UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN

Polish Journal of Natural Sciences

27
(1/2012)

PUBLISHER UWM
OLSZTYN 2012

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

The print edition is the primary version of the Journal

The Journal is also available in electronic form on the web site http://wydawnictwo.uwm.edu.pl (subpage *Czytelnia*)

PL ISSN 1643-9953

PUBLISHER UWM OLSZTYN

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Ark. wyd. 9,2, ark. druk. 7,5, nakład 125 egz.
Druk – Zakład Poligraficzny UWM w Olsztynie
zam. nr 189

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(1): 5–13, Y. 2012

FACTORS AFFECTING THE CHEMICAL COMPOSITION OF STRAWBERRY FRUITS

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K e y w o r d s: strawberry, fruit quality, chemical composition.

A b s t r a c t

Strawberry fruits (*Fragaria x ananassa* Duch.) are valued by consumers for their high nutritional qualities, taste and visual values. In this work the literature review was made about the influence of different factors on sugar, organic acids, vitamin C, poliphenols and antocyanins content in strawberry fruits.

It was found, that selection of strawberry cultivar combined with agricultural practices have the essential meaning to obtain the required yield parameters.

CZYNNIKI WPŁYWAJĄCE NA SKŁAD CHEMICZNY OWOCÓW TRUSKAWEK

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S ł o w a k l u c z o w e: truskawka, jakość owoców, skład chemiczny.

A b s t r a k t

Owoce truskawki (*Fragaria x ananasa* Duch.) są cenione przez konsumentów dzięki wysokim wartościom odżywczym, walorom smakowym, a także wizualnym. W pracy dokonano przeglądu piśmiennictwa na temat wpływu różnych czynników na zawartość cukrów, kwasów organicznych, witaminy C, polifenoli i antocyjanów w owocach truskawki.

Wykazano, że niezbędnym warunkiem zapewnienia wymaganych parametrów jakościowych plonu truskawek jest dobór właściwej odmiany i lokalizacja pola w połączeniu z odpowiednimi zabiegami agrotechnicznymi.

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Introduction

Strawberry (*Fragaria x ananassa* Duch.), a well known berry-bearing plant, was created by crossing Chilean strawberry (*Fragaria cgiloensis*) with Virginia strawberry (*Fragaria virginiana*) in the 18th century. Significant progress concerning cultivation of this plant and breeding highly productive varieties took place only in the 20th century (REJMAN 1994, PIEGRZA, SZCZYGIEŁ 1999). Strawberry is cultivated on a large scale in all countries of moderate climate and in cooler regions of subtropical countries (REJMAN 1994). The largest producers of strawberries include USA, Spain, Poland, Morocco and China (KUBIAK 2001). The most intense region of strawberry cultivation is California. Due to its specific climatic conditions, strawberries bear fruit there for 4–6 months a year, giving an average yield of 40–60 tones per 1 ha. For comparison, average yields of strawberries obtained in Poland amount on average to 6 tones per 1 ha. In recent years, as a result of the increasing requirements of food consumers, greater attention has been placed on yield quality. This aspect particularly concerns strawberries, which are characterized by high nutritive and health properties. Strawberry fruits are a valuable component of the diet, since they are of low calorie content and contain easily assimilable sugars, as well as many vitamins, pectines and fibre. As regards the content of iron and phosphorus, strawberries rank the highest among berry-bearing plants. They are also a good source of potassium, magnesium and calcium (HAKALA et al. 2003). Vitamins found in large amounts in strawberries include: vitamin A, B1, B2, C and PP. Their contents vary for different cultivars (REJMAN 1994). In terms of abundance in vitamin C in berry fruits, strawberries are second only to black currants. Strawberries are also valued for to their antioxidant content (SKUPIEŃ and OSZMIAŃSKI 2007). On the other hand, high water content in fruit determines their juiciness and refreshing taste.

One of the threats resulting from an intensive production of strawberries is the risk of contaminating the yield by harmful chemicals, like pesticides or fertilizers. Although monitoring research conducted in Poland does not show harmful amounts of residues of plant protection products, nonetheless, a detection of trace amounts in even 44.8% of the samples analysed should be considered alarming (NOWACKA et al. 2010). Insignificant amounts of pesticide residues in strawberries were also found in research conducted in Finland (HAKALA et al. 2003).

Factors related to the choice of cultivars, growing technology although harvesting and fruits trading are of crucial importance for ensuring quality parameters of the yield. Cultivars can be classified into traditional

varieties (short day cultivars) – early, medium-early and late, and everbearing (day neutral cultivars) – bearing fruit in elongated period of growing season). Generally, native cultivars are better adopted to the local environment, giving the best yield and fruit under the conditions in which they have been grown. The number of strawberry cultivars throughout the world already exceeds 2.000 (ŻURAWICZ 1997). In 2011, the register of orchard plant varieties in Poland contained 45 cultivars of strawberry (*Odmiany wpisane...* 2012).

The dominant cultivar in Poland is 'Senga Sengana', originated in Germany, particularly because of its good adaptation to prevailing climatic conditions and high yielding (ŻURAWICZ et al. 2005). Currently, the most demanded cultivars are dessert varieties, the fruits of which are tasty, well formed and are characterized by distinctive size, colour, high firmness and lower susceptibility to fungal diseases causing fruit rotting. Therefore, it is necessary to increase the market supply of strawberry cultivars that meet consumers' expectations and satisfy producers (MASNY et al. 1996).

This study has a review character. The aim of this work is to summarize researches data about the quality of strawberry fruits.

Strawberry yield quality parameters

Sugar content

Sugar content is one of the main parameters considered in the evaluation of the nutritive value of strawberries. Moreover, yield quality factors are included in the characteristics of cultivars registered in Poland. As demonstrated by some researchers, high sugar content was found for the 'Senga Sengana' cultivar (ROLBICKI and RZEKANOWSKI 1997, SKUPIEŃ 2003, KOSZAŃSKI et al. 2006). Low sugar content in fruits was determined for such cultivars, like: 'Pandora', 'Vicoda', 'Purpuratka', 'Dange', 'Saulene', 'Ydun' (REJMAN 1994, SKUPIEŃ 2003, KOPYTOWSKI et al. 2006, KOSZAŃSKI et al. 2006]. As regards accumulated sugars, particular attention should be given to: fructose, glucose and saccharose occurring in significant amounts in strawberry fruits (KALLIO et al. 2000, CORDENUNSI et al. 2002). The proportions of their content slightly varies, depending on the cultivar and agricultural conditions. The content of individual sugars within the cultivar seems to be inherited and independent of environmental conditions (OGIWARA et al. 1998) and thus it is important to cultivate and popularize varieties characterized by high sugar content, which will transfer this feature to subsequent generations (OHTSUKA et al. 2004). Sturm et al. (2003) also distinguish xylose, which is

found in trace amounts in strawberries. Its relatively high content was observed in fruits of the 'Northhaester' cultivar and it was very low in the 'Miss' cultivar. Literature data concerning sugar content in strawberries of individual cultivars are divergent depending not only on the cultivar, but also on many cultivation conditions, such as irrigation (which is particularly important in blossoming phase and fruit setting phase), fertilization (mainly nitrogen, phosphorus and potassium – NPK), as well as the health condition of the plantation (SKUPIEŃ 2003, OHTSUKA et al. 2004, KOSZAŃSKI et al. 2005]. The time between the harvest and fruit chilling also plays a significant role – a longer interval results in the greatest losses in nutritional value of strawberries (NUNES et al. 1995). Additionally, the period of storage frozen fruits affects the reduction of saccharose content (CORDENUNSI et al. 2003, SKUPIEŃ 2003). The research by KOSZAŃSKI et al. (2005) demonstrated that the 'Elsanta' cultivar contained lower amounts of sugars after the application of 2 NPK fertilization (40 kg of nitrogen, 80 kg of phosphorus and 100 kg of potassium ha^{-1}) and irrigation as compared to control objects. Different results were noted by CHEŁPIŃSKI et al. (2010), where fruits of cultivar 'Kent' contained more sugar after fertilization on 150 kg ha^{-1} NPK level. STURM et al. (2003) determined the highest total sugar content for the 'Mohawk' cultivar, depending on the degree of fruit maturity (mature fruits for processing purposes were characterized by lower sugar content than fully ripe fruits). Differences in sugar content can be also related to various factors occurring during the fruit ripening phase. TREDER (2003), after ROLBICKI and RZEKANOWSKI, demonstrated that drip irrigation had a significant effect on lowering sugar content in strawberries of the 'Senga Sengana' cultivar.

Organic acids content

The content of organic acids is a very important indicator affecting the taste of fruits, which influences both their consumer and processing value. This feature is variable and depends on the cultivar, weather conditions, as well as agricultural factors and regional differences between the studies conducted (SKUPIEŃ 2003). The content of organic acids in strawberries does not usually exceed 3%. The most important organic acids determined in strawberries include citric acid and apple acid (KALLIO et al. 2000), and the total acidity of fruit is determined on the basis of their content. The research by this author proved that the content of apple acid in strawberries fluctuated on a level that was 2–3 times lower than the level of citric acid. Similar results can be found in BASSON et al. (2010) research. Their studies showed that citrate content decreased slightly as the berries ripened, and sugar-to-acid

ratio increased during ripening. The highest values of total acidity were found for 'Aura', 'Dange' and 'Senga Sengana' cultivars (KOPYTOWSKI et al. 2006). STURM et al. (2003) demonstrated that fully ripe fruits were characterized by lower content of organic acids than technologically ripe fruit. In research by KOSZAŃSKI et al. (2005), the content of organic acids in the fruits of 'Senga Sengana' cultivar increased for objects fertilized with a double dose of NPK. Therefore, the acidity of strawberry fruits is not a constant feature of the cultivars under examination. It can change depending on many environmental parameters occurring during the cultivation and to a large extent depends on agricultural factors.

Content of biologically active compounds

In recent years, particular attention has been placed on the occurrence of biologically active compounds in strawberry fruits, such as vitamin C and antioxidant-type compounds, which include phenolic acids, flavonoids and anthocyans. A particular importance is attributed to the second group of compounds because of their anti-cancer, anti-inflammatory, neutron protective and anti-atherosclerosis properties (MILLER et al. 2008, KILLIAN et al. 2009, ROUSSOS et al. 2009). The accumulation of phenolic compounds is consistent with the proposed protective roles of these substances as antimicrobial metabolities (GIL et al. 1997).

Vitamin C

The content of ascorbic acid in fruits and vegetables is affected by many factors, such as genotypic differences, climatic conditions, cultivation and fruit ripening conditions and the time of storage (LEE and KADER 2000). In this regard, the research proved, for instance, the unfavourable effect of extending the period of fruit exposure to high temperature between the harvest and chilling (NUNES et al. 1995). HÄGG et al. (1995) reported that vitamin C content in the fresh mass of various cultivars of strawberry cultivated in Finland ranged between 56 and 99 mg% in 1992. Noticeable changes in content of vitamin C were noted between organic and conventional strawberry farms (REGANOLD et al. 2010). Fruits from organic farms contained more ascorbic acid. CORDENUNSI et al. (2002) found that all from studied cultivars showed increase in total ascorbic acid content from the early stages of development to full maturity. KAZUYOSHI et al. (1999) found that ascorbic acid content among the cultivars varied much during harvest. The first changes in vitamin C

content can already be observed in the period of supplying fresh fruit to the market (RUSSEL et al. 2009). Even short storage of frozen strawberries deprives them of a substantial amount of ascorbic acid (CORDENUNSI et al. 2003), but those changes are much more noticeable during long-term storage of frozen strawberries (SKUPIEŃ 2003). It was found that even subjecting strawberry fruit to irradiation does not prevent vitamin C losses during storage (GRAHAM and STEVENSON 1997). As results from the research conducted by KOPYTOWSKI et al. (2006), the highest content of this component was found in 'Saulene', 'Senga Sengana' and 'Aura' cultivars. The accumulation of vitamin C in vegetables, according to JABŁOŃSKA-CEGLAREK (1989), depends on the level of irrigation as well as on climatic, agricultural and varietal factors. KOSZAŃSKI et al. (2005) demonstrated a reduction of vitamin C content under the influence of irrigation and high levels of NPK fertilization.

Polyphenols and anthocyanins

Compounds demonstrating high antioxidant activity against free radicals include polyphenols and anthocyans. The content of those components in strawberries depends on many factors, such as the choice of cultivar, agricultural conditions, light availability, nitrogen content in soil, degree of fruit ripeness and storage temperature (KALT et al. 1999, BOJARSKA et al. 2006, BACCHELLA et al. 2009, ROUSSOS et al. 2009). BOJARSKA et al. (2006) demonstrated that the highest content of polyphenolic compounds in fruits was characteristic for the 'Polka' cultivar, and the lowest was for the 'Kent' cultivar. ROUSSOS et al (2009) observed a reduction in polyphenol content in fruits of the 'Camarosa' cultivar, obtained after previous treatment of plants with gibberellic acid and Fenotiol. Those differences prove the significant influence of the suppressing effect of growth stimulants on the content of antioxidants. FERREYRA et al. (2007) found that the decreasing antioxidant activity of fruit was negatively correlated with anthocyan synthesis. CORDENUNSI et al. (2005) recorded a decrease in the antioxidant activity of strawberries under conditions of lowered storage temperature. SHIN et al. (2007) demonstrated that strawberries revealed the highest antioxidant activity when they were stored at 10°C, regardless of the air humidity. SKUPIEŃ and OSZMIAŃSKI (2007) observed that the antioxidant activity against the DPPH radical grew significantly after applying fertilization with titanium only in fruits of the 'Elsanta' cultivar. For other cultivars, no significant influence of a growth regulator applied on the antioxidant activity of strawberry fruit was observed. In other studies (PANICO et al. 2009) was noted that bioactive compounds content was correlated with type of soil. REGANOLD et al. (2010)

found that strawberry fruits from organic farms had higher antioxidant activity and concentration of phenolic compounds in correlation to conventional ones.

Summary

The growing of strawberry cultivars distinguished by a high commercial yield, and at the same time rich in nutrients and other valuable components, is a very important aspect of the production of berry-bearing fruits. On the basis of research conducted to date, it can be claimed that between many factors affecting biological value of strawberry fruits, e.g. varietal, agricultural practices, weather conditions, the highest impact has their genotype and origin. Nevertheless, the quality of the yield obtained is conditioned by all factors accompanying the cultivation. It also applies to the production of fruit deprived of the remains of harmful chemical compounds, which is related to the preference of a protection system characterized by a low dependence on pesticides.

Translated by JOANNA JENSEN

Accepted for print 9.01.2012

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(1): 15–30, Y. 2012

MICROBIAL PREPARATION ENATIN: NEW APPLICATION ASPECTS

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K e y w o r d s: sporulating bacteria, cultivation, biological control, sanitation.

A b s t r a c t

Efficacy of biopreparation of Enatin to control pathogenic and facultative pathogenic microflora of stock farms has been established in laboratory and industrial conditions. Pilot-plant technology of Enatin production and application was elaborated.

As a result of investigations Enatin preparation was registered as an agent for preventive disinfection of pig sty complexes and recommended for application at swine-breeding farms of the Republic of Belarus.

PREPARAT MIKROBIOLOGICZNY ENATIN – MOŻLIWOŚCI NOWYCH ZASTOSOWAŃ

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S ł o w a k l u c z o w e: bakterie przetrwalnikujące, hodowla, ochrona biologiczna, warunki sanitarne.

A b s t r a k t

W warunkach laboratoryjnych i przemysłowych analizowano skuteczność preparatu biologicznego Enatin w zwalczaniu mikroflory patogennej i fakultatywnych bakterii chorobotwórczych na fermach hodowlanych. Opracowano pilotażową technologię produkcji preparatu Enatin oraz metody jego zastosowania.

Wyniki badań pozwoliły na zarejestrowanie preparatu Enatin jako środka do dezynfekcji prewencyjnej chlewni. Jest on także rekomendowany do stosowania na fermach zarodowych trzody chlewnej w Republice Białorusi.

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Introduction

Wide distribution of infectious diseases among farm stock caused by contamination of sties with pathogenic and facultative pathogenic bacterial species due to congestion and all-year-round indoor keeping of animals within a limited area results in sharp reduction of young stock survival rate, especially at swine-breeding complexes (MEDVEDEV 2001).

The use of disinfecting technologies based on chemical agents is accompanied by such adverse consequences as accumulation in the body of residual amounts of preparations; their pronounced immunodepressive effect, transformation in the environment to cancerogenic compounds and ecotoxins (dioxins, trihalomethanes); corrosive action; need in post-application air remediation; resistance to local microbiota determined by multiyear persistent use of the same preparations (IBRAHIMOVA 2000, KAMINSKY 2003, TARABUKINA and NEUSTROEV 2002).

It spurs interest in biological disinfectants ensuring both effective control of animal pathogens resistant to chemicals and output of ecologically safe agricultural products (CURTIS et al. 1995, POHILENKO and PERELYGIN 2007, SVERCHKOVA et al. 2007). In particular, preparations derived from bacteria of genus Bacillus find are used in stockbreeding to prevent and treat of gastrointestinal and respiratory diseases (HAENAL and BENDING 1985); raise fodder digestibility (TARAKANOV 1987); disinfect poultry droppings and manure contaminated with pathogenic microorganisms (TARABUKINA AND NEUSTROEV 2002), for sanitation of stock-rearing sections (POHILENKO and PERELYGIN 2007), remediation of water in fish ponds and prevention of contagious diseases in fish species (SIHARULIDZE 2006). It should be noted that strains *Bacillus subtilis* and *B. pumilus* are most widely applied for biological control of animal pathogens (TARABUKINA and NEUSTROEV 2002, TARAKANOV 1987).

We have developed biopesticide Enatin on the basis of sporulating bacterial strain *Bacillus pumilus* BIM B-263 to protect of cultivars from pathologies (ROMANOVSKAYA et al. 2002). Further investigation of Enatin properties indicated that this preparation was characterized by a broad spectrum of antimicrobial activity and ability to control spread of plant and animal pathogens. This substantiated studies on application prospects of Enatin as the effective agent for sanitation of stock farms – aim of this paper.

Materials and Methods

Strain *Bacillus pumilus* BIM B-263 (Meyer, Gottheils) characterized by high antimicrobial activity towards bacterial pathogens of plants, animals and

towards phytopathogenic fungi has been chosen as the object of studies. The strain was isolated from vine phylloplane (ROMANOVSKAYA et al. 2002) and deposited at Belarusian collection of non-pathogenic microorganisms (research collection of type and industrially valuable non-pathogenic microorganisms, Institute of Microbiology, National Academy of Sciences, Belarus). Submerged fermentation of bacteria *B. pumilus* BIM B-263 was carried out in Erlenmeyer flasks on the shaker (180–200 ± 10 rpm), in lab fermentors ANKUM-2M (aeration rate 0.5–2.0 l air/l · min, agitation rate 200 ± 10 rpm) and in pilot-plant fermentor of 0.3 m^3 volume (aeration rate 0.8–1.0 l air/l · min, agitation rate 250–270 ± 10 rpm) at temperature 30–33°C on the nutrient medium of the following composition (g/l): molasses – 20.0; $K_2HPO_4 \cdot 3H_2O$ – 7.0; KH_2PO_4 – 3.0; $MgSO_4 \cdot 7H_2O$ – 0.1; NH_4NO_3 – 1.0; Na-citrate · $3H_2O$ – 0.5; corn steep liquor – 2.5; H_2O – to 1 liter.

Percentage of introduced 1-day vegetative inoculum pre-grown on medium with molasses at 28–30°C is 10% (v/v).

Microbiological and biochemical control of culture development and accumulation of antimicrobial metabolites was performed by sampling every 12 hours and analyzing of cultural liquid specimens. Sporulation process was monitored by microscopy and by plating samples on meat peptone agar using finite dilutions technique (YEGOROV 1983). Biomass yield was estimated gravimetrically. The contents of free reducing substances was determined according to Miller (MILLER et al. 1959). Preliminary acid hydrolysis of cultural medium was conducted to evaluate the level of total sugars (YEMELYANOVA 1969).

Antagonistic activity of examined culture was assessed by wells (YEGOROV 1986) and replica (TIRRANEN 1989) methods.

Facultative pathogenic and strictly pathogenic bacteria *Escherichia coli* S3, *E. coli* Att 25, *Klebsiella pneumoniae* 9, *Proteus* sp 44, *Staphylococcus* sp. 7, *Staphylococcus aureus* 6538-p, *Streptococcus* sp. H2 responsible for animal pathologies of gastrointestinal tract and respiratory system, were provided by Research-Practical Center of Stock Breeding, National Academy of Sciences, Belarus and strains of phytopathogenic bacteria and fungi were our isolates or cultures supplied from Institute of Microbiology and Virology, National Academy of Sciences, Ukraine; Institute of Plant Protection, National Academy of Sciences, Belarus; Institute of Horticulture and Floriculture Scernevice, Poland; University of Opole, Poland and served as test-objects in assays of *B. pumilus* BIM B 263 antagonistic activity.

Bacterial pathogens of plant and animal species were grown on meat-peptone broth, phytopathogenic fungi were cultured on Hottinger broth diluted with water to 7° Balling in 100 ml Erlenmeyer flasks on the shaker (200 rpm) at 26–30°C during 1–2 days.

Laboratory tests of *B. pumilus* BIM B-263 antimicrobial activity against bacteria *E. coli* S3, *Staphylococcus* sp. 7 were based on qualitative suspension method and procedure using rubber tubes to evaluate antimicrobial action in accordance with regulations No 4718 of December 24, 1998 "Methods of testing antimicrobial activity of disinfection agents" issued by Principal Department of Hygiene, Epidemiology and Prophylaxis, Ministry of Health Care, Belarus (1998).

Chemical disinfectants recommended for sanitary treatment of stock farms were applied as references: – combined surface disinfectant (CSD) (incorporating aldehydes, quarternary ammonium salts, alcohols), Lysol (solution of purified soapy phenols), Sandim D (solution comprising stabilized hydrogen peroxide, peracetic and acetic acids).

Industrial trials of Enatin antimicrobial efficiency against sanitary-indicative microbiota (coliforms and staphylococcal-streptococcal groups) of stock farms were carried out at Borisovsky swine-breeding complex on sections of piglets at post-sucking stage. Empty test sections were treated by aerosol method, using pneumatic sprayers (jet spray generator CAG 1, CAG 2) or vacuum spray generator of series LOMA Cyclone 1 and working solutions of Enatin preparation at concentrations 2.5; 5 and 10%. Optimal application dose is 3 ml m^{-3} of air.

Efficiency of Enatin action was estimated via its impact on infectious background of piglet sections after temporary evacuation of reared animals, including ambient air and surface of sty facilities (breeding boxes, floor, walls).

Microbial contamination of air and surfaces before and after treatment was evaluated as the amount of sanitary-indicative (coliforms and staphylococcal--streptococcal groups) and total microbiota according to quality control directions for disinfection of stock farms (*Regulations*... 1988).

Analysis of air microflora was conducted by Koch sedimentation technique (KREMLYOV 2002) and aspiration method utilizing Kpotov device (GLASKOVICH 2000) when sets of 3 Petri plates containing selective nutrient medium for each microbial group were placed in 9 different points. The experiments were repeated thrice.

To assess microbial colonization of surfaces in sty sections, wash-off technology with subsequent transfer of obtained suspension onto diagnostic media was applied. Plating on Endo and selective salt agar media was required to isolate bacteria of coliforms and staphylococcal-streptococcal groups, respectively (R*egulations*... 1988).

Statistical processing of experimental results (ROKITSKY 1973) assumed calculation of arithmetical means whose confidence ranges for 95% probability level, using Microsoft Excel 2003 and Statistica for Windows, v 6.0 software. The least significant difference (LSD) was determined by dispersion analysis.

Results and Discussion

Interaction types between bacteria of genus *Bacillus* and phytopathogenic microorganisms (competition, hyperparasitism, antibiosis) describe so far do not cover the whole spectrum of links existing in biocenoses where various forms of antagonism are so closely correlated that a clear-cut discriminating line can not be drawn (SHABASHOVA 2005). The same microorganism may display different mechanisms of antagonistic action, and one interaction type may be replaced by another under the impact of environmental conditions.

Data presented in Table 1 indicates ability of strain *Bacillus pumilus* BIM B-263 to control development of a broad spectrum of plant and animal pathogens. Antimicrobial action of the culture is expressed as the formation of severe growth delay zones in pathogenic bacteria – up to 34 mm in diameter, in contrast to phytopathogenic fungi of genera *Alternaria* and *Fusarium* displaying only zones of slow growth lacking air mycelium (ROMANOVSKAYA et al. 2002, SHABASHOVA 2005). It should be noted that inhibitory effect of *B. pumilus* BIM B-263 on phytopathogenic fungi *Colletotrichum gloeosporioides* Sacc, *Pleiochaeta setosa* Kirchn, *Rhizoctonia solani* Kuhn is manifested as spread of invasion zones on test cultures which may be related to hyperparasitic activity of studied strain or a higher growth rate as compared to the fungi (KUPTSOV 2002).

Comparative evaluation of antimicrobial efficiency of *Bacillus pumilus* BIM B-263 cultural liquid (biopreparation Enatin) versus chemical disinfectants demonstrates (Table 2 and Table 3) that the liquid bacterial culture grown on Meynell nutrient medium ($1–5 \cdot 10^9$ CFU/ml) possessed marked bactericidal activity towards sanitary-indicative microbiota.

Complete decay of *Escherichia coli* S-3 culture was observed under the impact of 2.5% cultural liquid of *B. pumilus* BIM B-263 by 60–120 min of mixed incubation, whereas for bacteria *Staphylococcus* sp. 7 minimal bactericidal concentration of working solution of the antagonistic strain cultural liquid constituted 0.1% by 60 min of mixed fermentation (SVERCHKOVA et al. 2007). Thus, the efficiency of biopreparation action against E. coli S3 matches and with respect to Staphylococcus sp. 7 exceeds the efficiency of tested chemical disin

fectants, supporting to need for further studies aimed at elaboration of Enatin pilot-plant technology and its application for sanitation of stock farm facilities.

According to literature reports (OSADCHAYA et al. 1997, YEGOROV 1986) growth and antimicrobial activity of microbial antagonists depends on nutrient medium composition (presence of definite sources of energy and nutrition, promoters of growth and biosynthesis, precursors of synthesized product).

