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The comparison of probiotic monocultures influence on organochlorine pesticides changes in fermented beverages from cow and goat milk during cold storage

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Abstract

The effect of selected probiotic monocultures addition as a method of organochlorine pesticides (OCP) residues reduction in fermented beverages manufactured from cow and goat milk during cold storage was examined. Supplementation of traditional yogurt starter by single monoculture or a mixture of two monocultures has resulted in a decrease of OCPs in products during cold storage. Extension of the cold storage period up to 14 days resulted in further losses in the content of the analysed compounds. Regardless of the milk type used for beverage production, beverages prepared from the mixture of two monocultures (*Lb. acidophilus* LA-5 and *Bifidobacterium* BB-12) showed significantly greater decrease of pesticides content in comparison to beverages containing only LA-5. The maximum decrease (from 5.39 % to 20.7 %) in OCP was determined during the 14-day cold storage, and was few percent higher in beverages manufactured from goat milk.

Key words: **fermented beverages, probiotic bacteria, *Lb. acidophilus, Bifidobacterium,* organochlorine pesticides residues (OCPs)**

Introduction

Cow milk is the most widely manufactured and consumed product in the world wherein production in the EU countries constitutes ca. 24 % of the world production (Polish share equals 8.3 %) (Statistical Yearbook, 2016). Although in recent years, goat milk has become increasingly popular, as it is seen as an ecological and healthy product. At present, the global production of goat milk is estimated at 2025 million L of which 26 % is the EU production (Górska, 2015). Both, cow and goat milk can be used as raw materials for the production of yogurts and other fermented beverages. The most noticeable difference between goat and cow yogurts appears in the curd texture of the final product. Goat milk yogurt gel is more delicate and less viscous, compared to its cow milk equivalent (Park et al., 2007).

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In the age of healthy eating, the consumer draws particular attention to the composition of consumed products, amount and type of used additives and problem of the occurrence of many contaminants in dairy products. Particularly harmful and persistent are organochlorine xenobiotics, which significantly reduce the quality of the consumed products (Zhang et al., 2006; Abou Donia et al., 2010). The occurrence of organochlorine xenobiotics in caw and goat milk is one of biological indicators of environmental pollution and, consequently, food contamination.

Plant protection products (PPP), including organochlorine pesticides (OCP s), are a large group of diverse chemical compounds used to fight insects, weeds, parasitic fungi, rodents, etc. On the one hand, their long-term use has contributed to the growth of productivity of many agricultural crops. But on the other hand, it has created a significant threat to the environment and human health. Long-term studies have shown that the persistence of pesticides in the environment and their biodegradation resistance, as well as the potential for more toxic metabolites, are a decisive factor in considering the extent of their use and have significant health implications. Among others, they belong to neurotropic poisons, which compromise the balance of central neurotransmitter systems (catecholamine, indoleamine and GABA) and inhibit the activity of pyrophosphatases of central nervous tissue involved in the transmission of relay substances (Rafalska and Krauze, 2016).

Some chlorinated hydrocarbons (DDT, heptachlor) are inhibitors of respiratory cycle enzymes and carbohydrate phosphate metabolism. The acute neurotoxic effects of DDT are related to its direct effect on the sensory and motor nerves in the cerebral cortex and the inhibition of the sodium-potassium pump activity of the presynaptic membrane of neurons. Cyclodiene insecticides violate amino acid metabolism and cause an increase in the ammonia content in the brain. It is believed that different stimulation or depressive action of HCH isomers are due to their varying ability to penetrate axonal cell membranes (Rafalska and Krauze, 2016).

The compounds belonging to the OCPs, as part of multisystemic poisons, also damage other organs, primarily the liver and kidneys, which participate in detoxification and excretion of harmful metabolites from the body (Mrema et al., 2013). Due to the lipophilic nature of these compounds, they tend to accumulate in tissues and organs of dairy animals. Milk represents one of the main routes of excretion from the body, and the degree of penetration of the tested pesticides in the chain "feed - animal - milk" ranges from 2 to 80 %. Carry-over rate for some pesticides is estimated as: HCB≤79 %, αHCH in the range of <1-21 %, βHCH 15-54 %, γHCH 2-4 %, dieldrin 18-40 %, p,p'DDT 4 % and p,p'DDE 80 % (Blüthgen, 2000).

