UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN

Polish Journal of Natural Sciences

30

(1/2015)

PUBLISHER UWM
OLSZTYN 2015

The Polish Journal of Natural Sciences is indexed and abstracted in Biological Abstracts and Biosis Previews

The print edition is the primary version of the Journal

The Journal is also available in electronic form on the websites
http://www.uwm.edu.pl/polish-journal/ (home page)
http://wydawnictwo.uwm.edu.pl (subpage *Czytelnia*)

PL ISSN 1643-9953

PUBLISHER UWM OLSZTYN

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Ark. wyd. 7,38, ark. druk. 6,25, nakład 110 egz.
Druk – Zakład Poligraficzny UWM w Olsztynie
zam. nr 307

TABLE OF CONTENTS

Agriculture

Animal Breding and Husbandry

Biology

Food and Nutrition Sciences

SPIS TREŚCI

Rolnictwo

Chów i Hodowla Zwierząt

Biologia

Nauka o Żywności Żywieniu

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 30(1): 5–20, Y. 2015

THE EFFECT OF PLANT GROWTH REGULATORS ON THE INCIDENCE AND SEVERITY OF POTATO DISEASES

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K e y w o r d s: biological control, fungicides, potato, diseases, fungi.

A b s t r a c t

Four potato cultivars were grown: Volumia, Irga, Satina and Sylvana. Potato plants were sprayed three times with the growth biostimulator Asahi SL and growth regulators Bio-Algeen S-90 and Kelpak SL. During the growing season, the severity of late blight and early blight was evaluated. After five-month storage, the incidence of common scab, black scurf, soft rot, late blight and dry rot was determined on tubers. Fungi were isolated from potato tubers and were cultured on PDA medium. The severity of late blight was reduced in the treatments with growth regulators Bio-Algeen S-90 and Kelpak SL, and the incidence of early blight was reduced in the above treatments and in that with the biostimulator Asahi SL. Satina was the healthiest cultivar. Weaker symptoms of late blight and dry rot were observed on potato tubers harvested from plants treated with the analyzed growth regulators and biostimulator. *Colletotrichum coccodes* was the predominant pathogen colonizing potato tubers.

WPŁYW REGULATORÓW WZROSTU NA NASILENIE CHORÓB ZIEMNIAKA

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S ł o w a k l u c z o w e: biologiczna ochrona, fungicydy, ziemniak, choroby, grzyby.

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Abstrakt

Uprawiano 4 odmiany ziemniaka: Volumia, Irga, Satina i Sylvana. Rośliny opryskiwano trzykrotnie biostymulatorem wzrostu Asahi SL i regulatorem wzrostu Bio-Algeen S-90 i Kelpak SL. Podczas okresu wegetacji szacowano nasilenie zarazy ziemniaka i alternariozy. Po 5-miesięcznym przechowywaniu oceniano nasilenie parcha zwykłego, ospowatości bulw, mokrej zgnilizny, zarazy ziemniaka i suchej zgnilizny. Z bulw izolowano grzyby, a ich hodowlę prowadzono na podłożu PDA. Stwierdzono ograniczenie nasilenia zarazy ziemniaka na roślinach w obiektach doświadczenia ze stosowanym regulatorem wzrostu Bio-Algeen S-90 i Kelpak SL oraz ograniczenie alternariozy w wymienionych obiektach i dodatkowo w obiekcie ze stymulatorem wzrostu. Odmiana Satina okazała się najzdrowsza. Na bulwach pochodzących z poletek ze stosowanymi regulatorami wzrostu i stymulatorem Asahi SL zaobserwowano redukcję nasilenia objawów zarazy ziemniaka i suchej zgnilizny bulw. Wśród patogenów zasiedlających bulwy dominującym był gatunek *Colletotrichum coccodes*.

Introduction

Due to their widespread occurrence and genetic diversity, the fungus-like organism *Phytophthora infestans* and fungi of the genus *Alternaria* are among the most important potato pathogens (ABD-EL-KHAIR and HAGGAG 2007, KURZAWINSKA and MAZUR 2008, CWALINA-AMBROZIAK and TROJAK 2011). The symptoms of infections caused by the above pathogens are also observed on stored potato tubers. During storage, tubers are also affected by bacterial diseases – soft rot caused by *Pectobacterium carotovorum* subsp. *carotovorum* and common scab caused by *Streptomyces scabies* (MA et al. 2007, LUTOMIRSKA 2008), and fungal diseases – dry rot caused by *Fusarium* spp., black scurf caused by *Rhizoctonia solani* and silver scab caused by *Helminthosporium solani* (AVIS et al. 2010, GACHANGO et al. 2012). The above diseases reduce the market value and storage life of potato tubers, and the quality of seed potatoes (ERRAMPALLI et al. 2001). Disease management involves intercropping (BOUWS and FINCKH 2008), the use of biological and chemical control methods (STEPHAN et al. 2005, KURZAWINSKA and MAZUR 2008), and breeding of resistant varieties (OLANYA et al. 2006). Synthetic growth regulators, similarly as endogenous growth regulators, participate in the growth and development of plants, and contribute to cell wall strengthening, increasing tuber yield, improving tuber quality (including the content of dry matter, starch and phenolic compounds), extending storage life and inhibiting sprouting in storage (YADA et al. 1991, CALDIZ et al. 2001, ČERNÝ et al. 2002, CZECZKO and MIKOS-BIELAK 2004, MIKOS-BIELAK 2005). The influence of plant growth regulators on increasing resistance to adverse environmental conditions and pathogens in potatoes remains scarcely researched (MIKOS-BIELAK 2005, SAWICKA 2008).

The objective of this study was to determine the effect of growth regulators on disease severity in potato foliage and tubers.

Material and Methods

A three-year micro-plot field experiment was established in 2010 in Tomaszkowo (NE Poland), on brown soil developed from loamy sand, of quality class IVa and suitability complex 5 (slightly acidic pH of 5.32–5.70 in 1mol KCl dm^{-3}, high available phosphorus levels /69–72 mg P kg^{-1}/, moderate levels of available potassium /82–90 mg K kg^{-1}/ and magnesium /38–48 mg Mg kg^{-1}/). Four potato cultivars were grown: very early Volumia, and medium early Irga, Satina and Sylvana. Cereal crops were grown as forecrops (winter triticale in 2009 and 2012, oat in 2011). Manure was applied in the fall at 25 t ha^{-1}, and mineral fertilizers were applied in the spring: N – 40 (urea, 46% N), P – 60 (superphosphate, 17.45% P), K -100 (potash salt, 50% K) kg ha^{-1}. Seed potato tubers were planted at 67.5 x 40 cm spacing at the end of April, and were harvested at the beginning of September in 2010 and 2011, and on 21 August in 2012. Starting from the stage of crop cover complete (BBCH 39), at 10–14-day intervals, plants were treated three times with the growth biostimulator Asahi SL (0.1% solution; nitrophenols naturally occurring in plants: sodium o-nitrophenol, sodium p-nitrophenol, sodium 5-nitroguaiacol), the growth regulator Bio-Algeen S 90 (1.0% solution; marine brown algae extract, contains 90 groups of organic compounds including amino acids, vitamins, alginic acid and other active compounds found in marine algae, and small quantities of macroelements and microelements: nitrogen (N) – 0.02%, phosphorus (P_2O_5) – 0.006%, potassium (K_2O) – 0.096%, calcium (CaO) – 0.31%, magnesium (MgO) – 0.021%, boron (B) – 16 mg kg^{-1}, iron (Fe) – 6.3 mg kg^{-1}, copper (Cu) – 0.2 mg kg^{-1}, manganese (Mn) – 0.6 mg kg^{-1}, zinc (Zn) – 1.0 mg kg^{-1}, Mo, Se and Co) and the growth regulator Kelpak SL (0.2% solution; the brown alga *Ecklonia maxima*, contains 11 mg dm^{-3} auxins and 0.031 mg dm^{-3} cytokinins; based on the label, the auxin/cytokinin ratio is 350:1). In Poland, the use and approval of plant growth regulators are regulated by the Law on Fertilizers and Fertilization of 10 July 2007 and the Law on Plant Protection Products of 8 March 2013. In the control treatment, potato plants were not treated with growth regulators. Identical cultivation measures, recommended by the Institute of Soil Science and Plant Cultivation-National Research Institute in Pu awy, and plant protection measures, recommended by the Institute of Plant Protection-National Research Institute in Poznan (Infinito 687.5 SC, Pyton 450 SC, Toska 72.5 WP, Ridomil Gold 67.8 WG, Mospilan 20 SP, Calypso 480 SC), were applied in all plots. During the growing season, two weeks from the last

Table 1

Meteorological date according Meteorological Station in Tomaszkowo

Month	Mean	10-days	Temperature [°C]				Rainfall [mm]			
			2010	2011	2012	mean (1961–1995)	2010	2011	2012	sum (1961–1995)
May	for 10 days	I	10.8	8.7	13.1		25.4	10.8	0.8	
		II	12.2	14.2	12.0		46.1	32.5	48.6	
		III	12.9	16.2	14.8		60.4	7.8	2.3	
	monthly		12.0	13.1	13.4	12.4	131.9	51.1	51.7	56.7
June	for 10 days	I	18.2	18.8	12.2		25.3	39.9	33.8	
		II	15.5	15.4	16.5		36.0	30.7	18.5	
		III	15.6	16.9	16.4		23.5	11.1	50.9	
	monthly		16.4	17.1	15.0	15.7	84.8	81.7	103.2	68.3
July	for 10 days	I	20.0	17.1	21.6		31.3	124.8	6.7	
		II	23.3	19.2	15.5		6.2	36.4	32.1	
		III	20.1	17.7	19.9		42.9	41.6	12.2	
	monthly		21.1	17.9	19.0	15.3	80.4	202.8	121.0	81.3
August	for 10 days	I	20.7	18.2	18.6		26.6	20.4	29.2	
		II	21.0	16.9	17.0		17.4	44.9	9.5	
		III	16.6	17.7	17.4		51.3	16.8	6.4	
	monthly		19.3	17.6	17.7	17.9	95.3	82.1	45.1	78.1
Mean/Sum			17.2	16.4	16.3	15.3	392.4	417.7	321.0	284.4

treatment, the severity of late blight (*Phytophthora infestans*) and early blight (*Alternaria solani, A. alternata*) was evaluated three times on a nine-point scale, where 0º – no symptoms, 9º – most severe symptoms. After five-month storage at 4ºC, the incidence of common scab (*Streptomyces scabies*) and black scurf (*Rhizoctonia solani*) was determined on 100 randomly selected tubers. The results were presented as a percentage infection index (Ii,%). The severity of soft rot (*Pectobacterium carotovorum* subsp. *carotovorum*), late blight and dry rot (*Fusarium* spp.) was estimated on 5 kg samples, and the results were expressed as a percentage of the total weight of infected tubers. Fungi were isolated from potato tubers at the laboratory. Samples consisted of 30 tubers collected randomly in three replications per treatment. Tuber segments (0.5 x 0.5 x 0.5 cm) were disinfected with 50% ethanol and 1% sodium hypochlorite, and were transferred to PDA medium. After seven days, fungal colonies were inoculated onto agar slants and identified.

The results were verified statistically by ANOVA for a randomized block design (STATISTICA® 10.0), and means were compared by Duncan's test (significance level of 0.05).

Mean monthly temperatures in May-August in the analyzed growing seasons were comparable with the long-term average, except the warm June of 2011, July in all seasons and August of 2010 (Table 1). Rainfall amounts were above normal in all years. The wettest months were May of 2010, June in all seasons and July in the last two years.

Results and Discussion

The incidence and severity of late blight were high in the first two years of the experiment, and the highest infection rates were noted in 2011, from 62.7% on potato plants of cv. Satina treated with Kelpak SL to 72.4% on plants of cv. Volumia in the control treatment (Table 2). Significant differences in late blight intensity levels, relative to the control treatment, were observed in the growing season of 2011 on plants treated with growth regulators Bio-Algeen S-90 and Kelpak SL (except cv. Irga), and in 2012 on plants of cv. Volumia treated with Bio-Algeen S-90 and plants of cv. Sylvana treated with Kelpak SL. An analysis of average infection index values revealed that late blight severity was lower on potato plants treated with Bio-Algeen S-90 and Kelpak SL in the first two years of the study, and on plants treated with Kelpak SL in the last year, as compared with the infection rates noted in the Asahi SL and control treatments. CZECZKO and MIKOS-BIELAK (2004) demonstrated that growth regulators increase the concentrations of phenolic compounds in plants, which are involved in the defense mechanism against environmental stresses.

Table 2

The severity of infection caused by *Phytophthora infestans* on potato plants [infection index li, %]

Cultivar	2010				x	2011				x	2012				x
	Asahi	Bio-Alg	Kelpak	control		Asahi	Bio-Alg	Kelpak	control		Asahi	Bio-Alg	Kelpak	control	
Volumia	66.7^{a}	56.5^{bcd}	60.2^{ab}	63.0^{ab}	61.6^{a}	70.8^{ab}	67.0d^{ef}	67.8^{c-f}	72.4^{a}	69.5^{a}	49.5^{ab}	42.8^{b-e}	46.5^{abc}	51.1^{a}	47.5^{a}
Irga	61.2^{ab}	60.2^{ab}	58.8^{bc}	60.7^{ab}	60.2^{a}	70.3^{bc}	67.5^{def}	69.4^{bcd}	71.0^{ab}	69.7^{a}	43.5^{b-e}	38.1^{def}	37.8^{def}	40.8^{c-f}	40.1^{bc}
Satina	57.9^{bcd}	52.8^{cd}	51.4^{d}	56.1^{bcd}	54.6^{b}	66.4^{efg}	*64.0*^{gh}	62.7^{h}	67.3^{def}	66.4^{b}	38.8^{def}	39.0^{e-f}	35.0^{f}	40.3^{c-f}	38.3^{c}
Sylvana	57.9^{bcd}	58.8^{bc}	58.8^{bc}	60.2^{ab}	58.9^{a}	67.5^{def}	66.0^{efg}	65.4^{fg}	68.8^{b-d}	66.9^{b}	41.1^{c-f}	45.4^{a-d}	36.0^{ef}	45.5^{a-d}	42.0^{b}
Mean	60.9^{a}	57.1^{b}	57.3^{b}	60.0^{a}	–	68.8^{b}	66.1^{c}	66.3^{c}	70.5^{a}	–	43.2^{a}	41.3^{ab}	38.8^{b}	44.4^{a}	–

Key: Statistical data within years; Asahi – Asahi SL, Bio-Alg – Bio-Algeen S-90, Kelpak – Kelpak SL

Table 3

The severity of infection caused by *Alternaria solani* and *A. alternata* on potato plants [Infection index li, %]

Cultivar	2010				x	2011				x	2012				x
	Asahi	Bio-Alg	Kelpak	control		Asahi	Bio-Alg	Kelpak	control		Asahi	Bio-Alg	Kelpak	control	
Volumia	32.4^{b}	33.3^{ab}	33.8^{ab}	35.7^{ab}	33.8^{b}	23.0^{cd}	21.8^{cd}	22.5^{bcd}	23.5^{bc}	22.7^{b}	38.8^{a-d}	36.1^{a-d}	36.3^{a-d}	43.0^{ab}	38.6^{ab}
Irga	36.6^{ab}	37.0^{ab}	37.1^{ab}	38.4^{a}	37.3^{a}	23.0^{bcd}	22.0^{cd}	21.0^{d}	23.0^{bcd}	22.3^{b}	44.4^{a}	42.5^{abc}	40.5^{a-d}	44.9^{a}	43.1^{a}
Satina	32.0^{b}	32.4^{b}	33.3^{ab}	33.3^{ab}	32.8^{b}	22.0^{cd}	21.3^{cd}	18.5^{e}	22.5^{bcd}	21.1^{c}	33.4^{a-d}	35.8^{a-d}	30.1^{cd}	34.4^{a-d}	33.4^{b}
Sylvana	34.3^{ab}	35.6^{ab}	33.4^{ab}	35.7^{ab}	34.7^{b}	24.3^{ab}	22.8^{bcd}	22.0^{cd}	25.8^{a}	23.7^{a}	31.5^{bcd}	39.4^{a-d}	28.3^{d}	35.9^{a-d}	33.8^{b}
Mean	33.8^{a}	34.6^{a}	34.4^{a}	35.8^{a}	–	23.1^{a}	22.0^{b}	21.0^{c}	23.7^{a}	–	37.0^{b}	38.4^{ab}	33.8^{b}	39.5^{b}	–

Key: see Table 2

SAWICKA (2008) demonstrated that foliar application of the growth biostimulator Asahi SL increased tuber yield, inhibited the spread of late blight and early blight and suppressed the development of the causal agents of both diseases, which is an important consideration under heat stress conditions. According to the cited author, Asahi SL used in combination with foliar fertilizer Insol 7 provided effective disease control. In our study, the weakest symptoms of early blight were observed in 2011, and the infection index of 18.5% noted on plants of cv. Satina sprayed with Kelpak SL was significantly lower than in the control treatment (Table 3). Significant differences in disease severity were also found between plants of cv. Sylvana treated with growth regulators Bio-Algeen S-90 and Kelpak SL, and control plants. No significant differences in infection rates between treatments were observed in the other analyzed growing seasons. An analysis of average infection index values revealed that early blight severity was significantly lower in 2011 on potato plants treated with growth regulators Bio-Algeen S-90 and Kelpak SL, and in 2012 on plants sprayed with the growth biostimulator Asahi SL and the growth regulator Kelpak SL. The healthiest cultivars were Satina in the first two years of the experiment, and Sylvana in the last year.

The highest number of tubers showing symptoms of late blight (25% of the total weight of infected tubers) were harvested in 2010 in the Asahi SL treatment – cv. Volumia and Sylvana, in the Kelpak SL treatment – cv. Irga and in the control treatment – cv. Sylvana, and in 2011 in the Asahi SL treatment – cv. Volumia and in the control treatment – cv. Volumia and Sylvana (Table 4). Infection-free tubers were also collected in some treatments. The average values of the infection index show that disease severity was lower on tubers harvested from plants treated with the analyzed biostimulator and growth regulators. Tubers of cv. Satina were found to be healthiest. Infected tubers were encountered sporadically in the last year of the experiment, when the experimental site received less than half the average amount of rainfall towards the end of July and in August. A comparison of resistance to late blight in foliage and tubers revealed that tuber and foliage defense mechanisms against the late blight pathogen are similar in several potato varieties, particularly early and medium-early varieties that seem to be genetically predisposed to late blight (GAWINSKA-URBANOWICZ 2008). Tubers attacked by *P. infestans* are more prone to infections by other pathogens during storage, especially *Pectobacterium carotovorum* subsp. *carotovorum* and *Fusarium* spp. (KURZAWINSKA and MAZUR 2010). The effects of plant growth regulators on the incidence and severity of potato tuber diseases remains insufficiently investigated. In a study by REX (1992), foliar application of plant growth regulators chlormequat chloride (CCC) and ethephon (ETH), alone or in mixture, decreased the incidence of tubers with a hollow heart and increased the incidence

Table 4

The severity of infection caused by *Phytophthora infestans* after 5-month storage [% of the total weight of infected tubers]

Cultivar	2010				x	2011				x	2012				x
	Asahi	Bio-Alg	Kelpak	control		Asahi	Bio-Alg	Kelpak	control		Asahi	Bio-Alg	Kelpak	control	
Volumia	23.8^{ab}	9.1^{efg}	11.2^{de}	21.0^{b}	16.3^{b}	23.8^{a}	15.4^{cd}	16.1^{cd}	24.5^{a}	20.0^{a}	0^{c}	0^{c}	0^{c}	0^{c}	0^{b}
Irga	8.0^{fg}	11.2^{de}	22.4^{ab}	17.5^{c}	14.8^{c}	18.2^{cd}	10.5^{efg}	15.4^{cd}	21.0^{b}	16.3^{b}	0^{c}	0^{c}	0^{c}	3.2^{a}	0.8^{a}
Satina	0^{g}	8.4^{fg}	16.8^{c}	16.8^{c}	10.5^{d}	0^{g}	0^{g}	9.0^{efg}	6.0^{fg}	3.8^{d}	0^{c}	0^{c}	0^{c}	0^{c}	0^{b}
Sylvana	23.1^{ab}	17.5^{c}	8.0^{fg}	24.5^{a}	18.3^{a}	0^{g}	13.3^{de}	0^{g}	25.2^{a}	9.6^{b}	0^{c}	0^{c}	3.0b	0^{c}	0.8^{a}
Mean	13.7^{b}	11.6^{c}	14.6^{b}	20.0^{a}	–	10.5^{b}	9.8^{b}	10.1^{b}	19.2^{a}	–	0^{b}	0^{b}	0.8^{a}	0.8^{a}	–

Key: see Table 2

Table 5

The severity of infection caused by *Pectobacterium carotovorum* subsp. *carotovorum* after 5-month storage [% of the total weight of infected tubers]

Cultivar	2010				x	2011				x	2012				x
	Asahi	Bio-Alg	Kelpak	control		Asahi	Bio-Alg	Kelpak	control		Asahi	Bio-Alg	Kelpak	control	
Volumia	0^{f}	13.3^{e}	19.6^{cd}	12.6^{e}	11.4^{c}	0^{d}	0^{d}	0^{d}	4.4^{c}	1.1^{c}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Irga	0^{f}	0^{f}	0^{f}	0^{f}	0^{d}	0^{d}	0^{d}	0^{d}	7.0^{bc}	1.8^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Satina	0^{f}	18.2^{b}	21.0^{bc}	20.0^{bc}	13.3^{b}	0^{d}	0^{d}	0^{d}	0.6^{d}	0.2^{d}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Sylvana	0^{f}	22.4^{ab}	15.0^{e}	24.5^{a}	15.5^{a}	0^{d}	0^{d}	0^{d}	10.0^{a}	2.5^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Mean	0^{b}	13.5^{a}	13.9^{a}	12.8^{a}	–	0^{b}	0^{b}	0^{b}	5.5^{a}	–	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}

Key: see Table 2

of deformities. Davis and GROSKOPP (1981) reported that foliar sprays of maleic hydrazide (MH-30) had no influence on symptoms of the *Rhizoctonia* disease on potato tubers, but increased the overall tuber yield. REITER et al. (2012) also demonstrated that MH-30 and nitrogen application timing had an insignificant effect on deformity, tuber rots and yields of Russet potatoes. In the cited study, nitrogen rate and variety factors had the greatest impacts on the analyzed parameters.

In the first year of the present study, symptoms of infection caused by *Pectobacterium carotvorum* subsp. *carotovorum* were observed on tubers of all analyzed cultivars except cv. Irga, in the treatments with growth regulators Bio-Algeen S-90 and Kelpak SL, and in the control treatment (Table 5). The tested products did not reduce disease severity. Symptoms of soft rot were not noted on tubers harvested from plants sprayed with the growth biostimulator Asahi SL. In 2011 and 2012, *Pectobacterium* – infected tubers were harvested only from plants that were not treated with growth regulators in 2011, most probably due to low rainfall amounts between 20 and 31 August 2011 and between 10 and 31 August 2012. Tubers of cv. Sylvana represented the highest percentage of infected tubers. In Poland, two major causal agents of soft rot are *P. atrosepticum* and *P. carotovorum* subsp. *carotovorum*, but recently *Dickeya* spp. have also been identified as soft-rot bacteria (SAWIAK et al. 2009). In addition to causing rots of potato tubers, the above pathogens can latently infect seed potatoes and, under supportive environmental conditions, the developing plants (GARDAN et al. 2003).

The percentage of potato tubers infected by fungi of the genus *Fusarium* remained at a similar level throughout the experiment. The highest percentage (ca. 22%) of *Fusarium* – infected tubers was harvested from control plants of cv. Volumia in 2010, from control plants of cv. Irga in 2011 and from plants of cv. Sylvana treated with Bio-Algeen S-90 in 2012 (Table 6). The average values of the infection index show that the severity of dry rot varied between cultivars, and disease incidence was reduced by plant growth regulators only in the second year of the study. Dry rot is caused by *Fusarium* spp. (PETERS et al. 2008), in Poland by *F. sulphureum, Gibberella pulicaris* and *Haematonectria haematococca*, and mechanical damage during harvesting operations predispose tubers to attack by the above pathogens (KURZAWINSKA and GAJDA 2002, CWALINA-AMBROZIAK and BOGUCKA 2012).