Table 1

Antimicrobial activity spectrum of strain *Bacillus subtilis* BIM B-263

Test culture	Pathology	*Bacillus pumilus* BIM B-263	
		wells technique*	replica technique**
1	2	3	4
Bacterial pathogens of animals			
Escherichia coli S-3	infectious diseases of gastrointestinal tract	28.0 ± 0.5^{1}	95.1
Escherichia coli Att25		25.5 ± 0.4^{1}	93.5
Proteus sp. 44		23.0 ± 0.6^{1}	83.0
Klebsiella pneumoniae 9	infectious pathologies of respiratory system	17.5 ± 0.2^{1}	71.0
Staphylococcus sp. 7		28.5 ± 0.3^{1}	98.3
Staphylococcus aureus 6538-p		26.5 ± 0.4^{1}	95.5
Streptococcus sp. H-2		30.5 ± 0.6^{1}	92.5
Plant bacterial pathogens			
Erwinia carotovora pv. *atroseptica* 662	soft rot of vegetables	27.5 ± 0.8^{1}	85.5
Pseudomonas syringae pv. *atrofaciens* 239	bacterial diseases of grain crops	31.2 ± 1.0^{1}	85.7
Pseudomonas syringae pv. *lachrymans* 121	cucumber angular leaf spot	33.4 ± 0.8^{1}	98.2
Pseudomonas syringae pv. *syringae* 3-3	bacterial canker of fruit cultivars	34.0 ± 0.8^{1}	100.0
Xanthomonas beticola 8717	beet gall	30.0 ± 1.1^{1}	100.0
Xanthomonas campestris pv. campestris 1	cabbage black rot, carrot bacterial blight	32.0 ± 0.9^{1}	98.1
Plant fungal pathogens			
Alternaria alternata YKM F-15694	glume mould of cereals	32.0 ± 0.6^{2}	85.3
Alternaria brassica Pers.	Alternaria blight of cabbage	33.0 ± 1.3^{2}	75.6
Botrytis cinerea YKM F-15692	grey mould of legume and vegetable crops	35.0 ± 0.8^{3}	94.6
Colletotrichum gloeosporioides Sacc.	lupine anthracnose	47.0 ± 1.8^{3}	75.8
Fusarium culmorum YKM F-55043	scab of cereal crops, potato dry rot	32.0 ± 0.8^{2}	68.2
Fusarium oxysporum YKM F-55071	fusariose of vegetable and grain legume cultivars	27.5 ± 0.8^{2}	60.4
Pleiochaeta setosa Kirchn.	lupine brown spot	30.0 ± 1.0^{3}	75.0
Nectria galligena Bres.	European canker of fruit trees	20.5 ± 0.6^{1}	75.0
Rhizoctonia solani Kuhn.	cabbage root rot	32.0 ± 1.0^{3}	96.0

Note – Antagonistic activity was assayed: * – via diameter of growth inhibition zone in test cultures [mm], [1] – growth delay zone of test cultures, [2] – zone of retarded growth, [3] – zone of *B. pumilus* BIM B-263 invasion into test cultures; ** – as a size ratio [mm] of experimental and control colony of test culture [%]

Table 2

Comparative evaluation of inhibitory action of *Bacillus pumilus* BIM B-263 cultural liquid and chemical disinfectants on test cultures of *Escherichia coli* S-3 and *Staphylococcus* sp. 7 (qualitative suspension method)

Test culture	Time [h]	Control	Cultural liquid of *B. pumilus* BIM B-263 [%]				Combined surface disinfectant [%]				Lysol [%]				Sandim-D [%]			
			10.0	2.5	1.0	0.1	10.0	2.5	1.0	0.1	10.0	2.5	1.0	0.1	10.0	2.5	1.0	0.1
E. coli S-3	0.25	+++	–	++	+++	+++	–	+	++	+++	–	+	++	+++	–	–	–	+++
	0.5	+++	–	+	+++	+++	–	+	++	+++	–	+	++	+++	–	–	–	+++
	1.0	+++	–	+	++	+++	–	–	+	+++	–	–	+	+++	–	–	–	+++
	2.0	+++	–	–	+	+++	–	–	–	+++	–	–	–	+++	–	–	–	+++
	3.0	+++	–	–	+	+++	–	–	–	++	–	–	–	+++	–	–	–	++
	4.0	+++	–	–	+	+++	–	–	–	+	–	–	–	++	–	–	–	+
Staphylococcus sp.7	0.25	+++	–	–	–	+	–	–	++	++	–	–	++	+++	–	–	–	++
	0.5	+++	–	–	–	+	–	–	++	++	–	–	++	++	–	–	–	++
	1.0	+++	–	–	–	–	–	–	+	++	–	–	+	+	–	–	–	++
	2.0	+++	–	–	–	–	–	–	+	+	–	–	+	+	–	–	–	+
	3.0	+++	–	–	–	–	–	–	–	+	–	–	–	+	–	–	–	–
	4.0	+++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Notes: 1% neutralizing solution containing 3% tween–80, 0.1% saponin, 0.1% cysteine and 0.1% histidine served as the control; symbols: «+++» – active growth, «++» – moderate growth, «+» – slight growth, «–» – lack of growth

Table 3
Comparative assessment of inhibitory effect of *Bacillus pumilus* BIM B-263 cultural liquid and chemical disinfectants on test cultures of *Escherichia coli* S-3 and *Staphylococcus* sp. 7 (estimation of antimicrobial activity is based on application of rubber tubes)

Test culture	Time [h]	Control	Cultural liquid of *B. pumilus* BIM B-263 [%]				Combined surface disinfectant [%]				Lysol [%]				Sandim-D [%]			
			10.0	2.5	1.0	0.1	10.0	2.5	1.0	0.1	10.0	2.5	1.0	0.1	10.0	2.5	1.0	0.1
E. coli S-3	0.25	+++	–	+	++	++	–	+	++	+++	–	+	++	+++	–	–	–	++
	0.5	+++	–	+	++	++	–	+	+	++	–	+	++	+++	–	–	–	++
	1.0	+++	–	–	+	++	–	–	+	++	–	–	+	++	–	–	–	++
	2.0	+++	–	–	+	++	–	–	–	++	–	–	–	++	–	–	–	+
	3.0	+++	–	–	–	+	–	–	–	++	–	–	–	+	–	–	–	–
	4.0	+++	–	–	–	+	–	–	–	+	–	–	–	+	–	–	–	–
Staphylococcus sp.7	0.25	+++	–	–	–	+	–	–	++	++	–	–	++	+++	–	–	–	++
	0.5	+++	–	–	–	+	–	–	+	++	–	–	++	++	–	–	–	++
	1.0	+++	–	–	–	–	–	–	+	++	–	–	+	+	–	–	–	+
	2.0	+++	–	–	–	–	–	–	+	+	–	–	+	+	–	–	–	+
	3.0	+++	–	–	–	–	–	–	–	+	–	–	–	+	–	–	–	–
	4.0	+++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Explanations as in Table 2

The bacteria of genus *Bacillus* are known to utilize various carbohydrates (SMIRNOV et al. 1982), yet not all carbon sources show the same favourable effect on bacterial growth and generation of bioactive compounds with antimicrobial activity. For instance, readily digestible carbohydrares facilitating rapid growth of the cultures may inhibit production of antimicrobial metabolites (OSADCHAYA et al. 1997).

Earlier we found that optimal conditions for growth and synthesis of antimicrobial metabolites by the culture *Bacillus pumilus* BIM B-263 in laboratory fermentor were achieved at pH 7.0, temperature 30°C, aeration rate 1 l/l · min and agitation rate 200 rpm on the medium of the following composition (g/l): molasses – 20.0; $K_2HPO_4 \cdot 3H_2O$ – 7.0; KH_2PO_4 – 3.0; $MgSO_4 \cdot 7H_2O$ – 0.1; NH_4NO_3 – 1.0; corn steep liquor – 2.5; sodium citrate · $3H_2O$ – 0.5; tap water – adjusted to 1 liter (ROMANOVSKAYA et al. 2002).

These data was scaled up to semi-industrial level at research-technical center of the Novopolotsk protein-vitamin concentrate plant. Investigations performed resulted in optimization of *B. pumilus* BIM B-263 submerged culture parameters on modified Meynell medium in 300 l fermentor – aeration intensity 1 l/l · min, agitation rate – 250 rpm, temperature 33 ± 2C°, duration of the process 36–40 h (Table 4).

Table 4
Dynamics of growth and antagonistic activity of bacteria *Bacillus pumilus* BIM B-263 in pilot plant fermentor

Time	pH	Total sugar [g l^{-1}]	Free reducing substances [g l^{-1}]	Number of bacteria	Number of spores ml^{-1}	Diameter of growth delay zones in test cultures [mm]	
						E. coli S-3	*Staphylococcus* sp. 7
0	7.2	12.6	1.7	$1.1 \cdot 10^8$	$9.2 \cdot 10^6$		
12	7.4	8.9	3.2	$5.8 \cdot 10^8$	$4.6 \cdot 10^7$		
24	7.65	6.7	4.5	$2.5 \cdot 10^9$	$6.0 \cdot 10^7$	24.0 ± 0.3	26.5 ± 0.2
36	7.65	5.8	5.45	$3.2 \cdot 10^9$	$9.8 \cdot 10^8$	26.3 ± 0.5	28.4 ± 0.1
40	7.6	5.7	5.6	$4.0 \cdot 10^9$	$1.8 \cdot 10^8$	28.0 ± 0.7	31.5 ± 0.4
48	7.3	5.2	4.4	$3.7 \cdot 10^9$	$3.2 \cdot 10^8$	26.5 ± 0.5	29.8 ± 0.3

Decrease in the temperature to 28 ± 2°C and rate of air flow to 0.8 l/l medium per minute resulted in longer duration of the process – 48 h (caused by deceleration of sporulation process) and reduced antagonistic activity of *B. pumilus* BIM B-263. Enatin obtained with the optimized pilot plant technology is characterized by elevated the number of spores and high antagonistic activity exceeding activity of liquid preparation grown under lab

conditions by 8–14%. Based on obtained results pilot-plant technology of manufacturing Enatin preparation has been elaborated.

Veterinary-toxicological studies performed by researchers from the Institute of Experimental Veterinary Science have revealed that Enatin does not display toxic and toxicogenic action upon peroral and inhaled administration, it lacks skin-irritating properties, embryotoxic and teratogenic effects, does not impair quality of agroproducts.

One of the methods to reduce density of microbial contamination at stock breeding facilities contributing to prevention of outbreaks of contagious diseases assums a complex of veterinary-sanitary measures for disinfection of air and surfaces of stock farms (IBRAHIMOVA 2000, TARABUKINA and NEUSTROEV 2002).

According to investigations performed by IWANCZUK-CZERNIK et al. (2007), POHILENKO, PERELYGIN (2007), TEMMERMAN (2006), biological products may serve as effective preventive agents curbing spread of infections in animal herds caused primarily by the coliforms and staphylococcal--streptococcal groups.

For studies on dose-dependent effect in vitro, Enatin working solution at the concentrations 50, 20, 10, 5 and 2.5% was used. It follows from data presented in table 5 that its inhibitory action on test cultures – *E. coli* S3, *Staphylococcus* sp. 7, *Streptococcus* sp. H2 was established in all experimental variants. Diameter of growth suppression zones in test cultures ranged from 14.3 to 31.0 mm depending on concentration of biopreparation. Maximal growth delay zones of pathogens were recorded at Enatin concentrations 50, 20 and 10% (Table 5).

Table 5

Antagonistic activity of Enatin depending on its concentration

Tested concentration [%]	Diameter of growth suppression zone in test cultures [mm]			
	E. coli S-3	*Staphylococcus* sp. 7	*Streptococcus* sp. H-2	Total heterotrophic microbiota*
Undiluted Enatin (control)	28.6	29.0	28.5	31.0
50	25.5	27.4	27.0	28.0
20	24.5	26.5	25.5	27.0
10	23.8	26.0	25.0	25.2
5	19.5	20.3	20.0	18.0
2.5	17.5	18.5	18.5	14.3
HCP_{05}	2.1	1.4	1.2	1.9

* total heterotrophic microbiota: overall concentration of microorganisms on non-selective (MPA) medium

Table 6

Level of sanitary-indicative and total heterotrophic microbiota in air of stock section after Enatin treatment

Sampling time	Coliform group			Staphylococcal-streptococcal group			Total heterotrophic microbiota		
	CFU/plate	number of microorganisms [10^3 m^{-3}]	Treatment efficiency [%]	CFU/plate	number of microorganisms [10^3 m^{-3}]	Treatment efficiency	CFU/plate [%]	number of microorganisms [10^3 m^{-3}]	Treatment efficiency [%]
Before treatment	36.8 ± 1.5	4.69	–	65.2 ± 1.6	8.3	–	942.0 ± 9.5	120.0	–
Days after treatment:									
1	–	–	100	7.2 ± 0.2	0.92	89	188.4 ± 1.6	24.	80
2	–	–	100	10.5 ± 0.6	1.34	84	207.2 ± 3.2	26.4	78
4	1.8 ± 0.2	0.23	95	16.3 ± 0.5	2.08	75	348.5 ± 3.8	44.4	63
7	4.6 ± 0.5	0.59	87.5	21.2 ± 0.8	2.7	68	395.6 ± 3.9	50.4	58
10	4.8 ± 0.7	0.61	87	37.2 ± 1.1	4.74	43	763.0 ± 5.2	97.2	19
14	6.0 ± 0.8	0.75	84	130.0 ± 1.6	16.6	–	934.2 ± 8.0	119.0	–

Notes: differential-diagnostic Endo medium, selective mineral agar and MPA (2% of agar) were used for isolation of coliform bacteria, staphylococcal--streptococcal group and total microbiota, respectively; applied concentration of Enatin working solution – 10%

Table 7
Level of sanitary-indicative and total heterotrophic microbiota in air of stock section after Enatin treatment (aspiration method according to Kpotov)

Sampling time	Coliform group			Staphylococcal-streptococcal group			Total heterotrophic microbiota		
	CFU/plate	number of microorganisms [10^3 m^{-3}]	Treatment efficiency [%]	CFU/plate	number of microorganisms [10^3 m^{-3}]	Treatment efficiency	CFU/plate [%]	number of microorganisms [10^3 m^{-3}]	Treatment efficiency [%]
Before treatment	158.0+3.8	15.82	–	261.0+4.1	26.1	–	948.0+8.1	94.8	–
Days after treatment:									
1	1.6 ± 0.4	0.16	99.0	44.4 ± 1,9	4.44	83.0	237.0 ± 2.8	23.7	75.0
2	3.1 ± 0.9	0.31	98.0	46.9 ± 2,4	4.69	82.0	265.4 ± 3.1	26.5	72.0
4	17.4 ± 1.2	1.74	89.0	83.5 ± 2,6	8.35	68.0	398.2 ± 3.0	39.8	58.0
7	21.5 ± 1.5	2.15	86.4	91.3 ± 2.7	9.13	65.0	521.4 ± 4.2	52.1	45.0
10	26.9 ± 1.3	2.69	83.0	159.2 ± 3,1	15.92	39.0	824.8 ± 5.6	82.5	13.0
14	27.5 ± 1.5	2.75	82.6	442.0 ± 4.3	44.2	–	974.6 ± 8.4	97.5	–

Explanations as in Table 6

Table 8

Level of sanitary-indicative and total heterotrophic microbiota on the surface of stock section upon Enatin treatment

Sampling time	Stock box surface [CFU/cm^2]				Floor surface, CFU/cm^2				Wall surface, CFU/cm^2			
	Coliforms group [n · 10^2]	staphylococcal--streptococcal group, [n · 10^2]	total heterotrophic microbiota, [n · 10^3]	sporulating species, [n · 10^2]	Coliforms group [n · 10^2]	staphylococcal--streptococcal group, [n · 10^2]	total heterotrophic microbiota, [n · 10^3]	sporulating species, [n · 10^2]	Coliforms group [n · 10^2]	staphylococcal--streptococcal group, [n · 10^2]	total heterotrophic microbiota, [n · 10^3]	sporulating species [n · 10^2]
Before treatment	27.0+0.3	420.0+0.8	360.0+8.4	2.3+0.1	12.0+0.7	290.0+4.1	300.0+4.0	1.5+0.03	3.2+0.04	19.0+1.2	520.0+7.0	2.8+0.05
After treatment:												
1 day	–	44.9+0.3	57.6+4.3	318.0+2.5	–	37.7+1.2	102.0+5.2	265.0+4.1	–	1.6+0.09	179.2+6.3	195.0+3.2
Treatment efficiency [%]	100	89.3	84.0	–	100	87.0	66.0	–	100	91.5	68.0	–
2 days	–	63.0+0.5	82.8+5.3	300.0+1.9	–	41.0+0.9	140.0+9.5	250.0+3.2	–	2.1+0.05	250.0+12.1	180.0+2.7
Treatment efficiency [%]	100	85.0	77	–	100	86.0	53.0	–	100	89.0	52.0	–
4 days	4.7+0.05	75.6+0.7	118.8+7.7	224.0+2.1	0.96+0.03	72.5+1.7	153.0+7.2	168.0+2.7	0.4+0.05	4.4+0.3	255.0+8.1	127.0+1.5
Treatment efficiency [%]	82.5	82.0	67.0	–	91.0	75.0	49.0	–	87.4	77.0	51.0	–
7 days	5.1+0.08	130.0+0.8	180.0+9.5	80.0+1.4	1.3+0.02	78.0+1.5	180.0+12.9	130.0+1.5	0.5+0.02	5.1+0.1	340.0+13.1	36.0+1.7
Treatment efficiency [%]	81.0	69.0	50.0	–	89.0	73.0	40.0	–	84.0	73.0	35.0	–
10 days	7.8+0.07	222.6+0.7	225.0+7.2	69.0+1.8	3.8+0.09	165.3+2.3	228.0+8.5	79.0+1.8	1.4+0.04	9.3+0.6	369.2+9.2	12.5+2.2
Treatment efficiency [%]	71.0	47.0	37.5	–	68.0	43.0	24.0	–	56.3	51.0	29.0	–
14 days	8.9+0.09	320.0+0.9	310.0+4.2	60.0+1.1	4.4+0.04	190.0+4.8	320.0+10.5	10.0+3.7	1.5+0.02	14.0+1.3	460.0+10.7	2.7+0.1
Treatment efficiency [%]	67.0	24.0	14.0	–	63.0	34.0	–	–	53.0	26.0	12.0	–

It should be noted that antimicrobial activity of Enatin at large stock-breeding complex may differ significantly from that registered at laboratory level.

It seemed appropriate therefore to evaluate efficiency of different Enatin concentrations under industrial conditions.

It was shown that all tested concentrations of Enatin inhibited development of sanitary-indicative and total microbiota in air of stock sections. Yet, 10% working concentration of biopreparation proved preferential in efficiency, allowing to reduce air contamination with the bacteria of coliform group by 100%, staphylococcal-streptococcal group by 84%, total heterotrophic microflora by 78% in 2 days after treatment. In 7 days Enatin biological efficiency equaled 87%, 68% and 58%, respectively. Coliform bacteria were not detected even upon 14 days following treatment procedure (Table 6). Analysis of air microbiota in stock sections treated with Enatin using aspiration method with the Krotov device, demonstrated decrease in air contamination by 98 and 86.4% for *E. coli* by 82 and 65% for bacteria of staphylococcus-streptococcus group (the values were calculated 2 and 7 days after treatment, respectively) (Table 7).

Microbial colonization of farm stock boxes, floor and walls by coliforms, staphylococcal-streptococcal group and total heterotrophic microbiota 2 days after treatment declined by 100%, 87% and 61%, after 7 days – by 85%, 72% and 42%, respectively. Efficiency of the Enatin action against coliforms and staphyloccal-streptococcal group after 14 days averaged at 61 and 28%, respectively (Table 8).

Summary and Conclusions

Investigations have been completed to evaluate antimicrobial activity spectrum of sporulating bacterial strain *Bacillus pumilus* BIM B-263. It was shown that the examined strain displays high antagonistic activity towards a broad range of animal bacterial pathogens from genera *Escherichia*, *Klebsiella*, *Proteus*, *Staphylococcus*, *Streptococcus*, plant pathogens representing genera *Erwinia, Pseudomonas, Xanthomonas* and phytopathogenic fungi of genera *Botrytis, Colletotrichum, Nectria*.

Laboratory trials have demonstrated that the efficiency of *Bacillus pumilus* BIM B-263 action against *E. coli* S-3 matches, and with respect to *Staphylococcus* sp. 7 even exceeds the effect of the tested chemical disinfectants CSD and Lysol, indicating attractive prospects for a biological derived eco-safe product designed for antimicrobial treatment of farm stock breeding complexes.

Parameters were optimized for submerged fermentation of *Bacillus pumilus* BIM B-263 on laboratory and pilot-plant level. It was found that the

optimal conditions for culture growth in 300 l fermentor on modified Meynell medium were achieved at aeration rate 1 l/l min, agitation rate 250 rpm, temperature 33 ± 2^{o}C and process duration 36–40 h. Biopreparation Enatin produced according to optimized pilot-plant technology is characterized by the elevated numbers of tested microorganisms, their spores and antagonistic activity exceeding by 8–14% activity of the liquid product obtained under laboratory conditions. The collected data laid basis of pilot-plant regulations for a Enatin manufacturing process.

Veterinary-toxicological Enatin trials confirmed that the product is not toxic and toxigenic at peroral and inhalatory administration, it does not display skin-irritating properties, embryotoxic and teratogenic effects, will not affect quality of farm products.

High efficiency of Enatin action with regard to pathogenic and facultatively pathogenic of coliforms and staphylococcal-streptococcal groups under full-scale production conditions and its encouraging application prospects for sanitation of stock breeding complexes were demonstrated. Developed Enatin biopreparation in major parameters – spectrum of antimicrobial activity, technological flexibility and biological efficiency is the first of its kind in Belarus and matches recognized foreign analogs.

Biopreparation Enatin registered by State Veterinary Inspection of Principal Veterinary Department, Ministry of Food and Agriculture, Belarus as preventive disinfection agent for swine-breeding complexes (certificate nr 2795-10-08 BD of March 25, 2008) is recommended for application at pig farms located in the Republic of Belarus.

Translated by VLADIMIR KOLBUN

Accepted for print 20.10.2011

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(1): 31–39, Y. 2012

ANALYSIS OF RESULTS OF CONFORMATION EVALUATION OF THE STANDARD CHINCHILLA ACHIEVED ON THE BREEDING FARM

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K e y w o r d s: conformation evaluation, chinchilla, animal's age.

A b s t r a c t

The aim of the work was to assess the phenotypic value of conformation of standard chinchillas depending on their age groups. The research embraced a population of chinchillas (1986 individuals including: 1231 females and 755 males) from the breeding farm in the kujawsko-pomorskie voivodship within the years 2003–2010. Analyzed features included: animal size, color type, color purity, fur quality, belly-belt and total score achieved for the features mentioned above. Animals were divided into three age groups: I – up to 6 months, II – between 7 and 10 months, III – over 11 months.

As a result of the research it has been shown that the most stabilized features on the assessed farm were: animal size (71% of the population presented max. evaluation 4 pt.) and belly-belt (80% of the population presented max. evaluation 3 pt.). It has been pointed out that the older the animal was the higher score it obtained during the license evaluation. Other features obtained lower scores than the possible maximum, which proves that the special attention should be paid while conducting further breeding work on the considered farms.

ANALIZA WYNIKÓW OCENY POKROJU SZYNSZYLI ODMIANY STANDARD UZYSKANYCH NA FERMIE ZARODOWEJ

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S ł o w a k l u c z o w e: ocena pokroju, szynszyla, wiek zwierzęcia.

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Abstrakt

Celem pracy było oszacowanie wartości fenotypowej cech pokroju szynszyli odmiany standard w zależności od grupy wiekowej. Badaniami objęto populację szynszyli (1986 osobników w tym: 1231 samic i 755 samców) z fermy zarodowej w województwie kujawsko-pomorskim w latach 2003–2010. Analizowano takie cechy jak: wielkość i typ zwierzęcia, typ barwny, czystość barwy, jakość okrywy włosowej, pas brzuszny oraz sumę punktów uzyskanych za wymienione cechy. Zwierzęta podzielono na trzy grupy wiekowe: I – do 6 miesięcy, II – od 7 do 10 miesięcy i III – powyżej 11 miesięcy.

Stwierdzono, że najbardziej ustabilizowane cechy na ocenianej fermie to: wielkość i budowa zwierzęcia (max. ocenę 4 pkt. otrzymało 71% populacji) oraz pas brzuszny (max. ocenę 3 pkt., otrzymało 80% populacji). Wykazano, że im starsze były oceniane zwierzęta, tym wyższą otrzymywały punktację podczas oceny licencyjnej. Pozostałe cechy uzyskały punktację niższą od możliwego maksimum. Należy na nie zwrócić szczególną uwagę, prowadząc dalszą pracę hodowlaną na badanej fermie.

Introduction

The chinchilla is a fur animal of great worth. Its fur distinguishes among other furs by outstanding softness, density and original color. The chinchilla skins are utilized for manufacturing of luxury clothing, for example complete fur-coats as well as some supplementary items (BARABASZ 2003) (e.g. muffs and caps). The standard chinchilla is a species which is the most frequently used for farming in Poland. Within the last 10 years of the XX-th century, an interest in breeding of this breed of fur animals increased essentially in Poland, despite the fact that the farming activities are comparably difficult (FELSKA-BŁASZCZYK 2006). The immediate result of this interest was an increasing number of farms and – in consequence – the number of female chinchilla in foundation stock. In 2009, the chinchilla took the second place in Poland – after the mink (ZAWIŚLAK 2010) – among the fur animals subjected to evaluation of a conformation.

Husbandry of the chinchilla in Poland had been started within the mid-fifties of the XX-th century, however in the kujawsko-pomorskie voivodship it was done essentially later i.e. in nineties of the previous century. Within the period from 1994 till 2000 there were only 2 breeding farms. Then, the number of farms increased year by year. In 2010, there were as many as 11 farms (ZAWIŚLAK and WOJCIECHOWSKI 2011) in kujawsko-pomorskie voivodship.

The goal of this paper is to evaluate the fenotype value of the conformation of the standard chinchilla depending on the age group of animals for the chosen breeding farm in kujawsko-pomorskie voivodship.

Materials and Methods

Materials for investigations were extracted from the formal records concerning husbandry activities on the breeding farm involved in cultivation of the standard chinchilla. The farm is located in kujawsko-pomorskie voivodship, whereas the data was related to the period from 2003 till 2010. Within the whole mentioned period, the evaluation was performed by the same licensed judge. The evaluation of conformation was permanently carried out according to the valid pattern of evaluation which had been worked out by the Central Institution on Farming of Animals in Poland (*Wzorzec szynszyli*... 2000). The analyzed population consisted of 1986 individuals (among them: 1231 females and 755 males). Lower number of male individuals is connected with a harem-like system of breeding of the investigated animals. This system, due to economical reasons, is the most popular system on the chinchilla breeding farms.

There were several main subjects of the analysis e.g.: features like animal size (0–4 pt.), color type (0–5 pt.), color purity (0–9 pt.), fur quality (0–9 pt.) as well as belly-belt (0–3 pt.). The maximal sum of points assigned to all features was equal to 30 pt. Chinchillas which obtained 0 pt. for at least one feature, were disqualified and they were eliminated from further breeding.

Based upon the date of birth of an animal and the license date the age was calculated, which was taken into account during the evaluation of conformation of a chinchilla. Then the whole population was divided into three groups depending on their age: up to 6 months, from 7 till 10 months, above 11 months.

For the above listed features considered for a conformation evaluation, non-parametrical estimation of median value was performed. Standard deviation (S_x) was also calculated. During determination of the median – additionally the sex of investigated animals was taken into consideration. The complete results were statistically processed, presented and analyzed by means of the commercial software Statistica Pl (STANISZ 1998).

Results and Discussion

Based upon the performed analysis of the results of evaluation of conformation of the standard chinchilla (Table 1) – the following conclusions can be drawn: among the 1986 evaluated specimens the maximal number of points according to the feature: animal size was obtained by 71% of the population of animals. The specimens of age above 11 months had the values of this feature on the level 81% and it was the highest in comparison to the other

Table 1

Phenotype evaluation (in pt.) of standard chinchilla from the breeding farmin kujawsko-pomorskie voivodship within the period 2003–2010 – dependency on age group

Traits evaluation	Chinchillas' age during evaluation											
	up to 6 months (n = 336)			from 7 till 10 months (n = 1229)			11 and above (n = 421)			total (n = 1986)		
	median [pt.]	individual number [%]	S_x	median [pt.]	individual number [%]	S_x	median [pt.]	individual number [%]	S_x	median [pt.]	individual number [%]	S_x
Animal size	4	63	3.43	4	70	0.69	4	81	0.66	4	71	0.72
Color type	3	63	3.10	3	52	0.62	3	49	0.65	3	53	0.64
Color purity	7	81	6.96	7	82	0.84	7	84	0.79	7	82	0.85
Fur quality	7	70	6.87	7	68	1.13	7	68	1.13	7	68	1.12
Belly-belt	3	77	2.77	3	79	0.41	3	85	0.36	3	80	0.41
Total score	23	23	23.16	24	23	1.93	24	25	1.85	24	23	1.99

considered groups of animals. Within the period of investigations (Table 1), median of conformation was equal to 4 pt.

Animal size has an important meaning in breeding practice because the size of a fur just directly depends on this feature. Size of a fur is almost the most important factor which influences a price of a fur on the market which finally determinates profitability of husbandry business (BARABASZ 2008). Based upon the chosen scientific reports, one can expect that particular size of young animal gives highly probable forecast of a size of fur which could be achieved for the mature animal (BARABASZ 2008). BARABASZ et al. (2010) had determined the correlation coefficients between the body mass of a chinchilla and the fur parameters – especially they proved highly essential interaction with surface of skin ($r = 0.796$). LANSZKI (1999) in own, original investigations – performed in Hungary – has shown essential influence of the body mass on the fur length, where the obtained correlation coefficient for the chinchilla was equal to $r = 0.65$. However, the investigations performed by POYRAZ et al. (2005) on the chinchilla in Turkey had shown a correlation between a body mass and a length of a fur – on a relatively low level i.e.: only $r = 0.160$. Additionally, SULIK and CHOLEWA (1998a) proved weak correlation ($r = 0.18$; $p < 0,05$) between lengths of an ear and the body mass of a chinchilla.