It can be stated that the degradation of these compounds might be carried out not only by photochemical, chemical (e.g. oxidation, reduction, hydrolysis) or thermal decomposition (Biegańska, 2007), but also due to the activity of microorganisms (Phillips et al., 2005). Some studies, concerning initially the degradation of these compounds in contaminated soil (Sudharshan et al., 2013), also demonstrated the possibility of reducing the residue of these xenobiotics during food production (Abou-Arab, 1997, 1999).

Considering the possibility of causing many negative health effects to humans and the insufficient amount of research in this field, it was advisable to determine the possibility for reducing the residues of analysed pesticides by probiotic monocultures used in the production of milk-based fermented beverages manufactured from cow and goat milk during cold storage. Therefore, the main objective of this work was to examine the effect of adding probiotic monocultures *Lactobacillus acidophilus* LA-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 on the changes in content of selected organochlorine pesticides (αHCH, α-hexachlorocyclohexane, βHCH, β-hexachlorocyclohexane, γHCH - γ-hexachlorocyclohexane, heptachlor, heptachlor epoxide isomer B, dieldrin, endrin, op'DDE, pp'DDE, op'DDD, op'DDT, pp'DDD, pp'DDT) in the fermented beverages manufactured from cow and goat milk during cold storage (temp. 5±1°C).

Materials and methods

Preparation of fermented beverages as the test materials

Test materials were fermented beverages from cow and goat milk, prepared under laboratory conditions by the thermostatic method. The raw material for their production was cow and goat milk purchased from two farms in Poland (West Pomeranian Province). The milk was pasteurized with the tank method (85 °C/20-30 min) and then cooled to 42 °C. At the next stage, both types of milk were divided into three batches, which were inoculated with a suitable, previously activated culture (starter culture). A commercial yogurt culture YC-X16 (Chr. Hansen, Poland), containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus,* was added to the first batch of cow milk and obtained yogurt beverage was the control sample (A) (Table 1). The remaining two batches of milk, except of yogurt starter YC-X16, were inoculated respectively with monoculture *Lb. acidophilus* LA-5 and a mixture of two monocultures, i.e. *Lb. acidophilus* LA-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12, obtained two bio-yogurts were marked as A1 and A2. Similarly, fermented beverages from goat milk, i.e. yogurt (B), being also the control sample, as well as two bio-yogurts (B1 and B2), were also produced. All in all, 6 variants of fermented beverages were prepared (Table 1). In the prepared cow and goat milk bio-yogurts, the yogurt starter as well as LA-5 and BB-12 probiotic monocultures were used in a 1:1:1 ratio. The yogurt and probiotic starters were obtained by incubation of culture portion (0.6 g L^{-1}) in sterile skimmed milk, conducted at 42 °C for 6-8 h, and at 37 °C for 7-8 h, respectively.

Before proceeding to acidification (incubation) of the test beverages, each of the individual samples (200 mL) was contaminated with a selected group of organochlorine pesticides in the form of n-hexane solution (S-20168, AccuStandard USA). The target concentration of each of the analysed compound was set to 60 ng mL^{-1}. Incubation of experimental beverages was conducted at 42 °C to obtain a concise curd (pH 4.6-4.8) and then, the beverages were cooled to the 5±1 °C and kept under these conditions. The analysed samples were randomly collected at the 1st, 7th, 14th and 21st day of cold storage in 10 packages of each variant.

Parameters such as density, acidity, and total solids were determined directly after delivery to the laboratory. Density was determined by the aerometric method, and acidity (in °SH) - by the titration method according to the National Standard (AOAC, 1995). Fat content was determined using the Gerber's method (PN-ISO 488: 2010), while the dry mass content was measured gravimetrically. Protein content was determined using the spectrophotometric method (Cygański, 1993).