Tubers harvested in the first year of the experiment showed weak symptoms of common scab in storage, and disease-free tubers were collected in some treatments. The severity of infection caused by *Streptomyces scabies* was higher in the second and third year, and the highest infection index (ca. 19%) was noted on tubers of cv. Sylvana harvested in 2011 and 2012, treated with Asahi SL and Kelpak SL, respectively (Table 7). Throughout the experiment,

Table 6

The severity of infection caused by *Fusarium* spp. after 5-month storage [% of the total weight of infected tubers]

Cultivar	2010				x	2011				x	2012				x
	Asahi	Bio-Alg	Kelpak	control		Asahi	Bio-Alg	Kelpak	control		Asahi	Bio-Alg	Kelpak	control	
Volumia	16.2^{bc}	13.6^{c}	17.0^{b}	22.5^{a}	17.3^{a}	17.0^{bc}	13.0^{ef}	12.0^{efg}	16.0^{cd}	14.5^{a}	20.8^{ab}	17.0^{cd}	6.0^{i}	7.0^{i}	12.7^{b}
Irga	13.0^{c}	9.0^{d}	17.0^{b}	13.0^{c}	13.0^{b}	3.0^{h}	17.6^{b}	13.0^{fg}	22.4^{a}	14.0^{a}	6.5^{i}	6.5^{i}	15.4^{de}	10.0^{h}	9.0^{d}
Satina	3.4^{e}	16.2^{bc}	15.3^{bc}	14.5^{bc}	12.4^{b}	14.0^{de}	3.6^{h}	4.0^{h}	10.0^{g}	7.9^{b}	13.0^{fg}	17.0^{cd}	5.0^{i}	12.0^{g}	11.8^{c}
Sylvana	0^{f}	7.0^{d}	6.0^{de}	6.0^{de}	4.8^{c}	11.0^{fg}	19.2^{b}	13.0^{fg}	17.6^{b}	15.2^{b}	14.0^{ef}	22.0^{a}	18.0^{c}	20.0^{b}	18.5^{a}
Mean	8.2^{c}	14.5^{b}	13.8^{a}	14.0^{a}	–	11.3^{c}	13.4^{b}	10.5^{c}	16.5^{a}	–	13.6^{b}	15.6^{a}	11.1^{d}	12.3^{c}	–

Key: see Table 2

Table 7

The severity of infection caused by *Streptomyces scabies* after 5-month storage [infection index li, %]

Cultivar	2010				x	2011				x	2012				x
	Asahi	Bio-Alg	Kelpak	control		Asahi	Bio-Alg	Kelpak	control		Asahi	Bio-Alg	Kelpak	control	
Volumia	2.6^{b}	0^{f}	3.0^{a}	1.4^{d}	1.8^{a}	9.6^{ef}	13.2^{cd}	11.3^{de}	14.9^{bc}	12.0^{b}	23.3^{a}	8.4^{i}	16.7^{c}	4.9^{j}	13.3^{b}
Irga	1.4^{d}	0^{f}	0^{f}	0.7^{e}	0.5^{c}	5.9^{fg}	2.0^{h}	2.0^{h}	3.3^{gh}	3.3^{c}	10.4^{ghi}	9.6^{hi}	12.4^{efg}	10.4^{ghi}	10.7^{d}
Satina	0^{f}	0.7^{e}	3.0^{a}	1.6^{d}	1.3^{b}	14.9^{bc}	13.9^{cd}	16.0^{abc}	17.3^{abc}	15.5^{a}	9.1^{hi}	11.1^{fgh}	13.1^{ef}	14.4^{de}	11.9^{c}
Sylvana	2.2^{e}	0.6^{c}	0.7^{e}	2.0^{c}	1.4^{b}	18.2^{a}	15.7^{abc}	14.6^{bc}	13.6^{cd}	15.5^{a}	16.2^{cd}	13.6^{c}	19.2^{b}	8.4^{i}	14.4^{a}
Mean	0.3^{b}	0.3^{b}	1.7^{a}	1.4^{a}	–	11.9^{a}	11.2^{a}	11.0^{a}	12.3^{a}	–	14.8^{a}	10.7^{b}	15.4^{a}	9.5^{c}	–

Key: see Table 2

significantly lower infection rates were determined for tubers of cv. Irga, in comparison with the remaining cultivars. The plant growth regulators and growth biostimulator applied in the study exerted varied effects on the incidence and severity of potato tuber disease; in the first two years of the study, the tested products reduced infection rates or had no influence on disease spread, and in the last year Asahi SL and Kelpak SL contributed to infection progress. The incidence and severity of potato skin diseases were affected by variable weather conditions during the growing season, in particular by the amount and distribution of rainfall in the summer (LENC 2006). SAWICKA (1999) reported that the effects of plant growth regulators Mival and Moddus 250 ME on the severity of infections caused by *S. scabies* and *R. solani* were largely determined by weather conditions and disease resistance of the examined cultivars. In the cited study, high incidence of common scab was noted on tubers harvested from plants treated with growth regulators in years with moisture deficiency during tuberization and tuber setting, and high incidence of black scurf was observed in years with heavy precipitation at the end of the growing season.

In the current experiment, the infection index of black scurf ranged from 0.6% in the first growing season (tubers harvested from plants of cv. Satina sprayed with Asahi SL) to 10.4% in the second growing season (tubers harvested from plants of cv. Sylvana treated with Bio-Algeen S-90) (Table 8). Black scurf was most common on tubers harvested in 2011. The average values of the infection index indicate that black scurf was most effectively controlled with the growth biostimulator Asahi SL and the growth regulator Kelpak SL in 2010. In 2011 and 2012, the growth regulator Bio-Algeen S-90 had a stimulating effect on the infection spread. Infection rates varied significantly between potato cultivars and years of the study, and cv. Sylvana was found to be most affected. Cv. Volumia was the healthiest cultivar in the first two years, and cv. Satina in the last year. BAINS et al. (2002) also pointed to differences in the incidence and severity of black scurf between potato cultivars.

A total of 31 fungal species, yeast-like fungi and non-sporulating cultures were isolated from potato tubers stored for five months (Table 9). The pathogens, represented by *Alternaria alternata, Colletotrichum coccodes*, seven species of the genus *Fusarium,* and *R. solani*, colonized potato tubers in all treatments, except that *R. solani* was not isolated from control plants. The pathogens were most frequently isolated from tubers harvested from plants treated with Bio-Algeen S-90 (45%), followed by tubers harvested from control plants and plants sprayed with Kelpak SL, and least frequently from tubers harvested from plants treated with Asahi SL (Figure 1). The predominant pathogen was *C. coccodes* that accounted for 17 to 23% of all isolates. *Fusarium* species were isolated less frequently – they accounted for 8% of isolates

Table 8

The severity of infection caused by *Rhizoctonia solani* after 5-month storage [infection index li, %]

Cultivar	2010				x	2011				x	2012				x
	Asahi	Bio-Alg	Kelpak	control		Asahi	Bio-Alg	Kelpak	control		Asahi	Bio-Alg	Kelpak	control	
Volumia	1.3^{fgh}	2.0^{e}	0.7^{hi}	2.1^{e}	1.5^{c}	0.7^{j}	5.3^{de}	2.7^{hi}	2.0^{i}	2.7^{c}	2.2^{c}	4.0^{b}	2.7^{c}	4.0^{b}	3.2^{b}
Irga	0.9^{ghi}	0.7^{hi}	1.6^{ef}	4.5^{c}	1.9^{b}	6.7^{c}	4.7^{ef}	6.0^{cd}	4.0^{fg}	5.4^{b}	2.7^{c}	4.1^{b}	2.4^{c}	2.7^{c}	3.0^{b}
Satina	0.6^{i}	1.5^{efg}	1.0^{f-i}	3.3^{d}	1.6^{c}	6.7^{c}	4.0^{fg}	3.3^{ghi}	8.0^{b}	5.5^{b}	2.0^{cd}	1.3^{de}	1.3^{de}	2.7^{c}	1.8^{c}
Sylvana	1.1^{f-i}	9.3^{a}	1.3^{fgh}	6.5^{b}	4.6^{a}	6.0^{cd}	10.4^{a}	3.3^{ghi}	8.0^{b}	6.9^{a}	3.6^{b}	5.8^{a}	5.1^{a}	1.1^{e}	3.9^{a}
Mean	1.0^{c}	3.4^{b}	1.2^{c}	4.1^{a}	–	5.0^{b}	6.1^{a}	3.8^{c}	5.5^{b}	–	2.6^{b}	3.8^{a}	2.9^{b}	2.6^{b}	–

Key: see Table 2

Table 9

Fungi isolated from potato tubers after 5-month storage (Σ for 2010–2012)

Species	Control				Asahi SL				Bio-Algeen S-90				Kelpak SL			
	Vol	Irga	Sat	Syl	Vol	Irga	Sat	Syl	Vol	Irga	Sat	Syl	Vol	Irga	Sat	Syl
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Alternaria alternata (Fr.) Keissler	22	2	8	3	8	3	6	1	6	11	7	–	13	7	27	–
Ascus sp.	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–
Chrysosporium olivaceum (Link) J.J. Taylor	–	–	–	–	–	1	–	–	–	–	–	2	–	1	–	–
Cladosporium cladosporioides (Fres.) de Vries	1	–	–	–	5	3	–	–	–	–	–	–	–	–	–	–
Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams	12	26	–	–	1	17	1	13	–	26	–	–	–	1	–	–
Colletotrichum coccodes (Wallr.) S. Hughes	24	35	42	27	23	23	42	37	40	23	53	27	37	26	23	48
Endothia sp.	–	–	–	–	–	2	–	–	–	–	–	–	1	–	–	1
Epicoccum sp.	–	–	–	33	31	–	–	–	–	1	1	–	1	–	–	–
Fusarium chlamydosporum Wollenw. & Reinking	–	–	–	–	1	3	–	5	–	–	–	3	–	–	–	–
Fusarium concolor Reinking	2	–	–	–	–	–	–	–	–	–	–	1	–	1	–	–
Fusarium culmorum (W.G. Sm.) Sacc.	1	8	11	21	10	5	1	5	8	12	22	35	3	3	14	4
Fusarium oxysporum Schltdl	1	5	23	4	6	11	–	2	8	1	2	1	26	30	3	–

cont. table 9

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Fusarium poae (Peck) Wollenw.	–	–	–	3	–	1	1	1	–	1	1	–	1	4	1	2
Gibberella avenacea R.J. Cook	13	–	–	3	–	1	3	–	5	–	–	–	–	3	3	–
Haematonectria haematococca (Berk. & Broome) Samuels & Rossman	–	–	3	–	1	–	–	9	–	–	–	–	–	2	–	–
Humicola brevis Gilman & Abbott	1	–	–	–	2	1	–	–	–	–	–	–	–	1	–	–
Mortierella zonata Linnem.	–	–	–	–	–	7	–	4	–	–	8	–	–	–	–	–
Mucor circinelloides Tiegh.	–	–	2	–	2	2	–	–	–	1	2	–	–	–	4	–
Mucor hiemalis Wehmer	14	6	9	41	22	32	36	15	16	4	7	10	37	21	11	15
Myrothecium verrucaria (Alb. & Schwein.) Ditmar	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	3
Paecilomyces nivalens (Thom) Samson	1	–	–	–	–	3	–	–	6	–	–	–	–	3	–	–
Paecilomyces roseum (Thom) Samson	–	–	1	–	1	–	–	–	–	–	1	–	1	–	–	–
Paraconiothyrium minitans W.A. Campb. Verkley	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Penicillium sp.	73	69	74	47	69	53	70	87	90	67	44	38	117	87	77	53
Rhizoctonia solani Kühn	–	–	–	–	1	1	1	4	–	4	4	4	–	1	1	1
Rhizopus stolonifer (Ehrenb.) Vuill.	4	–	–	2	4	–	–	–	1	1	5	–	2	–	1	1
Sarocladium strictum W. Gams	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sphaerostilbella aureonitens (Tul. & C. Tul.) Seifert, Samuels & W. Gams	–	2	–	–	13	3	5	–	–	–	–	–	–	1	–	2
Sporormia sp.	–	–	–	–	1	–	–	–	1	–	–	–	–	1	–	–
Trichoderma hamatum (Bonord.) Bainier	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1	–
Trichoderma polysporum (Link) Rifai	–	–	–	–	–	–	–	–	–	4	–	–	–	–	–	–
Yeast-like fungi	–	1	11	–	–	1	–	–	–	–	3	1	–	–	5	–
Non-sporulating cultures	–	–	1	1	4	22	–	1	–	–	–	–	–	1	1	–
Total (number of isolates)	170	155	185	185	204	196	166	184	181	156	160	123	241	196	172	130

Key: Vol – Volumia, Syl – Sylwana, Sat – Satina

obtained from tubers harvested from plants treated with Asahi SL, and ca. 16% of isolates collected from tubers harvested from plants sprayed with Bio-Algeen S-90, which is consistent with data on dry rot incidence and severity. The causal agent of early blight was encountered less frequently, and only single isolates of black scurf were collected. Similar species composition of fungal communities isolated from potato tubers was reported by KURZAWIŃSKA and GAJDA (2002), and CWALINA-AMBROZIAK and BOGUCKA (2012). According to authors, tubers of medium-late and late potato cultivars are more frequently and abundantly colonized by fungi of the genus *Fusarium* than tubers of early cultivars.

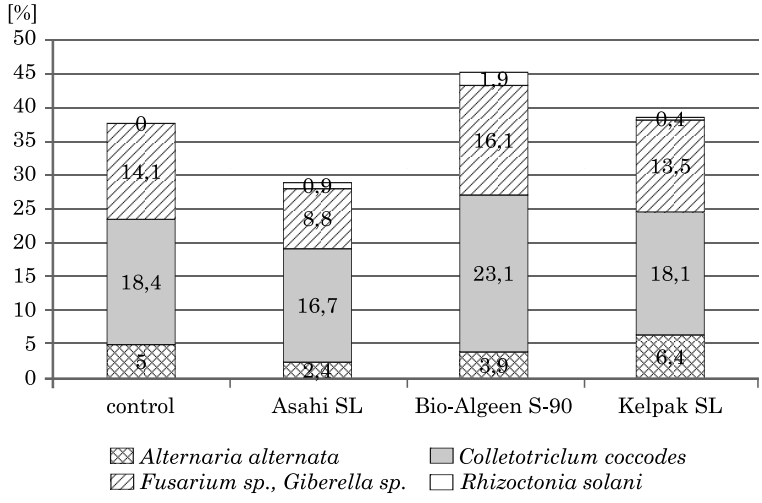

Fig 1. Percentages of pathogens isolated from potato tubers after 5-month storage (data for 2010–2012)

Conclusions

1. The highest severity of infection caused by *Phytophthora infestans* (ca. 70%) on potato plants was noted in the second year of the study. The highest rates of *Alternaria* spp. infection (40%) were observed on potato plants of cv. Volumia, Irga and Satina in the last year of the experiment.

2. The severity of late blight was reduced by plant growth regulators Bio-Algeen S-90 and Kelpak SL throughout the experiment, and the severity of early blight was reduced by Kelpak SL in 2011 and 2012.

3. Plant growth regulators Bio-Algeen S-90 and Kelpak SL and the growth biostimulator Asahi SL reduced the incidence and severity of late blight and

dry rot, but exerted varied effects on the development of the remaining diseases.

4. The analyzed pathogens were least frequently isolated from tubers harvested from potato plants sprayed with Asahi SL. *Colletotrichum coccodes* was the predominant species in the fungal community, whereas *Fusarium* spp., *Gibberella* spp., *A. alternata* and *Rhizoctonia solani* were less abundant.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 18.03.2015

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 30(1): 21–34, Y. 2015

MICE LINES DIVERGENTLY SELECTED FOR BODY MASS SHOW SIGNIFICANT DIFFERENCES IN SPATIAL LEARNING

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K e y w o r d s: Body weight, Behavior, Spatial memory, Learning, Mice.

A b s t r a c t

The present study was aimed to investigate the influence of multigenerational selection in the direction of low or high body weight on spatial learning and memory in mice. Light and heavy lines of rodents were selected from an outbred stock constructed from inbred strains; A/St, BN/a, BALB/c and C57BL/6J. Male mice selected at weaning for the low (L, n=13) or high (H, n=16) body weight for 94 generations have been evaluated for behavioral performance and cognition in the modified Morris water maze task. The unselected control line (Con, n=15) was run in parallel. Presented results lead to the conclusion that selection of mice for high and low body weight over 94 generations resulted in a significant differentiation in learning abilities. Our findings suggest improvement of learning of the hidden platform position in heavy line of mice.

LINIE MYSZY PODDANE SELEKCJI W KIERUNKU WYSOKIEJ I NISKIEJ MASY CIAŁA RÓŻNIĄ SIĘ ZNAMIENIE W UCZENIU PRZESTRZENNYM

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Abstrakt

Celem badania jest ocena wpływu wielopokoleniowej selekcji w kierunku niskiej lub wysokiej masy ciała na procesy uczenia się i pamięci przestrzennej u myszy. Obie linie gryzoni uzyskano w wyniku wielopokoleniowej selekcji z niekrewniaczej wsobnej hodowli myszy szczepów; A/St, BN/a, BALB/c i C57BL/6J. Zachowanie i funkcje poznawcze analizowano przy pomocy labiryntu wodnego Morrisa u 13 samców z linii lekkiej (L, n = 13) i 16 samców z linii ciężkiej (H, n = 16). Równolegle prowadzono linię kontrolną, z której do badania wybrano 15 samców (Con, n = 15). Prezentowane wyniki prowadzą do wniosku, że wielopokoleniowa selekcja myszy w kierunku wysokiej i niskiej masy ciała prowadzi do istotnego zróżnicowania w zakresie zdolności uczenia się. Nasze wyniki sugerują, że myszy z linii ciężkiej znacznie szybciej uczą się pozycji podwodnej platformy w teście labiryntu wodnego.

Introduction

Quantitative traits are conditioned by genes located on the same chromosome or on the added impact on many genes. Therefore simple selections based only on the one trait may produce changes in other functions, e.g. reproductive abilities. This phenomena may be associated with the preferential use of one gene product in regulation of expression of the others (LIU et al. 1994, REID et al. 1995).

The body weight is classified as a simple measured quantitative trait associated with multigene effects. Decades ago, correlations between body weight and longevity, processes of aging or certain physiological parameters have been proven.

So far, studies have shown that there is a relationship between body mass and life expectancy and the number of reproductive functions such as: time of puberty, gonadal weight, level of ovulation, the number of embryos, the fetus and placenta weight, litter size and mortality in the prenatal period (WIRTH-DZIECIOŁOWSKA et al. 2005). Positive correlation between this quantitative trait, fat content and size of many organs also has been found (BENIVAL et al. 1992, HASTINGS and HILL 1990, HASTINGS et al. 1991). Selection on body weight may affect also some aspects of animal behavior e.g. learning, memory and visuomotor skills. Study by Padeh and Soller (1976) demonstrated positive genetic correlations between body mass, weight of both cerebellum and cerebrum and learning abilities in inbred strains of mice.

This study focuses on the behavioral and cognitive processes in mice selected up to 94 generations in two lines with low and high body mass. The aim of our experiment was to examine the correlation between body weight the chosen lines of mice and spatial learning, memory, motivation and motor activity measured in a modified Morris water maze.

Our findings are important for the genetic and neural basis of spatial learning in laboratory rodents.

Material and Methods

Animals and method of selection based on body mass

The experiment was conducted on male mice that originated from rodents selected from 94 generations at a lower level (L line, n=13) or high (H line, n=16) body mass at the time of weaning at 21st postnatal day (PND).

Differences in body weight between L and H lines were created by outbred stock constructed from the inbred strains: A/St, BN/a, BALB/c and C57BL/6J. There was no selection in the parallel running control line (Con, n=15). In the 94 generation of selection 30 females were monogamically mated within their lines (avoiding inbreeding). Pairs were kept together until two months after the last delivery or till the death of the partner. Offspring were reared with parents until weaning at 21st postnatal day (PND 21). From among them, forty four young males were chosen and housed 5–8 in plastic breeding cages in a temperature-controlled room (23°C) with a constant photoperiod (12 h : 12 h light/dark cycle) and air humidity 60–70%. Animals were given free access to tap water and standard granulated feed (Murigran) ad libitum. The body weight of animals were measured at 21, 56, 70, 90 and 100 postnatal day. The effect of selection based on body mass was conducted on mice in 100st PND and analyzed in the modified Morris water maze task. All procedures were carried out according to the regulations Ethical Committee for Animal Experiments at Agricultural University of Warsaw in compliance with the ethical standards of the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Behavioral assessment

Water maze test

Mice were tested in the Morris water maze including acquisition trials and visual platform test (cued task) with minor modifications (WIDY-TYSZKIEWICZ et al. 1993). White, circular pool of 1.2 m diameter and 0.5 m deep was filed with water at 24±0.5°C. The experiment was performed during the light phase of the cycle (between 8.00–15.00 h). The pool was divided into four quadrants which were arbitrarily designed Northeast (NE), Northwest (NW), Southeast (SE) and Southwest (SW). The swimming pool was situated in the room with many objects that could be used by animals for spatial navigation. During training trials mice learned to escape from water by finding a submerged plexiglas platform (10 cm x 10 cm) hidden 0.5 cm below the water surface and placed in a fixed location in the center of SE quadrant. At the beginning of the

behavioral session each mouse was placed with its face toward the wall of the pool at one of three starting positions. During acquisition of the spatial navigation task mouse received 6 days of training with the hidden platform, each day included 4 training sessions (day 1–6; trial 1–24) with 15 s inter-session intervals. The starting location was diverse in each training trial and changed each day. The trial was terminated when the animal found the platform or until 60 s had elapsed. When the mouse found the platform it was allowed to remain there for 15 s. If the mouse didn't find the platform within this time it was placed on the platform by the experimenter for 15 s. At the end of day's session the mouse was removed from the pool, allowed to dry off and returned to its cage.

After completion of the hidden platform navigation task 24 h after last training trial, spatial memory was evaluated in the probe trial, on seventh day (day 7; trial 25). Memory test was performed after removing the platform and animals were allowed to swim for 60 s (probe trial).

Motor activity and motivation were evaluated in the cued task (day 8; trial 26–29). For the cued task the target platform was visible and placed 1 cm above the water line inside the pool. Data collection from Morris's water maze task that included escape latency, swim path length, swim speed, total time spend in the SE quadrant and the number of crossings over the former platform location, was automatically recorded using an HVS image analyzing system (Chromotrack, San Diego Instruments) and videotaping.

Statistical evaluation

Group differences were evaluated by applying analysis of variance – ANOVA with repeated measures (treatment × day × trial). Statistical analysis of the difference between groups was assessed with Student's t-test and two way ANOVA. Significant effects were further analyzed by post-hoc analyses (Newman-Keuls test and Student's *t*-test). All results are expressed as the mean ± SE for each experimental group. A value of $p<0.05$ was considered to be statistically significant.

Results

Body weight

Postnatal mean body mass of the light, heavy and control lines of mice are summarized in Table 1. Differences in the body mass between animals from the selected lines are presented on the 21st postnatal day (PND 21)

(Con: 13.35±0.13 g; L: 10.81±0.27 g; H: 14.42±0.17 g) ($F_{(2,41)}$=0.81) (p<0.001, Newman-Keuls) maintained at each time point where the body mass was measured (PND 56: $F_{(2,41)}$=1.58, p<0.001; PND 70: $F_{(2,41)}$=1.74, p<0.001; PND 90: $F_{(2,41)}$=1.68, p<0.001, Newman-Keuls). At 100st postnatal day, at the time of the behavioral experiment, the body mass of mice ranged between: 34.70±0.70 g for the control: 45.84±0.74 g for heavy line and 24.63±0.81 g for the low line and was significantly different between the control and experimental groups ($F_{(2,41)}$=2.11) (p<0.001, Newman-Keuls).

Table 1

Effect of divergent selection on the mean body mass (g ± SE) in light (L), heavy (H) and control (Con) line of mice on subsequent postnatal days (PNDs). ***L *vs* Con, p<0.001; $^{\#\#\#}$H *vs* Con, p<0.001; $^{\bullet\bullet\bullet}$L *vs* H, p<0.001 (Newman-Keuls test)

Mean body mass (g ± SE) in light, heavy and control line of mice on subsequent postnatal days					
Line	Postnatal day (PND)				
	PND 21	PND 56	PND 70	PND 90	PND 100
Light (L)	10.81±0.27$^{***\bullet\bullet\bullet}$	21.55±0.61$^{***\bullet\bullet\bullet}$	22.78±0.64$^{***\bullet\bullet\bullet}$	24.29±0.64$^{***\bullet\bullet\bullet}$	24.63±0.81$^{***\bullet\bullet\bullet}$
Heavy (H)	14.42±0.17$^{\#\#\#}$	40.25±0.97$^{\#\#\#}$	41.10±0.80$^{\#\#\#}$	44.41±0.91$^{\#\#\#}$	45.84±0.74$^{\#\#\#}$
Control (Con)	13.35±0.13	29.36±0.58	30.55±0.63	33.11±0.74	34.70±0.70

Behavior – water maze results

Acquisition trials (days 1-6; trials 1-24)

As shown in Fig. 1 the latency to escape to the platform in all groups of mice decreased following the training sessions. ANOVA for repeated measurements showed significant differences of place learning between the low and high body mass line ($F_{(2,41)}$=3.82, p<0.05, Newman-Keuls). The group with low body weight showed impairment of learning abilities compared to the high body mass line. Likewise significant effects in mean total escape latency for all experimental groups were found (Con: 40.75±0.90 s; L: 47.06±1.18 s; H: 36.17±1.11 s).

A statistically significant changes with considerable scatter of the results of latency were determined during all days of training. ANOVA analysis for a particular day of training and escape latency is as follows: Day 1: $F_{(2,173)}$=5.00, p<0.01; Day 2: $F_{(2,173)}$=6.16, p<0.001; Day 3: $F_{(2,173)}$=0.085, p>0.05; Day 4: $F_{(2,173)}$=3.69, p<0.05; Day 5: $F_{(2,173)}$=4.40, p<0.05; Day 6: $F_{(2,173)}$=4.38, p<0.05, Newman-Keuls.

