). To this end, measurements of the rheological properties were conducted at two different temperatures (slightly exceeding the process temperature), at different mass flows of the product flowing through the mixer and at different mass content levels of the concentrate (which is only a consequence of the difficulty of keeping them at the assumed level). The flow and viscosity curves of the components and mixtures were determined with a Rheotest-2 rotary rheometer with an S/S1 cylinder system, based on mean values of shear stress and viscosity, measured three times at an increasing and decreasing shear rate?, ranging from 1.5 to 121.5 s^{-1} (LIMANOWSKI, HAPONIUK 2003). This range was selected because it is associated with the processes of chewing and swallowing of food by humans (BARNES et al. 1989). The device readings were recorded after 60 seconds of the sample shearing. The dependence of shear stress on the shear rate was described using known mathematical models (BARNES et al. 1989, BASAK, RAMASWAMY 1994, CASTILLOA et al. 2006, WIŚNIOWSKI, SKRZYPASZEK 2006):

Ostwald de Waele	$\tau = K \cdot ?^n$	(1)
Herschel-Bulkley	$\tau = \tau_0^{HB} + K \cdot ?^n$	(2)
Casson	$\tau^{1/2} = (\tau_0^C)^{1/2} + (\eta \cdot ?)^{1/2}$	(3)

where:

τ – shear stress [Pa]
η – non-Newtonian viscosity [Pa · s]
? – shear rate [s^{-1}]
K – consistency coefficient [Pa · s^n]
n – flow behavior index [–]
η_C – Casson viscosity [Pa · s]
τ_0^{HB} – Herschel-Bulkley yield stress [Pa]
τ_0^C – Casson yield stress [Pa]

Results and Discussion

Mixing centrifugal cream cheese with strawberry-vanilla concentrate produced flavored cream cheese, whose shear stress and viscosity were lower than those measured for the separate components (Table 1, Figure 1, Figure 2). Calculations made with the Statistica software package showed that the highest flow curve correlation coefficients were obtained by describing them with the Herschel-Bulkley model (KUTCHMANN 1997). Calculations made with the use of Casson's model showed an uncertainty of estimation at the level of confidence of 95% despite using the maximum number of iterations and two independent methods of estimation: Gauss-Newton's and Levenberg-Marquardt's.

Table 1

Constants of equations of flow curves of a strawberry-vanilla cream cheese

Model	Constants, correlation coefficient R	Cream cheese	Strawberry-vanilla concentrate	Strawberry-vanilla cheese
Ostwald de Waele	K [Pa · s^n]	51.573 ± 6.786	39.513 ± 1.823	22.518 ± 2.506
	n [–]	0.296 ± 0.034	0.350 ± 0.012	0.450 ± 0.027
	R [–]	0.960	0.997	0.991
Herschel-Bulkley	τ_0^{HB} [Pa]	73.410 ± 5.434	30.575 ± 1.869	25.465 ± 6.671
	K [Pa · s^n]	3.517 ± 1.595	18.223 ± 1.144	8.543 ± 2.993
	n [–]	0.794 ± 0.092	0.484 ± 0.012	0.630 ± 0.069
	R [–]	0.994	0.999	0.997
Casson	τ_0^C [Pa]	insecure estimation	insecure estimation	27.837 ± 2.524
	η_C [Pa]			0.670 ± 0.053
	R [–]			0.995

The yield stress for a mixture of cream cheese and concentrate was lower than for the yield stress of the components, its consistency coefficient K was higher and the flow index n was lower than the initial values of K and n for the cream cheese. This means that addition of the concentrate reduced the inner stress, loosened the consistency of the cream cheese and made it more susceptible to flowing. In the range of shear rate which is most important in terms of organoleptic sensations, i.e. from 50 s^{-1} to 100 s^{-1}, the shear stress in fruit-flavored cream cheese was lower by 15.9% than the stress before mixing and was 8.9% lower than the stress in the concentrate. As the shear rate increased, the stress differences also increased, and the corresponding differences of viscosity decreased proportionally. The extent of the changes was estimated from the approximating curves.

Further studies showed that the direction of the changes of cream cheese, as described above, was consistent with the results of studies of a mixture

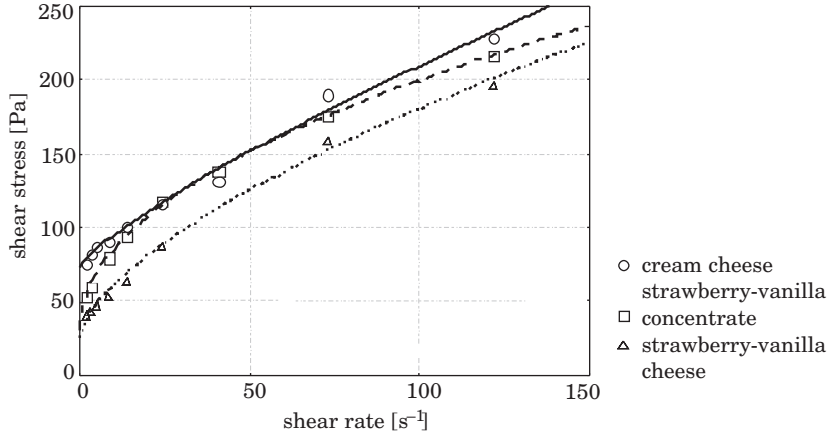

Fig. 1. Flow curves of a cream cheese produced with the centrifugal method, a strawberry-vanilla concentrate and their mixture

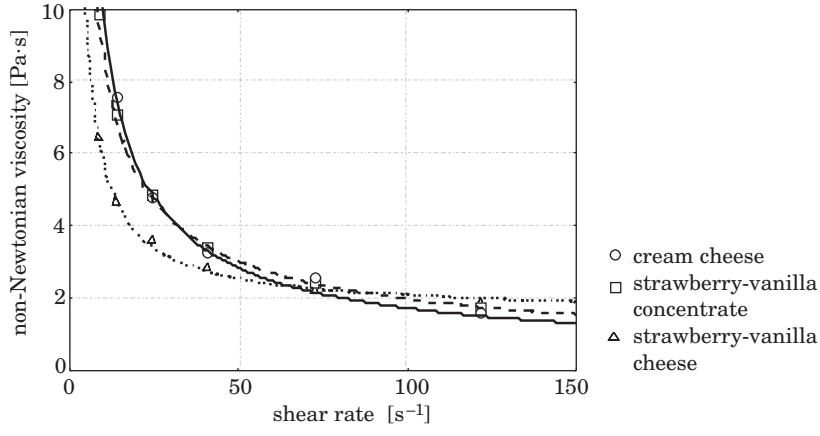

Fig. 2. Curves of changes in non-Newtonian viscosity of a cream cheese produced with the centrifugal method, a strawberry-vanilla concentrate and their mixture

of cream cheese manufactured by the ultrafiltration method and cherry concentrate (Figure 3). The most faithful mathematical reproduction of the measurement points which formed the flow curves for the components and the mixture was obtained again with the H-B model. The correlation coefficients for the curves described by the power-law model O-dW were smaller, and those calculated by the Casson model again showed uncertainty of estimation. A comparison of the constant values of the approximation equation H-B showed that adding the concentrate to the cream cheese slightly increased the yield stress of the mixture and reduced the consistency coefficient; it did not

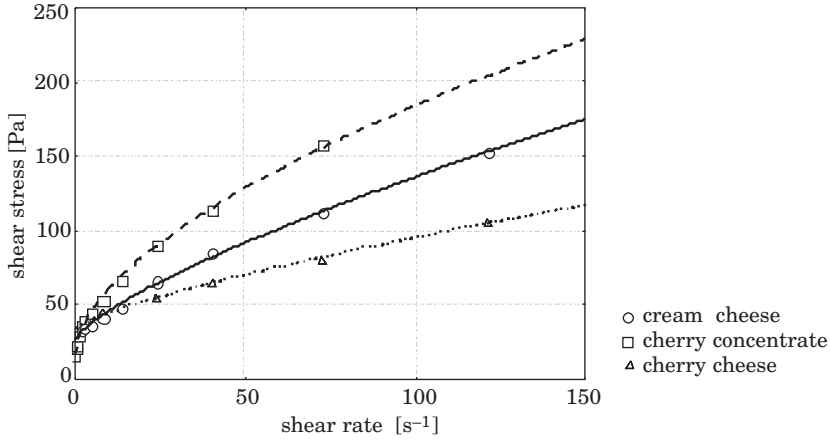

Fig. 3. Flow curves of a cream cheese produced with the ultrafiltration method, a cherry concentrate and their mixture

Table 2

Constants of equations of flow curves of a cherry concentrate

Model	Constants, correlation coefficient R	Cream cheese	Cherry cheese concentrate	Cherry
Ostwald de Waele	K [Pa · s^n] n [–] R [–]	17.025 + 2.308 0.449 + 0.033 0.986	24.459 + 0.821 0.304 + 0.015 0.993	24.809 + 2.680 0.285 + 0.028 0.970
Herschel-Bulkley	τ_0^{HB} [Pa] K [Pa · s^n] n [–] R [–]	26.336 + 1.727 3.527 + 0.577 0.747 + 0.033 0.999	16.313 + 2.093 11.624 + 1.549 0.580 + 0.030 0.998	33.706 + 0.895 1.976 + 0.298 0.748 + 0.030 0.999
Casson	τ_0^C [Pa] ηC [Pa] R [–]	21.122 + 0.825 0.499 + 0.017 0.999	insecure estimation	insecure estimation

change the flow capability (Table 2). In effect, the flow and viscosity curves for the mixture were below the corresponding curves of the components on the diagram. Based on the approximating curves, it was found that the tangent stress in the fruit-flavored cream cheese in the shear rate range from 50 s^{-1} to 100 s^{-1}, was about 27.9% lower than the stress in the cream cheese before mixing and by as much as 48.3% compared to the stress in the concentrate (Figure 3). An increase in the shear rate was accompanied by an increase in the differences between the shear stress of the components and the mixture and the tendency to equalize the viscosity (Figure 4). The results obtained for mixing the cream cheese with fruit concentrate found the same tendency for changes in the rheological properties of the cream cheese in both cases under

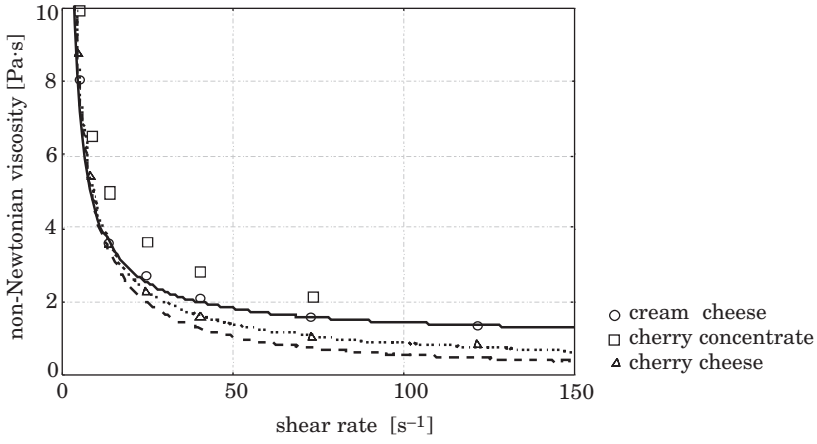

Fig. 4. Curves of changes in non-Newtonian viscosity of a cream cheese produced with the ultrafiltration method, a cherry concentrate and their mixture

study, despite the fact that the processes were conducted under different conditions. A rapid change in the mixture viscosity does not correspond to any known mathematical rules for its estimation. It can only be speculated that the observed changes may be caused by the content of dry matter and fat, the type and amount of used stabilizers, the methods of cold storage of milk and dairy products, the type of milk pre-processing (homogenization, pasteurization), process-related (type of starter cultures, conditions of incubation, temperature, pH) and technical factors (type of pumps used, method of mixing and type of mixer, diameter and length of the pipelines, type of the fixtures used, mass flow, methods of packaging and storage and others) (CHEN, CHENG 1998). Estimation of the effect of the factors and their interaction on the basic rheological properties of the product requires extensive methodological studies.

Conclusions

All of the products under study: cottage cheese, strawberry-vanilla concentrate, cherry concentrate, strawberry-vanilla cheese and cherry cheese have been found to be non-Newtonian, shear-thinned liquids, whose flow curves can be described most precisely by the Herschel-Bulkley model.

Increasing the shear rate increased the internal stress and decreased the difference in viscosity between the components and their mixture. The differencies between viscosity of strawberry-vanilla cheese and their components

were less than in the case of cheery cheese and their components in the middle area of shear rate (ca. 40–100 s^{-1}).

The mixing of cream cheese with fruit concentrate produced flavored cream cheese with higher flowability than those of its components.

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INTELLIGENT FOOD PACKAGINGS AS A LINK OF COMMUNICATION IN THE CHAIN OF SUPPLY

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K e y w o r d s: intelligent packagings, logistics, a chain of supply.

A b s t r a c t

The aim of this work is an attempt to define the role of intelligent packagings to make the course of logistic food processes more efficient. The implementation of the aim happened by conducting the surveys concerning the meaning of intelligent packagings advantages for the chosen participants of logistic processes in the chain of supply.

The studies were questionnaires and included the following elements of the supply chain: representatives of operational staff of transport companies, warehouses and consumers who are not professionally connected to transport and warehousing from the area of south-east Poland (sampling was accidental).

Statistical analysis was performed using an Anova packet of the Statistica softwear.

The surveys allowed to claim that the representatives of transport companies and warehouses have a high level of knowledge about intelligent packagings and their ratings. Consumers, however, do not know about them much. Varied results of the questionnaires allow to assume about a limited possibility of making logistic processes by using intelligent packagings more efficient.

INTELIGENTNE OPAKOWANIA ŻYWNOŚCI JAKO OGNIWO KOMUNIKACJI W ŁAŃCUCHU DOSTAW

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Abstrakt

Celem pracy jest próba określenia roli opakowań inteligentnych w usprawnieniu przebiegu procesów logistycznych żywności. Realizacja celu nastąpiła poprzez przeprowadzenie badań dotyczących znaczenia walorów opakowań inteligentnych dla wybranych uczestników procesów logistycznych w łańcuchu dostaw.

Badania miały charakter ankietowy i obejmowały następujące ogniwa łańcucha dostaw: przedstawicieli pracowników operacyjnych firm transportowych, magazynów oraz konsumentów niezwiązanych zawodowo z transportem i magazynowaniem z Polski południowo-wschodniej (dobór próby miał charakter przypadkowy).

Analizę statystyczną wyników wykonano z wykorzystaniem jednoczynnikowej analizy wariancji.

Badania pozwoliły na stwierdzenie wysokiego poziomu znajomości opakowań inteligentnych i ich wskaźników wśród przedstawicieli firm transportowych i magazynów oraz niskiego wśród konsumentów. Zróżnicowane wyniki badań pozwalają domniemywać o ograniczonej możliwości usprawnienia procesów logistycznych poprzez wykorzystanie opakowań inteligentnych.

Introduction

Contemporary market of packagings is characterized by the dynamic development, which results from the growth of number of products demanding packagings and the fact that new technologies which ensure product security and functionality of its packagings, come into being. New technologies enabled the development of so called intelligent packagings. Their main function is to inform a potential purchaser about the quality condition of a wrapped product. The main task of intelligent packagings is to monitor the conditions and inform about the changes which appear in a product from the moment it was wrapped until it is opened.

Controlling the conditions and changes which appear in food is particularly crucial in the logistic processes, mainly during its transport and storage.

Intelligent packagings monitor the inside or/and outside surroundings of a product. They deliver, at the same time, information about the product, which is inside the wrapping. Thanks to this type of packagings, detailed changes of the product quality, during storage, can be defined.

The main tasks of intelligent packagings are monitoring or delivering information about a product, its quality, security or localization during transport, warehousing, sale, and during its usage. Operation of these packagings is connected to the use of interactive indicators, most frequently variegated, enabling the valuation of the quality of a product being wrapped. Indicators appear inside the packaging or on it surface, giving the information about the quality state or the conditions of its storage. (BRODY et. al. 2008, CICHOŃ 1996, GILES 1999, KORZENIEWSKI et. al. 2011, LISIŃSKA-KUSNIERZ and UCHEREK 2003, LISIŃSKA-KUSNIERZ and KAWECKA 2012, UCHEREK 2003).

There are some types of indicators on the market. The most popular are the time and temperature indicators (TTI-Time-Temperature Integrators), freshness indicators and the RFID (Radio Frequency Identification) system. The working rule of the TTI is: it changes its qualities under the influence of the temperature which is higher than the quality requested or as a result of a thermal effect, accumulated during the warehousing and transport. The consequence of this change, which is irreversible, is a visual effect, the most frequently expressed by discolouration of a marked space of a label. Freshness indicators immediately inform about the product quality. They directly react to the changes of the atmosphere composition in the inside space of the packagings or to the changes which appear on the surface of the product itself. Their work is usually based on detecting the presence of microorganisms metabolites such as: carbon dioxide, sulphur dioxide, ammonia, amina, hydrogen sulphide, organic acids, ethanol, toxin and enzymes. Electronic and optical detectors, as well as variegated compounds, which are created in the reaction with the substance absorbed from the inside of the packaging, can be used in the in the indicator system of the indicator. RFID means identification of objects with the use of radio waves. The data stored in the tag memory is used in order to achieve the aim. The tags are composed of two main elements: the integrated circuit (chip) and the broadcasting-receiving aerial. The chip includes the memory from which we take the data with the use of the wireless transmission, realized by the aerial. The RFID system enables the observation of the way of the product in the supplies chain and the goods protection from the theft or forgery (KABAJA 2012, KOZAK 2007, MAJEWSKI 2006).

In spite of the fact that the concept of intelligent packagings is relatively new, it brings a lot of advantages in the whole world. Above all it creates a challenge for the wrappings producers who use it to increase the shares on the market. These types of packagings can also influence the improvement of the logistic processes.

The aim of this work is to know the opinion of the chosen participants of the supply chain about the role of intelligent packagings in order to make the course of logistic food processes more efficient.