TABLE 1. Variations of prepared fermented beverages

	Sample symbol	Sample description
Fermented beverages from cow milk	A	Cow milk yogurt with YC-X16 yoghurt starter
	A1	Cow milk bio-yogurt with YC-X16 yoghurt starter and *Lb. acidophilus* LA-5 monoculture
	A2	Cow milk bio-yogurt with YC-X16 yoghurt starter and *Lb. acidophilus* LA-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 monocultures
Fermented beverages from goat milk	B	Goat milk yogurt with YC-X16 yoghurt starter
	B1	Goat milk bio-yogurt with YC-X16 yoghurt starter and *Lb. acidophilus* LA-5 monoculture
	B2	Goat milk bio-yogurt with YC-X16 yoghurt starter and *Lb. acidophilus* LA-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 monocultures

Prior the analysis of organochlorine compounds, the samples were freeze-dried in a LyoLab 3000 apparatus, and stored at -18 °C in sealed glass containers until analysis. Determination of organochlorine compounds in yoghurts and bio-yogurts was done according to the methods described by Witczak et al. (2013). Analyses were performed in three replications, using the following GC/MS setting: carrier gas-helium; pressure: 0.061 Mpa (8.9 psi); flowrate: 0.8 mL min^{-1}, column (HP-5MS/60.0 m; ID 250 mm, 2.25 mm film thickness of the active phase) oven temperature: start from 90 °C (0.5 min), increase 7 °C min^{-1}, 220 °C (12 min), increase 6°C min^{-1}, 285 °C (7min), increase 5 °C min^{-1}, 295 °C (6 min) (post run). Time analysis of one sample was 54.9 min. Detector: mass spectrometer (HP5973).

The quantification of concentration and recovery was carried out by contaminated samples (with mixed OCP standards, S-20168, AccuStandard USA) and by the internal standard (*cis*-chlordane, 80 ng mL^{-1}, N11480-10MG, Supelco, USA). The limit of detection (LOD) for each compound was determined as the concentration in the extract which produced an instrumental response at two different ions to be monitored with a signal to noise ratio of 3:1 for the less sensitive signal (Commission Directive 2002/63/EC).

A blank method was included for every ten samples. The LOD for each pesticide was 0.01 ng mL^{-1} on average. The LOQ of OCPs were as follows: pp'DDE (0.06 ng mL^{-1}), pp'DDD (0.05 ng mL^{-1}), pp'DDT (0.01 ng mL^{-1}), α, β, γHCH (0.04 ng mL^{-1}). The average recovery of the organochlorine pesticides was ranged from 69.6 to 99.8 %.

Statistical analysis

The statistical analysis of study results was carried out with Statistica 10.0 software. The analysis of variance ANOVA was preceded by the Levene homogeneity test and Kolmogorov-Smirnov normal distribution test (K-S test). Coefficients of Pearson correlation were determined as well. The significance of differences between mean values was evaluated with the Tukey test (P<0.05).

Results and discussion

Basic chemical composition of the raw materials - cow and goat milk

Cow milk was richer in protein and fat compared to goat milk (1.2 and 1.4 times, respectively) while differences in acidity and density were not significant (P<0.05) (Table 2). The content of protein and fat in both raw materials was slightly different from the data available in the literature (Eissa et al., 2011; Costa et al., 2016). The composition of milk may, however, vary depending on the breed, lactation phase, genetic and environmental factors, animal health or feeding method. From a technological point of view, the appropriate protein and fat content in milk is extremely important because it favours obtaining the proper qualities of the final product. Furthermore, the titratable acidity and the pH of the tested raw materials (Table 2) were at similar levels as reported by other authors (Gomes et al., 2013 Costa, et al., 2016).

TABLE 2. The physicochemical properties of the raw material for the production of fermented beverages

Raw material	Total protein [%]	Fat [%]	pH	Total titratable acidity [°SH]	Density [g/cm³]
Cow milk	3.26 ±0.21	4.60 ±0.10	6.73 ±0.22	6.90 ±0.30	1.03 ±0.10
Goat milk	2.69 ±0.14	3.38 ±0.13	6.89 ±0.18	6.07 ±0.26	1.03 ±0.11

Residues of OCPs in cow and goat milk

Considering the raw material used for the production of fermented beverages, in addition to op'DDD, pp'DDT and heptachlor, goat milk was significantly richer (P<0.05) in pesticides, compared to cow milk. Lower pesticide concentrations were determined in goat milk (0.04 ng g^{-1} milk fat, op'DDD) and the highest (3.85 ng g^{-1} milk fat, heptachlor) in cow milk (Table 3).