As can be seen from Fig. 2 differences in total swim distance between all experimental groups (Con: 7.54±0.23 m; L: 8.33±0.26 m; H: 5.75±0.22 m;

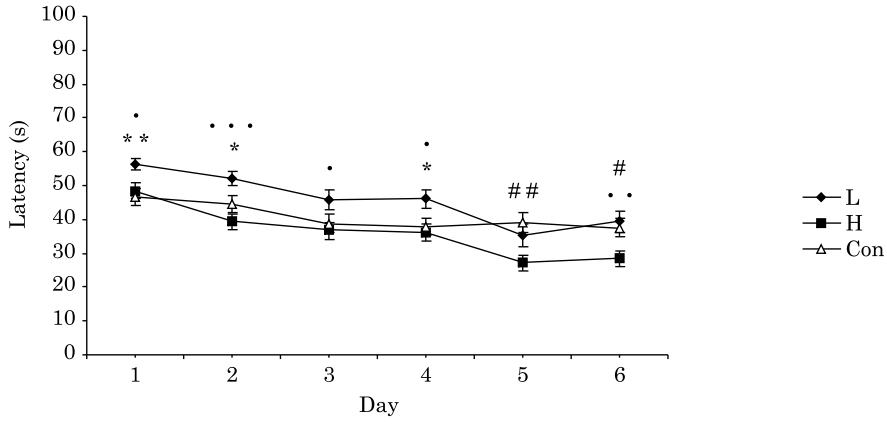

Fig. 1. Effect of divergent selection for body mass on spatial learning (time to escape from the water during acquisition trials using a submerged platform) in light (L), heavy (H) and control (Con) line of mice in the Morris water maze test. * L *vs* Con, $p<0.05$; ** L *vs* Con, $p<0.01$; $^{\#}$H *vs* Con, $p<0.05$; $^{\#\#}$H *vs* Con, $p<0.01$; •L *vs* H, $p<0.05$; ••L *vs* H, $p<0.01$; •••L *vs* H, $p<0.001$ (Newman-Keuls test)

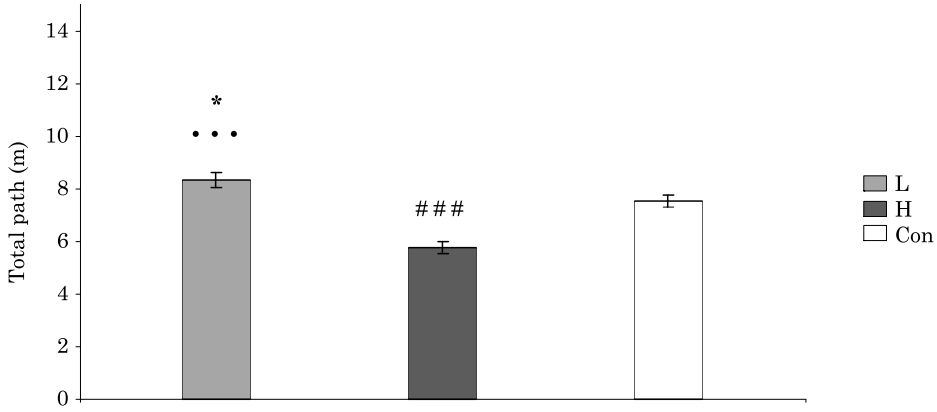

Fig. 2. Effect of divergent selection for body mass on mean swim distance during acquisition trials in light (L), heavy (H) and control (Con) line of mice in the Morris water maze test. * L *vs* Con, $p<0.05$; $^{\#\#\#}$H *vs* Con, $p<0.001$; •••L *vs* H, $p<0.001$ (Newman-Keuls test)

$F_{(2,1053)}=31.77$; $p<0.001$) have been found. The Newman-Keuls test also confirms the differences in swimming speed between the mice from heavy line, light line and the control group (Con: 0.19±0.003 m/s; L: 0.19±0.003 m/s; H: 0.17±0.04 m/s; $F_{(2,1053)}=18.34$; $p<0.001$, Newman-Keuls) (Fig. 3).

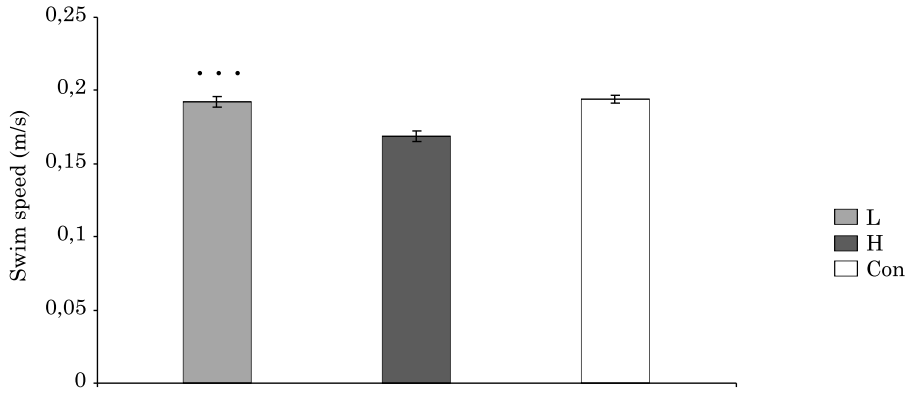

Fig. 3. Effect of divergent selection for body mass on mean swim speed during acquisition trials in light (L), heavy (H) and control (Con) line of mice in the Morris water maze test. •••L *vs* H, $p<0.001$ (Newman-Keuls test)

The probe trial-memory test (day 7; trial 25)

Platform crossings on the previous SE position did not show significant effects following line selection ($F_{(2,41)}=0.58$; $p>0.05$, Newman-Keuls). The time spent in target quadrant SE (earlier platform's position) did not differ between groups ($F_{(2,41)}=0.29$; $p>0.05$, Newman-Keuls). There wasn't any significant difference between groups in the percentage of time spent in the remaining quadrants (SW: $F_{(2,41)}=1.80$; NW: $F_{(2,41)}=0.26$; NE: $F_{(2,41)}=1.88$; $p>0.05$, Newman-Keuls). No significant effects in total swim distance ($F_{(2,41)}=2.18$) and speed ($F_{(2,41)}=2.24$; $p>0.05$, Newman-Keuls) for all experimental groups were found.

Visible platform test (day 8; trials 26–29)

An analysis of variance did not demonstrate significant difference between groups in the mean escape latency in the cued task on Day 8 ($F_{(2,173)}=1.51$; $p>0.05$). However we have seen an extended search time for the visible platform in the group of mice with the low body weight (47.60±3.00 s) compared to the heavy line (34.92±2.95 s) and the control (41.40±2.69 s). Results of the cued task on day 8 indicate an increase in the mean swimming speed in light line. The average swimming speed was lower in heavy line (0.12±0.007 m/s) and in the control group (0.16±0.012 m/s) *vs* light line (0.27±0.058 m/s) ($F_{(2,173)}=5.35$; $p<0.01$). The results did not show a significant main effect for mean distance travelled ($F_{(2,173)}=0.45$; $p>0.64$).

Discussion

In the present work we have demonstrated the consequences of multigenerational selection for the low or high body mass on spatial learning, memory, motivation and motor abilities in mice.

The body mass is a polygenic simple measured quantitative trait with moderate to high heritability. The present experiment demonstrates that multigenerational selection based on body weight results in the significant modulation of behavior with subtle changes concerning spatial learning in mice. Mice from two selected lines differ in body mass at the weaning and through the whole lifespan. The statistical analysis shows significant differences between the groups in the acquisition in the water maze test.

Our observations are partially compatible with the results of other investigators. Similar conclusions were reached by Padeh and Soller (1976), who found a positive genetic correlation between body mass in inbred strains of mice and T-maze learning ability as well as the relationship between body mass, cerebrum weight and learning evaluated in this assay. Numerous studies have documented that spatial memory depends on the combination of several genetic and environmental factors which contribute to spatial learning ability and cognition. One of the relevant factors which may be important for the proper functioning of the central nervous system and cognitive processes is the nutritional status (ACHAM et al. 2008).

In our experiment the ability of spatial learning in mice from heavy line evaluated in water maze was found to be increased. We notice frequently the restriction of the early postnatal development due to the large size of the litter in rodents. Based on observations made by the team of Jou and co-authors (2013) we can infer that, as in our experiment, early postnatal growth retardation leads to poorer developmental outcome and worse learning behavior.

Certain studies involving laboratory animals suggest that dietary restriction particularly early in life will not only reduce the incidence of genetic diseases and pathological changes, but also prolongs the mean life span (LLOYD 1984, MASORO 2000, WEINDRUCH 1996). Mice from the light line lived longer than rodents with high body mass (heavy line) (WIRTH-DZIECIOŁOWSKA and CZUMIŃSKA 2000). Results presented by Takahashi et al. (2006) suggested that the food reduction initiated at an early stage of life improves the acquisition of the passive avoidance task in animal model of accelerated aging. This beneficial effect in terms of memory improvement in mice scientists linked with changes in neuronal functions rather than with histological alteration in the brain (TAKAHASHI et al. 2006). Similarly, recent studies indicated that manipulation of caloric content produces learning and memory deficits in this distinctive strain of mice (KOMATSU et al. 2008).

A very interesting and intriguing conclusions were made by Cunnane and Crawford (2003) who developed theories that „fat babies were the key to evolution of the large human brain". According to this hypothesis, fetal fat deposits are required for the proper functioning of the developing brain and constitute the key to the evolution of the mammalian central nervous system.

On the other hand scientific evidence suggests that obesity may cause brain dysfunction (NILSSON and NILSSON 2009), changes in personality traits and anatomical variations in the brain structures in humans e.g. as increasing gray matter volume in the hippocampus (MORENO-LÓPEZ et al. 2012). It was also shown that a high body mass index (BMI) is correlated with decreased gray matter volume in the orbitofrontal regions and lower cognitive functioning (WALTHER et al. 2010). Interestingly, high BMI resulted in faster visuomotor speed. Study by GUNSTAD et al. (2007) based on studies involving 408 healthy subjects demonstrated that elevated BMI is negatively correlated with cognitive performance and other executive functions observed in overweight and obese adults. Poorer neurocognitive outcome was probably associated with a number of pathophysiological amendments within the cardiovascular system and impaired insulin regulation.

Integrative analysis of genetic factors related to complex traits such as body weight and obesity is extremely difficult. One of the technique that enables understanding the molecular mechanism and genetic basis of these complex traits is a module-guided Random Forest (mgRF). Many genes identified by this method may potentially contribute to the variation of the mouse body mass e.g. Mogat1, Cyp2c37, Gpld1 (CHEN and ZHANG 2013). Other studies analyzing the genetic basis of response to multi-generational selection on body weight in inbred mice were conducted by Keightley and Hill in 1989. In their experiment the high and low lines of mice derived from a subline of the inbred strain C3H/He were used. The authors emphasize the earlier observation of the increase of genetic variance of quantitative traits due to the accumulation of new mutations which may in turn be responsible for the artificial selection. At the same time, these studies suggest that between 38 and 50 generations a plateau, manifested in a little subsequent selection response is reached. Under the experimental conditions, selection can determine the extent of the inheritance of the complex quantitative traits and predict consequences of long-term selection for some characteristics in future. However, depending on the time scale, the effect and final goals of selection may vary. Long-term experiments may be useful for evaluating changes in response rates or differences caused by selection and provide details about the underlying inheritance of quantitative traits.

It should be noted that the lines of mice used in the experiment were created by outbred stock constructed from few inbred strains (A/St, BN/a,

BALB/c and C57BL/6J), which differ not only in the phenotypic traits but also in the neuroanatomical features, sensory and behavioral abilities (BROWN and WONG 2007). NGUYEN et al. (2000) indicated the existence of the strain-dependent variations in hippocampal long-term potentiation and spatial memory in inbred mice. Characteristic memory deficits in the Morris water maze were found in CBA/J mice, whereas both in DBA/2J and CBA/J strains impaired nonspatial learning and long-term memory in the contextual and cued fear conditioning tests were observed. As demonstrated *in vitro*, abnormalities in cognitive abilities in DBA/2 strain can be partly explained by reduction of the paired-pulse facilitation – the early phase of LTP in CA1 area of the hippocampus.

Even though, there was no line specific differences in the cued task, it is well known that mice with poor vision like BALB/c exhibited poor performance in the tests requiring distal location e.g. in radial arm maze (BROWN and WONG 2007).

It is reasonable to assume the existence of a connection between body size, longevity, diet and environmental factors, increased selective pressure for mental/social adaptation and behavioral innovation which eventually results in the increased brain size (WARD et al. 2004).

The relative brain weight (brain/body weight ratio) is considered to be one of the most important indicators of the evolutionary level of species. In the vertebrates, the brain size is affected and shaped by many factors operating separately. Despite the fact that having a larger brain (relative to body size) brings many benefits, the energy cost of maintaining this organ is very high and not always justified by evolution (FITZPATRICK et al. 2012, ROTH and DICKE 2005).

It is not exactly known which of the features: size of the brain, degree of encephalization or structural and functional specialization of cerebral cortex, most accurately reflects the animal intelligence (DEANER et al. 2007). However, previous studies show clearly and unequivocally the empirical link between brain size and mental and behavioral flexibility in primates (READER and LALAND 2002). Analysis of 100 postmortem human brains demonstrates that visuospatial skills and verbal intelligence is positively related to cerebral volume (WITELSON et al. 2006).

Jacobs and co-authors (1990) demonstrated that the evolution of spatial cognition and hippocampal size shows some differences based on gender. Moreover, behavioral differences between monogamous and polygamous laboratory strains of mice with larger hippocampus in the males have been shown. Another series of studies on the effect of selection for the body weight reported differences in the growth and changes occurring with age between the heavy and light lines. Analysis of differences in the longevity and aging of mice from

body weight selected lines show earlier mortality in the males from heavy line. This effect in this group was correlated with earlier weight loss and higher hyperplasia of the cortex cells in adrenals.. Selection for body weight induced also differences in exploratory behavior in mice measured in the open field and Lashley maze (WIRTH-DZIECIOŁOWSKA et al. 2005). The line with low body mass showed higher anxiety and impairment of spatial learning.

Salimov and co-authors (2004) pointed out that mice lines selected for different brain weight exhibit pronounced diversity in exploratory behavior and fear tendencies. The differences are particularly apparent with regard to the fear-anxiety and spontaneous stereotypic behavior. Mice with larger brain weight show higher scores of locomotion in peripheral parts of the open field arena, more rearing and less frequent freezing and grooming compared to the mice with smaller brain. Hybrid F2 mice with larger brain weight moved faster and more likely demonstrated stereotyped behavior in the cross-maze test (SALIMOV et al. 2004). Not unlikely, that it is related to higher sensitivity or increased response to the pain stimuli presented by those with lower brain weight. This proven relationship between phenotypes of the selected lines of mice and their behavior is a very interesting problem, but previous research has displayed divergent experimental data (FALCONER 1953, FOWLER 1962, HOLMES and HASTIGS 1995).

Body weight is a multigenic conditioning trait additively affected by genes, therefore simple divergent selection may easy lead to changes in the other features. Long-term selection for body composition produced significant differences in the proportion of gonadal fat and total body fat in the *Fat* and *Lean* lines of mice (MARTINEZ et al. 2000). These observations are confirmed by the results carried out by Eisen and co-authors (1978, 1987, 1988), which shows the particular relationship between the body weight and weights of the perigonadal fat.

The cumulative data indicate the occurrence of correlations of body weight with ovulation rate, mortality and embryo number, placental and fetuses weight and other reproduction traits. Selection of mice for the body weight for over 90 generations resulted in a differentiation in their sexual maturation rate. Determinations of the level of sexual hormones and histology of gonads confirm that mice from line L reached maturity later compared to the animals from H line. Changes in the weight of parovarian fat in females show clear connection with the period of reaching sexual maturity and length of the reproductive time (WIRTH-DZIĘCIOŁOWSKA et al. 1996). Evaluation of differences in the morphological structure and function of ovaries and testes in mice from line selected divergently for body weight show that degeneration of testicular stroma and inhibition of spermatogenesis were more advanced in mice from the light line. The divergent selection for body weight in rabbits

leads to the reduction of the semitendinosus muscle weight and decrease of the diameter of the constitutive myofibers in the low body weight line (LARZUL et al. 2005).

The results presented here lead to the conclusion that selection of mice for high and low body mass over 94 generations resulted in a differentiation of their cognitive abilities.

To sum up, long-term selection for body mass results in the changes in many behavioral traits including spatial learning and motor activity. There was a decrease in the ability to learn Water Maze test in mice from a light line. In the future, it will be necessary to perform profound analysis of the genetic background of the observed changes in the behavior and the identification of the molecular and cellular substrates of place learning in the selected lines of mice.

The observed differences in learning ability in the selected lines can generate practical consequences both for breeders and researchers.

Acknowledgments

The Authors declare that there is no conflict of interest. This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. All authors approved the final manuscript.

Translated by AUTHORS

Accepted for print 28.01.2015

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 30(1): 35–46, Y. 2015

ECOLOGICAL CHARACTERISTICS OF *POLYGONUM POLYSTACHYUM* POPULATION IN NORTH-WESTERN POLAND (WEST POMERANIA: NIEPOŁCKO)

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K e y w o r d s: *Polygonum polystachyum*, *Polygonaceae*, inter-individual attributes, population biology, West Pomerania.

A b s t r a c t

This paper presents the results of the study conducted on the *Polygonum polystachyum* population located in a manor park in Niepołcko (West Pomerania). This species in Poland has been recorded only in 11 localities so far, most of which are situated in the south of the country. The aim of the investigation was to identify the current status and edaphic conditions of the *P. polystachyum* population, as well as to recognize its selected inter-individual and group attributes. Number of specimens, their density per 1 m^2, and mean crowding was determined. Also the population's type of spatial structure was identified through observation and by calculation of the dispersion coefficient. On the basis of the conducted research it was found that the investigated population numbers 609 specimens and it currently occupies the area of 38 m^2. It has clustered spatial distribution type (dispersion coefficient>1) and is characterized with high value of mean shoot density, which amounts to 27.8 spec./1 m^2 (max. 38 spec./1 m^2). On the basis of the investigation of biometric attributes of 50 randomly selected specimens, it was found that shoots of *P. polystachyum* achieve the height from 163 to 222 cm, and width from 6.0 mm (lower height specimens) to 19.5 mm (taller specimens). Mean length of the examined bottom and top leaves is respectively 25.4 and 24.1 cm, while their mean width achieves 7.5 and 7.2 cm. The number of the inflorescence primary branches has the highest value of the coefficient of variation (V= 75.5%), whereas the height of the specimens has the lowest one (V=7.4%). Conducted correlation analysis revealed that there is a statistically significant positive correlation between the width of *P. polystachyum* shoots and the length of the leaves from the top of the stem (r_s=0.949367). On the basis of the conducted chemical analysis of the soil from the *P. polystachyum* habitat, the substrate was classified as alkaline soil (pH = 7.1), with low total nitrogen content (0.26%), as well as low organic carbon content (2.8%).

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CHARAKTERYSTYKA EKOLOGICZNA POPULACJI *POLYGONUM POLYSTACHYUM* W PÓŁNOCNO-ZACHODNIEJ POLSCE (POMORZE ZACHODNIE: NIEPOŁCKO)

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A b s t r a k t

Dotychczas w Polsce *Polygonum polystachyum* stwierdzono tylko na 11 stanowiskach, występujących w większości w południowej części kraju. Badania miały na celu rozpoznanie stanu i warunków edaficznych populacji *P. polystachyum* odnotowanej w parku podworskim w Niepołcku (Pomorze Zachodnie) oraz przedstawienie jej wybranych właściwości osobniczych, jak i grupowych. Określono liczbę osobników i ich zagęszczenie na 1 m^2, średnie zatłoczenie, rozpoznano także typ struktury przestrzennej populacji na podstawie obserwacji oraz obliczonego współczynnika dyspersji. Stwierdzono, ze populacja liczy 609 osobników i aktualnie zajmuje powierzchnię 38 m^2. Cechuje ją skupiskowy typ rozmieszczenia przestrzennego (Wsp. dysp.>1) i wysoka wartość średniego zagęszczenie pędów, która wynosi 27,8 osobn./1 m^2 (maks. 38 osobn./1 m^2). Na podstawie zbadanych cech biometrycznych losowo wybranych 50 osobników stwierdzono, iż pędy *P. polystachyum* osiągają wysokość od 163 do 222 cm i grubość od 6,0 mm (u niższych osobników) do 19,5 mm (u wyższych). Średnia długość zbadanych dolnych i górnych liści wynosi odpowiednio 25,4 i 24,1 cm, zaś ich średnia szerokość to 7,5 i 7,2 cm. Najwyższym współczynnikiem zmienności (V= 75,5%), charakteryzuje się liczba gałązek kwiatostanowych, najniższym zaś wysokość badanych osobników (V=7,4%). W przeprowadzonej analizie korelacji stwierdzono istnienie statystycznie istotnej dodatniej korelacji między grubością pędów osobników *P. polystachyum*, a długością liści z górnej części pędu (r_s=0,949367). Osobniki *P. polystachyum* występowały na glebach o odczynie obojętnym (pH = 7,1) o niskiej zawartości zarówno azotu ogólnego (0,26%) oraz węgla organicznego (2,8%).

Introduction

The genus *Polygonum* L. (*Polygonaceae* Juss.) in Poland is represented by 21 species, which mostly occur in grassland and ruderal habitats (RUTKOWSKI 2008). The majority of them are the common species with a wide range of ecological amplitude. However, within this genus, there are some species that are very rare in the flora of Poland. One of them is the Himalayan knotweed *Polygonum polystachyum* Wall. ex Meissner [=*Aconogon polystachyum* (Wall. ex Meissner) Haraldson, *Persicaria polystachya* (Wall. ex Meisn.) Gross, *P. wallichii* Greuter & Burdet, and *Reynoutria polystachya* (Wall. ex Meisn.) Moldenke]. To date, only 11 localities of this taxa have been recorded in Poland. Currently, only 10 of them have remained, 9 of which are situated in the south of the country: in Głuchołazy (Silesia) CF-31 – the first locality recorded in Poland (SCHUBE 1927), in Walim BE-94, Mąkolno BF-27, Budniki

BE-80, Górki Wielkie DF-91, Wielkie Drogi DF-78, Zawoja DG-17 (BARTOSZEK et al. 2006), near Duszniki-Zdrój BF-24 (SMOCZYK 2005), and in Potoczek village BF-54 (www.odkrywanie.bystrzyca.pl/20091011/relacja/20091011_rdest.html).

Polygonum polystachyum is a species easy to identify and it is different from the remaining invasive knotweeds recorded in Poland: *Reynoutria japonica* Houtt., *Reynoutria sachalinensis* (F. Schmidt) Nakai, and the hybrid between them – *Reynoutria* x *bohemica* Chrtek et Chrtková (Regulation of the Minister of the Environment 2011). Among them, *Polygonum polystachyum* is being distinguished by its lanceolate leaves with long-pointed tips, as well as by inflorescence panicles gathered atop the stem (TOKARSKA-GUZIK et al. 2009).

Polygonum polystachyum, unlike the taxa of *Reynoutria* genus, originates from the Central Asia. Its native range covers the Himalayas, whereas its colonized range includes central and north-west Europe, e.g.: Switzerland, Austria, Belgium, France, and Denmark (WEBB & CHATER 1964, RECHINGER 1957).

In Poland, *Polygonum polystachyum* currently has a status of an established neophyte. It was introduced to Poland in 1927 (TOKARSKA-GUZIK et al. 2012). It grows in anthropogenic habitats, e.g. along roadsides or in the ruins of old settlements (BARTOSZEK et al. 2006). It may also occur in marshy sites, along rivers and streams, as in the case of the investigated population in Niepołcko (SMOCZYK 2005, GUMIENIAK 2007).

It is difficult to estimate the time of the first introduction of *Polygonum polystachyum* to the examined area. Presumably, it was brought there in the first half of the 20th century, by the landowners of the mansion and park (von Kehler family), as an ornamental plant. Similarly, botanical gardens have significantly contributed to the propagation of *Reynoutria japonica* and *R. sachalinensis*, which are currently invasive species in Poland (BAILEY and CONOLLY 2000).

The only one *Polygonum polystachyum* locality in West Pomerania was recorded in the manor park in Niepołcko (BACIECZKO 1997). Its population was found in 1997 for the first time, then there were only 50 specimens. Currently it has remarkably increased in size, as well as its area of occurrence. In Poland there is lack of wider research on the distribution of this species, as well as population investigations – this study fills this deficit.

The aim of this study was to identify the status and edaphic conditions of the *Polygonum polystachyum* population, as well as to introduce its selected inter-individual and group attributes.

Material and Methods

Polygonum polystachyum was recorded in a manor park in Niepołcko. According to the administrative location, this village belongs to the Barlinek municipality, Myślibórz county, and West Pomerania voivodeship. According to the physical-geographical partition of Poland, it is situated in the Myślibórz Lakeland mesoregion (KONDRACKI 2001). According to the geobotanical regionalization of Poland it is located in the Lipiany Subregion of the Myślibórz Region (MATUSZKIEWICZ 2008). Finally, according to the ATPOL cartographic system, the site of investigation is positioned in the AC-27 square (ZAJĄC and ZAJĄC 2001).

Polygonum polystachyum population grows in the north-west section of the park, in the close vicinity of the Płonia river. Damaged drainage system influences the park's structure. Park's canopy and woody understory layer are mostly composed of native species, e.g.: *Quercus robur*, *Tilia cordata*, *Acer platanoides*, *Fraxinus excelsior, Corylus avellana*, *Euonymus europaeus,* and *Sambus nigra*. The population however, grows in the exposed spot, with the lack of tree cover. In the lush undergrowth, many herbaceous plant species are present, amongst which *Polygonum polystachyum* is being distinguished by its physiognomy. It coexists with such species, as: *Urtica dioica*, *Rubus caesius*, *Deschampsia caespitosa*, *Aegopodium podagraria*, *Cirsium oleraceum*, *Rumex obtusifolius*, *Galium aparine*, *Geum urbanum,* and *Acer platanoides* seedlings.

Field research and laboratory analysis was conducted in 2014, in two steps. In the first stage, the area covered by the population was estimated, number of specimens and their density per 1 m^2 was determined, population's type of spatial structure was identified through observation, and the dispersion coefficient (TROJAN 1975) was calculated. Also the mean crowding of specimens was determined, which was expressed in the Lloyd's index (COLLIER et al. 1978).