Methods

The research was done with the use of the questionnaire in the period from January to July 2012, on the territory of one of the south-east Poland's voivodeships. There were 186 respondents – the representatives of operational staff of transport companies (43 people), warehouses (33) and consumers (110). Sampling was accidental. All of the respondent groups answered the

same questions in the survey. The questionnaire included the questions concerning the knowledge about the particular indicators of the intelligent packagings, their usage and their meaning in the communication process in the supplies chain. The results of the survey presented in the elaboration are part of the research conducted on the territory of the whole country.

A variance analysis was performed using an Anova packet of the Statistica softwear. It was used in order to answer the question of whether the results of the answers received were varied depending on the sample. Further null hypothesis was verified F Snedecor test. The null hypothesis is rejected when the calculated value of F was higher than the limit (the accepted level of significance $\alpha = 0.05$), which means that the level of the test meter was significantly varied in the compared groups (SOBCZYK 2007).

Results and Discussion

The survey results have been presented below. They concern the answers of the chosen participants of the supplies chain, i.e. transport companies employees, warehouses employees and the consumers not connected professionally with transport and warehousing.

The first question concerned the respondents' knowledge about the „intelligent packaging" term (Figure 1).

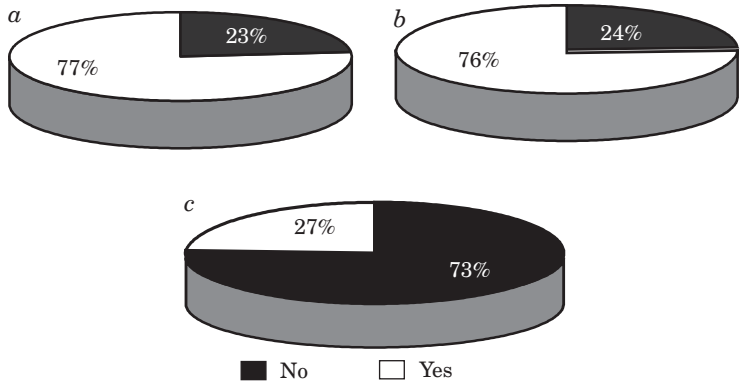

Fig. 1. Knowledge of the term „intelligent packaging": *a* – the transport companies employees; *b* – the warehouses employees; *c* – consumers

As it results from the above pie charts, the term „intelligent packaging" is the best known by the warehouses employees, slightly worse by the transport companies employees. Consumers, however, hardly know what the term means.

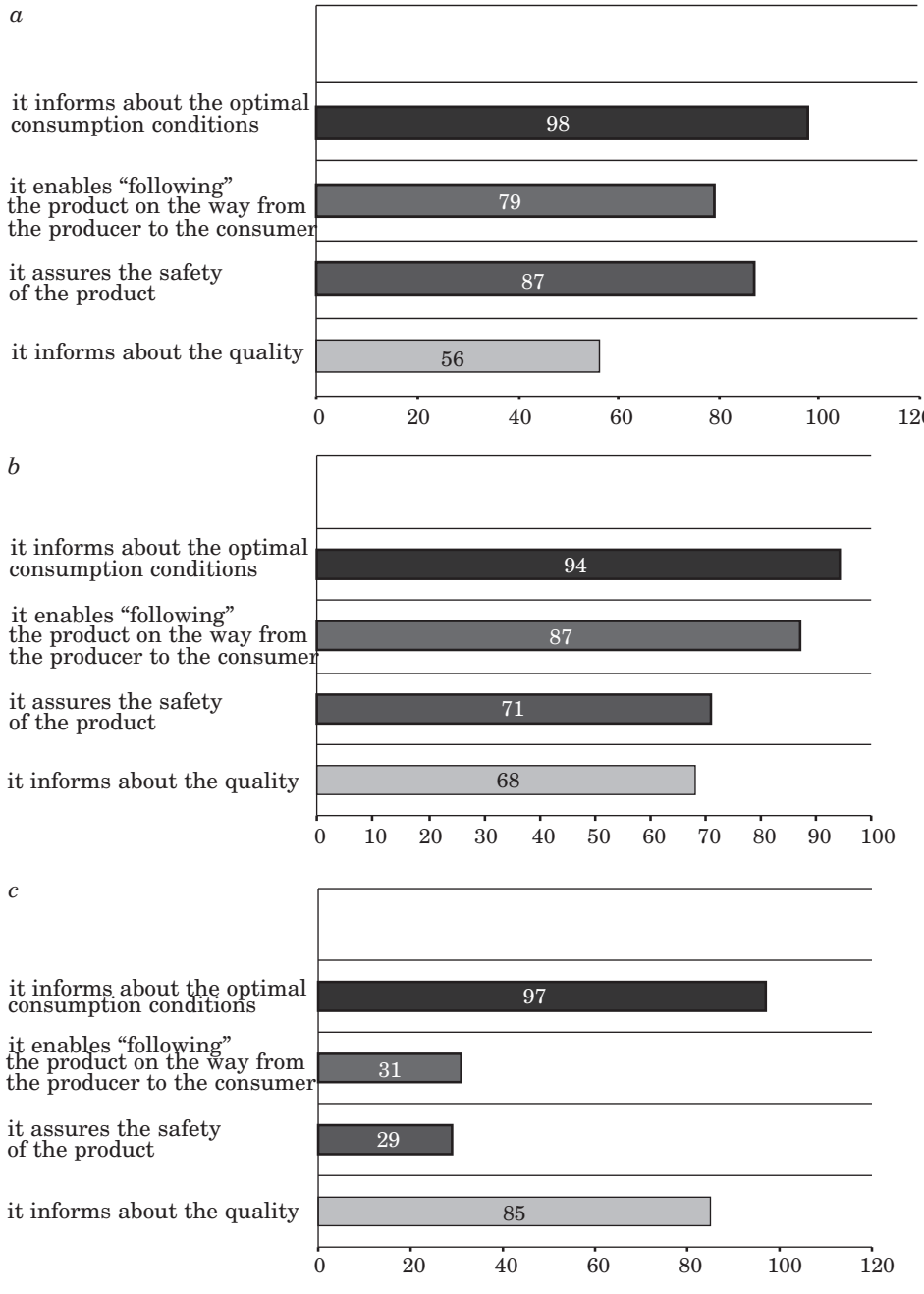

Fig. 2. The opinion on the role of intelligent packaging: *a* – the transport companies employees [%]; *b* – the warehouses employees [%]; *c* – the consumers [%]

As a result of statistical studies stated a statistically significant difference in this regard between the results of consumers answer and employees of stores and transportation companies.

The second question was about the role of the intelligent packagings (common answers were chosen of all the groups questioned) – Figure 2.

The above pie charts indicate that the certain groups of the respondents ascribe the same roles, however, their importance is varied.

The variance analysis allowed us to determine statistically significant difference between the results of employee responses storage and transport company employees and consumers' answer: „it enables following the product on the way from the producer to the consumer" and „it assures the safety of the product", and no significant difference between the results of other answer of all study groups.

The next question concerned the opinion of the respondents about the place of the intelligent food packagings in order to improve the logistic processes (Figure 3).

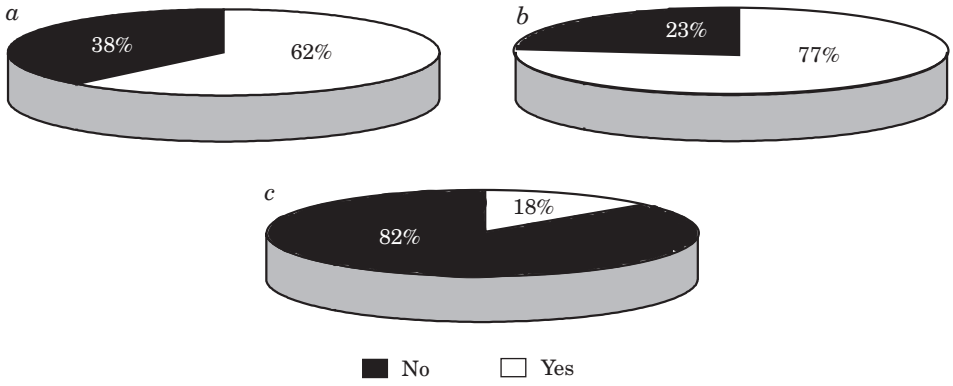

Fig. 3. Opinion on the impact of intelligent food packaging improve the process of movement of goods on the way from the producer to the consumer: *a* – the transport companies employees; *b* – the warehouses employees; *c* – consumers

Contrary to the assumptions, about 30% of employees of the transport companies and warehouses claim that the intelligent packagings do not influence the improvement of the goods transport process, what indicates a low level of awareness in the range of logistic function of the wrappings. A great majority (over 80%) of consumers have a similar opinion.

As a result of statistical research, again found a statistically significant difference (as I have in the case of question 1), in the opinions of consumers and employees of stores and personnel transport companies.

The aim of the next question was to define the influence of the intelligent packagings on the improvement of the logistic processes (Figure 4).

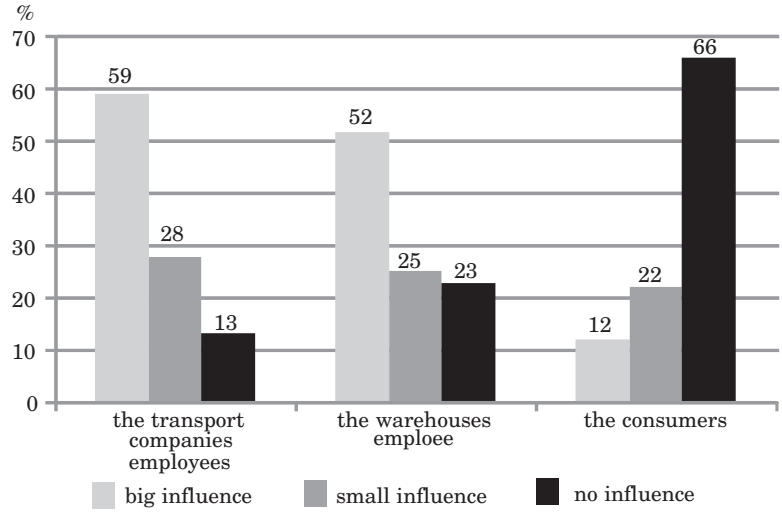

Fig. 4. The significance of the impact of intelligent food packaging process of moving goods on the way from the producer to the consumer

Over the half of the transport companies and warehouses employees indicate a big influence of intelligent packagings on the process of goods transport on the way form the producer to the customer. There are also many respondents in this group who do not see such influence. It is interesting because these employees are everyday participants of the logistic chain. As the results show, however, they are unaware. In the face of the earlier answers, the fact of the lack of awareness of this influence among the consumers is not surprising.

In the context of the impact of intelligent packaging on process of moving goods on the way from the producer to the consumer there was no statistically significant difference between the opinions of consumers and workers warehouses and transport company employees.

The last question concerned the influence of the indicators which are attributes of the intelligent packagings in order to improve the logistic processes (Figure 5). The respondents were presented the list of the chosen indicators (time-temperature; the opening; a quake and inclination; moisture; RFID), with the request of showing, which of them are the most crucial in the context of the logistic processes improvement.

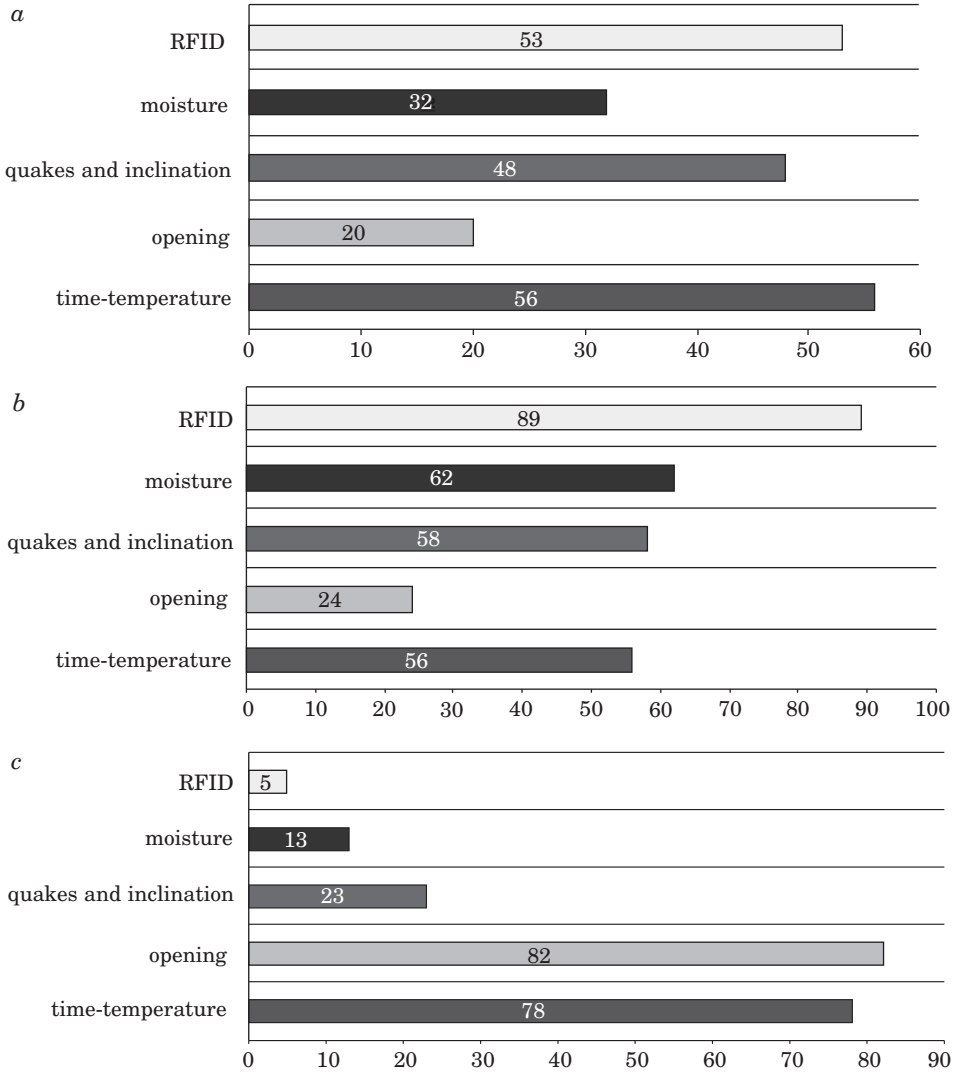

Fig. 5. The significance of the indicators as an attribute in the context of intelligent packaging improvements logistics processes: *a* – the transport companies employees [%]; *b* – the warehouses employees [%]; *c* – the consumers [%]

The results show that the most crucial attributes of the intelligent packagings proved to be: in the group of transport companies and warehouses employees – the time – temperature indicator and RFID, however, in the group of the consumers not professionally connected to transport and warehousing – the indicator of the opening and time – temperature.

As regards the impact indicators which are attributes of smart packaging to improve logistics processes were significantly statistical difference in the results of the answers given to this question between warehouses workers, transport companies workers and consumers.

Conclusions

The questionnaire done and the observations allow to formulate the following conclusions:

1. The knowledge about the term „intelligent packagings" and its role in the improvement of the processes of goods transport is varied, it depends on the logistic link chain.
2. The most aware participants of the logistic chain, in the range of the influence of the intelligent packagings on the process of goods transport on the way from the producer to the consumer proved to be the transport companies employees, the least, however, the consumers, not professionally connected to transport and warehousing.
3. The most crucial attributes of the intelligent packagings proved to be: the time- temperature indicator, RFID and indicator of the opening.
4. A variance analysis allowed us to verify the results of response groups.

Translated by AUTHORS

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CHARACTERIZATION OF SOME QUALITY PROPERTIES AND CHEMICAL COMPOSITION OF COLD-PRESSED OILS OBTAINED FROM DIFFERENT RAPESEED VARIETIES CULTIVATED IN POLAND

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Key words: rapeseed, *Brassica napus,* cold pressed oil, quality, fatty acids, tocopherols, sensory assessment, PCA.

Abstract

In this study comparison of quality parameters and chemical composition between cold-pressed oils obtained from 6 different rapeseed varieties, including double improved (RO), high-oleic (HORO) and yellow-seeded (YSRO), has been conducted. A clear correlation between fatty acid composition and oxidative stability of oils was observed. Variety-dependent variation in the content of individual tocopherols and slight differences in the content of total tocopherols was found. The results of oils sensory assessment based on PCA showed that the major sensory attributes assigned to ROs are seed-like and nutty, while sensory attributes like woody, strawy and astringent are strongly perceivable in HORO and YSRO.

Abbreviations: AV – acid value, CD – conjugated dienes, CT – conjugated trienes, IP – induction period, PV – peroxide value, *p*-AnV – *p*-anisidine value, RO – double improved „00" rapeseed oil, HORO – high-oleic rapeseed oil, YSRO – yellow-seeded rapeseed oil, α-T – alpha--tocopherol, γ-T – gamma-tocopherol, δ-T – delta-tocopherol, α-TE – α-tocopherol equivalent.

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CHARAKTERYSTYKA WYBRANYCH CECH JAKOŚCIOWYCH I SKŁADU CHEMICZNEGO OLEJÓW TŁOCZONYCH NA ZIMNO OTRZYMANYCH Z RÓŻNYCH ODMIAN RZEPAKU UPRAWIANEGO W POLSCE

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Słowa kluczowe: rzepak, *Brassica napus,* olej tłoczony na zimno, jakość, kwasy tłuszczowe, tokoferole, cechy sensoryczne, PCA.

Abstrakt

W pracy porównano parametry jakości i skład chemiczny olejów rzepakowych tłoczonych na zimno uzyskanych z 6 różnych odmian rzepaku, w tym: nasiona podwójnie ulepszone (RO), wysokooleinowe (HORO) i żółtonasienne (YSRO). Wyraźnie zaobserwowano korelację liniową między składem kwasów tłuszczowych a stabilnością oksydatywną uzyskanych olejów. W zależności od odmiany nasion użytych do tłoczenia stwierdzono różnice w zawartości poszczególnych form tokoferoli i niewielkie w ogólnej zawartości tokoferoli w otrzymanych olejach. Wyniki oceny sensorycznej olejów opartych na analizie PCA pokazały, że główne cechy sensoryczne olejów, takie jak: „typowy dla nasion" i „orzechowy" były przypisane odmianom podwójnie ulepszonym (RO), natomiast pozostałe cechy sensoryczne, takie jak: „typowy dla drewna, trawy, ściągający" były silnie odczuwalne w olejach z odmian HORO i YSRO.