The second feature which is evaluated during the license procedure is: color type. The median for this feature was equal to 3 pt. (Table 1). Simultaneously, it was shown that the male specimens had higher median in comparison to the female specimens (4 pt) – Figure 1. Taking into considerations the consecutive age groups of animals (in the light of evaluation procedure) – it was shown that the number of specimens which reached median on the level 3 pt was less and less in case of higher and higher age (Table 1). In accordance to the pattern of conformation of chinchillas (*Wzorzec szynszyli*... 2000), the specimens evaluated as 'excellent' according to this feature (color type) should have extensively dark, graphite black coloring evenly distributed on the back and the sides being in evident contrast in comparison to the belly-belt. Such an animal shall obtain 5 pt. Among the evaluated population, relatively low number of specimens reached this result, however median equal to 3 pt. was reached by only 53% of the investigated specimens and it was lower in comparison to all other features which have been taken into account. Some essential factors which have an influence on color of fur are e.g.: feeding and lighting of cages (FELSKA-BŁASZYK 2006, MACIEJOWSKI 1994).

In the investigated population of chinchillas, the essential majority of specimens has proper color purity. These specimens have median on the level 7 pt. (82% of the population), however the female specimens had greater standard deviation – Figure 1. According to MACIEJOWSKI (1994), proper concentration of different colorings existing in a particular fur depends just

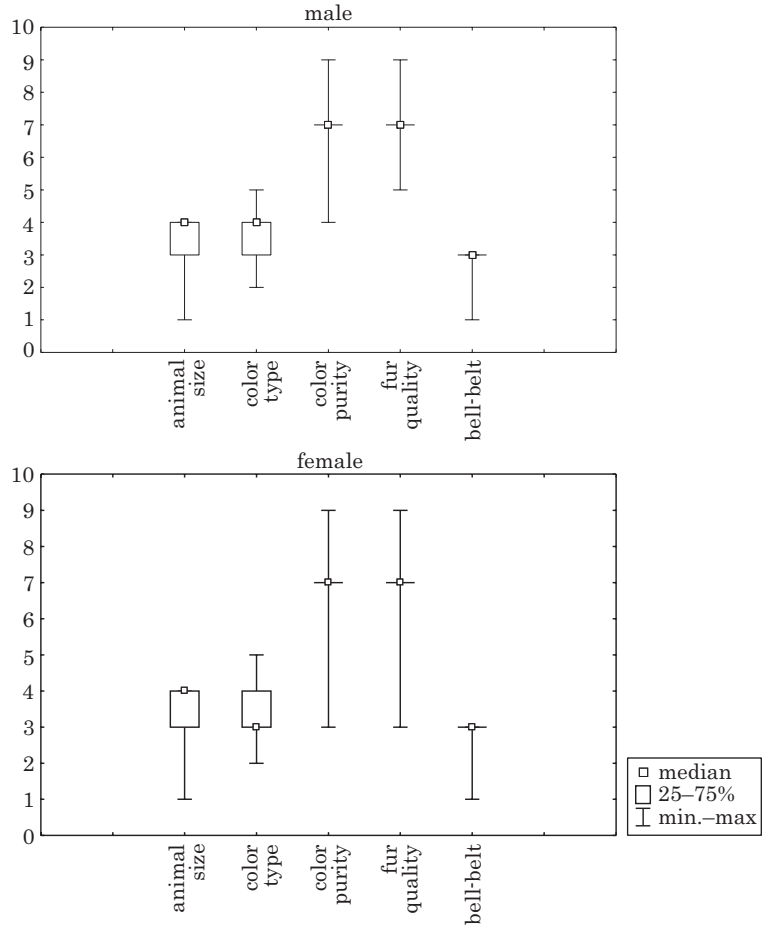

Fig. 1. The median value (pt.) obtained during conformation evaluation of standard chinchilla depending on sex

on the color purity. SULIK and CHOLEWA (1998b) had proved that the highest color purity for standard chinchillas is characteristic for the period winter/spring as well as they proved that the older a specimen is, the worse level of this feature is registered. During our investigations, the influence of age on the color purity was not confirmed. In our case percentage of maximal number of points assigned during the evaluation process was taken into account.

Analyzing data concerning fur quality – which was collected during the license procedure – it can be seen that the median value is reached by the number of animals within the range from 68% till 70%, whereas the value of median was equal to 7 pt. Moreover the span was from 3 to 9 pt. for female

specimens and from 5 to 9 pt for male specimens – Figure 1) was observed. However in the recent years (2008–2010), it was observed an essential decrease of number of specimens which obtained the score on the level of 7 pt. (during evaluation procedure). The above mentioned decrease was even down to 50%, which can be a warning sign pointing on the worsening structure of chinchillas' hair. Evaluation of fur quality includes such features as: density, silkiness and strain of hair as well as their length and glossiness. Therefore, it is a feature which has most sub-factors so its proper evaluation is most difficult (SULIK 2003). Fur quality is mainly determined by a guard hair (longer and stiffer hairs covering the under fur), even that their percentage in the whole fur hair is relatively low – as it was suggested by CAPPELLETTI and ROZEN (1995). FELSKA-BŁASZYK (2006) – based on her own investigations – it has been shown that increase of illumination above the 120 lx level can even cause diminishing of features connected with fur industry usefulness of these furs.

Belly-belt is a feature which has fairly evenly distributed evaluations. Its range was within the limits from 1 to 3 pt.; for both female and male specimens. Median for the whole population was equal to 3 pt. whereas the number of specimens which exactly reach this evaluation was equal to 80%. Moreover, it can be shown that there is a clear increasing tendency of these evaluations within the period of investigations (Table 1). This fact confirms the effectiveness of performed selection taking into account just this feature. Therefore the evaluated animals had snow-white belly-belt distinguishing (via a clear contrast line) from the sides of an animal. It is the most wanted feature of an animal fur (WOŹNY 2002).

However the overall evaluation (total score in pt.) is a factor which is considered as the most important by the farm owners. According to the pattern of evaluation of the chinchilla (*Wzorzec szynszyli*... 2000) – maximal possible sum of points assigned to all features is equal to 30 pt. Upon the analyses of the data concerning the investigated farms – median for the three investigated age groups was on the level 23–24 pt. (Table 1). So, it is essentially lower than the maximal value considered in the official pattern. Similarly low results for the average of the total score were observed by SOCHA and et al. (SOCHA et al. 2004) – for investigation of different breeds of chinchillas i.e: standard, black, silky and beige performed on farms of south Poland.

In our own investigations, number of specimens which reached the evaluation on median level (i.e. 24 pt.) was within the range of from 20 to 30% – Figure 2. However, it was observed a clear tendency of increase of the number of animals which reach higher and higher total evaluation of conformation. In the case of the investigated farm – special attention should be paid to the selection of animals (especially males) for mating, which have to have fur hairs of a very high quality as well as a proper body mass.

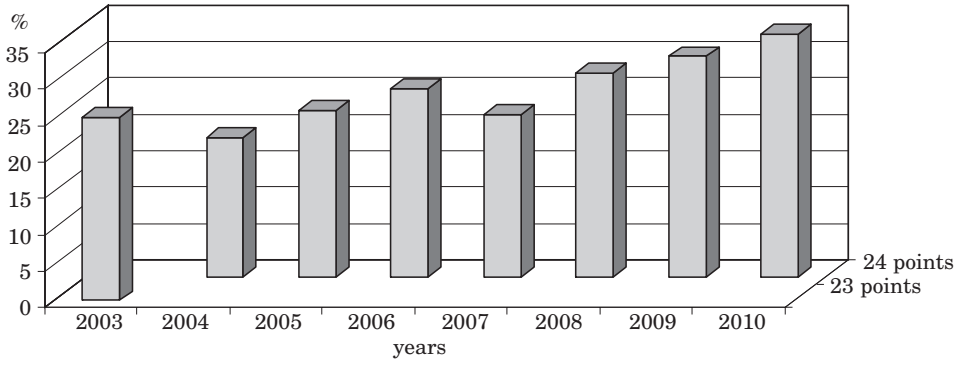

Fig. 2. Percentage distribution of the median for the total score of the standard chinchilla from the breeding farm in kujawsko-pomorskie voivodship within the period 2003–2010

Based upon the Table 2, one can analyze an influence of the animal age on the evaluation of conformation for the investigated chinchillas (5 features). The highest influence of age was observed on such features like e.g.: animal size, where 80,52% of chinchillas of age above 11 months obtained the maximal evaluation (4 pt.) as well as belly-belt, where approx. 85% of specimens obtained the maximal evaluation. In case of the remaining features, the number of specimens which obtained the maximal evaluations was low (color purity and fur quality) or even very low (color type).

Table 2

The dispersion of the maximum score for each features of confirmation evaluation of chinchillas depending on animal's age

Animal's age	Animal size	Color type	Color purity	Fur quality	Belly-belt
	percentage of the max number of points				
Up to 6 months	62.80	0.30	8.90	11.90	77.31
From 7 till 10 months	70.46	1.49	12.32	13.58	78.80
11 and above	80.52	0.71	10.71	12.83	84.80

To sum up, it can be stated that the standard chinchillas on the analyzed farm in the kujawsko-pomorskie voivodship have characteristic features like e.g: animal size and belly-belt on a satisfactory levels. There were observed some dependencies of these features e.g.: the older are the specimens (during license procedure) the higher is median. However, the remaining features like: color type, color purity and fur quality have the evaluations on the unsatisfactory levels. Therefore the breeding work on the investigated farm should be improved.

Translated by Stanisław Zawiślak

Accepted for print 5.01.2012

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(1): 41–51, Y. 2012

TOURISTIC AND RECREATIONAL USE OF THE SHORE ZONE OF UKIEL LAKE (OLSZTYN, POLAND)

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K e y w o r d s: lake, lake shores, shore accessibility, recreational impact, touristic use.

A b s t r a c t

Ukiel Lake (surface area 412 ha, maximum depth 43 m), the largest of the eleven lakes in Olsztyn, consists of four distinct basins, each with different environmental features. The presence of urban infrastructure, including a grid of streets, in the nearest proximity of the lake arises much interest in the recreational use of the lake. The present description of how the lake's shore zone around each basin is developed and managed relies on some observations gathered from monitoring the nature in the shore zone and access to the lake's water. The analysis of the actual use of the lake's tourist space was conducted on the basis of direct observations, including our assessment of the recreational pressure on the beaches, baths and near the water table. The results suggest that Ukiel Lake, owing to its size and diverse character, can be used for many different forms of recreation. Among the four basins, the most intensively developed and subjected to the highest recreational impact is Olsztyńskie Basin.

TURYSTYCZNE I REKREACYJNE ZAGOSPODAROWANIE STREFY BRZEGOWEJ JEZIORA UKIEL (OLSZTYN, POLSKA)

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S ł o w a k l u c z o w e: jezioro, linia brzegowa, dostępność strefy brzegowej, wpływ rekreacji, zagospodarowanie turystyczne.

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Abstrakt

Jezioro Ukiel (powierzchnia 412 ha, głębokość maksymalna 43 m) jest największym z jedenastu jezior Olsztyna. Składa się z czterech wyraźnie wyodrębnionych plos o odmiennych warunkach środowiskowych. Obecność zabudowy miejskiej, a także sieci dróg, w tym krajowych, w bezpośrednim otoczeniu zbiornika wpływa na znaczne zainteresowanie jego rekreacyjnym użytkowaniem. W oparciu o monitoring uwarunkowań przyrodniczych strefy brzegowej i dostępu do lustra wody dokonano charakterystyki zagospodarowania brzegów poszczególnych plos jeziora. Analizę wykorzystania przestrzeni turystycznej przeprowadzono w oparciu o jej bezpośrednią obserwację, określając natężenie ruchu rekreacyjnego w obrębie plaż, kąpielisk i lustra wody.

Uzyskane wyniki wskazują, że dzięki swej wielkości i zróżnicowaniu budowy jezioro Ukiel może być wykorzystywane do różnorodnych form rekreacji. Ploso Olsztyńskie jest częścią jeziora najbardziej zagospodarowaną i poddaną największemu natężeniu ruchu rekreacyjnego.

Introduction

Lakes make up a significant tourism industry resource and their recreational function creates the main economic base for many lake areas and even whole countries (BRAGG et al. 2003, COOPER 2006). Lakes and lake shores have other economical, social and cultural functions as well, but above all they serve an ecological role. Lake shores represent characteristic transitional habitats between terrestrial and aquatic ecosystems (SCHMIEDER 2004). Most recreational activities take place on the lake shore or at least use the lake shore for access to the water table (TIKKANEN 2003, SCHMIEDER 2004). Lakeshore habitats and ecosystems are of significant importance to the total biodiversity of landscapes owing to their high structural diversity and the resulting variety of ecological niches but also because of their expanse (KAJAK 2001, SCHMIEDER 2004). However, human involvement may lead to deterioration of lake shores, especially if a lake lies in a densely populated area. In order to protect lakes and lake shores, responsible management is required, which will rely, among other things, on continuous monitoring of the status of lake shores.

Ukiel Lake, which lies in the Masurian Lake District (north-eastern Poland) and, more specifically, in Olsztyn (Figure 1), a town with a population of 176 thousand, is evidently an example of a lake subjected to strong man-made pressure. The Masurian Lake District is one of the major tourist destinations in Poland and the lakes are the foundation for development of the tourism industry in this region (LIJEWSKI et al. 2008, *New 7 wonders* 2011). Olsztyn, the capital city of Warmia and Mazury Province, has eleven lakes, of which Ukiel Lake is the largest (LOSSOW et al. 2005). The surface area of the lake is 412 ha (Table 1), and the shoreline is strongly developed (the shoreline development factor equals 3.14) with numerous inlets, bays and peninsulas. Some of the lake shore rises quite high above the water

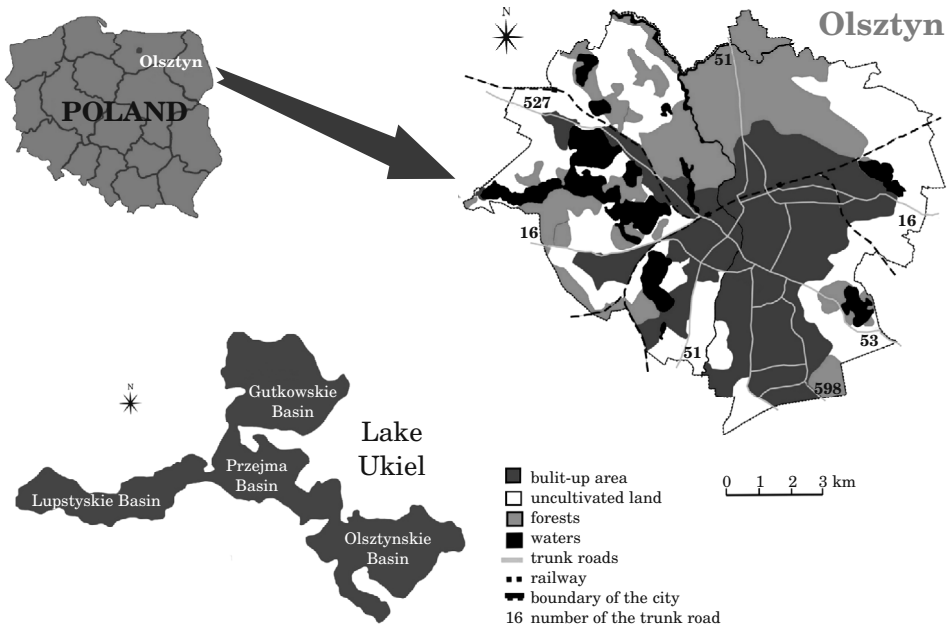

Fig. 1. Location of Olsztyn and Ukiel Lake

Table 1

Morphometric parameters of Ukiel Lake

Basin	Surface area [ha]	Maximum depth [m]	Average depth [m]	Lakeshore length [m]	Number of housing estates near lake shores
Olsztyńskie	120.4	20	7.0	6580.0	2
Gutkowskie	117.9	43	13.2	5187.8	2
Łupstyskie	85.4	32	10.1	4875.9	1
Przejma	84.9	41	12.7	5767.5	0
Ukiel Lake	412	43	10.6	22550	4

table, forming cliff banks up to 20 meters high. The natural watercourses supplying the lake with water are small streams, flowing through forests and fields. The Kortówka River is the only watercourse draining water from the lake. Ukiel Lake consists of four distinct basins, each with different environmental features (LOSSOW et al. 2005). The actual surface area of the lake's catchment basin is 1,675 ha, of which 62.7% is afforested, 21.6% is fallow land, 12.4% is arable land, 2.9% is developed and 0.4% is covered by surface waters. The drainage basin of the lake's basins Przejma and Łupstyskie is dominated by forests, whereas the catchment of the basins Gutkowskie and, especially, Olsztyńskie, are mainly developed areas or fallow land.

The presence of urban infrastructure, including a grid of streets, in the nearest proximity of the lake arises much interest in the recreational use of the lake. The main purpose of the present work has been to characterize the shoreline management and the actual use of the lake's tourist space.

Material and Methods

The present description of how the lake's shore zone around each basin is developed and managed relies on some observations gathered while monitoring the wildlife in the shore zone and the access to the water table around all the lake's basins. The method used for evaluation of the suitability of natural water bodies for angling was applied (SKRZYPCZAK 2005). Six properties (indices) were taken into consideration, as shown in Table 2, all of which define possible penetration of the shore line, not just by anglers but also by people pursuing other forms of recreation. The indices were assigned weights, which reflect varied importance attached to the possible recreational use of the lake shores. Four of these properties are termed stimulants, i.e. they identify the lake as more attractive and accessible; two are the so-called destimulants, which depress the lake's attractiveness and availability for recreation (Table 2). The preferences direction was made uniform by the method of a shift towards the maximum, thus changing destimulants into stimulants. Charac-

Table 2

Parameters of natural conditions of water bodies for performing the angling function (SKRZYPCZAK 2005)

Property	Measure unit	Character of property*	Weight
Shoreline development factor (K) $K = \frac{L}{2\sqrt{2\pi P}}$ L – shoreline length, P – surface	value of factor	S	0.10
Shoreline with a 1–5 m wide belt of emergent plants	% of shoreline length	S	0.25
Shoreline with a > 5 m wide belt of emergent plants	% of shoreline length	D	0.10
Drainage basin (up to 100 m) afforested, with the groundwater level < 1.0 m	% of shoreline length	S	0.20
Drainage basin (up to 100 m) covered with boggy forests, wetlands and swamps	% of shoreline length	D	0.10
Access to water table – anglers' paths (point access) and line access (width 1–10 m – 1 point 11–20 m – 2 points 21–30 m – 3 points, ect.)	points/100 m of shoreline	S	0.25

* Character of the property in the sense of stimulating (S – a stimulant) or limiting (D – a destimulant)

teristics which acted as stimulants were standardized by dividing the value of the factor by the value of the reference point (standard). The highest values of the characteristics were taken as standards. Next, the standardized characteristics were multiplied by the weights of the factors, and the results were added up. This way, a synthetic measure was obtained, which is a weighted average of the standardized characteristics.

The data for computing values of the above measure for all the four basins of Ukiel Lake were collected during field measurements and observations with additional information found in relevant literature and from a map of the lake's bathymetry.

The analysis of the actual use of the lake's tourist space consisted of direct observations lasting from 25 July to 7 August 2007 and 2008, which additionally included the authors' assessment of the recreational pressure on the beaches, baths and the lake's surroundings. The observations covered six beaches (3 around Olsztyńskie Basin, and one around each of the other three basins). The observations were carried out every day from 12.00 to 2.00 p.m. On each occasion, the number of people relaxing on or near the beach as well as the number of people using all types of water equipment (paddle boats, canoes, yachts, motor-boats, etc.) were counted. The information about the water and air temperature was provided by the Water Emergency Service lifeguards, who take such measurements every day on guarded beaches.

Results

Gutkowskie basin has the most favourable conditions for the development of water recreation (the value of the measure 0.7; Table 3). It has the best access to the water table, owing to the fact that this lake's basin lies in the nearest vicinity of two housing estates and near a main road (Table 1, Figure 1). There is a continuous stretch of the lake's shore with good access to open water near the town's developed area (Figure 1, Figure 2). Further, 14 fishing jetties, 2 recreational piers and a beach were inventoried along this part of the lake's shoreline. About 46.5% of the shoreline is overgrown with a belt of helophytes (emergent plants), although the belt of vegetation is over 5 meter wide just along 16.5% of the shoreline (Table 3).

The second most favourable natural conditions for water relaxation purposes can be found around Olsztyńskie Basin (Table 3). This basin has a large share of afforested land along the shoreline (46.15%) and a belt of emergent aquatic plants (48.9%). It is also characterized by a high water accessibility index (3.37 points). The open water is accessible from about 31% of the shoreline (Figure 2). The longest section of the shore where the water table

Table 3

Parameters of the natural conditions and value of the synthetic measure for particular basins of Ukiel Lake with respect to the recreational functions

Property	Basin			
	Olsztyńskie	Gutkowskie	Łupstyskie	Przejma
Shoreline development factor (K)	1.70	1.36	1.50	1.78
Shoreline with a 1–5 m wide belt of emergent plants	24.0	30.0	15.8	16.4
Shoreline with a > 5 m wide belt of emergent plants	24.9	16.5	10.0	15.1
Drainage basin (up to 100 m) afforested, with the groundwater level < 1.0 m	46.1	21.8	31.2	64.5
Drainage basin (up to 100 m) covered with boggy forests, wetlands and swamps	0	0	0	0
Access to water table	3.37	3.94	2.47	1.6
Value of synthetic measure	0.65	0.70	0.57	0.60

Measure units as in Table 2.

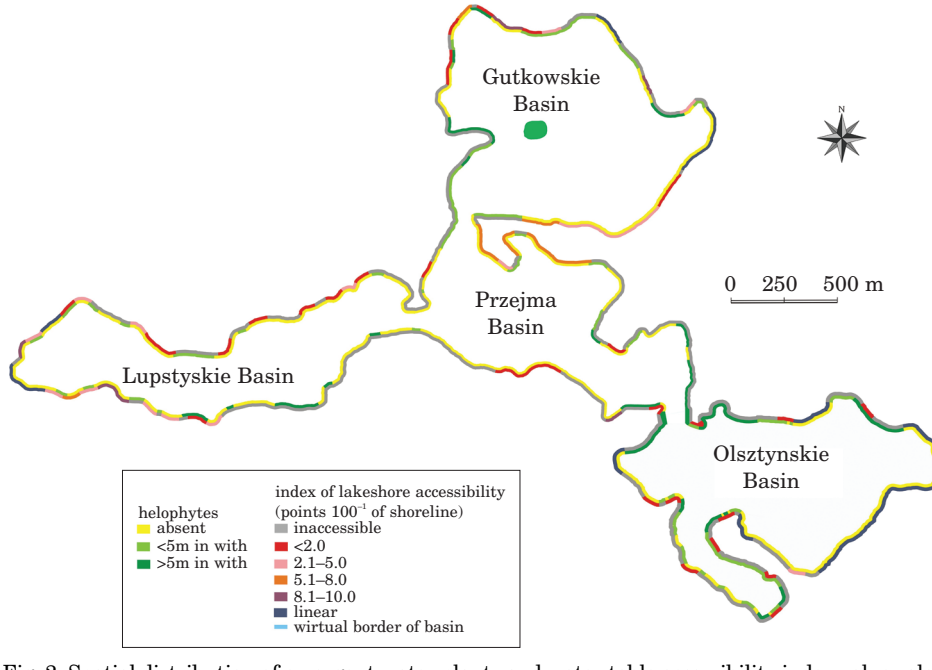

Fig. 2. Spatial distribution of emergent water plants and water table accessibility index values along the shoreline of Ukiel Lake

can be easily accessed by people (over 1,400 meters) is in the south-eastern part of the basin, where three beaches are located (including the largest one on Ukiel Lake, called the Municipal Beach). Other facilities located on the south-eastern shores of Olsztyńskie Basin are the water police base, the Volunteer Lifeguards Association base and three sports and recreation centres. Two other sports centres, a holiday centre with a water equipment rental and some water recreational facilities, such as 16 recreational and 9 angling jetties can be found around this basin of Ukiel Lake. Two housing estates and a main road are situated in the vicinity of this basin.

The basins Przejma and Łupstyskie have less easily accessible shores (Table 3). Access to the water table along some longer section of the shore can be found only near a housing estate by Łupstyskie Basin. There are also 12 fishing jetties and a small beach around this lake's basin. The fourth lake's basin called Przejma, where neither residential nor hydroengineering constructions can be found, has just one beach and over 64% of the shore zone is covered by forests. There is only point access to the water table (Table 2).

The rate of recreational movement during the analyzed period of time ranged from 0 to 4,366 persons (Table 4, Figure 3). It mainly depended on the weather and was higher in 2008 (Figure 3). The air temperature in 2007 ranged from 18 to 25°C, and water temperature varied from 18 to 19°C; in 2008, the respective temperatures were 19–29°C and 21–24°C. In 2008, there were more sunny, rainless days. More people tended to arrive at the lake

Table 4

The intensity of recreational traffic at Ukiel Lake

Observation time	Number of visitors					
	beaches and baths		water table		total	
	mean (± SD)	range	mean (± SD)	range	mean (± SD)	range
Weekdays	455.3 (± 543.2)	0–1902	65.2 (± 63.6)	0–208	520.5 (± 589.2)	0–2022
Weekend	1485.0 (± 1730.0)	8–4094	114.6 (± 100.8)	16–272	1599.6 (± 1801.6)	26–4366
Total	749.5 (± 1099.1)	0–4904	79.3 (± 77.5)	0–272	828.8 (± 1154.2)	0–4366

during weekends (mean 1599.6 persons) than on weekdays (mean 520.5 persons). One reason is that more events, e.g. sailing regattas, beach ball games or outdoor concerts, are organized at weekends, and they can attract more people, despite possibly worse weather conditions. On average, about 90% of the visitors stayed on the beaches; around 10% used all kinds of water equipment, mainly paddle boats and canoes (over 70%).

The recreational movement is mainly oriented towards Olsztyńskie Basin, where over 93% of all visitors come to relax (Figure 3), with 91% choosing the

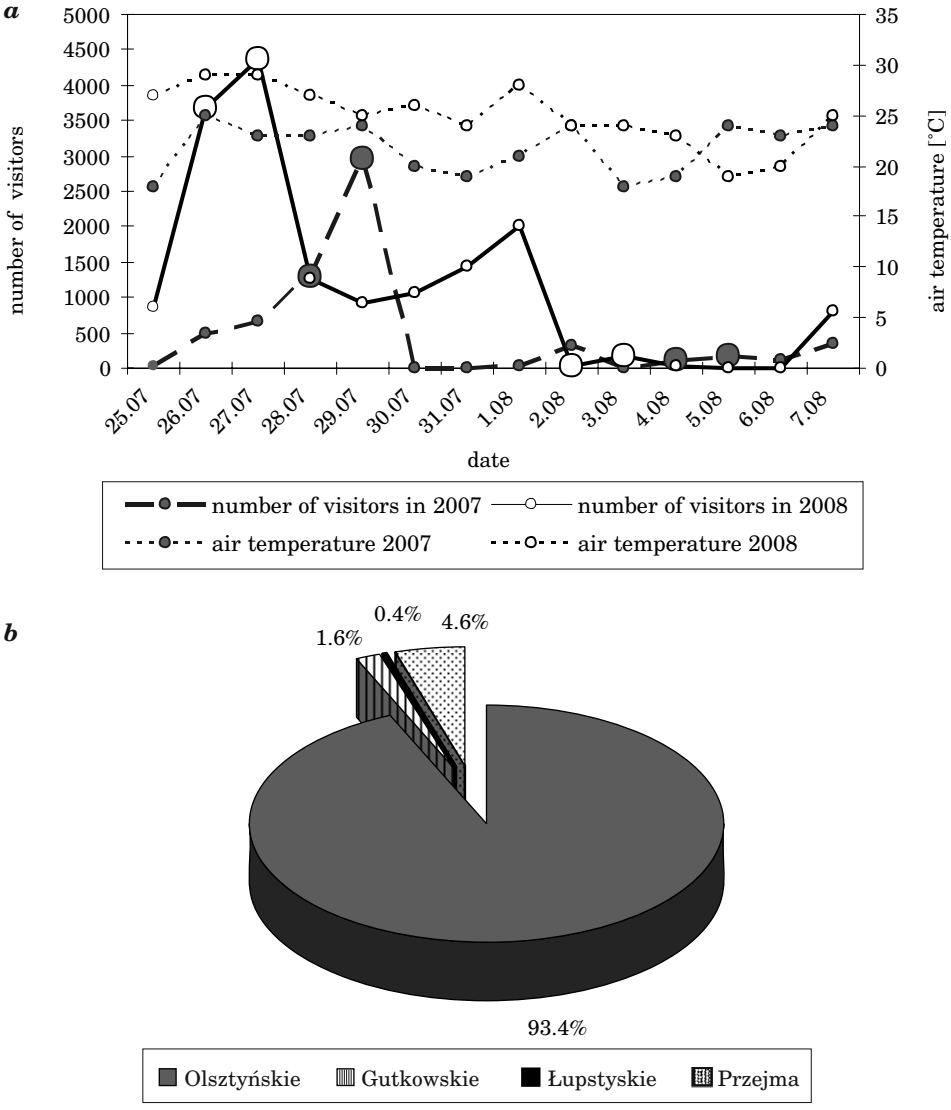

Fig. 3. The intensity of recreational traffic at Ukiel Lake: *a* – number of visitors resting by Ukiel Lake and the air temperature during the study. Large points stand for weekends, *b* – spatial distribution of recreational traffic rates around Ukiel Lake

Municipal Beach. Most of the events take place on the Municipal Beach because it has the best sports and recreational facilities. Likewise, most people using water sports equipment stay on Olsztyńskie Basin, where they can rent boats. The second most popular beach is the one by the basin called Przejma (4.6%). This beach owes its popularity to its location – near forests and far from

urban compact development. The other two basins, Gutkowskie and Łupstyskie, are only marginally developed to serve recreational purposes and consequently are visited mainly by residents of the nearby housing estates.