In the second step, the biometric measurements were conducted on 50 randomly selected adult and juvenile specimens of *Polygonum polystachyum*. The measured attributes were: 1 – height of plant shoot, 2 – width of plant stem, 3 – number of nodes on a stem, 4 – number of leaves on a shoot, 5a and 5b – length of the leaf from the bottom and the top of the shoot, 6a and 6b – width of the leaf from the bottom and the top of the shoot, 7a and 7b – length of the leaf petiole from the bottom and the top of the shoot, 8a and 8b – area of lamina of the leaf from the bottom and the top of the shoot, 9 – number of inflorescence primary branches. Photo-optical measurement of leaves' assimilation surface area was conducted in laboratory, with Delta

I Device Image Analysis System. Uniformed measurement process was used, in order to limit sources of errors.

Subsequently, *Polygonum polystachyum* intrapopulation variability was analysed on the basis of the calculated maximum, minimum values, arithmetic mean, standard deviation, and coefficient of variation. Pearson product-moment correlation was used to assess the relations between the selected morphological attributes. Statistical analyses were made using STATISTICA® 10.0 for Windows software (StatSoft, Inc 2010).

In order to determine edaphic conditions of the investigated population, Egner's sampling stick was used to collect the soil sample from the rhizosphere. 4 subsamples were collected and mixed into a composite soil sample. Afterwards, dried soil substrate was delivered to the Regional Chemical-Agricultural Station in Szczecin for physical-chemical analysis. Soil pH (pH_{KCl}) was assessed using the potentiometric method – with potassium chloride (KCl) of the concentration of 1 mole dm^{-3} C (KCl). Calcium carbonate content was calculated with Scheibler method, the carbon content – with Turin's method, whereas total nitrogen content – with Kjeldahl method. Bioavailable potassium forms (K_2) content was assayed using flame emission spectroscopy according to the Polish Standard PN-R-04022:1996, while bioavailable phosphorus (P_2O_5) content – using spectrophotometry according to the Polish Standard PN-R-04023:1996. Bioavailable magnesium (Mg) content was assayed using spectrophotometry with Titan Yellow according to the Polish Standard PN-R-04020:1994. Humus content was determined with gravimetric analysis.

Botanical names of vascular plants used in this study were adopted after MIREK et al. (2002), with the exception of *Polygonum polystachyum* which follows WEBB & CHATER (1964).

Results

Group attributes of the population of *Polygonum polystachyum* Wall. ex Meissner

Polygonum polystachyum population in the manor park in Niepołcko currently occupies the area of 38 m^2. It numbers 609 specimens grouped together in one spot. Their mean density amounts to 27.8 spec./1 m^2 (max. 38 spec./1 m^2). The population has a clustered spatial distribution type (dispersion coefficient>1). Mean crowding expressed in the Lloyd's index equals 26.2, which is slightly lower than the population mean density (tab. 1).

Table 1
Group attributes of the population of *Polygonum polystachyum* wall. et Meissner recorded in Niepołcko manor park

Total number of specimens	Density of specimens per 1 m^2			Mean crowding	Dispersion coefficient
	min.	max.	mean		
609	22	38	27.8	26.2	1.41

Population variability of *Polygonum polystachyum* Wall. ex Meissner

Mean values of the investigated inter-individual attributes, as well as the further population data, are presented in Table 2.

Examined *Polygonum polystachyum* shoots achieve the height from 163 to 222 cm and width from 6.0 mm (lower height specimens) to 19.5 mm (taller specimens). In order to precisely characterise the height of investigated population specimens, they were divided into 7 range classes (Figure 1). The majority of specimens' height is placed in the 170-200 cm range, which cumulatively constitutes 46% of the population. Participation of specimens in the remaining range classes is lower, it fluctuates from 1 to 8 individuals. Height of shoots attains the lowest value of the coefficient of variation (V=7.4%). This attribute follows normal distribution according to the Shapiro-Wilk test for normality (p=0.98).

Table 2
Main statistical characteristics of the specimens of *Polygonum polystachyum* Wall. et Meissner

No.	Characteristic	x_{min}	x_{max}	$\bar{x}$	SD	V
1	height of plant shoot	163.0	222.0	193.0	14.2	7.4
2	width of plant stem	6.0	19.5	10.2	2.3	22.6
3	number of nodes on a stem	18.0	28.0	23.0	2.1	9.1
4	number of leaves on a shoot	28.0	112.0	64.7	19.6	30.3
5a	length of the leaf from the bottom of the shoot	20.0	29.9	25.3	2.1	8.4
5b	length of the leaf from the top of the shoot	18.9	28.0	24.1	2.3	9.7
6a	width of the leaf from the bottom of the shoot	5.7	9.9	7.5	1.0	12.3
6b	width of the leaf from the top of the shoot	4.3	9.4	7.2	1.1	15.0
7a	length of the leaf petiole from the bottom of the shoot	1.2	2.5	1.8	0.3	17.0
7b	length of the leaf petiole from the top of the shoot	0.9	2.6	1.4	0.4	24.1
8a	area of lamina of the leaf from the bottom of the shoot	64.3	169.7	111.5	23.6	21.2
8b	area of lamina of the leaf from the top of the shoot	54.1	152.7	105.1	23.4	22.3
9	number of inflorescence primary branches	0	8	3.0	2.3	75.5

Explanations: x_{min} – minimal value, x_{max} – maximal value, $\bar{x}$ – arithmetic mean, SD – standard deviation, V – coefficient of variation

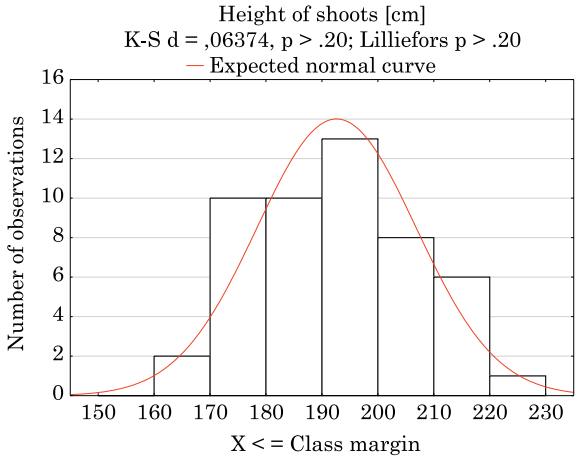

Fig. 1. Height structure of the population of *Polygonum polystachyum* Wall. et Meissner in Niepołcko

Number of leaves on a shoot is a subject to fairly large variability, with the coefficient of variation V=30.3%. Leaves from the bottom of the shoots are larger than the top leaves in terms of their length, width, and their assimilation surface area. The mean values of the length of the bottom and top leaves average 25.4 and 24.1 cm respectively. Higher coefficients of variation of the width of the examined leaves (V=12.3% and V=15%) indicates that their width variability is greater than their length variability (8.4% and 9.7%). Furthermore, investigated attributes achieve similar maximal and minimal values (Figure 2).

Great participation of blooming specimens in the examined sample (86%), including the great number of specimens with 4 inflorescence primary branches (53%), indicates the advanced age of the studied population. This attribute has the highest coefficient of variation (V=75.5%), because of the presence of non-blooming specimens in the tested sample.

Mean area of lamina of the bottom leaves equals 111.5 cm^2 and it is larger than the top leave's one (105.1 cm^2). Probability distribution of the specimens that are situated in each range class, in terms of the examined attribute, is fairly diverse. The majority of the specimens has both bottom and top leaves with the area of lamina situated in the 100-120 cm^2 range class, whereas the minority – in the marginal range classes (Figure 3). High coefficient of variation (V=21.2% and V=22.3%) and high standard deviation (SD=23.6 and SD=23.4) indicate a high dispersion of the examined attribute (Table 2).

The results of Pearson correlation analysis between the examined inter-individual attributes are presented in Table 3. It was found that there is a statistically significant positive correlation between the width of stems

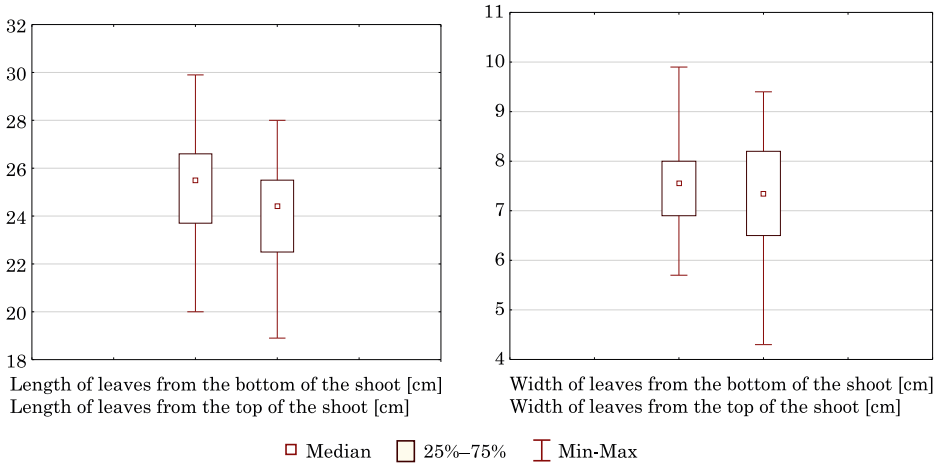

Fig. 2. Variability of length and width of leaves from the bottom and the top of the shoot of *Polygonum polystachyum* Wall. et Meissner

of *Polygonum polystachyum* specimens and the length of the leaves from the top of the shoot (r=0.949367). Furthermore, highly significant correlation was found between the number of nodes on a stem and the width of the leaves from the bottom of a shoot (r=0.928528), stem's width and top leaves' width (r=0,873273), top leaves' length and their width (r=0.827928), as well as between the width of top leaves and their petioles' length (r=0.772151).

Edaphic conditions

Chemical analysis of the soil surface layer from the *Polygonum polystachyum* locality revealed that soil pH is 7.1, therefore the examined soil was classified as alkaline. Soil reaction has influence as well on the growth of soil microorganisms, and on the mineralization process, which in the examined samples is being reflected by moderate degree of organic matter and humus accumulation. C_{org} content is correlated with the examined soil's fertility – it equals 2.8%. Total nitrogen content is related to organic matter and humus abundance. It reaches 0.26% in soil surface layer, which indicates that the substrate is nitrogen-poor. Proportion of C:N content equals 1.6, which implicates a difference in the mineralization rate of organic carbon and nitrogen compounds. The content of all bioavailable elements, according to the IUNG standards, is very high. Bioavailable potassium (K_2O) content reaches 38.5 mg · 100 g^{-1} soil, bioavailable phosphorus (P_2O_5) content amounts to 52.4 mg · 100 g^{-1} soil, whereas magnesium content equals 18.2 mg · 100 g^{-1} soil.

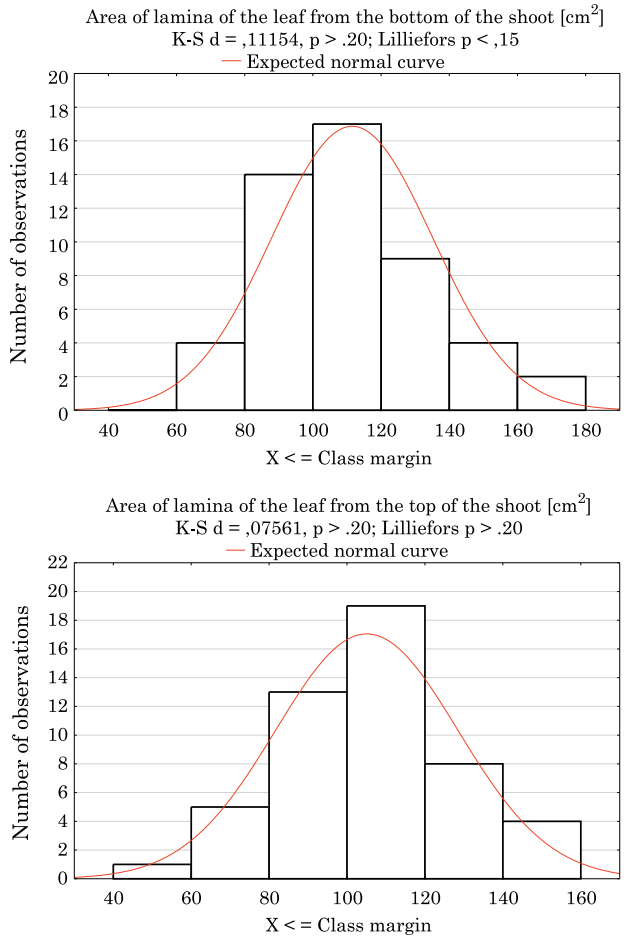

Fig. 3. Histograms of the area of lamina of the leaf from the bottom and the top of the shoot of *Polygonum polystachyum* Wall. et Meissner

Discussion

The investigated locality was recorded in 1997 for the first time. *Polygonum polystachyum* population numbered 50 specimens of approximately 1.3 m height back then (BACIECZKO 1997). The examined population have been devastated several times in the past 2 years due to restoration works performed on the 18th century mansion. Currently, the size of the population have increased over 30-times in comparison to 1997. The only *Polygonum polystachyum* population known in Poland, that is larger than the one recorded in Niepołcko, was observed in the Jałowiec ridge. It covers the area

Table 3
Coefficients of Pearson product-moment correlation between the examined biometric attributes of the specimens of *Polygonum polystachym* Wall. et Meissner (significance level $\alpha = 0.05$)

Characteristic	1	2	3	4	5a	5b	6a	6b	7a	7b	8a	8b
9	0.606474	0.327879	0.598474	0.033638	0.339386	0.342871	0.461009	0.417883	0.546524	-0.026906	0.325465	0.377246
8b	0.603192	0.503553	0.604612	0.216746	0.231369	0.512889	0.591650	0.488488	0.608260	0.419420	0.549813	–
8a	0.412106	0.478054	0.515731	0.345367	0.116941	0.400424	0.499244	0.489787	0.436342	0.459639	–	–
7b	0.259999	0.525182	0.417260	0.408019	0.091606	0.485321	0.468019	0.389072	0.425134	–	–	–
7a	0.629045	0.658139	0.850129	0.275599	0.231713	0.609994	0.772151	0.713412	–	–	–	–
6b	0.566029	0.873273	0.674329	0.422928	0.262278	0.827928	0.679822	–	–	–	–	–
6a	0.712456	0.745504	0.928528	0.270701	0.290227	0.709101	–	–	–	–	–	–
5b	0.585487	0.949367	0.678224	0.494379	0.338770	–	–	–	–	–	–	–
5a	0.428551	0.260075	0.323466	0.081858	–	–	–	–	–	–	–	–
4	0.265814	0.504944	0.268235	–	–	–	–	–	–	–	–	–
3	0.733532	0.723025	–	–	–	–	–	–	–	–	–	–
2	0.561850	–	–	–	–	–	–	–	–	–	–	–

Explanations (1–9) as in Table 2.

of 320 m^2 and numbers about 900 specimens (GUMIENIAK 2007). Rapid growth of the investigated population is mostly determined by the vegetative propagation of the *Polygonum polystachyum* specimens. Regardless of the intensive development, the population does not manifest the spontaneous long-distance expansion trend: no additional localities of the species have been found in the surroundings. It may be caused by the impossibility to produce fruit, related with the late flowering time (September-October: BARTOSZEK et al. 2006).

The number of *Polygonum polystachyum* shoots in randomly selected plots varied from 22 to 38, with the mean value of 27.8 per 1 m^2. Comparable with this, *Reynoutria japonica* specimens achieve the higher value – 41.8 individuals per 1 m^2 (maximum of 73 specimens per 1 m^2), according to ŚLIWIŃSKI and CZARNIECKA (2011).

Furthermore, the maximal height of *Polygonum polystachyum* shoots was 222 cm, which is higher than in the study of BARTOSZEK et al. (2006). Consequently, the leaves were also longer with the maximum of 29.9 cm. Only the leaves' width of the investigated specimens corresponds to the range presented in the study of BARTOSZEK et al. (2006).

Little is yet known about edaphic preferences of the investigated species. In India, its localities have been recorded at the elevation of 3000 m (KALA 2004), while in Afghanistan – above 3400 m.a.s.l (POLUNIN and STAINTON 1984). In Poland, the highest altitude *Polygonum polystachyum* locality that have been recorded is located in the Jałowiec ridge, at 500–599 m.a.s.l. (GUMIENIAK 2007). Unlike the investigated population in Niepołcko, in India this species grows on acid soils (pH 3.8–6.2), with significantly higher content of nitrogen (0.3–4.5%) and organic carbon (4–34%) (KALA 2004). This leads to the conclusion, that *Polygonum polystachyum* is able to adapt to soils with lower organic compounds content.

Conclusions

Since there is a limited number of *Polygonum polystachyum* localities in Poland, as well as restricted amount of data on the species, and its populations biology, it is recommended to ensure a proper monitoring of the only locality recorded in West Pomerania. Long-term observations would allow to determine whether the species should be classified as potentially invasive neophyte (epekophyte or hemiagriophyte), and whether it is required to use some control management in order to restrict its further spread.

Translated by EMILIA KASZYCKA

Accepted for print 25.02.2015

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 30(1): 47–57, Y. 2015

MITOTIC AND MEIOTIC CHROMOSOMES OF THE GREAT RAMSHORN SNAIL *PLANORBARIUS CORNEUS* (LINNAEUS, 1758) (GASTROPODA, PLANORBIDAE) FROM LAKE KORTOWSKIE

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K e y w o r d s: cytogenetics, molluscs, Planorbidae, chromosomes, spermatozoa.

A b s t r a c t

An analysis of meiotic and mitotic chromosomes of *P. corneus* inhabiting Lake Kortowskie was made in order to verify the use of different tissues and colchicine treatments, the hypotonization time and two methods of chromosome slide preparation. In total, 30 chromosomal slides of six individuals were analyzed. The well spread chromosomes were introduced onto the slides by dropping a cell suspension of the mantle epithelium, foot and intestine of each individual, directly injected with colchicine, after 20 min of hypotonization. The karyotype was composed of 2n=36 biarmed chromosomes, thirty metacentrics with the rest being submetacentrics, NF=72. In the slides of the gonads the meiotic chromosomes in spermatogenesis were observed as being in prophase I (leptoten, zygoten, and diakinesis) and in telophase I. In diakinesis 18 bivalents were formed. No disturbances were observed during meiosis. The spermatozoa were typical of aquatic molluscs; consisting of a spherical head, a short midpiece and a long tail.

CHROMOSOMY MITOTYCZNE I MEJOTYCZNE ZATOCZKA ROGOWEGO *PLANORBARIUS CORNEUS* (LINNAEUS, 1758) (GASTROPODA, PLANORBIDAE) Z JEZIORA KORTOWSKIEGO

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Abstrakt

Analiza chromosomów mitotycznych i mejotycznych zatoczka rogowego *P. corneus* z populacji w Jeziorze Kortowskim pozwoliła na określenie warunków wykonywania preparatów chromosomowych. Łącznie analizowano 30 preparatów chromosomowych wykonanych z sześciu osobników. Najlepiej rozproszone chromosomy uzyskano na preparatach wykonanych metodą nakrapiania utrwalonej zawiesiny komórek na szkiełko. Komórki te pochodziły z nabłonka płaszcza, jelita oraz nogi każdego z osobników, które poddano iniekcji kolchicyną. Najkorzystniejszy czas hypotonizacji wynosił 20 minut. Kariotyp składał się z 2n=36 chromosomów dwuramiennych, 30 metacentrycznych i sześciu submetacentrycznych, NF=72. W preparatach z gonad przeprowadzono obserwację chromosomów w różnych stadiach spermatogenezy: profazy I (leptoten, zygoten, diakineza) oraz w telofazie I. W profazie mejozy I chromosomy tworzyły 18 biwalentów. Proces mejozy przebiegał prawidłowo. Plemniki były typowe dla mięczaków wodnych; składały się z kulistej główki, krótkiej wstawki i długiej wici.

Introduction

Molluscs are represented by about 130,000 species. Knowledge of their cytogenetic features has grown with the development of research techniques. A review of the number of chromosomes in metazoans (HARVEY 1920, after NAKAMURA 1986) included only 44 mollusc species. In the years 1930–1969, papers were published describing the karyotypes of 622 species and sub-species of molluscs, and contained the chromosome number of taxa representing the following classes: gastropods Gastropoda, bivalves Bivalvia, chitons Polyplacophora, cephalopods Cephalopoda and scaphopods Scaphopoda. Although most of the data concerned Gastropoda; the karyotype of more than 300 species of snail have been described, including about 20 species analyzed using banding chromosome patterns (NAKAMURA 1986).

Karyotypes of snails are mainly composed of biarmed chromosomes, meta- and submetacentric (THIRIOT-QUIEVREUX 1994, 2003). Gastropods traditionally classified as Pulmonata contain about 36 thousand terrestrial and aquatic species (JÖRGER et al. 2010) and they are relatively well recognized karyologically. This is an informal group of snails and slugs characterized by their ability to breathe air, by virtue of having a pallial lung instead of a gill, or gills. The chromosome number and karyotype of about 70 species is known (less than 0.5% of all species have been described). Chromosomal measurements and chromosome banding patterns using AgNOR staining, C and G banding techniques of 34 species have been described (NAKAMURA 1986, THIRIOT-QUIEVREUX 2003, VITTURI et al. 2005).

The Pulmonata species of the family Planorbidae proved to be interesting due to the large morphological diversity and still unresolved phylogenetic relationships. Within this family about 40 genera and about 160 species are recognized (MEIER-BROOK 2002, ALBRECHT et al. 2007), including 20 species

that occur in Poland (BOGDANOWICZ et al. 2008). The genus *Planorbarius* contains two species, *P. corneus* (Linnaeus, 1758) and *P. metidjensis* (Forbes, 1838). However, because of the high morphological diversity within *P. corneus* sensu lato, between 5 and 8 sympatric species have been recognized as being distributed throughout Ukraine, with *P. corneus, P. banaticus, P. purple, P. grandis* and *P. stenostoma* commonly occurring (STADNICHENKO 1990, after GARBAR and GARBAR 2007). Comparative cytogenetic studies concerning the structure of karyotypes and the structure of chromosomes stained by banding techniques, for example, can provide the diagnostic data for these species.

The great ramshorn snail *P. corneus* is widely distributed in Europe and Northern Asia. It commonly occurs in Poland in standing and running freshwater reservoirs that are strongly overgrown. It is a polytypic species, which means that it is divided into subspecies: *P. corneus arabatzis* (Reischütz, Reischütz & Fischer 2008), *P. corneus grandis* (Dunker, 1850) and *P. corneus corneus* (species nominative) (Linnaeus, 1758) (SEDDON and VAN DAMME 2011).

Although several previous studies have described the number of chromosomes (BURCH 1961, BOTTKE 1982) and recently also the structural karyotypes of several species of *P. corneus* sensu lato (GARBAR and GARBAR 2007), the morphological plasticity of this species was the inspiration to undertake the research by the authors of this report. Chromosomal studies of gastropods, including *P. corneus* are difficult and complicated due to a relatively low number of metaphase samples suitable for analysis being observed (GARBAR and GARBAR 2007).

Aquatic organisms, including freshwater gastropods, are the first to suffer from the effects of environmental pollution that contaminates water bodies. Animals inhabiting them are exposed to a progressive degradation of their living environment that may lead to changes in their functional morphology, including the level of genomes and chromosomes. One of the effects of the pollution of aquatic environments may be a disturbance in the process of meiosis for living organisms, such as gastropods (BARSIENE 1994).

The aim of this present study was the analysis of meiotic and mitotic chromosomes of *P. corneus* individuals inhabiting Lake Kortowskie. This was preceded by a verification of the possibility of applying the techniques of snail chromosomal preparations as described in the literature.

Material and Methods

The study was performed by using six individuals of *P. corneus* (Fig. 1) collected from Lake Kortowskie. Chromosomal preparations were performed

using a modified technique described in the available literature (GILL and CAIN 1980, YARAYABHAND et al. 1998, VITTURI et al. 2004, GARBAR and GARBAR 2007, LEITAO et al. 2009) and our own experience (WOŹNICKI and BOROŃ 2003, BOROŃ et al. 2004). After collection from the environment and during the subsequent research, snails were kept in a well-aerated aquarium.

Fig. 1. Great ramshorn snail *Planorbarius corneus*

To inhibit cell division at the metaphase stage of mitosis, snails were subjected to colchicine. For this purpose, the snails were divided into two groups. Three individuals were injected directly with 0.05% colchicine solution in the amount of 0.1–0.3 ml/animal, and then left in a small aquarium containing about 600 ml of water, for about 20 hours. The remaining three individuals were placed in a 0.02% aquatic solution of colchicine in a small aquarium containing about 600 ml of water, in a dark place for about 20 hours. After a specified time, the individuals from both groups were processed using the same procedure. Snails were sacrificed by placing them in an aquatic solution of 2-phenoxyethanol. After that, they were dissected and the fragments of the following tissues were collected: the epithelial cells of the mantle edge, pallial lung, foot, intestine and gonad. Tissues were subjected to two ways of preparation:

a) small pieces of tissue (3–4 x 3–4 mm) were placed in 0.075M KCl hypotonisation solution for 20 or for 30 minutes, at room temperature,

b) small pieces of tissue were ground in a glass homogenizer and the obtained cell suspension was subjected to hypotonisation in a 0.075M KCl solution for 20 or for 30 minutes, at room temperature.

After this time, the tissue (a) or suspension cells (b) were fixed in a solution of methanol and glacial acetic acid at a ratio of 3:1. In the case of tissue

fragments (a) fixation consisted of changing the fixative solution three times at intervals of 10–15 minutes. Whereas, the cell suspensions (a) were centrifuged three times for 10 min at 1000 rpm/min and after each centrifugation the fixative solution was exchanged with freshly prepared solution.