Introduction

Production of rapeseed oil peaked in 2013/14 at 26.6 million tonnes, which placed it the third most important plant oil, after soy and palm oil (FAOSTAT 2015). Although the above figures do not distinguish the various types of rapeseed oil, there has been an increased interest by consumers for cold-pressed oils observed in recent years (MATTHÄUS and BRÜHL 2008). This trendis also noticeable in the Polish market, where an increased consumption of cold pressed oils, including rapeseed oil, is observed.

According to Codex Alimentarius Standard for Named Vegetable Oils (CODEX STAN 210–1999) oils „obtained, without altering the nature of oil, by mechanical procedures, e.g. expelling or pressing, without the application of heat" are defined as „cold-pressed oils". One of the most important parameter for the evaluation of the quality of cold-pressed oils is the sensory assessment, especially the intensity of sensory attributes. Typical cold-pressed rapeseed oil attributes are listed as follows: *seed-like*, *nutty*, *woody*, *astringent*, while off-flavours are described as *rancid*, *fusty*, *musty*, and *bitter* (BRÜHL and MATTHÄUS 2008).

Rapeseed/canola oil has unique health benefits than many other plant oils, primarily due to favourable fatty acid composition and the high concentration of bioactive compounds. Rapeseed oil is very low in SFA (< 7%), rich in oleic acid (< 60%), and contains linoleic to α-linolenic essential fatty acids ratio of 2:1, making it nutritious (O'BRIEN 2009). Moreover, rapeseed oil is rich source of natural antioxidants, including tocopherols, polyphenols and phytosterols. Crude rapeseed oil contains valuable amounts of tocopherols (approx. 770 mg kg^{-1}), primarily γ-T (65%), followed by α-T (35%), while β- and δ-T are present at very low or undetectable concentrations (PRZYBYLSKI 2011). Rapeseed contains more phenolic compounds than most of the other oilseed plants. The most significant of these are sinapic acid and its derivatives (NACZK et al. 1998, KOZLOWSKA et al. 1990). However, during oil cold-pressing only a small proportion of phenolic compounds is transferred to the crude oil, while the rest is retained in the meal (KOSKI et al. 2002). Crude rapeseed oil is also a good source of sterols (4500–11300 mg kg^{-1}) but when oil is processed further, especially under high temperatures, sterol levels in oil can be reduced (MÖLLERS 2002). The reported sterol distribution in rapeseed/canola oil is as follows: β-sitosterol (52%), followed by campesterol (28%) and brassicasterol (14%) (VERLEYEN et al. 2002).

Pigments represent the natural components of oilseeds, they are considered important factors because they can impart undesirable green/brown colour to vegetable oils or facilitate oxidation in the presence of light. The composition and content of chlorophyll pigments present in the seeds of rapeseed depends on seed maturity. During ripening the chlorophyll pigments are gradually degraded – physiologically mature seeds (35 days before maturity) contain an average of 1239 mg kg^{-1} total chlorophylls, while 4 mg kg^{-1} of chlorophylls can be found in fully matured seeds (WARD et al. 1994, MÖLLERS 2002). The chlorophyll content in crude canola oil should be less than 30 mg kg^{-1}, with chlorophyll *a* and chlorophyll *b* in the ratio of 3:1, and approximately 95 mg kg^{-1} of carotenoids, with ~ 90% xanthophylls and ~ 10% of carotenes (ENDO et al. 1992).

The objectives of this research were: (1) to evaluate the variation of some quality parameters, minor components (tocopherols and pigments), fatty acid composition and oxidative stability of cold-pressed rapeseed oils acquired from different rape varieties (double improved „00", high-oleic and yellow-seeded) cultivated in Poland, (2) to distinguish oils based on their sensory assessment performed by applying principal component analysis.

Materials and Methods

Material. Samples of six rapeseed varieties, including double improved *B. napus* species: Bogart, Bojan, Monolit and Starter (Plant Breeding Strzelce Ltd. Co. – IHAR Group, Poland), yellow-seeded *B. napus* line PNz022 and high-oleic *B. napus* line PN 1170 (The Plant Breeding and Acclimatization Institute, Poznan, Poland). The selected rapeseed varieties were cleaned and stored in paper bags at 15 ± 2°C.

Oil extraction by cold-pressing. Samples of rapeseed (1.5 kg) were cold-pressed with the use of screw press (Farmet, Czech Republic), the temperature of the outflowing oil was in the range from 38 to 42°C. After pressing oils were filtered to remove particles, and afterwards kept in dark glass bottles under refrigeration temperature (4 ± 2°C) until analysed.

Chemicals and solvents. Analytical standards of δ-, γ- and α-tocopherols and 5α-cholestane were purchased from Sigma-Aldrich, (USA). HPLC grade methyl *tert*-butyl ether (MtBE), acetonitrile (ACN) were obtained from POCH (Poland). Chloroform, a high-purity grade (~99.5%) acetic acid, potassium hydroxide and potassium iodide were supplied by Chempur (Poland), solvents: isooctane and n-hexane were acquired from Merck (Germany).

Oil quality analysis. The cold-pressed rapeseed oils were analysed for acid value (*Animal and vegetable...* ISO 660:2005), peroxide value (*Animal and vegetable...* ISO 3960:1996), *p*-anisidine value (ISO 6885:2008). The conjugated dienes and trienes, expressed by absorption coefficient $E^{1\%}_{1cm}$ at λ_{max} 232 and 286 nm (*Animal and vegetable...* ISO 3656:2011), were determined using Thermo-Spectronic Helios β spectrophotometer.

Pigments. The carotenoid and chlorophyll pigments were assayed spectrophotometrically using the ThermoSpectronic Helios β spectrophotometer. Total chlorophylls were determined according to AOCS Method (1997), by measuring the absorbance of oil at 630, 670 and 710 nm in 10 mm spectrophotometer cell against air. The content of chlorophyll pigments was expressed in mg of pheophytin a in 1 kg of oil. Total carotenoid pigments were determined in accordance with BSI Method (1977) by measuring the absorbance of oil samples diluted in cyclohexane at 445 nm. The results were calculated for total carotenoid pigments amount, expressed as mg of β-carotene in 1 kg of oil.

Determination of fatty acid composition. A mass of 0.2 g of oil was weighed and dissolved in 2 ml of hexane. The mixture was submitted for saponification with 0.5 ml of sodium hydroxide solution in methanol (2 M) at room temperature for 2 h. Then 200 µl of the hexane layer was transferred into 1.5 ml autosampler vial and dissolved in 1 ml of hexane. The diluted FAME (1 µl of the sample) were separated on a GC-MS system (Agilent 6890N GC,

Agilent Technologies, USA) equipped with a BPX 70 capillary column (60 m length, 0.22 mm i.d., 0.25 μm film thickness) and flame-ionization detector (FID). Helium was used as a carrier gas at a flow rate of 1.5 ml/min. The column temperature was programmed at 2°C/min with initial temperature 130°C and final temperature 235°C. The injector was set at 230°C with split ratio of 100:1 and the detector was set at 250°C. Fatty acids were identified by comparing their retention times with authentic standards, and the results were reported as weight percentages following integration and calculation using ChemStation Software (Agilent Technologies).

Determination of tocopherols. A sample of 0.2 g of oil was dissolved in 5 ml of ACN/MtBE mixture (4:6 by vol.). The mixture was filtered through a micro syringe filter (titan PTFE 0.2 lm). Then, 5 μl of the sample was injected into a VP Shimadzu HPLC system coupled with DAD detector (SPD-M10AVP, Shimadzu, Japan) and fluorescence detector (RF-10AXL, Shimadzu, Japan), reversed phase octadecyl silica Gemini C 18 column (150 mm × 2 mm × 3 μm) (Phenomenex Torrance, CA, USA) and suitable guard column. The isocratic mobile phase was a mixture of ACN and MtBE (4:6 v/v) at a flow rate of 0.15 ml/min, and the column oven temperature was 35°C. Tocopherols were detected by standard UV spectrum analysis (190–370 nm). Quantification of tocopherols was conducted using data from the fluorescence detector (FLD) with excitation/emission wavelengths of 290/330 nm, respectively. All samples were analysed in triplicate and the tocopherol/oil ratio was expressed in mg/100 g.

The vitamin E content, expressed in *d*-α-tocopherol equivalents (α-TE) was calculated by multiplying milligrams of α-T by 1.0 and γ-T by 0.1 (EITENMILLER, LEE 2004).

Harris coefficient, expressed as the ratio of α-tocopherol equivalent [mg] to the mass [g] of polyunsaturated fatty acids in 100 g of the oil, was calculated (WITTING 1972).

Oxidative stability determined via accelerated stability test (Rancimat). Oxidative stability of the oil sampleswas determined with a Rancimat apparatus (Metrohm model 743; Metrohm KEBO Lab AB, Herisau, Switzerland). Briefly, oil samples were weighed (2.5 g) into the reaction vessel and heated to 120°C under air flow of 20 L/h. The induction period (IP) was expressed in hours (h).

Sensory analysis. Sensory evaluation was performed in triplicate with a selected and trained panel consisting of 10 persons in accordance with *Sensory analysis...* ISO 4121:2003 standard. The oil samples (15 ml) were served in vessels at room temperature. The sensory profile of oils was determined in accordance with the reference-sensory assessment of virgin rapeseed oils (BRÜHL, MATTHÄUS 2008). Eight flavour attributes – seed-like,

nutty, woody, strawy, astringent, rancid, fusty, musty (Table 1) – were chosen. A quantitative sensory description was conducted using a graded 10-point scale to measure the intensity of attributes, leading from zero („not detectable") to ten („intense"). The obtained data, after conversion from linear scale into numerical data, were presented as graphic projection of PCA.

Table 1
Attributes used for sensory assessment of cold-pressed rapeseed oils and descriptors for perceived sensations

Attributes	Descriptors
Seed-like	green, cabbage, asparagus, fresh vegetable, sometimes with a sulphuric note
Nutty	hazelnut, nutty
Woody	wet wood, pencil, stem, pod but also sometimes resembling to chipboard possibly together with rancid
Strawy	straw, barn, throat feels rough
Astringent	rough mouth feeling, furred teeth, like tannins in red wine
Rancid	oxidised oil
Fusty	sour, fermented flavour, silage
Musty	musty smell, mouldy taste, french salami, especially white coated

Source: BRÜHL, MATTHÄUS (2008)

Statistical analysis. All experiments were carried out in triplicate. Statistical analysis was performed using Statistica 10 software. Data were expressed as Mean ± SD or as percentage. Variables were compared by T-test, one-way Anova; post hoc Tukey Test and the significance of differences among means were determined at $p<0.05$. The results obtained from the sensory assessment of oil samples were subjected to Principal Component Analysis (PCA) applying XLSTAT software (Addinsoft, France, Version 2014.6.04).

Results

The quality of the analysed cold-pressed rapeseed oils, assessed in terms of degree of hydrolysis and oxidation, was high, which testified to the appropriate technological value of the seeds used in the research. All oils fulfilled requirements pertaining to the AV (< 4 mg KOH g^{-1}) and PV (< 15 meq O_2 kg^{-1}) specified in the standard for cold-pressed and virgin oils (CODEX STAN 210–1999). The content of secondary oxidation products, resulting from the decomposition of hydro-peroxides, *p*-AnV of all oils did not exceed the value of 1.0, which testified to the insignificant influence of the cold-pressing process on the secondary degree of oxidation of the oil. These results are in agreement with previous studies (TAŃSKA et al. 2009, KRALJIC et al. 2013). The CD content ranged from 1.32 (% E) in HORO, up to 1.75 (% E) in YSRO (Table 2).

The lowest average CD concentration in HORO is related to its specific fatty acid composition – the amount of oxidisable PUFAs decreased to ~15%, and the amount of oxidation-resistant oleic acid increased up to ~76% (Table 3). Scarce concentration of CT detected in all oils (0.07–0.20% E) indicates negligible impact of cold-pressing on the formation of oxidation by-products, such as unsaturated α- and β-diketones and β-ketones.

Table 2

Tocopherols content α-tocopherol equivalent (mg/100 g) in ROs, HORO and YSRO produced by cold-pressing

Rapeseed oil variety	α-T	γ-T	δ-T	Total tocopherols	α-TE
RO Bogart	21.8 ± 1.41^{bc}	33.4 ± 1.95^{bc}	1.2 ± 0.04^{a}	56.4 ± 1.05^{c}	25.18 ± 1.61^{b}
RO Bojan	26.8 ± 2.56^{abc}	35.2 ± 0.21^{bc}	0.7 ± 0.17^{b}	62.7 ± 2.49^{ab}	30.34 ± 2.59^{ab}
HORO	21.3 ± 2.37^{c}	42.4 ± 0.72^{a}	0.1 ± 0.01^{c}	63.8 ± 2.70^{ab}	25.54 ± 2.44^{b}
RO Monolit	25.6 ± 1.85^{ab}	31.0 ± 2.48^{c}	0.6 ± 0.04^{b}	57.2 ± 2.78^{bc}	28.72 ± 2.10^{ab}
RO Starter	28.4 ± 1.34^{a}	37.4 ± 0.35^{ab}	1.3 ± 0.09^{a}	67.1 ± 1.10^{a}	32.18 ± 1.38^{a}
YSRO	22.7 ± 3.29^{abc}	41.8 ± 3.63^{a}	0.6 ± 0.05^{b}	65.1 ± 2.42^{ab}	29.60 ± 3.65^{ab}

Different superscript letters within each column indicate significant differences ($p<0.05$) between each rapeseed variety

Table 3

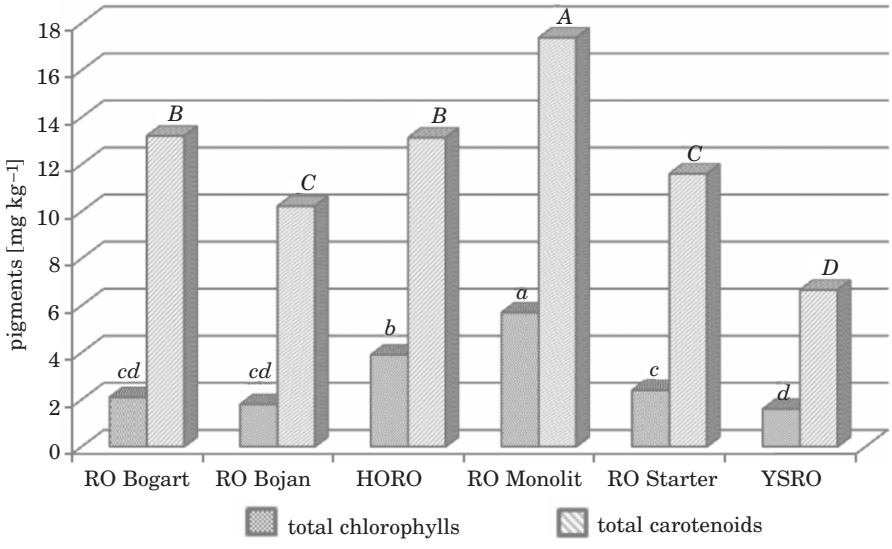
Quality characteristics of ROs, HORO and YSRO produced by cold-pressing

Specification	Rapeseed oil variety					
	RO Bogart	RO Bojan	HORO	RO Monolit	RO Starter	YSRO
AV [mg KOH g^{-1}]	1.08 ± 0.06^{b}	0.50 ± 0.04^{c}	0.42 ± 0.02^{d}	1.47 ± 0.03^{a}	0.55 ± 0.03^{c}	1.46 ± 0.00^{a}
PV [mEq O_2 kg^{-1}]	0.46 ± 0.07^{bc}	0.54 ± 0.08^{b}	0.84 ± 0.06^{a}	0.49 ± 0.01^{b}	0.38 ± 0.04^{cd}	0.56 ± 0.06^{a}
p-AnV	0.35 ± 0.12^{b}	0.26 ± 0.06^{b}	0.14 ± 0.03^{b}	0.37 ± 0.10^{b}	0.9 ± 0.17^{a}	0.4 ± 0.09^{b}
K_{232}	1.48 ± 0.03^{b}	1.47 ± 0.04^{bc}	1.32 ± 0.03^{d}	1.49 ± 0.03^{b}	1.42 ± 0.02^{c}	1.75 ± 0.03^{a}
K_{268}	0.11 ± 0.00^{b}	0.09 ± 0.00^{b}	0.07 ± 0.00^{c}	0.11 ± 0.00^{b}	0.07 ± 0.00^{c}	0.20 ± 0.01^{a}
Induction period [h]	3.75 ± 0.04^{bc}	3.80 ± 0.09^{b}	6.54 ± 0.10^{a}	3.60 ± 0.08^{cd}	3.91 ± 0.05^{b}	3.51 ± 0.07^{d}

Different superscript letters within each row indicate significant differences ($p<0.05$) between each rapeseed variety

Pigments are considered important factors as they exhibit antioxidant properties, but when oil is exposed to light and heat, they can act as pro-oxidants (YANG et al., 2013). In crude canola oil less than 30 mg kg^{-1} of chlorophyll pigments and approximately 95 mg kg^{-1} of carotenoids can be found (ENDO et al. 1992). The concentration of carotenoid pigments in analysed oils ranged from 6.66 to 17.39 mg kg^{-1} (YSRO and RO pressed from the seeds of Monolit variety,

respectively), while the average chlorophyll pigments content was 2.92 mg kg^{-1} (Figure 1), which is in agreement with previously published data (KRALJIC et al. 2013, YANG et al. 2013, GHAZANI et al. 2014).