Discussion

For water tourism and recreational use, the most suitable are large water reservoirs, covering over 100 ha, where many forms of water recreation are possible and which lend themselves to sailing (LIJEWSKI et al. 2008). Other factors which enhance the recreational attractiveness of lakes are presence of forests on lake shores, easy access to shores and good transport accessibility (DEDIO 1989, DEJA 2001). The results of our study suggest that Ukiel Lake, owing to its size and diverse character, can be used for many different forms of recreation (Table 1, Table 3, Figure 2). It is a lake with a well-developed shoreline, moderate water plant growth of the shores (37.6%) and a large share of afforested catchment basin (62.7%). It also possesses quite well developed tourist amenities (6 larger beaches, 6 sports and recreation centers). The fact that the lake lies in a town means that it is easily accessible, e.g. by public transport. The most easily accessible are the basins Gutkowskie and Olsztyńskie (Table 3, Figure 2). Because of its location inside a town, Ukiel Lake experiences recreational pressure not just during the summer season but all year round.

Lakes are vulnerable ecosystems, sensitive to changes caused by tourists and holidaymakers, and the type and extent of such changes are a function of the type and variety of tourist uses, the number of people who use the lake for recreation and the natural surroundings (COOPER 2006, HALL and HÄRKÖNEN 2006). All parts of Ukiel Lake can be classified as holomictic (dimictic) and eutrophic water bodies (LOSSOW at al. 2005). The trophic status increases from the least eutrophic Gutkowskie Basin, through Przejma and Olsztyńskie to the basin called Łupstyskie. Among the four basins, Olsztyńskie Basin is the most intensively developed and subjected to the highest recreational pressure (over 93% of all visitors, 3 beaches, 5 sports and recreation centers). At the same time, Olsztyńskie Basin is the least resistant to degradation (low average depth, small volume of the hypolimnion), whereas the other basins have better natural conditions (LOSSOW et al. 2005).

Because of the highly diversified use of lakes and their basins (e.g. water supply, fishing, transportation, agriculture, housing), and a variety of water recreation activities, it is difficult to assess the actual influence of tourist and recreational activities on changes in the natural environment. According to LOSSOW et al. (2005) recreational activities near or on Ukiel

Lake are responsible for around 3.3% of the external nitrogen load and 4.5% of the external phosphorus load reaching the lake. However, the changes are not limited to the inferior water quality but can also be noticed in the shore zone and littoral of lakes, that is the two components of the lake's environment which are most attractive and therefore most intensively used by holidaymakers (LIDDLE and SCORGIE 1980, COOPER 2006). It has been demonstrated that around 10% of the shoreline of Ukiel Lake is used for recreation, but the percentage goes up to 30% when Olsztyńskie Basin alone is considered. Such strong and continually increasing recreational pressure, exacerbated by the lack of a management plan based on good knowledge of the lake's environment, may lead to the degradation of Ukiel Lake.

SCHMIEDER (2004) emphasizes lack of information about the shoreline of lakes, while OSTENDORP (2004) suggests that principal quality components in the system for evaluation of the quality of lake shores that he is developing (the Integrated Lakeshore Quality Assessment) should comprise "forms and intensities of human activities in the lakeshore zone". To determine the impact of tourism on lake shores it is necessary to identify areas in the land water interface that were developed for the purpose of tourist accommodation, active recreation and other areas serving functions related to the tourism industry (FURGALA-SELEZNIOW et al. 2010). According to the World Lake Vision Committee (2003) "decision making for lake management should be based on sound science and best available information".

Translated by JOLANTA IDŹKOWSKA

Accepted for print 18.01.2012.

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(1): 53–66, Y. 2012

BIODIVERSITY AND INDICATIVE ROLE OF ZOOPLANKTON IN THE SHALLOW MACROPHYTE-DOMINATED LAKE ŁUKNAJNO

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K e y w o r d s: Lake Łuknajno, macrophyte-dominated lake, zooplankton biodiversity, biocoenotic indices.

A b s t r a c t

The biological diversity of zooplankton communities was studied in August 2006 and 2007 in Lake Łuknajno (Masurian Lakeland, NE Poland), a shallow, macrophyte-dominated water body. An analysis of the species composition, abundance, biomass and the values of biocoenotic indices revealed significant differences between the analyzed zooplankton groups. The noted differences were related to trophic levels, the presence of macrophytes and environmental conditions. A total of 20 zooplankton species were reported in 2006 and 2007. The average abundance and biomass of zooplankton in 2006 and 2007 reached 546 indiv./dm^3 and 0.3085 mg/dm^3, and 385 indiv./dm^3 and 0.6113 mg/dm^3, respectively. Rotifers dominated in terms of abundance (75% in 2006 and 70% in 2007), while crustaceans in terms of biomass (approx. 80% in 2006 and over 70% in 2007). In 2006 and 2007, indicator species of high trophicity accounted for 41% and 24%, respectively, of the total zooplankton abundance in Lake Łuknajno. The values of biocoenotic indices did not point to the predominance of any zooplankton group. There were no significant differences in biodiversity between sites with and without macrophyte cover, whereas such differences ($p < 0.05$) were observed between sampling sites.

RÓŻNORODNOŚĆ BIOLOGICZNA I INDYKACYJNA ROLA ZOOPLANKTONU W PŁYTKIM MAKROFITOWYM JEZIORZE ŁUKNAJNO

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S ł o w a k l u c z o w e: jezioro Łuknajno, jezioro makrofitowe, bioróżnorodność zooplanktonu, wskaźniki biocenotyczne.

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Abstrakt

Badania różnorodności biologicznej zooplanktonu przeprowadzono w płytkim, makrofitowym jeziorze Łuknajno (Pojezierze Mazurskie) w sierpniu 2006 i 2007 r. Na podstawie składu gatunkowego, liczebności, biomasy i wskaźników biocenotycznych stwierdzono duże zróżnicowanie grup zooplanktonu, zależne od trofi siedliska, makrofitów i warunków środowiskowych. Zarówno w 2006, jak i 2007 r. zaobserwowano po 20 gatunków zwierząt planktonowych. Średnia liczebność i biomasa w roku 2006 i 2007 wynosiły odpowiednio: 546 osobn./dm^3, 0.3085 mg/dm^3 oraz 385 osobn./dm^3 i 0.6113 mg/dm^3. Wrotki decydowały o liczebności zooplanktonu (75% w 2006 i 70% w 2007 r.), a biomasę kształtowały skorupiaki (około 80% w 2006 i ponad 70% w 2007 r.). Podczas badań odnotowano gatunki wskaźnikowe wysokiej trofii wód. W roku 2006 stanowiły one 41% udziału w ogólnej liczebności zooplanktonu jeziora Łuknajno, natomiast w roku kolejnym – 24%. Wskaźniki biocenotyczne nie wskazywały na dominację jakiejkolwiek z grup zooplanktonu. Między siedliskami porośniętymi makrofitami a siedliskami bez pokrywy roślinnej nie wykazano istotnych statystycznie różnic w różnorodności biologicznej. Różnice ($p < 0.05$) te stwierdzono natomiast między stanowiskami badawczymi.

Introduction

Biological diversity, or biodiversity, is defined as the variety of life forms at various levels. An analysis of biodiversity in a given environment enables to determine the species composition of the studied communities and the effects of external and internal factors, in both quantitative and qualitative terms. Biodiversity studies often focus on shallow water bodies where the composition of flora and fauna is considerably affected by environmental conditions. The specific character and ecological significance of shallow water bodies result from their morphology. Due to their relatively low depth, wind blowing over their entire surface thoroughly mixes the waters, thus preventing stratification (KUFEL and KUFEL 1997). Another distinct feature of shallow water bodies is the presence of macrophytes, with floating leaves or growing on the bottom. Macrophytes create specific habitats that support zooplankton development (CELEWICZ et al. 2001). Lake Łuknajno, a shallow water body located in the Masurian Lakeland (NE Poland), is overgrown with charophytes in 50%, which creates a suitable habitat for zooplankton development (BOWSZYS et al. 2006).

The objective of this study was to determine the effects of selected abiotic factors and the presence of macrophytes on zooplankton structure, and to analyze differences in biodiversity between habitats and sampling sites.

Materials and Methods

The study was conducted on the shallow, macrophyte-dominated Lake Łuknajno which – according to the typology proposed by KONDRACKI (2001) – is

located in the macroregion of Masurian Lakeland (NE Poland). This kettle, oval-shaped lake, elongated in the N-S direction, is connected to the moraine Lake Śniardwy by a narrow (approx. 500 m wide) inlet. Lake Łuknajno has a total surface area of 623 ha and a maximum depth of 3.0 m. The lake bottom is covered by a thick (approx. 15 m) layer of gyttja. Since the local climate is controlled by Arctic air masses, the growing season is short and the mean annual temperature is as low as + 6.5°C (DENISIUK and PROFUS 1990, BREYMEYER 1997, BOWSZYS et al. 2006). The lake has flat shores overgrown with dense rushes (a 30–150 m wide belt) comprising mostly the common reed (*Phragmites australis*) and the bulrush (*Typha latifolia*). Along the rush belt there is a sedge (*Carex* sp.) belt on the land side and a pondweed (*Potamogeton* sp.) belt on the water side. The lake bottom is covered by charophyte meadows, with *Chara aculeolata* and *Chara aspera* as predominant species (KRÓLIKOWSKA 1997). The littoral zone on the northern, western and southern side is swampy and marshy, with peat deposits. An alder forest stretches along the eastern side of the lake. The area is flat and morphologically uniform (BREYMEYER 1997). The lake's catchment area of 48.4 km2 comprises arable land (55%), grassland (26%) and forests (18%), (KUFEL and KUFEL 1997). Drainage reclamation works carried out in the catchment area of Lake Łuknajno during the late 1920s, including an artificial lowering of the water-table to 2.5 m, contributed to the eutrophication and shallowing of the lake (DENISIUK and PROFUS 1990).

An analysis of zooplankton communities (Rotifera, Crustacea) in Lake Łuknajno was based on materials collected at 20 sites in the water column (Figure 1), in August 2006 and 2007. Water samples were collected with a five-liter Patallas sampler, in the water column, from the surface to the bottom. A total of 25 liters of water was collected. Zooplankton samples were concentrated using an Apstein plankton net (30 μm), and they were fixed in Lugol's solution followed by 2–4% formalin. Water temperature and dissolved oxygen concentrations were determined with the use of the HACH HQd Field Case probe (RUGGED). Water transparency was measured with the Secchi disk (real-time assessment).

Plant communities were first identified based on previous studies (CIECIERSKA et. al. 2009), and next the sites were surveyed by boat during the growing season. A special anchor was used to collect macrophyte samples at deeper sites.

The species characteristic of high trophic levels were determined according to the procedure proposed by KARABIN (1985 a,b). A quantitative analysis of zooplankton was performed as described by STARMACH (1955), HILLBRICHT-ILKOWSKA and PATALAS (1967), BOTTRELL et al. (1976), and EJSMONT-KARABIN (1998). The values of biodiversity indicators (the Shannon-Weaver index based

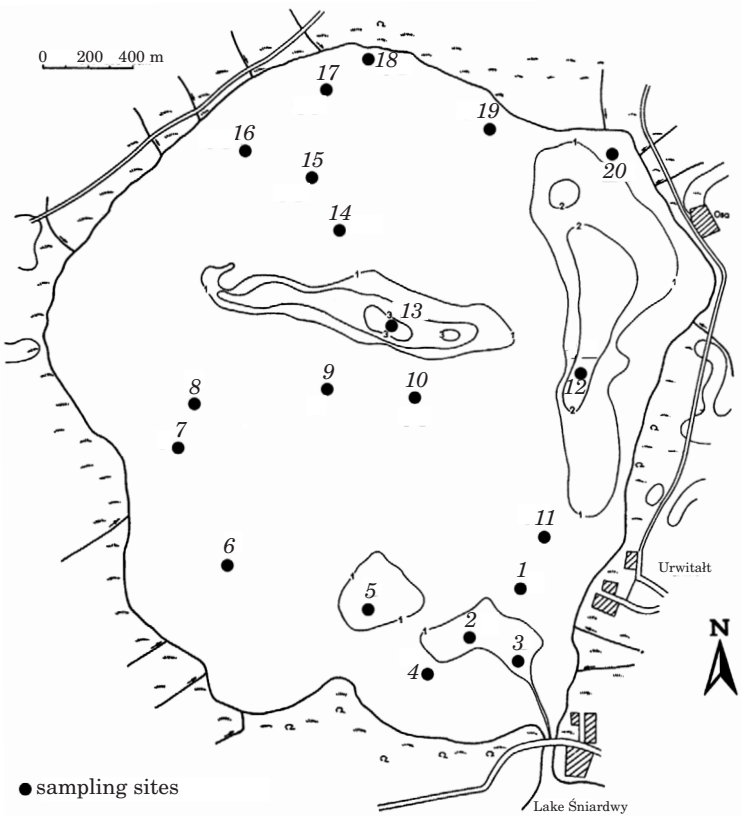

Fig. 1. Location of sampling sites on Lake Łuknajno

on abundance and biomass, the Simpson index based on abundance and biomass, Pielou's evenness index, species richness and density) were determined as recommended by ODUM (1982) and KREBS (1996).

The obtained results, in the form of measurable indices, were processed statistically using the Statistica PL 10.0 software package. The effects of abiotic environmental factors (temperature, oxygen saturation, water transparency) on the average abundance and biomass of zooplankton were determined by the Pearson correlation coefficient. The correlation between the average abundance and biomass of zooplankton and the presence of macrophytes was determined using Spearman's rank correlation coefficient.

In order to compare zooplankton biodiversity between sites with and without macrophyte cover, a *t*-test for two independent samples was performed. The following parameters were compared: the number, abundance and biomass of species, Shannon-Weaver index calculated based on abundance

and biomass, and the percentage share of indicator species in total zooplankton abundance. The data on biodiversity at different sampling sites, measured using the Shanonn-Weaver index based on abundance (HA), were subjected to a statistical analysis (*t*-test for two independent samples).

Results

A total of 25 Rotifera, Cladocera and Copepoda species were recorded in the zooplankton communities of Lake Łuknajno in August 2006 and 2007. In 2006, 20 zooplankton species were determined in water samples, including 11 Rotifera taxa, six Cladocera taxa and three Copepoda taxa. In 2007, also 20 species were identified, including seven Rotifera taxa, seven Cladocera taxa and six Copepoda taxa (Table 1).

Table 1

Species composition of zooplankton in Lake Łuknajno in August 2006 and 2007

2006	2007
ROTIFERA	ROTIFERA
Asplanchna priodonta priodonta (Gosse, 1850)	*Anuraeopsis fissa** (Gosse, 1851)
*Anuraeopsis fissa** (Gosse, 1851)	*Keratella cochlearis cochlearis* (Gosse, 1851)
*Brachionus angularis angularis** (Gosse, 1851)	*Keratella cochlearis tecta** (Gosse, 1886)
Keratella cochlearis cochlearis (Gosse, 1851)	*Keratella quadrata quadrata** (O.F. Müller, 1786)
*Keratella cochlearis tecta** (Gosse, 1886)	*Lecane luna luna* (O.F. Müller, 1776)
*Keratella quadrata quadrata** (O.F. Müller, 1786)	*Polyarthra dolichoptera dolichoptera* (Idelson, 1925)
Kellicottia longispina (Kellicott, 1879)	*Polyartha euryptera* (Wierzejski, 1891)
Lecane luna luna (O.F. Müller, 1776)	*Trichocerca capucina capucina* (Wierzejski & Zacharias, 1893)
Polyarthra dolichoptera dolichoptera (Idelson, 1925)	CRUSTACEA
Polyartha euryptera (Wierzejski, 1891)	Cladocera
Trichocerca capucina capucina (Wierzejski & Zacharias, 1893)	*Alona quadrangularis* (O.F. Müller, 1785)
Trichocerca similis (Wierzejski, 1893)	*Bosmina coregoni* (Baird, 1857)
CRUSTACEA	*Bosmina longirostris** (O.F. Müller, 1785)
Cladocera	*Bosmina longispina* (Leydig, 1860)
Alona quadrangularis (O.F. Müller, 1785)	*Chydorus sphaericus* * (O.F. Müller, 1785)
*Bosmina longirostris** (O.F. Müller, 1785)	*Diaphanosoma brachyurum** (Liévin, 1848)
*Chydorus sphaericus** (O.F. Müller, 1785)	*Leptodora kindti* (Focke, 1844)
Daphnia pulex (Leydig, 1860)	Copepoda
*Diaphanosoma brachyurum** (Liévin, 1848)	*Cyclops bohater* (Koźmiński, 1933)
Leptodora kindti (Focke, 1844)	*Cyclops kolensis* (Lilljeborg, 1901)
Copepoda	*Cyclops vicinus* (Uljanin, 1875)
Cyclops bohater (Koźmiński, 1933)	*Heterocope appendiculata* kop. (Sars, 1863)
Eurytemora lacustris (Poppe,1887)	*Mesocyclops leuckarti** (Claus, 1857)
*Mesocyclops leuckarti** (Claus, 1857)	*Mesocyclops oithonoides** (Sars,1863)

* indicator species of water bodies with high trophic levels (Karabin 1985a,b)

Indicator species of high trophicity were noted in both years of the study. A total of nine indicator species were identified, including: Rotifera – *Anuraeopsis fissa, Brachionus angularis angularis, Keratella cochlearis tecta, Keratella quadrata quadrata*, Cladocera – *Bosmina longirostris, Chydorus sphaericus, Diaphanosoma brachyurum*, and Copepoda – *Mesocyclops leuckarti, Mesocyclops oithonoides*. *B. angularis angularis* was present also in 2006, while *M. oithonoides* was noted only in 2007. In 2006 and 2007, taxa characteristic of high trophicity accounted for 41% and 24%, respectively, of the total zooplankton abundance in Lake Łuknajno.

The horizontal distribution patterns of zooplankton abundance and biomass in Lake Łuknajno were similar in 2006 and 2007. Single forms dominated in terms of abundance (Figure 2), whereas biomass varied over a wider range (Figure 3). In 2006 and 2007, Rotifera accounted for 75% and

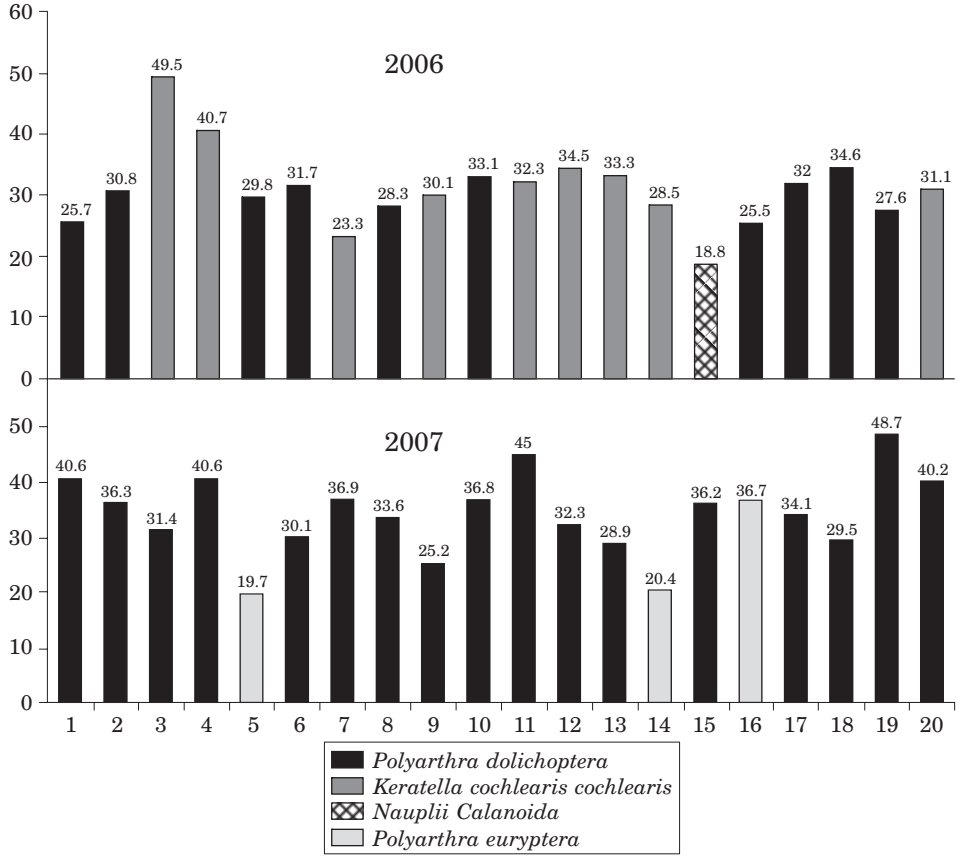

Fig. 2. Share (%) of predominant species in total zooplankton abundance at sampling sites on Lake Łuknajno in August 2006 and 2007

70%, respectively, of total zooplankton abundance. Several rotifer species (*Polyarthra dolichoptera* and *Keratella cochlearis cochlearis* in 2006, and *Polyarthra dolichoptera* and *Polyarthra euryptera* in 2007) formed large populations. Crustaceans accounted for 79% and 80% of total zooplankton biomass in 2006 and 2007, respectively. The predominant species were *Bosmina longirostris, Diaphanosmoma brachyurum* and *Mesocyclops leuckarti*. Juvenile crustaceans also had a high share of total zooplankton abundance and biomass.

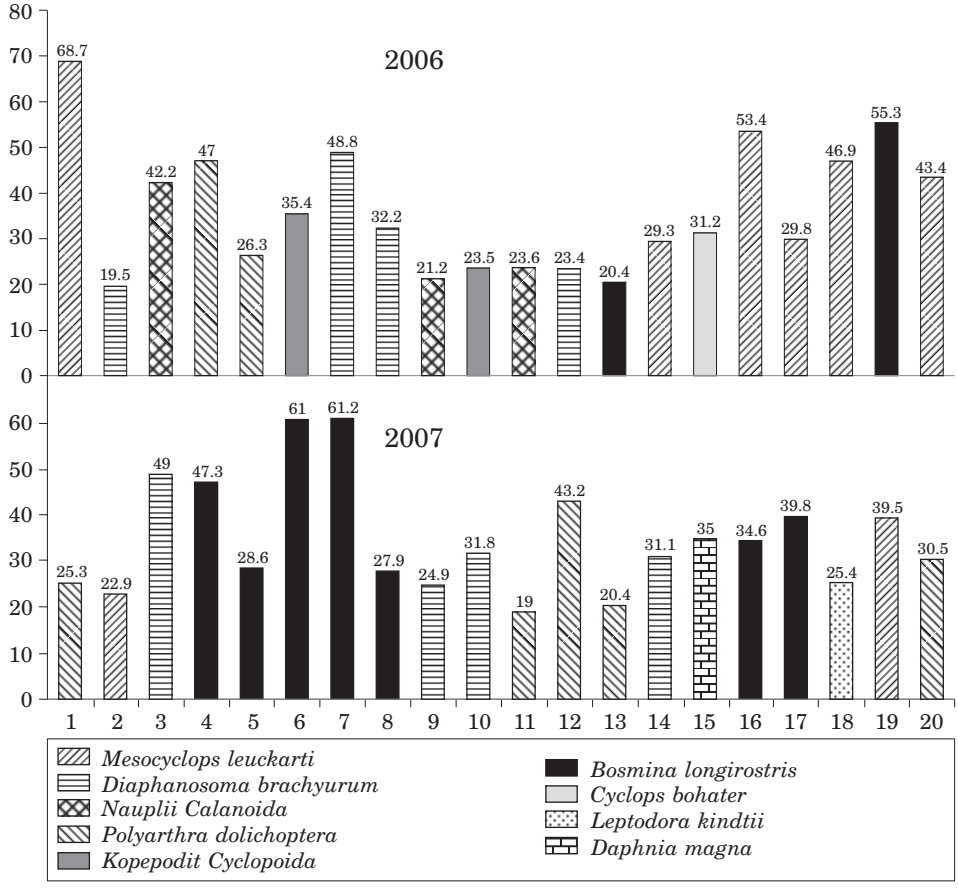

Figure 3. Share (%) of predominant species in total zooplankton biomass at sampling sites on Lake Łuknajno in August 2006 and 2007

Vegetation at the sampling sites showed low diversity levels. No vegetation cover was found at four sites: 2, 6, 9 and 15. The remaining sites were dominated by species of the genus *Chara* and *Stratiotes aloides*, as well as *Ceratophyllum demersum* at site 12, which partly or entirely covered the lake bottom (Table 2).

Table 2
Abiotic parameters of water and bottom cover in Lake Łuknajno in August 2006 and 2007

Sites	Depth [m]	Bottom cover	2006			2007		
			Temp. [°C]	Oxygen [mg O_2 dm^{-3}]	SDV [m]	Temp. [°C]	Oxygen [mg O_2 dm^{-3}]	SDV [m]
Sites with vegetation cover								
1.	0.5	*Chara (sand)*	21	9.42	1.8	22	9.23	1.7
3.	1.0	*S. aloides*	21	9.26	0.3	23	8.96	0.3
4.	1.2	*S. aloides (sand)*	21.5	8.78	1.4	23	9.04	1.3
5.	1.0	*sand (S. aloides)*	22	8.08	1.8	22.5	8.23	1.8
7.	0.3	*Chara (sand)*	22	9.98	0.6	22	9.90	0.6
8.	0.5	*sand (Chara)*	22	9.50	0.5	22	9.87	0.4
10.	0.8	*Chara*	21.5	9.35	0.8	22.5	9.23	0.7
11.	0.6	*Chara*	23	9.46	1.0	23	9.37	1.0
12.	0.8	*Chara (C. demersum)*	23	8.94	0.6	23	9.03	0.55
13.	0.85	*S. aloides*	21.5	8.01	1.0	22	8.12	1.0
14.	1.5	*S. aloides (sand)*	21	8.74	0.3	22.5	8.63	0.25
16.	0.5	*Chara*	21.5	9.52	0.5	22	9.48	0.5
17.	0.4	*Chara (sand)*	22	10.2	0.3	22	9.93	0.25
18.	0.5	*Chara (sand)*	22	9.44	0.2	23	9.37	0.2
19.	0.5	*S. aloides (Chara)*	22	9.17	0.5	23.1	9.15	0.4
20.	0.5	*S. aloides*	22.5	7.93	1.0	22.6	7.89	1.0
Sites without vegetation cover								
2.	0.5	*sand*	21.5	8.58	1.1	22.5	8.61	1.0
6.	0.4	*sand*	22	8.48	0.6	22.5	8.59	0.6
9.	0.9	*sand*	22	9.24	1.0	22	9.11	1.0
15.	2.5	*sand*	21	7.81	0.3	22.5	7.93	0.25

The abiotic factors affecting the environment of Lake Łuknajno were also studied (Table 2). In both study periods, water temperature remained relatively stable, in the 21.0–23.1°C range, oxygen saturation did not drop below 7.81 mg dm^{-3}, and water transparency varied from 0.2 m to 1.8 m. An analysis of the correlation between the average zooplankton abundance and biomass and abiotic environmental factors with the use of the Pearson correlation coefficient did not reveal significant differences between zooplankton abundance and biomass and temperature ($p=0.195$ and $p=0.172$, respectively), oxygen saturation ($p=0.819$ and $p=0.730$) and water transparency ($p=0.162$ and $p=0.688$) in Lake Łuknajno. The values of Spearman's rank correlation coefficient did not show relationships between zooplankton abundance ($p=0.3$) and biomass ($p=0.715$) and the presence or absence of vegetation at the sampling sites.

