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**SUPPORT OF THE GROWTH AND PROSPERITY
OF THE NORWAY SPRUCE (*PICEA ABIES*)
SEEDLINGS IN FOREST NURSERY BY THE
BIO-ALGEEN SYSTEM – PRELIMINARY RESULTS**

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Key words: Bio-Algeen, Norway spruce, forest nursery, growth of plants, root system.

A b s t r a c t

Besides the direct as well as indirect (basic rock powders) fertilizers, the different types of stimulative substances for production of high quality plantation stock as well as for support of young plantations *in situ* can be used. Aim of the presented study was the evaluation of the effects of different types of Bio-Algeen preparates on the growth and prosperity of planting stock, produced in the Forest Nursery Bukovina. Particular aims were defined: to stimulate the vitality and size of the root systems of planting stock produced, to improve the vitality of the planting stock, to accelerate the planting stock growth, to increase the resistance and vitality of out-planted material on the forest site. The obtained results showed very good production as well as economical effects of the Bio-Algeen preparates in the forest nursery. The biomass increased significantly and it can be assumed the better status and growth later after plantation on the forest lands.

**ZASTOSOWANIE PREPARATU BIO-ALGEEEN DO WSPIERANIA WZROSTU
I WARUNKÓW ROZWOJOWYCH SĄDZONEK ŚWIERKA POSPOLITEGO
(*PICEA ABIES*) W SZKÓLCE LEŚNEJ – BADANIA WSTĘPNE**

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Słowa kluczowe: Bio-Algeen, świerk pospolity, szkółka leśna, rozwój sadzonek, system korzeniowy.

A b s t r a k t

W praktyce leśnej często stosowane są zabiegi chemiczne i biologiczne mające na celu poprawienie jakości upraw. Jako zabiegi poprawiające wykorzystuje się m.in. wapnowanie oraz stosowanie mineralnych nawozów krzemianowych. Stosowane są także specjalistyczne nawozy o powolnym i długotrwałym okresie uwalniania związków mineralnych.

Celem pracy była ocena wpływu różnych sposobów aplikowania preparatu Bio-Algeen na wzrost i warunki rozwoju sadzonek, produkowanych w szkółce leśnej w Bukowinie (Republika Czeska). W ramach prac badawczych analizowano: stymulowanie zdrowotności i wzrostu systemów korzeniowych sadzonek, rozwój części nadziemnej materiału sadzeniowego, zwiększenie odporności i żywotności materiału sadzoniowego w lesie.

Uzyskane wyniki wykazały polepszenie produkcji, jak i pozytywny ekonomiczny efekt po zastosowaniu preparatu Bio-Algeen w szkółce leśnej.

Introduction

The growth of forest tree plantations is limited by severe climatic and site conditions in the Czech Republic in many cases. It is a consequence of severe site, ecological and anthropogenically changed conditions (BALCAR et al. 2012a, 2012b, BORŮVKA et al. 2005, VACEK and PODRÁZSKÝ 1994), leading to bad performance of plantations and bad growth and status of older stands (VACEK et al. 2009). The tree species have developed different strategies to overcome these unfavorable conditions (e.g. VACEK et al. 2012). In the forestry practice, particular treatments of the chemical as well as biological site improvement are used (BALCAR et al. 2011, KUNEŠ et al. 2011, PODRÁZSKÝ 1994, 2006a, 2006b). As the site improvement operations it was used the liming and silicate basic mineral rock powders (KUNEŠ et al. 2009), or specific slow-release fertilizers (KUNEŠ et al. 2004). Also some broad-leaved tree species can exhibit positive effects on the soil in mountain conditions (PODRÁZSKÝ et al. 2004). All abovementioned studies are especially important in the Polish-Czech border mountains.

Besides the direct as well as indirect (basic rock powders) fertilizers, the different types of stimulative substances for production of high quality plantation stock as well as for support of young plantations *in situ* can be used. One of them is the Bio-Algeen system, based on hydrolyzate of brown sea algae *Accophyllum nodosum*. It can be produced in the form of granules, spray or wet root coating material.

Aim of the presented study was the evaluation of the effects of different types of Bio-Algeen preparates on the growth and prosperity of planting stock, produced in the Forest Nursery Bukovina, property of the Czech Military Forests. Particular aims were defined:

- to stimulate the vitality and size of the root systems of planting stock produced,
- to improve the vitality of the planting stock,
- to accelerate the planting stock growth,
- to increase the resistance and vitality of out-planted material on the forest site.

Material and methods

Research area

The research was performed in the Center of Forest Nursery Bukovina, nursery Obrovce (eastern part of the military training area Hradiště (GPS N50.2821, E13.208473)). Altitude of the locality is 400 m a.s.l., mean annual temperature is 7.1°C, mean annual precipitation 607 mm, soil type is determined as gleyic Cambisol.

Preparates tested

There were used different forms and products of the Bio-Algeen system:

1) Bio-Algeen Granulate (B.A.G): Granulated form of the *A.n.* hydrolyzate, applied in soils and substrates, stimulating the uptake of nutrients, photosynthesis, chlorophyll formation and assimilates transport, leading to increase in the biomass and root systems.

2) Bio-Algeen S-90 (B.A.S): used for spraying (watering) as solution, containing concentrate of polyuron acids, amino-acids, phytohormones and trace elements.

3) Bio-Algeen Root Concentrate (B.A.RC): used for root coating before plantation

For testing and statistical evaluation of the Bio-Algeen preparates (B.A.) effects were designed following variants (Table 1).

Table 1

Variants of the B.S. application

Variant	Use of preparate	Nr of plants	Price	Expenses per plant CZK
B.A.root concentrate – B.A.RC	Root coating before plantation, diluted by water 1:20, mechanized planting	1000	200,- CZK/l	0.01
B.A. granulate B.A.G	150 g/m ² on the bed before planting (per 65 plants)	1000	150,- CZK/kg	0.35
B.A. RC+G	Combination of above described	1000		0.36
B.A. spray B.A.S	After planting irrigation by B.A.S-90; diluted 1:200, 1 l/m ²	1000	220,- /l	0.22
Control C	No treatment	1	000	0

Planting stock tested preparation

The first year, the seeds were sown and 1–1.5 years cultivated on the seed-beds. Second year, the seedlings were transplanted in the growing season (May and August) and the B.A. preparates were applied accordingly to the Table 1. The young plants were cultivated after transplant for next two years – normal planting stock production time.

Sampling

Each variant included 1000 seedlings planted on beds, in blocks, from each of them, 100 sample seedlings were selected for sampling, average in the growth. Sampling took place 20.6.2012 (3-years plantings) and 25.9.2013 (4-years plantings).

The following characteristics were determined:

- quantity (biomass in D.M.) of the root system, determined 2012 and 2013,
- quantity (biomass in D.M.) of the above-ground part, determined 2012 and 2013,
- diameter of the root collar and total height of the aboveground part, determined in 2013.

Dry mass (D.M.) was determined at 60°C drying the samples to the constant weight, height and root collar diameter were determined using measuring rod and mini caliper, both of corresponding size.

Data processing

Data were evaluated using the basic description statistics disposable in the Excel programme.

Results and discussion

All variants showed significant differences to the control plot, all types of the B.A. application positively and economically relevantly influenced the growth of the planting stock at low expenses (Table 2). In the 1st year, the most prominent results were observed at the B.A.RC+G and B.A.S variants, evaluating the dry matter of the below-ground as well as of above-ground parts of the plantings. Second year, the spraying showed the best effects on the biomass of the young plants. The root collar diameter and height were also the highest at the B.A.S variant. The spraying combines the easy application and the best results, respectively.

Table 2

Characteristics of the planting stock

Variant	Root system D.M. 2012		Above-ground D.M. 2012		Root system D.M. 2013		Above-ground D.M. 2013		Root collar 2013		Above-ground height 2013	
	g		g		g		g		mm		mm	
B.A.RC	68	194%	70	109%	341	105%	469	116%	6.6	97%	42.9	114%
B.A.G	37	106%	46	72%	444	137%	535	132%	7.2	106%	45.8	122%
B.A.R C+G	82	234%	82	153%	473	146%	509	125%	7.6	112%	43.4	115%
B.A.S	77	220%	98	153%	500	154%	587	145%	8.2	121%	44.4	118%
Control	35	100%	64	100%	325	100%	406	100%	6.8	100%	37.6	100%

Table 3

Quality parameters of the planting stock produced

Variant	Ratio A.G/B.G 2012	Ratio AG/BG 2013	Ratio H/D
B.A.RC	1.03	1.37	6.50
B.A.G	1.24	1.20	6.36
B.A.RC+G	1.00	1.08	5.71
B.A.S	1.27	1.17	5.41
C	1.93	1.25	5.30

AG/BG – above-ground/below ground ratio

H/D – height/diameter of root collar ratio

Also other variants of the B.A. system showed positive effects on the growth and prosperity of the planting stocks, support so better management and economic results. The B.A. system represents good way of the production of the higher quality of the planting stock.

The application of Bio-Algeen preparates showed very good effects, due to their complex affecting the physiological status of plants. Very similar results were reached at applying them to the agricultural, especially forage crops (SVOBODOVÁ and ŠANTRŮČEK 1998, ŠANTRŮČEK and SVOBODOVÁ 1995).

The support of plantations is necessary also in the afforestation activities during large land use changes, occurring in the past (SKALOŠ et al. 2012) as well as planned for the future (HATLAPATKOVÁ and PODRÁZSKÝ 2011). The importance increases at the reintroduction more site demanding tree species on site with long-term cultivation of conifers such as Norway spruce and Scots pine, where the natural regeneration (BÍLEK et al. 2014, VACEK et al. 2012) or standard planting stock is not fully successful. These conditions are determined mainly by the harsh environment in the mountain regions exposed to the immission calamity in the past, very often in the Polish-Czech border regions (BORŮVKA et al. 2005, BALCAR et al. 2012a, 2012b, PODRÁZSKÝ 2006a). Different supporting site improvement treatments were used there, such as liming, basic rock powder application as well as slow-releasing fertilizers (BALCAR et al. 2011, KUNEŠ et al. 2009, 2011, PODRÁZSKÝ 2006b, VACEK et al. 2009). Slow-releasing fertilizers showed very good efficiency at supporting site-demanding species on poor or degraded sites (KUNEŠ et al. 2004, PODRÁZSKÝ, REMEŠ 2007). Also in sandy regions with extremely low nutrient contents, the fertilization is well accepted by growing plantations (PODRÁZSKÝ 1994).

In these aspects, the use of preparates intensifying the physiological efficiency of the planting stocks seem to be very effective way in the restoration and reforestation of forest sites of very different characters.

Conclusions

The obtained results showed very good production as well as economical effects of the Bio-Algeen preparates in the forest nursery. The biomass increased significantly and it can be assumed the better status and growth later after plantation on the forest lands. It can be recommended for use in the forest nurseries to obtain more vital and prosperous planting stock for the next forestry operations.

In any case, this promising system needs testing in more differentiated site conditions, as well as in more differentiated aspects of the silviculture support: seed prosperity, plantation prosperity, reclamation.

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CONTENT OF MICROELEMENTS IN TUBERS OF POTATO TREATED WITH BIOSTIMULATORS

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Key words: potato cultivars, biostimulators, microelements content, microelements uptake.

Abstract

Three times every 10-14 days, starting from the BBCH 39 phase, four cultivars of edible potatoes were treated with the following bio-stimulants: Asahi SL, Bio-Algeen S90 or Kelpak SL. The control object was plants potato sprayed with distilled water. The large amount of precipitation in the first year of the research contributed to an increased content of Fe and Mn in the potato tubers. The tubers of potatoes treated with Kelpak SL had the highest content of Zn, Mn, Fe and Cu. The highest content of B was found after the potatoes were sprayed with Asahi SL. Compared to the control plants, Bio-Algeen S90 reduced the content of Zn, Mn, Fe and Cu. The content of microelements in the tubers was significantly dependent on the genotype. The highest concentration of Zn, Mn and B was found in the tubers of the cv. Volumia, the highest concentration of Fe and Cu – in the tubers of the cv. Sylvana, and the highest concentration of Ni – in the tubers of the cv. Satina. The uptake of microelements with the potato harvest depended more on the cultivar, in particular its yield, than on the biostimulators that were used.

ZAWARTOŚĆ MIKROELEMENTÓW W BULWACH ZIEMNIAKA TRAKTOWANEGO BIOSTYMULATORAMI

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Słowa kluczowe: ziemniak, odmiana, biostymulatory, zawartość i wynos mikroelementów.

Abstrakt

Począwszy od fazy BBCH 39 rośliny ziemniaka trzykrotnie, co 10-14 dni, traktowano biostymulatorami: Asahi SL, Bio-Algeen S90 lub Kelpak SL. Obiektem kontrolnym były rośliny ziemniaka opryskiwane wodą destylowaną. Duża ilość opadów w pierwszym roku badań przyczyniła się do zwiększenia zawartości żelaza i manganu w bulwach ziemniaka. Bulwy roślin traktowanych preparatem Kelpak SL charakteryzowały się największą zawartością cynku, manganu, żelaza i miedzi. Natomiast najwięcej boru stwierdzono po opryskiwaniu Asahi SL. W porównaniu do roślin kontrolnych, Bio-Algeen S90 zmniejszył zawartość cynku, manganu, żelaza i miedzi w bulwach. Zawartość mikroelementów w bulwach była również istotnie różnicowana genotypem odmiany. Największą koncentrację Zn, Mn i B stwierdzono w bulwach odmiany Volumnia, Fe i Cu u Sylvana, natomiast Satina zawierała najwięcej Ni. Wynos mikroskładników z plonem bulw ziemniaka w większym stopniu zależał od odmiany, a zwłaszcza jej plonu, niż od stosowanych biostymulatorów.

Introduction

In 2013, the surface area of land on which potatoes were grown in Poland was equal to 337,000 ha, and the average yield was equal to 21.1 t ha⁻¹ (*Statistical...* 2014). Potato tubers contain 1–1.2% of mineral components. Consumption of potatoes partly covers the demand for P, K, I, Fe and Cu (*The composition...* 2013).

Microelements control enzymatic processes, thus determining the chemical composition of tubers. The largest quantity of microelements (73–79%) comes from manure (KANIUCZAK et al. 2009). By stimulating living processes, growth regulators control the mineral management and increase the immunity of plants to stressful conditions, thus contributing to a higher quality and value of the yield (WIERZBOWSKA et al. 2010, GRZYŚ 2012). They influence the tubers yield, improve their biochemical parameters and increase their immunity to disadvantageous environmental conditions and pathogens (WIERZBOWSKA et al. 2015, SAWICKA and MIKOS-BIELAK 2002). An inexpensive source of natural growth regulators is algae extracts. They are used as biostimulators in agriculture and horticulture (PANDA et al. 2012). They can also reduce stress resulting from a shortage of nutrients, which enables reducing the doses of mineral fertilizers (PAPENFUS et al. 2013).

The purpose of the article is to study the impact of biostimulators on the content and uptake of microelements in potato tubers.

Materials and Methods

The micro-plot experiment in a system of randomly selected sub-blocks was established in four repetitions at the Experimental Facility in Tomaszkowo, which is owned by the University of Warmia and Mazury in Olsztyn. Potato

was grown on arable land classified as R IVa, i.e. of medium and better quality, on brown soil which was dystric cambisol developed from loamy sand. The soil was characterized by pH in the range of 5.32 to 5.70 in a 1 mol KCl dm⁻³ extract; the content of available components was equal to 69–72 mg P kg⁻¹, 82–90 mg K kg⁻¹, 38–48 mg Mg kg⁻¹, 0.33–0.42 mg B kg⁻¹ (in a hot water extract) and 0.90–1.05 mg Cu kg⁻¹, 4.50–5.59 mg Zn kg⁻¹ and 110–139 mg Mn kg⁻¹ in a 1 mol HCl dm⁻³ extract).

Four cultivars of edible potato was grown: the early cv. Volumia and the medium early Irga, Satina and Sylvana cultivars. The forecrop were cereals (oat in 2011 and triticale in 2012). In the autumn, manure was used in the quantity of 25 t ha⁻¹, and in the spring – mineral fertilizers in the following quantities: N – 40 (urea 46% N); P – 26,2 (superphosphate 17.45% P); K – 100 (potassium salt 50% K) kg ha⁻¹. The potato tubers were planted at intervals of 67.5 x 40 cm in late April. All the potato cultivars were harvested on the same days (6 September 2011 and 21 August 2012).

Starting from the BBCH 39 phase (complete coverage of the space between the rows), the plants potato were treated with biostimulators three times, at intervals of 10–14 days:

Asahi SL – 0,1% solution – contains phenols naturally occurring in plants: sodium ortho-nitrophenol, sodium para-nitrophenol, sodium 5-nitroguaiacol

Bio-Algeen S90 – 1,0% solution – extract from marine algae, contains amino acids, vitamins, alginic acid as well as macro- and micronutrients

Kelpak SL – 0,2% solution – extract from *Ecklonia maxima* (11 mg dm⁻³ auxins and 0.031 mg dm⁻³ cytokinins; the auxin to cytokinin ratio is 350:1

The control object was plants potato sprayed with distilled water. More information on the chemical composition of used biostimulators is presented in an article by CWALINA-AMBROZIAK et al. (2015).

During the harvest, the yield of potato tubers was determined, and samples were taken for the purpose of chemical analysis. Dried and ground plant material was subject to wet mineralization in a mixture of nitric(V) and chloric(VII) acid (at a ratio of 4:1), with an addition of hydrochloric acid. The content of Cu, Zn, Mn, Fe and Ni was determined using the atomic absorption spectrophotometric method using an AA-6800 Shimadzu instrument. In order to determine the content of boron, the plant material was subject to dry mineralization (520°C) in the presence of calcium oxide, and the ash was dissolved in a 0.5 mol HCl dm⁻³. The boron content was determined colorimetrically using azomethine-H (BENEDYCKA and RUSEK 1994).

The results of the tests were analysed statistically by way of variance analysis (STATISTICA 10 package), and the difference between the averages were compared using the Tukey's system at a significance level of $p = 0.05$.

Results and discussion

During nearly the entire period of vegetation of the potato, the temperature was higher than the multi-year average temperature. A significant excess of precipitation was observed in 2011: after a dry spring (April-May), there was an excess of precipitation in the summer, especially in July (269% of the multi-year average) and in August (120%). In 2012, after a dry April, the quantity of precipitation in May was slightly lower than the multi-year average for that month. On the other hand, in June and July, there was an excess of precipitation (130% and 160% of the multi-year average, respectively). The deficit of rain re-appeared in the late plant growing season.

The variance analysis demonstrated that the contents of Zn, Mn, Fe, Cu and Ni in potato tubers were significantly modified by the genotype of the cultivars and the bioregulators used (Table 1–3). The years of the study and of the interactions of the factors had a highly significant impact on the contents of the aforementioned microelements. The content of B was highly dependent on the characteristics of the studied cultivars and significantly dependent on the bioregulators used and the interaction between these factors and the years of the study.

Table1

Results of the variance analysis on the contents of microelements in the potato tubers

Factor	The significance of the impact					
	Zn	Mn	Fe	Cu	Ni	B
A – year	xx	xx	xx	xx	xx	ns
B – biostimulator	xx	xx	xx	xx	xx	x
C – cultivar	xx	xx	xx	xx	xx	xx
A x B	xx	xx	xx	xx	xx	x
A x C	xx	xx	xx	xx	xx	x
B x C	xx	xx	xx	xx	xx	ns
A x B x C	xx	xx	xx	xx	xx	ns
Significant at $\alpha \leq 0,05$ – x, $\alpha \leq 0,01$ – xx						

A x B; A x C; B x C; A x B x C – interaction; ns – non significant

In the first year of the study, the large amount of precipitation caused the potato tubers to contain 10% more Mn and nearly two times more Fe, while in the second year, the content of Zn, Ni and Cu was higher (by 4.8, 6.3, and 18.2%, respectively). No significant difference in the content of B was observed (Table 2). In the first year of the study, the tubers of the potatoes that were sprayed with Kelpak SL contained the largest quantity of iron (91.85 mg kg⁻¹ DM). Compared to the control plants, Kelpak SL increased the content of Mn,

Table 2
Content of microelements in tubers of potatoes depending on the biostimulator used

Factor	Zn	Mn	Fe	Cu	Ni	B
	mg kg ⁻¹ DM					
2011						
Control	13.34	8.00	74.33	3.02	0.88	7.45
Asahi SL	14.91	8.02	73.42	3.34	1.16	8.01
Bio-Algeen S90	13.68	8.04	56.31	2.81	1.18	7.63
Kelpak SL	16.03	9.21	91.85	3.61	1.21	7.38
2012						
Control	14.84	8.01	43.25	3.79	1.10	7.48
Asahi SL	15.30	7.74	41.38	3.79	1.14	8.13
Bio-Algeen S90	14.48	7.01	34.04	3.77	1.18	7.70
Kelpak SL	16.14	7.43	39.45	3.74	1.32	7.36
Mean for year						
2011	14.49	8.31	73.98	3.19	1.11	7.62
2012	15.19	7.55	39.53	3.77	1.18	7.67
Mean of biostimulator						
Control	14.10	8.00	58.79	3.41	1.00	7.46
Asahi SL	15.11	7.88	57.40	3.56	1.15	8.07
Bio-Algeen S90	14.08	7.53	45.17	3.29	1.18	7.67
Kelpak SL	16.08	8.32	65.65	3.67	1.26	7.37
LSD _{0.05} A	0.36	0.14	0.05	0.07	0.06	ns
B	0.68	0.26	0.18	0.13	0.12	0.02
A x B.	1.15	0.44	0.51	0.22	0.20	ns
A x C	1.15	0.44	3.20	0.22	0.20	0.03

A – year; B – biostimulator; C – cultivar; A x B; A x C; B x C; – interaction; ns – non significant

Cu, Fe, Zn and Ni (by 4.0, 7.8, 11.6, 14.1 and 27.9%, respectively), while reducing the content of B. Asahi SL, on the other hand, significantly increased the concentration of Ni (by 16.7%), B (by 8.1%) and Zn (by 7.2%) and also, to a lesser extent, of Cu. Bio-Algeen S90 had a negative impact on the concentration of Zn, Mn, Fe and Cu and a positive impact on the quantity of Ni and B. Significantly highest concentration of Fe and Cu was measured in tubers of the cv. Sylvana, and the highest concentration of Zn, Mn and B – in the tubers of the early cv. Volumia (Table 3). On the other hand, the lowest quantity of Zn and Mn was found in the tubers of the cv. Satina, and the lowest quantity of Cu and B – in the cv. Irga. Significantly largest content of Zn (19.36 mg kg⁻¹ DM) was measured in the tubers of the cv. Volumia treated with Kelpak SL, and the smallest (11.32 mg kg⁻¹ DM) – in the tubers of the cv. Satina from the control object. The highest content of Mn was measured in the tubers of the cv. Volumia from the control object and from the field treated with Kelpak SL. Moreover, the use of Kelpak SL resulted in the highest content of Fe

Table 3
Content of microelements in tubers of potatoes depending on the cultivar and the biostimulatore used

Factor	Cultivar	Zn	Mn	Fe	Cu	Ni	B
		mg kg ⁻¹ DM					
Control	Volumia	17.27	9.61	68.10	3.30	1.41	8.16
	Irga	14.55	7.19	52.60	3.17	0.85	6.23
	Satina	11.32	6.96	55.40	3.31	0.82	7.41
	Sylvana	13.25	8.23	59.07	3.85	0.87	8.04
Asahi SL	Volumia	16.88	8.27	36.90	3.26	1.36	8.60
	Irga	14.52	7.22	57.21	3.30	1.12	6.91
	Satina	12.14	6.93	60.09	3.15	0.83	8.60
	Sylvana	16.89	9.12	75.41	4.54	1.30	8.16
Bio-Algeen S90	Volumia	17.57	8.78	57.37	3.26	1.22	7.98
	Irga	14.85	7.35	31.67	3.01	1.36	6.98
	Satina	11.93	7.06	35.45	3.34	1.53	8.41
	Sylvana	11.99	6.91	56.20	3.56	0.61	7.29
Kelpak SL	Volumia	19.36	9.44	60.52	3.95	0.85	7.54
	Irga	15.90	7.80	48.60	3.15	1.35	6.79
	Satina	13.22	7.51	58.40	3.68	1.78	7.85
	Sylvana	15.86	8.52	95.07	3.91	1.07	7.29
Mean for cultivar	Volumia	17.77	9.03	55.72	3.44	1.21	8.07
	Irga	14.96	7.39	47.52	3.15	1.17	6.73
	Satina	12.15	7.11	52.34	3.37	1.24	8.07
	Sylvana	14.49	8.19	71.44	3.97	0.96	7.70
LSD _{0.05} C B x C		0.68	0.26	1.89	0.13	0.12	0.02
		1.15	0.71	5.18	0.36	0.33	ns

Explanations as Table 1

(cv. Sylvana – 95.07 mg kg⁻¹ DM) and Ni (cv. Satina – 1.78 mg kg⁻¹ DM) and the smallest content of Cu (cv. Irga – 3.15 mg kg⁻¹ DM). The use of Bio-Algeen S90 resulted in the lowest content of Fe in the tubers of the cv. Irga (31.67 mg kg⁻¹ DM) and of Ni in the tubers of the cv. Satina (0.61 mg kg⁻¹ DM). Asahi SL had a positive impact on the content of Cu in the tubers of the cv. Sylvana (4.57 mg kg⁻¹ DM).