Mean values denoted by the same letter by the columns do not constitute statistically significant differences at $p<0.05$

Fig. 1. Pigments [mg kg^{-1}] in ROs, HORO and YSRO produced by cold-pressing

According to the sources, regular rapeseed varieties contain approximately 60% of C18:1 fatty acid, while C18:1 fatty acid content in HORO range from 69 to 77% (BARTH 2009), which is consistent with the results obtained in this study (Table 4). HORO clearly differ from oils pressed from regular rapeseed varieties (Bogart, Bojan, Monolit and Starter), and from YSRO in terms of PUFAs concentration (15.1% vs. 29.0–30.9%). Modifying fatty acid composition by decreasing the amount of oxidisable fatty acids such as C18:2 and C18:3 fatty acids, and increasing the amount of oxidation-resistant fatty acids, such as C18:1 fatty acid, disrupted nutritionally favourable C18:2 to C18:3 essential fatty acids ratio of 2:1 in HORO. As it could be seen from Table 4, HORO contain nearly the same level of C18:2 and C18:3 fatty acids (7.7 and 7.4%, respectively), in contrast to ROs and YSRO, exhibiting desirable 2:1 ratio of ω-6 and ω-3 fatty acids. Samples of YSRO and HORO had the lowest SFAs concentration (5.5 and 5.8%, respectively), while ROs showed typical SFAs content of ~7%.

Typically, tocopherol ratio of 65% γ-T and 35% α-T is commonly found in rapeseed oil (MÖLLERS 2002). However, the amounts of total and individual tocopherols in extracted oil may fluctuate within one rapeseed variety, since

Table 4

Fatty acid composition [%] of ROs, HORO and YSRO produced by cold-pressing

Fatty acid	Composition [%]					
	RO Bogart	RO Bojan	HORO	RO Monolit	RO Starter	YSRO
C16:0	3.89 ± 0.05^{b}	4.11 ± 0.01^{b}	3.62 ± 0.06^{a}	4.42 ± 0.03^{c}	4.62 ± 0.02^{c}	3.53 ± 0.05^{a}
C18:0	1.59 ± 0.04^{a}	1.88 ± 0.03^{b}	1.71 ± 0.05^{b}	2.02 ± 0.04^{c}	1.52 ± 0.05^{a}	1.52 ± 0.02^{a}
C18:1	61.07 ± 0.05^{a}	61.03 ± 0.05^{a}	76.64 ± 0.01^{b}	61.04 ± 0.06^{a}	61.14 ± 0.06^{a}	61.02 ± 0.03^{a}
C18:2	19.18 ± 0.04^{d}	18.82 ± 0.07^{c}	7.74 ± 0.03^{a}	18.91 ± 0.04^{c}	18.11 ± 0.05^{b}	21.04 ± 0.01^{e}
C18:3	10.57 ± 0.01^{c}	10.63 ± 0.04^{c}	7.42 ± 0.06^{a}	10.14 ± 0.06^{b}	11.45 ± 0.04^{d}	9.92 ± 0.08^{b}
C20:0	0.48 ± 0.07^{a}	0.64 ± 0.03^{c}	0.52 ± 0.04^{b}	0.62 ± 0.05^{c}	0.61 ± 0.03^{c}	0.54 ± 0.05^{b}
C20:1	1.17 ± 0.04^{a}	1.32 ± 0.02^{c}	1.32 ± 0.05^{c}	1.23 ± 0.05^{b}	1.23 ± 0.05^{b}	1.21 ± 0.04^{b}
C22:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Others	1.18 ± 0.03^{b}	1.93 ± 0.05^{e}	1.23 ± 0.05^{b}	1.71 ± 0.06^{d}	1.54 ± 0.06^{c}	0.94 ± 0.03^{a}
SFA	6.00 ± 0.04^{c}	6.62 ± 0.06^{d}	5.82 ± 0.03^{b}	7.03 ± 0.01	6.73 ± 0.05^{d}	5.54 ± 0.06^{a}
MUFA	62.27 ± 0.03^{a}	62.31 ± 0.03^{a}	77.95 ± 0.04^{b}	62.41 ± 0.06^{a}	62.33 ± 0.03^{a}	62.21 ± 0.04^{a}
PUFA	29.78 ± 0.02^{b}	29.44 ± 0.02^{b}	15.12 ± 0.06^{a}	29.04 ± 0.04	29.53 ± 0.04^{b}	30.91 ± 0.04^{c}
n-6/n-3	1.78 ± 0.04^{c}	1.81 ± 0.07^{c}	1.03 ± 0.03^{a}	1.93 ± 0.03^{c}	1.62 ± 0.05^{b}	2.14 ± 0.05^{d}
Harris coefficient	0.84 ± 0.03^{a}	1.03 ± 0.05^{b}	1.69 ± 0.05^{c}	1.06 ± 0.05^{b}	1.09 ± 0.06^{b}	0.87 ± 0.04^{a}

SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, n.d. – not detected

Values (means ± SD) bearing different superscripts are statistically significantly different ($p<0.05$)

their presence in oil is influenced by many factors, such as climate conditions, genotype, content of PUFAs in oil, and processing/storage conditions (GHAZANI et al. 2014). There are also noticeable variety-dependent differences in the ratio between individual tocopherols, as well as slight variation in the total tocopherols content. Regular canola oil contain on average 695 mg kg^{-1} of total tocopherols, while 901 mg kg^{-1} of tocopherols can be found in high-oleic low-linolenic canola oil (PRZYBYLSKI 2011). The largest amounts of γ-T were detected, followed by α-T, trace amounts of δ-T, and no β-T, but the amount of individual tocopherols varied significantly ($p<0.05$), depending primarily on the rapeseed variety (Table 2). In HORO and YSRO γ-T was present in the largest concentration (42.4 and 41.8 mg/100 g, respectively), while the highest amount of α-T was detected in RO produced from seeds of Starter variety (28.4 mg/100 g). Despite the differences in the amounts of individual tocopherols, there was no significant difference in the total tocopherol content between HORO, YSRO and RO pressed from seeds of Bojan variety. The lowest amount of tocopherols were found in RO acquired from seeds of Bogart variety (56.4 mg/100 g), and the highest in RO obtained from seeds of Starter variety (67.1 mg/100 g). KRALJIC et al. (2013) found similar concentration of total tocopherols in cold-pressed oils, in contrary to GHAZANI et al. (2014), who found nearly 2-fold lower total tocopherol content (~36 mg/100 g) in the studied cold-pressed oils. The amount of vitamin E (α-tocopherol equivalents) in the

analysed oil samples varied from 25.18 to 32.18 mg/100 g, which is typical for low erucic acid rapeseed (LEAR) oils (GUGAŁA et al. 2014).

In order to determine nutritional value of examined oils, Harris coefficient was calculated. HORO was marked by the highest Harris coefficient (1.69), which is a result of decreased PUFAs content, while Harris coefficient calculated for ROs and YSRO ranged from 0.84 to 1.09. However, all oils exhibited proper physiological value (α-TE to PUFA ratio (mg g^{-1}) of 0.6:1, as a minimum to protect against PUFA peroxidation) (VALK, HORNSTRA 2000).

The oxidative stability of vegetable oils is determined by their fatty acid composition and antioxidants, mainly tocopherols but also other non-saponifiable constituents. The effect of fatty acids on stability depends mainly on their degree of unsaturation and, to a lesser degree, on the position of the unsaturated functions within the triacylglycerol molecule (KAMAL-ELDIN 2006). The fatty acid composition of vegetable oils is affected by botanical source, as well as by genetical variations. Traditional plant breeding and genetic manipulations of conventional oilseed crops have resulted in high-oleic oil varieties. Modifying fatty acid composition by decreasing the amount of oxidisable fatty acids such as α-linolenic and linoleic acids and increasing the amount of oxidation-resistant fatty acids such as oleic acid improved the oil's oxidative stability (MERRILL et al. 2008). From the results shown in Table 3 it can be concluded that the IP length differences of the examined oils arise mainly from to differences in the fatty acid composition, with superior oxidative stability of HORO (6.54 h) compared to YSRO (3.51 h), and ROs and (3.60–3.91 h). The oxidative stability of cold-pressed ROs in studies conducted by KOSKI et al. (2002) ranged from 2.1 to 4.5 h, while the IP of HORO examined by MATTHÄUS (2006) was 7.3 h.

PCA was performed for the mean ratings of each rapeseed oil across the 8 chosen attributes (Table 1). As presented in Figure 2 the first two PCs accounted for 85.20% of variability (51.70% and 33.50%, respectively). PC1 was highly contributed by the following sensory attributes: astringent (0.815), strawy (0.808), seed-like (-0.796) and nutty (-0.795) while PC2 was highly contributed by woody sensory attribute (0.817) – Table 5. The score plot of PCA shows a clear differentiation of oils obtained from different rapeseed varieties. As shown in Figure 2, YSRO and HORO are placed in the right portion of the score plot, in contrast to oils obtained from regular rapeseed varieties, which are in the left portion. Figure 2 also showed the positioning of the oil samples with respect to the intensity of the sensory attributes. ROs were similarly characterized by nutty and seed-like sensory attributes, the most noticeable sensory attributes in YSRO were woody and strawy, while astringent sensory attribute was strongly perceivable in HORO.

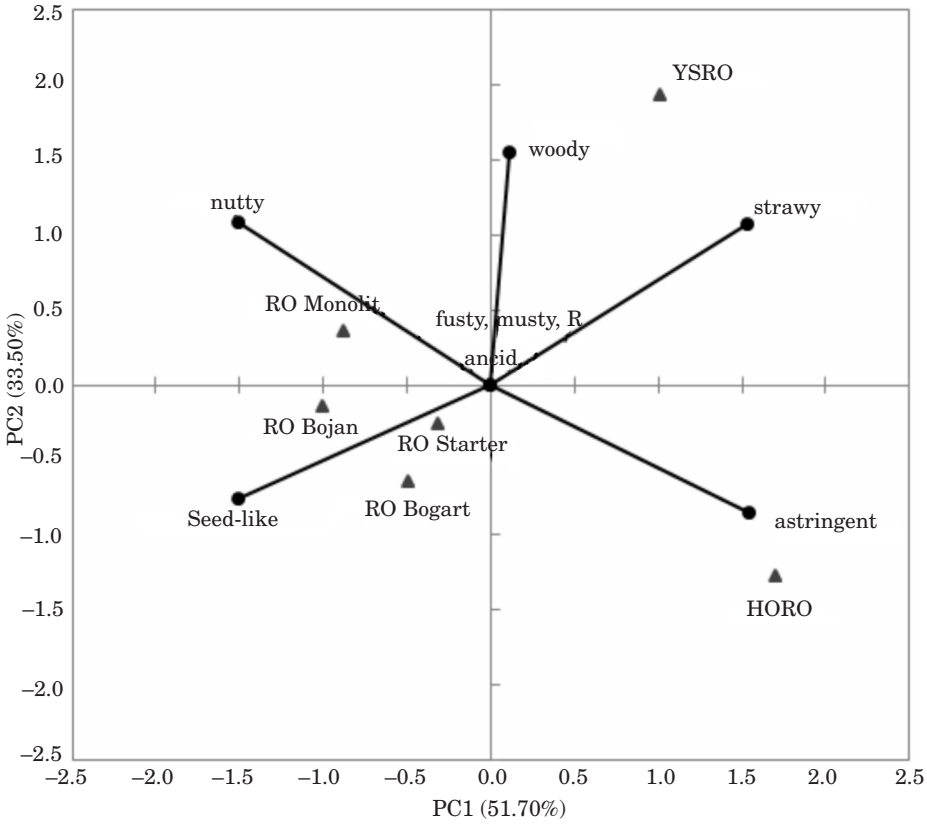


Fig. 2. Principal component analysis (PCA) based on sensory attributes profiling analysis of ROs, HORO and YSRO produced by cold-pressing

Table 5

Principal component analysis (PCA) factor loadings for the sensory attributes of ROs, HORO and YSRO produced by cold-pressing

Sensory attributes	PC1	PC2
Seed-like	**-0.796**	-0.398
Nutty	**-0.795**	0.571
Woody	0.056	**0.817**
Strawy	**0.808**	0.567
Astringent	**0.815**	-0.448
Rancid, fusty, musty	0.000	0.000

Values in bold are loadings with an absolute value greater than 0.70

Conclusions

The quality parameters of all cold-pressed rapeseed oils were within Codex Alimentarius limits which testifies to the high quality of the seeds used in the research. HORO was marked by the highest oxidative stability (IP = 6.54 h), most likely due to the lowest amount of PUFAs (15.1%), in contrast to YSRO, which has the lowest induction period (3.51 h) and the highest PUFAs concentration (30.9%). The highest pigments content was found in RO obtained from seeds of Monolit variety (23.09 mg kg^{-1}), while YSRO had nearly 3-fold lower pigments concentration (8.26 mg kg^{-1}). The highest total tocopherols content was found in conventional RO acquired from seeds of Starter variety (67.1 mg/100 g), which was also marked by the highest α-tocopherol concentration (28.4 mg/100 g), while γ-tocopherol was present in the largest concentration in HORO and YSRO (42.4 and 41.8 mg/100 g, respectively). Principal component analysis differentiated cold-pressed oils based on their sensory assessment.

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CHARACTERISTICS OF SORPTION PROPERTIES OF SELECTED POWDERED FOOD PRODUCTS

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K e y w o r d s: full-fat milk powder, powdered soybean drink, sorption isotherms, water activity, sorption kinetics.

A b s t r a c t

The goal of this study was to evaluate sorption properties of two selected powdered food products – full-fat milk powder and powdered soybean drink.

The sorption properties of these products were determined with the static method based on the evaluation of water vapor sorption isotherms and with the dynamic method by assaying water vapor sorption kinetics. The mathematical interpretation of the course of water vapor sorption isotherms was conducted with the use of the Brunauer, Emmett and Teller (BET) equation in a water activity range of $0.07 \leq a_w \leq 0.33$.

The kinetics of water vapor sorption was determined in the environment with relative humidity of – a_w = 0.33, 0.64, 0.98, within 48 h.

Results achieved in the study demonstrated that the differences in the sorption properties of the analyzed products were determined, most of all, by various technological processes applied by particular producers and by different chemical composition of the investigated products.

CHARAKTERYSTYKA WŁAŚCIWOŚCI SORPCYJNYCH WYBRANYCH PRODUKTÓW ŻYWNOŚCIOWYCH W PROSZKU

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Abstrakt

Celem badań była ocena właściwości sorpcyjnych dwóch wybranych produktów żywności w proszku – mleka w proszku pełnego oraz proszku napoju sojowego.

Oceny właściwości sorpcyjnych dokonano metodą statyczną na podstawie oceny izoterm sorpcji pary wodnej i metodą dynamiczną przez wyznaczenie kinetyki sorpcji pary wodnej. Do matematycznej interpretacji przebiegu izoterm sorpcji pary wodnej zastosowano równanie BET w zakresie aktywności wody $0{,}07 \leq a_w \leq 0{,}33$.

Kinetykę sorpcji pary wodnej wyznaczono w środowisku o wilgotności względnej – a_w = 0,33; 0,64; 0,98 w czasie 48 godzin.

Na podstawie przeprowadzonych badań stwierdzono, że różnice właściwości sorpcyjnych badanych produktów w proszku determinowane były przede wszystkim odmiennym procesem technologicznym zastosowanym przez poszczególnych producentów badanych produktów, jak również różnicami w składzie chemicznym badanych produktów.

Introduction

Food powders available on the food market are examples of convenient and stable foodstuffs characterized by diversified composition as well as physical and hygroscopic properties. Their assortment is being increased through multiple modifications of the already existing products and through the introduction of novel products targeted at a specified segment of consumers, e.g. vegetarians.

Plant-based powdered beverages are gluten-free and lactose-free products used to prepare hot or cold dairy drinks or applied as coffee additives. An example of such an innovative plant product is a powdered soybean drink being an alternative for lactose-intolerant consumers unable to use food products based on cow's milk powder.

Food powders produced by, e.g. spray drying are characterized by a low initial water activity at a level of $a_w = 0.15 \div 0.40$, as well as by hygroscopicity and easy water absorption from the environment, which affects their quality and storage stability (KOWALSKA et al. 2011). This study was aimed at evaluating sorption properties of two selected food powders – full-fat milk powder (product of animal origin) and powdered soybean drink (product of plant origin), by determining sorption kinetics and plotting water vapor sorption isotherms.

Material and Methods

The experimental materials were powdered food products: product I – full-fat milk powder produced by Dairy Cooperative Mlekpol' Grajewo, and product II – powdered soybean drink, Soymil produced by SVEN, made in the Czech Republic and imported by GOMIX enterprise from Białystok.

The purchased products were stored in their original packages, in a dry and cool place (following producers recommendations provided on packages, temp. ≥ 20°C, the relative air humidity ≥ 75%). The experimental part of this study was carried out at the laboratory of the Maritime Academy in Gdynia.

The investigated products were characterized by different chemical composition and ingredients, as declared by respective producers on the package (Table 1). Product II was characterized by a lower energy value and protein content and by higher contents of carbohydrates and fat, compared to product I.

Table 1
Chemical composition of the analyzed powdered dairy drinks, declared by producers on the unitary package

Product	The raw material composition	Parametr	The value (100 g)
I	–	energetic value [kcal] protein [g] carbohydrates [g] fat [g]	496 26.5 38.0 26.5
II	corn syrup, soybean oil, sodium caseinate, mono- and diglycerides of fatty acids, soybean lecithin, emulsifier, natural dye – annatto	energetic value [kcal] protein [g] carbohydrates [g] fat [g]	450 2.4 66.1 28.1

Methods

Determination of water content. Water content was determined by drying the samples (ca. 2 g ± 0.0001 g) at a temperature of 105°C for 1 h (KREŁOWSKA-KUŁAS 1993).

Determination of water activity. Water activity was determined in the AquaLab apparatus, with an accuracy of ± 0.003 (Series 3 model TE, Decagon Devices USA) at a temperature of 25 ± 1°C.

Determination of sorption kinetics. Kinetics of water vapor sorption in products I and II were was determined by the dynamic method, using a measuring stand that enabled keeping a constant temperature of the measurement at 25°C and stable water activity of the environment at the level of a_w = 0.33, 0.64, and 0.98. The water vapor sorption kinetics were determined for 48 h. Kinetic curves represented graphic description of changes in the quantity of water (g/100 g d.m.) adsorbed in time.