Table 3

Bioconotic indices of zooplankton communities at sampling sites on Lake Łuknajno in August 2006 and 2007

Sites	Biocoenotic indices													
	2006							2007						
	H_A	H_B	S_A	S_B	e	d	A_g	H_A	H_B	S_A	S_B	e	d	A_g
Sites with vegetation cover														
1.	1.98	1.2	0.83	0.51	0.83	1.62	1.1	1.9	2.1	0.76	0.84	0.79	1.6	1.1
3.	1.22	1.35	0.65	0.71	0.63	1.05	0.7	2.3	1.7	0.85	0.72	0.93	1.85	1.2
4.	1.34	1.47	0.67	0.82	0.69	1.05	0.7	1.98	1.8	0.78	0.73	0.77	1.9	1.3
5.	1.8	1.94	0.79	0.83	0.93	0.89	0.7	2.45	1.8	0.9	0.85	0.96	2.22	1.3
7.	2.24	1.71	0.86	0.7	0.87	1.8	1.3	1.93	1.41	0.79	0.6	0.84	1.48	1.0
8.	1.28	1.92	0.96	0.82	0.58	1.35	0.9	2.15	1.85	0.87	0.81	0.86	1.81	1.2
10.	2.0	2.34	0.81	0.88	0.78	1.87	1.3	1.98	2.0	0.8	0.83	0.8	1.83	1.2
11.	2.0	2.11	0.82	0.85	0.83	1.56	1.1	1.9	2.2	0.75	0.86	0.76	1.76	1.2
12.	2.1	2.0	0.82	0.84	0.91	1.49	1.0	2.05	1.6	0.82	0.72	0.82	1.72	1.2
13.	1.93	2.28	0.98	0.88	0.80	1.47	1.1	2.15	2.1	0.84	0.85	0.81	2.22	1.4
14.	2.13	2.1	0.85	0.84	0.93	1.44	1.0	2.4	2.1	0.88	0.84	0.91	2.3	1.4
16.	2.17	1.62	0.86	0.68	0.94	1.52	1.0	2.1	1.8	0.82	0.79	0.84	1.93	1.2
17.	1.95	2.1	0.82	0.83	0.89	1.24	0.9	2.1	1.7	0.83	0.75	0.91	1.52	1.0
18.	1.9	1.64	0.81	0.72	0.91	1.2	0.8	2.05	1.9	0.84	0.81	0.82	1.94	1.2
19.	1.94	1.54	0.81	0.66	0.93	1.14	0.8	1.6	1.5	0.71	0.73	0.77	1.28	0.8
20.	2.1	1.82	0.84	0.78	0.96	1.39	0.9	1.8	1.7	0.78	0.79	0.78	1.66	1.0
Sites without vegetation cover														
2.	1.96	2.17	0.81	0.87	0.82	1.5	1.1	1.8	2.0	0.78	0.84	0.78	1.52	1.0
6.	1.88	2.1	0.79	0.82	0.76	1.68	1.2	2.2	1.25	0.85	0.57	0.83	2.1	1.4
9.	1.9	2.33	0.82	0.89	0.83	1.4	1.0	2.1	1.9	0.84	0.83	0.91	1.55	1.0
15.	2.15	1.74	0.87	0.78	0.98	1.31	0.9	1.99	1.7	0.81	0.76	0.86	1.6	1.0

H_A – Shannon-Weaver index of species diversity based on abundance, H_B – Shannon-Weaver index of species diversity based on biomass, S_A – Simpson index based on abundance, S_B – Simpson index based on biomass, e – Pielou's evenness index, d – species richness index (species diversity), A_g – species density index

The biodiversity of zooplankton in Lake Łuknajno was evaluated using biocoenotic indices (Table 3). As regards zooplankton abundance, the Shannon-Weaver index ranged from 1.22 to 2.24 in 2006, and from 1.6 to 2.45 in 2007, and its value reached or exceeded 2.00 at many sites. Predomination of a single species was observed at the lowest values of the Shannon-Weaver index, e.g. *Keratella cochlearis cochlearis* dominated at site 3 in 2006. This taxon accounted for 50% of total zooplankton abundance and therefore the Shannon-Weaver index was as low as 1.22. In 2007, a similar situation was observed at site 19 where the predominant species was *Polyarthra dolichoptera*

(49%), and the studied index reached 1.6. There were significant ($p < 0.05$) differences in zooplankton abundance between the sampling sites (63% in 2006 and 64% in 2007). The Shannon-Weaver index based on zooplankton biomass was in the range of 1.2 – 2.34 in 2006 and 1.25 – 2.2 in 2007. The values of this index exceeded 2.00 at many sites. Similarly as with abundance, lower values of the studied index were correlated with the predomination of a single species at sampling sites – e.g. in 2006 *Mesocyclops leuckarti* dominated (69%) in terms of biomass at site 1. The Simpson index, calculated for zooplankton abundance and biomass, reached relatively high values (0.65 – 0.98 and 0.51 – 0.88, respectively). The values of species richness ranged from 0.89 to 1.87 in 2006 and from 1.48 to 2.22 in 2007. Pielou's evenness index varied between 0.58 and 0.98, indicating relatively high species evenness within the analyzed zoocoenosis. The density of zooplankton per unit volume ranged from 0.7 to 1.3. High values of this index were noted at sites 7 and 10, while low values – at sites 3, 4 and 5 where the number of planktonic animals was lower.

An analysis of biodiversity at sites with and without macrophyte cover revealed no significant differences with respect to the number of species ($p=0.8731$), abundance ($p=0.2994$), biomass ($p=0.3519$), values of the Shannon-Weaver index calculated based on abundance ($p=0.7640$) and biomass ($p=0.5272$), and the percentage share of indicator species in the total zooplankton abundance ($p=0.6513$). Differences in zooplankton biodiversity, measured using the Shanonn-Weaver index calculated based on abundance (H_A), were noted between sampling sites. Such differences were recorded at 64% of the sites in 2006, and at 66% of the sites in 2007.

Discussion

Shallow water bodies overgrown with vegetation, such as Lake Łuknajno, are of high ecological importance with regard to the preservation of high biodiversity of zooplankton communities, affected by both biotic and abiotic factors.

The effect of macrophytes on zooplankton abundance and biomass has been widely discussed in literature. Dense vegetation cover provides shelter and food for planktonic animals (BOWSZYS et al. 2006). According to KUCZYŃSKA-KIPPEN (2007), the presence of macrophytes has a direct influence on zooplankton biodiversity. The cited author studied the distribution of rotifers among emergent macrophytes (*Typha latifolia*), submerged macrophytes (*Chara tomentosa*) and in the open water area. The highest zooplankton biodiversity was noted in habitats with *Chara* beds, while the lowest biodiversity was observed at open water sites. The higher biodiversity of zooplankton

in charophyte meadows, compared with *Typha* sp. communities, is due to the more complex morphological and spatial structure of the former, which offers a more effective refuge and access to multiple food sources. Similar results were reported by KUCZYŃSKA-KIPPEN and NAGENGAST (2006a) who studied the shallow, macrophyte-dominated Lake Wielkowiejskie. The values of the Shannon-Weaver index determined for vegetation cover were generally high, and they were lowest in the open water area. Among the analyzed plant communities, the lowest biodiversity was noted at localities with *Utricularia vulgaris* for cladoceran species, and in habitats with *Nymphaea alba* for rotifer species. The highest biodiversity was recorded at localities with *Myriophyllum verticillatum* for Rotifera, and in habitats with *Nymphaea alba* for Cladocera. A trend towards higher zooplankton biodiversity in plant communities than in open water habitats was also reported by other authors (KUCZYŃSKA-KIPPEN and NAGENGAST 2006b, KUCZYŃSKA-KIPPEN and BASIŃSKA 2008, CELEWICZ-GOŁDYN et al. 2010, KUCZYŃSKA-KIPPEN and JONIAK 2010), which suggests that the species richness of planktonic animals increases along with an increase in habitat heterogeneity.

During the current study, conducted in August 2006 and 2007 on Lake Łuknajno, the absence of vegetation cover was reported at four sites only. No significant correlation was found between zooplankton abundance and biomass, and the presence or absence of vegetation, most probably due to a too small number of measurements. Further investigations are therefore required to determine whether such a relationship exists.

In shallow water bodies overgrown with vegetation, zooplankton biodiversity cannot be analyzed in view of a single factor (the presence of macrophytes), since the problem is much more complicated. Plant communities differ considerably with respect to environmental conditions including the physicochemical parameters of water (temperature, dissolved oxygen content, the concentrations of biogenic elements). KUCZYŃSKA-KIPPEN and KLIMASZYK (2007) investigated the effect of oxygen saturation on zooplankton in charophyte beds and found that only rotifers were significantly affected by this factor. The oxygen concentrations were lowest in the middle of charophyte mats (30% lower than at the remaining sites), mostly due to high vegetation density which hindered the flow of oxygen-carrying water. The above resulted in worse light conditions, a slower rate of photosynthesis and a faster rate of organic matter decomposition. The lowest abundance of Rotifera was also noted in the middle of charophyte meadows, which indicates that the density of rotifers decreases along with a drop in oxygen levels. Such a relationship was not observed in the present study since zooplankton samples were collected only in August. However, the effect of temperature on zooplankton abundance in Lake Łuknajno was reported by KARABIN et al. (1997) who noted that

cold-water, oxygen-loving rotifer species, such as *Polyarthra dolichoptera*, *Filinia terminalis* and *Synchaeta pectinata*, dominated at low temperatures (ca. 7°C), accompanied by juvenile crustaceans (Cyclopoida), while cladocerans were nearly absent. A rapid increase in water temperature was followed by an unexpected appearance of large populations of *Bosmina longirostris* and the disappearance of species which prefer lower temperatures. DAR et al. (2009) also observed a positive correlation between temperature rise and the abundance of Copepoda, which suggests that copepods, in contrast to rotifers, develop better at higher temperatures.

The present study was conducted on Lake Łuknajno only in August, in 2006 and 2007, when water temperature and dissolved oxygen concentrations remain relatively constant. Therefore, no significant effect of the examined abiotic environmental factors on zooplankton abundance was noted. A more detailed analysis should be performed, preferably over an annual cycle, to determine the impact of temperature and oxygen availability on the growth of planktonic animals.

Zooplankton biodiversity can also be evaluated in view of trophic conditions. Numerous researchers (KUCZYŃSKA-KIPPEN et al. 2004, PINTO-COELHO et al. 2005, PATUREJ 2006, WANG et al. 2007) found a close correlation between the trophic status of a water body and the abundance of planktonic animals. As demonstrated by PACE (1986), the taxonomic structure of zooplankton changes along with changes in the trophic state of a water body. Oligotrophic waters are usually dominated by copepods of the order Calanoida, whereas smaller copepods of the order Cyclopoida, rotifers and cladocerans predominate in eutrophic waters. Certain zooplankton species may act as bioindicators of the trophic state of a water body (KARABIN 1985a,b). Such a relationship was observed in many lakes (PATUREJ 2006, ČEIRĀNS 2007, PIASECKI and WOLSKA 2007, PATUREJ 2008), including in Lake Łuknajno where species typical of high trophicity were present. Yet the bioindicative role of zooplankton in Lake Łuknajno could be minimized by the effects of other factors. EJSMONT-KARABIN et al. (1996) reported that bacteria and macrophytes, which have the capacity to accumulate phosphorus, may decrease the amount of this element in the water column, thus maintaining lower trophicity. KUFEL and OZIMEK (1994) also demonstrated that owing to their specific ecological and physiological properties (relatively slow decomposition rates and the ability to grow during the winter season), stable *Chara* populations contribute to maintaining mesotrophic conditions in Lake Łuknajno through phosphorus uptake and storage in their biomass. Thus, the presence of bioindicator zooplankton species could not reflect the actual trophic state of the analyzed water body. Further studies are required to determine whether this is a general trend characteristic of all shallow lakes.

Statistical analyses showed no significant correlation between zooplankton abundance and biomass in Lake Łuknajno, and the abiotic parameters of the environment. There were no significant differences in biodiversity between sites with and without macrophyte cover, whereas such differences were observed between sampling sites. According to the findings of other authors, zooplankton biodiversity in Lake Łuknajno is affected by a variety of abiotic environmental factors and the presence of macrophytes. The fact that no such relationships were noted in this study suggests scarcity of data and the need for further investigations.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 14.11.2011

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(1): 67–79, Y. 2012

EVALUATION METHOD OF LABELLING OF VEGETABLE OILS ON THE OLSZTYN MARKET

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K e y w o r d s: vegetable oil, labelling, quality, fatty acids, degree of hydrolysis and degree of oxidation.

A b s t r a c t

The aim of the study was to evaluate the labelling of selected vegetable oils and the provision of required and up-to-date information about their quality. Eight vegetable oils, including 2 rapeseed oils and 6 mixtures, were used as the study material. Evaluation of labelling involved analysing information provided on oil labels in terms of its compliance with the requirements set out in *Tłuszcze roślinne*... PN-92/A-86932, particularly as to whether it was up-to-date and whether it complied with the quality determined in the analyses. The quality of oils was described by the degree of hydrolysis (acid value), degree of oxidation (peroxide number, anisidine number, diene and triene content) and fatty acid composition.

Labelling of the analysed vegetable oils was found to be unsatisfactory since not all the required information was provided; for example, there was no information about the preservation or flavouring of the oils, only sporadically was any information provided on vitamin content (1 out of 8 oils) and other information was missing. Labelling was also unsatisfactory in terms of the accuracy of information because it did not provide data about the ratio of omega-6 and omega-3 acids or about how long an oil had been stored – which is associated with the content of fat oxidation products. It was established in analyses of oil quality that the acid value and the peroxide number did not exceed the minimum adopted for refined oils (*Oleje i tłuszcze*... PN-A-86908:2000) and that the values of anisidine number in 3 oils were close to, or exceeded, the value mentioned in the standard. The percentage of saturated fatty acids complied with the producers' claims, whereas the percentage of monounsaturated fatty acids was below standard for one oil and the percentage of polyunsaturated fatty acids was below standard for two oils.

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OCENA SPOSOBU ZNAKOWANIA OLEJÓW ROŚLINNYCH Z RYNKU OLSZTYŃSKIEGO

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S ł o w a k l u c z o w e: olej roślinny, znakowanie, jakość, kwasy tłuszczowe, stopień hydrolizy i utlenienia.

A b s t r a k t

Celem badań była ocena znakowania wybranych olejów roślinnych w kontekście dostarczania wymaganych i aktualnych informacji o ich jakości. Materiał badawczy stanowiło 8 olejów roślinnych, w tym 2 rzepakowe oraz 6 mieszanin. Oceniając znakowanie, analizowano informacje zamieszczone na etykietach opakowań olejów pod kątem ich zgodności z wymaganiami określonymi w *Tłuszcze roślinne...* PN-92/A-86932, aktualności informacji oraz zgodności z jakością ustaloną w badaniach. Jakość olejów określono poprzez wyznaczenie stopnia hydrolizy (liczba kwasowa) i utlenienia (liczba nadtlenkowa, anizydynowa, zawartość dienów i trienów) oraz składu kwasów tłuszczowych.

Stwierdzono, że znakowanie analizowanych olejów roślinnych jest niezadowalające z uwagi na brak wszystkich wymaganych informacji, np. całkowity brak informacji o konserwowaniu i aromatyzowaniu, sporadyczne informowanie o zawartości witamin (1 na 8 olejów) oraz brak innych danych. Znakowanie jest niezadowalające także w kontekście trafności informacji, gdyż konsumenci nie mogą poznać proporcji kwasów omega-6 i omega-3 oraz długości okresu przechowywania, związanej z zawartością produktów utlenienia lipidów. W badaniach jakości olejów ustalono, że wartości liczby kwasowej i nadtlenkowej wszystkich olejów nie przekraczały maksimum przyjętego dla olejów rafinowanych (*Oleje i tłuszcze...* PN-A-86908:2000) oraz, że wartości liczby anizydynowej w 3 olejach zbliżone były do wartości normatywnej lub ją przekraczały. Oznaczony udział kwasów tłuszczowych nasyconych we wszystkich olejach był zgodny z deklaracjami producentów, natomiast udział kwasów jednonienasyconych – niezgodny w przypadku jednego oleju, a wielonienasyconych – w dwóch olejach.

Introduction

The beneficial effect of vegetable oils on human health has been known for a long time. It was common knowledge by the end of the 20th century that these oils owe their nutritional value to a high content of polyunsaturated fatty acids from omega-6 and omega-3 families (EUFA). The best oils were soybean, sunflower and grapeseed oil, which contained over 60% of polyunsaturated acids. Omega-6 and omega-3 acids are metabolised in the human body to units with a higher number of double bonds and a longer carbon chain, which are precursors of two groups of eicosanoids with complementary, beneficial effects on the body (BARTNIKOWSKA 2008, JELIŃSKA 2005). Omega-6 and omega-3 acids are metabolised with the help of Δ-6desaturase, hence, an excess of one acid inhibits or prevents transformations of the other (KOLANOWSKI 2007).

The diet of people living in industrialised countries contains an excess of omega-6 acids and a deficit of omega-3 ones, which results in an improper ratio of 15.0:1–16.7:1 (SIMOPOULOS 2008). An excessive amount of omega-6 acids in a diet (like an increased consumption of saturated fatty acids and trans unsaturated acids) is regarded as a cause of increased incidence of cardiovascular diseases (BARTNIKOWSKA 2008, FERNANDES and VENKATARAMAN 1993). Edible oils should contain a proper ratio of omega-6:omega-3 acids, lying within the range from 1:1 to 5:1 (SIMOPOULOS 2008, KOLANOWSKI 2007, ACHREMOWICZ and SZARY-SWORST 2005, JELIŃSKA 2005). With the average demand for energy of an adult, estimated to be about 3000 kcal/day, consumption of omega-3 acids should amount to about 1.4 g/day (BARTNIKOWSKA 2008). According to other sources, the daily demand for omega-3 is estimated to be 2-3 g/day (MOZAFFARIAN 2005, DE LORGERIL and SALEN 2004).

Of the edible oils, the right ratio of omega-6 to omega-3 acids, i.e. 2:1, was found only in rapeseed oil (AMBROSEWICZ et al. 2011). Recognising the importance of the right ratio of polyunsaturated fatty acids, the oil industry started producing mixtures of vegetable oils with an increased content of omega-3 acids. Following that, a need appeared to label oils with the acid. The European Commission found it appropriate to introduce standards for nutritional claims for foods containing omega-3 acids. This is specified in Regulation no. 116/2010, which proposes that such phrases as "source of omega-3 fatty acids" or "high in omega-3 fatty acids" should be used. The level of 0.3 g of α-linolenic acid in 100 g of a product was adopted as the minimum value.

ŚWIDERSKI (2003) claimed that "a food package should play an informative and educational role". To fulfil this requirement, it is necessary to label food products properly. This paper is devoted to labelling oil products. Its aim is to evaluate the labelling of vegetable oils in the following aspects: requirements specified in the standard PN-92/A-86932 (*Tłuszcze roślinne*... PN-92/A-86932), accuracy of the requirements with respect to the current knowledge and compliance of the selected features claimed on packages with those found in this study.

Materials and test methods

Characterisation of the study material

Eight samples of oil, including 2 samples of rapeseed oil and 6 samples of vegetable oil mixtures, purchased in retail outlets (Table 1, 2) were used as the study material.

Table 1

Composition of the oil mixture used in the study, according to producers' claims

Mixtures of vegetable oils	Vegetable oils									
	rapeseed	sunflower	soybean	olive	linseed	wheat germ	grapeseed	rice	corn	sesame
M_1*	+	+	–	–	–	–	–	–	–	–
M_2	+	+	–	+(1.5%)	–	–	–	–	–	–
M_3*	+	+	+	–	–	–	–	–	–	–
M_4	+(97.0%)	–	–	–	+(2.8%)	+(0.2%)	–	–	–	–
M_5*	+	–	–	–	–	–	+	+	+	+
M_6	–	+(98.5%)	–	+(1.5%)	–	–	–	–	–	–

* no data on the percentage of oils

Table 2

Commercial characterisation of oils

Oil samples	Package		Remaining shelf life at the time of analysis (months)
Rapeseed oils	O_1	light plastic bottle	5
	O_2	dark plastic bottle	2
Mixtures of vegetable oils	M_1	light plastic bottle	1
	M_2	dark plastic bottle	7
	M_3	light plastic bottle	8
	M_4	clear bottle of emerald glass	4
	M_5	clear, light, glass bottle	16
	M_6	light plastic bottle	11

Study methods

Evaluation of labelling of unit packages. The principle of evaluation included comparison of data provided on unit packages of oils with the requirements of standard PN-92/A-86932 (*Tłuszcze roślinne*... PN-92/A-86932) as well as their accuracy/reliability with respect to current knowledge on nutritional value and demand from a contemporary consumer.

Testing oil quality. Oil quality was evaluated based on the following: the content of water and volatile compounds (*Tłuszcze roślinne*... PN-73/A-86912), acid value (*Oleje i tłuszcze*... PN-ISO 660:1998), peroxide number (*Oleje i tłuszcze*... PN-ISO 3960:1996), anisidine number and Totox index (*Tłuszcze roślinne*... PN-93/A-86926) as well as diene and triene content (*Ultraviolet*... AOCS. Official method Cd 7-58:1973). The fatty acid composition was determined in accordance with PN-EN ISO 5508:1996 (*Analiza estrów*... PN-EN

ISO 5508:1996), by preparing methyl esters by the method described by ZADERNOWSKI and SOSULSKI (1978). Methyl esters were separated on a GC 8000 gas chromatography analyser, series FISONS Instrument, with: an FID, a column (type DB-225 [30 m x 0.25 mm]) with packing of chromosorb GP and helium as a carrier gas. Fatty acids were identified based on retention times determined for fatty acid standards.

Statistical analysis. Obtained results of researches were statistically analyzed using the Statictica 9.0 PL (StatSoft Poland) program. In order to indicate significance of differences between oils of peanut samples unvaried analysis of variance (ANOVA) with Tukey's test of $p \leq 0.05$ significance level was used.

Results and Discussion

Evaluation of the oil labelling showed that the labels placed on packages were written in the Polish language, in a clear and legible manner. As regards the requirements of standard PN-92/A-86932 (*Tłuszcze roślunne*... PN-92/A-86932), it was found that the labelling of the oils was varied and incomplete (Table 3).

The packaging of all oils bore all the information required by standard PN-92/A-86932 (*Tłuszcze roślinne*... PN-92/A-86932) with respect to points 1–7 and 12–14, whereas the other points, 8–11 and 15–18, were not included by all the producers (Table 3). Only one out of 8 oil packages provided information on vitamin content, 3 oil packages provided quality class, 6 oil packages provided method of use, EUFA and cholesterol content and 7 provided calorific value. None of the oil packages bore any information on preservation or flavouring. If the oils were not preserved or flavoured, it was the producers' duty to provide such information. Lists of the components of most mixtures were too general. Only the packages of mixtures M_4 and M_6 provided data on the percentage of each oil in the mixture. A package of mixture M_2 provided only information about the percentage of olive oil, without providing any data on the other oils, i.e. rapeseed oil and sunflower oil (Table 3). The composition of that mixture (Table 4) only slightly deviated from the fatty acids' composition in rapeseed oil, which indicates a dominant share of the oil in the mixture. Of all the oils used in the study, the best labelling was found on packages of mixture M_5 and rapeseed oil O_1, which met 16 and 15 requirements, respectively, of the 18 set out in the standard. The least amount of data was provided by the producers of mixtures M_1 and M_3, whose packages met 12 requirements each. Labels on the packages of those oils did not have the important information on flavouring or preserving the oils, or the content of vitamins, EUFA or cholesterol.

Table 3
Conformity of information on unit packages of oils with the requirements of PN-92/A-86932

Requirements	Oil samples							
	O_1	O_2	M_1	M_2	M_3	M_4	M_5	M_6
List of components	+	+	+	+	+	+	+	+
Date of minimum durability or use-by date	+	+	+	+	+	+	+	+
Net content	+	+	+	+	+	+	+	+
Storage conditions specific to the product	+	+	+	+	+	+	+	+
Name and address of the producer	+	+	+	+	+	+	+	+
Name and address of the bottling and packaging entity	+	+	+	+	+	+	+	+
Country or place of origin	+	+	+	+	+	+	+	+
Method of use	+	–	+	+	+	+	+	–
Quality class	+	–	+	–	–	–	+	–
Preserved	–	–	–	–	–	–	–	–
Flavoured	–	–	–	–	–	–	–	–
Labelling a production batch which makes it traceable	+	+	+	+	+	+	+	+
Type of material used for plastic packages	+	+	+	+	+	+	+	+
EAN barcode in conformity with PN-90/O-79004 and PN-90/O-79005	+	+	+	+	+	+	+	+
Vitamin content	–	–	–	–	–	–	+	–
EUFA content	+	+	–	+	–	+	+	+
Calorific value	+	+	–	+	+	+	+	+
Cholesterol content	+	+	–	+	–	+	+	+

Labelling oils with total percentage of EUFA is inappropriate, according to the latest knowledge. Attributing a beneficial effect of the oil on human health to a high percentage of EUFA has resulted in an increase in the consumption of oils containing an excess of such acids which, in turn, has resulted in an increase in cardiovascular disease, cancer incidence and mortality rates (KARŁOWICZ-BODALSKA and BODALSKI 2007, JELIŃSKA 2005).

The results of an *IJHARS* inspection in 2009 revealed numerous non-conformities in the labelling of selected agri-food products, including vegetable oils and fats. Labels of 29.8% vegetable oils and fats were found to contain information which implied that the product has special properties, e.g. "no preservatives", "contains natural OMEGA-3 acids" and that the photographs on those labels were not in any way associated with the manufacturing of the product (*IJHARS* 2009).

It is regarded as very useful that detailed information about the content of omega-6 and omega-3 acids or the ratio of their content should be provided on the label. It would also be appropriate to provide information about vitamin content with respect to vitamin E, which is the sum of the content

Table 4

The share [%] of fatty acid of oils [%]

No.	Fatty acids											
	[C16:0]	[C16:1]	[C18:0]	[C18:1]	[C18:2]	[C18:3]	[C20:0]	[C20:1]	[C22:0]	[C22:1]	others	share of omega-6 do omega-3
O_1	4.78^{a} ± 0.085	0.31^{a} ± 0.064	1.57^{ab} ± 0.007	62.92^{h} ± 0.09	20.07^{a} ± 0.03	8.13^{e} ± 0.10	0.61^{b} ± 0.007	1.43^{f} ± 0.021	0.00^{a} ± 0.000	0.09^{b} ± 0.021	0.17^{a} ± 0.017	2.5:1
O_2	4.75^{a} ± 0.049	0.47^{d} ± 0.092	1.56^{a} ± 0.021	61.86^{g} ± 0.06	19.97^{a} ± 0.08	8.60^{a} ± 0.14	0.56^{e} ± 0.042	1.67^{a} ± 0.014	0.28^{f} ± 0.014	0.12^{c} ± 0.021	0.14^{a} ± 0.014	2.3:1
M_1	5.02^{b} ± 0.057	0.41^{c} ± 0.007	1.68^{c} ± 0.021	59.75^{d} ± 0.14	22.55^{d} ± 0.021	8.50^{a} ± 0.089	0.07^{c} ± 0.007	0.08^{b} ± 0.000	0.14^{c} ± 0.000	0.08^{b} ± 0.007	1.75^{e} ± 0.035	2.7:1
M_2	4.71^{a} ± 0.127	0.39^{c} ± 0.014	1.85^{d} ± 0.007	59.94^{e} ± 0.04	21.51^{c} ± 0.07	8.60^{a} ± 0.18	0.64^{b} ± 0.064	1.68^{a} ± 0.021	0.31^{g} ± 0.007	0.12^{c} ± 0.021	0.28^{c} ± 0.049	2.5:1
M_3	8.43^{d} ± 0.035	0.26^{ab} ± 0.054	2.55^{e} ± 0.000	43.90^{c} ± 0.10	36.12^{e} ± 0.14	6.53^{d} ± 0.028	0.49^{a} ± 0.007	0.79^{e} ± 0.000	0.25^{e} ± 0.014	0.06^{e} ± 0.007	0.63^{b} ± 0.014	5.5:1
M_4	4.90^{ab} ± 0.361	0.28^{a} ± 0.024	1.60^{b} ± 0.049	61.17^{f} ± 0.26	20.31^{b} ± 0.26	9.43^{f} ± 0.13	0.66^{f} ± 0.014	1.50^{g} ± 0.007	0.00^{a} ± 0.000	0.00^{d} ± 0.000	0.18^{a} ± 0.007	2.2:1
M_5	8.78^{e} ± 0.200	0.27^{a} ± 0.000	3.67^{g} ± 0.000	32.76^{a} ± 0.00	51.08^{f} ± 0.28	1.77^{c} ± 0.00	0.35^{d} ± 0.000	0.41^{d} ± 0.000	0.12^{b} ± 0.000	0.03^{a} ± 0.000	0.79^{d} ± 0.000	29:1
M_6	7.12^{c} ± 0.085	0.21^{b} ± 0.007	3.47^{f} ± 0.007	34.31^{b} ± 0.07	51.77^{g} ± 0.21	1.57^{b} ± 0.16	0.47^{a} ± 0.014	0.24^{c} ± 0.007	0.21^{d} ± 0.007	0.02^{a} ± 0.000	0.65^{b} ± 0.022	1.4:1

of α-tocopherol and the content of the other isomers (NOGALA-KAŁUCKA et al. 2003). Moreover, it is regarded as important to inform consumers whether the oil was preserved or not. If it was, information should be provided about the preservatives used and their doses. The only source which allows for preserving vegetable oils is the Regulation of the Minister of Health and Social Care (*Zarządzenie Ministra Zdrowia*... 1993), which approves of the use of lactic acid at 0.1 g/kg; however, producers do not inform about its use. According to the position of the Polish Oil Producers Association on *Labelling vegetable oils*, it is not necessary to provide the eight-item nutrient table, with the content of sugar, fibre, sodium and cholesterol, when it is known that the level of those substances is equal to zero (*PSPO* 2007).