In the studies described here, the Fe:Mn ratio in potato tubers was at a level of 4.3–11.2:1. In the opinion of ROGÓŻ (2009), a Fe: Mn ratio higher than 2.5:1 indicates an excess of Fe, which is accompanied by a shortage of Mn. ROGÓŻ and TRĄBCZYŃSKA (2009) assume that Cu content below 5 mg Cu kg⁻¹ DM indicates a shortage of this element in potato tubers. In the research conducted by WIERZBICKA and TRAWCZYŃSKI (2011), the average contents of micro-elements in the tubers of organic potatoes were equal to: Cu – 4.5; Fe – 46.9; Mn – 7.3; Zn – 12.4; B – 4.9 mg kg⁻¹ DM.

The spatial distribution of minerals in potato tubers is not even (SUBRAMANIAN et al. 2011, PETRYK and BEDLA 2010, ŠREK et. al. 2012). The

concentrations of most minerals were higher in the peel than inside the tuber. The potato peel contained about 17% of the total content of Zn and 55% of Fe. In the fresh tuber matter, the concentration of these elements was found to be higher in the stolon part of the tuber. The concentration of Cu decreases from the outside toward the centre of the tuber.

The cultivation system may affect the „mineral profile” of potato tubers (LOMBARDO et al. 2014). Early varieties from organic farms contained more P and quantities of Mg and Cu in comparison to ones from fields with traditional cultivation systems. On the other hand, more K, Ca, Fe, Na and Mn were found in tubers of potatoes grown in a conventional manner. Then, American research demonstrated that potatoes from organic farms contained more Mg and Cu and less Fe and Na, while the content of Ca, K and Zn was similar to that in tubers from conventionally cultivated fields (GRIFFITHS et al. 2012). In the opinion of ZARZECKA (2004), certain herbicides cause an increase in the Fe content and, to a lesser degree, also in the manganese content in potato tubers. Moreover, the concentration of these elements depends on the weather conditions: wet and cold weather is conducive to accumulation of Fe and Mn in the tubers. The genotype of the cultivar is an important factor that affects the content of metals in potato tubers (PROŚBA-BIAŁCZYK and MYDLARSKI 2000).

In the opinion of LIPIŃSKI et al. (2006), the concentration of Fe in wheat grains was also correlated with the quantity of clay particles in the soil, while a higher pH of the soil and higher content of humus resulted in a reduction of Fe content in the grains. On the other hand, the factors that reduced the content of Mn were increased quantity of particles larger than 0.02 mm, an increasing share of organic matter and high pH. In literature, there is the opinion that sulfur has an antagonistic action in relation to the availability and metabolism of Fe in crops (YOUSFI et al. 2007, KOZŁOWSKA-STRAWSKA 2010). In the opinion of KANIUCZAK et al. (2009), liming significantly reduced the contents of Mn and Zn, while increasing mineral fertilization with the NPK fertilizer increased the content of Mn in potato tubers.

Asahi SL mitigated the effects of stress caused by a shortage of water in maize (GRZYŚ 2012). Moreover, Asahi SL and 2-AE (2-aminoethanol – naturally occurring in plant cell) limited the effects of salt stress, which caused a reduction of the contents of K, Ca, Mg, Cu and Fe in the dry matter of the epigeal parts of maize. The preparations used resulted in a particular increase in the concentration of K, Ca and Mg, while reducing the accumulation of sodium in the epigeal parts of corn grown in salty conditions.

In the opinion of WESTERMANN (2005), the yield of potato tubes equal to 56 t ha⁻¹ results in a uptake of about 2,000 g of Fe, 1,000 g of Mn, 200 g of B, 120 g of Zn and 100 g of Cu. In the studies described here, the uptake of Fe with

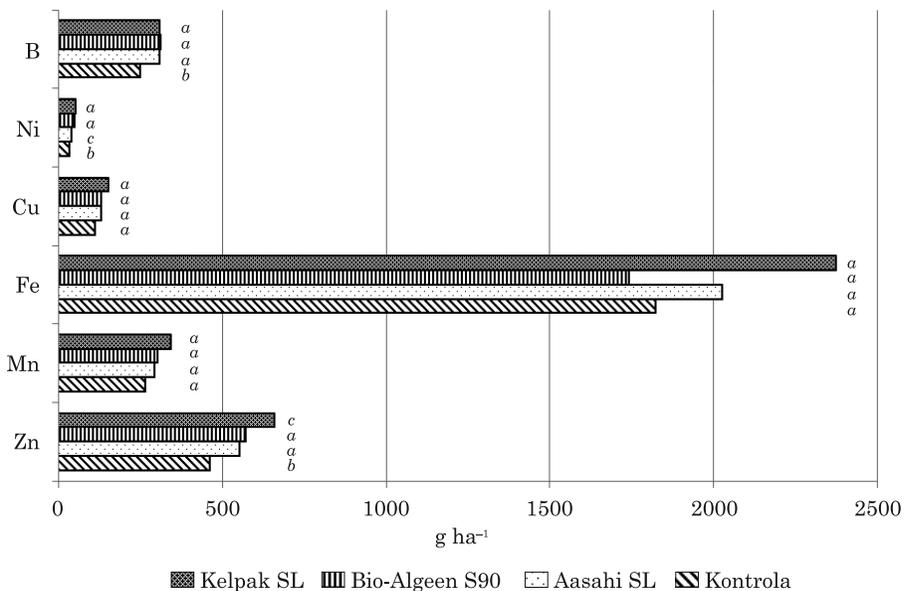


Fig. 1. The uptake of micro-elements with the potato tuber harvest depending on the biostimulator – averages of the two years (the data for individual elements marked with different letters are significantly different, at a level of $p \leq 0.05$)

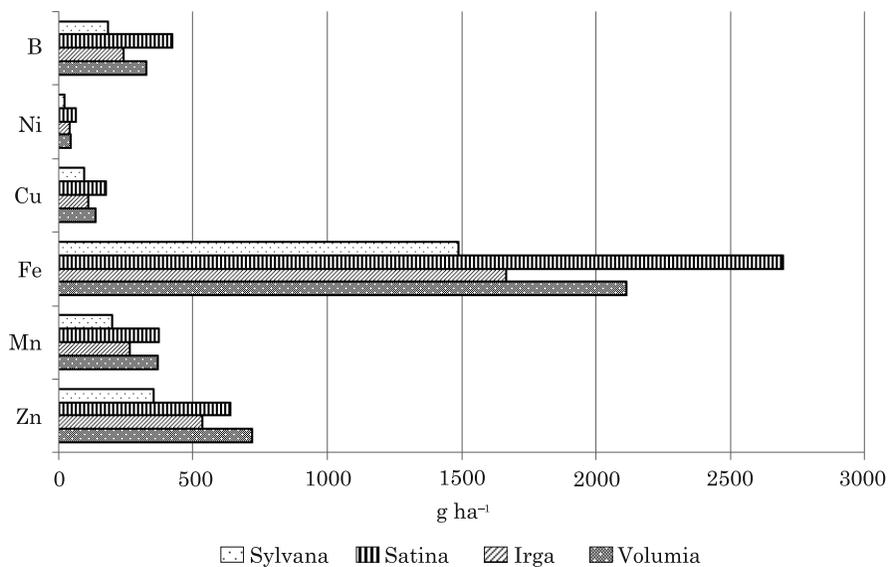


Fig. 2. The uptake of microelements with the potato tuber harvest depending on the cultivar – averages of the two years (Explanations as Figure 1)

the potato tuber harvest (WIERZBOWSKA et al. 2015) was at a level of 1,491–2,697 g ha⁻¹, while the uptake of Mn was only equal to 200–375 g ha⁻¹ (Figure 1 and 2). On the other hand, the concentration of Zn in the harvested tubers was at a level of 355–720 g ha⁻¹, and the removal of B was at a level of 185–425 g ha⁻¹. The elements with the lowest concentration in the potato tubers were Cu (96–197 g ha⁻¹) and Ni (24–64 g ha⁻¹). Only in the case of Zn, Ni and B did the bioregulators significantly increase the removal of those metals compared to the control object. The smallest amount of microelements, mostly due to the smallest yield, was accumulated in the tubers of the cv. Sylvana.

Conclusion

The weather conditions, in particular the large amount of rain in the first year of the research, contributed to an increased content of Fe and Mn in the potato tubers. The tubers of potatoes treated with Kelpak SL had the highest content of Zn, Mn, Fe and Cu. The highest content of B was found after the potatoes were sprayed with Asahi SL. Compared to the control plants, Bio-Algeen S90 reduced the content of Zn, Mn, Fe and Cu in the tubers. The content of microelements in the tubers was also significantly different depending on the genotype of the cultivar. The highest concentration of Zn, Mn and B was found in the tubers of the cv. Volumia, the highest concentration of Fe and Cu – in the tubers of the cv. Sylvana, and the highest concentration of Ni – in the tubers of the cv. Satina. The uptake of microelements with the potato harvest depended more on the cultivar, in particular its yield, than on the biostimulators that were used.

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**CLICKER TRAINING EFFICIENCY IN SHAPING
THE DESIRED BEHAVIOUR IN THE FOLLOWING DOG
BREEDS: BOXER, CHOW CHOW AND YORKSHIRE
TERRIER**

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Key words: clicker training, shaping behavior, Boxer, Chow Chow, Yorkshire Terrier.

Abstract

Works comparing the trainability of dog breeds mainly comprised indirect assessment methods; however, little empirical research has targeted the behavioural characteristics of breeds. The goal of this paper was to study the reaction of Boxer, Chow Chow and Yorkshire Terrier breeds to the shaping method with the use of the clicker in acquiring the desired behaviour. The dogs' task was to pass by the first (proximal) and second (distal) cones counter-clockwise. Initially, Boxers coped worst. However, after achieving the first success, achieving two consecutive successes was relatively easy for them. Chow Chows were relatively successful initially, but to repeat the success twice they required a much higher number of sessions with the clicker than the remaining two breeds. The results achieved by this breed show that, contrary to the opinion of many dog coaches, it is possible to successfully train Chow Chows.

**SKUTECZNOŚĆ METODY KLIKEROWEJ W KSZTAŁTOWANIU POŻĄDANEGO
ZACHOWANIA U PSÓW RAS: BOKSER, CHOW CHOW I YORKSHIRE TERRIER**

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Słowa kluczowe: szkolenie klikerowe, kształtowanie zachowań, bokser, chow chow, Yorkshire terrier.

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Abstrakt

Prace porównujące stopień wyszkolenia różnych ras psów opierały się głównie o pośrednie metody oceny, natomiast niewiele przeprowadzono badań empirycznych. Celem pracy było zbadanie reakcji psów ras bokser, chow chow i Yorkshire terrier na metodę kształtowania zachowania z użyciem klikera w uzyskaniu pożądanego zachowania. Zadaniem psów było okrążyć pierwszy (bliższy) i drugi (dalszy) pacholek. Początkowo najgorzej zadanie wykonywały bokserzy, ale po osiągnięciu pierwszego sukcesu wykonanie następnych zadań stało się dla nich relatywnie proste. Chow chowy szybko osiągnęły pierwszy sukces, ale jego powtórzenie wymagało znacznie większej ilości sesji klikerowych niż u bokserów i Yorkshire terrierów. Wyniki uzyskane przez tę rasę wskazują jednak, że – w przeciwieństwie do opinii wielu treserów – chow chowy można wytrenować.

Introduction

Dog training methods are grounded in the fact that dogs learn through classical conditioning, non-associative learning and through instrumental conditioning. The Koehler method, dominance-based training, negative reinforcement, relationship-based training, and clicker training are the most popular (MEHRKAM and WYNNE 2014).

The clicker is a small plastic box with a metal plate inside, which upon pressure produces a characteristic, invariable sound. This sound may indicate to the animal very precisely that at this moment it approximates the desired behaviour. Immediately after clicking, the dog's coach offers treat or another reward to the dog. The reward becomes an unconditional stimulus, and clicking becomes a conditional stimulus. This process of learning is termed classical conditioning, or Pavlovian conditioning (FUGAZZA and MIKLÓSI 2014). In this process the auditory stimulus (clicking) after a certain number of repetitions becomes a reliable predictor of the unconditioned stimuli, and conditions the dog to recognize the clicking sound as reinforcement itself.

Developing the desired behaviour in a dog with the use of a clicker may be realised in three basic ways: luring (where apart from clicking, the dog receives additional hints from the coach, e.g. pointing to something with a hand), capturing (where independent behaviour presented by the dog is captured), and shaping (skilful reinforcement with the clicking sound of each small step towards the target behaviour) (ALEXANDER 2003). In shaping, the animal learns to solve problems independently, because the animal is the causal factor here (the dog has to independently associate which behaviour is going to be reinforced by the coach).

Despite the increasing popularity of clicker training (DONALDSON 1996, PRYOR 1999, PARSONS 2005), there are no proofs based on scientific research showing that it is better (faster) for achieving the final desired behaviour in dogs (SMITH and DAVIS 2008). However, it seems that the clicker, as a tool

which in all conditions produces the same sound, devoid of the coach's emotions, may constitute a good tool allowing for discovering differences in predispositions for realising specific tasks in dogs belonging to various user groups and breeds. Works comparing the trainability of dog breeds mainly comprised survey-based studies and other indirect assessment methods; however, relatively little empirical research has targeted the behavioural characteristics of breeds (MEHRKAM and WYNNE 2014). Attention should be paid to the fact that observation of dogs' reactions to specific training methods may have practical implications for further training.

FCI (Federation Cynologique Internationale – World Canine Organisation based in Belgium) recognizes 332 dog breeds from around the world. One of them is Boxer. This German dog can be defined as a working breed with good trainability (SPITZER 2006). Another breed is Yorkshire Terrier which comes from northern England. Yorkshire Terrier seems to be a social breed, dependent on humans (LANE 2001). Another, very characteristic breed, is Chow Chow. This ancient breed came from China. Chow chow was often bred for meat production. In fact, this breed has not historically developed relationship with a man (REED 2014). It can be assumed that the three above breeds will exhibit different reactions on the certain training methods. Therefore, the goal of this paper was to study the reaction of Boxer, Chow Chow and Yorkshire Terrier breeds to the shaping method with the use of the clicker in acquiring the desired behaviour.

Material and methods

Animals and their initial conditioning

Three breeds of dogs: Boxer (B), Chow Chow (CC) and Yorkshire Terrier (YT) participated in the research (Table 1). The dogs, which are taken for a walk every day, and which, during the walks, for at least thirty minutes stay without the leash, were selected. None of the dogs was castrated or sterilized. The research developed following SMITH and DAVIS (2008), that is: 1) none of the dogs had previous contact with a clicker, 2) prior to the beginning of each session the dogs hadn't received any meal for at least 3 hours (all sessions were held in the afternoon), 3) the sessions took place in a room in each dog's home, 4) all the dogs were trained by the same person (it was a man experienced in a clicker training for three years; he did not know the dogs before the experiment started). Air-dried ZiwiPeak Lamb bits (pieces) were provided as treats.

Table 1

Breed, age and sex of dogs

Dog breed	Number of males	Number of females	Total number of dogs	Age of dogs (years)
Boxer (B)	3	4	7	4.27±2.73
Chow chow (CC)	4	3	7	4.10±2.18
Yorkshire terrier (YT)	4	3	7	4.41±2.55

No significant differences

The initial period lasted 4 days. Initially, the dogs were conditioned to the clicker for two consecutive days – after every click a treat was offered. During those two days every dog received 60 treats after clicking. On the third day, the dogs were rewarded for a vertical movement of the head (3 sessions with 10 treats), and on the fourth day the dogs were guided with the treat to take their place next to the coach's left leg (3 sessions with 10 treats).

Tasks

The exact period of research lasted until the realisation of all the tasks described here by a given dog. The first yellow cone, 0.25 m in height, was placed at a distance of 0.8 m in front of a person sitting on a chair, and the second yellow cone was placed at a distance of 1.6 m from the sitting person (Figure 1). This equipment was placed there for the period of the clicker sessions. The dogs' task was to pass by the first cone counter-clockwise (task 1), repeat it twice (task 1a), and next, pass by the second cone (task 2) and repeat it twice (task 2a). If the dog completed the given task, the session was always finished.

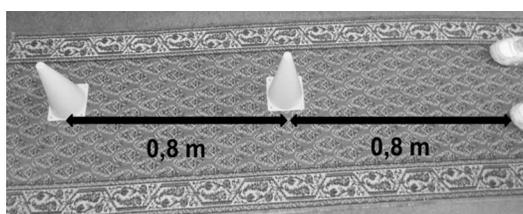


Fig. 1. Equipment used in clicker sessions

During the research, the dogs' reactions and their willingness to cooperate with the person conducting the tests were observed. If the dog started to clearly look to the side or clearly freeze on the spot, looking at the floor (the

reaction was observed in Chow Chows), the session was finished. There were at most 3 sessions a day. If the dog was willing to work, up to 20 treats were allowed per session. The pieces of food offered to the dog, and the number of sessions needed to accomplish tasks were counted. This constituted the basis for calculating the pieces/session ratio.

Statistical analysis

Data were presented as arithmetic means and standard deviations. The significance of differences among groups was marked with the Kruskal-Wallis test with the use of the Statistica (StatSoft 2011) software.

Results

Table 2 presents the results acquired by Boxer breed (B), Chow Chow (CC) and Yorkshire Terrier (YT) at the first cone. In order to achieve the first success, B required 22.29 ± 3.77 sessions on average, and YT required only 16.57 ± 3.55 sessions ($p < 0.05$). Moreover, in order to achieve the first success, Boxers on average required 8.88 reinforcements per session and YT required 13.57 ± 1.75 ($p < 0.01$). CC's results were in between the two other breeds. In order to repeat the success twice (from 1 to 3 successes) Chow Chows required 8.43 ± 1.99 sessions, constituting a significant difference ($p < 0.05$) compared to the number of sessions in the case of B (4.43 ± 1.72) and YT (4.71 ± 2.06). To perform the task, B required 7.23 ± 1.46 pieces/session on average, and this was less than in the case of CC (13.91 ± 4.71 , $p < 0.05$), and YT (14.44 ± 3.21 , $p < 0.01$).

Table 2

The results obtained by dogs during performing tasks 1 and 1a

Dog breed	Task 1		Task 1a	
	number of sessions	pieces/session ratio	number of sessions	pieces/session ratio
Boxer (B)	22.29 ± 3.77^a	8.88 ± 1.69^A	4.43 ± 1.72^a	7.23 ± 1.46^{Aa}
Chow chow (CC)	18.14 ± 2.91	11.71 ± 0.91	8.43 ± 1.99^b	13.91 ± 4.71^b
Yorkshire terrier (YT)	16.57 ± 3.55^b	13.57 ± 1.75^B	4.71 ± 2.06^a	14.44 ± 3.21^B

Means with different letters in columns differ significantly at $p \leq 0.05$ and $p \leq 0.01$

Table 3 shows the results achieved by dogs at the second cone. B passed by the cone for the first time (first success) after 39.29 ± 18.73 sessions, CC after 51.43 ± 19.52 , and YT after 35.00 ± 8.81 sessions. However, the differences

among groups were not statistically significant. Moreover, no differences in the pieces/session ratio, which would be only numerically highest in YT (12.70 ± 2.76), and lowest in CC (11.16 ± 1.86), were observed. Additional passing by the cone, repeated twice, took place during 7.14 ± 2.61 sessions in YT, up to 8.71 ± 5.44 sessions in CC, and the average number of pieces per session amounted to 8.31 ± 4.09 in B, up to 11.98 ± 12.81 in CC. Similarly to achieving the first success at this cone, no statistically significant differences were observed here.

Table 3

The results obtained by dogs during performing tasks 2 and 2a

Dog breed	Task 2		Task 2a	
	number of sessions	pieces/session ratio	number of sessions	pieces/session ratio
Boxer (B)	39.29 ± 18.73	12.59 ± 1.71	7.29 ± 2.50	8.31 ± 4.09
Chow chow (CC)	51.43 ± 19.52	11.16 ± 1.86	8.71 ± 5.44	11.98 ± 3.78
Yorkshire terrier (YT)	35.00 ± 8.81	12.70 ± 2.76	7.14 ± 2.61	9.50 ± 3.51

No significant differences

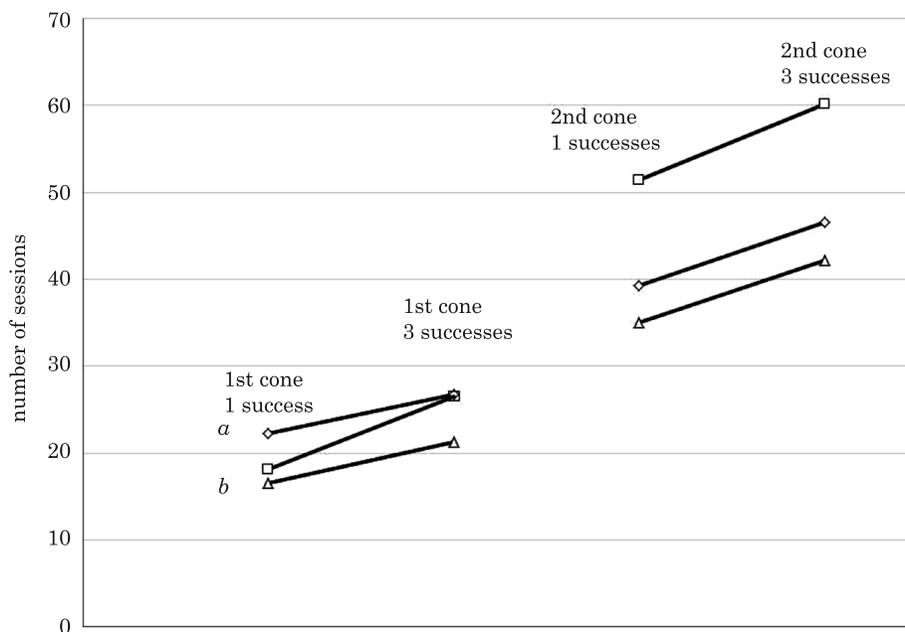


Fig. 2. The results obtained by dogs – on each cone summed ascending
Means with different letters differ significantly at $p \leq 0.05$

Picture 2 presents the number of sessions for the sum of successes at each cone (success = single passing by the cone). Achieving the first success at the first cone was significantly simpler for YT than for B ($p < 0.05$). However, no statistically significant differences were observed at consecutive measurements. In order to achieve success at the second cone, CC needed the highest number of sessions, but it was only a tendency ($p < 0.1$).

Discussion

In research comparing breeds in privately owned dogs it is difficult to collect comprehensive statistical material. Consequently, it is not a surprise that often only a few animals participate in research comprising various breeds of dogs (WEISS and GREENBERG 1997). It is widely known that previous training can affect the outcome. Therefore, we tried to choose dogs with similar age and training experience. Age of the animals varied between 4.10 ± 2.18 in CC and 4.41 ± 2.55 in YT breed. Moreover, all the dogs had the opportunity to stay without the leash during daily walks so it can be assumed that they respected at least the basic commands. The breeds of dogs studied by us from the perspective of their utility have relatively few common features. Boxers are moloses. They are assistance dogs, and are defensive and utility dogs. They work as patrol dogs, rescue dogs, and they assist the disabled. During the First World War they were used as guard, patrol and report dogs. Boxers are known for being disciplined. They were bred for close cooperation with people and for performing orders (PALIKA 2007). In contrast, many dog coaches believe that Chow Chows are a breed in which it is impossible to achieve success in training. This may stem from the fact that Chow Chows do not tolerate training techniques based on violence (REED 2014). They are Asian Spitzes. They are very territorial dogs with a strong hunting instinct. On the other hand, Yorkshire Terriers, miniature terriers, were originally owned by working-class people and were used for vermin control as well as companionship (PALIKA 2007).

Differences among the three breeds under research occurred during passing by the first cone (tasks 1 and 1a). Initially, Boxers coped worst (Table 2). They needed over 22 training sessions to perform the first task. Moreover, training sessions in this breed were significantly ($p < 0.01$) shorter than YT sessions, because Boxers quite soon started to look to the side, indicating dropping interest in the training. However, it is characteristic that after achieving the first success, achieving two consecutive successes was relatively easy for them, despite the fact that the training sessions were shorter and comprised around 7.23 pieces of food in each session. During performing tasks

at the second cone, this breed started to show increased willingness to work (around 12.59 pieces/session); however, their willingness to work after achieving the first success (second task) dropped again (8.31 pieces/session).

Chow Chows were relatively successful at the first task, but to repeat the success twice they required a much higher number of sessions with the clicker than the remaining two breeds. After the change from the first to the second cone, Chow Chows required an increased number of sessions needed to achieve the first success (51) compared to B (39) and YT (35). Initially, the change of the cone to a further one was difficult, but after performing the task it was quite easy for them to repeat the success at the second cone. The results achieved by this breed show that, contrary to the opinion of many dog coaches, it is possible to successfully train Chow Chows.