Chromosomal preparations were performed using the following techniques adequate for the tissue samples (1) and the cell solutions (2):

1. The tissues samples were placed on a microscope slide moistened with a fixative and then they were turned using preparative needles and forceps so that the surface of the tissue touching the slide left the exterior cells exposed to hypotonisation and fixation.

2. The 'splash' technique was used as follows:

a) cell suspensions were dropped with a pipette onto microscope slides from a height of about 30 cm,

b) a small volume of the cell suspensions were fixed in a mixture of a fixative composed of 55% methanol and 45% acetic acid.

After that, the cell suspension was dropped with a pipette onto a microscope slide but in a different way. A drop of cell suspension was placed on a microscope slide with a pipette and after a few seconds it was withdrawn back into the pipette. In this way we observed the cells arranged concentrically in rings formed after the evaporation of the fixative.

The chromosome slides were dried at room temperature for 24 hours, and then were stained with 5% Giemsa solution for 15 min. After that, they were rinsed in running water and then twice in distilled water and air dried at room temperature.

Analysis of chromosomal preparations and photographic documentation were performed using an Olympus BX51 light microscope equipped with a camera and MultiScan Karyotype software. Chromosomes were classified into morphological categories such as metacentric (Ms) and submetacentric (SMs) according to the method proposed by LEVAN et al. (1964).

Results

The best samples for observation and chromosomal spread were obtained by using the dropping cell suspension technique derived successively from the mantle edge (Fig. 2), the foot and the intestine of individuals which were directly injected with colchicine solution. The shorter hypotonisation time of 20 minutes was better than the longer time of 30 minutes. On the other hand, more metaphase plates were observed using the longer hypotonisation time, but most of them contained chromosomes that were rather clustered and not well spread out, which were not suitable for counting.

Finally, we found four metaphase plates with relatively well spread chromosomes for counting. The karyotype of *P. corneus* inhabiting Lake Kortowskie was composed of 2n = 36 biarmed elements; 30 metacentrics and 6 submetacentrics (30Ms + 6SMs), and the number of chromosome arms was NF=72 (Fig. 2).

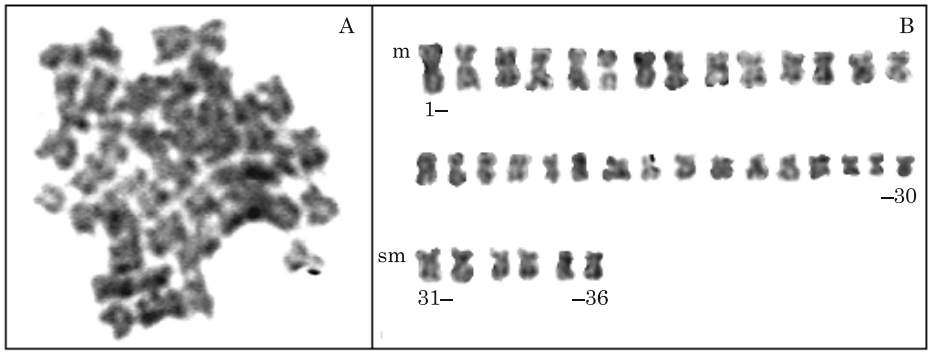

Fig. 2. Metaphase plate (A) and karyotype (B) of *P. corneus* from the cell of mantle edge. Chromosomes; M – metacentric; SM – submetacentric. Magnification 1000x

In the chromosomal slides of the gonads, obtained both by the dropping cell suspension technique and by turning a piece of tissue on the microscope slide, the different stages of meiotic chromosomes in spermatogenesis were observed as follows: prophase I (leptoten, zygoten, chromosomes in an early diakinesis with visible centromeric constrictions and bivalents visible during diakinesis) (Fig. 3 A – G), and the chromosomes during the telophase I stage (Fig. 3 G). In the prophase of meiosis I, the chromosomes formed 18 bivalents, and their number confirmed the diploid number of 2n = 36 chromosomes as a characteristic of this species. In the meiotic chromosomal slides, spermatozoa were also observed, which consisted of a spherical head, a short midpiece and a long tail (*flagellum*) (Fig. 3 H, I).

Discussion

The preparation of chromosomal slides of *P. corneus*

The chromosomes may be obtained from any cells that are actively dividing. Usually in molluscs, they can be obtained from the cells of various tissues, e.g., the mantle epithelium, the kidney, the gonads, tissues of the embryo as

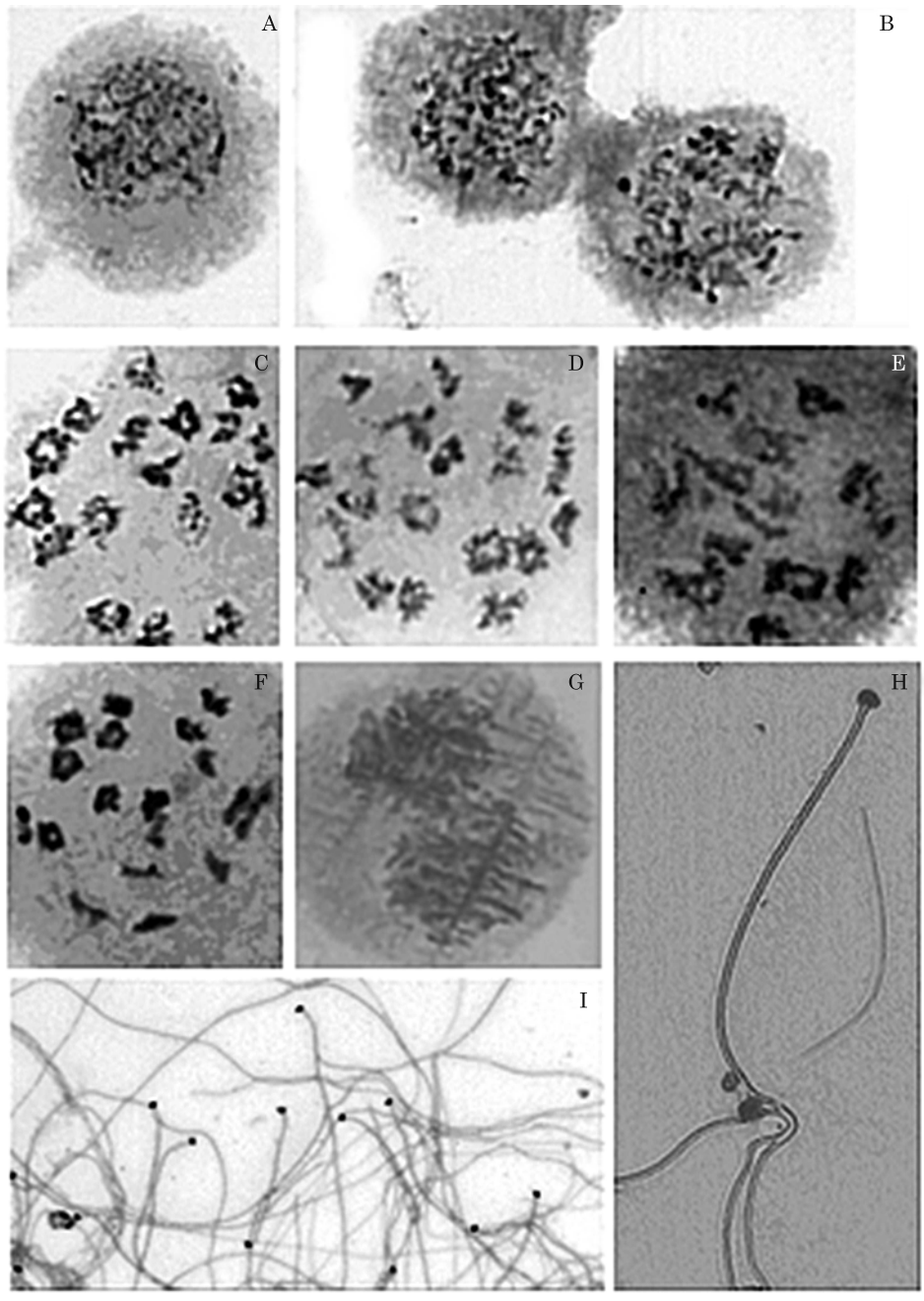

Fig. 3. Meiotic chromosomes in spermatogenesis. Prophase I: leptoten (A), zygoten (B), chromosomes in an early diakinesis (C-D) with visible centromeric constrictions, diakinesis (E-F) with visible bivalents. Telophase I (G). Spermatozoa (H), magnification 400x. Spermatozoa (I), magnification 200x

well as others (NAKAMURA 1986). We had prepared chromosomal slides from five different tissues and most metaphase plates were observed in cells derived from the mantle edge, the intestine and the foot. A good source of cells is also the gonad used mainly for the observation of meiotic chromosomes (GARBAR and GARBAR 2007), which were used in the studies presented here.

Most of the cytogenetic studies of molluscs, including gastropods, refer to the number of chromosomes. There is much less data relating to the karyotype structure of these animals, and even less is known about the structure of the chromosomes. The relatively poor state of knowledge of molluscs is related to the small size of the chromosomes (~ 10 mm) and the lack of research techniques that allow a large number of metaphase plates to be obtained (NAKAMURA 1986).

An analysis of the available literature indicates that in karyological studies *P. corneus* achieved a relatively low number of metaphase plates, which is similar to what was observed in the present paper. In the chromosomal slides prepared from 5 to 35 individuals, there were between 1 and 13 metaphase plates analyzed (GARBAR and GARBAR 2007). In the present study, a relatively large number of 30 chromosomal slides made from the cells of different tissues (the epithelial edge of the mantle, the pallial lung, the foot, the intestine and the gonad) of six individuals were analyzed. Despite the fact that a relatively high number of metaphase plates were observed, only four of them were suitable for a determination of the karyotype.

The best metaphase cells were obtained from the mantle edge, the gonad and the foot after 20 minutes of hypotonisation, and perhaps that time could be shorter. Getting a low number of metaphase plates may be a result of a lack of colchicine activity which could not reach all the tissues. What is important is the mode of how the colchicine is introduced into the snail body; colchicine injection directly into the body was a better method of obtaining proliferating cells than incubating the whole animal in an aquatic colchicine solution.

Mitotic and meiotic chromosomes of *P. corneus*

In snails, cytogenetic observations of both mitotic and meiotic chromosomes are important (NAKAMURA 1986) as described in the presented study. Although in several metaphase plates the chromosomes were clearly visible, which allowed the determination of their characteristic diploid 2n = 36, and four of them were sufficient enough spread to arrange a karyotype. That being said, the results obtained can be regarded as good since the cytogenetic data of *P. corneus* published so far clearly show that it is rather difficult to obtain mitotic metaphase chromosomes of this species (MAKSIMOVA 1995, after GAR-

BAR and GARBAR 2007). The experimentally determined conditions presented in this study for making the chromosome preparations of this species seem to be highly recommendable.

Chromosomes of *P. corneus* in prophase of the first meiotic division, and chromosomes in the metaphase stage of the second meiotic division were for the first time described by MAKSIMOVA (1995), after GARBAR and GARBAR (2007). The number of chromosomes in the haploid set ranged from n = 15 to n = 20, with a predominance of n = 18. According to the author of this cited paper, the variation of this number might be due to the presence of additional chromosomes, but it has not been confirmed by GARBAR and GARBAR (2007) and similarly by the results under this study.

The karyotype of *P. corneus* obtained in this present work contained 2n = 36 chromosomes, and has been previously described in detail by GARBAR and GARBAR (2007). The relative length of chromosomes ranged from 8.42 (1 pair) to 3.69% (18 pair), whereas their total length TCL (total complement length) was 156.56 ± 5.91 microns. The karyotype was arranged according to the size of the chromosomes containing six submetacentric chromosomes (pair numbers: 2, 14 and 17) and thirty metacentric chromosomes (other pairs) and can be presented using the following formula: 2n = 30M + 6SM. The number of chromosome arms was FN = 72. This same karyotype was found in the individuals collected from Lake Kortowskie.

A comparative meiotic chromosome analysis of four morphologically distinct species of the genus *Planorbarius*, viz. *P. corneus, P. banaticus, P. purpura* i *P. grandis* from Ukraine showed no differences in the karyotypes of these species. The karyotype pattern of all species was the same: 2n = 36; 30M + 6SM and FN = 72. This karyotype did not differ significantly in terms of the total and the relative length of the chromosomes and the centromeric index (GARBAR and GARBAR 2007).

In the chromosomal slides made from the gonads, the relatively rare mitotic divisions of the oogonia or spermatogonia were observed, while it is relatively easy to obtain and visualize the snails' meiotic chromosomes forming bivalents (BURCH and PATTERSON 1965, after NAKAMURA 1986). The observed number of bivalents during meiosis, amounting to 18, confirmed the number of mitotic chromosomes. The disturbances in the process of meiosis of gastropods living in the polluted aquatic environments have been detected (BARSIENE 1994). However the observed different stages of meiotic chromosomes in spermatogenesis of investigated individuals indicated the normal process of meiosis.

The documented appearance of sperm which is characterized by a simplified construction should be emphasized. Spermatozoa of the great ramshorn snail are typical for animals using external fertilization, these aquatic animals

use so-called „primitive" sperm. Generally, „primitive" sperm have a head, a short midpiece and a long tail (FRANZÉN 1970). In the current literature, data on sperm morphology among the various groups of molluscs has been used in systematics and phylogeny (DROZDOV et al. 2012).

The results presented here do not reveal any differences between the karyotype of the great ramshorn snail from Lake Kortowskie and the karyotypes formerly reported in the published literature, but only confirmed data on the karyotype of this species. However, the results contributed new data on meiotic chromosomes, and the spermatozoa of this species. Insightful observation of meiosis may in the long-run perspective allow the recording of disturbances in this process among snails, caused by water pollution.

Conclusions

The great ramshorn snail is characterized by a karyotype containing 2n = 36 biarmed chromosomes, *viz.* 30 metacentric and 6 submetacentrics, NF = 72. During the prophase stage of meiosis I, 18 bivalents are observed in this species. The meiosis process in the gonads of *P. corneus* inhabiting Lake Kortowskie did not show any disturbances. The sperm of this species consists of a spherical head, a short midpiece and a long tail and are typical of aquatic molluscs.

Translated by ALICJA BOROŃ

Accepted for print 24.03.2015

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 30(1): 59–72, Y. 2015

CLOUDY RED-FLESHED APPLE JUICE PRODUCTION AND QUALITY

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K e y w o r d s: cloudy juice, red-fleshed apples, polyphenols, anthocyanins, juice production.

A b s t r a c t

The aim of the study was to examine the possibility of processing red-fleshed apples into cloudy juices. In a series of experiments on laboratory and semi-technological scale, the effect of enzymatic treatment of the pulp, an addition of ascorbic acid, centrifugation of the must and storage conditions on the physicochemical quality of the finished juice were investigated. The following red-fleshed apple cultivars were used: „Trinity", „Maypole", „Alex Red", and as the control: „Shampion" and „Idared" cultivars.

Studies have shown that an addition of ascorbic acid during pressing, in the amount of 500–100 mg kg^{-1} of pulp, increased anthocyanins extraction into juice and prevented their oxidation. The enzymatic treatment of the pulp did not affect the pressing yield, and adversely affected the content of phenolic compounds and antioxidant activity. It was found that due to the rapid degradation of anthocyanins the red-fleshed apple juices should be stored at low temperatures.

PRODUKCJA I JAKOŚĆ MĘTNYCH SOKÓW Z JABŁEK CZERWONOMIĄŻSZOWYCH

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S ł o w a k l u c z o w e: mętny sok, jabłka czerwonomiąższowe, polifenole, antocyjany, produkcja soku.

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Abstrakt

Celem pracy było sprawdzenie możliwości przetworzenia jabłek czerwonomiąższowych na mętne soki. Przeprowadzono cykl eksperymentów w skali laboratoryjnej i półtechnicznej. Badano wpływ obróbki enzymatycznej miazgi, dodatku kwasu askorbinowego (AA), wirowania moszczu oraz warunków przechowywania gotowego soku na jakość produktu. Materiał badawczy stanowiły jabłka odmian czerwonomiąższowych: „Trinity", „Maypole", „Alex Red", a dla porównania odmiany: „Szampion" i „Idared".

W badaniach stwierdzono, że dodatek AA, w ilości 500–1000 mg kg^{-1} miazgi, podczas tłoczenia zwiększył ekstrakcję i zapobiegał oksydacji antocyjanów. Obróbka enzymatyczna miazgi nie wpływała na wydajność tłoczenia, natomiast niekorzystnie oddziaływała na zawartość związków fenolowych i aktywność przeciwutleniającą. Stwierdzono, że ze względu na szybką degradację antocyjanów soki z jabłek czerwonomiąższowych powinny być przechowywane w warunkach chłodniczych.

Introduction

In the past years, consumers have become more aware of the positive effects of a diet rich in fruit and vegetables, especially in the prevention of many civilization diseases, such as CVD, obesity and cancer (CHUN et al. 2005, PASCUAL-TERESA and SANCHEZ-BALLESTA 2008, SLAVIN and LLOYD 2012, PHILLIPS 2013). This is all due to propagation of knowledge concerning the significance of nutrients and bioactive compounds in fruit, such as vitamins, minerals, dietary fibre and polyphenols.

Apples play an important role in the human diet because they are consumed often and are one of the best sources of bioactive compounds, whose health benefits are well documented (HYSON 2011). The consumption of fresh fruit is often replaced by the intake of fruit juices. In Europe, the consumption of apple juice takes the second place after orange juice (AIJN 2012). Due to higher content of polyphenols, especially proanthocyanidins and dietary fibre (MARKOWSKI et al. 2007, OSZMIAŃSKI et al. 2007) cloudy apple juice revealed a higher cancer-prevention potential than the clear product (BARTH et al. 2005, BARTH et al. 2007).

The dynamically developing juice market is providing consumers with a more and more attractive range of juices, nectars and especially cloudy fruit products and those containing puree such as smoothies. Therefore, in many countries there is an interest in red-fleshed apples. New such cultivars have been developed through selective breeding. Now several splendid culinary fruits with red flesh are available. These apples, on the contrary to white or yellow flesh apple cultivars, contain anthocyanin compounds in the flesh (MAZZA and VELIOGLU 1992, RUPASINGHE et al. 2010, BALÁZS et al. 2012), which are strong antioxidants (CASTAÑEDA-OVANDO 2009, MIGUEL 2011). Red-fleshed apple cultivars are interesting for processing due to the red or pink colour of

the products obtained (RUPASINGHE et al. 2010). However, there is still scarce information on their quality.

The examination of processing red-fleshed apples into cloudy juices were the basis of experiments on both a laboratory and semi-technological scale. The effect of enzymatic maceration, addition of ascorbic acid (AA), juice centrifugation and effect of storage conditions on physical and chemical quality of cloudy apple juices were the aim of these experiments.

Materials and Methods

Plant materials

The apple cultivars used in the experiments were red-fleshed genotypes: „Trinity", „Alex Red", „Maypole" and standard apple cultivars: „Shampion" and „Idared" from the Experimental Orchard of the Research Institute of Horticulture in Brzezna, Poland. Apples were picked at harvest maturity in 2010 (only „Trinity") and in 2011 season.

Enzyme preparations: Rohapect PTE and Rohament PL were provided by AB Enzymes (Darmstadt, Germany).

Analytical methods

Soluble solids contents (°Brix) were measured with a digital refractometer (Mettler-Toledo, type RE50) and density by Mettler-Toledo, type DE51 meter. Total titratable acidity (pH 8.1, expressed as citric acid equivalents in g 100 mL^{-1} of juice) was determined potentiometrically (Mettler-Toledo, type DL 58).

Turbidity (T_0) of juices was measured nephelometrically using a HACH turbidimeter (Hach Company). Turbidity results are given in NTU (nephelometric turbidity units). Stable turbidity (T_S) and the stability of turbidity (T%) were determined according to STÄHLE-HAMATSCHEK and GIERSCHNER (1989) after centrifugation using a laboratory centrifuge (4200 × g for 15 min at room temperature) and calculating a percentage of cloud stability according to the following formula: ($T\% = T_S/Tox100$).

Organic acids (ascorbic ac., malic ac. and citric ac.) content was determined by reversed-phase HPLC with HP 1100 system (Hewlett-Packard, Waldbronn, Germany) equipped with a Diode Array Detector (DAD) using two Supelco LC-18 columns (25 cm x 4.6 mm; 5 μm). A 73.5 mmol L^{-1} water solution of KH_2PO_4 buffer at pH 2.5 was used as the mobile phase. The column temperature was kept at 30°C with a flow rate of 0.8 mL min^{-1}. Detection of acids was

performed at 210 nm and 244 nm. The results were expressed in mg 100 mL^{-1}. The total anthocyanins content was quantified spectrometrically (Varian, UV/Vis CARY 300E) by the pH differential method according to WROLSTAD (1976). Anthocyanins content was calculated using the molar absorptivity of cyanidin-3-galactoside (26900 L mol^{-1} cm^{-1}) and the molecular weight of 445 g mol^{-1}.

The polyphenolic compounds were determined by a modified version of the HPLC method of TSAO and YANG (2003) using a Phenomenex Fusion RP column (250 nm x 4.6 mm; particle size 4 μm) with a guard column. An Agilent 1100 series HPLC (Hewlett-Packard) system equipped with a DAD detector was used.

Free-radical scavenging activity was determined according to the method described by RE et al. (1999), using $ABTS^{\bullet+}$ radical cation, and the detailed measurement procedure was according to OSZMIAŃSKI and WOJDYŁO (2007).

Juice preparation

Two experiments were conducted on a laboratory scale using „Trinity" *cv*. in season 2010. The apple juice was pressed using Instron 4303 texture press equipped with special attachment (load cell 5000 N, cylindrical container and tightly fitting plunger) using a small samples (200 g of fruit mash). During the pressing cycle the plunger was moved at a speed of 8 mm/min. Time of pressing was 15 minutes. In the first experiment effect of addition of ascorbic acid (AA) into the mash at the dose of 0, 200, 500 and 1000 mg kg^{-1} of fruit was investigated. The second experiment concerned the effect of enzyme maceration using: Rohapet PTE, Rohament PL and combination of this two enzyme preparations (in ratio 1:1) at the dose of 50 g t^{-1}, for enzymation time 30 min at 20°C. The apple mash was enriched with 500 mg of AA per kg of fruit just before pressing. Obtained juices were subjected to action of microwave (900 W, 70 s) in order to inactivate enzymes, than cooled in cold tap water. The juice productions on a laboratory scale were carried out in triplicate.

The third experiment was carried out on semi-technological scale using 3 red-fleshed and 2 control apple cultivars in season 2011. The fruit was stored in normal atmosphere at +1.5°C until processing, which took place after obtaining a negative results for starch using iodine test. Healthy apples, 10 kg per treatments were selected for processing, washed up and disintegrated using Fryma perforated disc mill equipped with 6 mm openings disc. Obtained mash was pressed using rack and cloth Bucher press for 10 min. Juices were produced with centrifugation at 1500 rpm (all cultivars) or without (only „Alex Red", „Shampion" and „Idared") before pasteurization at 92–96°C for 30 s using plate

heat exchanger, than filled into 0.25 L screw cap bottles and cooled down to ambient temperature. Two technological replications of juice production were carried out for each apple cultivar. The juices were analyzed directly after production and after storage for 3, 6 and 12 months at 2°C and 20°C.

Statistical analysis

Results were subjected to one or two way analysis of variance (ANOVA) using Statistica V. 8.0 software (Statsoft Inc., Tulsa, OK, USA). The significance of the differences between sampling dates was estimated with Tukey's HSD test at p=0.05.

Results and discussion

Experiments on laboratory scale

Studies on laboratory scale have shown that addition of ascorbic acid (AA) during pressing, in the amount of 500–100 mg kg^{-1} of pulp, increased anthocyanins extraction into juice and/or prevented their oxidation. The same effect of AA addition was reported in case of blackcurrant and plum juices production (MIESZCZAKOWSKA-FRĄC et al. 2012). The juice pressed with addition of 500 mg AA kg^{-1} had a clear red colour and level of anthocyanins was 22.8 mg L^{-1} (Table 1). The addition of AA had a positive effect on antioxidant activities of juices that increased from 0.18 mg Trolox mL^{-1} to 0.92 mg Trolox mL^{-1}. On the other hand, the addition of AA did not change a soluble solids and acidity of red-fleshed apple juices. The added ascorbic acid in the amount of

Table 1
The effect of ascorbic acid (AA) addition during juice pressing on physico-chemical parameters of cloudy apple juices on a laboratory scale

Addition of AA mg kg^{-1}	SS °Bx	TA %	Ascorbic acid mg 100 mL^{-1}	ABTS mg mL^{-1}	Anthocyanins* mg L^{-1}	Turbidity NTU	Stability of turbidity %
0	9.87^{a}	0.74^{a}	0.0^{a}	0.18^{a}	8.2^{a}	908^{a}	22.6^{a}
200	9.78^{a}	0.75^{a}	0.0^{a}	0.17^{a}	8.1^{a}	1488^{b}	15.6^{a}
500	9.76^{a}	0.76^{a}	15.4^{b}	0.38^{b}	22.8^{c}	1287^{b}	16.7^{a}
1000	9.93^{a}	0.77^{a}	62.8^{c}	0.92^{c}	16.6^{b}	1243^{ab}	18.6^{a}

Means in the same column marked by the same letter do not differ significantly at $p = 0.05$ (n=3).
Abbreviations: SS – soluble solids, TA – titratable acidity, ABTS – antioxidant activity.
* quantified spectrophotometrically by the pH differential method

200 mg kg^{-1} of pulp was completely oxidized during juice production. About 31% or 63% of the added AA remained in juices when the levels of AA addition were 500 mg and 1000 mg per kg of pulp, respectively. The juices pressed with AA addition possessed also higher total turbidity (1243–1488 NTU) than the juice without addition (908 NTU). Summarizing the results, the addition of AA in the amount of 500 mg kg^{-1} was chosen for the next experiments.