Evaluation of the oils quality indicated considerable variation of the values of individual features (Table 5).

The content of water and volatile substances in the oils under study ranged from 0.04 to 0.15%. According to standard PN-A-86908:2000, the acceptable content of those compounds is 0.05% (*Oleje i tłuszcze*. PN-A-86908:2000). Of the oils under study, $O1$ and M_1–M_3 were found to contain the highest concentration of those substances (Table 5).

The study results show that the acid values for the oils, ranging from 0.33–0.44 mg KOH g^{-1} of oil, exceeded the acceptable values, which for refined oils was 0.30 mg KOH g^{-1} (*Oleje i tłuszcze*. PN-A-86908:2000) (Table 5). Excessively high acid values of rapeseed oils (O_1 and O_2) and the mixtures M_2 and M_4 probably resulted from wholesalers' and retailers' failure to meet the storage conditions.

The values of the peroxide number ranged from 0.40 to 1.05 mEq O_2/kg of oil, which indicates a very low degree of the oils' oxidation (Table 5). According to standard PN-A-86908:2000, the acceptable value of the peroxide number for refined oils is 5 mEq O_2/kg of oil (*Oleje i tłuszcze*... PN-A-86908:2000). The oil oxidation rate depends on the fatty acid composition, pro- and antioxidant content, as well as on storage conditions, e.g. on exposure to oxygen, light and high temperature (JELNICKA and PTASZNIK 2008, WRONIAK 2008, WRONIAK et al. 2006, DROZDOWSKI et al. 1998, KRYGIER et al. 1998). The rate of oxidation of polyunsaturated fatty acids (PUFA) has been found to be higher than for monounsaturated acids (MUFA) (DROZDOWSKI 2002, FREGA et al. 1999). The results presented in the current study have confirmed this. Despite the longest shelf life period (16 months), the mixture of grapeseed, rapeseed, corn and sesame oils (M_5), containing a lot (about 60%) of PUFA and stored in a transparent package (clear glass bottle) had the highest peroxide value (Table 5). The lowest value was found for rapeseed oil O_1, which had the lowest percentage of those acids (28.20%).

Table 5

Discriminate of oils technological value

No.	Water and volatile substances [%]	Acid value [mg KOH g^{-1}]	Peroxide value [mEq O_2 kg^{-1}]	Anisidine value	Totox index	Conjugated compounds [%]	
						diene	trienes
O_1	$0.04^a \pm 0.001$	$0.44^b \pm 0.000$	$0.40^c \pm 0.008$	$2.38^a \pm 0.06$	3.18	$0.03^a \pm 0.002$	$0.00^a \pm 0.000$
O_2	$0.09^a \pm 0.013$	$0.44^b \pm 0.001$	$0.61^a \pm 0.030$	$2.43^{ab} \pm 0.12$	3.65	$0.03^a \pm 0.001$	$0.00^a \pm 0.000$
M_1	$0.06^a \pm 0.000$	$0.33^a \pm 0.001$	$0.61^a \pm 0.030$	$6.01^d \pm 0.11$	7.23	$0.21^d \pm 0.006$	$0.06^c \pm 0.002$
M_2	$0.09^a \pm 0.016$	$0.44^b \pm 0.001$	$0.59^a \pm 0.001$	$3.70^c \pm 0.03$	4.88	$0.02^b \pm 0.003$	$0.06^c \pm 0.002$
M_3	$0.15^a \pm 0.012$	$0.33^a \pm 0.000$	$0.60^a \pm 0.000$	$7.22^e \pm 0.20$	8.42	$0.03^a \pm 0.000$	$0.00^a \pm 0.00$
M_4	$0.11^a \pm 0.013$	$0.44^b \pm 0.001$	$0.46^b \pm 0.021$	$2.61^b \pm 0.08$	3.53	$0.23^e \pm 0.000$	$0.07^d \pm 0.002$
M_5	$0.05^a \pm 0.013$	$0.33^a \pm 0.001$	$1.05^d \pm 0.015$	$7.62^f \pm 0.05$	9.72	$0.03^a \pm 0.001$	$0.00^a \pm 0.00$
M_6	$0.05^a \pm 0.001$	$0.33^a \pm 0.000$	$0.50^b \pm 0.018$	$9.13^g \pm 0.03$	10,13	$0.15^c \pm 0.005$	$0.04^b \pm 0.006$

The anisidine value, which describes the content of secondary oxidation products (JERZEWSKA 1991), differed statistically for individual oils and lay within a broad range from 2.38 to 9.13 (Table 5). Of all the oil samples, the highest anisidine value was found in the mixture of sunflower oil and olive oil (M_6), which also contained the highest concentrations of PUFA (53.34%). The lowest value (2.38) was found in rapeseed oil O_1 with a 5-month shelf life.

WRONIAK et al. (2006) examined the stability of refined oils and observed a high degree of oxidation of sunflower oil, for which the anisidine value exceeded the highest acceptable value of 8, as set out in PN-A-96908:2000 (*Oleje i tłuszcze*... PN-A-86908:2000). Elevated values of the anisidine value of oils with over an 8-month shelf life could result from improper storage conditions, e.g. excessive temperature or exposure to light, which is possible when a colourless glass package (M_5) or plastic package (M_3 and M_5) is used.

The value of the Totox oxidation index ranged from 3.18 to 10.13 (Table 5). According to reports, the value of the Totox index of good quality oil should not exceed 10 (JERZEWSKA 1991 after: ALLEN and HAMILTON). Only sunflower oil mixed with olive oil (M_5) did not meet the criterion despite a long shelf life (11 months). The formation of secondary oxidation products in the oil may have been caused by both a high percentage of EUFA and exposure to light, which initiated photosensibilised oxidation.

The content of coupled diene acids in oils ranged from 0.02 to 0.23% and it was statistically differentiated for most oils (Table 5). The generally higher values of those substances were found in mixtures of rapeseed oil with sunflower oil (M_1) as well as with the addition of linseed oil and wheat germ (M_4). The presence of sunflower oil and linseed oil in those mixtures, whose oxidative stability is low, may have intensified the transformation of PUFA into compounds with coupled double bonds.

The content of coupled triene acids in the oils under study was low and it did not exceed 0.07% (Table 5).

An analysis of the fatty acids composition showed considerable differentiation (Table 4).

Oleic acid, which accounted for 43.90 to 62.92% of the total fatty acids, was the dominant acid in rapeseed oils O_1 and O_2 and in oil mixtures M_1–M_4 (Table 4). The oil samples mentioned above contained a majority of rapeseed oil, therefore they contained oleic acid at a level which is typical for the oil (AMBROSEWICZ et al. 2011, TAŃSKA et al. 2009). Linoleic acid dominated in mixtures M_5 and M_6, where it accounted for about 51%. Oleic acid in those samples accounted for about 33% of the total fatty acids (Table 4).

The percentage of monounsaturated fatty acids determined in this study confirmed the content claimed by the producers in 5 out of 8 samples. The exception was mixture M_6, which claimed a percentage of 27% MUFA,

whereas in fact it was 35% (Table 6). Furthermore, the producers of mixtures M_1 and M_3 did not provide information about the percentage of those fatty acids (Table 6).

Table 6

Claimed and actual percentage of mono-, polyunsaturated and saturated fatty acids [%]

No.	Fatty acids					
	MUFA		EUFA		saturated	
	claimed	in this study	claimed	in this study	claimed	in this study
O_1	65	65	27	28	7	7
O_2	66	64	27	29	7	7
M_1	·	60	·	31	·	7
M_2	64	62	28	30	8	8
M_3	·	45	·	43	·	12
M_4	63	63	30	30	7	7
M_5	33	33	46	53	13	13
M_6	27	35	62	53	13	13

Oils containing high concentrations of MUFA are regarded as products with beneficial effects on human health. They lower the concentration of triacylglycerols and LDL fraction of cholesterol, thereby reducing the risk of cardiovascular disease (KRIS-ETHERTON et al. 1999).

PUFA, such as linoleic and α-linolenic acids together accounted for 28.20–53.34% of total fatty acids in the oils under study. Mixtures M_5 and M_6 contained the highest amounts of linoleic acid (51.4% on average). A high percentage of linoleic acid in mixture M_5 shows that the mixture contains a majority of grapeseed, rice and corn oil, while the label contains no information about their content. Rapeseed oils O_1 and O_2 and mixtures M_1, M_2 and M_4 contained similar amounts of linoleic acid of 21%. The percentage of α-linolenic acid in the oils varied and ranged from 1.53 to 9.43% (Table 4). The highest percentage of the acid – 8.13–9.43% – was found in samples of rapeseed oils (O_1 and O_2) and mixtures M_1, M_2 and M_4. According to claims, rapeseed oil dominated only in mixture M_4 (97%). The labels of mixtures M_1 and M_2 mentioned the presence of the oil but without giving any information about its content (Table 2). The high content of α-linolenic acid in those mixtures, typical of rapeseed oil, is proof of the absolute domination of the oil.

The percentage of EUFA found in this study complied with the values claimed by the producers for samples O_1 and O_2 as well as M_2 and M_4. There were no data on the EUFA content on the labels of mixtures M_1 and M_3. The content of EUFA found in mixtures M_5 and M_6 was lower than the claimed value by 7 and 9 percentage points, respectively (Table 6).

The ratio of omega-6 and omega-3 acids in the oils under study varied and ranged from 1.4:1 to 29.0:1 (Table 5). A lower, and more beneficial, ratio of the acids was found in most oils (O_1, O_2, M_1, M_2, M_4 and M_6). Mixture M_3 contained slightly too much omega-6 acid (omega-6:omega-3 = 5.5:1), whereas the omega-6 acid ratio in M_5 was clearly disadvantageous (29.0:1).

Saturated acids: palmitic, stearic, arachidic and behenic, accounted together for the smallest portion of total fatty acids (6.90-12.92%) – Table 4.

Conclusion

Labelling vegetable oils is not satisfactory because of the incompleteness of the information provided by the producers (and required by *Tłuszcze roślinne...* PN-92/A-86932) such as that about preserving or flavouring oils and the content of vitamins and other essential nutrients. It was also observed that the labels do not contain some important information, which is not required by the standard, such as the ratio of omega-6 or omega-3 fatty acids or the vitamin E content, while providing information about the shelf life and date of production and the total durability period. Moreover, it is regarded as important to provide information on packages on the beneficial properties of the components present in oils. Information was given on the package of BENECOL, for example, that the presence of sterols lower the level of cholesterol. An analysis of the content of secondary products of oxidation indicated that oils with the longest shelf life period at the time of analysis had the highest anisidine values, close to or exceeding the highest acceptable levels for refined oils. The composition of fatty acids in six oils (two of them were not labelled with the feature) complied with the claim only for saturated fatty acids. The content of MUFA did not comply with the claim for one oil and the content of EUFA did not comply for two oils.

Translated by Marta Ambrosiewicz

Accepted for print 24.11.2011

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(1): 81–92, Y. 2012

THE APPLICATION OF PROBIOTIC BACTERIA IN THE FERMENTED VEGETABLE, CEREAL AND MEAT PRODUCTS*

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K e y w o r d s: non-dairy products, probiotic bacteria, functional probiotic products.

A b s t r a c t

Probiotic microorganisms are known to have the beneficial effects on people's health. Literature data show that the best source of probiotic bacteria are dairy fermented products. Not only dairy fermented foods contain probiotic cells. There are also non-dairy products from fermented vegetables, cereals, soy and meat where a growth of probiotic bacteria takes place. The interest in the application of probiotic bacteria in the food production comes from their positive influence on human organisms. They are applied in the treatment of gastrointestinal infections, virus and bacteria infections. Probiotic bacteria improve the lactose metabolism and decrease cholesterol. They possess anti-mutagenic and anti-carcinogenic properties. There is a need of widening a variety of non-dairy products in which it will be possible to apply probiotic bacteria.

The purpose of this paper was to discuss the possibilities of application of probiotic bacteria in new and traditional non-dairy products on base of literature data.

ZASTOSOWANIE BAKTERII PROBIOTYCZNYCH W FERMENTOWANYCH PRODUKTACH WARZYWNYCH, ZBOŻOWYCH I MIĘSNYCH

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S ł o w a k l u c z o w e: produkty niemleczarskie, bakterie probiotyczne, funkcjonalne produkty probiotyczne.

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* This work was supported by grant no. N N312 427639.

Abstrakt

Mikroorganizmy probiotyczne mają korzystny wpływ na zdrowie człowieka. Dane z piśmiennictwa wskazują, że najlepszym ich źródłem są fermentowane produkty mleczne. Nie tylko one zawierają bakterie probiotyczne. Istnieją również fermentowane produkty otrzymane z warzyw, zbóż, soi oraz mięsa, w których występuje wzrost bakterii probiotycznych. Zainteresowanie zastosowaniem tego rodzaju bakterii w produkcji żywności wynika z ich prozdrowotnego wpływu na organizm ludzki. Stosuje się je w leczeniu infekcji układu pokarmowego, zwalczaniu infekcji wirusowych i bakteryjnych. Bakterie probiotyczne wspomagają metabolizm laktozy oraz obniżają poziom cholesterolu. Mają właściwości antymutagenne i antykancerogenne. Istnieje potrzeba powiększenia oferty produktów niemleczarskich, w których możliwe będzie zastosowanie bakterii probiotycznych.

Celem pracy było omówienie możliwości zastosowania probiotyków do wyrobu nowych i tradycyjnych produktów uzyskanych z warzyw, zbóż, soi i mięsa na podstawie danych literaturowych.

Introduction

Probiotics are commonly known to have the beneficial influence on the host organism (BROWN and VALIERE 2004). They positively affect the gastrointestinal infections, possess antimicrobial activity, help with lactose metabolism, decrease serum cholesterol, stimulate immune system, possess antimutagenic properties, anti-carcinogenic properties, anti-diarrheal properties, alleviate the inflammatory bowel disease symptoms, suppress the infection caused by *Helicobacter pylori* (AGERHOLM-LARSEN et al. 2000, GOTCHEVA et al. 2002, NOMOTO 2005, IMASSE et al. 2007, SHAH 2007).

They are usually added to yogurt and other fermented dairy products (LOURENS-HATTINGH and VILJOEN 2001, PENNA et al. 2007). However, in the recent years, there has been the increasingly observed demand for so called non-dairy-based probiotic products. Such microorganisms are often added to drinks or sold in the form of tablets, capsules and freezedried formula (SCHREZENMEIR and DE VRESE 2001). Probiotic microorganisms inhabit human and animal intestines. It is known that fermentation is used to preserve products of plant and animal origin, improve their quality, change their flavor. Traditional fermented foods contain a great number of probiotic microorganisms (DE VALDEZ et al. 1990, ASHENAFI and BUSSE 1991, BOTES et al. 2007, LEE and LEE 2006, LEI and JACOBSEN 2004, PSANI and KOTZEKIDOU 2006, TODOROV et al. 2008). The consumption of these products is strongly recommended due to their ability to help with lactose intolerance and cholesterol decrease.

Probiotic bacteria

Probiotic bacteria are commonly consumed with fermented products, and dairy food is a main source of probiotics (HELLER 2001). These microorganisms act beneficially when they are delivered as viable cells. They need to survive

in non-fermented food matrices as well as non-dairy products (SHEEHAN et al. 2007). Probiotics belong not only to the genera *Bifidobacterium* and *Lactobacillus,* but also to the genera *Lactococcus, Enterococcus, Saccharomyces* and *Propionibacterium* (BLANDINO et al. 2003, VINDEROLA and REINHEIMER 2003). They inhabit human and animal gastrointestinal tract. The intestines of newborns are exclusively colonized with *Bifidobacterium* within a few days after birth. The ability of probiotic bacteria to colonize the intestines and influence unwanted intestinal bacteria is dependent on many factors. The optimum pH for the growth of *Bifidobacteria* is in a range of 6.0–7.0 which means that no growth takes place below of 4.5 and above 8.5. The optimum growth temperatures are 37–41°C, the minimum are 25–28°C, and the maximum are 43–45°C. Among *Bifidobacterium* there are the most commonly mat strains which are *B. adolescentis, B. longum, B. infantis*, and *B. breve*.

Probiotic bacteria are often belong to the LAB genera and are classified on the base of their morphology and an ability to glucose fermentation. They are commonly applied in the food industry due to their ability to convert fermentable sugars into lactic acid, ethanol and some other metabolites, which are responsible for lowering the pH value and preventing the potential growth of pathogenic microorganisms in food products as well as in the human intestines. These bacteria are classified as homofermentative producing lactic acid as main metabolite, and heterofermentative producing ethanol and carbon dioxide. It is known that some LAB strains may be used as probiotics because they tolerate the conditions present in the host gastrointestinal track and they are able to prevent the growth of undesirable pathogenic bacteria (CHIU et al. 2007). Among LAB the most significant are *Leuconostoc, Lactobacillus, Streptocccus* and *Pediococcus*. In the genera *Lactobacillus*, *Lactobacillus acidophilus* is known to be in a majority in the intestinal tract of healthy people. It is very often applied in probiotic products (ARIHARA et al. 1998). It is able to grow in temperature 45°C and its optimum is in a range of 35 and 40°C. *L. acidophilus* multiplies in slightly acidic conditions in pH of 6.4–4.5, and survives in pH of 4.0–3.6. It stays alive in 0.3% to 1.9% titrable acidity, while its optimum pH is 5.5–6.0 (CURRY and CROW 2003).

Probiotics are used for the production of functional foods which means that they have to be viable and available at a high concentration, usually at least 10^8–10^9 per gram of product. They also need to tolerate the presence of human gastric juice in the stomach and be able to colonize the small intestine and the colon. They influence beneficially the intestinal immune system, eliminate enteric pathogens, deliver antimutagens and antioxidants (PARK et al. 2007). It should be taken into consideration that each strain possesses completely different beneficial features (SHAH 2007). Probiotic bacteria should tolerate the severe conditions in the gastrointestinal track. They must be resistant to acid, bile, enzymes, low levels of oxygen. Such the resistance to bile acids ensures

that probiotic bacteria reach the intestinal tract as alive cells (TARANTO et al. 2006). Probiotic bacteria must be able to adhere to mucosal surfaces of the intestines.

The antimicrobial features of LAB and *Bifidobacterium* are related to the ability to compete for nutrients and the ability to produce the inhibitory substances including organic acids, hydrogen peroxide and bacteriocins. The capability of decreasing pH value as a result of the production of organic acids contributes to a bacteriocidal and bacteriostatic effect (SHAH 2007). Probiotic bacteria due the acid production eliminate the pathogens from the host intestines (OUWEHAND and SALMINEN 1998). The presence of lactic acid in combination with bile salts causes the growth inhibition of Gram-negative pathogenic bacteria (BEGLEY et al. 2005). The biological activity of probiotics added to fermented foods is dependent on some factors. They include the physiologic state of the probiotics given by the logarithmic or the stationary growth phase; the concentration at the time of consumption related to the fact that some commercial products do not contain a proper number of viable probiotic bacteria during their shelf-life (DAVE and SHAH 1997, SCHILLINGER 1999); the chemical composition of the product including its pH, water activity, the carbon, nitrogen, mineral and oxygen content, the possible interactions of the probiotics with the starter cultures (TRACHOO et al. 2006).

Vegetable products

Dairy products are regarded to be the current industrial probiotic foods (HEENAN et al. 2004). Due to the technological advances it is possible to change some structural features of fruits and vegetables by the introduction of some component modifications in a controlled way (BETORET et al. 2003). Such modification makes it possible to be ideal substrates for the probiotic bacteria because of a high content of minerals, vitamins, dietary fibers, and antioxidants (YOON et al. 2004). There is a great need to produce fruit functional drinks containing probiotics. They enjoy a huge popularity due to their taste suitable for all age groups and because they are considered to be healthy and refreshing (TUORILA and CARDELLO 2002, SHEEHAN et al. 2007). When drinks are added with *Lactobacillus plantarum*, the unsuitable aromas (perfumery, dairy) and flavors (sour, savory) are felt (LUCKOW and DELAHUNTY 2004). Unfortunately, it has been discovered that customers more eagerly consume juices having conventional favour in comparison to the ones containing probiotics). Their interest grows when they are provided with the information on the beneficial value of such juices.

It is known that LAB are a group of bacteria having special requirements and they need essential amino acids and vitamins for their growth (SALMINEN

and VON WRIGH 1993). On the other hand, it has been discovered that there are some probiotic bacteria which can grow in fruit products. The ability of cells to grow is dependent on the strains, feature of the substrate, the oxygen content as well as the final acidity of product. The addition of *Lactobacillus* and *Bifidobacterium* into orange, pineapple and cranberry juices caused huge differences in the characteristics of juices. All of the strains added to the juices had an ability to survive for a longer period of time in orange and pineapple juices in comparison to cranberry. *Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus paracasei* were able to survive at levels above 7.0 log cfu/ml in orange juice and above 6.0 log cfu/ml in pineapple juice for at least 12 weeks. However, the thermal pasteurization at 76°C for 30 s and 90°C for 1 min in combination with a high-pressure treatment of 400 MPa for 5 min, *Lb. casei, Lb. rhamnosus* and *Lb. paracasei* were not able to withstand a level above 6.0 log cfu/ml (SHEEHAN et al. 2007).

LAB cause the fermentation in vegetables and their composition depends on the quality of the raw material, temperature, as well and harvesting conditions (ROBERTS and KIDD 2005, YOON et al. 2006). The fermentation in vegetables is mainly carried out by *Lb. plantarum, Leuc. mesenteroides* (CLEVELAND et al. 2001, YAN et al. 2008) and *Lb. paracasei/casei, Lb. delbrueckii* and *Lb. brevis* (CZYZOWSKA et al. 2006). Different strains of probiotic bacteria have been isolated from vegetables. Fermented olives have contained *Lb. casei* in its majority (RANDAZZO et al. 2004). Species belonging to genus *Leuconostoc* encourage *Lactobacilli* and *Bifidobacteria* to grow in the intestines due to its ability to produce dextransucrase (EOM et al. 2007, SANZ et al. 2006). It has been proved that tomato juice constitutes a good substrate for *Lb. acidophilus, Lb. plantarum, Lb. casei* and *Lb. delbrueckii* to carry out the lactic acid fermentation despite its low pH value (YOON et al. 2004). After fermentation pH of tomato juice decreases from an initial pH value of 4.1 the pH 3.5 after 72 h. Bacteria multiply very rapidly and in 30°C after 48 h are able to achieve a number of 10^8 cfu/ml from in comparison to an initial number of 10^5 cfu/ml.

It has been indicated that *Lb. acidophilus, Lb. plantarum, Lb. casei* and *Lb. delbrueckii* were viable and in a proper number in fermented tomato juice having high acidity. Such juice may be called a probiotic beverage because it contains viable LAB after 4 weeks of keeping it at 4°C, and *Lb. acidophilus* and *Lb. delbrueckii* stay in dominance. YOON et al. (2006) applied the same LAB in a form of *Lb. plantarum, Lb. casei, Lb. delbrueckii* to check if cabbage may constitute a good substrate for their life and thus if cabbage may be used for production of probiotic cabbage juice. It was observed that *Lb. plantarum, Lb. casei*, and *Lb. delbrueckii* were able to multiply fast in sterilized cabbage juice. Their number increased up to 10^8 cfu/ml after 48 h of fermentation at 30°C. For better fermentation in vegetable juices and better multiplication of probiotic bacteria, they are recommended to be enriched with brewer's yeast

autolysate (RAKIN et al. 2007). It was found that red beets are a good substrate for probiotic bacteria. KYUNG et al. (2005) investigated if *Lb. acidophilus* and *Lb. plantarum* could multiply in the beet juice. The experiment proved that both strains decreased the pH value of beet juice from 6.3 to 4.5 after 48 h of fermentation. They produce significantly more lactic acid in comparison to *Lb. casei* and *Lb. delbrueckii*.

To sum up, the growth of probiotic bacteria is dependent on a vegetable used, on a strain used, on the final acidity of the product as well as the concentration of lactic and acetic acid in the vegetables (DAVE and SHAH 1997, YOON et al. 2004). Probiotic bacteria due to their beneficial features for human organisms should be commonly added to vegetable products.

Cereal and soya products

Cereals are commonly known to be an extremely rich source of protein, carbohydrates, vitamins, minerals and fiber in a diet of people (CHAVAN and KADAM 1989). What is more, due to their high content of non-digestible carbohydrates they play a role of prebiotics encouraging the growth of *Lactobacilli* and *Bifidobacteria* in the intestines (ANDERSSON et al. 2001). Their prebiotic features are supported by the fact that they contain water-soluble fiber, oligosaccharides and resistant starch (SHAH 2001, ANDERSSON et al. 2001). Apart from these components they also contain phytoestrogens, phenolic compounds, antioxidants, phytic acid and sterols (KATINA et al. 2007). It is known that cereals possess a lower nutritional value in relation to milk and its products. They contain a lower protein content, they lack some essential amino acids, they show low starch availability. The fermentation process causes a decrease in amount of carbohydrates and oligosaccharides, causes some protein degradation leading to releasing some amino acids like lysine and helps with the synthesis of vitamins from B group. The fermentation with its relatively high acidity is responsible for releasing mineral compounds like manganese, iron, zinc as well as calcium which help LAB grow (BLANDINO et al. 2003).

As cereal grains are a rich source of fermentable carbohydrates, amino acids, B vitamins, nucleic acids and minerals, they may constitute an ideal substrate for bacteria belonging to *Lactobacillus* genus. Moreover, fermented cereal products may be called functional products containing viable cells of probiotic bacteria. Some strains such as *Lb. plantarum, Candida rugosa* and *Candida lambica* were identified from microflora living in a traditional Bulgarian cereal-based fermented beverage giving it a status of a probiotic product. These strains were found not to be sensitive to 2% bile concentration and such feature makes them possible to stay viable in the gastrointestinal

system (GOTCHEVA et al. 2002). The cereal products constitute a good medium for the growth of probiotic bacteria thus making them functional products. They possess a suitable chemical composition as their malt, wheat and barley extracts are ideal for the growth of *Lb. plantarum, Lb. fermentum, Lb. acidophilus* and *Lb. reuteri*. It should be noted that cereal grains are a good environment not only for the growth of LAB but also for yeast. The relationships between these microorganisms need to be discussed as they have a huge influence on themselves causing the stimulation or inhibition of some strains. There is a competition among microorganisms about nutritional substances and they release some metabolites which may inhibit or encourage the growth of other microorganisms. It is known that yeast may be producers of vitamins which help LAB grow.

Oat belongs to cereals rich with beta-glucane, which plays a role of prebiotic for probiotic bacteria. *Lb. reuteri, Lb. acidophilus* and *Bifidobacterium bifidum* are known to grow rapidly in an oat medium. Oat was used as a substrate for the growth of *Lb. plantarum* in order to produce a fermented beverage containing both probiotic bacteria and beta-glucane as a prebiotic. A number of the viable cells after 24 days of keeping this drink at temperature 4°C was about 10 log cfu/ml. The shelf life of this beverage containing probiotic bacteria was 21 days. Oat cereals have been applied for production of so-called yogurt with *Lb. rhamnosus*. New functional products should contain probiotic bacteria in combination with a prebiotic which is beta-glucane responsible for a decrease in cholesterol. The presence of LAB including probiotic bacteria influence beneficially on the intestinal balance of people.