Training results certainly depend on the dogs' willingness to work. Willingness to work cannot be measured directly, but the pieces/session ratio may constitute an indicator. During performing work at the first cone, YT showed the highest willingness to work with the clicker, but at the second cone the results are not as clear. This shows that skilful training may improve dogs' commitment to the performed work. DONALDSON (1996) emphasizes this fact.

A comparison of the results achieved by us with Coren's list, describing the differences in intelligence between dog breeds (COREN 1994), is very interesting. Coren created a ranking focusing on the working and obedience intelligence (it may consequently be described as trainability) of dogs, based on the opinions of American Kennel Club and Canadian Kennel Club obedience trial judges. According to this ranking, Yorkshire Terriers are „above average working dogs”, which means that understanding new command takes 15–25 repetitions, and that they obey the first command $\geq 70\%$ of the time. Boxers have „average working/obedience intelligence” – they understand a new command after 25–40 repetitions, and obey the first command $\geq 50\%$ of the time. Chow Chows belong to the last group, with „the lowest degree of working/obedience intelligence” (they need more than 80 repetitions, and obey the first command less than 30% of the time). In fact, Chow Chows take the 4th place from the end of the list comprising 131 classified breeds. Despite restrictions concerning interpretation of Coren's research results (COPPINGER and COPPINGER 2001), many authors agree with the ranking of dog breeds (DAVIS and CHEEKE 1998, MIKLÓSI 2009). Although in our research the results for the first cone were different from those achieved by Coren, the order of breeds at the second cone was compliant to Coren's list: YT was the first, B was the second, and CC was the last one. Despite this, we showed that Chow Chows are much more trainable than Coren asserted.

As stated by COPPINGER and COPPINGER (2001), the results achieved by dogs cannot be interpreted in the manner that one breed is more intelligent

than another. The differences observed in present research do not stem from the fact that some breeds are more intelligent than others according to rankings developed by people, but they result from differences in specific behavioural conformation among breeds. YT achieved the best results in our tests which was natural since they were bred as companion dogs for years. In addition, in their history, they had to be independent when the situation demanded (they often hunted for rodents) (LANE 2001). B achieved not such as good results as YT, but, except task 1, their results were not statistically worse. B are known for their honesty and loyalty. In training sessions, they react well to humans (SPITZER 2006). In general, it can be concluded that the comparatively worst results were obtained by CC. It was not surprising if their history into account will be taken – this breed was not dedicated to work with humans (REED 2014). In other words, the breeds studied by us are dedicated to completely different goals, and they succeed in their roles. Moreover, not only the type of task, but also the manner of training (here: positive reinforcing – shaping with the clicker) certainly influenced the results achieved by dogs.

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AGE-RELATED CHANGES IN THE DISTRIBUTION OF LEAN MEAT, FAT WITH SKIN, AND BONES IN TURKEY CARCASSES

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K e y w o r d s: turkeys, age-related changes, meat, fat, bones.

Abstract

The experimental materials comprised 600 BIG-6 turkeys (360 ♂ and 240 ♀). Females were reared to 16 and males to 22 weeks of age. Day-old poults were sexed and randomly allocated to 16 pens (eight pens of males and eight pens of females, 45 males per pen and 30 females per pen). Starting from one day of age, the turkeys were weighed at two-week intervals, and starting from two weeks of age, three birds selected randomly of each pen (24 ♂ and 24 ♀) were slaughtered at two-week intervals. Chilled carcasses were divided into parts (neck, legs, wings, breast and back) which were dissected to separate lean meat, skin with subcutaneous fat, inter-muscular fat, and bones. At two weeks of age, in both males and females, over 76% of the total muscle content was found in the breast and legs, and the remaining meat was located in the wings, back and neck. Until 16 weeks of age in males and until approximately 12 weeks of age in females, the rate of muscle deposition in the legs and wings was relatively slow, while a faster rate of muscle deposition was observed in the breast and back. Age-related changes in the distribution of skin with fat in turkey carcasses were greater than changes in muscle distribution. The content of skin with fat increased considerably in the breast in males and in the legs in females, while it decreased in the wings. The bone content of carcass parts changed to the lowest degree in growing turkeys.

ZMIANY W ROZMIESZCZENIU MIĘSA, TŁUSZCZU ZE SKÓRĄ I KOŚCI W POSZCZEGÓLNYCH CZĘŚCIACH TUSZEK INDYKÓW WRAZ Z WIEKIEM PTAKÓW

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Słowa kluczowe: indyki, zmiany z wiekiem, mięso, tłuszcz, kości.

Abstrakt

Materiał do badań stanowiło 600 indyków (360 ♂ i 240 ♀) Big 6, odchowywanych do 16 tygodnia – samice i 22 tygodnia – samce, według ogólnie przyjętej technologii. Jednodniowe pisklęta po zważeniu i oznakowaniu, rozmieszczono losowo w 16 kojcach (po 8 kojców samców i samic; w jednym kojcu samców – 45 sztuk a samic – 30 sztuk). W okresie od jednego dnia w odstępach 2-tygodniowych indyki ważono, a poczynając od 2-tygodnia pobierano losowo po 3 ptaki z każdego kojca (24 ♂ i 24 ♀) do uboju. Tuszki po wychłodzeniu (ok. 24 h w temp. 4°C) dzielono na części (szyja, nogi, skrzydła, część piersiowa i grzbiet), które poddano szczegółowej dysekcji wyodrębniając mięso, skórę z tłuszczem podskórnym, tłuszcz międzymięśniowy i kości. Stwierdzono, że w wieku 2 tygodni zarówno w tuszkach samców jak i samic duża ilość mięsa (powyżej 76%) zlokalizowana była w części piersiowej i w nogach, a pozostała część w grzbiecie, skrzydłach i szyi. Do wieku 16 tygodni u samców i do około 12 tygodni u samic tempo odkładania tego składnika było stosunkowo wolne w nogach i skrzydłach, a większe w części piersiowej i grzbietowej. Rozmieszczenie skóry z tłuszczem w poszczególnych częściach tuszki ulegało wraz z wiekiem ptaków większym zmianom niż lokalizacja mięsa. Znaczna tendencja wzrostowa wystąpiła w udziale skóry z tłuszczem z części piersiowej u indorów i z nóg u indyczek, a malejąca w ilości tych składników tkankowych ze skrzydeł. Najmniejszym zmianom wraz z wiekiem indyków ulegał w ich tuszkach udział kości z poszczególnych części.

Introduction

Whole turkey carcasses are usually perceived by consumers as less attractive, compared with retail and market-ready cuts (breast and legs, or breast fillets, thighs and drumsticks) which do not require additional cutting prior to processing.

The growth rate of turkeys is an important consideration for both researchers and producers. Age-related changes in the body weight of birds are closely correlated with changes in the proportions of body parts and tissue components (BOCHNO et al. 2003, BOCHNO et al. 2006, MURAWSKA 2012, MURAWSKA 2013a,b, MURAWSKA and BOCHNO 2008, WILKIEWICZ-WAWRO et al. 2005).

Domestic fowl species differ considerably with respect to their growth rate. At the early stages of life, turkeys and broiler chickens grow more slowly

(LEESON and SUMMERS 1980a,b, LEWCZUK et al. 1994, MICHALIK 1994, PASTERNAK and SHALEV 1983, KNIŽETOVA et al. 1991b, BOCHNO and BRZOWSKI 1998) than ducks and geese (LEESON et al. 1982, KNIŽETOVA et al. 1991a, JANISZEWSKA 1993, WIEDERHOLD and PINGEL 1997). The growth curves for major carcass parts are also different in different species. In all domestic fowl, the most dynamic changes are observed in the legs and breast, while changes in the back and neck are non-significant. At the end of the growth period, ducks are characterized by the lowest content of breast and legs in the carcass, and the highest proportion of the back (LEESON et al. 1982). In turkeys (LEESON and SUMMERS 1980 b) and Muscovy ducks (WILKIEWICZ-WAWRO et al. 2005), the breast, legs and back have the highest share of the carcass, with the lowest percentage of the wings. Broiler chickens are marked by the highest content of breast and legs in the carcass (LEESON and SUMMERS 1980a). In day-old chickens, leg weight and breast weight account for approximately 33% and only 17% of the total carcass weight, respectively. Over the subsequent weeks, the proportion of the breast increases rapidly to around 32%, whereas the proportion of the legs decreases, to reach around 29% at 10 weeks of age (BOCHNO and BRZOWSKI 1998). The dynamics of changes in the percentage content of carcass parts, relative to total body weight or total carcass weight, is similar in geese (JANISZEWSKA 1993) and Pekin ducks (BOCHNO et al. 2005). In Muscovy ducks, from 7 to 11 weeks of age, the relative content of legs in the carcass decreases non-significantly, while the percentage of the breast increases (WILKIEWICZ-WAWRO et al. 2005).

Carcass tissue composition is also species-dependent. Turkey carcasses have a high lean content (69.43% at 18 weeks of age) and a low content of fat with skin (10.51%, BOCHNO et al. 1993). The carcasses of broiler chickens and Muscovy ducks also have a high lean content, at 59.62% and 52.5% in males, respectively, and approximately 51.7% in females. Fat and skin account for 21.36% in broiler chickens (BOCHNO et al. 2003), and for more 25% in Muscovy ducks aged 12 weeks (WILKIEWICZ-WAWRO et al. 2005). The carcasses of geese slaughtered at 14 weeks of age contain less muscle (44.02%) and more fat with skin (27.56%) (BOCHNO et al. 2006) than the carcasses of broiler chickens and Muscovy ducks. The least desirable proportions of lean and fat with skin in the carcass has been reported for Pekin ducks slaughtered at 7 weeks of age (39.78% and 35.7%, respectively) (BOCHNO et al. 2003). Age-related changes in the percentage content of lean meat, fat with skin and bones occur at different rates in different poultry species. The percentage of muscle and fat with skin increases gradually, while the proportion of bones decreases (BOCHNO et al. 1986, BOCHNO et al. 1993, LEWCZUK et al. 1994). The rate of the above changes is faster in ducks (BOCHNO and LEWCZUK 1986) and geese (JANISZEWSKA 1993), and slower in chickens (BOCHNO and BRZOWSKI 1998) and turkeys (LEWCZUK et al. 1994).

A review of the available scientific literature provides scant information on the distribution of lean meat, fat and bones in turkey carcasses. In view of the above, the objective of this study was to determine age-related changes in the distribution of tissue components in the carcasses of heavy-type turkeys.

Materials and Methods

The experimental materials comprised 600 BIG-6 turkeys (360♂ and 240♀). Females were reared to 16 weeks of age, and males to 22 weeks of age, in accordance with universally accepted technological standards. Day-old poults were weighed, marked with wing tags, sexed and randomly allocated to 16 pens (eight pens of males and eight pens of females, 45 males per pen and 30 females per pen). The birds were fed *ad libitum* complete diets (the chemical composition of diets is given in Table 1). Starting from one day of age, the turkeys were

Table 1

Chemical composition of diets

Specification	Diet						
	R 280	R 281	R 282	R 283	R 284	R 285	R 286
	Feeding period (weeks)						
	1-2	3-5	6-8	9-11	12-14	15-17	> 18
Dry matter (g/kg)	910.7	908.0	899.6	903.3	902.8	899.4	901.2
Crude ash (g/kg)	92.7	89.8	72.0	65.2	58.0	51.2	49.5
Total protein (g/kg)	272.3	254.7	230.0	225.7	194.8	182.1	165.0
Crude fat (g/kg)	48.5	53.9	58.0	68.9	85.2	84.2	85.0
Crude fiber (g/kg)	27.5	32.0	33.0	34.8	41.0	35.0	35.0
Metabolizable energy (MJ kg ⁻¹)	11.61	11.44	12.16	12.18	12.24	12.76	12.96

weighed at two-week intervals (females at 2, 4, 6, 8, 10, 12, 16 week, males at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 week), and starting from two weeks of age, three birds selected randomly of each pen (24 ♂ and 24 ♀). The birds were fasted for 12 hours, weighed and sacrificed by cervical dislocation. Following slaughter, the turkeys were bled for about five min., and then scalded in water at about 55°C for 2 min., to facilitate plucking. The birds were defeathered manually. The carcasses were plucked and eviscerated, and the heads, shanks and wingtips were cut off. Chilled carcasses (approx. 24 h at 4°C) were weighed and divided into the following parts: neck – along the line connecting the cephalad borders of the coracoids; wings – at the shoulder joints; legs – at the hip joints (from the process of the pubis through the groin towards the back, and along the vertebral column, starting from the anterior border of the pelvis, to separate thigh muscles from the carcass); breast portion – from the process of the pubis, through the cartilaginous

adhesions of the ribs, to the coracoid process; back and loin – the remaining part of the carcass (referred to as the back). Carcass parts were dissected to separate lean meat, skin with subcutaneous and inter-muscular fat (referred to as skin with fat), and bones. Lean weight is the weight of muscular tissue without inter-muscular fat, which was separated during dissection. Bone weight includes the weights of all bone elements of the carcass, separated during detailed dissection – the meat was separated from the bones using scissors and a scalpel.

The growth rate of birds (g_r , %) was calculated using the following formula:

$$g_r = \frac{w_2 - w_1}{0.5 (w_1 - w_2)} \cdot 100\%$$

where:

w_1 – initial body weight,

w_2 – final body weight,

0.5 – constant value

Statistical Analysis

The results were processed statistically (Statsoft 9.0, INC 2010), including the determination of:

1. arithmetic means and coefficients of variation (\bar{x} and v) for carcass weight and the weights of tissue components in the carcass; the significance of differences in the mean values of the above traits between age groups and between males and females (one-way ANOVA); F and D tests, t-test,
2. the percentage content of tissue components in the carcass,
3. age-related changes in the content of lean meat, fat with skin, and bones in carcass parts, as a percentage of the total weight of respective components in the whole carcass; the total weight of each tissue component in the carcass was assumed to be 100%.

Results

The average body weight of male turkeys increased by approximately 21 700 g from hatching to 22 weeks age (Figure 1a). The average body weight of female turkeys increased by 10 600 g until 16 weeks of age. Sexual dimorphism in body weight was noted as early as in week 4. Both in males and females, the highest relative body weight gains (above 100%) were observed during the first four weeks (Figure 1b). Lower, but still significant body weight gains were noted until week 8.

At later stages of rearing, the body weights of birds increased at a slower rate, particularly in females.

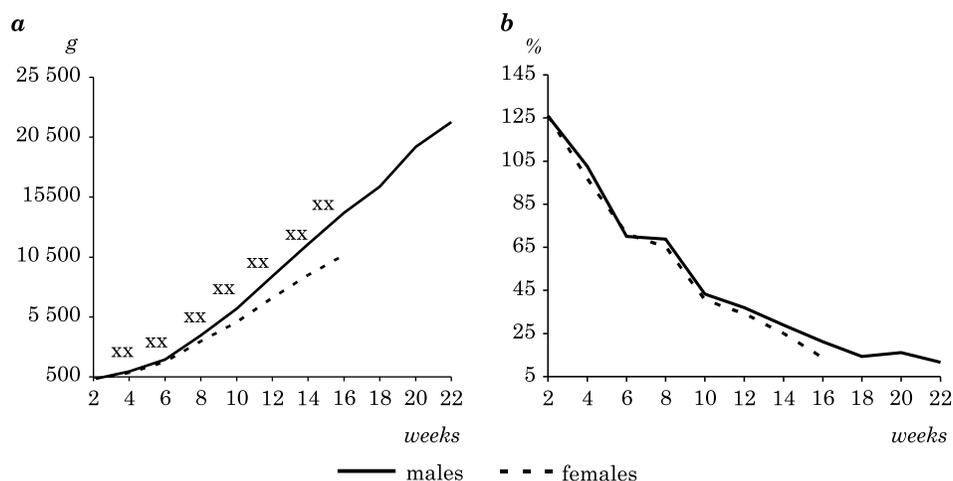


Fig. 1. Body weights (a) and body weight gains (b) of male and female turkeys

Carcass weight and carcass tissue composition (g) are presented in Table 1. Carcass weight, identical in males and females at two weeks of age ($\bar{x} = 180$ g), increased to over 17 460 g in 22-week-old males, and to over 8 300 g in 16-week-old females. Similarly as with body weight, sexual dimorphism in carcass weight was noted already in week 4.

The average meat content of the whole carcass exceeded 100 g (Table 1) in two-week-old birds, and it increased to 12 448 g in males and to 5 748 g in females at slaughter. The weight of bones and skin with subcutaneous and inter-muscular fat also continued to increase throughout the study.

To better illustrate the discussed age-related changes in growing turkeys, the weights of tissue components from Table 1 were expressed as a percentage of total carcass weight (Figure 2). In males, the muscle content of the carcass increased at a relatively fast rate until week 12 – from 57.64% in two-week-old birds to 68.61% in 12-week-old birds. In the subsequent 10 weeks, the lean content of carcasses increased by only 2.67%, to reach 71.28% in turkeys slaughtered at 22 weeks of age. In females, a rapid increase in muscle content (by around 10%) was observed for the first 10 weeks, to reach 68.23%. In the next six weeks, the meat content of the carcass increased by around 1%. The content of skin along with separable fat (skin with fat) remained at a stable level of approximately 8% in both males and females for the first four weeks. At later stages of the study, the content of skin and fat in the carcass increased gradually to reach around 14.5% in males aged 20 and 22 weeks, and 15.59% in females aged 16 weeks.

Table 2

Means (\pm SE) tissue component weight in turkey carcasses over the experimental period

Specification	Sex	Age (weeks)										
		2	4	6	8	10	12	14	16	18	20	22
Weight (g): - carcass (including neck)	♂	179.7 ^{Aa} +5.83	611.7 ^{Abxx} +13.32	1369.7 ^{Bxx} +23.62	2881.1 ^{Cxx} +54.15	4604.0 ^{Dxx} +50.83	6749.1 ^{Exx} +64.60	9015.6 ^{Fxx} +89.60	11249.4 ^{Gxx} +150.24	13100.8 ^H +209.35	15839.8 ^I +134.48	17462.3 ^J +240.55
	♀	179.5 ^A +6.38	554.9 ^B +11.81	1229.1 ^C +26.96	2517.3 ^D +50.09	3778.8 ^E +61.23	5346.0 ^F +62.30	6986.6 ^G +92.24	8308.7 ^H +140.74			
- lean meat	♂	103.7 ^{Aa} +3.62	373.1 ^{Ab} +8.86	879.7 ^B +16.45	1894.1 ^C +36.49	3066.6 ^D +35.05	4631.1 ^E +47.82	6244.2 ^F +64.74	7855.5 ^G +93.95	9203.2 ^H +157.39	11093.2 ^I +95.08	12448.4 ^J +179.36
	♀	105.7 ^A +4.24	344.2 ^B +7.16	799.6 ^C +18.83	1704.1 ^D +33.77	2578.3 ^E +43.09	3730.7 ^F +46.90	4838.0 ^G +60.82	5747.9 ^H +106.63			
- bones	♂	47.6 ^A +1.42	150.2 ^B +3.06	319.0 ^C +6.23	625.3 ^D +11.87	970.9 ^E +10.94	1317.3 ^F +16.29	1579.6 ^G +18.86	1804.1 ^H +24.45	1934.7 ^I +23.33	2145.8 ^J +14.50	2181.4 ^J +30.90
	♀	46.4 ^A +1.52	131.7 ^B +3.13	278.7 ^C +5.59	495.1 ^D +9.73	701.0 ^E +10.30	871.0 ^F +10.01	999.6 ^G +16.55	1117.1 ^H +19.04			
- skin and fat	♂	14.4 ^{Aa} +0.55	46.9 ^A +1.38	111.0 ^{Ab} +2.44	261.3 ^B +7.39	430.0 ^C +9.17	636.3 ^D +13.95	1005.2 ^E +26.18	1375.0 ^F +37.15	1746.8 ^G +45.92	2339.3 ^H +43.78	2521.4 ^I +47.39
	♀	13.9 ^{Aa} +0.56	43.0 ^{Ab} +1.43	103.3 ^{Bb} +2.99	232.2 ^C +8.07	389.8 ^D +10.84	614.7 ^E +18.52	1015.0 ^F +36.25	1296.7 ^G +34.32			
Offal and dissection losses	♂	14.0 ^A +0.65	41.4 ^{Ba} +1.21	60.0 ^{Bb} +1.86	100.4 ^C +2.80	136.5 ^D +4.72	164.4 ^{Bc} +6.49	186.6 ^{Bd} +4.91	214.7 ^F +5.45	216.0 ^F +6.99	261.5 ^G +8.70	311.1 ^H +10.60
	♀	13.4 ^A +0.51	36.1 ^B +1.13	47.5 ^C +1.36	86.0 ^D +3.06	109.7 ^E +3.14	129.6 ^F +4.23	133.9 ^F +3.99	147.0 ^G +4.60			

Mean values denoted by different superscript letters or x (sex) in the same row are significantly different: capital letters or xx - at $P \leq 0.01$, small letters or x - at $P \leq 0.05$

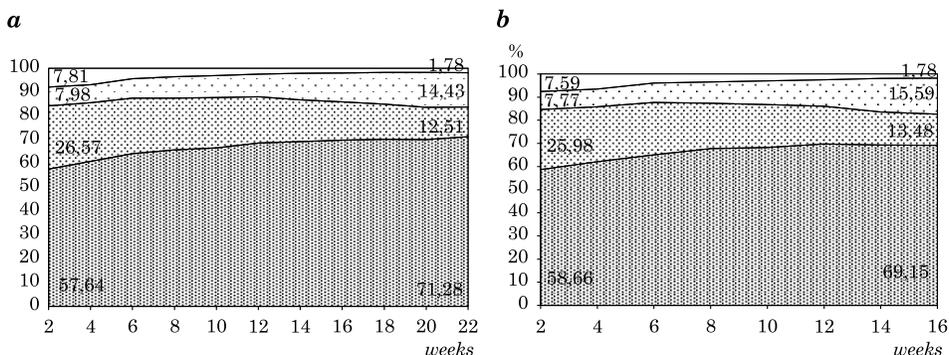


Fig. 2. Percentage content of tissue components in the carcasses of males (a) and females (b). *loss=slaughter offal

The bone content of the carcass decreased gradually with age, from 26.57% and 25.98% in two-week-old males and females, respectively, to 12.51% in males aged 22 weeks and 13.48% in females aged 16 weeks. At two weeks of age, in both males and females, over 76% of the total muscle content was found in the breast (42.53% in males and 42.98% in females) and legs (34.16% in males and 33.79% in females), and the remaining meat was located in the wings, back and neck (Figure 3). Muscle distribution in turkey carcasses changed inconsiderably until slaughter (22 weeks in males, 16 weeks in females). In both males and females, the lean content of the breast increased by only around 3%, while the lean content of the legs and wings decreased by 2% and 3%, respectively. Over this period, the meat content of the back increased by approximately 2.0%, whereas the meat content of the neck remained relatively unchanged throughout the experiment.

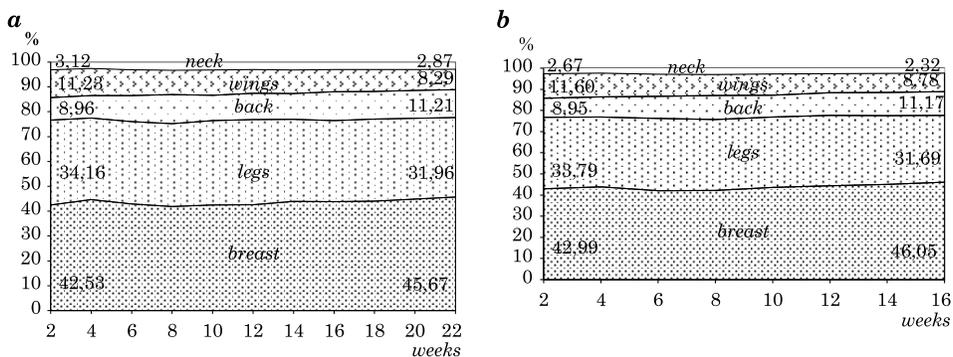


Fig. 3. Lean meat content of carcass parts as a percentage of total lean weight in the carcasses of males (a) and females (b)

Age-related changes in the distribution of skin with fat in carcass parts were greater than changes in muscle distribution (Figure 4). In males, the content of skin with fat in the breast increased from 21.12% in week 2, to 33.72% at slaughter. In females, the content of skin with fat in the breast increased by over 3% between weeks 2 and 16. As regards the content of skin with fat in the legs, it was increasing until 14 – 18 weeks of age in males, to reach 25%, and then it decreased significantly to 22.57% at 22 weeks. In females, an approximately 9% increase in the content of skin with fat in the legs was recorded by week 16. The content of skin with fat in the wings varied considerably over time. It had the highest share of the total weight of skin and fat in the carcass in week 2, at 25.83% in males and 25.43% in females (Figure 4). In the next two weeks, the content of skin with fat in the wings increased to 27.19% and 28.57% in males and females, respectively. At later stages of rearing, the proportion of skin with fat in the wings decreased gradually, to reach 10.40% in males and 12.52% in females at the end of the study.