Determination of sorption isotherms. The course of water vapor sorption isotherms in products I and II was determined with the static-exsiccator method. The assay was conducted in a water activity range of 0.07 ÷ 0.98, at a temperature of 25°C, in 3 replications (TYSZKIEWICZ 1987).

The time necessary to reach system equilibrium reached 45 days. Crystalline thymol was introduced into the exsiccators with water activity above 0.7 to prevent microflora growth in the samples. The initial weight of the product and changes in water content enabled calculating the equilibrium water content and plotting sorption isotherms with the use of EXCEL program.

Determination of sorption properties based on BET model. For the mathematical description of empirically-determined sorption isotherms and for the determination of sorption properties of the analyzed food powders, the sorption isotherms were transformed using the Brunauer, Emmett and Teller (BET) equation in a water activity range of $0.07 \leq a_w \leq 0.33$ (PADEREWSKI 1999, TYSZKIEWICZ 1987).

$$a = \frac{\nu_m \cdot c \cdot a_w}{(1 - a_w)[\ + (c - 1) \cdot a_w]} \tag{1}$$

where:
a – adsorption [kg kg^{-1}];
ν_m – water content in the monolayer [g H_2O/100 g d. m.];
c – constant, related in an exponential way with the difference between adsorption heat on the first and following layers, accepted as stable and equal to the condensation heat;
[Pa]; a_w – water activity [-] (OŚCIK 1983).

The specific surface of adsorbent was computed based on the following formula:

$$a_{sp} = \omega \frac{\nu_m}{M} N \tag{2}$$

where:
a_{sp} – sorption specific surface [m^2/g d. m];
M – water molecular mass [18 g/mol];
N – Avogadro number, (6.023×10^{23} molecules/mol);
ω – water setting surface, (1.05×10^{-19} m^2/molecule) (PADEREWSKI 1999).

Results and Discussion

The study included determinations of water content and water activity in the analyzed products I and II. Based on the evaluation of the products sampled directly from packages, a higher initial water content and a lower water activity were determined in product II – powdered soybean drink (Table 2).

Table 2

Moisture content and water activity of investigated powder products I and II

Product	Water content [g /100 g d. m.]	SD	Coefficient of variation	Water activity [–]	SD	Coefficient of variation
I	2.57	0.02	0.78	0.254	0.002	1.00
II	3.35	0.02	0.66	0.151	0.005	3.67

SD – standard deviation

In the analyzed products the above parameters were, probably, determined by technological processes applied by their producers as well as by the resultant of water content in the products and the extent of its binding with a product's matrix.

The kinetics of water vapor sorption is influenced by many factors. A powdered food product is a heterogenous material in terms of both the chemical composition and water vapor sorption ability. The kinetics of sorption is additionally affected by the character of raw material, diversified surface of the product as well as by the relative air humidity and temperature (MARZEC and LEWICKI 2004, RUSZKOWSKA 2010).

The evaluation of the course of sorption kinetic curves, in the environment with water activity of a_w = 0.64; 0.98, within 48 h of measurement, demonstrated an increase in water content compared to its initial value in both analyzed products (Figure 1).

In the case of product I, the course of water vapor sorption kinetics indicated the occurrence of water vapor adsorption within 48 h in the medium with water activity of a_w = 0.33 and 0.98. Product I was characterized by a higher water vapor adsorption ability compared to product II. In the environment with water activity of a_w = 0.64, the greatest changes in water content of product I, corresponding to the process of adsorption, were observed within the first 9 h of the measurement (9.04 g/100 g d.m.). Afterwards, the product was successively releasing water and after 48 h its water content reached 8.09 g /100 g d.m. (Figure 1).

The evaluation of the course of water vapor sorption kinetics in product II demonstrated that in the environment with water activity of aw = 0.33 within 48 h of measurements, water vapor was subject to the desorption process and the analyzed soybean milk powder reached a lower water content (3.28 g /100 g d.m.) compared to its initial level (3.35 g/100 g d.m.). In the studied water activities of the environment: a_w = 0.64; 0.98, it was noted that the course of water vapor sorption kinetics of product II was similar to that of product I (Figure 1). However, at the evaluated water activities (a_w = 0.69; 0.98) product II was characterized by an insignificantly lower kinetics of the water vapor

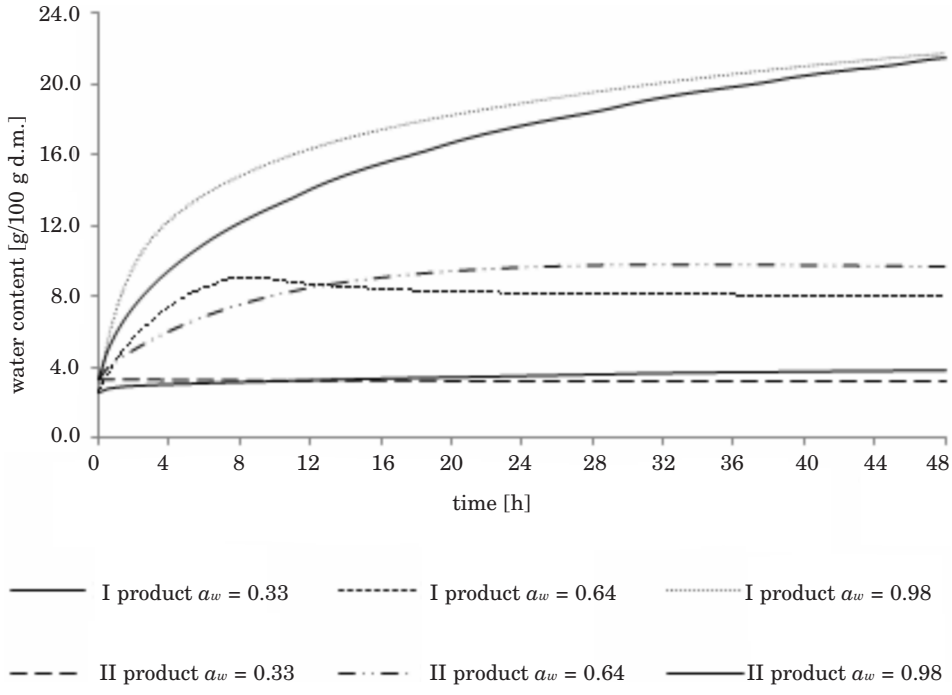

Fig. 1.Vapor sorption kinetics of product I and II, in an environment with a water activity of a_w = 0.33; 0.69; 0.98

sorption process, compared to product I. Consequently, after 48 h of measurements of water vapor sorption kinetics, a lower water content was demonstrated in product II (powdered soybean drink) – Figure 1.

Based on the comparative analysis of the kinetic curves (Figure 1), it was concluded that their course was significantly affected by the ratio of product water content and environment humidity, as the differences in the water content of a food product and humidity of the environment determine the difference in humidity potential and propelling force of the process. Therefore, the higher the water activity of the environment (a_w = 0.64; 0.98) the samples were kept in, the higher their water absorbability.

The course of the sorption process kinetics was also, probably, influenced by the thermodynamic status of the analyzed system that had been determined by the affinity of the investigated products to water, whereas diversity of raw materials used in the production process of product II was affecting the intensity of the course of the sorption phenomenon (Table 1) (OCIECZEK et.al. 2015).

This study included also determinations of water content in products I and II that was assayed after 48 h of sorption kinetics from water vapor sorption isotherm.

The comparative evaluation of experimental results (Table 3) demonstrated that after 48 h of the process in the environment with water activity of $a_w = 0.33, 0.64, 0.98$, the content of water in the analyzed products I and II was lower than the equilibrium water content determined from the course of the sorption isotherm (Table 3). The results achieved showed that after 48 h, the examined products did not reached the state of the real thermodynamic balance with the environment. This indicates that the state of the real equilibrium of relative humidity of the analyzed products I and II can only be reached in a longer process.

Table 3

The data of equilibrium water content achieved in the steam kinetics process determined from the steam adsorption isotherms in product I and II

Product	$a_w = 0.33$		$a_w = 0.64$		$a_w = 0.98$	
	A	*B*	*A*	*B*	*A*	*B*
I	3.28	4.64	9.64	11.15	21.56	27.70
II	3.91	4.87	8.09	14.86	21.75	32.50

A – Water content after 48 h sorption kinetics [g/100 g d. m.]
B – Equilibrium water content calculated from the sorption isotherms [g/100 g d. m.]

Sorption isotherms constitute graphical display of correlations between moisture content of the analyzed product and water activity in a specified temperature, thus being a tool to determine thermodynamic interactions between water molecules and a food matrix. The preliminary evaluation of sorption isotherms of products I and II was conducted by comparing the mutual location of the isotherms (Figure 2). The analyzed products I and II were characterized by the sigmoidal course of water vapor sorption isotherm.

In the water activity range of $a_w = 0.07 \div 0.98$, the highest sorption capacity was determined for product II – soybean drink powder (Table 4). The shape of sorption isotherm of product I, in the water activity range of $a_w = 0.33 \div 0.44$, was characterized by the interruption of curve continuity manifested by a decreased level of equilibrium moisture content of this product (Table 4). It is likely that water absorption was accompanied by structural changes resulting from the increased degree of ordering of particular components of product I. The water content and water activity achieved by product I in the environment with water activity of $a_w = 0.33 \div 0.44$, were indicative of the ongoing process of lactose crystallization, i.e. its transition from the amorphous into the crystalline state. The decrease of the equilibrium water content in product I occurred at the simultaneous increase of water activity in the product (Table 4). A possible reason for the discontinuity was a phase transformation of lactose as one of the most important milk constituents. Storage stability and its deriva-

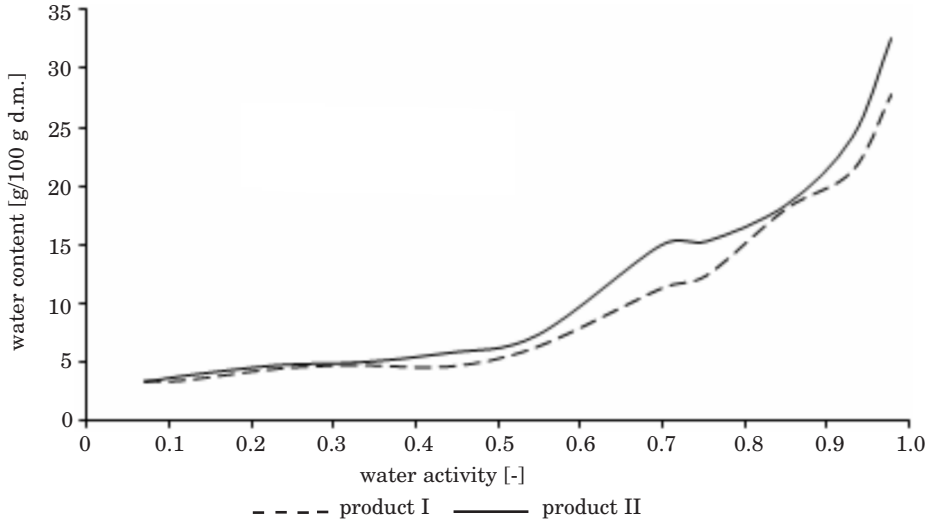

Fig. 2. Sorption isotherms of product I and II

tive, i.e. the nutritional quality of powdered milk, largely depend on the physical state of lactose which may be amorphous or crystalline. As a result of the rapid removal of water from milk during drying, fully-hydrated lactose in an amorphous state and of low viscosity is stabilized while transformed into a glassy amorphous state. This state is unstable at temperatures below the temperature of glass transition (T_g). Hydration of a product or exceeding the T_g temperature is a factor that favours glass transition and crystallization of lactose. Under such conditions, a glassy state transforms into a viscous-elastic state, which is also called rubber-like, which results in a substantial increase in the mobility of lactose particles and leads to its gradual crystallization (OCIECZEK 2014, OLKOWSKI et al. 2012). The occurrence of crystalline lactose results in a balance between water content and its activity being set at a new level, which is reflected in a disruption of the continuity of a sorption isotherm. The phase transformation of lactose causes substantial changes in the nutritional quality of powdered milk due to an increase in the oxidation rate of other milk constituents, for instance, milk fat, non-enzymatic browning and a reduced capacity for rehydration (OCIECZEK 2014, FITZPATRICK et al. 2007). As a result of lactose crystallization, water is released, which favours lactose hydrolysis to glucose and galactose.

No decrease in water content was observed in product II in the environment with the water activity of $a_w = 0.33 \div 0.44$, whereas after 45 days of water vapor sorption isotherms determination this product reached higher content and activity of water, compared to product I (Table 4).

The course of sorption isotherms in the water activity range of $a_w = 0.07 \div 0.33$ enabled determining parameters of the BET equation (V_m, a_w) by assaying the degree of its fit (r^2, FitStdErr) to empirical data. Respective results were presented in Table 5. The capacity of the monolayer (V_m) determined based on the BET equation describes the accessibility of polar sites to water vapor and indicates the volume of water that is strongly adsorbed by the specific sites and is considered optimal to ensure a high stability of food products. The analysis of the achieved capacity of the monomolecular layer of the powdered soybean drink (product II) suggest that changes proceeding in this product during storage will be less intensive than in product I – full-fat milk powder.

Table 4

The moisture and water activity of the products I, II, after 45 days of storage

Water activity of the environment [–]	Product I		Product II	
	ąwater content [g/100 g d. m.]	water activity [–]	water content [g/100 g d. m.]	water activity [–]
0.07	3.311	0.126	3.161	0.113
0.11	3.293	0.140	3.654	0.131
0.23	4.317	0.285	4.626	0.279
0.33	4.636	0.341	4.874	0.330
0.44	4.514	0.461	5.667	0.434
0.53	6.200	0.551	7.178	0.521
0.64	11.154	0.743	14.857	0.773
0.75	12.290	0.781	15.187	0.805
0.84	17.974	0.833	18.298	0.841
0.93	21.200	0.880	24.066	0.889
0.98	27.698	0.937	32.499	0.952

Table 5

The BET equation parameters

Product	ν_m	a_w	R^2	FitStdErr	Specific surface of sorption asp [$m^2\ g^{-1}$]
I	3.19	0.220	0.936	0.250	112
II	3.39	0.216	0.934	0.218	119

R^2 – determination coefficient; FitStdErr – standard error

The observed differences in monolayer capacity (V_m) were probably a consequence of the physical state of particular components rich in polar sites, but also a consequence of their varying physical state and to the occurring interactions that contribute to the formation of additional hydrogen bonds.

The V_m value enabled calculating the specific surface of sorption. The results achieved (Table 5) demonstrated product II (a_{sp} = 119 $m^2\ g^{-1}$) to be characterized by a greater specific surface, than in product I (a_{sp} = 112 $m^2\ g^{-1}$).

Conclusions

The study demonstrated that the course of kinetic curves was determined by the ratio of products moisture content and environment humidity, which constituted the propelling force of the process. The course of sorption process kinetics enabled concluding that the examined powders I and II tended to reach the state of humidity balance with the surrounding atmosphere.

The shape of water vapor sorption isotherms plotted for the analyzed products was typical of the products with a complex composition. Minimal differences in the shape of sorption isotherms of the products were, probably, due to changes in the conformation of macromolecules determined by various technological processes. The process of lactose crystallization in product I, proceeding in the environment with $a_w = 0.33 \div 0.44$, contributed to diminished equilibrium water content and increased water activity in the analyzed products.

Results achieved in the study enable concluding that the differences in the sorption properties of the analyzed products were determined, most of all, by various technological processes applied by particular producers and by differences in their chemical composition. It can be expected that the sorption properties of the tested products were determined, inter alia, type of protein in the milk powder – casein, and caseinate and soy product having improved properties of affinity for water.

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THE EFFECT OF LOW- AND HIGH-PRESSURE HOMOGENIZATION ON *IN VITRO* MILK FAT DIGESTION BY LIPASE

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Key words: milk fat, homogenization, lipase, digestion.

Abstract

The object of the study was to compare the effect of low- and high-pressure homogenization of milk on fat digestion in vitro. The extent of lipid digestion by lipase with and without addition of bile salts and phospholipid was monitored by determining the release of fatty acids. Additionally changes in the milk fat emulsion were monitored during the process of lipolysis. Higher amounts of fatty acids were released from samples of homogenized milk fat globules as compared to native fat globules in raw milk. Bile salts caused an increase in the quantity of released fatty acids in both samples of milk, while this increase was more pronounced in raw milk. However, considering the available surface area of milk fat globules, the amount of released fatty acids was higher in the case of raw milk, which can reflect the adaptation of the structure of the interface to the lipase-colipase-bile salts digestive system. An important role in the digestion of triglycerides under the influence of lipase can play both the structure of the interface and the degree of dispersion of milk fat.

WPŁYW HOMOGENIZACJI NISKO- I WYSOKOCIŚNIENIOWEJ NA TRAWIENIE TŁUSZCZU MLEKOWEGO W WARUNKACH *IN VITRO*

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Słowa kluczowe: tłuszcz mlekowy, homogenizacja, lipaza, trawienie.

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Abstrakt

Przedmiotem badań było porównanie wpływu homogenizacji nisko- i wysokociśnieniowej na trawienie tłuszczu mlekowego metodą *in vitro*. W badanych próbkach mleka surowego i homogenizowanego oznaczano ilość uwalnianych kwasów tłuszczowych pod wpływem lipazy, dodatkowo w obecności soli żółciowych i fosfolipidu. W trakcie procesu lipolizy monitorowano także zmiany w układzie emulsji tłuszczowej mleka. Stwierdzono większe ilości uwalnianych kwasów tłuszczowych w przypadku kuleczek tłuszczowych poddanych homogenizacji w porównaniu z natywnymi kuleczkami tłuszczowymi w mleku surowym. Sole żółciowe wpłynęły na wzrost ilości uwolnionych kwasów tłuszczowych w obu próbkach mleka, przy czym wzrost ten był wyraźniejszy w mleku surowym. Jednak biorąc pod uwagę dostępną powierzchnię międzyfazową, ilość uwolnionych kwasów tłuszczowych była wyższa w przypadku mleka surowego, co może świadczyć o wzajemnym dopasowaniu struktury otoczki do systemu trawiennego lipaza–kolipaza–sole żółciowe. Istotną rolę w trawieniu triacylogliceroli pod wpływem lipazy może odgrywać zarówno struktura powierzchni międzyfazowej, jak i stopień dyspersji tłuszczu mlekowego.