The enzymatic treatment of the pulp did not influence the pressing yield, however adversely affected the content of phenolic compounds and antioxidant activities (Table 2). The content of polyphenols in the juices, obtained after enzymatic maceration, was about 12–31% lower, depending on the enzyme preparation, as compared to the juice pressed without enzyme treatment. MARKOWSKI et al. (2009) also observed lower content of phenolic compounds after apple mash enzymation. Similarly, after enzymatic maceration, the antioxidant activity of juices decreased by 14–35%. Therefore the further juice productions were carried out without the enzyme treatment of apple pulp. Furthermore, it was observed that in the first experiment juice produced with 500 mg AA kg^{-1} addition exhibited antioxidant activity as 0.38 mg Trolox mL^{-1} (Table 1), while a similar juice in the second experiment (500 mg AA kg^{-1}, no enzymatic treatment) had antioxidant activity as 0.99 mg Trolox mL^{-1} (Table 2). In the second experiment the inactivation of enzymes by microwave energy was used. According to literature, microwave pasteurization is not only efficient way to eliminate bacteria, but also effective method of inactivating the enzymes (NIKDEL and MACKELLAR 1992), including native enzymes, such as peroxidase (POD) and polyphenols oxidase (PPO) (MATSUI et al. 2007). It is known that PPO causes an oxidation of compounds responsible for antioxidant activity of fruit and their products (TOMÁS-BARBERÁN and Esptn 2001, LI et al. 2008). Consequently, the use of microwave energy results in higher antioxidant properties of apple juice.

Table 2

The effect of enzyme treatment on physicochemical parameters of cloudy apple juices on a laboratory scale

Enzyme preparation	SS °Bx	TA %	ABTS mg mL^{-1}	Polyphenols mg L^{-1}	Pressing yield %
No enzyme	10.4^{b}	0.83^{b}	0.99^{c}	108.9^{c}	77.0^{a}
Rohapect PTE	10.2^{ab}	0.80^{ab}	0.85^{b}	96.2^{bc}	78.9^{a}
Rohament PL	10.1^{a}	0.80^{ab}	0.68^{a}	85.7^{ab}	77.5^{a}
R.PTE+R.PL	10.1^{a}	0.79^{a}	0.64^{a}	74.8^{a}	78.4^{a}

Means in the same column marked by the same letter do not differ significantly at $p = 0.05$ (n=3).
Abbreviations: see in Table 1.

Table 3

The effect of centrifuge on the physicochemical parameters of apple juices directly after production

Cultivar	Centr.	TA %	SS °Bx	TA/SS ratio	Turbidity NTU	Asc. ac. mg 100 mL^{-1}	ABTS mg mL^{-1}	Anth* mg L^{-1}	Phenols mg L^{-1}
„Alex Red"	Yes	1.34^{c}	10.0^{a}	7.5^{a}	204^{a}	44.7^{bc}	0.90^{b}	18.8^{b}	114^{a}
	No	1.33^{c}	10.0^{a}	7.5^{a}	286^{a}	43.5^{b}	0.91^{b}	19.1^{b}	115^{a}
„Shampion"	Yes	0.33^{a}	12.7^{c}	38.7^{c}	1944^{c}	48.2^{cd}	1.35^{c}	0.0^{a}	273^{b}
	No	0.33^{a}	12.8^{c}	38.3^{c}	1978^{c}	51.8^{d}	1.38^{c}	0.0^{a}	276^{b}
„Idared"	Yes	0.62^{b}	12.4^{b}	20.0^{b}	425^{b}	0.0^{a}	0.81^{a}	0.0^{a}	308^{c}
	No	0.61^{b}	12.3^{b}	20.4^{b}	440^{b}	0.0^{a}	0.89^{a}	0.0^{a}	410^{d}
„Maypole"	Yes	1.10	11.2	10.2	851	7.6	0.66	9.6	462
„Trinity"	Yes	1.38	10.3	7.5	316	41.7	0.96	21.8	146

Means in the same column marked by the same letter do not differ significantly at $p = 0.05$ (n=2). Abbreviations: Centr. – centrifuged or not; Asc. Ac. – ascorbic acid, Anth -anthocyanins; TA, SS and ABTS see in Table 1.
* quantified spectrophotometrically by the pH differential method

Experiments on semi-technological scale

The centrifugation, one of the technological step in the production of juices, had no significant effect on most of the measured parameters. Only juice from „Idared", produced without centrifugation, contained more phenolic compounds (Table 3) than the juice after centrifugation. The red-fleshed apple juices were characterized by the about 3–4-times higher acidity than the „Shampion" juices and about 2-times higher compared to the „Idared" juices (Table 3). On the other hand, the red-fleshed juices contained lower soluble solids, within the range of 10.0–11.2 °Bx, compared to the control juices: 12.3–12.8 °Bx. Therefore they had a very low sugar-acid-ratio (7.5–10.2), what is in agreement with the value obtained for „Weirouge" apple – 7.1 (SADILOVA et al. 2006). The highest juice turbidity was observed for „Shampion", above 1940 NTU, while the highest turbidity of red-fleshed juices was found in juices from „Maypole" – 851 NTU.

Red-fleshed apple juices had lower concentration of flavan-3-ols (13.2–53.9 mg L^{-1}) than the control cloudy juices (69.7–210.0 mg L^{-1}) (Table 4). Moreover, red-fleshed juices did not contain (+)-catechin and concentration of (-)-epicatechin was below 5 mg L^{-1}. This is inconsistent with the data shown by SUN-WATERHOUSE et al. (2013), who reported that red-fleshed apple juice contained a significantly greater amount of (+)-catechin than white-fleshed apple juice, which may be explained by the diversity of cultuvars. On the other hand, red-fleshed juices had more phloretin xyloglucoside and as opposed to control juices contained anthocyanins at the level of 6.1–20.3 mg L^{-1}, mainly

Table 4
The composition of phenolic compounds (mg L^{-1}) in cloudy apple juices directly after production (semi-technological scale)

	„Maypole"	„Trinity"	„Alex Red"	„Shampion"	„Idared"
(+)-Catechin	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	9.2 ± 0.4^{c}	6.1 ± 0.2^{b}
(-)-Epicatechin	4.9 ± 0.0^{a}	4.6 ± 0.2^{a}	4.1 ± 0.2^{a}	64.8 ± 0.9^{c}	20.3 ± 0.3^{b}
Oligomeric procyanidins	49.0 ± 0.4^{d}	15.3 ± 0.4^{b}	9.1 ± 0.2^{a}	136.0 ± 4.8^{e}	43.3 ± 1.5^{c}
Phloretin xyloglucoside	27.6 ± 0.5^{e}	13.6 ± 0.9^{d}	10.5 ± 0.3^{c}	8.7 ± 0.1^{b}	3.2 ± 0.1^{a}
Phloridzine	11.1 ± 0.5^{c}	10.4 ± 0.2^{c}	8.5 ± 0.3^{b}	7.1 ± 0.6^{a}	13.8 ± 0.3^{d}
Chlorogenic ac.	318.4 ± 5.8^{d}	62.5 ± 3.9^{b}	49.1 ± 1.1^{b}	29.7 ± 12.2^{a}	180.4 ± 4.6^{c}
Derivative of chlorogenic ac.	33.7 ± 0.7^{c}	3.5 ± 0.2^{a}	3.8 ± 0.2^{a}	3.2 ± 0.1^{a}	21.7 ± 0.6^{b}
p-Coumarylquinic ac.	5.7 ± 0.1^{a}	7.8 ± 0.4^{c}	6.5 ± 0.1^{b}	9.6 ± 0.1^{d}	14.0 ± 0.6^{e}
Glycoside of quercetine	5.3 ± 0.1^{ab}	7.8 ± 0.1^{c}	7.3 ± 0.8^{c}	4.6 ± 0.3^{a}	5.7 ± 0.2^{b}
Anthocyanins	6.1 ± 0.1^{b}	20.3 ± 0.5^{d}	15.3 ± 1.4^{c}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Total	462 ± 7.3^{e}	146 ± 5.2^{b}	114 ± 4.2^{a}	273 ± 12.8^{c}	308 ± 5.8^{d}

Means in the same line marked by the same letter do not differ significantly at $p = 0.05$ (n=2).

cyanidin-3-O-galactoside (MAZZA and VELIOGLU 1992, SADILOVA et al. 2006, RUPASINGHE et al. 2010). The highest concentration of the total polyphenols was in the juice from „Maypole": 462 mg L^{-1} which resulted from very high concentration of chlorogenic acid 318.4 mg L^{-1}. SADILOVA et al. (2006) reported that chlorogenic acid and phloretin xyloglucoside were the major phenolic compounds in the juice from red-fleshed apple „Weirouge;. However, juices from other red-fleshed cultivars, such as „Trinity" and „Alex Red" were characterized by a lower content of polyphenols than the control juices.

The extension of storage time at 2°C resulted in a decrease of total phenols content by about 12% after 3 months, 23% and 38%, respectively after 6 and 12 months (Figure 1). Similar tendency was observed when juices were stored at 20°C, except from „Trinity" and „Alex Red" juices, for which, after 12 months of storage, the increasing of phenols content was observed. The higher temperature of storage increased degradation of phenolic compounds by about 2–13% after 3 months of storage, 3–17% after 6 months and 5–8% after 12 months in juice from „Shampion" and „Idared" *cvs*. compared to juices stored at 2°C. However, in case of red-fleshed juices stored for 12 months at 20°C the concentration of phenols was even 44% higher than in juices at 2°C. This phenomenon can be explained by the fact that higher temperature accelerates the Maillard reaction process resulting in formation of non-enzymatic browning products. These compounds might falsify the real concentration of phenolic compounds.

The juice from „Shampion" exhibited the highest antioxidant activitiy directly after production (1.35 mg Trolox mL^{-1}) whereas the lowest (0.66 mg Trolox mL^{-1}) was for juice from „Maypole" in spite of the highest concentration of phenolic compounds (Figure 2). RUPASINGHE et al. (2010) reported both higher and lower antioxidant activities for red-fleshed apple juices compared with juices from commercial apple.

There was some fluctuations of the antioxidant activity during storage. The juices from red-fleshed apples possessed the highest scavenging properties after 3 months of storage, and there were no differences between juices kept at 2°C and 20°C. However, juices stored for 6 or 12 months at 20°C had about 11% lower antioxidant activities than juices stored at 2°C. Storage temperature had the most significant influence on antioxidant properties in case of juices from „Shampion;, for which a higher temperature has reduced radical scavenging properties, about 9% after 6 months and 21% after 12 months compared to juices stored at 2°C.

Among the analyzed red-fleshed apple juices the highest concentration of anthocyanins was observed in the juices from „Trinity" (21.8 mg L^{-1}), and the lowest for „Maypole" (9.6 mg L^{-1}) (Figure 3). The greatest alterations of anthocyanins content during storage was found in „Maypole" juices. Already,

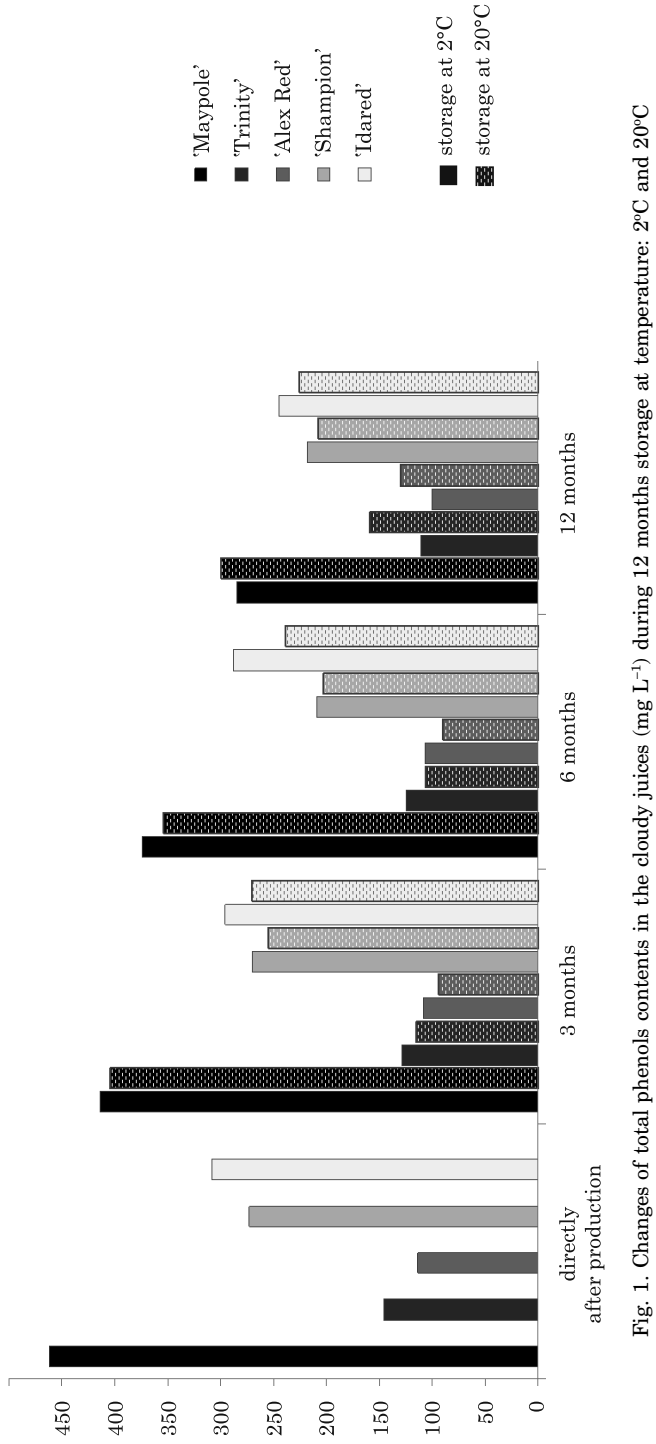

Fig. 1. Changes of total phenols contents in the cloudy juices (mg L^{-1}) during 12 months storage at temperature: 2°C and 20°C

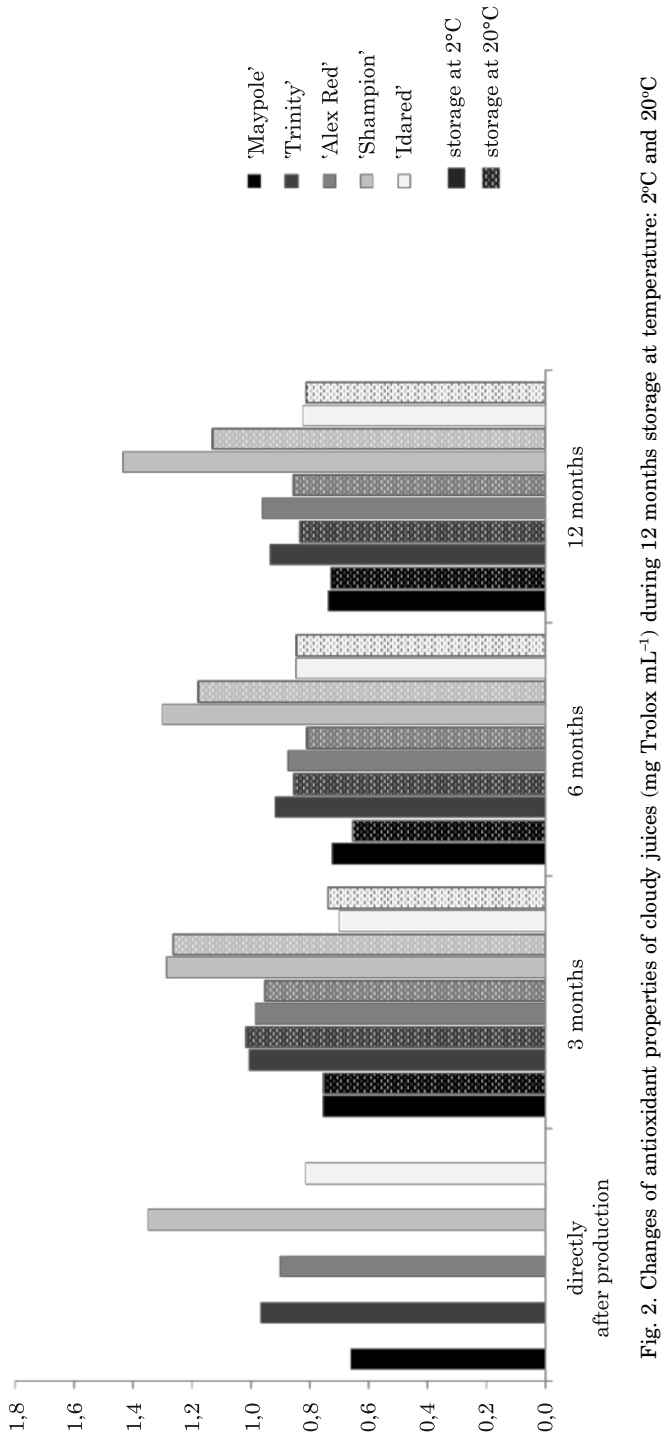

Fig. 2. Changes of antioxidant properties of cloudy juices (mg Trolox mL^{-1}) during 12 months storage at temperature: 2°C and 20°C

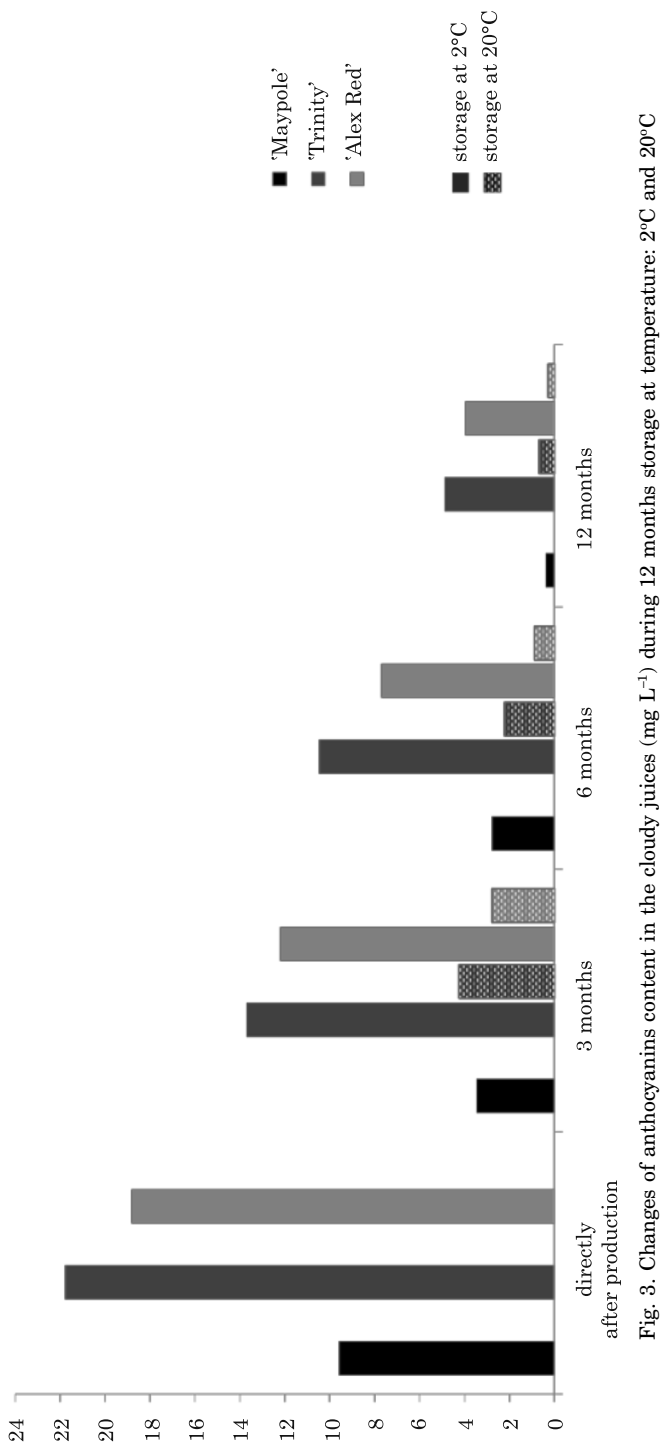

Fig. 3. Changes of anthocyanins content in the cloudy juices (mg L^{-1}) during 12 months storage at temperature: 2°C and 20°C

after 3 months of storage at 20°C they did not contain anthocyanins, while the juices at 2°C possessed only 36% of an initial amount, and after 6 months – 29%, whereas after 12 months, there were only traces of anthocyanins. The juices from other cultivars: „Trinity" and „Alex Red" after 3 months of storage at 2°C possessed about 63–65% of the initial content of anthocyanins, after 6 months 41–48% and after 12 months, only 21–22%. Higher storage temperature resulted in a substantial reduction in an amount of anthocyanins in the juices, with retention at about 15–19%, 5–10% and 2–3%, respectively after 3, 6 and 12 months of storage.

Conclusions

The cloudy red-fleshed apple juices are a rich source of bioactive compounds and on the contrary to those produced from white or yellow flesh apple juices, contain anthocyanin compounds, which are strong antioxidants. The addition of ascorbic acid during pressing, in the amount of 500–100 mg kg^{-1} of pulp, increased anthocyanins extraction into juice and/or prevented their oxidation. Thanks to anthocyanins presence the red-fleshed juices had an interesting dark red colour. The enzymatic treatment of the pulp did not influence the pressing yield, however, adversely affected the content of polyphenolic compounds and antioxidant activities. The juice from „Maypole" contained the highest amount of total phenolic compounds and juices from „Trinity" were the most abundant in anthocyanins, mainly cyanidin-3-O-galactoside.

Due to the rapid degradation of anthocyanins, the red-fleshed apple juices should be stored at cold storage conditions. The cloudy red-fleshed apple juices production may be an interesting proposition for small and medium-sized enterprises.

Translated by Monika Mieszczakowska-Frąc

Accepted for print 18.03.2015

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 30(1): 73–80, Y. 2015

CONJUGATED LINOLEIC ACID (*cis*9*trans*11 C18:2, CLA) AND *trans* ISOMERS OF C18:1 AND C18:2 ACIDS IN YOGHURTS, KEFIRS AND MOLD CHEESES

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K e y w o r d s: yoghurt, kefir, mold cheeses, CLA, *trans* isomers.

A b s t r a c t

The aim of this study was to determine contents of *cis*9*trans*11 C18:2 acid (CLA) and *trans* isomers of C18:1 and C18:2 acids in fat of yoghurts, kefirs, cheeses with bloomy molds and blue-veined cheeses. Fatty acid composition of the analyzed dairy products was assayed with the gas chromatography method using a 100-m capillary column with CP Sil 88 phase.

The study demonstrated that contents of *cis*9*trans*11 C18:2 acid (CLA) and *trans* isomers of C18:1 and C18:2 acids in the analyzed yoghurts and kefirs were very similar. Slightly higher contents of CLA and *trans* isomers were determined in fat isolated from the investigated mold cheeses.

SPRZĘŻONY KWAS LINOLOWY (*cis*9*trans*11 C18:2, CLA) ORAZ IZOMERY *trans* KWASU C18:1 I C18:2 W JOGURTACH, KEFIRACH I SERACH PLEŚNIOWYCH

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S ł o w a k l u c z o w e: jogurt, kefir, sery pleśniowe, CLA, izomery *trans*.

A b s t r a k t

Celem pracy było określenie zawartości kwasu *cis*9*trans*11 C18:2 (CLA) oraz izomerów *trans* C18:1 i C18:2 w tłuszczu jogurtów, kefirów oraz serów z porostem i przerostem pleśni. Skład kwasów tłuszczowych tłuszczu badanych produktów oznaczano metodą chromatografii gazowej na 100 m kolumnie kapilarnej z fazą CP Sil 88.

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Przeprowadzone badania wykazały, że zawartość kwasu *cis*9*trans*11 C18:2 (CLA) oraz izomerów *trans* kwasu C18:1 i C18:2 w badanych jogurtach i kefirach była bardzo zbliżona. Nieco wyższą zawartość kwasu CLA i izomerów *trans* stwierdzono w tłuszczu wydzielonym z badanych serów pleśniowych.

Introduction

The term „linoleic acid with conjugated bonds" (CLA) refers to a group of positional and geometric isomers of linoleic acid (C18:2), in which two double bonds are separated with only one single bond. In fat of the ruminants, the highest concentration has been reported for *cis*9*trans*11 C18:2 acid which in milk fat constitutes from 75 to over 90% of the sum of C18:2 acid isomers with conjugated bonds (CHIN et al. 1992, PARODI 2003, PRECHT and MOLKENTIN 2000). This acid exhibits many activities beneficial to health like anticarcinogenic, anti-atherosclerotic, antioxidative and anti-inflammatory (CICHOSZ 2007, MOLKENTIN 1999, PARIZA 1991, PARODI 1994, PARODI 1997). A rich natural source of CLA in a human diet is milk and its products. CLA concentration in milk fat is highly diversified depending mainly on the feeding season of cows. According to ŻEGARSKA et al. (2006), the content of conjugated linoleic acid in milk originating from the pasture feeding (summer) ranged from 1.06% do 1.76% of total fatty acids and in milk from the stall feeding (winter) – from 0.32% to 0.52%. In turn, LIPIŃSKI et al. (2012) reported that the contribution of CLA in the total fatty acid composition ranged from 0.38% in March and April to 1.68% in August.