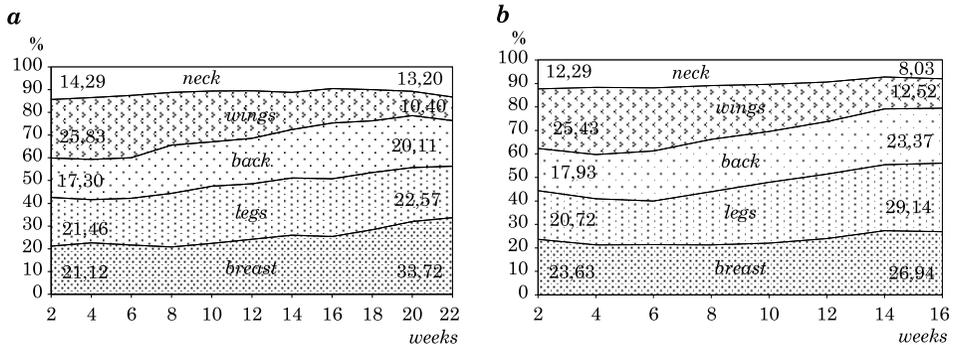


Fig. 4. Content of skin with fat in carcass parts as a percentage of the total weight of skin with fat in the carcasses of males (a) and females (b)

The content of skin with fat in the back and neck also changed with age. In males, the content of skin with fat in the back was found to increase during the first 16 weeks (24.5%), and then it decreased to 20.11% in week 22. The proportion of skin with fat in the neck was decreasing until week 16 (9.55%), and next it increased to reach 13.20% in week 22. In females, a steady increase in the content of skin with fat in the back was noted, from around 18% in week 2 to over 23% in week 16, while the proportion of skin with fat in the neck decreased by more than 4% over this period.

The bone content of carcass parts changed to the lowest degree in growing turkeys (Figure 5). In the breast, it increased by only around 2% in males and less than 1% in females. Changes in the bone content of the legs and wings were equally non-significant. The proportion of bones in the neck decreased by approximately 4% in both males and females over the experimental period.

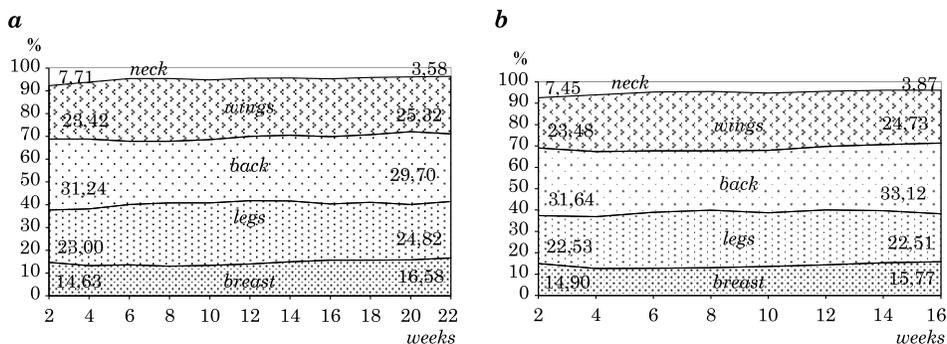


Fig. 5. Bone content of carcass parts as a percentage of total bone weight in the carcasses of males (a) and females (b)

Discussion

Domestic fowl species vary widely with respect to the growth rates of body weight, muscle, skin with fat and bones. Waterfowl species, including Muscovy ducks, Pekin ducks and geese, grow fast in the first weeks of their life (BAEZA et al. 1998, BOCHNO et al. 2006, BOCHNO et al. 2006, GILLE et al. 1999, WIEDERHOLD and PINGEL 1997, WILKIEWICZ-WAWRO et al. 2005). Many years have passed since the domestication of ducks and geese, yet it seems that their fast initial growth rate is an atavistic feature. Fast-growing ducklings and goslings stand a greater chance of survival in the aquatic environment. The initial growth rate of broiler chickens (BOCHNO and BRZOWSKI 1998, BOCHNO et al. 2003, KNIŻETOWA et al. 1991b, LESSON and SUMMERS 1980a, PASTERNAK and SHALEV 1983), turkeys (LESSON and SUMMERS 1980 b, LEWCZUK et al. 1994, LEWCZUK et al. 2003) and laying hens (MURAWSKA and BOCHNO 2006) is much slower, compared with waterfowl. The growth rate of broiler chickens and turkeys increases with age.

Carcass tissue composition also varies considerably among poultry species. Research results (MURAWSKA 2013a), including our study, show that turkey carcasses are characterized by the most desirable proportions of muscle and skin with fat. Our findings corroborate those of MICHALIK (1994) and POUR (1991), who studied carcass tissue composition in turkeys, chickens, ducks and geese.

The main aim of this study was to determine age-related changes in the distribution of lean meat, skin with fat, and bones in carcass parts in growing turkeys. Similarly as in other animal species, also in birds tissue growth is non-uniform. Rapid development of nervous tissue and osseous tissue occurs during prenatal life. Muscle tissue starts to grow fast after hatching, and adipose tissue is the last to develop. Nesting birds, including poultry, are born with well-developed legs and pelvic bones.

In poultry species, the proportions between various body and carcass parts change with age. The growth patterns of tissue components (muscle, skin with fat and bones) in carcass parts also vary during the growing period (MURAWSKA and BOCHNO 2007). In turkeys, the growth of neck and wings is complete first, and breast development takes the longest time (LEWCZUK et al. 2003). Over the entire rearing period, breast muscle weight increases to the highest, and back muscle weight to the lowest degree.

In our study, two-week-old poults had well-developed breast and leg muscles, which accounted for 43% and 34% of the total muscle weight, respectively. Leg muscle weight decreased by around 2% over time. A slight decrease in leg muscle weight, accompanied by an increase in breast muscle weight, have also been observed in broilers (BOCHNO et al. 2003) and layers (MURAWSKA et al. 2005), and in an earlier study of turkeys (LEWCZUK et al. 1993).

Different trends have been reported for geese (BOCHNO et al. 2006). In two-week-old goslings, leg muscles and breast muscles accounted for nearly 61% and only 8.78% of the total muscle weight, respectively. At 10 weeks of age, the proportion of breast muscles increased to 35.79%, while the proportion of leg muscles decreased to 30.61%. Such changes were also noted by BOCHNO et al. (2005), and GILLE and SALOMON (1998) in Pekin ducks, and by WILKIEWICZ-WAWRO et al. (2005) in Muscovy ducks.

The muscle content of the wings, expressed as a percentage of the total lean meat content of the carcass, decreased in turkeys from over 11% at two weeks to over 8% at slaughter. As demonstrated by BOCHNO et al. (2006), muscle weight in the wings of geese increased by over 8%, relative to total muscle weight, during the first eight weeks. According to the cited authors, a fast growth rate of muscles, bones and feathers in the wings, accompanied by rapid breast muscle growth at early life stages, is associated with the flying ability of wild geese. Similar changes have also been observed in Muscovy ducks (WILKIEWICZ-WAWRO et al. 2005) and ducks (BOCHNO et al. 2005). In broiler chickens, the lean content of the wings remained at a stable level of 8% during the entire rearing period (BOCHNO et al. 2003).

In heavy-type turkeys, approximately 77% lean meat is deposited in the most valued carcass parts, i.e. the breast and legs, while the neck, wings and back contain only 23% lean meat. In broiler chickens (BOCHNO et al. 2005) and medium-heavy turkeys (LEWCZUK et al. 1993) the neck, wings and back contain 26% and 25% lean meat, respectively. In geese (BOCHNO et al. 2006), Pekin ducks (BOCHNO et al. 2005) and Muscovy ducks (WILKIEWICZ-WAWRO et al. 2005), muscles are located in less valuable carcass parts, i.e. the neck, wings and back (over 33% in total).

In poultry, skin and a subcutaneous fat layer are very difficult to separate, which is why they were analyzed in combination in our study, although it could

distort the picture of carcass tissue composition. In growing turkeys, the distribution of skin with fat in carcass parts is affected by gender. Over the growth period, the content of skin with fat in the breast increased by over 12% in males and by only 3% in females. The opposite trend was noted with regard to the content of skin with fat in the legs, which increased by over 8% in females and by only approximately 1% in males.

According to BOCHNO et al. (2006), age-related changes in the distribution of skin with fat in goose carcasses are relatively small, except that the deposition rate of these tissue components is faster in the wings and slower in the back and legs. In the present experiment, the content of skin with fat in the wings, relative to the total weight of skin and fat in the carcass, decreased considerably from around 26% in turkeys aged two weeks to around 10% in males and 12.5% in females at slaughter. The content of skin with fat in the back increased over this period by 3% in males and by 5% in females.

The bone content of carcass parts, expressed as a percentage of total bone weight, changed non-significantly in growing turkeys. In males and females, most of the bones were found in the back (approx. 31%), legs and wings (approx. 23% each), both at two weeks of age and at slaughter. In Muscovy ducks (WILKIEWICZ-WAWRO et al. 2005), the proportion of bones in the legs and back decreased by approximately 10% – 12% over the rearing period, whereas the bone content of the wings increased almost three-fold, from 6.1% in week 2 to 22.2% in males and 23.5% in females at slaughter. According to PINGEL (1990), a fast growth rate of bones, skin with fat and feathers in the wings of ducks is a flight adaptation. However, Muscovy ducks have largely lost their ability to fly due to domestication and selection for increased body weight.

It can be concluded that age-related changes in the distribution of lean meat, skin with fat and bones in the carcass are less significant in heavy-type turkeys than in broiler chickens. According to the cited published sources, the greatest changes in carcass tissue composition occur in growing ducks and geese.

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**MICROBIAL AIR CONTAMINATION
IN THE CENTER AND IN THE FORDON DISTRICT
OF BYDGOSZCZ**

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Key words: microbial air contamination, mold fungi, heterotrophic bacteria, actinomycetes.

Abstract

Air sampling by impaction was conducted at eight sampling sites in a seasonal cycle (sites I–IV located in the center of Bydgoszcz, sites V–VIII located in the Fordon district). The results indicate the dominance of mold fungi (77%). Heterotrophic mesophilic bacteria were the second most numerous (22%) while actinomycetes accounted for a small percentage of the total number of microorganisms (1%). The following genera contributed to the population of mold fungi: *Cladosporium* (84%), *Alternaria* (5%), *Penicillium* (3%), *Fusarium* (3%), *Aspergillus* (1%). According to Polish Standard air contamination with microorganisms belonging to all investigated groups did not exceed limit values. The number of the investigated microorganisms varied seasonally and depended on the sampling site. The highest numbers were typically recorded in summer and autumn, while the lowest, in winter. Fungi and actinomycetes were the most numerous in the Old Market Place in the Fordon District and heterotrophic bacteria, in the center of Bydgoszcz at ul. Gdańska.

**MIKROBIOLOGICZNA JAKOŚĆ POWIETRZA W CENTRUM I DZIELNICY FORDON
MIASTA BYDGOSZCZY**

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Słowa kluczowe: zanieczyszczenie powietrza, grzyby pleśniowe, bakterie heterotroficzne, promieniowce.

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Abstrakt

Badanie mikroorganizmów występujących w powietrzu prowadzono na ośmiu stanowiskach badawczych (I–IV w centrum Bydgoszczy i V–VIII w dzielnicy Fordon), w cyklu sezonowym, przy użyciu metody zderzeniowej. Analizy wykazały, że wśród drobnoustrojów powietrza dominowały grzyby pleśniowe (77%), nieco mniejszą grupę stanowiły heterotroficzne bakterie mezofilne (22%), zaś promieniowce, stanowiły niewielki procent wśród ogółu mikroflory (1%). Wśród grzybów pleśniowych stwierdzono obecność grzybów z rodzaju: *Cladosporium* (84%), *Alternaria* (5%), *Penicillium* (3%), *Fusarium* (3%), *Aspergillus* (1%). Stopień skażenia powietrza poszczególnymi grupami mikroorganizmów zgodnie z Polską Normą przeważnie nie przekraczał dopuszczalnych wartości. Liczebność badanych drobnoustrojów była zróżnicowana i zmieniała się sezonowo oraz w zależności od miejsca poboru prób. Maksimum liczebności badanych grup drobnoustrojów stwierdzono zazwyczaj latem i jesienią, natomiast zimą notowano spadek ich liczby. Grzyby i promieniowce dominowały na Starym Rynku w Fordonie, natomiast bakterie heterotroficzne najliczniej występowały na stanowisku zlokalizowanym w centrum Bydgoszczy przy ul. Gdańskiej.

Introduction

The air is a mixture of gases which does not constitute an adequate environment for microorganisms as it fails to provide nutrients and good physico-chemical conditions. Failing to provide nutrients and good physico-chemical conditions, the air, a mixture of gases, does not constitute an adequate environment for microorganisms. However, microorganisms suspended in the air in the form of bioaerosol significantly affect air quality. Bioaerosol is a collection of biological particles dispersed in the air or the gas phase. It consists of single spores, pollen, bacterial cells and viruses, aggregates of spores, cells, and other biological material, products or fragments of mycelium, fungal spores and bacterial cells (endotoxins and mycotoxins), biological material lifted from the ground on its own accord or carried by bigger non-biological particles (CHMIEL et al. 2015). Biological particles get into the atmosphere in many ways: removed from the surface of soil and plants, carried by the wind or lifted by means of thermal convection, released (either spontaneously or by rainfall) from natural bodies of water, and as a result of storing and processing solid and liquid waste (KULKARNI et al. 2011).

Qualitative and quantitative composition of bioaerosol depends on time and location.

Constituting 70% of the microbial population, filamentous fungi (*Cladosporium*, *Alternaria*, *Penicillium*, *Aspergillus*, *Mucor*, *Rhizopus*) are typically the main component of bioaerosols (D'AMATAGO et al. 2000). Saprophytic bacteria belonging to the genera *Micrococcus* and *Bacillus* constitute another numerous group. In contrast, actinomycetes and yeast (belonging to the genera *Torulopsis*, *Rhodotorula*, *Candida*, *Saccharomyces*) represent only 5% of the microorganisms isolated from the air.

Epidemiological studies show that high concentration of microorganisms in the air can be allergenic. Sometimes, however, even very low concentrations of particular microorganisms can cause serious diseases (STRYJKOWSKA-SEKULSKA et al. 2007).

Fungal allergens may trigger symptoms of respiratory disorders and skin diseases in susceptible patients (TWAROCH et al. 2015). Exposure to molds may have multiple health effects: it can cause allergies in the form of rhinitis and bronchial asthma, allergic aveolitis, and in people with poor resistance can result in severe opportunistic infections (WISZNIEWSKA et al. 2004).

Fungal conidia present in the air contain extremely high amounts of mycotoxins (KARWOWSKA 2005). They cause skin infections and toxemias characterized by headaches, diarrhea, changes to immunological mechanisms and damage to the liver, kidneys and central nervous system, and they can also be carcinogenic (GOLOFIT-SZYMCZAK and SKOWRON 2005).

For the above reasons, it is important to monitor air quality in places of increased risk of biological contamination (hospitals and particularly isolation wards), in storage premises where food items are stored, in places where people spend time every day, like dwelling places, public utility buildings and atmospheric air in the streets of towns with heavy traffic (DONDESKI et al. 2005).

This study is aimed at evaluating air quality in two crucially different parts of the town of Bydgoszcz, i.e. the centre and the Fordon district located in the east. The areas differ in terms of population, urban development, traffic density and number of tourists.

Materials and Methods

Sampling sites

We evaluated microbial air quality in the center of Bydgoszcz and in the Fordon district (Figure 1).

Bydgoszcz has an area of 176 km² and a population of 358 337 (CSO 2014). Due to unique natural values, 35% of its territory is located within the protected landscape area while 9% is covered by the Vistula Landscape Park (in the north and north-east of the city). Bydgoszcz can boast having a large number of parks (31 parks with an area of more than 2 ha) which cover the area of 879 hectares. It is known for well-developed tourist traffic (390 499 tourists in 2013), services and industries including food, chemical, (rolling stock and tram production), and information technology industries.

Fordon is a district located in the east of Bydgoszcz, in the Fordon Valley on the Vistula. It has a surface area of 3063 hectares and a population of 72 160.

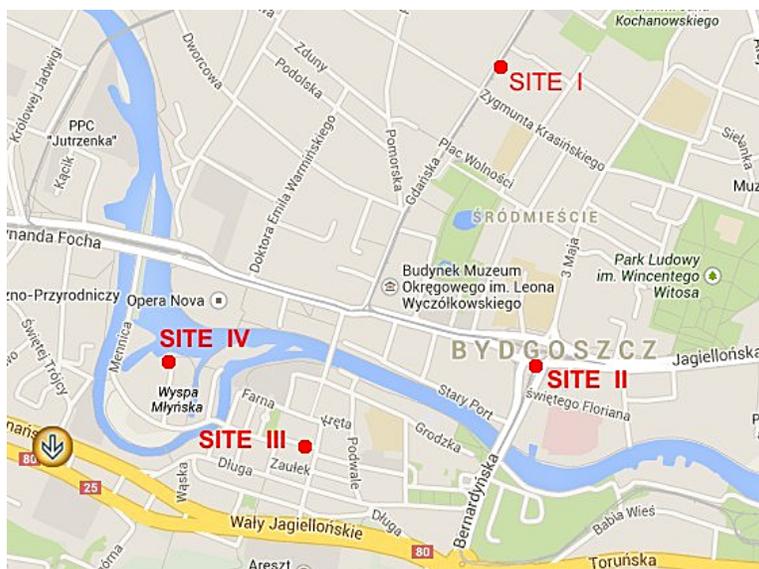


Fig. 1. The location of sites (Bydgoszcz)



Fig. 2. The location of sites (Fordon district)

Although green areas cover one third of its surface, it is principally a residential district comprising 20 housing estates, filled mainly with apartment blocks and single-family houses.

Sampling sites were located in the areas of both compact and scattered development.

Sampling sites were located in the center of Bydgoszcz: sampling site I – in ul. Gdańska, sampling site II – Rondo Jagiellonów, sampling site III – Wyspa Młyńska (historic river island within the Old Town district, famous for a wide

range of cultural and recreational facilities, surrounded by boulevards and hydro facilities: canals, dams, bridges), sampling site IV – Old Market Place (Figure 1), and in the Fordon district: sampling site V – UTP Campus in ul. Fordońska, sampling site VI – Fordońska Dolina Śmierci (Fordon Death Valley – the site of mass killing and a mass grave of 1,200 – 1,400 Poles and Jews murdered between October and November 1939 by Nazi Germans), sampling site VII – Białe Marketplace (Tatra housing estate), sampling site VIII – Marketplace in Stary Fordon (Figure 2).

Sampling

The research was conducted in a seasonal cycle (spring, summer, autumn, winter), by the impaction method using Merck MAS – 100 air sampler.

The amount of 50–100 liters of air (depending on the expected contamination level) was filtered in the sampler's chamber containing a Petri dish filled with a suitable nutrient medium. The microflora from the air stream was sucked in by the air sampler and deposited on the surface of the medium. At all sampling sites sampling was conducted in three parallel repeats. The air samples were transported to the laboratory, placed in a thermostat and incubated for a specific time at an appropriate temperature. After the incubation grown colonies were counted and the number was expressed as colony forming units per cubic meter of air (CFU/1 m³).

Air temperature, relative humidity and wind velocity were measured during sampling using Nielsen-Kellerman anemometer, Kestrel 3500 (Table 1).

Table 1
Meteorological parameters during sample collection

Date of sampling	Temperature (°C)	Humidity (%)	Wind speed (km/h)	Wind direction
13.05.2011	14	49	11	W
01.07.2011	33	30	7	SE
03.10.2011	18	56	8.5	S
18.02.2012	-1.4	83	5	N

Microbial Research

The microbial research was aimed at determining the following: 1) the total number of heterotrophic bacteria, 2) the number of mannitol-positive bacteria of *Staphylococcus* genus, 3) the number of *Pseudomonas fluorescens*, 3) the number of actinomycetes, 4) the number of mold fungi and their identification.

The total number of heterotrophic bacteria was determined using TSA agar medium. The bacteria were incubated at 37°C for 48 hours, then grown colonies were counted and their number was expressed as colony forming units per cubic meter of air (CFU/1 m³).

The presence of mannitol-positive staphylococci was detected according to Polish Standard PN 89/Z-04111/02 using Chapman's nutrient medium. Bacterial cultures were incubated at 37°C for 48 hours, then grown colonies were counted. Bright yellow zones around a grown colony indicated a positive result. Additionally, the strains were Gram stained and identified under a microscope.

The number of *Pseudomonas fluorescens* was determined in accordance with Polish Standard PN 89/Z-04111/02 using Kinga's B nutrient medium. The bacterial cultures were incubated at 26°C for 5 days.

The number of actinomycetes and mold fungi was determined using Pochon's and Sabouraud's nutrient mediums accordingly. The microorganisms were incubated at 26°C for 5 days, after which time grown colonies were counted and their number was expressed as colony forming units per cubic meter of air (CFU/1 m³). Mold fungi were identified on the basis of their macro- and microscopic features using the SAMSON et al. (2000) key.

Microbial air quality in Bydgoszcz was evaluated in accordance with Polish Standards PN 89/Z-04111/02 and PN-89/Z-04111/03. The results were analyzed in STATISTICA 6.0. Statistical analysis was based on Kruskal-Wallis test (one-way ANOVA on ranks), which assesses statistically significant differences between groups of data.

Results

The number of microorganisms belonging to all investigated microbial groups are presented in Tables 2 and 3 and Figure 3 and 4. The results indicate that the highest average number of heterotrophic bacteria was identified at sampling site I located in the center of Bydgoszcz in ul. Gdańska (467 CFU/m³ of mesophilic bacteria). In the Fordon District, the highest average number of heterotrophic bacteria was identified at sampling site VII (Białe Marketplace). A slightly lower number was recorded at sampling sites II (Rondo Jagiellonów) – 165 CFU/m³ and III (Wyspa Młyńska) – 161 CFU/m³. The remaining sampling sites (i.e. IV- Old Market Place, V – UTP Campus, VI – Fordońska Dolina Śmierci (Fordon Death Valley), VIII – Market Place in Stary Fordon) were characterized by the lowest average number of heterotrophic bacteria (under 70 CFU/m³). According to Polish Standards N-89/Z-04111/02 and 03 all sampling sites can be regarded as uncontaminated. We observed seasonal fluctuations in the number of heterotrophic bacteria with the highest numbers recorded in the

summer, and lower and similar in the remaining seasons, both in the center of Bydgoszcz and the Fordon District (Table 2).

Table 2
The number of microorganisms and level of microbial air contamination at different sampling sites in the center of Bydgoszcz and in the Fordon district according to Polish Standards PN-89/Z-04111/02 and PN-89/Z-04111/03

Sampling site	Number of microorganisms in 1 m ³ of air				
	Heterotrophic bacteria	<i>Staphylococci</i>	<i>Pseudomonas fluorescens</i>	<i>Actinomycetes</i>	Mold fungi
I	467 *	7 **	6 **	13 **	705 *
II	165 *	11 **	2 **	7 *	523 *
III	161 *	4 **	1 **	17 **	543 *
IV	60 *	1 **	4 **	6 *	511 *
V	58 *	3 **	6 **	4 *	562 *
VI	56 *	2 **	4 **	6 *	685 *
VII	193 *	4 **	21 **	8 *	721 *
VIII	69 *	0 *	4 **	21 **	748 *
Polish Norm No pollution *	<1000	No	No	10	3000-5000
Medium pollution**	1000-3000	< 25	< 50	10-100	5000-10000
Heavy pollution ***	>3000	> 25	50	> 100	> 10000

Table 3
An average number of microorganisms in 1 m³ of air in the center of Bydgoszcz and in the Fordon district, depending on the season

Date of sampling	Heterotrophic bacteria	<i>Staphylococci</i>	<i>Pseudomonas fluorescens</i>	<i>Actinomycetes</i>	Mold fungi
	[CFU · m ⁻³]				
13.05.2011	121* 0-668**	1* 0-6**	3* 0-10**	3* 0-10**	694* 213-1526**
1.07.2011	259* 90-600**	5* 0-23**	16* 0-80**	9* 0-20**	997* 88-1610**
3.10.2011	109* 30-240**	8* 0-23**	6* 0-15**	24* 3-60**	709* 413-1230**
18.02.2012	114* 0-668**	0,5* 0-4**	0* 0-0**	1* 0-2**	99* 34-228**

Explanations: * mean, ** range

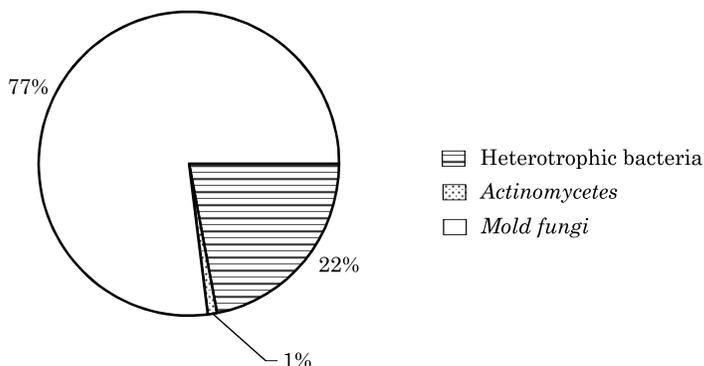


Fig. 3. Percentage fraction of microorganisms in the air

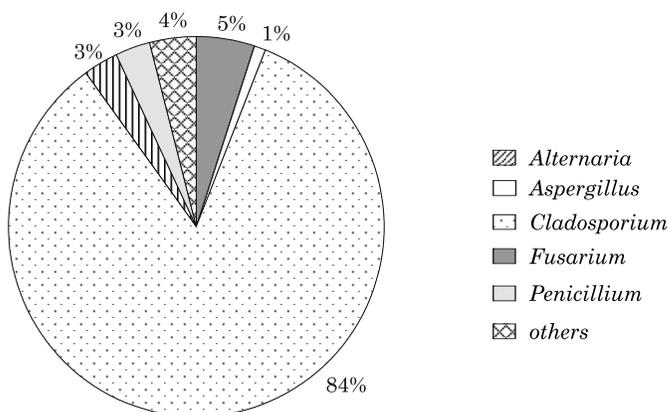


Fig. 4. Dominant genera of mold fungi

The results show that mannitol-positive *Staphylococci* were not identified only at sampling site VIII (Market Place in Stary Fordon) thereby indicating that the air was not contaminated. The remaining sites were moderately contaminated with *Staphylococci* whose number did not exceed 11 CFU/m³ (Table 1). The most numerous in the summer and the fall, *Staphylococci* were sparse in the winter and the spring (Table 2).