Introduction

Milk fat is an essential component of human diet. It is found in milk in the form of milk fat globules ranging in size from 0.2 to 15 μm, with an average size of 4 μm. The structure of milk fat globules consists of a triacylglycerol „core" surrounded by so-called, the milk fat globule membrane of a thickness of approx. 10–20 nm. It protects against coalescence of the globules, but also affects the process of hydrolysis by lipases. The membrane is characterized by a complex and ordered structure. It consists of triple layer of phospholipids, proteins, cholesterol and vitamins (MATHER 2000, SMOCZYŃSKI et al. 2012).Digestion of milk fat in the human body is a complex process. It begins in the stomach under the influence of gastric lipase, but the main process of digestion takes place in the duodenum and upper small intestine under the influence of pancreatic lipase. This enzyme acts together with colipase at the fat-water interface, where changes in the conformation of the enzyme lead to the unveiling of the catalytic triad for the substrate (BROCKMAN 2000).

An important role in the digestion of fat components plays bile, a fluid secretions of the liver, stored in the gallbladder. It includes components such as bile salts, cholesterol, phospholipids and minerals. They support the digestive process by modifying the structure of the fat-water interface, thus enabling lipase access to triacylglycerols located inside the milk fat globules. Studies on artificial emulsions have shown that hydrolysis of fat in the emulsion depends on both the particle size distribution of the emulsion (i.e. the available interfacial area), the type of substrate, but also on the structure and composition of the interface (BERTON et al. 2012, MUN et al. 2007, SINGH et al. 2009). Because milk as a mammary gland secretion evolved to provide optimal nutrition for the offspring, hence the composition and structure of the milk fat

globule membrane should play an important role in the digestion and bioavailability of milk fat (SMOCZYNSKI 2014).

Homogenization during milk production causes drastic changes to the structure of the milk fat globule membrane. The process consists of pushing milk between the valve seat and the homogenizing valve under pressure of 8–20 MPa. The sharp increase in the flow rate and rapid decline of pressure rupture the fat globules. As the area of the droplets increases, the interfacial material is not sufficient to cover it. So it is covered by other surface active constituents of milk, mainly casein and whey proteins (CANO RUIZ, RICHTER 1997). This results in a new, modified structure of the milk fat globule interface.

A better understanding of the relationship between changes in the native structure of the interface and the digestion of milk fat may be important not only for infants, but also adults who consume dairy products. Studies on the effect of the milk fat structure on its lipolysis, absorption and lipid profile have been widely discussed recently (MICHALSKI 2009), and may be particularly relevant to the problems of modern society such as obesity and type II diabetes. Despite the progress, many mechanisms involved in digestion of fat, particularly in complex matrices, such as food products, are not completely understood.

In studies on natural emulsions it has been shown that the increase in the interfacial area by homogenization leads to an increase in the amount of released fatty acids (BERTON et al. 2012). However, little attention was paid to the high-pressure homogenization. This process may lead to a different structure than that obtained with low-pressure, due to increased shear forces. Hence, the aim of this study was to compare the availability of milk fat in globules surrounded by native membrane with membranes changed under the influence of high- and low-pressure homogenization. In the study, an *in vitro* model was used, simulating the intestinal phase of digestion under the influence of pancreatic lipase with and without the addition of bile salts and phospholipid (phosphatidylcholine). During the process of lipolysis the amount of free fatty acids released was determined and also changes in the milk fat emulsion were monitored.

Materials and Methods

Raw cow's milk was purchased from a local supplier. To minimize changes to the native globules milk samples were stabilized by the addition of sodium azide (0.05% w/w) instead of pasteurization. Porcine pancreatic lipase (type II, comprising a complex of lipase and colipase), bile salts (mixture of cholic (50%) and deoxycholic (50%) sodium salts) and phosphatidylcholine (from egg yolk, 60%) were purchased from Sigma-Aldrich. All other chemicals were analytical grade. Solutions were prepared using deionized water.

Part of the milk was heated to a temperature of 60 ± 2°C and homogenized at 200 ± 5 bar (low pressure homogenization) and 1000 ± 50 bar (high pressure homogenization) using a two-stage high-pressure homogenizer Panda Plus 2000 (Niro-Soavi, Parma Italy). Analyses were carried out within 48 hours after preparation of milk samples. In the further course the raw milk was designated as milk 1, low-pressure homogenized milk as milk 2 and high-pressure-homogenized milk as milk 3.

Procedure for simulating the intestinal phase of digestion and intestinal fluid composition was adapted from the literature (MALAKI NIK et al. 2010, NEUMANN et al. 2006, WULFF-PEREZ et al. 2010, YE et al. 2010). Porcine pancreatic lipase (showing also activities of amylase and protease) was used for the in vitro digestion of fat globules. This enzyme has already been used to simulate the intestinal phase of digestion in vitro (MUN et al. 2007, TORCELLO-GOMEZ et al. 2011). The simulated intestinal fluid (SIF), pH 6.8 contained 6.805 g of KH_2PO_4 and 0.896 g of NaOH in 1 liter (United States Pharmacopeia 2003). The process of simulated digestion proceeded in 300 ml plastic bottles in a shaking water bath at 37°C to mimic conditions of the intestinal tract. The milk samples were pre-adjusted to pH 7.5 using 0.25 mol l^{-1} sodium hydroxide. The temperature of 100 ml of milk sample was stabilized for 15 min and then 50 ml of SIF containing the enzyme with/without the addition of bile salt and phospholipid was added. The final enzyme, bile salt and phospholipid concentration in the milk sample were 1.5, 5 and 3.8 mg ml^{-1}, respectively. The concentrations were chosen based on the literature and corresponded to the fed state conditions (MCCLEMENTS et al. 2009). The enzyme was used within 30 minutes after preparation to avoid protein denaturation. Samples from the reaction mixture were periodically selected for analysis. Lipase activity was determined by measuring the amount of free fatty acids released from the milk samples by titration with a 0.1 mol l^{-1} sodium hydroxide to pH 7.5 with the use of a Titro Line easy titration unit (Schott Instruments, Mainz, Germany), a pH-meter (Mettler Toledo, Schwerzenbach, Switzerland) and a magnetic stirrer. The lipase activity was expressed as miliequivalents of fatty acids released from 1 l of milk sample defined as:

$$mEq = \frac{V \cdot C}{1}$$

where:
V – ml of sodium hydroxide,
C – molar concentration of sodium hydroxide.

The particle size distribution in the milk samples was determined by measuring laser light scattering using the apparatus Mastersizer 3000 (Malvern Instruments Ltd, Worcestershire, UK). The test sample was added to a measuring cell to obtain obscuration in the range of 10 ± 3%. The refractive index for the dispersion medium and the fat globules was 1.33 and 1.46, respectively. From the size-distribution the volume/surface average diameter of fat globules (defined as $D_{3,2} = \Sigma n_i d_i^3 / \Sigma n_i d_i^2$) and the specific surface area were calculated by the software.

The analysis was performed in duplicate on three batches of milk. Results are presented as the mean of at least 5 determinations ± standard deviation. The results were statistically analyzed using one-way analysis of variance (ANOVA). To test the differences ($P < 0.05$) between the average values Fisher's test was applied. The analyzes were performed using Statistica version 10 for Windows.

Results and Discussion

Triacylglycerols are non-polar and requires the development of appropriate mechanisms for their effective management in a hydrophilic environment of the cell. During digestion and subsequent absorption by the epithelial cells in the intestine, they must be converted to the more polar compounds, such as fatty acids or monoacylglycerols. These compounds as more polar are able to diffuse through the aqueous diffusion layer on the epithelial cells of the intestine and can be subsequently absorbed (BAUER et al. 2005, SMOCZYNSKI et al 2012).

Homogenization leads to significant changes in the structure the milk fat globule membrane. Modified interface may affect the access of the lipase to the core of fat globules, thereby affecting the lipolysis and the amount of released fatty acids. During digestion of fat the process of lipolysis takes place mainly in the duodenum and upper small intestine. Therefore in this study a model simulating the intestinal digestion was used. Figure 1 shows the amount of released free fatty acids with/without the presence of bile salts in the course of the experiment.

The differences in the amount of released fatty acids can be observed. For both samples of homogenized milk, regardless of the presence of bile salt and phospholipid, the amount of released fatty acids was higher than in the raw milk. Particularly significant difference was observed in the absence of bile salt and phospholipid. After 30 min of lipolysis amount of acids released in the homogenized milk was almost two times higher than in raw milk (23.4 for raw milk and 42.9 and 42.7 $mEq\ l^{-1}$ for low and high pressure homogenized samples of milk, respectively). The addition of bile salt and phospholipid to a significant degree caused an increase in the amount of released fatty acids

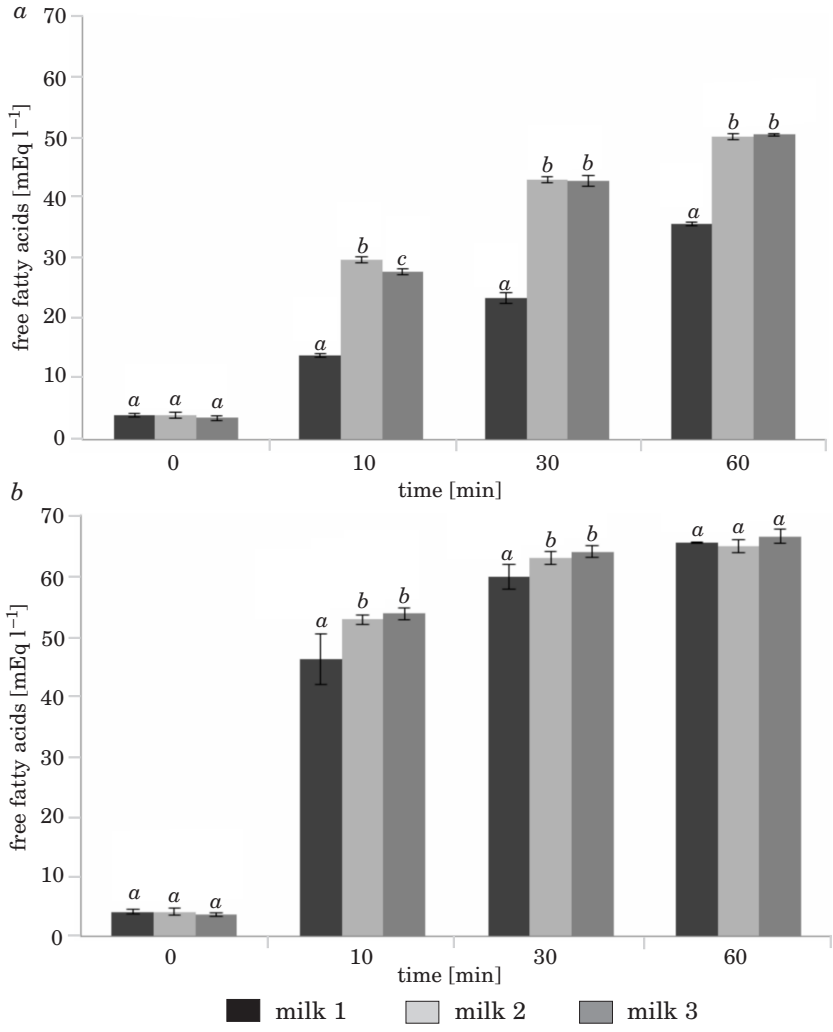

Fig. 1. Free fatty acids measured during lipolysis without (*a*) and with (*b*) bile salt and phospholipid. Values with the same letter are not significantly different ($P<0.05$)

in all milk samples. In the case of raw milk the amount of released fatty acids increased to a greater extent, especially after first 10 min. After 60 min of lipolysis in the presence of bile salt and phospholipid there was no difference in the amount of released fatty acids in all three milk samples examined. This may indicate an advanced degree of hydrolysis and reduction in the availability of substrate for lipase.

Changes in the emulsion system were additionally monitored with the use of the Mastersizer apparatus. Figure 2 shows changes in the distribution of milk fat globules sizes during the process of lipolysis.

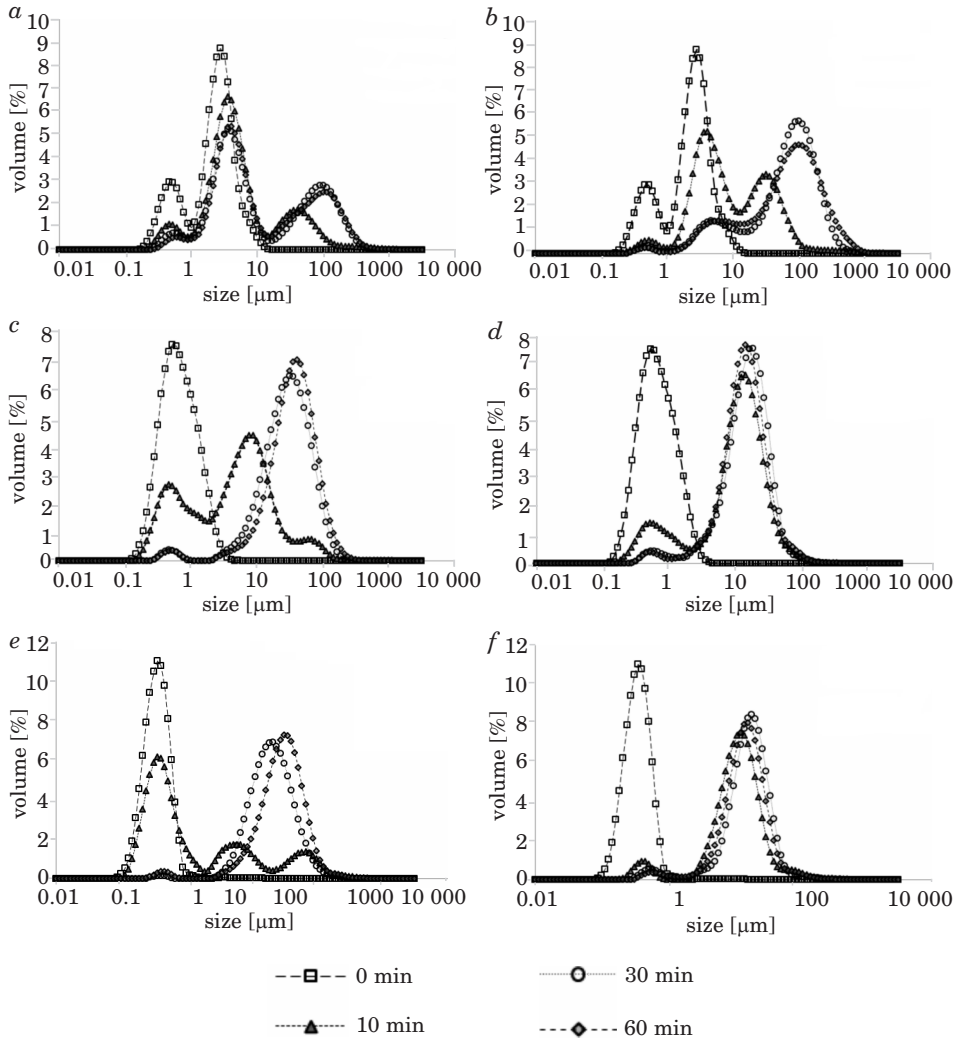

Fig. 2. The changes of sizes of milk fat globules during lipolysis: *a, c* and *e* – milk 1, 2 and 3, respectively; *b, d* and *f* – the same types of milk with the addition of bile salt and phospholipid

The results in Figure 2 show that the smallest changes in the emulsion droplet sizes occurred in raw milk in the absence of bile salt and phospholipid. After 10 min of lipolysis small amount of particles with sizes in a range of 10–100 μm appeared, most likely due to flocculation and eventually coales-

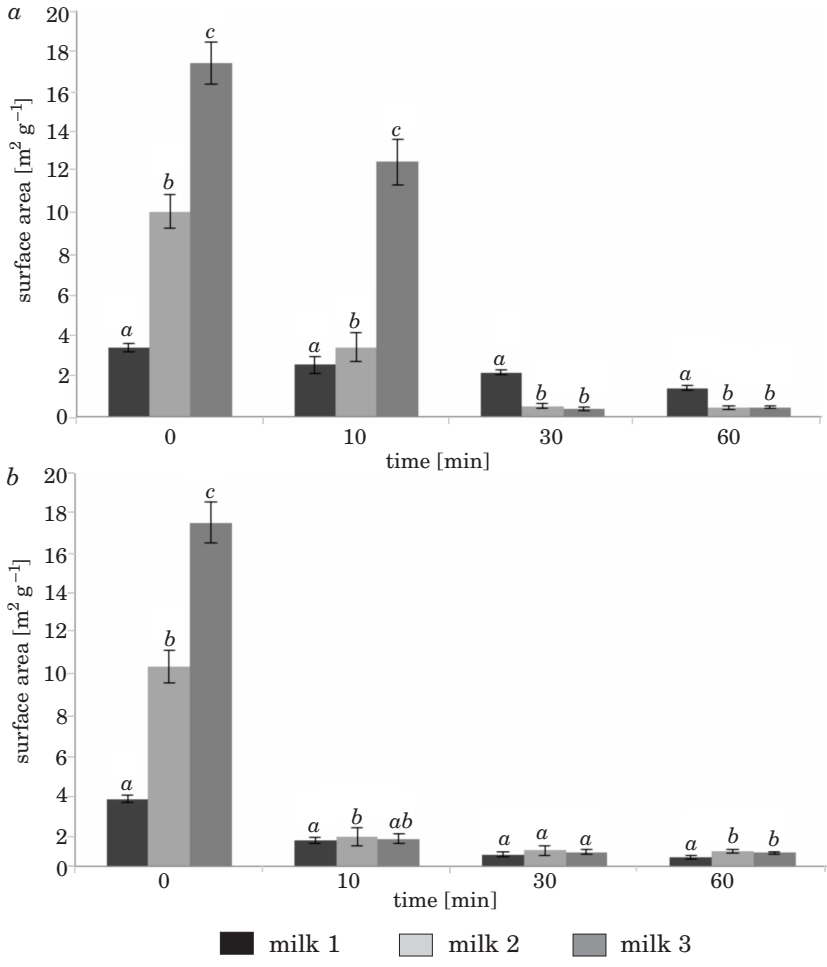

Fig. 3. Changes of surface area of milk fat globules during lipolysis without (*a*) and with (*b*) bile salt and phospholipid. Values with the same letter are not significantly different (P<0.05)

cence of fat globules. After 30 min both the quantity and size of these aggregates increased. In the presence of bile salt and phospholipid the changes were more pronounced. It can be seen a greater loss of native particles present in raw milk, and more significant increase in the number of aggregates with sizes ranging from 50 to 150 μm. At this time most of native fat globules were aggregated, which may indicate more advanced process of lipolysis. This is in agreement with the results of determination of released fatty acids.