TABLE 3. Residues of OCPs in raw milk intended for fermented beverages production

Compounds	Cow milk		Goat milk	
	Content, ng·g⁻¹ milk fat	CV, %	Content, ng·g⁻¹ milk fat	CV, %
αHCH	0.12±0.01[1]	4.92	0.17±0.01	7.19
βHCH	0.44±0.03	7.27	0.81±0.01	1.11
γHCH	0.24±0.04	14.8	0.68±0.05	7.65
pp'DDE	1.95±0.22	11.4	3.49±0.10	2.89
op'DDE	0.04±0.01	11.4	0.10±0.01	8.00
pp'DDD	0.07±0.01	8.82	0.15±0.01	5.88
op'DDD	0.05±0.004	8.00	0.04±0.003	7.14
pp'DDT	1.03±0.03	2.92	0.68±0.02	3.52
op'DDT	0.37±0.03	7.24	0.50±0.04	8.33
heptachlor	3.85±0.24	6.32	2.12±0.13	5.95
heptachlor epoxide	1.36±0.16	11.9	1.81±0.21	11.4
dieldrin	1.19±0.13	11.3	0.93±0.06	6.44
endrin	0.85±0.02	2.22	0.79±0.08	10.5

[1]x±SD; αHCH - α-hexachlorocyclohexane; βHCH - β-hexachlorocyclohexane; γHCH - γ-hexachlorocyclohexane;
pp'DDE - 1,1-*bis*-(4-chlorophenyl)-2,2-dichloroethene; pp'DDD - 1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene;
pp'DDT - 1,1'-(2,2,2-Trichloroethane-1,1-diyl)bis(4-chlorobenzene)

Changes of OCPs in fermented beverages

Based on the obtained results, it was found that the addition of both bacterial starter YC-X16 and bacterial monocultures (Table 1) did not significantly affect (P>0.05) changes in the fat content of the tested products, with an average content of 3.38 % in goat milk beverages and 4.60 % in cow milk beverages.

Effect of yogurt starter and monoculture supplement on OCPs changes during cold storage

Finally, only products obtained after 7 and 14 days of cold storage were considered, and those after 21 days were rejected because they were unsuitable for consumption, from the consumer point of view (mold formation was observed at the surface of fermented beverages).

According to Tukey test (P<0.05), after the first day of storage there were no significant changes the analysed pesticides' content (0.4 % to 0.07 %) in contaminated samples with regard to the applied microflora ranging from. The changes recorded after 7th and 14th days of cold storage indicated significant (P<0.05) losses (or increases) of the analysed compounds in the tested fermented beverages, are shown in the Table 4.

Based on the results of the experiment, it was noted that in beverages (marked A and B) fermented with the traditional yogurt culture YC-X16 (Table 1), significant losses in the analysed compounds were observed during cold storage. In the case of cow milk products, these variations ranged from -5.17% (heptachlor) to -69 % (αHCH) (after 7 days) and from -9.2 (pp'DDT) to -96 % (αHCH) after 14 days of cold storage. In the case of fermented beverages obtained from goat milk, the changes ranged from -5.83 % (pp'DDT) to -52.5 % (βHCH) after 7 days of storage and from -9.27 % (pp'DDT) to -89.4 % (op'DDT) after 14 days of cold storage (Table 4).

It could also be noticed that monoculture *Lb. acidophilus* LA-5 used in bio-yogurts from cow milk (A1) and goat milk (B1) caused an additional reduction in the test compounds (Table 4).

After 7 days of storage significant (P<0.05) losses in pesticide content (from -0.89 % pp'DDD to -30.6 % αHCH) could be observed in beverages from cow milk (A1), and after 14 days from 9.18 % (op'DDT) to -36.6 % (heptachlor) (Table 4).