The results indicate moderate air contamination with *Pseudomonas fluorescens* in Bydgoszcz. The highest average number of these microorganisms (21 CFU/m³) was recorded in the Fordon District at sampling site VII (Białe Marketplace). They were identified from spring to fall but they were not identified in the winter (Table 3).

Actinomycetes were the most numerous at the following sampling sites: I (in ul. Gdańska) – 13 CFU/m³, III (Wyspa Młyńska) – 17 CFU/m³ and VIII

(Market Place in Stary Fordon) – 21 CFU/m³, which, according to Polish Standards, indicates their moderate contamination with these microorganisms. At the remaining sites the air was classified as uncontaminated with actinomycetes (Table 2). Actinomycetes had the highest percent contribution to the microbial community in summer and fall and significantly lower in spring and winter (Table 3).

Similarly to other microorganisms, mold fungi were the least numerous in the winter (Table 3). According to Polish Standards the air in the center of Bydgoszcz and in the Fordon District was uncontaminated with these microorganisms. Their average highest number (over 700 CFU/m³) was recorded at sampling sites I (in ul. Gdańska), VII (Białe Marketplace, and VIII (Market Place in Stary Fordon). At the remaining sites the average number of mold fungi was lower (Table 2).

The following genera contributed to the population of mold fungi: *Cladosporium* (84%), *Alternaria* (5%), *Penicillium* (3%), *Fusarium* (3%), *Aspergillus* (1%) (Figure 4).

Statistical analysis did not indicate statistically significant differences between the numbers of investigated microorganisms and sampling sites. Statistically significant differences were found only between the number of staphylococci, actinomycetes and fungi during some research seasons (Table 4).

Table 4. Statistical differences between the numbers of identified groups of microorganisms in different research seasons

Microorganisms		13.05.2011	1.07.2011	3.10.2011	18.02.2012
Heterotrophic bacteria	13.05.2011	–	ns	ns	ns
	01.07.2011	ns	–	ns	ns
	03.10.2011	ns	ns	–	ns
	18.02.2012	ns	ns	ns	–
<i>Pseudomonas fluorescens</i>	13.05.2011	–	ns	ns	ns
	01.07.2011	ns	–	ns	ns
	03.10.2011	ns	ns	–	ns
	18.02.2012	ns	ns	ns	–
<i>Staphylococci</i>	13.05.2011	–	ns	ns	ns
	01.07.2011	ns	–	ns	ns
	03.10.2011	ns	ns	–	*
	18.02.2012	ns	ns	*	–
Actinomycetes	13.05.2011	–	ns	*	ns
	01.07.2011	ns	–	ns	ns
	03.10.2011	*	ns	–	**
	18.02.2012	ns	ns	**	–
Mold fungi	03.05.2011	–	ns	ns	*
	01.07.2011	ns	–	ns	***
	03.10.2011	ns	ns	–	**
	18.02.2012	*	***	**	–

Explanations: ns – differences statistically non-significant, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Discussion

Air pollution is an important problem facing the world. Rapid industrial and transport development together with aggressive urban expansion lead to the menacing concentration of the air pollutant emission sources within relatively small areas. The atmosphere must absorb substantial amounts of harmful contaminants including different powders, organic compounds, non-organic compounds of nitrogen, sulphur, coal and other compounds as well as various microorganisms including bacteria, viruses, fungal spores etc. Since the air's ability to self-clean is vastly limited, it is necessary to supervise its quality and take measures which would protect it from excessive pollution (MICHALKIEWICZ 2002).

However, the results presented in this study should be regarded as temporary values, valid only at the time of the measurement. The physico-chemical parameters of the air as well as its contamination level can change dramatically within minutes (COX 1987). Hence, the results provide merely an approximate estimation, allowing the evaluation of the microbial number at a given moment.

When evaluating microbial air quality one should take into account a range of factors that affect the composition of microflora and therefore the results of the research: the type of emission sources, intensity of microbial emission, meteorological conditions (temperature, wind force, humidity, precipitation, sunlight, UV radiation) and the season of the year (CHMIEL 2015, KRZYSZTOFIK 1992, BREZEA-BORUTA 2010).

High temperatures and lack of precipitation foster their growth, which explains why we usually recorded a higher number of bacteria and fungi in the summer than in the winter, when weather conditions are unfavorable for microbial growth. Similarly to other cities including Toruń in Poland (DONDESKI et al. 2005) and Castilla La-Mancha (SABARIEGO et al. 2012) and Cartagena (ELVIRA-RENDULES et al. 2013) in Spain, at a landfill site (MIAŚKIEWICZ-PĘSKA et al. 2015), the number of bacteria and fungi in the investigated air varied seasonally, reaching their maximum in summer.

Fluctuations of the total number of microorganisms depend largely on ground conditions, such as the availability of nutrients, and the intensity of microbial growth in soil and in water as well as on garbage, plant and animal remains (KRZYSZTOFIK 1992).

The results indicate that traffic, that raising dust which contains microorganisms, has a great impact on their number in the air. After some time, floating aerosol settles and accumulates on the surface of soil, water, plants, and buildings, constituting a potential source of secondary emission that can be triggered by wind or traffic (KOŁWZAN et al. 2012). Hence we believe that

heavy road traffic pedestrian traffic contributed to the increased number of heterotrophic bacteria and mold fungi in the air along the main street in the center of Bydgoszcz (ul. Gdańska) and in the Białe Marketplace as well as to the increased number of mold fungi in the the Old Market Place in Fordon.

In addition, the air at sampling sites located in the center of Bydgoszcz generally contained a higher number of microorganisms than at sites located far from the center, i.e. the Fordon district. Mold fungi were the only microorganisms whose number was similar at all sampling sites. The same pattern was also noted by DONDESKI et al. (2005) who recorded higher numbers of bacteria in the Old Town in Toruń than at Rubinkowo housing estate. Similarly, BURKOWSKA et al. (2012) recorded maxima of mesophilic bacteria, molds, mannitol-positive staphylococci and hemolytic bacteria in the urbanized part of Ciechocinek and their minima around the graduation towers.

The microbial population in the air in the center of Bydgoszcz and in the Fordon district was dominated by mold fungi. Heterotrophic mesophilic bacteria were the second most numerous while actinomycetes accounted for a small percentage of the microbial population. Similar results were obtained at the municipal landfill site Żółwin-Wypaleniska (MAŁECKA-ADAMOWICZ 2007) and the sewage treatment plant Kapuściska (MAŁECKA-ADAMOWICZ 2011) in Bydgoszcz.

According to Polish Standards PN-89/Z-04111/02 and 03 the air in the center of Bydgoszcz and the Fordon district can be considered uncontaminated with heterotrophic bacteria. Similar number of heterotrophic bacteria (ranging from 111 CFU/m³ to 189 CFU/m³) was recorded in the Old Town in Toruń (DONDESKI et al. 2005). Other authors including BUGAJNY et.al. (2005) recorded 10-fold higher number of mesophilic bacteria (i.e. 13,000 CFU/m³) in large urban areas such as Poznań. FANG et al. (2007) during the research conducted in Beijing in areas with high level of traffic and human activity, recorded even 22,000 CFU/m³).

Regarded as air quality indicators, *Staphylococci* species indicate possible air contamination with pathogenic microorganisms (AKERMAN et al. 2003). Even without producing spores, they have the ability to remain in the air for long periods of time. This quality is of great importance as it indicates that infections can easily spread by airborne transmission (PILLAI and RICKE 2002). High sensitivity of pathogenic organisms to negative meteorological factors combined with the absence of emission sources may be responsible for their low number in the air (DONDESKI et al. 2005). The low number of mannitol-positive staphylococci in the air in the center of Bydgoszcz (1–11 CFU/m³) and Fordon (0–4 CFU/m³) district seems to confirm this observation, indicating moderate air pollution. Similar results were obtained by MAŁECKA-ADAMOWICZ et al. (2011) in the Forest Recreation Park in Myślęcinek, where the air was

moderately contaminated with these microorganisms at all sampling sites (5–25 CFU/m³).

The presence of *Pseudomonas fluorescens* in the air indicates its contamination with bioaerosols originating from water bodies. The air in the center of Bydgoszcz and the Fordon district was moderately contaminated with these microorganisms, which may be related to the fact that River Brda cuts through Bydgoszcz and flows into River Vistula in the Fordon district and both rivers can constitute emission sources of these bacteria.

Actinomycetes, air quality indicators originating from soil, are always present in the air, both in urban areas and in the vicinity of municipal facilities (KALISZ 1994). The air in the center of Bydgoszcz and in the Fordon district contained small numbers of actinomycetes, i.e. from 4 to 21 CFU/m³. Similar results were obtained in urban parts of Ciechocinek (BURKOWSKA and DONDESKI 2007). In contrast, a large number of actinomycetes was recorded in the vicinity of the Kapuściska sewage treatment plant (MAŁECKA-ADAMOWICZ et al. 2011) and in the municipal landfill site in Żółwin-Wypaleniska (MAŁECKA-ADAMOWICZ et al. 2007).

In considering the presence of actinomycetes in the air, it should be emphasized that KAZIMIERCZUK et al. (2004) found these bacteria to be common organisms in atmospheric aerosols, which therefore cannot be treated as valuable indicators of air quality. However, GRZYB and FRĄCZEK (2013) maintain that actinomycetes are one of the most important components of bioaerosols and that they may pose a health risk as even very low concentrations may trigger allergies.

The microbial community in the air in the center of Bydgoszcz and in the Fordon district was dominated by mold fungi, which confirms the thesis that they are well-adapted to spreading in the air. However, although they can survive in almost all environments, they thrive indoors affecting health and well-being of residents (KRZYSZTOFIK 1968). The surface of soil and plants as well as building facades also provide habitat for these microorganisms. Mold fungi produce large numbers of spores, transmitted over thousands of kilometers and found even in stratosphere.

Mold fungi have also been isolated from the air in caves, from cave rocks, sediments and biota present in caves (BASTIAN et al. 2010, JURADO et al. 2010, WANG et al. 2010, OGÓREK et al. 2013). More than 600 CFU/m³ of mold fungi were isolated from the air outside Niedźwiedzia Cave (OGÓREK et al. 2014).

The microbial air quality is influenced not only by the number of molds in the air, but also by the species composition of the fungal community.

The following fungal genera were identified in the air in the center of Bydgoszcz and the Fordon district: *Cladosporium* (84%), *Alternaria* (5%), *Penicillium* (3%), *Fusarium* (3%), *Aspergillus* (1%). A similar composition was noted in the center of Polish cities: Ciechocinek (BURKOWSKA and DONDESKI

2008), Poznań (Bugajny et al. 2005) and in the southern Iraq in Basrah (MUHSIN and ADLAN 2012). In the atmosphere of EL – Beida City (Libya), *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium* and *Penicilium* were recorded in all months of the year (EL-GALI and ABDULLRAHMAN 2014).

Cladosporium species have a world-wide distribution and are amongst the most common air-borne mold fungi. As has been confirmed by earlier findings, *Cladosporium* species prevail in the outdoor air, constituting between 50 and 90% of the fungal population (BUGAJNY et al. 2005), (ELVIRA-RENDUELES 2013). Some species are commonly found on living and dead plant material, some parasitize other fungi (DOLIŃSKA et al. 2011).

Microbial air contaminants may emit secondary metabolites including mycotoxins, endotoxins, enterotoxins, and enzymes affecting human health. Molds of the genera *Aspergillus*, *Candida* and *Alternaria* which enter the body through the respiratory tract with spore-infected air also pose a threat to human health including allergy risk (GOŁOFIT-SZYMCZAK and SKOWRON 2005). Sensitivity to fungi is not only typically diagnosed in patients suffering from asthma but may also represent a risk factor for developing this disease (JO et al. 2014). Regular monitoring of microbial air quality is therefore necessary.

The information on the airborne fungi in an area would be useful to determine geographical patterns of asthma and hay fever (ADHIKARI et al. 2004). Daily monitoring of the number of molds in the air seems a practical solution. It is also recommended that researchers determine health effects caused by fungi and establish exposure thresholds and guidelines for the medical community (PORTNOY 2004).

Conclusions

Microbial community in the center of Bydgoszcz and the Fordon district was dominated by mold fungi, followed by heterotrophic mesophilic bacteria. The number of the investigated microorganisms varied over time and depended on the sampling site. According to Polish Standards (PN-89/Z-04111/02 and PN-89/Z-04111/03) air contamination with all microbial groups in the center of Bydgoszcz and Fordon District did not exceed limit values.

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CHEMICAL COMPOSITION AND pH OF PROCESSED PORK MEAT PRODUCTS SUPPLIED BY A RENOWNED POLISH MANUFACTURER

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Key words: processed pork meat products, chemical composition, pH.

Abstract

The aim of this study was to determine the proximate chemical composition and pH values of selected processed pork meat products popular among consumers, supplied by a renowned Polish manufacturer. All products were purchased in the city of Olsztyn.

The experimental materials comprised pork meat products manufactured during a standard production process in a meat processing plant. Branded pork products available in stores across Poland were randomly selected and purchased at monthly intervals over two years. Pork deli meats of the following categories were analyzed: smoked pork products (smoked pork sirloin – „Połędwica sopocka”), finely ground sausages (Vienna sausage – „parówki”), semi-coarsely ground sausages („Podwawelska”), semi-coarsely ground dry sausages („Żywiecka”), semi-coarsely ground scalded sausages („Mielonka tyrolska”), coarsely ground ham sausages („Szynkowa”), dry sausages („Krakowska sucha”) and pork meat blocks (canned ham – „Szynka konserwowa”). The products were analyzed in a laboratory to determine their proximate chemical composition, collagen content and pH values.

In the examined pork products, fat content and water content varied within the widest and the narrowest range, respectively. Smoked pork sirloin („Połędwica sopocka”) and Vienna sausage („parówki”) were characterized by the least stable and the most stable proximate chemical composition, respectively. Collagen content was highest in Vienna sausage („parówki”), and lowest in smoked pork sirloin („Połędwica sopocka”) and canned ham („Szynka konserwowa”). The products with the lowest collagen content were characterized by the highest variation in collagen concentrations. Average pH values were lowest in the semi-coarsely ground dry sausage („Żywiecka”) and in Vienna sausage („parówki”), and highest in the semi-coarsely ground sausage („Podwawelska”) and semi-coarsely ground scalded sausage („Mielonka tyrolska”).

SKŁAD CHEMICZNY I WARTOŚĆ pH WYBRANYCH WĘDLIN WIEPRZOWYCH ZNANEJ KRAJOWEJ MARKI

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Słowa kluczowe: wędliny wieprzowe, skład chemiczny, wartość pH.

Abstrakt

Celem przeprowadzonych badań była ocena podstawowego składu chemicznego oraz wartości pH wybranych, popularnych wśród konsumentów wędlin wieprzowych, znanej krajowej marki, zakupionych na terenie Olsztyna.

Materiał do badań stanowiły wędliny pochodzące z normalnego cyklu produkcyjnego, prowadzonego w warunkach przemysłowych. Na przestrzeni dwóch lat, 12-krotnie w ciągu roku (raz w miesiącu), losowo wybierano i zakupywano wędliny marki znanej i dostępnej na terenie całego kraju. Badaniami objęto wędliny reprezentujące ich następujące grupy technologiczne: wędzonki (połędwica sopocka), kielbasy drobno rozdrobnione (parówki), kielbasy średnio rozdrobnione (podwawelska), kielbasy średnio rozdrobnione podsuszone (żywiecka), kielbasy średnio rozdrobnione parzone (mielonka tyrolska), kielbasy grubo rozdrobnione (szynkowa), kielbasy suszone (krakowska sucha), produkty blokowe (szynka konserwowa). Produkty, po przewiezieniu do laboratorium, zostały poddane badaniom mającym na celu określenie w nich zawartości podstawowych składników chemicznych, kolagenu oraz ustalenie wartości pH produktu.

Stwierdzono, że składnikiem chemicznym, którego udział w wędlinach wykazywał największą zmienność był tłuszcz, a najmniejszą woda. Największą zmiennością podstawowego składu chemicznego charakteryzowała się połędwica sopocka, natomiast najbardziej stabilny był skład parówek. Analiza zawartości kolagenu wykazała jego największy udział w parówkach, a najmniejszy w połędwicy sopockiej i szynce konserwowej. Produkty, charakteryzujące się najmniejszą zawartością kolagenu, odznaczały się jednocześnie największą zmiennością jego udziału. Najniższe średnie wartości pH stwierdzono w kielbasie żywieckiej i parówkach, a najwyższe w kielbasie podwawelskiej i mielonce tyrolskiej.

Introduction

Processed meat products, in particular sausages, have a high market share in Poland (Central Statistical Office 2013). Research studies (PODOLEC et al. 2011, SYGNOWSKA et al. 2005) have shown that the average consumption of cold cuts and sausages is significantly higher than the recommendations in the current dietary guidelines. Cold cuts are popular among consumers who can choose from a wide range of deli meats tailored to meet their specific tastes and preferences with regard to price, organoleptic properties, nutritional value and originality (traditional products, regional specialties, products containing wild game meat, etc.).

In order to meet high quality standards and consumer expectations, the manufacture of processed meat products has to be carefully monitored at each stage of the process, including final product quality control. The main quality indicators are the proximate chemical composition and pH of the analyzed meat product. The overall quality of the end product is largely determined by the concentrations and proportions of various chemical components, and active acidity (MAKALA et al. 2008).

The objective of this study was to determine the proximate chemical composition and pH values of selected processed pork meat products popular among consumers, supplied by a renowned Polish manufacturer and purchased in the city of Olsztyn.

Materials and Methods

The experimental materials comprised pork meat products manufactured during a standard production process in a meat processing plant. Branded pork products available in stores across Poland were randomly selected and purchased in a retail outlet in Olsztyn at monthly intervals over two years. Pork deli meats of the following categories were analyzed (PN-A-82007:1996Az1:1998): smoked pork products (smoked pork sirloin – „Polędwica sopocka”), finely ground sausages (Vienna sausage – „parówki”), semi-coarsely ground sausages („Podwawelska”), semi-coarsely ground dry sausages („Żywiecka”), semi-coarsely ground scalded sausages („Mielonka tyrolska”), coarsely ground ham sausages („Szynkowa”), dry sausages („Krakowska sucha”) and pork meat blocks (canned ham – „Szynka konserwowa”). The products were analyzed in a laboratory to determine their proximate chemical composition, collagen content and pH values.

The analysis of the proximate chemical composition of processed pork meat products included the determination of dry matter content, total protein content by the Kjeldahl method, fat content by the Soxhlet method (diethyl ether as the solvent) and ash content (AOAC 1990). The hydroxyproline content of cold cuts (PN-ISO 3496:2000) was measured and converted into total collagen content using a conversion factor of 8.

The pH of samples was measured in the water homogenates of cold meats (product to redistilled water ratio of 1:1, m/v) with the use of a combination Polylite Lab electrode (Hamilton Bonaduz AG, Bonaduz, Switzerland) and a 340i pH-meter equipped with a TFK 325 temperature sensor (WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany).

The data were processed statistically using STATISTICA 9.0 (2009) software (StatSoft, Inc., Tulsa, OK, USA, 2009). The quality of processed pork

meat products manufactured in different seasons (spring – March, April, May; summer – June, July, August; autumn – September, October, November; winter – December, January, February) was compared by one-way analysis of variance (ANOVA). The statistical significance of differences between means in groups was estimated by Duncan's multiple range test at $P \leq 0.05$ and $P \leq 0.01$.

Results and Discussion

The study revealed significant variations in the chemical composition of the analyzed cold cuts (Table 1). Throughout the year, the fat content and the water content of the examined pork products varied within the widest and the narrowest range, respectively. The smoked pork product („Polędwica sopocka”) and the finely ground sausage („parówki”) were characterized by the least stable and the most stable proximate chemical composition, respectively.

Variations in the chemical composition of cold cuts available on the domestic market were also reported by other authors (MAKAŁA et al. 2008, MAKAŁA et al. 2007, WALCZYCKA 2007). The above information is important for consumers who expect uniform quality every time they buy a given product. The stability of chemical composition (and nutritional value) of food products is an important consideration for many consumers who carefully plan their meals (including the energy content and salt content of products) based on a dietitian's or a physician's recommendations.

None of the analyzed products had a nutrition facts label, including information on chemical composition and nutritional value, on its package. However, it should be noted that currently nutritional labeling is mandatory only in selected groups of food products (Regulation of the Minister of Health of 25 July 2007). The situation will change in the nearest future due to the Regulation (EU) No. 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, which entered into force on 13 December 2014.

The branded deli meats analyzed in our study complied with the requirements of Polish Standard PN-A-82007:1996/Az1:1998 for proximate chemical composition. Polish food producers are no longer legally obliged to follow national quality standards (PN), but many of them continue to make efforts to meet the requirements contained in those standards. An analysis of the quality of cold cuts conducted by, among others, TYBURCY et al. (2005) and WĘSIERSKA et al. (2012) supports the above hypothesis.