Other raw material which might be a source of nutritional components for probiotic bacteria is maize. It is also known to possess a low nutritional value for people. However, some researchers have been made to confirm that some probiotics are able to survive and carry out the fermentation process (HELLAND et al. 2004). Previously sterilized maize porridge was separately inoculated with probiotic bacteria such as *Lb. reuteri, Lb. acidophilus* and *Lb. rhamnosus* and kept at 37°C for 24 h. The maize porridge was enriched with barley malt. The fermented products possessed similar pH values, however, these strains released lactic acid in a relatively different amount in a range of 1360 and 4000 mg/kg. *Lb. rhamnosus* was known to release the biggest amount of lactic acid. Only *Lb. reuteri* was able to use maltose but all the strains used glucose and fructose as a source of carbohydrate. *Lb. rhamnosus* was the strain which released 6 mg/kg diacetyl, which in comparison to fermented milk (1–2 mg/kg) was a significantly high amount. Certain amounts of ethanol were identified in the oat porridge fermented by *Lb. rhamnosus* and in the porridge fermented *Lb. reuteri*. It means that both strains release the dehydrogenase of alcohol. The porridge inoculated with *Lb. acidophilus* was rich with diacetyl and acetaldehyde as this strain produces alcohol dehydrogenase in a relatively

small amount (GONZALEZ et al. 1994). Probiotic bacteria including *Lb. fermentum* and *Lb. plantarum* due to their ability to produce bacteriocins possess the inhibitory features against *Klebsiella pneumoniae* which was indicated in the research made by VON MOLLENDORF et al. (2006). Lactic acid fermentation causes the inhibition of *Bacillus cereus* growth. It is a widely known pathogen present in cereals, whose growth is suppressed when pH value amounts to 2.6 after 72 h.

There is one more raw material such as rice which is also a good substrate for probiotic bacteria. Apart from the fermentation process which causes a rise in amount of nutrients in cereals, there is also a process of germination increasing the quality of nutrients and bioactive compounds. During the germination process there is an increase in amount of proteins, amino

acids, sugars as well as vitamins which is vital for multiplication of probiotic bacteria (TRACHOO et al. 2006). Germinated rice grains were inoculated with *Lb. acidophilus, Lb. pentosus, Lb. plantarum* and *Lb. fermentum*. It was observed that they were able to increase a content of reducing sugars, total protein contents and vitamins.

Soybean is known to be an essential legume in the Asian diet which contains a high amount of high-quality protein (WANG et al. 2006). Soybean is popularly considered to prevent from chronic diseases like menopausal disorder, cancer, atherosclerosis, and osteoporosis (LIU et al. 2006). However, soybean possesses some disadvantages in terms of bean flavor and its content of raffinose and stachyose which lead to flatulence. The process of fermentation is often applied to enhance the digestibility of soybean and make it more tasty (HAN et al. 2001). The soymilk is known to be a good substrate for probiotic bacteria including *Lb. casei* (GARRO et al., 1999), *Lb. helveticus* (MURTI et al. 1993), *Lb. fermenti* (CHUMCHUERE and ROBINSON 1999), *Lb. fermentum* (GARRO et al. 2001, 2004), *Lb. reuteri* (TZORTZIS et al. 2004), and *Lb. acidophilus* (WANG et al. 2002, 2003, 2006). Soy beans are also known to possess phenolic compounds which show antioxidative features. It occurred that such features are significantly stronger in fermented soy products. Soymilk finds its application in the production of a cheese-like product fermented by LAB. Such cheese was produced by LIU et al. (2006). It was fermented by soy cheese bacterial starter cultures and *Lb. rhamnosus* possessing probiotic features. *Lb. rhamnosus* achieved a level of 10^8–10^9 cfu/ml after 6 h of fermentation at 10°C. 30 days of keeping at 10°C leaded to a little reduction of pH value and a slight decrease in a number of viable cells. The numbers of the viable cells of *Lb. rhamnosus* and the starters amounted to 10^7 and 10^6. It can be concluded that *Lb. rhamnosus* was able to use soybean oligosaccharides constituting a carbon source.

It is commonly known that cow milk does not constitute a substrate in which probiotic bacteria multiply fast in comparison to soymilk (CHAMPAGNE

et al. 2005). It was observed that some probiotics were able to grow faster with yogurt cultures in soymilk. FARNWORTH et al. (2007) added a combination of yoghurt starters consisting of *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* to both cow milk or soymilk containing either the probiotic bacteria *Lb. rhamnosus, Lb. johnsonii* or human derived *Bifidobacteria*. After the incubation at 41°C pH of soymilk dropped faster than in case of cow milk. It meant that in soymilk there was a higher amount of organic acids in comparison to cow milk. The presence of probiotic *Lb. rhamnosus* and *Lb. johnsonii* did not influence the growth rates of the yogurt starters. *Lb. delbrueckii* subsp. *bulgaricus* did not multiply fast in soymilk at the beginning. However, after 4 h of fermentation it started growing rapidly. It was a result of the reduction of pH value which enhanced the growth of lactobacilli. Both yoghurt starters possess the beneficial influence on themselves. *Lb. delbrueckii* subsp. *bulgaricus* is known to have the proteolytic activity, and releases free amino acids which enhance the growth of non-proteolytic bacteria.

Meat products

Meat is considered to be an ideal substrate for probiotic bacteria. Meat prevents LAB from the lethal action of bile. It is particularly used for the production of the different dry sausages made by the fermentation without heating. Minced meat is put in casings and is left to ferment and mature (LEROY et al. 2006). Meat is the natural environment for LAB, Gram-positive, catalasepositive cocci, moulds and yeasts which start growing first (LEBERT et al. 2007). Meat in order to be fermented in a controlled way should be inoculated with a starter culture containing of selected LAB. Such microorganisms are facultative heterofermentative strains which are able to transform hexoses including glucose and lactose into lactic acid. When the glucose content is insufficient in meat, it is added in amount of 0.4–0.7% to minced meat. LAB strains which are commonly applied as starters include *Lb. casei, Lb. curvatus, Lb. pentosus, Lb. plantarum, Lb. sakei, Pediococcus acidilactici* and *P. pentosaceus*. Such functional strains allow to achieve the product which is tastier, safer and healthier (AMOR and MAYO 2007). It is known that probiotic bacteria do not grow eagerly in fermented products. In order to guarantee a proper number of probiotic bacteria in meat products, the encapsulation is used. *Lb. reuteri* and *B. longum* are alginate-microencapsulated (MUTHUKUMARASAMY and HOLLEY 2006, 2007). ARIHARA et al. (1998) indicated that LAB strains including *Lb. acidophilus, Lb. crispatus, Lb. amylovorus, Lb. gallinarum, Lb. gasseri,* and *Lb. johnsonii* should be applied in the meat fermentation to increase the safety of product. *Lb. rhamnosus* and *Lb. paracasei* subsp. *paracasei* are suitable for meat fermentation.

Conclusion

The fermented dairy products are commonly believed to constitute the best source of probiotic bacteria due to their high nutritional value. However, non-dairy products such as vegetables, different cereals, soy and meat also constitute good substrates for the growth of very demanding probiotic bacteria. Probiotic bacteria are encouraged by the starter cultures to grow. There is a growing need to make functional probiotic products using not only milk, but also some other matrices. They have proved to possess the beneficial features for people. Despite the fact that there is a big variety of probiotic products in the market, it is still very little known about the identity and the source of some probiotic strains. As probiotic products are considered to be beneficial for human health, more researches should be carried out to confirm such features.

Translated by Milena Alicja Stachelska

Accepted for print 10.11.2011

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(1): 93–101, Y. 2012

CONJUGATED LINOLEIC ACID (CLA) AND *TRANS* ISOMERS OF C18:1 AND C18:2 ACIDS IN MOULD CHEESES

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K e y w o r d s: mould cheeses, conjugated linoleic acid (CLA), *trans* isomers.

A b s t r a c t

This study was aimed at determining the content of *cis*9*trans*11 C18:2 (CLA) acid and of *trans* isomers of C18:1 and C18:2 acids in fat of mould cheeses originating from various producers (from different countries) available on the market in the city of Olsztyn.

Analyses were carried out for 20 types of mould cheeses originating from different EU Member States, i.e. Poland (8 types) as well as Denmark, Germany, France and Italy (3 types from each country).

The content of *cis*9*trans*11 C18:2 acid in fat of the analyzed mould cheese originating from Polish producers ranged from 0.25 to 0.64% and did not differ significantly ($p>0.05$) from that noted in fat extracted from cheeses originating from the other EU Member States. The content of this acid in cheeses from Denmark ranged from 0.50 to 0.54%, in those from Germany – from 0.44 to 0.81%, in those from Italy – from 0.48 to 0.51%, and in those from France – from 0.41 to 0.54% of the total fatty acid composition.

Contents of *trans* isomers of C18:1 acid in cheeses from Poland accounted for 1.94 to 2.64% of the total fatty acid composition. A similar content of those isomers was reported in the French cheeses. In turn, in the cheeses originating from Denmark, Germany and Italy the total content of C18:1 *trans* isomers was found to be significantly ($p\leq0.05$) higher.

Trans isomers of C18:2 acid analyzed in the Polish cheeses constituted from 0.57 to 0.73%. In the cheeses from Denmark and France they were at a similar level, whereas in the cheeses from Germany and Italy the total content of those isomers was significantly ($p \leq 0.05$) higher than in the Polish cheeses.

The study demonstrated that the investigated mould cheeses originating from various producers and from various countries, and available on the Olsztyn market, were characterized by similar contents of CLA and diversified contents of *trans* isomers of C18:1 and C18:2 acids.

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SPRZĘŻONY KWAS LINOLOWY (CLA) I IZOMERY *TRANS* KWASU C18:1 I C18:2 W SERACH PLEŚNIOWYCH

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S ł o w a k l u c z o w e: sery pleśniowe, sprzężony kwas linolowy (CLA), izomery *trans*.

A b s t r a k t

Celem badań była ocena poziomu kwasu *cis*9*trans*11 C18:2 (CLA) oraz izomerów *trans* kwasu C18:1 i kwasu C18:2 w tłuszczu serów pleśniowych pochodzących od różnych producentów (z różnych krajów), dostępnych w Olsztynie.

Oceniano 20 rodzajów serów pleśniowych pochodzących z różnych krajów UE. Analizie poddano sery wyprodukowane w Polsce (8 rodzajów) oraz sery pochodzące z Danii, Niemiec, Francji i Włoch (po 3 rodzaje serów).

Zawartość kwasu *cis*9*trans*11 C18:2 w tłuszczu badanych serów pleśniowych pochodzących od polskich producentów wynosiła od 0,25 do 0,64% i nie różniła się istotnie (p>0,05) od zawartości tego kwasu w tłuszczu wydzielonym z serów produkowanych w innych krajach UE. Zawartość tego kwasu w serach z Danii wynosiła od 0,50 do 0,54%, w serach z Niemiec od 0,44 do 0,81%, w serach z Włoch od 0,48 do 0,51%, a w serach z Francji od 0,41 do 0,54% ogólnego składu kwasów tłuszczowych.

Zawartość izomerów *trans* kwasu C18:1 w serach z Polski wynosiła od 1,94 do 2,64% ogólnego składu kwasów tłuszczowych. Zbliżoną zawartością tych izomerów charakteryzowały się sery francuskie. W serach pochodzących z Danii, Niemiec i Włoch stwierdzono istotnie wyższą ($p \leq 0,05$) sumaryczną zawartość izomerów *trans* C18:1.

Izomery *trans* kwasu C18:2 w serach z Polski stanowiły od 0,57 do 0,73%. W serach z Danii i Francji były na zbliżonym poziomie. Z kolei w serach z Niemiec i Włoch ich sumaryczna zawartość była istotnie wyższa ($p \leq 0,05$) w porównaniu z zawartością tych izomerów w serach polskich.

W badaniach wykazano, że badane sery pleśniowe pochodzące od różnych producentów i z różnych krajów, znajdujące się na olsztyńskim rynku, charakteryzowały się zbliżoną zawartością sprzężonego kwasu linolowego CLA i zróżnicowaną zawartością izomerów *trans* kwasu C18:1 i kwasu C18:2.

Introduction

The term “linoleic acid with conjugated bonds (CLA)” refers to a group of positional and geometric isomers of linoleic acid (C18:2), in which two double bonds are separated with only one single bond. Out of that group of isomers, in fat of ruminants the highest content is reported for *cis*9*trans*11 C18:2 acid which in milk fat constitutes from 75 to over 90% of total isomers of C18:2 acid with conjugated bonds (CHIN et al. 1992, PRECHT and MOLKENTIN 2000, PARODI 2003). The *cis*9*trans*11 C18:2 acid exhibits a number of properties being beneficial to health, including anticarcinogenic, antiatherosclerotic, anti-

oxidative and anti-inflammatory ones (PARIZA 1991, PARODI 1994, 1997, MOLKENTIN 1999, CICHOSZ 2007). The main natural source of CLA in a human diet is milk and dairy products. The content of CLA in milk fat fits within a very wide range depending on the feeding period, lactation period, breed and individual determinants of cows. Out of these factors, the greatest significance is attributed to the mode of feeding. Higher contents of this acid occur in fat of milk originating from pasture feeding, whereas lower ones in milk fat from the period of stall feeding (ŻEGARSKA et al. 1996, PRECHT and MOLKENTIN 1997). According to a research by ŻEGARSKA et al. (2006), the mean content of *cis*9*trans*11C 18:2 acid in fat of milk originating from the summer accounted for 1.40% and that in milk fat from the winter period – for 0.40% of the total fatty acid composition. In the case of dairy products (cheeses or fermented drinks), apart from the animal diet, the level of CLA is significantly affected by conditions occurring during technological processing and by the activity of the starter cultures added (SHANTHA et al. 1992, JIANG et al. 1998, LIN et al. 1998, KIM and LIU 2002, BZDUCHA-WRÓBEL and OBIEDZIŃSKI 2009, DOMAGAŁA et al. 2009). As reported by LIN et al. (1995), the content of conjugated linoleic acid in cheeses ranged from 3.59 to 7.96 mg/g fat. The highest contents of CLA were noted in Blu and Brie cheeses (mould cheeses), whereas the lowest ones in processed melted cheeses. In French cheeses analyzed by LAVILLONNIER et al. (1998), the concentration of linoleic acid with conjugated bonds ranged from 5.3 to 15.8 mg/g fat of cheeses. In turn, in German cheeses FRITSHE and STEINHART (1998) reported CLA content at the level of 0.40 to 1.70% of the total fatty acid composition, whereas Turkish cheeses investigated by GÜRSOY et al. (2003) contained from 0.29 to 0.97% CLA in the total fatty acid composition.

The objective of this study was to determine the content of *cis*9*trans*11 C18:2 (CLA) acid and of *trans* isomers of C18:1 and C18:2 acids in fat of mould cheeses originating from various producers (from different countries) available on the market in the city of Olsztyn.

Materials and Methods

Material to be analysed were 20 types of mould cheeses from different manufactures from different EU countries are available on the market in Olsztyn. Cheeses produced in Poland (8 types) and cheese from Denmark, Germany, France and Italy (after the 3 types of cheese) were analysed. The study included cheeses on the market in winter (January – February). All products were tested during their shelf life.

Fat from the analysed products was extracted according to the Folch's method (CHRISTIE 1973).

Fatty acid methyl esters of the fat were determined with the IDF method using a methanolic KOH solution (*IDF standard.* 182:1999).

Separation of methyl esters of fatty acids of the isolated fat was conducted with the gas chromatography (GC) method using a Hewlett Packard 6890 chromatograph with a flame-ionization detector (FID).

Determinations were carried out under the following conditions: capillary column – 100 m x 0.25 mm i.d. (Chromopack), film thickness – 0.20 μm, stationary phase – CP Sil 88, column temperature: 60°C (1 min) – 180°C, $\Delta t = 5^{o}C/min$; the injector and detector temperatures: 225 and 250°C, respectively; carrier gas: helium, flow rate: 0.8 cm^3/min, split 100:1.

The identification of CLA and *trans* isomers was based on retention times of standards (Sigma and Supelco) and literature data (LUND and JENSEN 1983, HENNINGER and ULBERTH 1994, WOLFF 1994, PRECHT and MOLKENTIN 1995, 1996, 1997).

The results for the contents of CLA and *trans* isomers were given in reference to the total fatty acid composition (weight percentage). All samples were analysed in duplicate and mean values reported.

Statistical calculations were made based on the program STATISTICA PL (6.0).

Results and Discussion

The contribution of *cis*9*trans*11 C18:2 acid (CLA) and the sum of *trans* isomers of C18:1 and C18:2 acids in the total fatty acid composition of fat extracted from the investigated mould cheeses was presented in Table 1. The exemplary chromatogram separation of *trans* and *cis* isomers C18:1 and *cis,trans*/*trans,cis* C18:2 acids is presented in Figure 1.

Data presented in Table 1 demonstrate that the content of *cis*9*trans*11 C18:2 acid (CLA) in the total fatty acid composition of fat extracted from the investigated mould cheeses produced in Poland ranged from 0.25% (Hochland a la Brie) to 0.64% (Valbon fit jogurtowy). The mean content of CLA in fat extracted from the mould cheeses originating from Polish producers reached 0.43% and did not differ significantly from the respective values assayed in the cheeses produced in the other EU Member States. In fat of Danish cheeses, the mean content of CLA accounted for 0.53%, in that of German cheeses – for 0.58%, in that of Italian cheeses – for 0.50%, and in that of French cheeses – for 0.46% of the total fatty acid composition.

A similar CLA content of the total fatty acid composition in fat of mould cheeses available on the Olsztyn market in the winter period (February and March) was reported by ŻEGARSKA et al. (2008), i.e. from 0.42 to 0.54% (mean:

Table 1

The content of *cis*9*trans*11 C18:2 acid (CLA) and the sum of *trans* isomers of C18:1 and C18:2 acids in the total fatty acid composition of fat extracted from the investigated mould cheeses

Izomery *trans*	Country of origin																			
	Poland (n=8)				Denmark (n=3)				Germany (n=3)				Italy (n=3)				France (n=3)			
	min.	max.	$\bar{x}$	s	min.	max.	$\bar{x}$	s	min.	max.	$\bar{x}$	s	min.	max.	$\bar{x}$	s	min.	max.	$\bar{x}$	s
*Cis*9*trans*11 C18:2 (CLA)	0.25	0.64	**0.43**^{a}	0.12	0.50	0.54	**0.53**^{a}	0.02	0.44	0.81	**0.58**^{a}	0.16	0.48	0.51	**0.50**^{a}	0.01	0.41	0.54	**0.46**^{a}	0.06
Σ *trans* C18:1	1.94	2.64	**2.34**^{a}	0.22	2.58	3.63	**3.18**^{b}	0.44	2.59	3.08	**2.77**^{b}	0.22	3.51	4.46	**3.79**^{b}	0.48	2.29	3.01	**2.67**^{a}	0.29
Σ *trans* C18:2	0.57	0.73	**0.63**^{a}	0.05	0.65	0.67	**0.66**^{a}	0.01	0.71	0.86	**0.78**^{b}	0.06	0.75	0.85	**0.80**^{b}	0.04	0.60	0.71	**0.65**^{a}	0.05

Values in rows marked by the same letter are not significantly different (p>0.05)

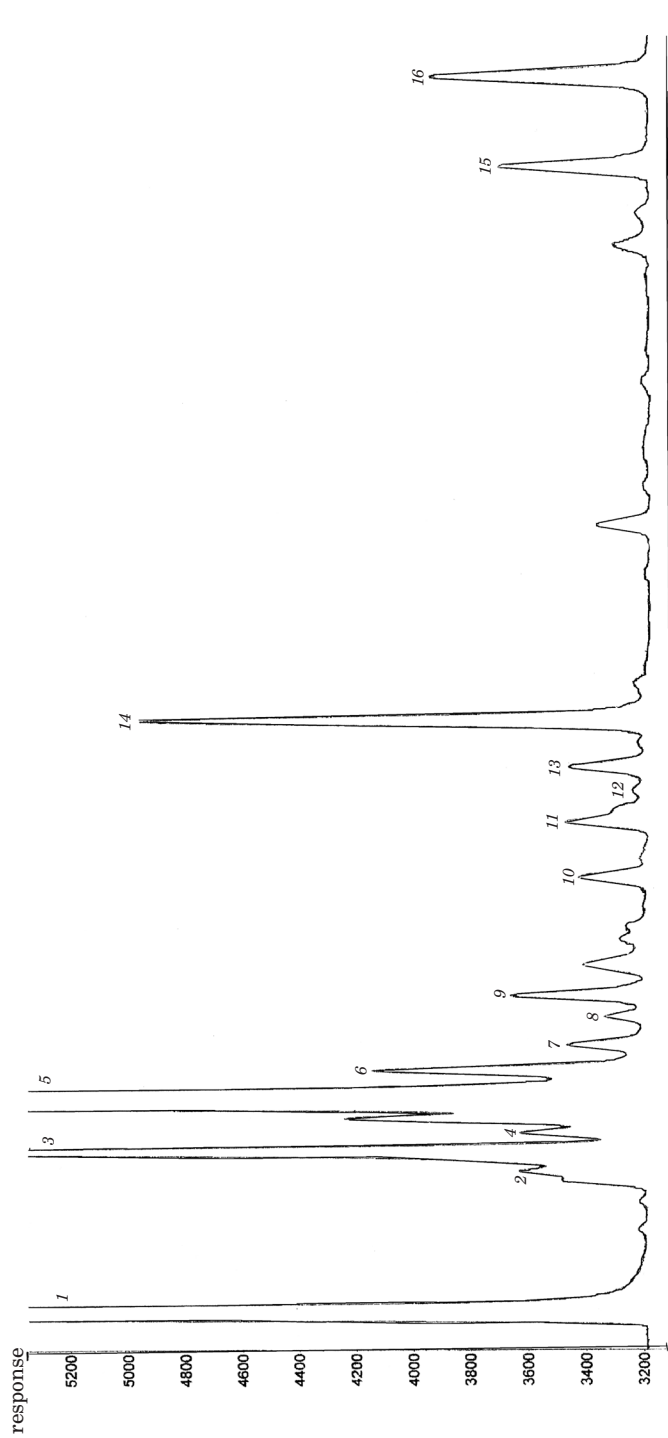

Fig. 1. Separation of *trans* and *cis* isomers of C18:1 and *cis, trans*/*trans*, *cis* of C18:2 fatty acids of mould cheese (produced in Poland) by GC. Identification: *1.* C18:0; *2. trans*6–9 C18:1; *3. trans*10+11 C18:1; *4. trans*12 C18:1; *5. cis*9 C18:1; *6. cis*11 C18:1; *7. cis*12 C18:1; *8. cis*13 C18:1; *9. trans*16 C18:1; *10. cis*9*trans*13 C18:2; *11. cis*9*trans*12 C18:2; *12. trans*9*cis*12 C18:2; *13. trans*11*cis*15 C18:2; *14. cis*9*cis*12 C18:2; *15. cis*9*cis*12*cis*15 C18:3; *16. cis*9*trans*11 C18:2 (CLA).

0.48%). A significantly higher content of that acid, ranging from 0.59 to 1.24%, was noted by those authors in fat of mould cheeses available on the market in November. The content of CLA in German cheeses investigated by FRITCHE and STEINHART (1998), ranged from 0.49% in Brie cheese to 0.69% in Gorgonzola cheese.

The mean content of *trans* isomers of C18:1 acid in the total fatty acid composition of fat of the examined mould cheeses produced in Poland ranged from 1.94 to 2.64%, with the mean value accounting for 2.34%. Those values are similar to the ones reported for milk fat originating from the intermediate feeding period (May, October and November) (ŻEGARSKA et al. 2006). Alike total contents of *trans* isomers of C18:1 acid in mould cheeses were determined by ŻEGARSKA et al. (2008). According to those authors, in cheeses purchased in February-March, the total content of C18:1 *trans* isomers constituted from 1.40 to 2.47%, and in cheeses purchased in November it was higher and ranged from 2.52 to 3.91% of the total fatty acid composition.

Cheeses originating from France and available on the Olsztyn market in January and February were characterized by a similar content of those isomers. The mean total content of C18:1 *trans* isomers in fat of the investigated French cheeses reached 2.67% and did not differ significantly ($p>0.05$) from the mean content of those isomers noted in the Polish cheeses. A significantly ($p\leq0.05$) higher total content of *trans* isomers was assayed in Danish, German and Italian cheeses (Table 1). The total content of *trans* isomers in the analyzed cheese ranged from 2.58 to 3.63% in cheeses from Denmark, from 2.59 to 3.08% in cheeses from Germany, and from 3.51 to 4.46% in cheeses from Italy. German cheeses investigated by FRITSCHE and STEINHART (1997) contained from 1.23 to 2.30% of *trans* isomers of C18:1 acid.

The content of *trans* isomers of C18:2 acid in fat of the analyzed mould cheeses originating from Polish producers ranged from 0.57 to 0.73%, with the mean value accounting for 0.63% of the total fatty acid composition (Table 1). The cheeses originating from Denmark and France were characterized by a similar mean total content of *trans* isomers of C18:2 acid. In contrast, a significantly ($p\leq0.05$) higher mean content of those isomers was assayed in fat extracted from the German and Italian cheeses, i.e. 0.78% and 0.80% of the total fatty acids, respectively.

In mould cheeses purchased in February and March and examined by ŻEGARSKA et al. (2008), the total content of *trans* isomers of C18:2 acid was in the range of 0.39 to 0.69% of the total fatty acid composition. In cheeses purchased in November, it was significantly higher.

Conclusions

The study demonstrated that the investigated mould cheeses originating from various producers and from various countries, available on the Olsztyn market, were characterized by similar contents of *cis*9*trans*11 C18:2 acid (CLA). The mean total content of *trans* isomers of C18:1 acid in fat of mould cheeses originating from Poland reached 2.34%. Alike content of those isomers was assayed in French cheeses. In turn, significantly higher contents of those isomers were reported in cheeses originating from Denmark, Germany and Italy. The mean total content of *trans* isomers of C18:2 was similar in cheeses originating from Poland, Denmark and France, whereas contents of those isomers assayed in German and Italian cheeses were significantly higher.

Translated by JOANNA MOLGA

Accepted for print 10.12.2011

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(1): 103–113, Y. 2012

ASSESSMENT OF THE KNOWLEDGE OF KUJAWY REGIONAL DISHES

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K e y w o r d s: regional and traditional food, Kujawy, questionnaire research.

A b s t r a c t

This study analysed the level of knowledge of regional dishes among the residents and restaurant owners in Kujawy. This objective was realized through questionnaire surveys conducted from October till December 2010. Two questionnaires were used, with closed alternative and open-ended questions covering the concepts of traditional and regional products, the availability and range of these products on the market and the factors influencing food shopping motives.

Although 43% of respondents could distinguish between traditional and regional products, respondents considered that regional dishes were still little-known and, above all, not available. There is a lack of promotion and advertising in the region. Local restaurants also have limited menus of particular Kujawy dishes. The most popular were: żurek kujawski, czernina, ryba po kujawsku, szynka z kością, kuleśniak, polewka kujawska and pierogi.

OCENA ZNAJOMOŚCI POTRAW REGIONALNYCH KUJAW

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S ł o w a k l u c z o w e: żywność regionalna i tradycyjna, Kujawy, badania ankietowe.

A b s t r a k t

Celem pracy było określenie znajomości potraw regionalnych wśród mieszkańców i właścicieli restauracji na terenie Kujaw. Cel ten zrealizowano, prowadząc badania ankietowe od października do grudnia 2010 r. Posłużono się dwoma kwestionariuszami ankietowymi, w których zastosowano

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pytania zamknięte alternatywne, dysjunktywne i koniunktywne oraz pytania typu otwartego. Dotyczyły one znajomości pojęć produkt tradycyjny i regionalny, dostępności i asortymentu tychże produktów na rynku oraz czynników wpływających na ich wybór żywności podczas zakupów.

Prawie 43% respondentów rozróżniało pojęcie produkt tradycyjny i regionalny. Ankietowani uznali, że potrawy te są jednak ciągle mało znane, a przede wszystkim niedostępne. Brakuje w regionie promocji i reklamy, a lokale gastronomiczne dysponują ubogim menu potraw kujawskich. Do najbardziej znanych dań należą: żurek kujawski, czernina, ryba po kujawsku, szynka z kością, kuleśniak, polewka kujawska i pierogi.