The content of CLA in dairy products may differ from that in milk. According to literature data (BZDUCHA-WRÓBEL and OBIEDZIŃSKI 2009, DOMAGAŁA et al. 2009, JIANG et al. 1998, KIM and LIU 2002, LIN et al. 1998, LIN 2003, SHANTHA et al. 1992, SIEBER et al. 2004), the level of CLA in dairy products (cheeses or fermented dairy drinks) may be influenced by parameters of technological processes and by the activity of starter cultures added.

Therefore, the aim of this study has been to determine contents of *cis*9*trans*11 C18:2 acid (CLA) and *trans* isomers of C18:1 and C18:2 acids in fat of yoghurts, kefirs, cheeses with bloomy molds and blue-veined cheeses.

Material and Methods

Material

The experimental material included fermented dairy drinks (6 yoghurts and 6 kefirs) and mold cheeses (7 cheeses with bloomy molds and 7 blue-veined cheeses). The analyzed products originated from various producers and were

purchased (one sample of each) between February and April in retail stores in Olsztyn. All analysed were performed in duplicate. All products were tested within their shelf life.

Analytical methods

Fat content in yoghurts and kefirs was determined with the Roese-Gottlieb's method (PN-75/A-86130), whereas in the analyzed mold cheeses – with the Schmidt-Bondzynski-Ratzlaff's method (PN-73/A-86232).

To determine fatty acid composition, fat of yoghurts and kefirs was isolated with the Roese-Gottlieb's method (PN-75/A-86130), and fat of cheeses – with a modified Folch's method (CHRISTIE 1973).

Methyl esters were prepared from the isolated fat acc. to the IDF method using a methanolic solution of KOH (IDF standard 182:1999).

Determinations of fatty acid composition, CLA and *trans* isomers of C18:1 and C18:2 acid were carried out with gas chromatography method using a Hewlett Packard 6890 chromatograph with a flame-ionization detector and capillary column with CP Sil 88 phase (100 m x 0.25 mm i.d., liquid phase film thickness 0.20 mm). Separation conditions were as follows: column temp.: 60°C (1 min) – 180°C, Δt = 5°C/min; injector temp.: 225°C; detector temp.: 250°C; carrier gas: helium, flow rate: 1.5 cm^3/min, injector: split 50:1.

Peaks of individual fatty acids were identified by comparing their retention times with those of methyl esters of reference fat with known fatty acids profile (BCR Reference Materials, symbol CRM 164). For identification of positional *trans* isomers of C18:1, use was made of the standards of methyl esters of those isomers (*trans* 6, *trans* 9, *trans* 11) (Sigma) and literature data. In turn, the *trans* isomers of C18:2 acid (*cis,trans* and *trans,cis*) were identified with the use of a mixture of standards of C18:2 isomers (Supelco), *cis*9, *trans*11 CLA – with a mixture of CLA methyl esters (Sigma) and literature data.

Percentage contents of conjugated linoleic acid *cis*9*trans*11 C18:2 and assayed *trans* isomers of C18:1 acid and C18:2 acid were calculated relative to the total content of fatty acids (weight %). Statistical calculations were made using STATISTICA PL software.

Results and discussion

The analyzed products were characterized by diversified contents of fat. In yoghurts its content ranged from 1.1% to 3.4% and in kefirs – from 1.3% to 2.0%. The percentage content of fat in cheeses with bloomy mold ranged from 19.3% to 31.5%, whereas in blue-veined cheeses – from 27.9% to 33.6%.

The examined yoghurts and kefirs were characterized by similar contents of particular groups of fatty acids. The content of short-chain fatty acids ranged from 10.00% to 11.33% of the total fatty acid composition in fat of yoghurts, and from 10.02% to 11.05% in fat of kefirs. The contents of saturated fatty acids ranged from 59.66% to 63.14% in fat of yoghurts, and from 60.40 to 62.47% in fat of kefirs. In fat of yoghurts, the mean content of monoenoic fatty acids accounted for 25.37%, and that of polyenoic fatty acids for 3.12% of the total fatty acid composition. In fat of kefirs, the respective values reached 25.45% and 3.05%. Greater differences in contents of the particular groups of fatty acids were determined in the analyzed mold cheeses. In fat extracted from cheeses with bloomy mold, the content of short-chain fatty acids ranged from 7.26% to 12.40%, that of saturated fatty acids from 58.95% to 81.56%, that of monoenoic acids from 8.81% to 27.39%, and that of polyenoic fatty acids from 2.39% to 3.48%. In the blue-veined cheeses, the respective values were as follows: from 9.67% to 16.29%, from 57.20% to 67.36%, from 18.54% to 25.32%, and from 2.52% to 4.19%.

In this study, we paid special attention to contents of CLA and *trans* isomers of C18:1 and C18:2 acids in fat separated from the analyzed dairy products.

Contents of *cis*9*trans*11 C18:2 acid (CLA) and assayed *trans* isomers of C18:1 acid and C18:2 acid in the total fatty acid composition of fat isolated from the analyzed fermented drinks and mold cheeses were presented in Table 1.

Data in Table 1 demonstrate that CLA content in fat of the analyzed yoghurts ranged from 0.38% to 0.46% of total fatty acids. The mean content of this isomer was at 0.42%. The mean content of this acid in kefirs available on the market in the same period was at the same level. These results are similar to findings reported by PASZCZYK et al. (2006), who showed that in commercial yoghurts originating from January to March the mean CLA content reached 0.42% (range: from 0.35 to 0.50% of total fatty acids). Similar contents of CLA in yoghurts and kefirs were also determined by ŻEGARSKA et al. (2008). Kefirs purchased by these authors in winter (from January to February) contained from 0.34 to 0.48%, and yoghurts from 0.37 to 0.49% of CLA. Kefirs and yoghurts analyzed by these authors in June and July were characterized by a higher CLA content.

Compared to fermented dairy drinks, a significantly ($p \geq 0.05$) higher content of CLA was reported in the analyzed cheeses with bloomy mold, i.e. on average 0.71% of total fatty acids (range: from 0.54 to 0.88%) in cheeses produced from milk from the winter feeding period.

The analyzed blue-veined cheeses were characterized by the greatest differences in CLA content. In fat of this group of cheeses, the content of *cis*9*trans*11 C18:2 acid ranged from 0.35% to 1.10% of total fatty acid composition. Its mean content in fat of these cheeses reached 0.56% of total fatty acids

and did not differ significantly from values reported in the analyzed fermented dairy drinks and cheeses with bloomy molds (Table 1).

In various mold cheeses analyzed by ŻEGARSKA et al. (2006), the mean CLA content accounted for 0.48% of total fatty acids (from 0.42 to 0.54%) in fat of cheeses purchased in February and March, and for 0.89% of total fatty acids (from 0.59 to 1.24%) in fat of cheeses bought in November. As demonstrated by PASZCZYK et al. (2012), mold cheeses originating from different EU Member States (Poland, Germany, Italy and France) available on the Olsztyn market in January and February were characterized by a similar mean content of *cis*9*trans*11 C18:2 acid (CLA), ranging from 0.43 (in Polish cheeses) to 0.58% of total fatty acids (in German cheeses). According to a study by LIN *et al*. (1995), the content of conjugated linoleic acid varied in different types of cheeses, i.e. the highest CLA content was found by these authors in Blue and Brie cheeses (mold cheeses) and the lowest one in processed melted cheeses. In turn, when investigating German mold cheeses, FRITHE and STEINHART (1998) showed CLA content to reach 0.55% of total fatty acids in Blue cheese and 0.49% in Brie cheese.

The total content of *trans* isomers of C18:1 acid in fat extracted from the analyzed yoghurts ranged from 1.99 to 2.61% of total fatty acids (Table 1). The mean content of these isomers was at 2.25% and did not differ significantly ($p \geq 0.05$) from the value reported in the analyzed kefirs. In fat of kefirs the *trans* isomers of C18:1 acid constituted on average 2.08% (i.e. from 1.82% to 2.32% of total fatty acids) (Table 1). Similar contents of these isomers, i.e. from 1.66 to 2.34%, were determined by PASZCZYK et al. 2006) in yoghurts purchased from January till March. According to research by ŻEGARSKA et al. (2008), yoghurts purchased in January and February contained on average 1.64% (from 1.24 to 1.87%), and kefirs 1.54% of *trans* isomers of C18:1 acid (from 1.18 to 1.98%). Higher contents of these isomers were determined by these authors in yoghurts and kefirs analyzed in June and July.

In the analyzed cheeses with bloomy molds, the content of *trans* isomers of C18:1 acid ranged from 2.32 to 3.34% of total fatty acids. The mean summary content of these isomers in these cheeses reached 2.92% and was significantly higher than their content in the analyzed kefirs ($p \geq 0.01$) and yoghurts ($p \geq 0.05$) (Table 1). The content of these isomers in the investigated blue-veined cheeses ranged from 1.71 to 3.45% of total fatty acids. Their average total content (2.36% of total fatty acids) did not differ significantly from respective values determined in fermented dairy drinks and cheeses with bloomy molds (Table 1). The mean content of C18:1 acid *trans* isomers in various mold cheeses analyzed by ŻEGARSKA et al. (2008) accounted for 1.88% (i.e. from 1.40 to 2.47%) in cheeses from January and February, and for 3.08% (i.e. from 2.52 to 3.91%) in cheeses from November. Mold cheeses from the winter period

Table 1
The content of *cis*9*trans*11 C18:2 acid (CLA) and *trans* isomers of C18:1 and C18:2 acids in fat of analyzed dairy products (% of total fatty acids)

*Trans i*somers	Products							
	Yoghurts (n=6)		Kefirs (n=6)		Cheese with bloomy mold (n=7)		Blue-veined cheese (n=7)	
	Min. – Max.	$\bar{x} \pm s$	Min. – Max.	$\bar{x} \pm s$	Min. – Max.	$\bar{x} \pm s$	Min. – Max.	$\bar{x} \pm s$
*cis*9*trans*11 C18:2(CLA)	0.38 – 0.46	$0.42^{a,A} \pm 0.03$	0.37 – 0.48	$0.42^{a,A} \pm 0.04$	0.54 – 0.88	$0.71^{b,A} \pm 0.15$	0.35 – 1.10	$0{,}56^{a,b,A} \pm 0.25$
t6 – t9	0.38 – 0.46	$0.42^{a,A} \pm 0.04$	0.35 – 0.43	$0.40^{a,A} \pm 0.03$	0.31 – 0.43	$0.40^{a,A} \pm 0.05$	0.32 – 0.58	$0.39^{a,A} \pm 0.09$
*t*10 ±*t*11	1.10 – 1.48	$1.26^{a,A} \pm 0.14$	1.02 – 1.37	$1.15^{a,A} \pm 0.14$	1.55 – 2.28	$1{,}94^{b,B} \pm 0{,}28$	0.97 – 1.53	$1.43^{a,A,B} \pm 0.47$
t12 C18:1	0.24 – 0.34	$0.28^{a,A} \pm 0.04$	0.20 – 0.26	$0{,}25^{a,A} \pm 0{,}02$	0,20 – 0,28	$0.26^{a,A} \pm 0.03$	0.17 – 0.33	$0.23^{a,A} \pm 0.06$
t16 C18:1	0.27 – 0.33	$0.30^{a,A} \pm 0.02$	0.25 – 0.30	$0.28^{a,A} \pm 0.02$	0.26 – 0.36	$0.32^{a,A} \pm 0.03$	0.24 – 0.38	$0.30^{a,A} \pm 0.06$
Σ *trans* C18:1	1.99 – 2.61	$2.25^{a,A,B} \pm 0.23$	1.82 – 2.32	$2.08^{a,A} \pm 0.18$	2.32 – 3.34	$2.92^{b,B} \pm 0.36$	1.71 – 3.45	$2.36^{a,b,A,B} \pm 0.54$
c9 t13	0.14 – 0.21	$0{,}18^{a,A} \pm 0{,}03$	0,11 – 0,20	$0.16^{a,A} \pm 0.03$	0.15 – 0.22	$0.19^{a,A} \pm 0.03$	0.12 – 0.31	$0.19^{a,A} \pm 0.06$
c9 t12	0.24 – 0.30	$0{,}27^{a,A} \pm 0{,}02$	0.23 – 0.28	$0.25^{a,A} \pm 0.02$	0.22 – 0.30	$0.27^{a,A} \pm 0.03$	0.13 – 0.35	$0.25^{a,A} \pm 0.08$
t9 c12	0.01 – 0.04	$0.02^{a,A} \pm 0.01$	0.01 – 0.03	$0.01^{a,A} \pm 0.01$	0.02 – 0.04	$0.03^{a,A} \pm 0.01$	0.01 – 0.14	$0.05^{a,A} \pm 0.08$
t11 c15	0.09 – 0.12	$0.11^{a,A} \pm 0.01$	0.08 – 0.15	$0.12^{a,A} \pm 0.03$	0.13 – 0.25	$0.19^{a,A} \pm 0.05$	0.06 – 0.40	$0.16^{a,A} \pm 0.11$
Σ *trans* C18:2	0.51 – 0.61	$0.57^{a,A} \pm 0.04$	0.51 – 0.63	$0.54^{a,A} \pm 0.05$	0.55 – 0.80	$0.76^{a,A} \pm 0.09$	0.48 – 1.06	$0.66^{a,A} \pm 0.19$

a,b – Values in rows denoted with the same letter are not significantly different (p>0.05)
A,B – Values in rows denoted with the same letter are not significantly different (p>0.01)

analyzed by PASZCZYK et al. (2012) contained on average from 2.34% (Polish cheeses) to 3.79% (Italia cheeses) of these isomers.

The mean content of the analyzed *trans* isomers of C18:2 acid in fat of kefirs and yoghurts was alike. In fat isolated from yoghurts these isomers constituted on average 0.57% (from 0.51 to 0.61% of total fatty acids) and in fat of kefirs – 0.54% (from 0.51 to 0.63%) (Table 1). Their similar contents were reported by ŻEGARSKA et al. (2008) in fat of commercially-available kefirs and yoghurts purchased in winter, i.e. 0.47% in both. Slightly higher contents of these isomers were determined by these authors in fermented dairy drinks purchased in June and July, i.e. 0.74% in fat from kefirs and 0.72% in fat from yoghurts.

A slightly higher mean content of *trans* isomers of C18:2 acid was determined in mold cheeses. In cheeses with bloomy molds they constituted 0.76% (from 0.55 to 0.80%) and in blue-veined cheeses – 0.66% (from 0. 48 to 1.06%) of total fatty acids. Slightly lower sums of these isomers were reported by ŻEGARSKA et al. (2008) in different mold cheeses purchased since February till March, i.e. from 0.39 to 0.69% with an average content of 0.54% of total fatty acids. According to PASZCZYK et al. (2012), in mold cheeses originating from different EU Member States and purchased in January-February the mean content of *trans* isomers of C18:2 acid ranged from 0.63% (cheeses from Poland) to 0.80% (cheeses from Italy).

Conclusions

The content of conjugated linoleic acid *cis*9*trans*11 C18:2 in the total fatty acid composition of fat isolated from the analyzed yoghurts ranged from 0.38% to 0.46%. The content of this isomer in fat of the investigated kefirs available on the market in the same period was at the same level.

The analyzed yoghurts and kefirs were also characterized by similar contents of *trans* isomers of C18:and C18:2 acids.

The content of CLA in the investigated mold cheeses was higher than in yoghurts and kefirs. In fat of cheeses with bloomy mold it ranged from 0.54% to 0.88%, whereas in fat of blue-veined cheeses it ranged from 0.35% to 1.10% of the total fatty acid composition.

The analyzed mold cheeses were additionally characterized by higher contents of *trans* isomers of C18:1 and C18:2 acids compared to yoghurts and kefirs.

Translated by JOANNA MOLGA

Accepted for print 14.01.2015

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 30(1): 81–89, Y. 2015

INACTIVATION OF THE NATIVE MICROFLORA IN BEETROOT JUICE BY HIGH PRESSURE CARBON DIOXIDE COMBINED WITH TEMPERATURE

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K e y w o r d s: high pressure carbon dioxide, beetroot juice, spoilage microorganisms, lactic acid bacteria, yeasts and moulds.

A b s t r a c t

Commercially available unpasteurized, freshly-squeezed beetroot juice with a 24–72 hour shelf-life in cold storage, retains its natural flavour and nutritional value but can be a source of undesirable microflora. In this paper, the suitability of high pressure carbon dioxide (at 20 and 60 MPa, and a temperature of: 20, 35 and 60°C) to inactivate the native microflora in this juice has been studied.

The results show that high pressure carbon dioxide was effective in the inactivation of the studied groups of microorganisms only when combined with increased temperature. The reduction in the total count of spoilage microorganisms and lactic acid bacteria was 5–6 log when 20 or 60 MPa and 60°C for at least 30 min were used. Yeasts treated with carbon dioxide at 20 MPa and 60°C were totally (>6 log) inactivated. The reduction in moulds count above 3 log, was observed in this conditions.

INAKTYWACJA NATURALNEJ MIKROFLORY SOKU Z BURAKÓW ĆWIKŁOWYCH DITLENKIEM WĘGLA POD WYSOKIM CIŚNIENIEM W POŁĄCZENIU Z TEMPERATURĄ

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S ł o w a k l u c z o w e: ditlenek węgla pod wysokim ciśnieniem, sok z buraków, mikroorganizmy psujące, bakterie fermentacji mlekowej, drożdże i pleśnie

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Abstrakt

Dostępny w handlu niepasteryzowany, świeżo wyciskany sok z buraków ćwikłowych posiada 24–72 godzinny okres przydatności do spożycia. Zachowuje swój naturalny smak i walory odżywcze, ale może być źródłem niepożądanej mikroflory. W artykule przedstawiono ocenę przydatności ditlenku węgla pod wysokim ciśnieniem (20 i 60 MPa w temperaturze: 20, 35 i 60°C) do inaktywacji naturalnej mikroflory soku z buraków ćwikłowych.

Wyniki badań wskazują, że działanie ditlenku węgla jedynie w połączeniu z podwyższoną temperaturą skutecznie eliminuje poszczególne grupy badanych drobnoustrojów. Przy zastosowaniu ditlenku węgla pod ciśnieniem 20 lub 60 MPa i temperatury 60°C przez co najmniej 30 min, osiągnięto redukcję ogólnej liczby drobnoustrojów psujących i bakterii fermentacji mlekowej o 5–6 log. Drożdże ulegały całkowitej inaktywacji (>6 log) po zastosowaniu ditlenku węgla pod ciśnieniem 20 MPa i temperatury 60°C. W tych samych warunkach redukcja liczby pleśni wynosiła powyżej 3 log.

Introduction

High pressure carbon dioxide (HPCD) processing has been developing rapidly over the past decades as an innovative, non-thermal pasteurization method for the preservation of liquid food. It has the ability to inactivate different microorganisms without exposing foods to the adverse effects of heat and therefore they can retain their fresh-like physical, nutritional, and sensory qualities (DAMAR and BALABAN 2006, KINCAL et al. 2006, GARCIA-GONZALES et al. 2007, CHEN et al. 2009, FERRENTINO et al. 2009b). HPCD processing has been also proven effective to inactivate certain enzymes, including polyphenol oxidase and peroxidase which cause fruit, vegetable and juice browning (LIU et al. 2008 a, LIU et al. 2010, XU et al. 2011).

The efficacy of HPCD on gram-positive bacteria, gram-negative bacteria, bacterial spores, fungi (SPILIMBERGO et al. 2002, ZHANG et al. 2006, LIAO et al. 2007, BAE et al. 2009, LIAO et al. 2010a, YUK et al. 2010, YUK and GEVEKE 2011), and the native microflora in juices (LIM et al. 2006, FERRENTINO et al. 2009a, FERRENTINO et al. 2009b, LIAO et al. 2010b, XU et al. 2011) has been studied over the past few years. It is known that, microbial inactivation is accelerated with increasing CD pressure. As a consequence, at higher pressure, a shorter exposure time is needed to inactivate the same level of microbial cells. The microbial inactivation is also sensitive to the applied temperature. In general, the inactivation rate increases with increasing temperature.

Beetroot juice is a popular beverage in Poland and is commercially available as an unpasteurized, freshly-squeezed juice with a 24–72 hour shelf-life (depending on the season) in cold storage, or as a product preserved by pasteurization. The attractive colour of beetroot juice is associated with betalain pigments, which belong to the group of cation antioxidants. Red betalain pigments, betacyanins, show anticancer activity and play an important role in preventing degenerative diseases (KANNER et al. 2001). Due to the

lack of heat treatment, freshly-squeezed beetroot juice retains most betacyanins, as well as its fresh taste and odour, but it can be a source of undesirable microflora.

The number of spoilage microorganisms in Polish commercial beetroot juice, supplemented with 5% apple juice, ranged from 2.1 x 10^6 to 2.4 x 10^7 cfu/mL, most of which were lactic acid bacteria and the rest were yeasts and moulds (SOKOŁOWSKA et al. 2011). The 25 g juice samples analyzed were negative for *Salmonella* spp. *Listeria monocytogenes* was found in 66.7% of the tested samples, but did not exceed 100 cfu/mL, which is the legal limit for such bacteria in food. The contamination of beetroot juices with *E. coli* was 1–800 cfu/mL. All the tested-juice samples met the microbiological safety criteria for raw juices as required by Commission Regulation (EC) No 2073/2005.

The elimination of pathogenic microorganisms and the reduction in the risk of microbial spoilage of industrially processed juices are usually accomplished by means of thermal pasteurization. Thermal preservation has some disadvantages such as biochemical and nutritional changes in processed products; in the case of beetroot juice the main problem is a decrease in betalains and betacyjanins content resulting in the degradation of the colour of the juice (CZAPSKI 1990, KIDOŃ and CZAPSKI 2007, CHANDRAN et al. 2012).

For these reasons new alternatives to the thermal treatment technologies of beetroot juice have been studied. The use of high hydrostatic pressure of 400 MPa at 20°C for 10 min, extended the shelf-life of freshly-squeezed beetroot juice from 1 day to 10 days, in refrigerated storage (SOKOŁOWSKA et al. 2014 a). The reduction in the *E. coli* cell number was 6.2 log under the same conditions and *L. innocua* cells were completely inactivated after 1 min at 400 MPa, 20°C (SOKOŁOWSKA et al. 2014b). The process conditions provided satisfactory product quality and safety.

The HPCD preservation method has several advantages. The CO_2 used in this method is inert, non-toxic, accessible, and inexpensive. In ambient conditions, CO_2 is a gas and does not leave any residues in the treated product, and furthermore, it is considered to be a GRAS solvent, which means it can be used in food products.

The aim of this work was to evaluate the suitability of the HPCD technique for the inactivation of native microflora in beetroot juice.

Material and Methods

Commercial unpasteurized beetroot juice, supplemented with 5% apple juice, pH 4.35, produced by Marwitt Sp. z o.o., was used. The juice samples (7–8 mL) were treated with HPCD at 20 and 60 MPa at a temperature of 20, 35 and

60°C with holding time of up to 40 min, using modified Applied Separations *Spe-ed* SFE device. The HPCD treatment times reported do not include the come-up and come-down times. The temperature was measured in the chamber. To evaluation impact of temperature 60°C, juice samples were held under the same treatment time, but without the flow of carbon dioxide.

Samples of the HPCD-treated juice were analyzed immediately after treatment. Raw juice was also used as a control. Samples were serially diluted in maximum recovery diluents (Merck), and spread on DRBC agar (Oxoid) in duplicate for the enumeration of yeasts and moulds (incubation: 5 days at 25°C, in accordance with PN-ISO 21527-1:2009). Mesophilic lactic acid bacteria was determined using the pour plate technique in duplicate on MRS agar (Merck) according to PN-ISO 15214:2002 (incubation: 3 days at 30°C). To enumerate the total count of spoilage microorganisms the pour plate technique was used in duplicate on Orange Serum Agar (Merck). In accordance with IFU Method no. 2:1996, 3 days incubation at 30°C was carried out.

An analysis of the variance and the Tukey multiple-range test, using StatSoft&Statistica 7.1, was used to test the significance of the differences ($p < 0.05$) between the mean log values of the survival microflora count.

Results and discussion

The inactivation of native microflora in beetroot juice samples exposed to HPCD treatment are presented in Figures 1–4. The reduction in the number of living microorganisms depended on their type, the pressure, time and temperature of the HPCD treatment.