In case of homogenized milk samples similar changes occurred, but much faster. After 30 min even in the absence of bile salts and phospholipid, most of the fat globules were aggregated. However, in this case the size of the resulting aggregates was smaller, most of them in the size range from 8 to 30 μm.

Figure 3 shows changes in available surface area during the process of lipolysis. The values of Sauter diameter for raw, low- and high-pressure homogenized milk samples were 1.75, 0.59 and 0.34 μm, respectively. During lipolysis these values increased for all milk samples.

The highest specific surface (17.46 $m^2 g^{-1}$) was obtained for the high pressure homogenized milk sample. Large shear forces during homogenization allowed to obtain fat globules of smallest sizes. The surfaces of fat globules in low-pressure homogenized and raw milk sample were 10.15 and 3.43 $m^2 g^{-1}$, respectively. Statistically significant differences between the raw milk and homogenized milk samples can be observed during the whole time of lipolysis in the absence of salt and phospholipid. However, in the presence of these substances small differences can be visible only after 10 min.

The results show that under the influence of lipase fatty acids are released and at the same time flocculation and/or coalescence of milk fat globules occurs. The amount of fatty acids released depends mainly on the available interface, but also on the structure of the surface. After homogenization Sauter diameter decreased and available surface area of milk fat globules increased. Generally in both samples of homogenized milk greater amounts of fatty acids were released, which mainly depended on the greater available interface. This allows for efficient access of the enzyme to the substrate. Similar results were obtained by BERTON et al. (2012). They studied the kinetics of reaction under the influence of lipase using an automatic pH-stat device and showed a higher degree of lipolysis in homogenized milk, which was related to the available interfacial surface.

Also, YE et al. (2010) reported a lower level of lipolysis in pasteurized milk, compared to recombinant homogenized milk. However, BERTON et al. (2012) also pointed out, that given the available surface area of milk fat globules, lipolysis is more efficient for raw milk. Similarly, in this study the amount of fatty acids released per available surface area after 30 min of lipolysis without the presence of bile salt and phospholipid in raw milk was 6.8 Eq m^{-2}, while for the samples of low and high pressure homogenized milk these values were 4.25 and 2.45 Eq m^{-2}, respectively. In the presence of salt this difference is even higher. These values for samples of raw, low and high pressure homogenized milk were 17.4, 6.2 and 3.65 Eq m^{-2}, respectively. So considering the available interfacial area lipolysis occurs more efficiently in raw milk, which is particularly evident in the presence of bile salts and phospholipid. It is known that the lipase is an enzyme acting on the fat-water interface (VERGER 1997). It requires also colipase and bile salts. Bile salts and phospholipids also help to remove products of lipolysis from the interface, because this surface active particles can block the access of lipase to the substrate. Free fatty acids, mono- and diacylglycerols together with bile salts and phospholipids form a so-called mixed micelles, and in this form can be absorbed by the enterocytes in the intestine.

The results show on one hand a faster lipolysis in homogenized milk samples, but no significant differences can be observed between low and high pressure homogenization. This may affect further absorption of lipid substances in the intestine and also subsequent metabolism of fat, however, this requires further study. In contrast, the differences in the amount of fatty acid released per unit of the available surface area point to more efficient lipolysis in the case of native milk fat globules.

The results indicate a relative matching between the structure of the milk fat globule membrane and the lipase-colipase-bile salts digestive system. This adjustment of interfacial properties and the enzyme system could develop in the course of evolution to allow for efficient lipolysis. On the other hand, excessive emulsification of the substrate after homogenization may increase the role of diffusion of the enzyme and thus impede the efficient lipolysis. Also, the newly formed interface formed under the influence of large shear forces during homogenization may present a more compact structure. The slowest lipolysis per unit of interfacial area in high pressure homogenized milk may support this thesis. On the other hand a highest degree of dispersion of fat in this milk can slow down the process of lipolysis. In this context, the degree of dispersion in raw milk should perhaps be considered optimal for effective lipolysis.

After homogenization the increased surface area, besides fragments of native milk fat membrane (which are insufficient), is covered by other surface active particles, mainly casein and whey proteins. These proteins may be more difficult to remove from the interface and reduce the lipase access to the substrate, as demonstrated in other studies (Malaki Nik 2011, SARKAR et al. 2010). All of this points to the complexity of the hydrolysis of milk fat in the gastrointestinal tract and the need for further research in order to fully understand the role of the interface in the digestion of fat.

Conclusions

1. The study showed a significant role of both the structure and available interface in the process of milk fat digestion.

2. Homogenization of milk increases the release of fatty acids from the fat globules, without significant differences between the low and high pressure homogenization.

3. Given the available interfacial area lipolysis occurred most efficiently in the case of raw milk and native milk fat globules, which may indicate the compatibility of the milkfat globule membrane and the lipase-colipase-bile salts system.

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HYGIENE ASSESSMENT OF MILK SUPPLIED THROUGH DIRECT SALES

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K e y w o r d s: milk, microbiological quality, direct sales.

A b s t r a c t

The aim of this study was to determine the state of hygiene of milk supplied through various forms of direct sales; purchased from the individual producer, a dairy delivering products to the consumer's home, and from the milkomats located in the Tri-City. The milk samples were marked to determine the OLD at 30°C, the presence and enumeration of *Staphylococcus aureus* and the presence and number of E. coli and coliform. In total, 164 samples were examined. Milk from milkomats and the individual producer was characterized by a similar level of total microorganisms ratio of 3.4×10^6 cfu ml^{-1}. However, the biggest amount of *S. aureus* was detected in milk from individual suppliers ($1.44 \cdot 10^2$ cfu ml^{-1}), and the presence of coliform at the highest level ($7.1 \cdot 10^3$ cfu ml^{-1}) was noted in milk from a dairy plant.

OCENA STANU HIGIENY MLEKA POCHODZĄCEGO ZE SPRZEDAŻY BEZPOŚREDNIEJ

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S ł o w a k l u c z o w e: mleko, jakość mikrobiologiczna, sprzedaż bezpośrednia.

A b s t r a k t

Celem pracy była próba określenia stanu higienicznego mleka pochodzącego z różnych form sprzedaży bezpośredniej. Badano mleko nabywane od producenta indywidualnego, z zakładu mleczarskiego oferującego produkty do domu konsumenta oraz pochodzące z mlekomatów znajdujących się na terenie Trójmiasta.

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W próbkach mleka oznaczano ogólną liczbę bakterii mezofilnych tlenowych (OLD) w 30°C, obecność i liczbę *Staphylococcus aureus* oraz obecność i liczbę *E. coli* i pałeczek z grupy coli (coliform). Przebadano łącznie 164 próbki. Mleko pochodzące z mlekomatów i od producenta indywidualnego charakteryzowało się zbliżonym poziomem ogólnej liczby drobnoustrojów, wynoszącym $3{,}4 \cdot 10^6$ jtk ml^{-1}. Największe zanieczyszczenie *S. aureus* wykazywało natomiast mleko od dostawcy indywidualnego ($1{,}44 \cdot 10^2$ jtk ml^{-1}), a obecność coliform na najwyższym poziomie ($7{,}1 \cdot 10^3$ jtk ml^{-1}) cechowało mleko pochodzące z zakładu mleczarskiego.

Introduction

Milk is an important component of the diet of both children and adults. It is the base product of nutrition in childhood. Qualities of milk have many aspects, with the most important being milk as a source of protein with a well-balanced amino acid composition, easily absorbable calcium and group B vitamins. In addition to significant amounts of riboflavin, milk also contains fat-soluble vitamins. Strong bone structure developed during childhood is the best way to prevent osteoporosis in adulthood. Calcium is essential in development of bones and teeth. It also regulates the nervous system and musculature, acts as enzyme activator, and a clotting factor. A very important nutritional value of milk is the advantage of nutritional milk is the predominance of alkaline-forming elements over the acid-forming which determines the stability of the acid-base balance of blood (ZIAJKA 2008). According to worrying opinions of nutritionists and paediatricians, children, adolescents and adults drink less and less milk, and as a result their daily calcium intake does not exceed 50% of the RDA (KARCZMAREWICZ et al. 2002, LORENC, KARCZMAREWICZ 2001, SZYMELFEJNIK et al. 2005).

Due to its specific chemical composition, milk is a good medium for microbial growth whose main source of building material and energy is lactose. Components influencing the microbiological purity of milk are; sanitary conditions of obtaining the raw material, state of health of the cows, as well as the storage conditions in the place of production, transportation and sales. Microorganisms responsible for the udder inflammation (mastitis) are mainly *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, while *Escherichia coli, Pseudomonas aeruginosa, Listeria monocytogenes* and *Candida* and *Cryptococcus* are less likely to cause this condition. Significant contamination of raw milk with coliform rods signifies a lack of hygienic milking. These microorganisms, along with lactic acid bacteria contribute to the acidification of milk (ZIAJKA 2008).

Milk which has not been heat treated above 40°C ought to comply with all the requirements set out in the Regulations of the Minister of Agriculture and Rural Development of 18 May 2005 amending the regulation on veterinary

requirements for milk and milk products (Official Journal of 2 June 2005) and of 29 December 2006 on animal health requirements for the production of products of animal origin intended for direct sale (Journal of Laws of 2007. no. 5, poz. 38) controlling the size of the sales area, the requirements for places of production and sales, and the requirements which are to be met by the products themselves. Raw milk intended for direct sale must comply with the requirements outlined in Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004. According to these documents, the total of milk's microflora at 30°C should be less than 100.000 cfu per ml, with the number of *Staphylococcus aureus* up to 500 cfu per ml. Temperature of raw milk intended for direct sales can not be higher than 6°C, and its increase by 2°C is allowed only during transport, provided it does not take more than 2 hours. Raw milk intended for direct sale should be stored in clean, sealed containers, allowing sales under hygienic conditions directly to consumers; own containers (Dz.U. z 2007 r. nr 5, poz. 38, Rozporządzenie (we) nr 853/2004, Dz.U. z dnia 2 czerwca 2005 r.). At markets, these specific temperature requirements are only met during autumn and winter.

The specificity of the milk and dairy products production and promoted dietary trends are determined around the expectations of consumers.

According to estimates provided by National Reseach Institute – Institute of Agricultural and Food Economics (IAFE – NRI) for the year 2011, Poland's milk consumption per capita was 193 liters, while the statistical consumption of this product in the EU is almost 360 liters, with around two or three-fold higher consumption in the Scandinavian countries compared with that of Poland's (SYCH-WINIAREK 2012, Hurt & detal 7(41)/2009). The consequence of such a low consumption of milk and dairy products is an unmet daily requirement for calcium in the diets of both children and adults (Hurt & detal 7(41)/2009, KOSIORKOWSKA 2012).

A wide range of dairy products allows consumers to make choices that will meet their need of diverse and easy to prepare food which is safe, tasty and which positively affects the body. A noticeable trend of consumers seeking fresh milk has emerged in recent years. This direction is a part of a general trend of consumers seeking minimally processed food. The market offers raw milk via the so-called direct sales, which can include inter alia the distribution of this product by milkomats. This offer also includes the supply of milk to the consumer's home directly from the farmer or producer, omitting retail distribution. Dairy, which, among others, directly supplies milk that has not undergone any heat treatment to the consumer's home area, covers approx. 30 km^2 Tri-City. Delivered products are transported in refrigarated vans while the sales volume is adjusted to the number of individual orders. In many countries, the direct sale of milk includes not only the markets, but also

milkomats that have gained popularity among consumers. In the European Union milkomats are popular in Austria (over 1,500 such devices), Belgium (500) Czech Republic (300), France (400), Italy (1,500) and the United Kingdom, as well as non-European countries including, Japan and the U.S. (WIECZORKIEWICZ 2012). In Poland, the milk distributed in this way is a milk with fat content of about 4%, non-heat-treated in a thermisation, pasteurization or sterilization. Currently there are 60 milkomats our country, with the largest number located in the south-western region. The owners of these devices are mostly individual farmers or groups of milk producers who promote their holdings without incurring expenditures on advertising.

The aim of this study was to determine the state of hygiene of milk supplied through various forms of direct sales; it was a milk offered by the individual producer, a dairy delivering products to the consumer's home, and from the milkomats located within the Tri-City.

Materials and Methods

A total of 164 milk samples (n = 164) derived from milkomats (n = 50), dairy delivering the dairy products to the consumer's home (n = 58) and from the individual producer (n = 56) were examined. The microbiological analysis of the milk samples was carried out two times.

The study determined the total number of aerobic mesophilic bacteria on nutrient agar medium produced by Merck and population of staphylococci on selective medium Baird-Parker RPF by bioMérieux as well as the population size of *Escherichia coli* and coliform in the chromogenic substrate Cola ID by bioMérieux. Incubation of aerobic mesophiles was carried out at at 30°C for 72 h, staphylococci, *E. coli* and coliforms at 37°C for 48 h Microbiological analyses were performed using traditional plate. The results obtained were analyzed statistically. The study was performed in the period from October 2012 to February 2013. Analyses were performed in each case within 1–2 hours after purchasing the milk from milkomat or from an individual producer, and milk from the dairy was delivered in a car-cold store. Microbiological tests were performed through dilution according to PN (*Mleko i przetwory...* PN-A--86034-02:1993, *Mleko i przetwory...* PN-A-86034-03:1993, *Mleko i przetwory...* PN-A-86034-04:1993, *Mleko i przetwory...* PN-A-86034-09:1993, *Mleko i przetwory...* PN-A-86034-13:1993).

Results and Discussion

The development of a population of selected micro-organisms in milk depending on the forms of direct sales is shown in Table 1.

Table 1
The population of selected microorganisms in milk depending on the form of direct sales

Population size [cfu ml^{-1}]	Milkomat	Individual producer	Dairy plant
Number of aerobic mesophilic bacteria	$9.8 \cdot 10^3$–$2.01 \cdot 10^7$	$2.61 \cdot 10^5$–$8.27 \cdot 10^6$	$1.6 \cdot 10^3$–$2.71 \cdot 10^6$
Average	$3.5 \cdot 10^6$	$3.3 \cdot 10^6$	$1.26 \cdot 10^6$
SD	$4.8 \cdot 10^6$	$2.6 \cdot 10^6$	$1.0 \cdot 10^6$
Staphylococcus aureus	ab. – $6.7 \cdot 10^2$	ab. – $1.3 \cdot 10^3$	ab. – $8.7 \cdot 10^2$
Average	$5.4 \cdot 10^1$	$1.44 \cdot 10^2$	$5.2 \cdot 10^1$
SD	$1.2 \cdot 10^2$	$2.1 \cdot 10^2$	$1.4 \cdot 10^2$
Coliform	$1.05 \cdot 10^2$–$3.05 \cdot 10^4$	$1.0 \cdot 10^1$–$3.0 \cdot 10^2$	$4.2 \cdot 10^1$–$3.05 \cdot 10^4$
Average	$5.6 \cdot 10^3$	$7.2 \cdot 10^1$	$7.1 \cdot 10^3$
SD	$7.1 \cdot 10^3$	$9.2 \cdot 10^1$	$8.3 \cdot 10^3$

ab – absent

The obtained results showed that the highest number of aerobic mesophilic bacteria was characteristic to milk purchased from an individual producer. The population of these organisms was at the level from $2{,}61 \cdot 10^5$ to $8{,}27 \cdot 10^6$ cfu per ml of tested milk. None of the 56 samples met the requirements of the Regulation of the Minister of Agriculture and Rural Development of 29 December 2006 on animal health requirements for the production of products of animal origin intended for direct sale. Slightly lower levels of aerobic mesophilic population were observed in milk samples originating from milkomats ($9.8 \cdot 10^3$ – $2.01 \cdot 10^7$ cfu ml^{-1}). Only one sample of milk from milkomat showed the presence of aerobic mesophiles at the level permitted by the above mentioned document. However, these criteria were met by almost 20% of the samples from dairy plants which deliver goods directly to consumers; homes. The tested samples showed the presence of aerobic mesophiles at $1.6 \cdot 10^3$ to $2.71 \cdot 10^6$ cfu ml^{-1}. Similar results in terms of the presence of aerobic mesophiles bacteria in milk derived from direct sales were determined by Bis and Mędrela-Kuder. The authors carried out a microbiological analysis of the milk supplied by individual producers at a market in Krakow. The population of aerobic mesophiles exceeded 10^6 cfu ml^{-1} (Bis, Mędrela-Kuder 2011). However, research results by Czerwińska and Piotrowski indicate a contamination with aerobic mesophilic bacteria only in milk originating from

a young cow derived in the autumn ($1.1 \cdot 10^5$ cfu ml^{-1}), while the samples obtained in winter contained an allowable number of these micro-organisms (CZERWIŃSKA, PIOTROWSKI 2011).