Introduction

Diet is a major factor in human health and is the basis of its existence. Besides the fulfilment of biological functions, it also has a social, cultural and religious dimension. In Poland and around the world there is a growing trend towards ethno-regionalism (TUL-KRZYSZCZUK, KRAJEWSKI 2003). This is related to maintaining the regional identity of individual countries as well as within smaller administrative units (WRITGHEN et al. 1999). There is also a growing trend towards popularization and promotion of traditional food, not necessarily as pro-health, but characterized by unusual organoleptic characteristics (TREGEAR et al. 1998). Kujawy is a region which is rich in multiculturalism. Like Warmia and Mazury, these areas have an ethnic mix of Germans, Russians, Ukrainians, Poles, Jews and even Dutch and Mennonites (KRZEMIŃSKI 2004, WECKWERTH 2010). The Roma who inhabited mainly Włocławek (and now Ciechocinek) should also be mentioned (KRZEMIŃSKI 2004).

The culinary richness of the Kujawsko-Pomorskie region reflects the growing number of products registered as traditional products. As of 07.06.2011 there were 24 products registered (*Ministerstwo Rolnictwa i Rozwoju Wsi*... 2011). Kujawy is a poor region and hence the cuisine is characterized by the simplicity of prepared dishes and monotony. This was associated with the periodicity of production and food processing which, in turn, is associated with the available resource base and is influenced by the seasons, a poor harvest, or imperfect methods of storage (KALINOWSKA 2002). The nomenclature of dishes related to the dialect (kulis (kuleśniak), gzik, polewka, gomółki, pirzok etc.) is also quite distinctive

After the Polish accession to European Union, the authorities of Kujawy and other provinces embarked on a development strategy for culinary regionalism. This resulted in an increase in regional awareness and interest in the acquisition, consumption and distribution of traditional food. To date, issues related to consumer behaviour in relation to food and regional products have not been a subject of many studies (ŻAKOWSKA-BIEMANS, KUC 2009). Such food,

although different from today's dietary recommendations and preferred consumption methods, can also be a potentially healthy food. This has been confirmed by studies of the knowledge and acceptability of regional and traditional food and basic analysis of chemical composition, calorific value etc. (VASILOPOULOU, TRICHOPOULOU 2009, BABICZ-ZIELIŃSKA, ZABROCKI 2003, JEZNACH 2008, JEŻEWSKA-ZYCHOWICZ 2008, SPIEL, BOROWSKI 2010).

The purpose of this study was to determine knowledge of local food among the inhabitants and owners of restaurants in the Kujawy region. In addition, food or products which may serve as a showcase for the region were analysed and consumer preferences and the purchasing motives of this kind of food were determined.

Material and Methods

The aim of the study was carried out based on questionnaire surveys conducted during October-December, 2010. Two questionnaires were used with closed-alternative and open-ended questions. The questions covered, among others, knowledge of traditional and regional product concepts, the availability and assortment of these products on the market and the factors influencing the purchasing motives. Surveys were addressed to randomly--selected residents of Kujawy and to restaurant owners. Restaurateurs were also asked about the type of gastronomic sites, the type and quantity of served traditional food, sources of supply of raw materials and recipes and the type of technology used in food preparation. The questionnaires contained 21 questions, of which 7 represented socio-demographic data describing the socio--economic situation of respondents. The results were analysed statistically using STATISTICA 9.0. The significance of the relationship between the two features were verified using a Chi^2 test. Results were was considered statistically significant when $p<0.05$.

Characteristics of the respondents

Consumer research involved a survey of a group of 140 persons; including 67 men and 73 women (representing 48% and 52%, respectively). The respondents were aged 18–30 years (27%), 31–40 years (29%), 41–50 years (28%) and over 51 years of age (16%). It was noted that older people were unwilling to answer survey questions.

Forty-six percent of respondents reported having higher education (including incomplete higher education), 25% of respondents declared secondary

education, 18% had basic-vocational education and nearly 11% had primary education.

About 24% of respondents came from rural areas, while the rest came from urban areas over 50 thousand inhabitants. 60% of the respondents were employed, while 16% were unemployed. Less than 11% of respondents were students while 12% were pensioners or retirees. Net income attributed to a family member averaged 1000–1500 PLN. Almost 8% of respondents declared incomes exceeding 2500 PLN.

Studies among restaurateurs were conducted in 46 gastronomic sites, where 63% of the owners were women and 37% men. The owners of the restaurant were mainly from the age groups of 31–40 (35%) and 41–50 (33%).

Approximately 57% had a university degree, 37% secondary education, 4% had basic-vocational education and 2% had primary education.

19.6% of respondents came from a rural area. 94% of owners of gastronomic places declared having a private form of property. Most of the companies were registered in 2002–2010 (61%), the oldest site was registered in 1970. In these places, there were from 1 to 73 people employed. Frequently, the number of employees was 6, with predominantly secondary education (63%).

Results

Consumer survey

The ability to distinguish regional and traditional product concepts was declared by 43% of respondents aged from 31–40, with higher education who were urban residents. These concepts are associated mainly with food products or dishes. For 71% of the respondents, products/regional dishes could be a symbol of the region. The major products included: żurek kujawski, czernina, szynka z kością, kuleśniak (kulis), ryba po kujawsku and polewka. The percentage rate of selected answers is presented in Table 1.

According to respondents, distinguishing between food groups is mainly affected by factors such as: the culinary traditions (27.8%), availability of products (19.1%), promotion (18.7%) knowledge of food preparation methods (17.1%).

The detailed shares of growth determinants in the recognition of traditional foods is presented in Figure 1.

Respondents felt that they were aware of promotional activities conducted in their region. The main methods of promoting traditional food included: folklore events (31%), festivals and ceremonies (29%) and exhibitions in museums (19%). Respondents' knowledge associated with regional cuisine

Table 1

Dishes considered to be regional dishes by respondents in the Kujawy region

The names of dishes (entered by the respondents)	Response [%]
Żurek kujawski	23.3
Czernina/czarnina	17.7
Szynka z kością	14.4
Kulis/kuleśniak	10.5
Ryba po kujawsku	10
Polewka kujawska	6.6
Piernik	2
Brukowiec	1.5
Krupnik, zupa z bani	1.2*
Smażonka, jadwiżanki, gomółki, parzybroda, zagraj, prażucha, szablok ze śliwkami	0.8*
Zacierka, placki ziemniaczane, kiełbasa, kapuśniak, pirzok, kluski z makiem, kopytka fasolowe, pierogi, półgęsek, golonka	0.5*
Zupa królewska, smalec, zupa owocowa, kujawiak, orzechowiec, okrasa z gęsi	0.3*

* percentages for each dish separately

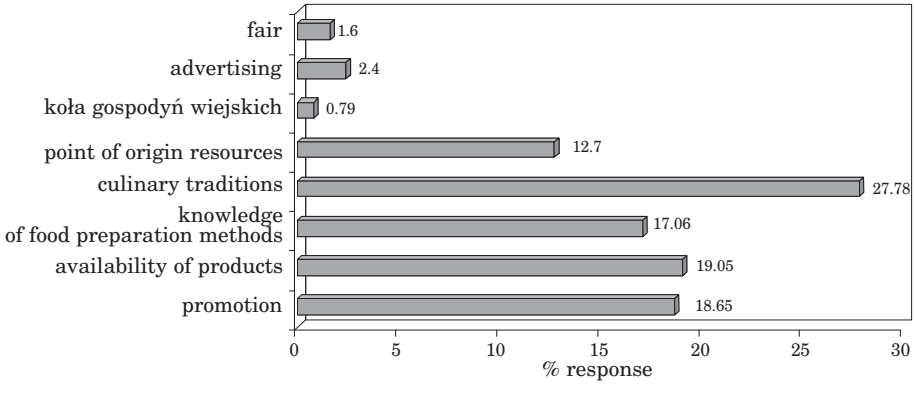

Fig. 1. Growth determinants of knowledge of regional Kujawy cuisine dishes

originated from: events held in the place of residence and neighbourhood fairs (31%), local farmers (29%), the press (18%) and advertising (14%). To a lesser extent, such knowledge is acquired in the workplace, through the Internet, from tourist information or during special events. Paradoxically, events which have an abundance of regional foods also provide little knowledge of it.

While regional food itself was considered difficult to obtain by 62% of respondents, only 5% said that it is very good. This could include people who are either manufacturers of certain products or have a permanent source

of procurement. It was also noted that women have a problem with finding a traditional food at markets, although men believe that the availability of such products is good. An analysis of these relationships showed that they were statistically significant ($p<0.05$). A similar relationship was observed for education. It was considered that the higher education a respondent had, the higher their knowledge of the market was and, thus, such persons were more resourceful in finding places where regional food could be bought.

In addition, consideration should be given to the fact that while 76% of respondents declared a purchase of regional food, others did not purchase it, therefore, their knowledge of the availability and range of traditional products could be insufficient.

In turn, age, education and employment had a statistically significant influence on the purchasing capacity of the respondents. Most buyers were people of both genders aged 41–50 years, with secondary education, were employed, had an income above 1000 PLN per person in the household, and had families between 3 and 5 persons with up to two children. The most often purchased products were fresh meat, meat products, honey, alcoholic beverages, soft drinks, cheese and other dairy products (Figure 2).

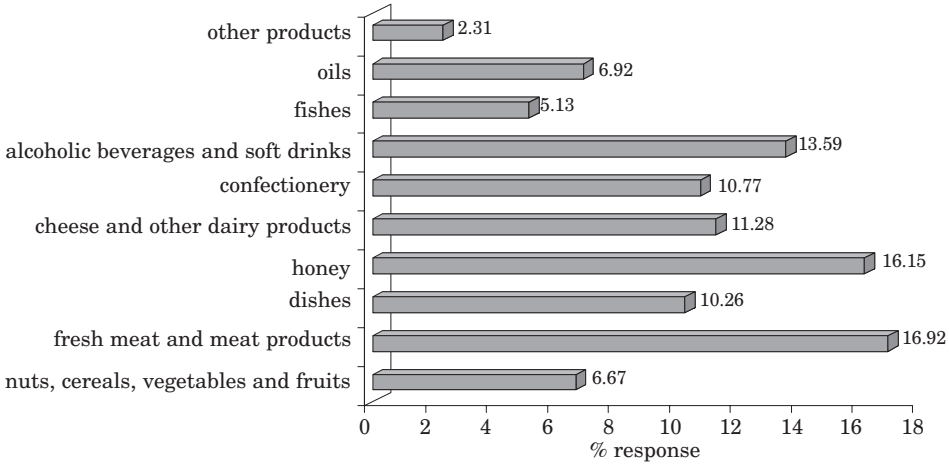

Fig. 2. Regional products most frequently purchased by respondents

Approximately 39% of respondents often paid attention to the origin of raw materials and food production methods. Slightly fewer (33%) admitted that they do it very rarely. In turn, almost 16% had never considered it. These were primarily rural young people under 30 years old with an education up to secondary, studying or working, with an income per one member of the family

from 500–1000 PLN. There was a statistically significant relationship ($p<0.05$) between employment status and the kind of purchased products. However, as the most important reasons for purchasing regional food, the respondents indicated (it was possible to select several answers as the key and important factors): quality (86% of responses), health benefits (83%), sensory (81%), price (60%) and opinion of friends (50%), habit (28% of responses), coincidence or out of curiosity (19%) and fashion (10%).

The survey of restaurant owners

The starting point in the analysis of restaurateurs; knowledge and familiarity with regional dishes was to determine the type of gastronomical site. The percentage distribution of the type of facility is shown in Figure 3. As shown in Figure 3., these were restaurants (58.7%), bars (19.6%), inns (15.2%) and confectioneries (6.5%).

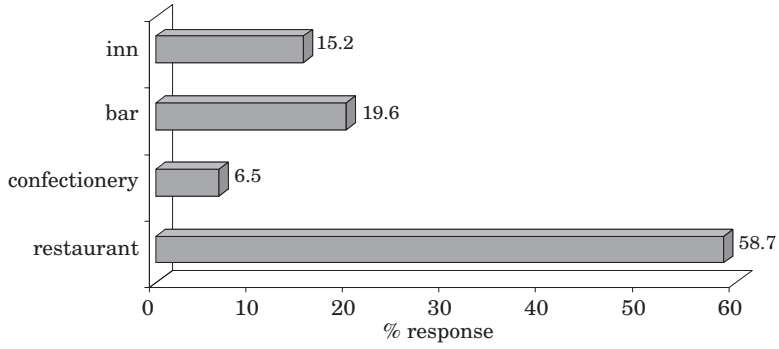

Fig. 3. Kind of gastronomical site

The concept of regional dish or product is associated by restaurateurs with the region of manufacturing (30% of responses), traditional recipe (29%) or method of production (25%). The specific organoleptic properties are the hallmark of traditional dishes for only 12.3%, and quality for less than 2.4% of the respondents. According to Council Regulation (EC) No 509/2006 of 20 March 2006 (*Rozporządzenie Rady*... Dz.Urz. WE 2006, L 93/1), the term traditional product means a product documented in use on the Community market for at least a time period showing transmission from generation to generation. This period should correspond to the period generally ascribed to one generation, i.e. at least 25 years. Traditional

products are distinguished by a specific nature, which means a feature or set of features which clearly distinguishes an agricultural product or foodstuff from similar products in the same category (O.J. EU 2006, L 93/1). A correct and full definition was given by almost 38% of respondents. These were primarily urban women aged 31–40 with higher education, from cities with populations over 100 thousand. 62.5% of restaurant owners declared serving between 1 to 5 traditional or regional dishes on their menus. The dishes served are shown in Table 2.

Table 2

Dishes recognized as regional, served in gastronomical sites in Kujawy

The names of dishes (entered by the respondents)	Response [%]
Żurek kujawski	19.2
Czernina/czarnina	13.5
Ryba po kujawsku	7.7
Szynka z kością	7.1
Kulis/kuleśniak	6.4
Polewka kujawska	5.1
Pierogi	3.9
Piernik, sandacz po kujawsku, dziczyzna, ryby, kaczka faszerowana, zapiekanka	1.9*
Brukowiec, zalewajka, kotlet po chłopsku, ciasto miodowe, drób w potrawce, kapuśniak, kluski z serem, pasztet z fasoli, krupnik, tort śmietanowy, golonka, barszcz biały	1.3*
Bigos, orzechowiec, wątróbka kujawska, zupa serowa, naleśniki, żeberka w kapuście, zupa owocowa, gołąbki, sernik, karkówka w sosie, półgęski, jabłecznik, mazurek kujawski	0.6*

* percentages for each dish separately

The source from which the restaurant owners acquired raw materials was often from a single provider (53% of restaurateurs), including regional providers (43%). Raw materials also originated from organic farms (22%) and from own production (13.8%).

When purchasing raw materials, such factors as quality (92% of responses considered it very important or important), origin (78%), sensory (62%) and price (53%) were taken into account. Dishes were prepared based on recipes from family transfers (34%), cookbooks (30%), their own ideas (19%), Internet (6%), materials provided by the Agricultural Advisory Agency (5%), from personal experience and the press (both 3%). Approximately 41% of restaurateurs claimed that serving regional dishes in the menu increased the attractiveness of the restaurant, while other motivations included: satisfaction of consumer needs (34%), attachment to tradition (13%), income (6%) and

passion (6%). But involvement in the promotion and sale of traditional products was not felt to be important (81% of respondents). For respondents who did declare such a commitment, the main promotion instrument was advertising. Less than 59% declared that they were willing to broaden their knowledge in this field. Women predominated in this regard (70%).

Discussion

According to GRZYBEK (2009), the main focus of the food market are the customers who determine the requirements for products to meet their consumption needs. In recent years, global markets have seen a trend towards a return to regional food as an alternative to highly-processed or genetically-modified foods (BATYK, SMOCZYŃSKI 2009). Previous studies have confirmed an increased interest in such products. The respondents from Kujawy are familiar with the regional product concept: 42% associate it with food products and 39% with dishes. POMIANOWSKI (2009) suggested that 2/3 of respondents were familiar with the regional product concept and that they identified regional foods even without the word "regional" in the product name. ŻAKOWSKA-BIEMANS and KUC (2009) studied supermarkets (Bomi and Carrefour in Warsaw) and found that knowledge of regional food was declared by 96% of consumers. A questionnaire conducted in Lublin and the surrounding area by STADNIK (2010) indicated a 50% knowledge of regional food by consumers. Regional food is associated by the inhabitants of Kujawsko-Pomorskie province with the region of residence and traditional methods of production. Similarly, LATOCH (2010) found that predominantly men associate regional products with traditional recipes and the manufacturing process. In addition, SPIEL and BOROWSKI (2010) found that 53% of respondents in Warmia and Mazury indicated a knowledge of regional products. PIENIAK et al. (2009) conducted an international study on a representative sample of 4828 people from Belgium, France, Italy, Norway, Poland and Spain and noted that knowledge of regional products was strongly correlated with attachment to tradition, which follows traditional methods of production and methods of consumption.

Forty-five percent of respondents from Kujawy could distinguish between traditional and regional products. Approximately 38% of the restaurants owners, where such dishes are served, were able to give a full definition of a traditional product. The lowest level of this knowledge was among restaurant owners aged 18–30 with lower education. This may result from a lack of experience or lack of interest. It seems unusual that 81% of restaurant owners do not even try to promote traditional food. Thanks to promotion, they could both inform consumers about regional products and also influence their

purchasing decisions. But restaurateurs hold the view that advertising does not correlate with increased sales and is expensive. The study did not establish the reasons for the consolidation of such beliefs, although it is clear that ordinary consumers do notice the promotional activities in their region. Hence, it could be assumed that properly employed advertising, folklore events, fairs, celebrations, festivals and ceremonies could increase the purchase and consumption of regional food. An increase in regional food knowledge could also contribute to the cultivation of family traditions; increasing the availability and assortment of dishes and information on the raw materials and their origins and preparation technology. In addition, 76% of respondents declared a willingness to purchase such products. The most popular would be meat and meat products, honey, alcoholic beverages, soft drinks, cheese and other dairy products.

Conclusions

By analysing and comparing the two surveys it can be concluded that the regional market has two conflicting factors. Randomly-chosen respondents seeking traditional food, have a knowledge of its organoleptic values and quality and are willing to pay more for it. However, restaurateurs and food producers, rather than meet the demands of such consumers, prefer to maintain the usual, conservative methods of marketing. Although they show a willingness to improve knowledge in this field, they are reluctant to provide direct interviews (only 46 were willing to participate in the survey).

Despite the development of the regional food market, it is still considered to be under-developed and not very well-advertised. In the Kujawy area, the range of these products is poor. Many of the dishes thought of as regional products by respondents are actually not. The most frequently-mentioned dishes which could be regarded as a symbol of the Kujawy region were żurek kujawski, czernina and szynka z kością. They are on the List of Traditional Products in Kujawy and Pomerania. Ham on the bone was officially registered as a traditional product by the Ministry of Agriculture and Rural Development on 18 June 2009, żur kujawski on 9 May 2008 and czarnina kujawska on 18 January 2010.

Translated by Joanna Jensen Accepted for print 13.12.2011

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POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(1): 115–119, Y. 2012

TRICHOTILLOMANIA WITH LATE RECURRENCE IN CAT

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K e y w o r d s: cat, trichotillomania recurrence.

A b s t r a c t

Trichotillomania (TTM) is most frequently characterized by small bald spots on the posterior part of the body of dogs and cats. The disease arises on a psychogenic basis and is a consequence of distress: the animal deprives itself of hair and brings about skin lesions by intensively licking its body. It has been relatively rarely described in cats and, in the case discussed here, is unique in view of the described recurrence. This study presents TTM occurring in a female cat in the second month of life with a recurrence after four years.

TRICHOTILLOMANIA Z PÓŹNYM NAWROTEM U KOTA

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S ł o w a k l u c z o w e: kot, trichotillomania nawracająca.

A b s t r a k t

Trichotillomania (TTM) charakteryzuje się najczęściej drobnymi ogniskami łysienia w tylnej okolicy ciała psów i kotów. Powstaje na tle psychogennym, jest następstwem dystresu – zwierzę intensywnie wylizując się, pozbawia się włosów i doprowadza do zmian w skórze. U kotów jest stosunkowo mało opisywana, a prezentowany przypadek jest unikalny w związku z przedstawionym nawrotem. Opisano TTM występującą u kotki w drugim miesiącu jej życia i nawrót po 4 latach.

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Introduction

Trichotillomania (TTM) – is a term indicating hair removal by a given specimen (from Greek tricho – hair, and till – to pull). It is also referred to as psychogenic baldness (WOLTAN 1986). It occurs in humans and in animals (ANZIETA et al. 2008, MORALS et al. 2010, SEHGAL, SRIVASTAVA 2006, WILLEMSE et al. 1989). In dogs, it is most often characterized by small bald spots in the lumbosacral area, the abdominal surface of the body or the medial part of the thighs (WILKINSON, HARVEY 1996). It also accompanies inflammation of the distal parts of the limbs. In cats, the aforementioned disease can be preceded by a primary condition, such as e.g. atopic dermatitis, atopy or food intolerance (DODMAN 1994). TTM can also result in formation of trichobezoars in the gastrointestinal system (ANZIETA et al. 2008, MORALS et al. 2010, SEHGAL, SRIVASTAVA 2006).

Idiopathic appearance of this psychodermatosis occurs more frequently in cats of oriental breeds. According to WILLEMSE et al. (1989), European short-haired cats can also suffer from this disease. The appearance of pathological changes is related to the distress caused by a new child in the family, a change of furniture or carpets, a threat to its own territory caused by a new cat or even a change in the food bowl location, as well as many other reasons that are difficult to ascertain. The animal escapes to a safe place, where it "rechannels" the tension, i.e. provides an outlet for its frustration by licking a "selected" part of the body. Sometimes it does it only in hiding, so its carers believe that the animal is not licking the pathologically changed skin area.

Case report

A crossbred female cat was found in the street as a four-week-old homeless animal. From the first moments of staying with new owners, it showed deviations from the standard behaviour. The cat demonstrated increased reactivity, anxiety and excessive distrust towards new carers, who were experienced breeders. Frequently, as a result of fear, excessive jumping was observed, similar to the behaviour depicted in animated cartoons.

A gentle approach and attempts to win the trust of the cat were impeded by the presence of two dogs within the boundaries of the property – a boxer and a female dachshund, the latter revealing a particularly hostile attitude towards cats. In spite of these difficulties, the process of the cat's adaptation to the new environment proceeded relatively quickly and normally over the following weeks. The cat grew and developed well and was fed pursuant to formulas provided for young, growing kittens of its age.

At the end of its second month of life, the cat was bitten by a dog – a 3 cm x 1.5 cm bite wound was noticed in its lumbosacral area. The treatment of this wound, carried out for about six days, resulted in its complete healing. However, about two weeks later, excoriations appeared in the same area, followed by increasing bald spots (Figure 1). No parasites or primary or secondary lesions were found on the skin. The hair cover, apart from the discussed area, was normal, sleek and soft, and the skin did not bear any noticeable lesions.

The therapeutic approach consisted of the oral administration of sedatives (Relanimal (Diazepam) in a dose of 3 mg/kg b.m. – i.e. the lowest dose recommended by the manufacturer), along with vitamin and mineral preparations and resolving stressful situations in a controlled manner, pursuant to the general principles for treating neuroses (FONBERG 1971).

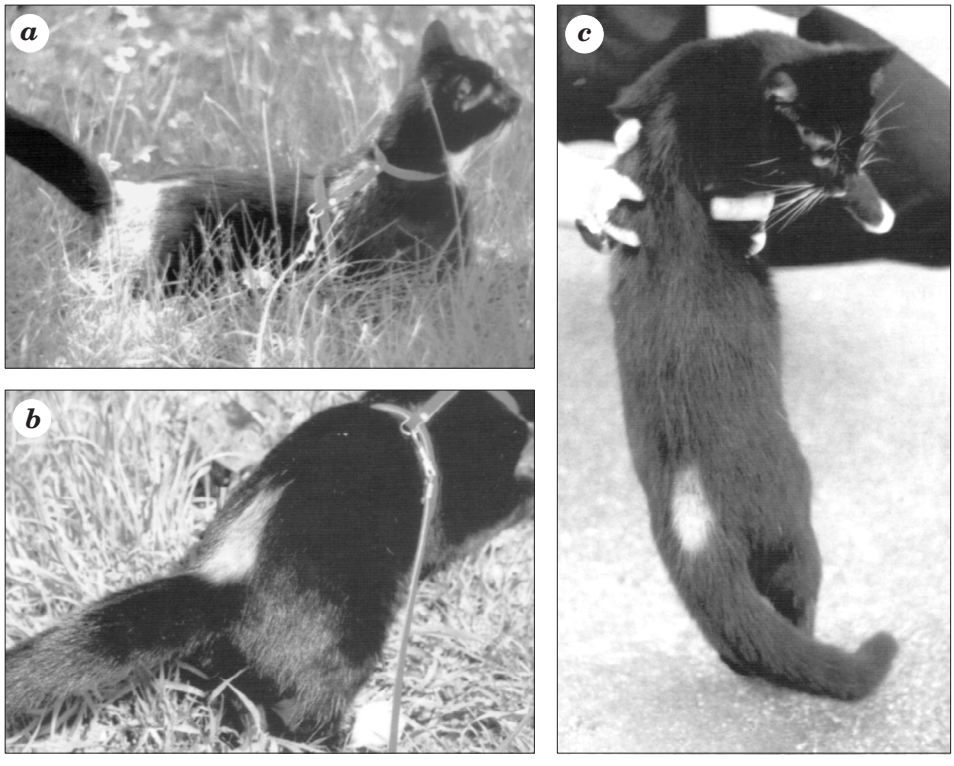

Fig. 1. The cat with skin lesions in the lumbosacral area, as a consequence of TTM: *a* – two weeks after being bitten by a dog; *b* – four weeks after getting bitten by a dog, ten days after applying the TTM – oriented treatment; *c* – six weeks after applying the TTM – oriented treatment

In that period, after some successful escapes, the cat calmly passed the dogs at a distance it considered as safe. The anxiety intensified when the animal was carried by its carers on their arms, which, as it seems, was associated with the fact that it had been attacked by the above-mentioned dachshund in such circumstances several times before. The anxiety was also caused by a noise, e.g. the "clicking" of a camera. However, the recovery continued at a good rate. At about the sixth month of life, the cat had its first oestrus cycle, with inhibited symptoms and a shortened course, which is of importance for distinguishing the TTM discussed here with baldness-related hormonal disorders which can occur at this stage.

The described TTM-related situation recurred about four years later (Figure 2), the new stimulus being the birth of the first child in the family,

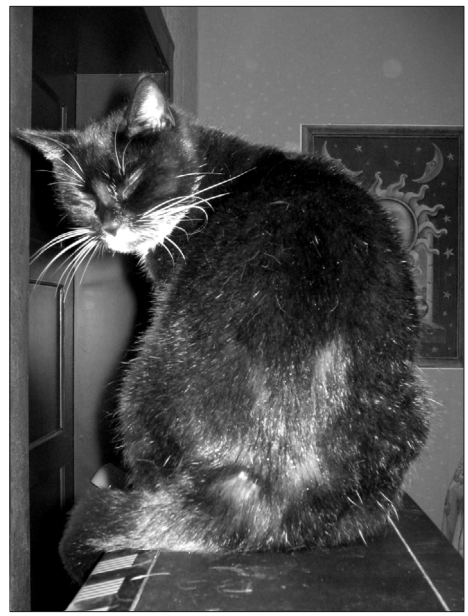

Fig. 2. TTM recurrence in a 4-year old cat – skin lesions in the lumbosacral area in the third week of the recurrence

towards which the previously castrated cat approached with distrustful interest. In this case, the previously-described therapeutic approach was extended and brought about noticeable results only at the end of the fourth week after the beginning of the treatment. The treatment involved the application of Acepromazine in a dose of about 1 mg/kg of body mass once a day, in the evening. To date, the cat has displayed limited trust, although no hostile intent, towards the child.

The case of TTM described here, with the recurrence of symptoms after about four years, is the first study in the Polish literature concerning this subject and demonstrates how strong the process of "imprinting" habits in the early stage of life can be and how difficult it is to "recondition" created behavioural stereotypes. Nevertheless, a proper approach before the animal reaches maturity makes it possible to mitigate the effects of a pathological stereotype of behaviour and subsequently, facilitates proper relations with the carers.

It should be also emphasized that in the case of a similar disease occurring in humans, which is definitely psychological in origin, it suggests a similar approach to take in relation to cats suffering from TTM.

Translated by JOANNA JENSEN

Accepted for print 15.11.2011

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