Collagen content was highest in Vienna sausage („parówki”), and lowest in smoked pork sirloin („Polędwica sopocka”) and canned ham („Szynka konser-

Table 1
Results of processed pork meat products quality evaluation (mean ± standard deviation)

Product	Season of the year	Trait																	
		Dry matter [%]			Total protein [%]			Fat [%]			Ash [%]			Collagen [%]			pH		
		\bar{x}	SD	CV	\bar{x}	SD	CV	\bar{x}	SD	CV	\bar{x}	SD	CV	\bar{x}	SD	CV	\bar{x}	SD	CV
1	2	25.32	1.14	4.50	17.45	1.36	7.79	2.95	46.10	3.84	12.80	0.56	15	16	17	18	19	20	0.81
Smoked pork products (smoked pork sirloin - „Poledwica sopočka”)	spring	25.64	1.95	7.61	18.55	1.57	8.46	2.57	40.86	3.84	11.46	0.52	14	15	16	17	18	19	2.92
	summer	26.13	1.28	4.90	18.49	2.06	11.14	2.93	27.30	4.15	7.23	0.55	16	17	18	19	20	0.10	1.64
	autumn	24.04	2.34	9.73	17.31	1.79	10.34	1.81	43.65	4.04	2.22	5.45	0.41	16	17	18	19	20	2.40
	winter	25.33	1.85	7.30	18.04	1.74	9.65	2.55	40.00	3.98	3.37	9.30	0.51	0.18	35.29	6.17	0.14	2.27	
Finely ground sausages (Vienna sausage - „parówki”)	spring	40.64 ^a	0.98	2.41	13.42	0.42	3.13	21.17	1.23	5.81	3.06	1.12	3.92	2.55 ^a	0.16	6.27	6.08	0.07	1.15
	summer	40.67 ^a	0.89	2.19	13.68 ^a	0.38	2.78	20.34 ^a	0.86	4.23	3.05	0.13	4.26	2.57 ^a	0.17	6.61	6.08 ^a	0.09	1.48
	autumn	41.62	2.26	5.43	13.13 ^b	0.18	1.37	21.96	2.34	10.66	3.04	0.04	1.32	2.39	0.16	6.69	6.04	0.19	3.15
	winter	42.97 ^b	1.57	3.65	13.24	0.53	4.00	23.05 ^b	1.06	4.60	3.12	0.10	3.21	2.28 ^b	0.20	8.77	6.20 ^b	0.13	2.10
average	41.52	1.76	4.24	13.37	0.43	3.22	21.63	1.78	8.23	3.06	0.10	3.27	2.44	0.20	8.20	6.10	0.14	2.30	
Semi-coarsely ground sausages („Podwawelska”)	spring	38.12 ^a	0.63	1.65	12.17	0.26	2.14	21.21	0.84	3.96	3.12 ^a	0.10	3.21	1.18	0.03	2.54	6.37	0.04	0.63
	summer	38.57 ^a	1.38	3.58	12.81	0.37	2.89	18.08	7.27	40.21	3.21	0.10	3.12	1.23	0.22	17.89	6.32 ^a	0.07	1.11
	autumn	37.97 ^A	0.85	2.24	12.53	0.55	4.39	20.53	1.26	6.14	3.16	0.19	6.01	1.36	0.14	10.29	6.36	0.08	1.26
	winter	40.25 ^{Bb}	1.64	4.07	12.68	0.69	5.44	22.51	1.91	8.49	3.30 ^b	0.11	3.33	1.32	0.17	12.88	6.43 ^b	0.05	0.78
average	38.74	1.47	3.79	12.59	0.53	4.21	20.42	4.24	20.76	3.20	0.14	4.38	1.28	0.17	13.28	6.37	0.07	1.10	
Semi-coarsely ground dry sausages („Żywiecka”)	spring	40.12 ^A	0.94	2.34	21.43	1.89	8.82	14.84 ^a	2.53	17.05	3.29	0.17	5.17	1.18	0.26	22.03	5.98 ^a	0.06	1.00
	summer	45.10 ^B	2.58	5.72	21.49	1.31	6.10	19.66 ^b	4.03	20.50	3.29	0.26	7.67	1.18	0.13	11.02	6.08 ^b	0.08	1.32
	autumn	44.11 ^B	2.25	5.10	20.92	1.90	9.08	19.23 ^b	4.00	20.80	3.28	0.29	8.84	1.22	0.15	12.30	6.07 ^b	0.05	0.82
	winter	43.93 ^B	2.14	4.87	22.15	2.17	9.80	18.32	1.69	9.22	3.38	0.16	4.73	1.18	0.25	21.19	6.07 ^b	0.04	0.66
average	43.69	2.66	6.09	21.48	1.76	8.19	18.40	3.57	19.40	3.34	0.23	6.89	1.19	0.18	15.13	6.06	0.07	1.16	
Semi-coarsely ground scalded sausages („Mielonka tyrolska”)	spring	33.70	0.80	2.37	11.72	0.29	2.47	15.15	1.08	7.13	3.32	0.09	2.71	1.08 ^a	0.14	12.96	6.41 ^a	0.03	0.47
	summer	33.26	1.38	4.15	12.10	0.34	2.81	14.26	1.85	12.97	3.25	0.08	2.46	1.27	0.25	19.69	6.38	0.02	0.71
	autumn	33.48	0.80	2.39	11.98	0.18	1.50	14.45	1.12	7.75	3.26	0.11	3.37	1.47 ^b	0.15	10.20	6.36 ^b	0.05	0.39
	winter	32.63	1.16	3.56	11.42	1.04	9.11	14.82	2.02	13.63	3.26	0.16	4.91	1.38 ^b	0.26	18.84	6.40	0.02	0.31
average	33.26	1.08	3.25	11.85	0.56	4.73	14.60	1.51	10.34	3.27	0.10	3.06	1.32	0.24	18.18	6.38	0.04	0.63	

cont. Table 1

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Coarsely ground ham sausages („Szynkowa”)	spring	26.30	2.01	7.64	15.48	1.40	9.04	4.21	1.47	34.92	3.28	0.09	2.74	0.73 ^a	0.16	21.92	6.14	0.03	0.49
	summer	26.97	0.99	3.67	16.09	1.25	7.77	4.74	1.30	27.43	3.29	0.20	6.08	0.97	0.16	16.49	6.15	0.07	1.14
	autumn	26.63	1.22	4.58	15.89	0.59	3.71	4.24	0.96	22.64	3.36	0.25	7.44	0.92	0.20	21.74	6.15	0.04	0.65
	winter	27.22	1.47	5.40	16.47	0.82	4.98	4.64	0.45	9.70	3.26	0.12	3.68	1.01 ^b	0.24	23.76	6.19	0.07	1.13
	average	26.82	1.32	4.92	16.02	1.00	6.24	4.48	1.03	22.99	3.30	0.18	5.45	0.93	0.20	21.51	6.16	0.06	0.97
Dry sausages („Krakowska sucha”)	spring	45.62	1.78	3.90	25.89 ^a	1.78	6.88	14.71	2.18	14.82	4.07	0.31	7.62	1.11	0.09	8.11	6.21	0.08	1.29
	summer	45.13	1.99	4.41	24.62	1.28	5.20	15.95	1.55	9.72	3.88	0.29	7.47	1.26	0.31	24.60	6.17 ^a	0.04	0.65
	autumn	44.78	1.95	4.35	24.47	1.53	6.25	15.23	2.43	15.96	4.11	0.25	6.08	1.35	0.24	17.78	6.18	0.07	1.13
	winter	43.80	3.62	8.26	23.42 ^b	2.25	9.61	15.70	2.24	14.27	3.87	0.48	12.40	1.24	0.13	10.48	6.26 ^b	0.06	0.96
	average	44.78	2.39	5.34	24.49	1.79	7.31	15.47	2.02	13.06	3.98	0.34	8.54	1.26	0.23	18.25	6.21	0.07	1.13
Pork meat blocks (canned ham – „Szynka konserwowa”)	spring	23.45	0.79	3.37	15.43	0.91	5.90	2.78	0.45	16.19	3.43	0.11	3.21	0.43	0.06	13.95	6.29	0.04	0.64
	summer	24.42	0.80	3.27	15.71	0.55	3.50	3.26	0.53	16.26	3.47	0.09	2.59	0.58	0.14	24.14	6.29	0.07	1.11
	autumn	24.14	0.94	3.89	15.19	0.97	6.39	3.54	1.21	34.18	3.38 ^a	0.09	2.66	0.56	0.14	25.00	6.16	0.36	5.84
	winter	23.52	0.58	2.47	15.18	0.68	4.48	2.69	0.64	23.79	3.51 ^b	0.05	1.42	0.48	0.11	22.92	6.35	0.07	1.10
	average	23.95	0.85	3.55	15.38	0.77	5.01	3.12	0.84	26.25	3.44	0.10	2.91	0.53	0.13	24.53	6.27	0.21	3.35

For each product, values followed by different superscript letters in the same column are significantly different, AB – $P \leq 0.01$; ab – $P \leq 0.05$

wowa”) (Table 1). The products with the lowest collagen content were characterized by the highest variation in collagen concentrations. The lowest variation in collagen content was noted in Vienna sausage.

The packaging and labels of the examined products did not contain information on connective tissue proteins used in the production process. The collagen content of the cold meats was low (as confirmed by a laboratory analysis) and connective tissue levels did not exceed the limits set out in the legislation, therefore connective tissue proteins did not have to be listed separately in the list of ingredients (Regulation of the Minister of Agriculture and Rural Development of 10 July 2007). Nevertheless, the information on the collagen/total protein ratio in meat products should be provided to consumers since collagen is considered an incomplete protein with a lower nutritional value because it lacks tryptophan and is low in tyrosine, histidine and total sulfur-containing amino acids (KOŁCZAK 2008). According to LAWRIE (1985), the nutritional value of meat decreases significantly if the ratio of connective tissue nitrogen to total muscle tissue nitrogen is greater than 1.

Average pH values were lowest in the semi-coarsely ground dry sausage („Żywiecka” – 6.06) and in Vienna sausage („parówki” – 6.10), and highest in the semi-coarsely ground sausage („Podwawelska” – 6.37) and semi-coarsely ground scalded sausage („Mielonka tyrolska” – 6.38) (Table 1). The highest variation in pH was noted in canned ham, and the lowest – in „Mielonka tyrolska”. In some of the analyzed products, average pH values varied throughout the year. Vienna sausage, „Podwawelska” sausage and „Krakowska sucha” sausage purchased in winter had a higher pH than the same items bought in summer. „Żywiecka” sausage had the lowest average pH in spring. The average pH of „Mielonka tyrolska” was higher in spring than in autumn. However, it should be stressed that differences in the average pH values between the sausages were small, and the observed statistical significance of differences between means in groups ($P \leq 0.05$) resulted from low variation in the analyzed parameter. The differences in average pH levels noted between the same products purchased during the year and between different products were most probably due to differences in the content and quality of raw materials and functional additives used in the production process (MAKAŁA et al. 2007).

Conclusions

1. The nutritional value of processed pork meat products varied depending on their proximate chemical composition (water, protein, fat and mineral content) and collagen content. The observed differences resulted from the

content and quality of raw materials and functional additives used in the production process.

2. The examined products differed in their content of basic chemical components and collagen. Fat content and water content varied within the widest and the narrowest range, respectively.

3. The results of this study indicate that consumers should be informed about the chemical composition and nutritional value of cold cuts available on the market.

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THE USE OF OXYGEN ABSORBERS FOR PACKAGING RIPENED CHEESE¹

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Key words: ripened cheese, shelf-life of cheese, vacuum packaging, modified atmosphere packaging, active packaging, oxygen absorbers.

Abstract

Oxygen absorbers (AP) were used for packaging portioned Gouda cheese. Vacuum packaged cheese (VP), cheese packaged under modified atmosphere (MAP) and cheese packaged in ambient air (control samples) were compared. Cheese samples were chill stored for 30, 60 and 90 days. The gas composition in cheese packaging was analyzed, the counts of coliform bacteria, yeasts and molds in cheese were determined, and the sensory attributes of cheese were evaluated in fresh cheese and in samples stored for 30, 60 and 90 days. The oxygen content of all types of packaging remained very low throughout the experiment, ranging from 0.00% in the packaging with oxygen absorbers to 2.67% in the control packaging. Oxygen absorbers effectively lowered the oxygen content of packaging to trace amounts. The results of microbiological analyses and sensory evaluations of stored cheese samples indicate that active packaging is nearly as effective in extending the shelf-life of ripened cheese as vacuum packaging and modified atmosphere packaging.

WYKORZYSTANIE ABSORBERÓW TLENU W PAKOWANIU SERÓW DOJRZEWAJĄCYCH

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Słowa kluczowe: sery dojrzewające, trwałość serów, pakowanie próżniowe, pakowanie w modyfikowanej atmosferze, pakowanie aktywne, absorbery tlenu.

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Abstrakt

W pracy zastosowano absorbery tlenu (AP) do pakowania kawałków serów typu Gouda. Dla porównania zapakowano sery próżniowo (VP), w modyfikowanej atmosferze (MAP) oraz powietrzu (próbki kontrolne). Próbki przechowywano w warunkach chłodniczych przez 30, 60 i 90 dni. W tych odstępach czasu oznaczano skład atmosfery w opakowaniach serów, a w serach oznaczano liczbę bakterii z grupy coli, liczbę drożdży i pleśni oraz oceniono cechy sensoryczne tych produktów. Wyniki badań wykazały, że w całym okresie przechowywania zawartość tlenu we wszystkich opakowaniach była bardzo niska i wahała się w zakresie 0,00% w opakowaniach z absorberem tlenu do 2,67% w opakowaniach próbek kontrolnych. Pochłaniacze tlenu skutecznie obniżały zawartość tlenu w opakowaniu do ilości śladowych. Wyniki badań mikrobiologicznych serów przechowywanych oraz zmiany cech sensorycznych tych serów wykazały, że pakowanie aktywne może być wykorzystane do pakowania serów dojrzewających prawie z takim samym skutkiem jak pakowanie próżniowe i w modyfikowanej atmosferze.

Introduction

Vacuum packaging and modified atmosphere packaging are popular packaging methods in the food processing industry (ROSENTHAL et. al. 1991, FLOROS et. al. 2000, GONZALEZ-FANDOS 2000, MORTENSEN et. al. 2003, DEV-LIEGHERE et. al. 2004, PANFIL-KUNCEWICZ et. al. 2012), which are also used to package portioned and sliced ripened cheese. In vacuum packaging, excess air is pumped out and the packaging is tightly sealed. In the modified atmosphere method, excess air is removed and a neutral gas or a mixture of neutral gases is introduced to the packaging to control or destroy undesirable microflora and prevent the oxidation of cheese ingredients. Vacuum packaging is a simple technique, but it can lead to undesirable changes in the structure and appearance of cheese (SEVERINI et. al. 1998). For this reason, modified atmosphere packaging is becoming an increasingly popular alternative. The atmosphere inside packaging can be modified with the involvement of various methods, including by:

- creating a vacuum inside the packaging and reducing it with neutral gases,
- filling the packaging with neutral gas and tightly sealing the packaging,
- use of packaging materials with selective gas permeability,
- placing an inedible substance which absorbs and/or emits gas inside the packaging (PANFIL-KUNCEWICZ et. al. 2012).

The latter method is used to produce a new generation of active packaging which is becoming increasingly popular in the food processing industry. In Poland, the active packaging concept has not yet been applied in practice. In this type of packaging, various substances which absorb or release oxygen, carbon dioxide and ethylene (iron powder, ascorbic acid, calcium oxide, calcium hydroxide, oxidases, etc.) are placed inside the packaging. In the latest

solutions, those substances are built into the packaging material. Attempts have also been made to use gas absorbers and emitters in cheese packaging (SUPPAKUL et. al. 2003, RODRIGUEZ-AGUILERA and OLIVEIRA 2009, PANFIL-KUNCEWICZ et. al. 2012).

The aim of this study was to evaluate the effectiveness of oxygen absorbers in extending the shelf-life of portioned ripened cheese.

Materials and Methods

The experimental materials comprised Gouda ripened cheeses supplied by three dairy plants. In the laboratory of the Department of Dairy Science and Quality Management, the products were divided into portions of approximately 150 g and were packaged in PA//PA//PE/PE-EVA bags, 70 μ m thick (as recommended by Multivac). Cheese samples were packaged in the Multivac C300 single-chamber machine with the use of four different methods: I – active packaging (AP) (the air atmosphere was modified by placing a sachet of ATCO FT 210 oxygen absorber inside the packaging), II – modified atmosphere packaging (MAP) (40% CO₂ and 60% N₂, gas volume – approximately 300 cm³), III – vacuum packaging (VP), IV – packaging in ambient air (control samples). Packaged cheese portions were chill stored (6 \pm 0.5°C) for up to 90 days.

Samples of cheese stored for 24 hours (referred to as „fresh cheese”) and for 30, 60, 90 days were analyzed to determine the percentage gas composition of packaging using the PBI-Dansensor CheckMate3 gas analyzer. Plate counts of coliform bacteria were determined on VRBL medium (Merck) according to Standard PN-ISO 4832:2007, and yeast and mold counts were determined on YGC selective medium (Merck) according to Standard PN-ISO 6611:2007. An organoleptic evaluation was performed using the grading approach, based on a six-point rating scale (1–6) (PN-ISO 4121:1998; PN-ISO 6658:1998). Five quality attributes were evaluated in the sensory analysis: color, eye formation, consistency, aroma and taste, in accordance with Polish Standard PN-68/A-86230 and IDF Standard 99C:1997. Coded cheese samples were assessed by a team of five panelists trained in sensory analysis. The results of the above analyses were expressed as mean values for three groups of cheese samples.

Data were analyzed statistically using Microsoft Excel 2007 software package, and means and standard deviations were calculated. The significance of the effects exerted by the experimental factors on the analyzed parameters was estimated by two-way analysis of variance (ANOVA) at a significance level of $p \leq 0.05$.

Results and Discussion

The gas composition of cheese packaging varied subject to the packaging method and storage time (Table 1). After 24 hours of storage, the oxygen content of control samples decreased (in comparison with air) from 21.00% to 17.90%, and carbon dioxide content increased to 5.65%. The nitrogen content was determined at 76.45%. After 30 days of storage, oxygen content decreased to 2.67%, and after 60 and 90 days, trace amounts of oxygen were determined at 0.1% and 0.02%, respectively. The drop in oxygen content was accompanied by an increase in carbon dioxide concentrations that reached 22.70%, 21.58% and 19.92% after 30, 60 and 90 days of storage, respectively. Nitrogen content, which was determined by O₂ and CO₂ concentrations, was similar to that found in air, and it ranged from 74.63% to 80.06%. The observed changes in gas composition inside the packaging resulted from the metabolic changes of cheese microflora, in particular aerobic microorganisms – yeasts and molds.

The oxygen content of packages containing oxygen absorbers was determined at 0.12% after 24 hours of storage, and oxygen was completely eliminated from samples stored for 30, 60 and 90 days. The average carbon dioxide

Table 1
Changes in the gas composition of cheese packaging subject to the packaging method and storage time

Packaging method	After 24 h		30 days			60 days			90 days			
	Gas content [%]											
	$\bar{x} \pm s$											
	O ₂	CO ₂	N ₂	O ₂	CO ₂	N ₂	O ₂	CO ₂	N ₂	O ₂	CO ₂	N ₂
Control	17.90 ±1.09	5.65 ±1.34	76.45 ±0.25	2.67 ±2.51	22.70 ±5.74	74.63 ±3.64	0.10 ±0.10	21.58 ±4.36	78.32 ±4.43	0.02 ±0.02	19.92 ±1.89	80.06 ±1.89
AP	0.12 ±0.02	0.37 ±0.37	99.51 ±0.40	0.00 ±0.00	1.51 ±1.26	98.49 ±1.26	0.00 ±0.00	1.38 ±0.64	98.62 ±0.64	0.00 ±0.00	1.14 ±0.92	98.86 ±0.92
MAP	0.27 ±0.01	33.80 ±2.26	65.93 ±2.25	0.43 ±0.67	33.38 ±3.89	66.19 ±4.23	0.24 ±0.31	31.65 ±2.50	68.11 ±2.79	0.23 ±0.36	28.98 ±0.72	70.79 ±0.86
VP	not analyzed											
statistical results												
Type of gas						Packaging method			Storage time			
Oxygen						*			*			
Carbon Dioxide						*			*			
Nitrogen						*			*			

Explanatory notes:

Mean value + standard deviation $\bar{x} \pm S.D$

* – statistically significant differences at $p \leq 0,05$

NS – no significant differences at $p \leq 0,05$

Source: Own study

content of packaging was determined at 0.37% in samples and at 1.51%, 1.38% and 1.14% in samples stored for 30, 60 and 90 days, respectively. Cheese samples in packages containing oxygen absorbers were practically stored in a nitrogen atmosphere (approximately 98.50%).

The composition of modified atmosphere in the packaging after 24-hour storage of cheese was as follows: O₂ – 0.27%, CO₂ – 33.80%, N₂ – 65.93%. During storage, no significant changes in oxygen levels were observed in the modified atmosphere, whereas CO₂ content decreased gradually to 33.38% after 30 days, 31.65% after 60 days and 28.98% after 90 days of storage. The above can be probably attributed to the dissolution of carbon dioxide in cheese and its permeation through the packaging material. The content of nitrogen inside the packaging increased with a decrease in carbon dioxide concentrations.

A statistical analysis of the gas composition of cheese packaging, depending on the packaging method and storage time, revealed significant ($p \leq 0.05$) relationships between the experimental factors and the O₂, CO₂ and N₂ content of packaging in cheese samples stored for 90 days (Table 1) .

The growth rates of the analyzed microbial populations were also affected by the applied packaging method. Coliform bacteria counts, which are an indicator of the hygienic quality of cheese, were determined at 1.93 log CFU/g in fresh cheese samples (Figure 1). In general, the growth rates of coliform bacteria in stored cheese packaged with the use of different methods (AP, MAP, VP) were somewhat lower than in control samples. None of the analyzed packaging methods had a clear influence on the proliferation of coliform bacteria.

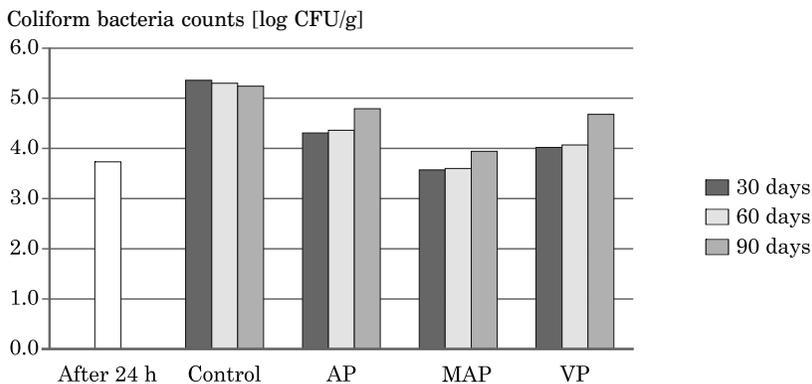


Fig. 1. Changes in coliform bacteria counts in cheese samples subject to the packaging method and storage time

Source: Own study

Greater variations were noted in yeast cell counts (Figure 2). In fresh cheese samples, the average yeast counts were determined at 4.23 log CFU/g. After 30 days of storage, the greatest increase in the number of yeast cells was observed in control samples where it reached 6.01 log CFU/g on average and remained fairly stable after 60 and 90 days of storage. The lowest increase in yeast populations was reported in cheese samples packaged under modified atmosphere. After 30 and 60 days of storage, yeast counts were similar to those determined in fresh cheese, and a minor increase in the number of yeast cells was noted only after 90 days of storage (4.77 log CFU/g on average). In cheese packaged with oxygen absorbers and under vacuum, the size of yeast populations increased steadily to 5.19 and 5.33 log CFU/g, respectively, after 90 days of storage. Modified atmosphere packaging (40% CO₂, 60% N₂) had the most inhibitory effect on the proliferation of yeasts.

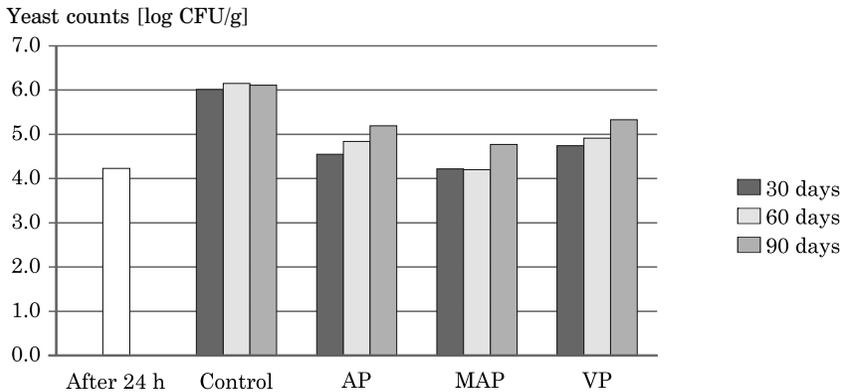


Fig. 2. Changes in yeast counts in cheese samples subject to the packaging method and storage time
Source: Own study

Changes in mold counts in stored cheese packaged with the use of different methods were similar to those noted in yeast counts (Figure 3). Average mold counts in fresh cheese samples were determined at 3.73 log CFU/g. In samples packaged in air, mold counts increased to 5.36, 5.30 and 5.24 log CFU/g after 30, 60 and 90 days of storage, respectively. The growth of molds was most effectively suppressed by modified atmosphere packaging. The number of mold filaments in samples packaged under modified atmosphere and stored for 60 days was similar to that observed in fresh cheese, and a minor increase was reported only after 90 days of storage. Vacuum packaged samples were also characterized by a small increase in mold counts in comparison with fresh cheese. In VP samples, mold counts were determined at 4.02 log CFU/g after 30 days, 4.07 log CFU/g after 60 days of storage, and an increase to 4.68 log CFU/g was reported after 90 days. In packages containing oxygen absorbers, mold

proliferation proceeded at a slower rate than in control samples, but it was somewhat more intense than in MAP and VP samples.

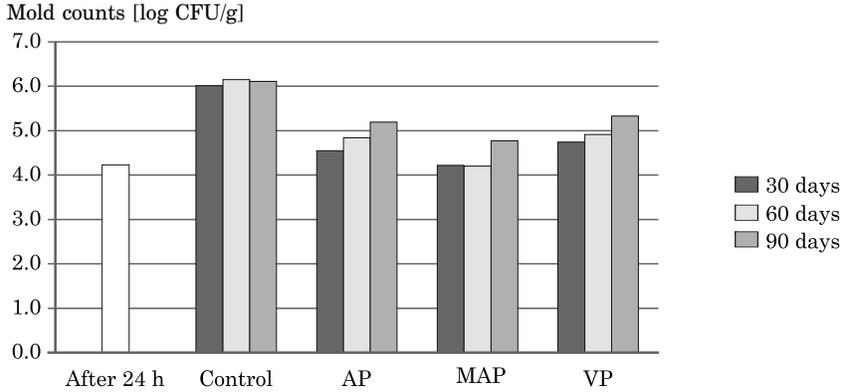


Fig. 3. Changes in mold counts in cheese samples subject to the packaging method and storage time
Source: Own study

A statistical analysis of the counts of contaminating microflora in cheese samples indicated that only the packaging method had a significant effect on yeast and mold counts in the analyzed cheeses. Storage time had no significant effect on changes in the counts of undesirable microflora in cheese samples throughout the experimental period (Table 2).