The population of *Staphylococcus aureus* in the milk samples purchased from milkomats reached a level to $6.7 \cdot 10^2$ cfu ml^{-1} and 40% of the samples displayed an absence of the organism. The criteria of the microbiological evaluation concerning the presence of staphylococci in milk were met by 90% of this type of milk samples. A similar degree of contamination by *Staphylococcus aureus* was noted among the milk samples from the dairy plant, at a value to $8.7 \cdot 10^2$ cfu ml^{-1}. There was no presence of staphylococci in every fifth milk sample. Only 3% of the samples subjected to this microbiological analysis did not meet the criteria of the presence of *Staphylococcus aureus*. The highest level of contamination by staphylococci was evident among milk samples purchased from an individual producer. The absence of this micro-organism was reported in only 4% of the analysed material. However, 5% did not meet the criteria of the permitted number of *Staphylococcus aureus*. Such state of hygiene of the tested milk could have been determined by numerous factors: its collection, transport and distribution.

The presence of staphylococci in food is, next to *Salmonella*, one of the most common causes of food poisoning in humans. Simultaneously, the presence of *Staphylococcus aureus* in the transient hand and mucous membranes microflora is ascertained in 20–40% of the healthy human population. Enterotoxins produced by strains of *Staphylococcus aureus* enter the consumer's body through consumption of food contaminated by staphylococci U.S. statistics document more than 186 thousand cases of *Staphylococcus aureus* intoxication per year, while the national data records more than 600 similar cases. These figures, however, should be taken as an underestimate due to the large number of non-reported or undiagnosed cases of staphylococcal food poisoning (BLACKBURN, MCCLURE 2002, BILEK 2004, HASSE-CIEŚLIŃSKA 2007).

The study also evaluated the level of contamination by *Escherichia coli*. All of the analysed milk samples showed absence of the organism. However, belonging to the coliform bacteria called coliform *Enterobacter*, *Citrobacter* and *Klebsiella* which signify the lack of hygiene during milking, storage or transport were present in all analysed milk samples. The highest number of these microorganisms was found in samples of milk from a dairy plant (average $7.1 \cdot 10^3$ cfu ml^{-1}), slightly lower values were obtained by examining milk from milkomats (average $5.6 \cdot 10^3$ cfu ml^{-1}), while the milk purchased from individual producer showed the lowest degree of contamination by coliform bacteria ($7.2 \cdot 10^1$ cfu ml^{-1}). Research by CZERWIŃSKA, PIOTROWSKI on the presence of *Escherichia coli* in raw chilled milk during the winter season showed the presence of this organism in 0,001 ml of the test samples. The authors assessed

the quality of the milk as unsatisfactory due to the presence of both *Escherichia coli* and *Staphylococcus aureus* (CZERWIŃSKA, PIOTROWSKI 2011). Similarly, BIS, MĘDRELA-KUDER also pointed out the poor hygienic quality of the milk from individual producer which showed a significant degree of contamination by aerobic mesophilic bacteria, proteolytes, fungus, while the presence of coliform bacteria were found in almost 70% of the samples (BIS, MĘDRELA-KUDER 2011). The presence of pathogens and microorganisms that suggest low hygienic quality of milk pose a risk to consumer's health. (ZIARNO, MOLSKA 1999).

Conclusions

1. Nearly all of the tested milk samples indicated a significant level of contamination by the aerobic mesophilic bacteria.

2. The best microbiological quality was determined in the samples of milk provided by the dairy plant offering its products via direct delivery to customers' houses

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AN ATTEMPT TO EVALUATION OF THE LAKE TROUT STOCKING EFFECTIVENESS IN LAKE WDZYDZE WITH THE USE OF THERMAL SHOCK AS THE METHOD OF THE FISH MASS MARKING

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K e y w o r d s: *Salmo trutta* m. *lacustris*, fish mass marking, thermal shock, otolith.

A b s t r a c t

The aim of the study was to compare the share of hatchery-reared lake trout *Salmo trutta* m. *lacustris* in catches of fingerlings in the upper Wda and the Pilica Rivers in Autumn, following the stocking fish in spring 2005. Larvae in number of 40 thousand had been previously exposed to the thermal shock procedure (temperature decrease from 8.0 to 2.5°C for 2 hours, and back to initial value), then released to both the Wda and Pilica Rivers, connected with the Lake Wdzydze, when the source of the endemic lake trout population exist. The readability of the thermal mark on otoliths was good and was visible as an expanded dark band within the daily increments. Thermal marks were found in every otolith in sample of larvae dedicated for stocking. The percentage of marked otoliths in fingerlings sampled from the Wda and Pilica rivers was, respectively, 79.3% and 85.0%. Results indicate the natural recruitment of lake trout in both tested rivers. Further research on necessity of supplemental stocking should be continued. Mass marking procedure presented here could be useful in such investigations.

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PRÓBA OCENY EFEKTÓW ZARYBIEŃ JEZIORA WDZYDZE TROCIĄ JEZIOROWĄ Z WYKORZYSTANIEM SZOKU TERMICZNEGO JAKO METODY MASOWEGO ZNAKOWANIA RYB

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S ł o w a k l u c z o w e: *Salmo trutta* m. *lacustris*, masowe znakowanie ryb, szok termiczny, otolit.

A b s t r a k t

Celem badań było porównanie udziału troci jeziorowej *Salmo trutta* m. *lacustris*, wyhodowanej w wylęgarni i pochodzącej z naturalnego tarła w odłowach narybku z górnej Wdy i Pilicy wykonanych jesienią, po zarybieniach przeprowadzonych wiosną 2005. Larwy (40 tys.), wcześniej poddane procedurze znakowania szokiem termicznym (obniżenie temperatury z 8,0 do 2,5°C na 2 godziny i powrót do temperatury wyjściowej), wypuszczono do Wdy i Pilicy, cieków połączonych z jeziorem Wdzydze, gdzie znajduje się endemiczna populacja troci jeziorowej. Czytelność znaczka termicznego na otolitach była dobra. Znaczki były widoczne w postaci rozszerzonego ciemnego pasma w obrębie przyrostu dobowego. Znaczki termiczne znaleziono na wszystkich otolitach w próbie larw przeznaczonych na zarybienia. Procent znakowanych otolitów w próbie pobranej jesienią z Wdy i Pilicy wynosił odpowiednio 79,3% i 85,0%. Wyniki wskazują na istnienie naturalnej rekrutacji troci jeziorowej w obu badanych ciekach. Badania dotyczące konieczności dodatkowego zarybiania jeziora Wdzydze powinny być kontynuowane. Zastosowana procedura znakowań masowych ryb szokiem termicznym może być użyteczna w badaniach tego typu.

Introduction

Autochthonic form of the lake trout *Salmo trutta* m. *lacustris* inhabits 10 lakes in Poland, located in the upper Wda, Brda and Drawa rivers basins. In Europe, the species is found in some alpine and pre-alpine reservoirs and costal lakes located along the Baltic sea moreover, in Scandinavia, Russia and in some Scottish lakes (BARTEL 2000). The population in Poland belongs to smaller ones, with an estimate number below 10 000 specimens. The most numerous stock presumably occurs in the Lake Wdzydze in the Wda River basin (the part of the drainage area of the Vistula River), estimated at 3 to 5 thousand specimens including 50 to 100 spawners (RADTKE 2001).

Mature specimens of Lake Wdzydze trout migrate to the Wda River and Trzebiocha stream for spawning. The stocking material for Lake Wdzydze and for lake trout reintroduction measures taken in many Polish water bodies

originates from these two watercourses. Biology of the lake trout population from Lake Wdzydze is similar to pre-alpine populations (e.g. this inhabiting the Lake Vättern) or some of Finnish populations (KAJ 1961).

Due to the regulation of the Wda River and the Trzebiocha stream the water level of these watercourses significantly decreased and they became unsuitable for trout's spawning. In 1993–1994, a partial renaturalization of spawning grounds was done using the felled trees for the obstacles creation in rivers' beds as well as by removing the fascine fences (RADTKE 1994).

Spawners (more than half of the total population of female) migrating to the Wda River and the Trzebiocha stream for spawning in late October and November have been caught and gathered in The Wdzydze fish farm every year since 1951. Eggs incubation was carried out in a hatchery in Grzybowski Młyn, supplied with water of the Trzebiocha stream (RADTKE 2004).

Since 1952, Lake Wdzydze catchment area has been regularly stocked with hatchery-reared lake trout larvae to sustain this valuable population. Due to high mortality of larvae, observed during rearing in the past, only freshly hatched larvae are used for stocking. The mortality was caused mainly by parasitic diseases (*diplostomulosis* and *saprolegniosis*) (RADTKE, DĘBOWSKI 1996). Since 1971, irregularly, tens of thousands of larvae has been used for stocking. Due to the deterioration of environmental conditions in the lower part of the Trzebiocha Stream, larvae have been released only into Pilica Stream (the upper part of the River) since 1991 (RADTKE 1997).

The evaluation of stocking results had been difficult until the mass marking methods of fish larvae (including thermal shock) were developed. Marking of fish otoliths is an reliable tool for gathering the data on the effectiveness of supporting the natural populations with larvae obtained in hatchery (KOZŁOWSKI et al. 2009). The aim of the present study was to attempt the evaluate the effectiveness of stocking with lake trout larvae in the upper Wda River and the Pilica River, using the thermal shock as a technique for mass marking fish.

Material and Methods

Marking procedure was carried out in the Grzybowski Młyn hatchery in April 2005, when the hatchery-reared free swimming larvae constituted up to 50% of the stock. The hatchery was supplied with water from the Trzebiocha Stream (temp. 8.0 ± 0.1°C, an oxygen saturation above 93%). Thermal shock was carried out in flow through tanks. The bath marking was cooled by adding the crushed ice until the temperature dropped to 2.5 ± 0.1°C. Larvae were exposed to this temperature continuously for 2 hours. After that, an intensive

stream of water was passed through tanks, until the temperature reached the initial value of 8°C. More than 40 thousand larvae were treated with this procedure. Three days later the sample of 30 specimens (starting sample 1; S_1), previously anesthetized with MS-222 (300 mg · l^{-1}) and fixed in 70% ethanol, were examined on the thermal mark presence.

On the 4th day after the bath-marking, larvae were released at two different sites: the first at the upper Wda near the Płocice locality, and the second at the Pilica near the Łubiana village, in number of 20 thousand specimens in each site. In October the fish (age 0+) were caught by electrofishing from the Wda River (sample 2; S_2) as well as from the Pilica River (sample 3; S_3) in number of, respectively: 29 and 20 specimens. Prior to the otolith dissection and preparation, the fish were anaesthetized in MS-222 (300 mg · l^{-1}), fixed in 70% ethanol and measured (with an accuracy up to 0.1 mm) and weighted (up to 0.01 g). The sagittal otolith of each fish was mounted on a glass slide with entellane resin. Then, otoliths were manually polished with the fine-grain sandpaper. The thermal marks were identified under a light microscope. Fish measurements were analyzed using the Kruskal-Wallis test (non-parametric ANOVA). Significance of differences in proportion of marked fish between groups were tested with Statistica 8 software (test of significance of difference between two structure coefficients). The differences were regarded as significant at $p \leq 0.05$.

Results

A thermal mark, in the form of the expanded dark band within the daily increment, was found in every otolith of the starting sample 1 (S_1), and marks were clearly readable in the entire sample (Figure 1).

Fish sampled in Autumn 2005 at Wda River (S_2) achieved on average 99.5 ± 16.2 mm in length and 8.69 ± 4.5 g in weight (the marked individuals (n-23) 95.9 ± 24.7 mm and 8.6 ± 4.6 g, unmarked (n-6) 101.1 ± 17.9 mm and 9.1 ± 4.5 g). Fish caught at Pilica River (S_3) achieved 105.5 ± 15.6 mm in length and 10.22 ± 5.1 g in weight (the marked fish (n-17) 108.5 ± 15.1 mm and 11.1 ± 5.0 g, the unmarked fish (n-3) – 89.0 ± 3.7 mm and 5.1 ± 1.6 g). Differences in both, the fish length and weight between sites (S_2 and S_3) as well as between hatchery reared and fishes of natural recruitment were not significant ($p > 0.05$).

The percentage of marked otoliths in starting sample (S_1) was 100%, while in fingerlings sampled from the Wda and Pilica Rivers reached, respectively, 79.3% and 85.0% . The difference in percentage of otoliths with thermal mark between S_2 and S_3 samples was not statistically important (Table 1).

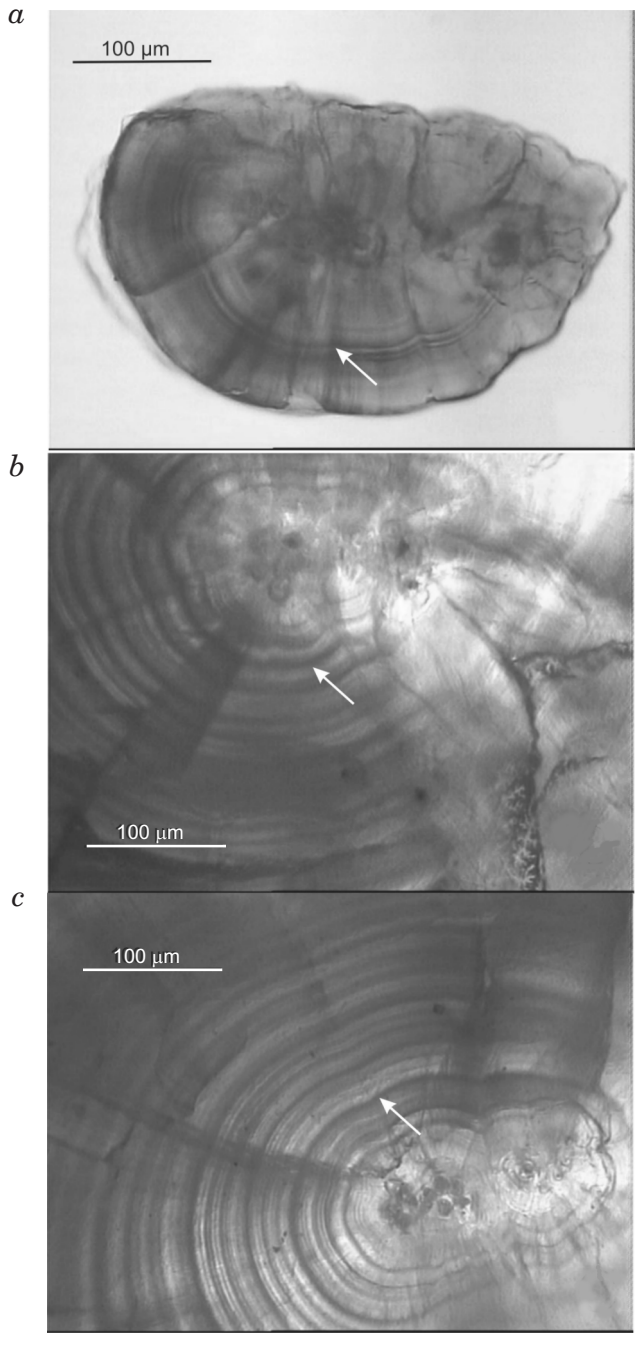

Fig. 1. Lake trout otoliths: *a* – larval, *b, c* fingerling's. The thermal mark is indicated with the arrow

Table 1

Percentage of lake trout larvae and fingerlings with the thermal mark

Group	Number of sampled otoliths	Otoliths with thermal mark [%]
S_1	30	100.0^a
S_2	29	79.3^b
S_3	20	85.0^b

Values marked with the same letter index are not statistically different ($p \leq 0.05$)

Discussion

Stocking with fry is a commonly used method to sustain or build up the number of salmonid populations. However, there is still luck of reliable information on the effectiveness of such measures. Results of the fish stocking are easily observed during introduction and reintroduction of a species in habitats where natural breeding does not exist. The evaluation of stocking in waters, where natural habitat does not provide the appropriate conditions for young fish is quite hindered. The mass marking of fish intended for stocking provides the tool potentially useful to evaluate stocking effectiveness in such a cases.

Mass marking of embryos or larvae is the common procedure (BROTHERS 1990). Nowadays, year by year millions of specimens are marked and released to natural habitats. They belong mainly to species for which the controlled breeding and stocking is crucial to sustain populations in natural water bodies. Common methods used for mass marking of juvenile fish stages are: (1) immersion in a fluorochrome solution (HATTLER 1984), (2) using stable strontium salts as markers (OPHEL, JUDD 1968) and (3) short-term thermal shock. During these all procedures marks are „written" into otoliths permanently (VOLK et al. 1990). The use of short-term thermal shock is probably the simplest, cheapest and the least harmful procedure (KRUSZNIEWSKI et al. 1998, VOLK et al. 1999, SKALSKI, GRISWOLD 2006).

CAMPANA and NEILSON (1985) showed that in fish exposed to an optimal and stable temperature, aragonite crystals in otoliths are deposited in a regular mode, but changes in environmental conditions may influence the microstructure of an otolith. Thermal shock is the factor which is able to change appearance of daily increments. BROTHERS (1990) and VOLK et al. (1994) showed that it is possible, by means of a combination of subsequent thermal shocks, to obtain unique patterns on otoliths of lake trout *Salvelinus namaycush* and Pacific salmon *Oncorhynchus* sp. The pattern, unique for each hatchery, potentially enables the origin of fish used for stocking to be recognized.

However, during the bath marking it is very important to keep a stable temperature following the thermal shock, because the temperature alterations could result in additional „unplanned marks". In consequence, the marks become unreadable. Also, the 100% effectiveness of marking is very important because it is crucial for reliable distinguishing the stocked fish from the fish of natural recruitment.

No mortality and maximal possible effectiveness gained in the marking procedure in presented study indicates, that marking parameters including the temperature gradient (a decrease of 5.5°C) and the time of exposure (2 hours) were appropriate for lake trout larvae. The high share of marked fish in samples from the Wda and Pilica rivers (respectively, 79.3 % and 85.0%) suggest that the supplemental stocking is necessary and should be continued. On the other hand, the presence of fish from natural spawning may indicate the positive effects of the rivers' re-naturalization program. However, one has to remember that the number of spawners of Wdzydze lake trout is also limited (up to 50–100 individuals) (RADTKE 2001) and thus, catching of mature specimens for artificial spawning may affect significantly the natural spawning results. If, the natural reproduction of Wdzydze lake trout is sufficient there is no need for additional stocking. Further research is needed to obtain a more complete picture of stocking results. The method of thermal shock presented here is appropriate to mass marking of lake trout larvae and could be useful in such investigations.

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