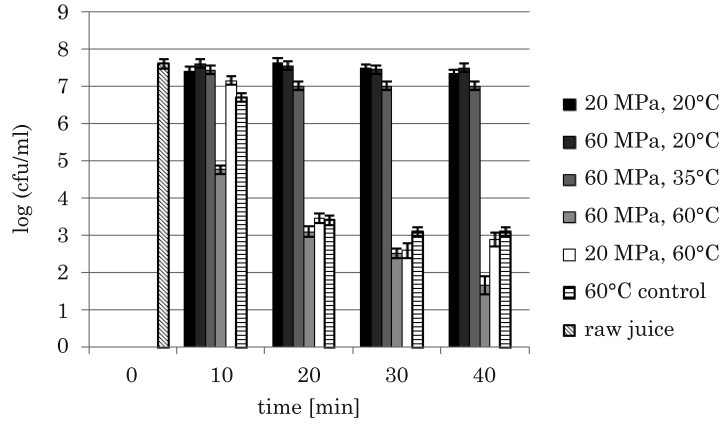

Fig. 1. Survival of spoilage microflora in beetroot juice supplemented with 5% apple juice treated with HPCD. The bars on the figures indicate the mean standard deviation for data points

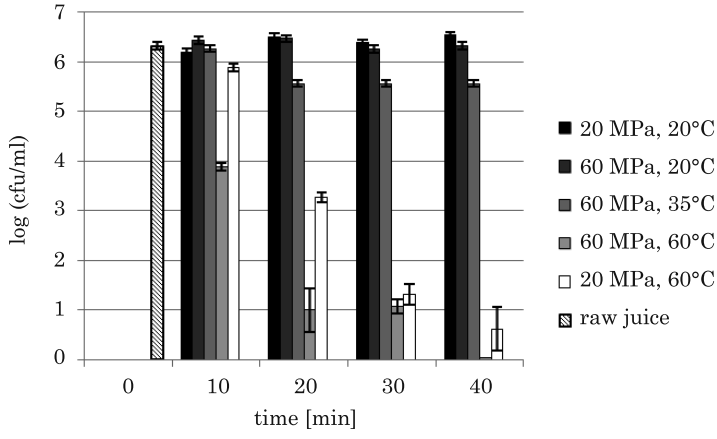

Fig. 2. Survival of lactic acid bacteria in beetroot juice supplemented with 5% apple juice treated with HPCD. The bars on the figures indicate the mean standard deviation for data points

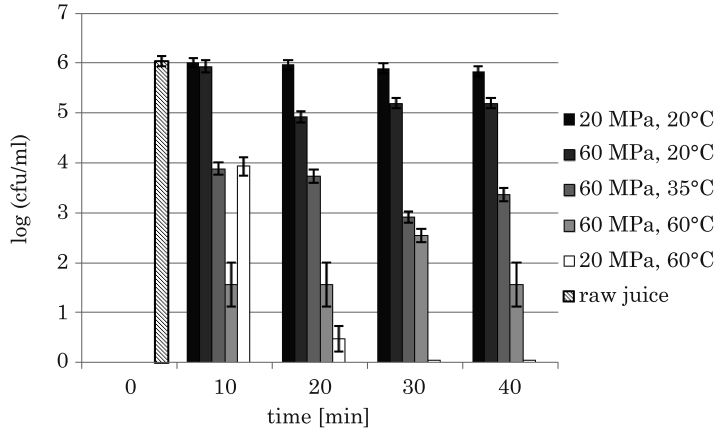

Fig. 3. Survival of yeasts in beetroot juice supplemented with 5% apple juice treated with HPCD. The bars on the figures indicate the mean standard deviation for data points

Only slight changes in the population of spoilage microorganisms were observed when HPCD under 20 MPa at 20°C and 60 MPa at 20 or 35°C was used (Fig. 1). When the HPCD treatment was combined with a temperature of 60°C, a significant reduction in spoilage microorganisms was achieved. The reduction in the total count of spoilage microorganisms was 2.8, 4.5, 5.1 and 5.9 log after 10, 20, 30 and 40 min treatment with HPCD at 60 MPa. Unfortunately, the reduction was mainly caused by temperature treatment. They achieved 0.9, 4.2, 4.5 and 4.5 log after 10, 20, 30 and 40 min treatment at 60°C, respectively. Perhaps it could be related to small volume of sample in our device. Decreasing the HPCD pressure to 20 MPa resulted in a similar

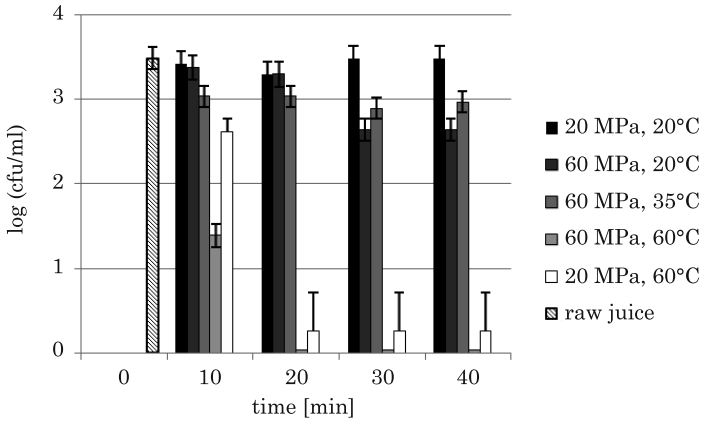

Fig. 4 Survival of moulds in beetroot juice supplemented with 5% apple juice treated with HPCD. The bars on the figures indicate the mean standard deviation for data points

reduction in the total count of spoilage microorganisms. Under these conditions the reductions were significantly lower ($p< 0.05$) than when using 60 MPa, except for the result after 30 min, which did not differ significantly ($p>0.05$). These results show that temperature plays a prominent role in the inactivation of spoilage microorganisms when combined with HPCD whereas the impact of the pressure value was less relevant.

Similar observation were reported in earlier studies carried out on apple juice. The microbial inactivation of 5 log of natural flora was achieved using the HPCD process at 16 MPa, 60°C and 40 min (FERRENTINO et al. 2009 a). LIAO et al. 2010 a obtained a 3.9 log reduction in the natural microorganisms in apple juice with HPCD at 20 MPa and a temperature of ≥52°C. The total aerobic flora in apple juice was reduced by 3.72 log after 10 min treatment at 22 MPa and 60°C (XU et al. 2011). Similar results were also observed in other juices. In litchi juice, a reduction of 4.19 log of aerobic bacteria was achieved with HPCD at 10 MPa and 52°C for 15 min (LI et al. 2012). A 5 log reduction for total aerobic microorganisms occurred with 34.5 MPa at 40°C and 7 min treatment in red grapefruit juice (FERRENTINO et al. 2009b).

The use of the temperature of 60°C could have serious disadvantages to beetroot juice. The stability of beetroot pigments such as betanin and isobetanin under HPCD was affected by both pressure and temperature. The study LIU et al. 2008 b shown, that treatments with a pressure above 30 MPa and temperature more than 55°C, led to a more rapid loss of betanin and isobetanin, and color change from violet to orange-red, when the aqueous solution of pigments was used.

As shown in Fig. 2, a significant reduction in lactic acid bacteria in beetroot juice was achieved, similarly to spoilage organisms, when HPCD was combined

with a temperature of 60°C. Inactivation reached 5.0 and 5.7 log after 30 and 40 min treatment with 20 MPa and respectively 5.2 and 6.3 log when 60 MPa was used. After 30 min treatment the results did not differ significantly ($p>0.05$).

Only a few articles concerning the inactivation of lactic acid bacteria with HPCD were found. The inactivation of *Lactobacillus plantarum* by 8 log was observed within 120 min under HPCD of 6.9 MPa at 30°C (HONG and PYUN 1999). The inactivation of *Lactobacillus plantarum* in apple cider reached 5 log after 20 min treatment at 7.6 MPa and 42°C, using a continuous system (YUK and GEVEKE 2011). The authors concluded that both CO_2 concentration and temperature contributed to microbial inactivation. Another lactic acid bacteria – *Leuconostoc dextranicum* was inactivated by at least 8 log at 35°C in 15–20 min under CO_2 pressure 6.9 or 20.7 MPa (LIN et al. 1993).

The results of our study confirmed the thesis that it is more difficult to inactivate natural microorganisms in real foods than inoculated microorganisms in real foods or buffers, which could be due to the complexity of natural microflora in real foods and the fact that they differ from inoculated pure strains in their susceptibility to HPCD.

Yeasts in beetroot juice were inactivated, when 60 MPa HPCD was conducted for 20 min even at 20°C, but at 35°C the reduction was higher and reached 2.7 log after 40 min (Fig. 3). The greatest reduction in yeasts, reaching 6.0 log, was achieved after HPCD treatment for 30 and 40 min at 20 MPa and 60°C. Contrary to previously described results, increasing the pressure to 60 MPa did not result in the higher inactivation of yeasts.

A significant reduction in moulds, above 3 log, was observed when HPCD both at 20 and 60 MPa was applied at a temperature of 60°C for at least 20 min (Fig. 4). In this case also, temperature played a predominant role in the inactivation of moulds; the pressure value was less significant.

In recent years there have been several studies on the inactivation of fungi using HPCD. The yeasts and moulds in apple juice treated with HPCD at ≥42°C were totally inactivated after 30 min, with a 3.9 log reduction (LIAO et al. 2010 b). XU et al. 2011 showed that yeast and moulds were completely inactivated (>4 log) in apple juice after 3 min treatment at 22 MPa and 60°C. The inactivation of yeast and moulds in litchi juice was complete (2.60 log reduction) at 10 MPa and 32°C for 30 min, or at 42°C for 15 min and at 52°C for 5 min (LI et al. 2012). Five log reduction for yeasts and moulds occurred at 34.5 MPa at 40°C and 7 min treatment in red grapefruit juice (FERRENTINO et al. 2009 b).

Studies on the effect of HPCD on yeasts were also conducted using *Saccharomyces cerevisiae* as the target microorganism. VALVERDE et al. 2010 reported that a 5 log inactivation of *S. cerevisiae* with HPCD in the Conference pear took place at 55°C. The required pressure and exposure times were

relatively low ≥6 MPa and 10 min. SPILIMBERGO et al. 2007 achieved an above 4 log reduction of *S. cerevisiae* in apple juice treated with HPCD 20 MPa at a temperature in the range of 25–50°C.

Conclusion

Studies of the effectiveness of preserving techniques, in which natural microorganisms in real foods are considered the targets, are very important for process design. A significant reduction in the native microflora in beetroot juices with HPCD using laboratory apparatus has been achieved. The study showed that the inactivation of native microflora in beetroot juice using HPCD was greatly affected by the treatment temperatures. The results indicate that HPCD combine with temperature may be a useful technique for preserving beetroot juices, but further studies on a larger half technical scale and shelf-life study are needed.

Translated by BARBARA SOKOŁOWSKA and SYLWIA SKĄPSKA
Corrected by ANNE-MARIE FABIANOWSKI Accepted for print 12.03.2015

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 30(1): 91–97, Y. 2015

USEFULNESS OF SELECTED BROCCOLI VARIETIES FOR FREEZING

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K e y w o r d s: broccoli, freezing, vitamin C, carotenoids, polyphenols.

A b s t r a c t

The purpose of this investigation was to evaluate the usefulness for freezing of new broccoli varieties. The evaluation was based on chemical composition of fresh and frozen broccoli stored for three months. The study was conducted on seven broccoli varieties, namely: SVR 97, SVR 99, SVR 1002, SVR 1017, Ironman, Beneforte and Bellaverde. The broccoli were obtained directly from the producer (Moszna, near Nałęczów). Physico-chemical analysis which were conducted on the samples involved determination of: dry mass, soluble solids, acidity, vitamin C, total carotenoids, total sugars and polyphenols content. Our results let us to conclude that it is unable to type the variety that would have the highest values of all analysed parameters. Beneforte variety could be of particular interest as it possesses high levels of sugars, carotenoids, polyphenolics, acidity, soluble solids and dry mass. In this variety, the smallest drop of analysed chemical compounds was noticed, as the result of blanching and freezing.

PRZYDATNOŚĆ WYBRANYCH ODMIAN BROKUŁA DO MROŻENIA

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Abstrakt

Celem pracy było określenie przydatności technologicznej nowych odmian brokuła do mrożenia w oparciu o charakterystykę fizykochemiczną surowca i produktu zamrożonego, przechowywanego przez trzy miesiące. Do badań wykorzystano 7 odmian brokuła: SVR 97, SVR 99, SVR 1002, SVR 1017, Ironman, Beneforte i Bellaverde. Surowiec uprawiano w prywatnym gospodarstwie ogrodniczym w Mosznej koło Nałęczowa. Analiza obejmowała oznaczenie zawartości suchej masy, ekstraktu ogółem, kwasowości, witaminy C, sumy karotenoidów, cukrów ogółem i związków fenolowych ogółem. Na podstawie przeprowadzonych analiz stwierdzono, iż nie można jednoznacznie wskazać jednej odmiany, która charakteryzowałaby się najwyższymi wartościami wszystkich analizowanych parametrów. Niemniej jednak, na szczególną uwagę zasługuje odmiana Beneforte, charakteryzująca się wysoką zawartością cukrów, karotenoidów, polifenoli, kwasów ogółem, ekstraktu i suchej masy. W odmianie tej stwierdzono najmniejsze straty poszczególnych związków chemicznych, będące wynikiem obróbki wstępnej surowca oraz przebiegiem procesu mrożenia i 3 miesięcznego przechowywania.

Introduction

Cultivation of broccoli in Poland dates to the beginning of the XX century when they started to be grown thank to appropriate climate conditions. Broccoli were accepted by consumers due to their extraordinary nutritional value which results from the high content of provitamin A (β -carotene), vitamin B_1, and vitamin C, minerals and fiber. Additionally, good sensory features (mild taste and flavor) along with anticancer properties make them highly attractive (SZYDŁOWSKA and CZARNIECKA-SKUBINA 2006, GĘBCZYŃSKI 2003, KRALA et al. 2007, ZALEWSKA-KORONA 2004).

Spain is the biggest broccoli producer is Europe (the annual production is approximately 280.000 tons) followed by Italy and Great Britain. In Poland its cultivation covers approximately 5.000 hectares, whereas the crop is estimated to be 50.000 tons. The production is concentrated mainly near the cities like Cracow, Warsaw and Kujawy region (GRABOWSKA and KUNICKI 2013).

However, seasonal character of the crop causes the necessity to ensure the continuity of delivery during the late autumn, winter and spring. The most useful method to preserve broccoli is freezing as it enables to retain attractive appearance along with the high quality (LEE and KEDDAR 2000, BĄKOWSKI 2002).

The most important features that affect the usefulness for freezing are: the size, density and colour of flower heads and lack of woody stems. Another relevant factor is capability to retain high nutritional value, appropriate consistency, mild taste and flavour and intense green colour.

The aim of this investigation was to evaluate the suitability for freezing of new broccoli varieties and to assess the influence of freezing on chemical composition and the content of biologically active compounds after three months storage.

Materials and Methods

The study was conducted on seven broccoli varieties, namely: SVR 97, SVR 99, SVR 1002, SVR 1017, Ironman, Beneforte and Bellaverde. The broccoli were obtained directly from the producer (Moszna, near Nałęczów). They were washed and divided into florets. One part of the material, referring as the fresh, was analysed two hours after the harvest. The second part was blanched at 95°C for four minutes (blanching parameters were set according to peroxidise test) cooled and frozen in a chest freezer. The samples were kept at -20°C for three months. Physico-chemical analyses, which were conducted on the samples, involved determination of: dry mass, soluble solids, acidity, vitamin C and total carotenoids content, all of which were done according to Polish Norms. Additionally, total sugar and polyphenolics content were determined according to Somogyi-Nelson and Folin-Ciocalteu methods, respectively (Staniec and Bojarska 1997, Radzki et al. 2014). All measurements were done in triplicate and the obtained data was expressed as mean ±SD (standard deviation). The data was compared using analysis of variance ANOVA with a level of significance set at $\alpha = 0.05$. Statistically different data was evaluated by Tukey's test.

Results and Discussion

The results on fresh broccoli are presented in table 1, while the data on frozen broccoli in table 2. All the data were calculated on fresh weight basis. Dry mass content of the seven broccoli varieties differed statistically and ranged from 12.20% to 14.19% in fresh samples and from 9.72% to 12.26% in the frozen ones. The highest dry mass content was observed in Beneforte and Bellaverde varieties (14.19% and 14.05%, respectively) (Table 1). The highest level of dry mass noticed for the frozen samples was 12.26% (Beneforte), while the lowest was 9.72% (Ironman). The decrease of dry mass content in frozen broccoli could result from blanching. Similar effect was noticed by GĘBCZYŃSKI and LISIEWSKA (2006). An analogue relation was observed in the content of soluble solids, total sugars, and vitamin C. According to GĘBCZYŃSKI (2003) dry mass content in fresh and frozen broccoli amounted of 10.4% and 9.01%, respectively. In different work researchers observed that dry mass content in fresh and frozen broccoli (stored for four months) reached 10.34% and 9.17–11.22, respectively. Other author noted that dry mass content amounted of approximately 10% and 8.5%, correspondingly in fresh and frozen broccoli (WIECZOREK 2007).

Table 1

The basic quality parameters in fresh broccoli

Variety	Dry matter [%]	Total soluble solids [%]	Acidity [%]	Total sugars [%]	Vitamin C [mg 100 g^{-1}]	Total corotenoids [mg 100 g^{-1}]	Polyphenols [mg 100 g^{-1}]
Bellaverde	$14.05^{c} \pm 0.88$	$6.57^{b} \pm 0.15$	$0.36^{b} \pm 0.08$	$4.75^{c} \pm 0.06$	$17.05^{b} \pm 0.00$	$5.16^{b} \pm 0.00$	$97.53^{b} \pm 0.55$
Beneforte	$14.19^{c} \pm 0.20$	$5.40^{a} \pm 0.17$	$0.32^{a-b} \pm 0.00$	$5.53^{d} \pm 0.05$	$14.20^{a} \pm 0.00$	$4.30^{a} \pm 0.00$	$173.41^{d} \pm 0.61$
Ironman	$12.54^{a-b} \pm 0.15$	$5.33^{a} \pm 0.12$	$0.30^{a-b} \pm 0.07$	$2.99^{a} \pm 0.04$	$16.10^{a-b} \pm 1.65$	$4.99^{b} \pm 0.00$	$87.33^{a} \pm 1.51$
SVR 97	$12.72^{a-b} \pm 0.25$	$7.40^{c} \pm 0.10$	$0.20^{a} \pm 0.03$	$4.23^{b} \pm 0.13$	$14.20^{a} \pm 0.00$	$7.17^{d} \pm 0.08$	$103.26^{c} \pm 0.46$
SVR 99	$13.49^{b-c} \pm 0.14$	$8.37^{e} \pm 0.06$	$0.31^{a-b} \pm 0.02$	$4.27^{b} \pm 0.05$	$17.05^{b} \pm 0.00$	$6.39^{c} \pm 0.28$	$85.48^{a} \pm 0.84$
SVR 1002	$12.74^{a-b} \pm 0.10$	$5.33^{a} \pm 0.12$	$0.25^{a-b} \pm 0.00$	$3.00^{a} \pm 0.03$	$20.84^{c} \pm 1.64$	$7.37^{d} \pm 0.41$	$102.49^{c} \pm 0.61$
SVR 1017	$12.20^{a} \pm 0.31$	$7.87^{d} \pm 0.06$	$0.26^{a-b} \pm 0.05$	$5.81^{e} \pm 0.04$	$17.05^{b} \pm 0.00$	$7.13^{d} \pm 0.11$	$102.42^{c} \pm 0.90$

Mean values denoted by different letters in the columns differ statistically significantly ($p \leq 0.05$).

Table 2

The basic quality parameters of frozen broccoli

Variety	Dry matter [%]	Total soluble solids [%]	Acidity [%]	Total sugars [%]	Vitamin C [mg 100 g^{-1}]	Total corotenoids [mg 100 g^{-1}]	Polyphenols [mg 100 g^{-1}]
Bellaverde	$10.04^{a} \pm 0.25$	$5.20^{c} \pm 0.10$	$0.18^{b} \pm 0.01$	$2.45^{c-d} \pm 0.05$	$14.2^{b-c} \pm 0.00$	$11.25^{b} \pm 0.13$	$68.74^{c} \pm 0.61$
Beneforte	$12.26^{b} \pm 0.77$	$5.33^{c-d} \pm 0.15$	$0.30^{c} \pm 0.01$	$3.38^{e} \pm 0.12$	$12.31^{a-b} \pm 0.00$	$11.72^{b} \pm 0.03$	$111.45^{d} \pm 0.55$
Ironman	$9.72^{a} \pm 0.17$	$5.53^{d-e} \pm 0.15$	$0.19^{b} \pm 0.00$	$2.39^{c} \pm 0.04$	$15.15^{c} \pm 1.54$	$13.95^{c} \pm 0.20$	$69.39^{c} \pm 0.57$
SVR 97	$10.18^{a} \pm 0.27$	$4.60^{b} \pm 0.00$	$0.13^{a} \pm 0.01$	$1.98^{b} \pm 0.04$	$11.36^{a} \pm 0.00$	$16.75^{d} \pm 0.55$	$51.75^{a} \pm 0.54$
SVR 99	$10.31^{a} \pm 0.07$	$4.27^{a} \pm 0.58$	$0.19^{b} \pm 0.01$	$1.33^{a} \pm 0.06$	$14.2^{b-c} \pm 0.00$	$21.84^{e} \pm 0.54$	$68.53^{c} \pm 0.80$
SVR 1002	$11.71^{b} \pm 0.22$	$5.83^{e} \pm 0.12$	$0.18^{b} \pm 0.01$	$2.43^{c} \pm 0.11$	$18.00^{d} \pm 1.41$	$4.41^{a} \pm 0.13$	$65.12^{b} \pm 0.49$
SVR 1017	$10.57^{a} \pm 0.54$	$5.20^{c} \pm 0.10$	$0.18^{b} \pm 0.00$	$2.73^{d} \pm 0.20$	$11.37^{a} \pm 0.00$	$11.64^{b} \pm 0.44$	$66.45^{b} \pm 0.49$

Mean values denoted by different letters in the columns differ statistically significantly ($p \leq 0.05$).

The amount of soluble solids showed wide variation among the frozen samples and ranged from 5.33 to 8.37%. The highest soluble solids level in fresh broccoli was observed of SVR 99 variety (Table 1). In frozen product the highest content was observed in SVR 1002 varieties (the values reached above 5.83%) (Table 2).

An average total sugar content in the fresh samples ranged from 2.99% for Ironaman variety to 5.81% for SVR 1017 variety (Table 1). The frozen broccoli contained lower amounts of total sugars which varied between 1.33% (SVR 99 variety) to 3.38% (Beneforte variety) (Table 2).

Total acidity in the analysed fresh samples varied from 0.20 to 0.36%. The highest values were noticed for Bellaverde and Beneforte varieties (0.36 and 0.32%, respectively) (Table 1). Among the frozen samples Beneforte variety contained the highest level of total acids (0.30%), while the lowest was observed for SVR 97 variety (Table 2). Our data are in agreement with KOWALCZYK et al. (2010) who reported that acidity content of fresh broccoli reached up to 0.3%.

With respect to vitamin C contents in fresh broccoli, the highest value was noticed for SVR 1002 variety (20.84 mg 100 g^{-1}), while the lowest for SVR 97 and Beneforte varieties (14.20 mg 100 g^{-1}). In frozen broccoli its quantity reached the maximum for SVR 1002 variety (18.00 mg 100 g^{-1}), whereas the lowest content was noticed for SVR 97 and SVR 1017 varieties (11.36 mg 100 g^{-1} and 11.37 mg 100 g^{-1}, respectively). Storing at -20°C lead to substantial loss of vitamin C content. The observed decrease ranged from 5.9% for Ironman variety to 33.3% for SVR 1017 variety (Table 1, 2). According to GĘBCZYŃSKI fresh broccoli contained 11.4 mg 100 g^{-1}d.w. of vitamin C, while frozen ones 7.01 mg 100 g^{-1}d.w. Other studies reported that the content of vitamin C in fresh and frozen broccoli amounted of 106 mg 100 $g^{-1}$and 29 to 60 mg 100 g^{-1}, respectively (GĘBCZYŃSKI and LISIEWSKA 2006). On the other hand, other researchers, who investigated 80 different varieties grown in California, observed much higher vitamin C content, ranging from 57.35–131.35 mg 100 g^{-1} fresh weight (KOH et al. 2009). According to SINGH at al. (2007) the levels of vitamin C in broccoli cultivated in India varied from 25.5 to 82.3 mg 100 g^{-1}fresh weight.

The content of total carotenoids in fresh and frozen broccoli varied between 4.30–7.37 mg 100 g^{-1} and 4.41 to 21.84 mg 100 g^{-1}, respectively. Among the fresh samples, SVR 1002 variety contained the highest amounts of carotenoids, while Beneforte variety showed the lowest value. In frozen broccoli the highest concentration of caroteoids was noticed for SVR 99 variety and the lowest for SVR 1002 (Table 1, 2). The increase of carotenoids content in frozen samples could be attributed to the applied thermal processing. As a result of blanching, carotenoids that were bound to tissue structure could be released. According to

DRUŻYŃSKA et al. (2009) an average content of total carotenoids measured in fresh broccoli was 27.2 mg 100 g^{-1} dry weight, while in cooked broccoli 36.4 mg 100 g^{-1} d.w. Other authors demonstrated that fresh and frozen broccoli contained 3.5 mg 100 g^{-1} and 2.8–3.6 mg 100 $g^{-1}$of carotenoids, respectively (GĘBCZYŃSKI and LISIEWSKA 2006).

Regarding to the total polyphenolics content, its quantity ranged from 85.48 to 173.41 mg 100 g^{-1} in fresh samples and from 51.75 to 111.45 mg 100 g^{-1} in frozen samples. The highest level of phenolics was noticed in Beneforte variety, both fresh and frozen. WIECZOREK (2007) stated that average content of phenolics in fresh broccoli amounted of 129 mg 100 g^{-1}, whereas frozen broccoli contained 90 mg 100 g^{-1} of phenolics. Our data are higher than those obtained by GĘBCZYŃSKI and LISIEWSKA (2006) who reported that the level of phenolics in fresh and frozen broccoli reached 87.4 mg 100 g^{-1} and 47.4 to 64.5 mg 100 g^{-1}, respectively.

Conclusions

1. The analysed varieties differed in the tested physico-chemical parameters.

2. Beneforte variety could be of particular interest as it possess high contents of sugars carotenoids, polyphenolics, acidity, soluble solids and dry mass.

3. In Beneforte variety, the smallest drop of analysed chemical compounds was noticed, as the result of blanching and freezing.

Translated by WOJCIECH RADZKI

Accepted for print 12.02.2015

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