Table 2

Counts of coliforms, yeasts and molds

Parameter	Statistical results	
	Packaging method	Storage time
Coliforms	NS	NS
Yeasts	*	NS
Molds	*	NS

Explanatory notes as in Table 1
Source: Own study

A sensory evaluation of cheese samples packaged by different methods revealed that the sensory attributes of cheese packaged in air deteriorated most rapidly in comparison with AP, MAP and VP samples (Table 3). In general, the above samples received similar scores in the evaluation of sensory attributes performed at 30-day intervals, and the greatest similarities were observed between packages containing oxygen absorbers and modified atmosphere packages. Vacuum packaging was somewhat more effective in preserv-

Table 3
Sensory attributes of cheese samples subject to the packaging method and storage time (days)

Weight coefficient W_w	Quality attributes	Control			AP			MAP			VP		
		30	60	90	30	60	90	30	60	90	30	60	90
statistical measures $\bar{x} \pm s$													
0.15	color	4.30 ± 0.36	4.10 ± 0.52	4.17 ± 1.37	4.33 ± 0.35	4.27 ± 0.55	4.27 ± 1.10	4.00 ± 0.78	3.70 ± 1.11	3.50 ± 0.92	4.33 ± 0.31	4.47 ± 0.45	4.80 ± 0.20
0.15	eye formation	4.07 ± 0.12	4.13 ± 0.23	4.03 ± 0.06	4.37 ± 0.35	3.87 ± 0.81	4.00 ± 0.00	4.40 ± 0.17	4.13 ± 0.32	4.17 ± 0.29	3.83 ± 0.12	4.00 ± 0.10	3.80 ± 0.10
0.25	consistency	4.23 ± 0.59	4.47 ± 0.45	4.03 ± 0.81	4.47 ± 0.45	4.50 ± 0.46	4.37 ± 0.71	4.33 ± 0.35	4.47 ± 0.42	4.40 ± 0.44	4.50 ± 0.40	4.87 ± 0.57	4.33 ± 0.64
0.20	aroma	5.07 ± 0.31	4.53 ± 0.15	4.07 ± 0.36	4.73 ± 0.15	4.77 ± 0.21	4.53 ± 0.15	4.73 ± 0.32	4.17 ± 0.70	3.90 ± 0.66	4.77 ± 0.15	4.50 ± 0.20	4.40 ± 0.10
0.25	taste	4.77 ± 0.15	3.80 ± 0.10	3.53 ± 0.47	4.33 ± 0.06	4.07 ± 0.21	3.63 ± 0.15	4.37 ± 0.35	3.87 ± 0.72	3.40 ± 0.53	4.43 ± 0.15	4.37 ± 0.35	3.77 ± 0.38
$\Sigma=1$	total score	4.52	4.21	3.43	4.45	4.32	4.15	4.38	4.09	3.88	4.41	4.48	4.20
statistical results													
Factor	Parameter												
	color	eye formation			consistency			aroma			taste		
Packaging method	NS												
Storage time	NS												

Explanatory notes as in Table 1

Source: Own study

ing the sensory attributes of cheese. Samples packaged with oxygen absorbers scored relatively high in respect of their taste and aroma. Sensory attributes were not significantly correlated with the microbiological quality of the studied cheese. The lowest increase in coliform bacteria, yeast and mold counts during storage was observed in samples packaged under modified atmosphere, and MAP samples received the lowest scores for taste and aroma, which could be attributed to high CO₂ concentrations inside the packaging.

A statistical analysis of the results of sensory evaluation of cheese samples revealed no significant correlations between the color, eye formation and consistency of cheese and the packaging method throughout the storage period (Table 3). The packaging method and storage time had a significant ($p \leq 0.05$) effect on the aroma and taste of stored cheese.

The results of this study suggest that active packaging, modified atmosphere packaging and vacuum packaging effectively extend the shelf-life of cheese. Modified atmosphere packaging had the most inhibitory effect on undesirable microflora, and the effectiveness of VP and AP methods in reducing the proliferation of undesirable microorganisms was only insignificantly lower.

The gas composition of cheese packaging has been studied intensively in recent years, and the reported results are often contradictory. The earliest studies demonstrated that the packaging containing 100% CO₂ effectively prolongs the shelf-life of cottage cheese, although some authors argued that high concentrations of carbon dioxide deteriorate the taste and aroma of cheese (KOSIKOWSKI and BROWN 1973, MANIAR et. al. 1994, ALVES et. al. 1996, MANNHEIM and SOFFER 1996, SEVERINI et. al. 1998, PANFIL-KUNCEWICZ et. al. 2006). Subsequent research revealed that hard and semi-hard ripened cheese is most effectively stored in a mixture of nitrogen and carbon dioxide where the proportion of CO₂ ranges from 10% to 50%. The above atmosphere inhibits the growth of undesirable microflora and slows down enzymatic changes in cheese without affecting the taste and aroma of packaged products (ELIOT et. al. 1998, PINTADO and MALCATA 2000, FAVATI et. al. 2007, PANTALEAO et. al. 2007, PAPAIOANNOU et. al. 2007). The relatively high differences in the proposed gas composition are due to the fact that a wide variety of cheeses produced in many countries and regions differ significantly in their physicochemical, microbiological and sensory attributes. Those differences are taken into account when designing the gas composition of packaging to extend the shelf-life of packaged foods.

In our study, oxygen absorbers eliminated oxygen from packages, thus contributing to an increase in nitrogen concentrations to approximately 98.50%. Carbon dioxide content was reduced below 2%. The degree to which oxygen absorbers inhibited the growth of yeasts, molds and coliform bacteria

in cheese was comparable with that noted in vacuum packaging, and somewhat lower than in modified atmosphere packaging (40% CO₂ and 60% N₂). Oxygen absorbers are recommended for removing residual oxygen from modified atmosphere packages. Our findings indicate that oxygen absorbers can also be used independently for extending the shelf-life of portioned ripened cheese.

The analyzed fresh cheese was characterized by low counts of coliform bacteria, which suggests that cheese of high microbiological quality can be effectively packaged with the use of oxygen absorbers without prior atmosphere modification. The results of a sensory evaluation confirmed the above observation. It should be noted, however, that this study analyzed only three types of Gouda cheese supplied by different manufacturers. Further work involving a greater number of cheese products is needed to investigate the effectiveness of oxygen absorbers in more detail.

Conclusions

1) The gas composition of cheese packaging was affected by the packaging method and storage time.

2) Modified atmosphere packaging was most effective in reducing microbial counts in cheese.

3) Oxygen absorbers can be used independently to modify the composition of the internal atmosphere in packages of portioned ripened cheese. Their effectiveness in prolonging the shelf-life of cheese is comparable to that of vacuum packaging and modified atmosphere packaging.

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**EFFECT OF MECHANICAL TREATMENT
ON THE PARTICLE SIZE DISTRIBUTION
AND RHEOLOGICAL PROPERTIES OF TOMATO
CONCENTRATE**

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Key words: tomato concentrate, mechanical treatment, particle size distribution, rheology.

Abstract

In this study effect of mechanical treatment on the particle size distribution and rheological properties of tomato concentrate has been investigated. Tomato paste samples after dilution to 17% dry matter content were treated in blender for the time of 1, 3, 6, 10 and 15 minutes. The Bostwick consistency, particle size distribution, steady-shear viscosity and dynamic rheological measurements were performed. As expected, the mechanical treatment reduced the mean particle diameter, but the shape of distribution curve did not change substantially, it was only shifted towards small particles. The disruption in a blender improved tomato concentrate consistency and also an increase of apparent viscosity, values of G' and G'' moduli and the Bostwick value were observed.

**WPLYW OBRÓBKI MECHANICZNEJ NA ROZKŁAD WIELKOŚCI CZĄSTEK
I WŁAŚCIWOŚCI REOLOGICZNE PRZECIERU POMIDOROWEGO**

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Słowa kluczowe: przecier pomidorowy, obróbka mechaniczna, rozkład wielkości cząstek, reologia.

Abstrakt

Celem pracy było ustalenie wpływu obróbki mechanicznej na rozkład wielkości cząstek i właściwości reologiczne przecieru pomidorowego. Próbki koncentratu pomidorowego po rozcieńczeniu do 17% zawartości suchej substancji poddano obróbce mechanicznej za pomocą blendera

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w czasie 1, 3, 6, 10 i 15 minut. Dokonano oceny konsystencji Bostwick'a uzyskanych próbek i przeprowadzono pomiary rozkładu wielkości cząstek, a w ramach badań reologicznych wyznaczono krzywe płynięcia i wykonano testy oscylacyjne. Jak można się było spodziewać, w wyniku obróbki mechanicznej zmniejszyła się średnia średnica cząstek, ale rozkład wielkości cząstek zachował swój kształt, został tylko przesunięty w kierunku małych cząstek. W wyniku ścinania zachodzącego w blenderze stwierdzono poprawę konsystencji przecieru pomidorowego – zaobserwowano wzrost lepkości pozornej, wyższe wartości modułów G' i G'' , oraz konsystencji Bostwick'a.

Introduction

Tomato is one of the most widely grown and willingly consumed vegetable all over the world. Only a part of the global tomato production is consumed as fresh, the remaining part is directed to the food industry where it is processed into tomato paste (SÁNCHEZ et al. 2003). Concentrated – i.e. dehydrated paste, prepared for a long storage, is a stable material which contains not less than 24% total solids. After dilution, the obtained suspension, defined as „tomato concentrate”, is used to produce ketchup, soups, sauces and juices (DOGAN et al. 2002).

Quality of tomato paste is very important, because quality of the finish product depends in a large degree on its properties. There are many factors affecting the quality of the paste: tomato variety and ripeness, water insoluble/soluble solids content, particle size distribution and particle shape (TIBÁCK et al. 2009, BAYOD et al. 2008). Consistency and other flow properties of tomato products -i.e. the rheological properties decide about acceptability of tomato products by consumers (SHAROBA et al. 2005). These properties are also substantial for several unit operations involved in tomato processing, such as heating, pumping and mixing (SHARMA et al. 1996). Consistency of tomato products is typically determined using the Bostwick consistometer, a traditional device used for quality control (PERONA 2005, PROBOLA et al. 2011). However, in order to assess the complex rheological properties of tomato products it is necessary to carry out precise instrumental measurements (TABILO-MUNIZAGA and BARBOSA-CÁNOVAS 2004).

Concentrating tomato juice to paste during the tomato season makes it possible to preserve it and store, whereas subsequent dilution enables formulation of various value-added products. However, this operation is known to result in a loss of consistency. Heating after reconstitution restores consistency to the same level as measured before storage (ANTHON and BARRETT 2010). The objective of the present study was to evaluate to what degree shearing in a blender will improve consistency of tomato concentrate and what effect this method of treatment will have on the particle size distribution and rheological properties of tomato concentrate.

Materials and methods

The commercial tomato paste, of nominal dry matter content 28–30%, was purchased in a local supermarket. After determination of total solids content (measure by drying 2 grams of tomato paste in an oven at 100°C for 24h – TSC=28,49±0,39%), tomato paste was diluted with distilled water. Obtained 17 g/100 g (w/w) tomato concentrate was divided into 6 samples weighting 300 g each: a control one and the remaining five were submitted to homogenization in a blender (MMB1001, Bosch, Germany) for the time of 1, 3, 6, 10 and 15 minutes. The samples were marked as follows: TP00 (the control sample without mechanical treatment) and TP01, TP03, TP06, TP10, TP15 respectively.

The particle size distribution in the samples was measured by light scattering method (Malvern Mastersizer 2000, Malvern Instruments Ltd., UK), for the pastes sheared for 1, 3, 6, 10 and 15 minutes. Following magnitudes were determined:

D(v, 0.1) – the value of particle size below which there is 10% of sample volume,

D(v, 0.5) – the value of particle size below which there is 50% of sample volume,

D(v, 0.9) – the value of particle size below which there is 90% of sample volume,

Span – evaluation of distribution width, calculated as the difference D(v, 0.9) and D(v, 0.1) divided by D(v, 0.5),

D[3,2] – the area-based mean diameter,

D[4,3] – the volume-based mean diameter,

SSA (Specific Surface Area) – the total surface area of the particles per mass unit.

The consistency of the concentrate was measured using the Bostwick consistometer (Central Scientific, USA). The distance travelled by the concentrate in the time of 30 s was measured in cm and expressed as the Bostwick consistency.

Rheological measurements were carried out using rotational rheometer (Rheostress RS1, Haake). For steady state measurements a concentric cylinder system Z34 DIN 53019 was used. Flow curves were obtained at increasing shear rates from 0 to 500 s⁻¹ during 150 s and decreasing from 500 s⁻¹ to 0 at the same time.

The relationship σ vs. $\dot{\gamma}$ obtained at increasing shear rates was approximated with the Herschel-Bulkley model:

$$\sigma = \sigma_{HB} + K \cdot \dot{\gamma}^n \quad (1)$$

where σ is shear stress (Pa), σ_{HB} is yield stress (Pa), K is consistency coefficient (Pa sⁿ), $\dot{\gamma}$ is shear rate (s⁻¹) and n is flow behaviour index.

The viscoelastic properties of the samples were evaluated by oscillatory tests, which were carried out using a stainless steel cone-plate geometry (60 mm diameter and angle 2°). The frequency sweep measurements were performed within the linear viscoelastic region, at constant stress amplitude 0.1 Pa in the range of frequencies 0.01–10 Hz.

All measurements were made in triplicate for each sample, results presented were the means and standard deviations of each experiment. A one-way analysis of variance (ANOVA) and Tukey's test were used to establish the significance of differences among the averages at the 0.95 level of confidence. The statistical analysis were performed using STATISTICA 9 (StatSoft, Inc., Tulsa, USA).

Results and discussion

Figure 1 shows the effect of mechanical treatment time on particle size distribution in the tomato concentrate, whereas particle size distribution data of all samples were collected in Table 1. To preserve readability of the diagrams the results for three samples only are presented – i.e.: TP00 – without mechanical treatment, T06 and TP15 – treated for 6 and 15 minutes. As expected, the mechanical treatment reduced the mean particle diameter: the area-based mean diameter $D[3,2]$ has been reduced by 83%, while the volume-based mean diameter $D[4,3]$ has been reduced by 43% (Table 1). Similar reduction occurred after high-pressure homogenization at 50 MPa (AUGUSTO et al. 2012). Because the $D[4,3]$ is greatly influenced by large particles and the $D[3,2]$ is more influenced by smaller ones (LOPEZ-SANCHEZ et al. 2011, BENGTTSSON and TORNBERG 2011), the result obtained indicates a considerable increase in the number of small particles. As shown on Figure 1 the shape of distribution curve did not change substantially after mechanical treatment (the span of the distribution for all the samples was similar – Table 1), however with an increase of mechanical treatment time the distribution was shifted towards small particles. For sample TP00 the value of $D(v, 0.5)$ is equal 313 μm , decreasing with time increase of mechanical treatment, whereas for sample TP15 it reaches the value of 182 μm . With the increasing time of mechanical treatment values of parameters the characteristic diameters of particles decrease and simultaneously the Specific Surface Area (SSA) becomes greater.

Figure 2 shows flow curves at increasing and decreasing shear rate of samples T00, T06 and T15. The tomato concentrate flow was well described ($R^2 > 0.98$) by the Herschel-Bulkley model, the rheological parameters of this model σ_{HB} , K and n are collected in Table 2. All samples tested show non-Newtonian,

Table 1

Particle size distribution parameters of all tomato pulp samples tested

Sample	D(v, 0.1) μm	D(v, 0.5) μm	D(v, 0.9) μm	Span	D[3,2] μm	D[4,3] μm	SSA m/g
TP00	99.3±1.2 ^a	313±3 ^e	661±7 ^a	1.81±0.02 ^a	170±2 ^a	351±5 ^a	0.034±0.001 ^a
TP01	95.6±1.8 ^b	297±6 ^b	596±19 ^b	1.66±0.06 ^b	169±3 ^a	326±12 ^b	0.036±0.002 ^{ab}
TP03	94.3±3.1 ^b	275±4 ^c	543±13 ^c	1.61±0.08 ^b	160±2 ^b	301±6 ^c	0.039±0.002 ^b
TP06	77.6±0.8 ^c	241±11 ^d	470±11 ^d	1.63±0.04 ^b	133±2 ^c	260±7 ^d	0.049±0.001 ^c
TP10	55.2±0.5 ^d	207±2 ^e	426±4 ^e	1.77±0.03 ^a	38±1 ^d	228±15 ^e	0.157±0.005 ^d
TP15	41.1±1.1 ^e	182±4 ^f	379±8 ^f	1.83±0.05 ^a	29±1 ^e	200±4 ^f	0.204±0.003 ^e

Averages on the same column which are significantly different ($p < 0.05$) are indicated with a different superscript letter

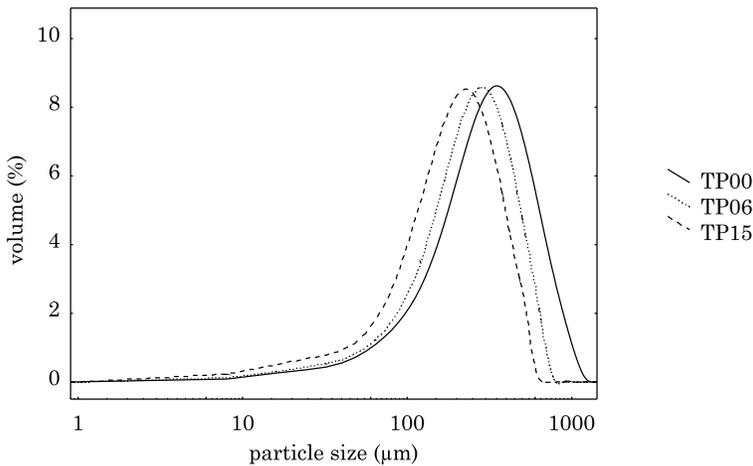


Fig. 1 Effect of mechanical treatment on the particle size distribution in tomato concentrate

shear-thinning behaviour with yield stress. Flow curves at decreasing shear rate unveiled thixotropic behaviour of the concentrate. This property is typical for fruit and vegetables suspensions and has been described for jaboticaba pulp (SATO and CUNHA 2009), chilli puree (AHMED et al. 2000) and mango pulp (BHATTACHARYA S. 1999). As shown on Figure 2, the increase in time of mechanical treatment caused increase in apparent viscosity of the tomato concentrates. It is confirmed by increased values of consistency coefficient K (Table 2), which can be considered as a measure of the resistance to flow (HEYMAN et al. 2010). YOO and RAO (1996) found increased viscosities for reduced particle sizes. The authors explained this as due to the shorter interaction distance between small particles. As shown in Table 2, all flow behaviour index values (n) were below unity, which indicates pseudoplastic properties of the studied concentrates. Those values decreased with the increase in time of mechanical treatment. Similarly, with the increase in time of disruption, weakened thixotropy behaviour of tomato concentrate – the thixotropy area decreased.

Table 2

Tixotropy area and Herschel-Bulkley parameters of all samples tested

Sample	σ_{HB} [Pa]	K [Pas ⁿ]	n	R ²	Thixotropy area [Pa/s]
TP00	24.7±0.4 ^a	0.76±0.06 ^a	0.675±0.015 ^a	0.99	3282±67 ^a
TP01	26.7±0.9 ^b	0.90±0.28 ^b	0.622±0.022 ^b	0.98	3231±25 ^a
TP03	27.6±1.2 ^{b,c}	1.64±0.02 ^c	0.580±0.008 ^c	0.99	3100±15 ^b
TP06	28.9±1.6 ^c	3.53±0.75 ^d	0.473±0.019 ^d	0.99	2643±46 ^c
TP10	26.6±1.1 ^b	7.14±0.43 ^e	0.379±0.031 ^e	0.98	2327±49 ^d
TP15	27.7±1.7 ^{b,c}	5.05±0.21 ^f	0.421±0.012 ^f	0.99	1852±16 ^c

Averages on the same column which are significantly different ($p < 0.05$) are indicated with a different superscript letter

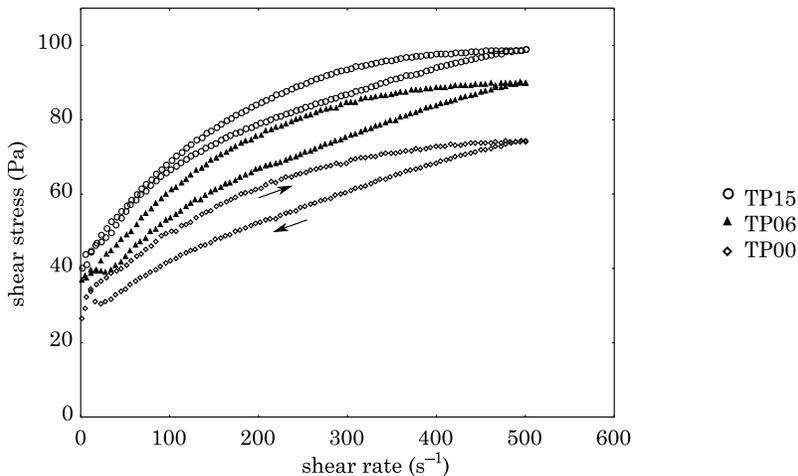


Fig. 2 Flow curves of tomato concentrates for samples TP00, TP06 and TP15

Figure 3 shows the effect of mechanical treatment on Bostwick consistency of tomato concentrate samples. An increasing time of mechanical treatment produced smaller Bostwick values, which means a thicker consistency of the product. It is compatible with increased values of consistency coefficient K , high coefficients of correlation of apparent viscosity and Bostwick values were repeatedly described (MILCZAREK and MCCARTHY 2006, MAZAHARI TEHRANI and GHANDI 2007).

Figure 4 shows storage (G') and loss (G'') moduli as a function of applied frequency for samples TP00, TP06 and TP15. For those and all remaining samples both G' and G'' moduli increased slightly with increasing frequencies. Storage moduli (G') were always greater than the loss moduli (G''), which is typical of gels (TONON et al. 2009). Since the ratio G'/G'' was greater than 1 and less than 10, all concentrates could be characterized as a material with weak

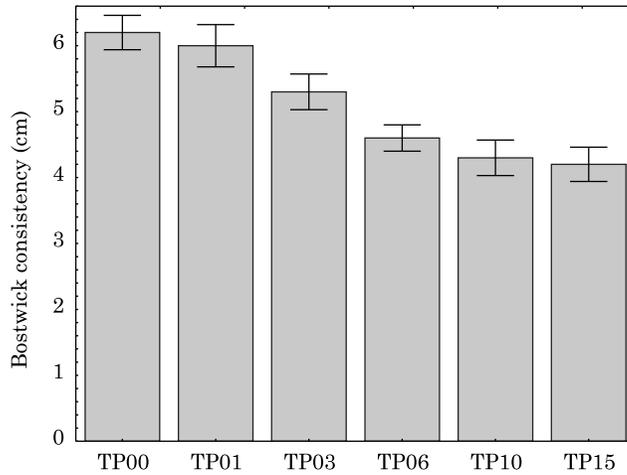


Fig. 3 Effect of mechanical treatment on Bostwick consistency of all tomato concentrate samples. Error bars represent standard deviation

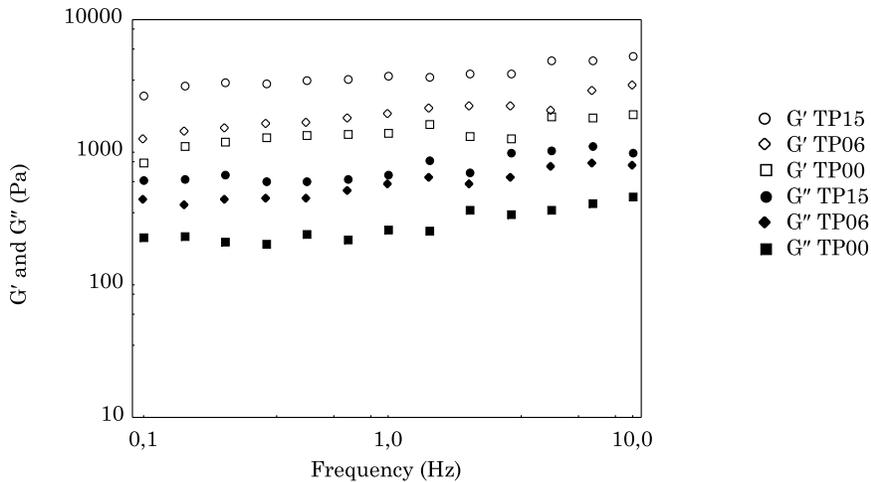


Fig. 4 Storage (G') and loss (G'') moduli for samples TP00, TP06 and TP15 as a function of applied frequency

gel-like behavior (ALVAREZ and CANET 2013). For all samples of 17% tomato concentrate an increasing time of mechanical treatment caused greater values of both G' and G'' moduli.

In conclusion, the process of mechanical treatment had a considerable effect on particle size distribution, which affected microstructure of the concentrate and in result caused changes of rheological properties of the

suspensions formed. The increase in time of mechanical treatment of 17% tomato concentrate caused increase in apparent viscosity, Bostwick consistency, values of storage (G') and loss (G'') moduli and decrease of the thixotropy area.

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INFORMATION ON FOOD FORTIFICATION WITH BIOACTIVE COMPOUNDS IN OBSERVATION AND CONSUMER STUDIES

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Key words: food fortification, bioactive compounds, labeling, consumer knowledge.

Abstract

This study was aimed at evaluating the load of information displayed on packages of fortified food products and at assessing consumer knowledge on fortified foods and information about them provided via package.

The study demonstrated that on the Olsztyn market the most frequently fortified groups of food products were juices and non-alcoholic drinks as well as dairy and cereal products. Labels of the evaluated packages were comprehensive and in most cases correct. The nutritive value was provided on packages of all analyzed products. The products were fortified with permitted components including mainly vitamins and minerals. The consumer survey showed that the selected target group knew the concept of fortified food however in the analyzed group of respondents this knowledge was not reflected in the consumption of these food products. Women had richer knowledge on food fortification.

INFORMACJA O WZBOGACANIU ŻYWNOSCI SKŁADNIKAMI BIOAKTYWNYMI W BADANIACH OBSERWACYJNYCH I KONSUMENCKICH

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Słowa kluczowe: wzbogacanie żywności, znakowanie, wiedza konsumentka.

Abstrakt

Celem niniejszej pracy była ocena warstwy informacyjnej opakowań produktów wzbogaconych, a także poznanie opinii konsumentów na temat żywności wzbogaconej i informacji o niej przekazywanej przy pomocy opakowania.

Otrzymane wyniki wykazały, że najczęściej wzbogaconymi grupami produktów spożywczych na rynku olsztyńskim są soki i napoje bezalkoholowe, przetwory mleczne oraz produkty zbożowe. Etykiety ocenianych opakowań były czytelne i w większości przypadków poprawnie oznakowane. Wszystkie poddane analizie produkty posiadały podaną wartość odżywczą oraz do wzbogacania użyto dozwolonych składników. Głównymi składnikami wzbogacającymi używanymi podczas fortyfikacji były przede wszystkim witaminy i składniki mineralne. Z przeprowadzonych badań konsumenckich wynika, iż wybrana grupa docelowa zna pojęcie żywności wzbogaconej, jednak w badanej grupie respondentów nie przekłada się to na jej spożycie. Wyższy poziom wiedzy na temat wzbogacania żywności posiadały kobiety.

Introduction

The approach to nutritional issues has recently been subject to considerable changes. Proper nutrition affects health status and contributes to the prevention of many diseases. It is responsible for providing nutrients (including vitamins and minerals) and influences proper body functioning. For this reason, apart from the primary role of hunger satisfaction, consumers increasingly often expect food products to serve additional functions. They pay great attention to a high nutritive value and health-promoting properties of food. In order to meet these expectations producers extend their assortment with, e.g., fortified foods (ACHREMOWICZ, JAWORSKA 2009, CANNON 2006).

The fortification of food products with vitamins or minerals enables preventing nutrients deficiency in a man's diet. Products fortified with nutrients represent a segment of food with a health-promoting character, but available in many branches of the food industry. Breakfast cereals with the addition of vitamins, fruit juices with vitamin C or milk desserts with calcium are commonly available in retail. Apart from food products fortified with vitamins and minerals, consumers search for innovative additives with health-promoting effects, e.g. omega 3 and omega 6 fatty acids, dietary fiber, probiotics or components with stimulating effects. But, are they aware of what they buy? In this case a significant role is ascribed to a food producer and to product's package. Often, labels placed on product's package are the only carriers of information about it. Apart from obligatory elements they frequently contain additional information placed by producers in order to attract consumers' attention, e.g. nutritional and health claims which are formulas used to emphasize special properties of a given product. However, producers not always adhere to labeling guidelines. Sometimes "promises" made are not confirmed by scientific research and information provided on products' labels

is far from truth (DZIĘCIOŁ et al. 2007, GÓRSKA-WARSEWICZ 2006, KUDELKA 2011, KUNACHOWICZ et al. 2007, KUNACHOWICZ, RATKOVSKA 2009, SZAMREJ 2011).

In view of the above, the aim of this study has been to evaluate the load of information displayed on packages of fortified food products and to assess consumer knowledge on fortified foods and information about them provided via package.

Material and methods

The study was divided into two parts. In order to assess labels of selected groups of fortified products the marketing research technique of observation was used. These observations were done by two people trained to conduct observation research. An identification card of fortified foods label has been elaborated that consisted of 6 groups of questions related to: food product name, its composition, its nutritional value, nutritional claims, health claims, and promotional mottos/information (EU Register of nutrition and health claims www.ec.europa.eu/nuhclaims, Rozporządzenie Ministra Zdrowia. Dz.U.10.174.1184, Rozporządzeniu (WE) 1924/2006. Dz.U. L.404 z 2006 z późn. zm., Rozporządzeniu (WE) 1925/2006. Dz.U. L.404 z 2006 z późn. zm., Rozporządzenie Parlamentu Europejskiego i Rady 1169/2011. Dz.U. L.304 z 2011).

Each group contained open and closed type questions. Products analyzed in the study included fortified foods selected according to assortment groups available in retail on the Olsztyn market. In total, analyses were conducted for labels of 72 packages of fortified food products. The packages were divided into 3 groups: fortified juices and non-alcoholic drinks (n=30), fortified dairy products (n=25), and fortified cereal products (n=17). Data collected from identification cards were transferred to an Excel calculation sheet. Next, results were subjected to a quantitative analysis.

The survey on the perception of fortified food and information about it provided via package was conducted among 150 young consumers, aged 19-15, living in Olsztyn. Non-probability sampling, i.e. a convenience sampling, was applied. Survey questionnaire consisted of 10 questions referring to fortified foods and to information displayed on their packages, including 9 closed questions and 1 question with a 5-point frequency scale, where 1 denoted – “I do not buy at all” and 5 – “I buy very often”.

Results were processed using a Statistica 9.0 calculation sheet, based on basic statistical measures: mean values and percentage values.

Results and discussion

Evaluation of information conveyed via package in selected groups of fortified food products – observation study

A nation-wide survey shows that in Poland the most frequently fortified group of food products includes juices and non-alcoholic drinks that have the largest share in the market of fortified foods (KUNACHOWICZ, RATKOVSKA 2009). Our study confirmed this observation in respect of the Olsztyn market. The survey evaluated 113 products divided into the following groups: fruit juices and beverages, dairy products, cereal products, food concentrates, margarines and vegetable oils, confectionery as well as coffees and teas. The largest group consisted of fruit juices and beverages (26.55%), followed by dairy products (22.12%) and cereal products (15.04%). The smallest group of fortified food products included coffees and teas (4.42%).

Information displayed on packages of the fortified food products was evaluated in three groups of enriched products having the greatest contribution in the market, i.e. juices and non-alcoholic drinks (n=30), dairy products (n=25) and cereal products (n=17).

The first evaluated element was product's name. The survey demonstrated that in many cases the name of the product did not correspond to its type. Labels of 74% of products from the group of juices and non-alcoholic drinks displayed only some fancy name not related to product type and did not describe precisely the product to a potential consumer. Both fancy and informative (precise) name of the product was visible on barely 26% of labels of juices and non-alcoholic drinks, but on as many as 72% and 65% of labels of dairy and cereal products, respectively. This form of expressing product's name is not only consistent with binding regulations, but also allows the consumer to know the food product by conveying reliable information on its type and properties.

In 50% of juices and non-alcoholic drinks, 50% of cereal products and 65% of dairy food products the name was suggesting their specific properties. Such information was usually linked with product fortification with nutrients, for example „*Multivitamin classic juice with vitamins*”, „*Carbonated drink: 3 vitamins A, C, E*”, „*Milk with the addition of omega 3 polyunsaturated fatty acids*”, „*Sugar-free bread made with prebiotic and dietary fiber*”, or „*Cereal beads with chocolate flavor fortified with vitamin, iron and calcium*”.

Another evaluated information was the composition of a food product. All analyzed juices and non-alcoholic drinks were multi-component products and all were fortified. In 10% of the products no information was provided on the content of components indicated in product's name, which is inconsistent with

respective legal regulations. Labels of all products from this group were also lacking information on allergenic components. Similar situation occurred in the group of dairy products, of which all were multi-component. In the case of all products the name of which indicated a flavor additive, information on additive content was also displayed in product composition, and in 64% of the packages additional components were listed. The fortifying substances were displayed on 96% of packages of products from this group. Such information was missing in the label of 1 product only. In 24% of the packages, labels contained comprehensive information on allergenic components. All analyzed cereal products were multi-component and all had labels informing about fortification (in “Composition” part). Fortifying substances were added in forms described in the appendix of Regulation (EC) 1925/2006, of 20 December 2006 on the addition of vitamins and minerals and selected other substances to food products, for instance calcium in the form of calcium carbonate or magnesium in the form of magnesium carbonate. Some labels contained information only about the fortifying component (e.g. iron or magnesium) but did not specify its form. In the case of one product, its label (“Composition” part) did not confirm the information about flavor additive indicated in product’s name. Most of packages (88%) contained information about allergens, provided as the last element of product composition.

Another analyzed element was information about the nutritive value. All analyzed products had this information clearly displayed on packages. In packages of all analyzed juices and non-alcoholic drinks it was provided per 100 mL of product, and in 63% of packages it was additionally displayed per product portion. In the case of dairy products, in 92% of packages it was expressed per 100 g and in 20% of packages per 100 mL. In addition, over 50% of packages provided information on the nutritive value per product portion. In the case of all cereal products, their nutritive value was provided per 100g of the product, and in most of them additionally per product portion.

Pursuant to Regulation (EC) 1925/2006, packages of food products fortified with vitamins or minerals should contain information on their nutritive value considering group II of nutrients. In all three analyzed groups not all packages of products fortified with minerals or vitamins had this information provided on their labels.

Fruit juices and drinks were fortified exclusively with such vitamins as: C, B1, B2, B6, B12, A, E, folic acid, pantothenic acid, and niacin, and with minerals: magnesium (Figure 1). Vitamin C addition was visible in the list of fortifying components in all analyzed juices and non-alcoholic drinks. In compliance with requirements provisioned in the Regulation of the Minister of Health of 16 September 2010 on fortifying substances added to food products, the fortifying substance ought to cover at least 15% of recommended daily

allowances. The evaluated products met this criterion in 29 cases. Such information was missing at package of one product only. According to Polish regulations, the maximum quantity of a fortifying agent (apart from folates and vitamin C) should cover 50% of recommended daily allowances (RDA). Only one product failed to meet this requirement.

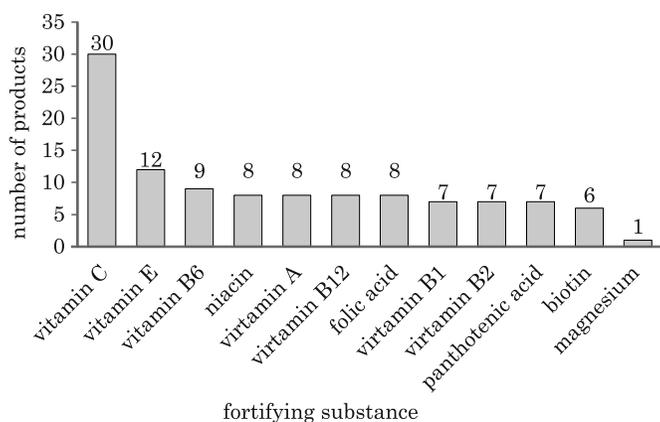


Fig. 1. Fortifying substances applied in the group of juices and non-alcoholic drinks (n=30)

Dairy products (Figure 2) – apart from vitamins and minerals – were fortified with dietary fiber, unsaturated fatty acids, probiotics as well as coenzyme Q 10 and ginseng. All of them contained fortifying agents listed in the Regulation (EC) 1925/2006, e.g. vitamins: D, E, A, B1, B2, and additional minerals: calcium and magnesium. Dairy products were most often fortified with vitamin D (9 products) and calcium (8 products). Frequently also mixed fortification

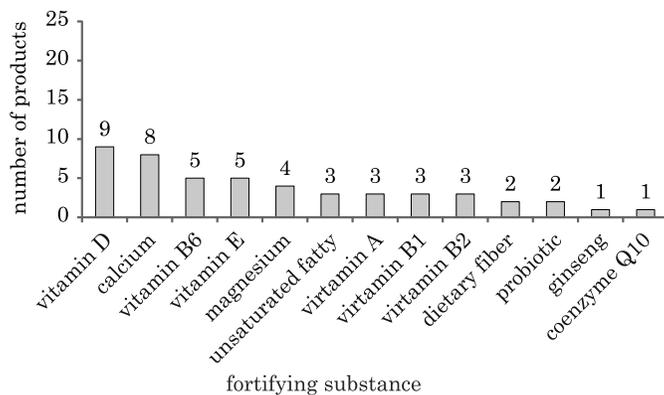


Fig. 2. Fortifying substances applied in the group of dairy products (n=25)

was applied, which included at least two different fortifying components. In accordance with legal regulations, in this group of products all added minerals and vitamins covered at least 15% and did not exceed 50% of RDA.

Likewise fruit juices and beverages, cereal products were fortified mainly with vitamins (B6, B1, B2, B12, C, A, E, D, niacin, biotin, folic acid and pantothenic acid) and minerals (iron, calcium, zinc and magnesium) (Figure 3). A prebiotic was also used in one product. All applied components were listed in the EC Regulation 1925/2006. Most frequently, these products were fortified with niacin, folic acid, vitamins B1 i B2 and iron. The most common form of fortification was a vitamin complex, a single fortifying agent was applied very seldom. Packages of three products had no information on meeting the recommended daily allowances, and in as many as 8 packages the declared doses were higher then the permissible levels. Some vitamins and minerals covered even up to 100% of RDA.

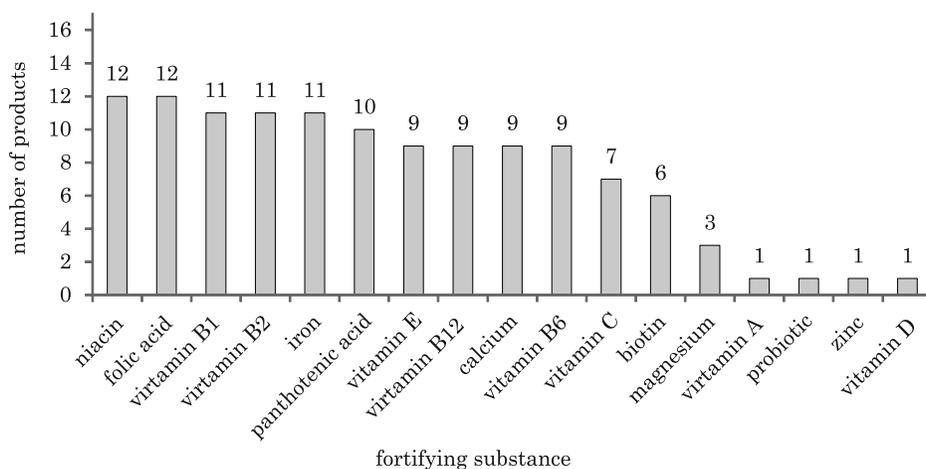


Fig. 3. Fortifying substances applied in the group of cereal products (n=17)

Pursuant to Regulation (EC) 1925/2006, packages of food products fortified with vitamins and minerals should contain information on the fortification only if they meet provisions of Regulation (EC) 1924/2006 which says that packages of food products with the addition of vitamins and minerals may contain nutritional or health claims. In the analyzed group of juices and non-alcoholic drinks, 53% of the packages contained nutritional and health claims supported by information on the nutritive value. The claims, if any, were linked with the applied fortifying components. The most frequently appearing declarations were: „rich in vitamin C”, or „a source of vitamin C”. Nutritional claims occurred also on 64% of packages of dairy products and

referred to their fortification, e.g.: „*a source of calcium*”, „*high content of omega 3 and omega 6*”, and „*a source of vitamin*”. In the group of cereal products, the nutritional claims were mainly related to dietary fiber, e.g. „*high content of dietary fiber*” or „*a source of dietary fiber*”.

Another analyzed informative element of the package was health claim. In the group of juices and non-alcoholic drinks, none of the products contained such claim. In the other two groups, such declarations appeared on packages of few products only (12% of dairy products and 1% of cereal products). The following declaration, *calcium and vitamin D are necessary for the proper growth and development of children*” – approved by the European Food Safety Authority (EFSA), as well as the following promises: „*omega 3 and omega 6 are necessary for the proper growth and development of children*” and „*actively enhances digestive processes*” appeared in the group of dairy products. In turn, the following claim: „*supports digestion, facilitates gut peristalsis*” appeared in the group of cereal products (EU Register of nutrition and health claims... www.ec.europa.eu/nuhclaims).

Apart from nutritional and health claims, producers place various promotional mottos on product packages, often implying non-specific benefits, namely statements that suggest the impact of a given component on health status. The non-specific benefits may be used on the package when they occur together with health claims consistent with binding regulations. In the group of juices and non-alcoholic drinks, slogans used on 4 packages were on the verge of nutritional claims and health claims, e.g., „*extra C*” or „*improves mental and physical capability*”. Promotional slogans, e.g. „*Everyday portion of health*” or „*bottle full of life*” were placed on packages of dairy products where they appeared more frequently (in 60% of packages) than on packages of juices and non-alcoholic beverages. In the group of cereal products, as in the group of juices and non-alcoholic drinks, the promotional slogans occurred rarely. Only packages of 3 products contained declaration with a character of nutritional or health claims, but not being the real claims, e.g., „*Healthy nutrition may be delicious*”. One of the packages contained the following recommendation: „*Good choice – I recommend it, Ewa Wachowicz*”.

Fortification of food in the opinion of consumers

Respondents participating in the survey were asked to indicate components with a fortifying character. Over half of them declared that they were paying attention to contents of nutrients. Further on, they were listing contents of minerals (25% of answers) and vitamins (23% of answers). The respondents were least interested in contents of plant sterols and stanols and contents of

unsaturated fatty acids. Every fourth respondent claimed not to pay any attention to food components. The study proved also that women were paying greater attention to contents of nutrients (62% of this group), dietary fiber (24% of all women) and salt (17% of this group). In turn, men were more often considering contents of vitamins (28% of this group) and minerals (34% of this group).

Respondents were also asked to indicate information which – in their opinion – implied the fortification of a food product. The survey showed that consumers related fortified foods mainly with products having the addition of dietary fiber (67% of answers), vitamins (61%) and with products containing minerals as well as omega 3 and omega 6 fatty acids (54%). Only a small percentage of respondents was declaring to know the concept of bioactive compounds, 21% of respondents were linking this concept with improved digestion, whereas 25% – with the addition of preserving agents. The analysis of results in terms of respondents gender showed that women knew better the idea of food fortification. A higher percentage of women than men related fortified foods with such terms as “with the addition of vitamins”, “with the addition of minerals”, “with the addition of dietary fiber”, “with the addition of omega 3 and 6 fatty acids” as well as “with bioactive components”. In addition, men were more frequently perceiving fortified foods as products with the addition of preserving agents (30% of answers) and as “light” products (8% of answers).

When asked about where they would search for information about food fortification the respondents indicated most of all the “Composition” of a food product (69%). Additional declarations of producers were indicated by 34% whereas nutritive value – by 22% of the respondents. The lowest number of respondents indicated product’s name (13%) as the source of information on product fortification. The target group was also asked if they pay attention to the coverage of daily recommended allowances by fortifying components declared by the producer. The study showed that 64% of the respondents (including 62% of women and 68% of men) paid no attention to this information.

Furthermore, survey results (Figure 4) showed that the respondents were most often identifying fortified foods with such products as fruit juices and nectars, dairy products (82% of answers), and breakfast cereals (77% of answers). In contrast, they had problems with indicating obligatorily-fortified food products. In accordance to Polish regulations, obligatory fortification applies to salt (fortification with iodine) and vegetable oils (with vitamins A and D). In this case, only half of the respondents indicated vegetable oils and only 11% indicated salt, and some of them were listing products that were not subjected to the fortification process, i.e. vegetables (8% of answers) and rice (9% of answers).

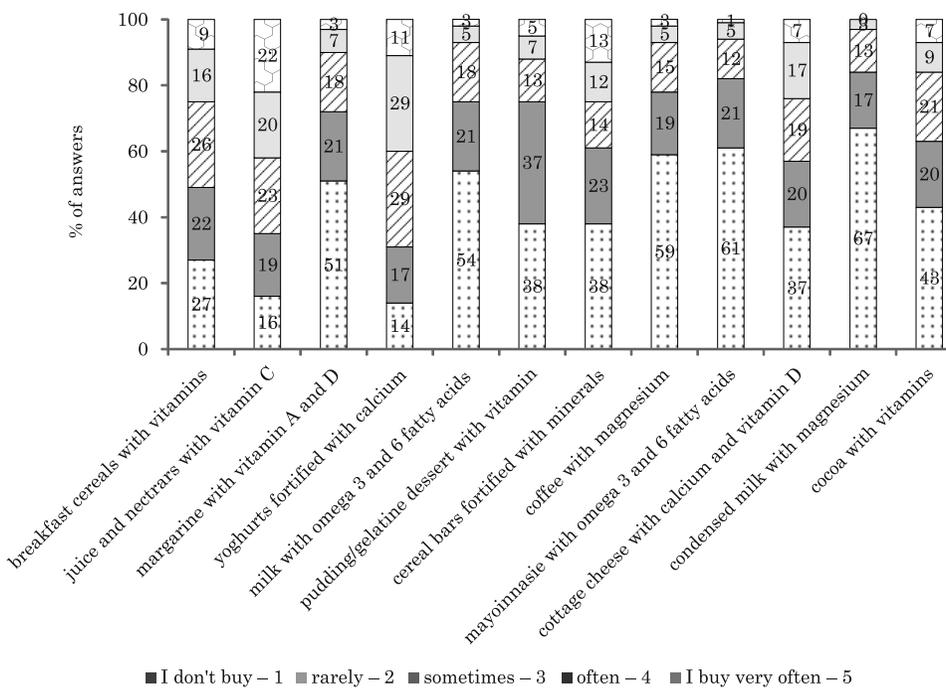


Fig 4. Frequency of purchase of fortified food products by respondents (% of answers)

The analysis of results (Figure 4) demonstrated that the selected target group was purchasing the fortified food products very rarely. In the case of 5 products (margarine with vitamin A and D, milk with omega 3 and 6 fatty acids, coffee with magnesium, mayonnaise with omega 3 and 6 fatty acids, and condensed milk with magnesium) over half of the respondents declared not to buy such products. Also other fortified products, like: puddings and gelatin desserts with vitamins, cereal bars with minerals, or cocoa with vitamins, were rarely selected by the respondents. Most often purchased fortified food products, as declared by the respondents, included: fruit juices and nectars (22% of answers) and dairy products (29% of answers).

The survey demonstrated (Figure 5) also that the main reason of purchasing fortified food products by respondents was the fact that they provide vitamins and minerals (64% of answers). Almost 40% of the respondents declared to buy these products owing to higher declared content of nutrients compared to conventional foods. Other important factors influencing the purchase of this type of food turned out to be: curiosity (27% of answers) and advertisement (22% of answers).

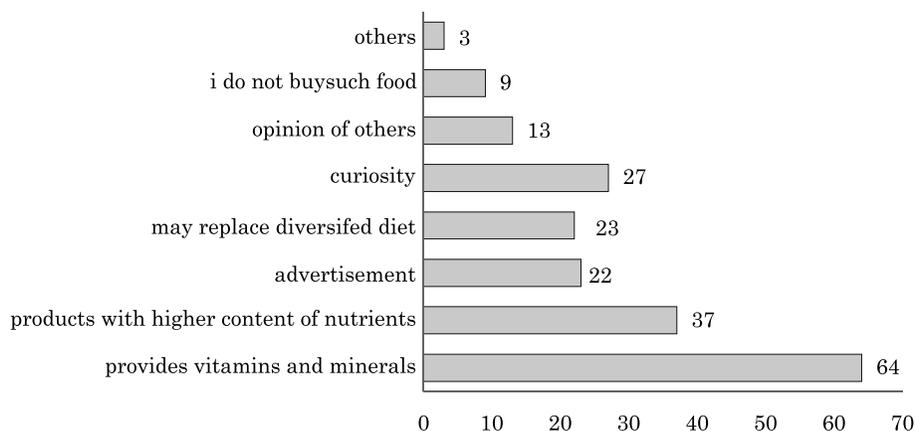


Fig. 5. Reasons influencing the purchase of fortified foods (% of answers)

Women significantly more often than men were declaring to buy fortified foods owing to their content of vitamins and minerals (72% of answers). In turn, men were more frequently claiming that “fortified foods may replace diversified diet” (28% of answers) and that they do not buy such food products at all (18%).

When asked about the reasons behind the production of fortified foods, half the respondents have stated that they are produced in order to make the market assortment more attractive. In this case, over 40% of the respondents indicated also: “to increase the nutritive value of food” and “to reduce deficiencies of nutrients in man’s diet”. Only 28% of the respondents “answered” “to restore the natural nutritive value of food products”.

Conclusions

The conducted survey proved that the analyzed groups of food products were most often fortified with vitamins and minerals. Fruit juices and beverages were mainly fortified with vitamin C, cereal products – with a vitamin complex and iron, and dairy products – with calcium and vitamin D. Other fortifying agents were applied rarely. The evaluated labels in most cases met the requirements provisioned in Polish and EU regulations. All analyzed food products had nutritive value indicated on their labels and were fortified with permitted components.

The names of the evaluated cereal and dairy products were in most cases clear and precise (informative). In contrast, the names of fruit juices and drinks were usually fancy. The nutritional and health claims appearing on

labels of the assessed food products of each group referred most of all to the properties of the added fortifying component.

Most of the surveyed respondents were able to correctly indicate concepts linked with fortified foods. Women more often than men were indicating the correct answers.

In turn, both female and male respondents had no knowledge on the products fortified obligatorily in Poland. Groups of fortified foods most frequently indicated by the respondents included: fruit juices and drinks, dairy products and breakfast cereals. The choice of fortified food products by respondents was mainly driven by their health-promoting properties. Men more often than women were declaring to buy such products owing to curiosity.

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