TABLE 4. Effect of addition of probiotic culture to the fermented beverages with YC-X16 yogurt culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) on changes in content of analysed compounds

OCPs	after 7 days of storage				after 14 days of storage			
	A1	B1	A2	B2	A1	B1	A2	B2
	Changes in content, %							
αHCH	-30.6	-25.1	-29.3	-35.3	4.2	-7.32	4.23	-5.0
βHCH	-14.1	-2.33	6.73	3.82	8.54	0.35	15.6	0.63
γHCH	-22.3	-9.77	-36.3	-25.5	-14.2	-20.0	-14.4	-39.5
heptachlor	-17.3	-22.6	-23.7	-12.5	-36.6	-42.7	-46.4	-48.6
heptachlor epoxide	-7.29	-33.3	-19.3	-28.3	-1.04	-15.9	-5.07	-16.8
op'DDE	-9.89	-4.97	-11.3	-2.98	-2.92	4.01	-6.63	-9.49
pp'DDE	-3.47	-27.9	-17.6	-38.0	-4.6	-54.7	-9.84	-56.7
Dieldrin	-5.58	2.21	-28.3	-1.8	-2.61	-24.2	-25.2	-11.6
op'DDD	-2.19	-11.8	-11.1	-15.5	3.33	-5.8	-8.49	-10.6
Endrin	-3.6	5.77	3.46	-32.0	-10.1	-3.92	-24.5	-19.8
op'DDT	-4.7	-4.86	-18.9	-10.0	9.18	-0.86	-20.3	-0.77
pp'DDD	-0.89	-5.16	-5	-10.6	-5.96	-2.35	-9.81	-13.1
pp'DDT	-5.42	-10.9	-10.5	-17.8	-17.4	-17.7	-26.9	-38.0
Average	-9.79	-11.6	-15.5	-17.4	-5.39	-14.7	-13.7	-20.7

A1 - cow milk bio-yogurt with LA-5 monoculture, A2 cow milk bio-yogurt with LA-5 and BB-12 monoculture, B1 - goat milk bio-yogurt with LA-5 monoculture, B2 - goat milk bio-yogurt with LA-5 and BB -12 monoculture)

In goat milk beverages (B1) an additional decrease from 2.21 % (dieldrin) to -33.3 % (heptachlor epoxide) after 7 days of cold storage was determined, and after 14 days from 4.01 % (op'DDE) to -54,7 % (pp'DDE) (Table 4).

In bio-yogurts obtained using the mixture of probiotic monocultures LA-5 and BB-12, it was found that, microflora (*Lb. acidophilus* LA-5 and *Bifidobacterium* BB-12) contributed to the changes in the content of tested pesticides in both types of bio-yogurts (cow (A2) and goat (B2) milk), during storage (Table 4).

In beverages from cow milk (A2), the pesticide content changes ranged from -6.73 % (βHCH) to -36.3 % (γHCH) after 7 days of storage, and after 14 days from 15.6 % (βHCH) to -46.4 % (heptachlor) (Table 4).

In goat milk beverages (B2), additional decrease from 3.82 % (βHCH) to -38.0 % (pp'DDE) was noticed after 7 days of cold storage, and after 14 days from 0.63 % (βHCH) to - 56.7 % (pp'DDE) (Table 4).

The correlation coefficients r between changes in the content of the analysed pesticides and the storage time of fermented beverages ranged from -0.99 to 0.45.

Significantly greater loss of pesticides in the probiotic beverages prepared from the mixture of two monocultures (*Lb. acidophilus* LA-5 and *Bifidobacterium* BB-12) - A2 and B2 were detected in both milk types, as compared to the beverages containing only LA-5 (Table 4). Taking into account the type of raw material (cow and goat milk) used for the production of fermented beverages, it was noted that the average content losses of the analysed compounds under the influence of the bacterial microflora were higher by several per cents in beverages manufactured from goat milk. This was probably due to differences in the properties of cow and goat milk.

In Poland, there is a constantly growing interest in fermented beverages, which are considered as a healthy part of the diet. In addition to many pro-health qualities, they also tend to accumulate toxic substances from the environment.

The OCPs analysed in this work are lipophilic. However, fat content in milk is not the only factor influencing the amount of accumulated contaminants (Witczak, 2017). The degree of the environmental pollution and general conditions on the cattle farms might be of a greater importance. Other individual factors influencing the contamination of animal organisms with chemicals and their release mechanisms along with secretions of milk glands (Salem et al., 2009) cannot be excluded. In the case of goat farms, where free range flocks are preferred, pollution of environmental origin cannot be avoided, for example ingesting it with more diverse, often accidental food. As several authors have already pointed out, many of these compounds are legacy of the last century, and they are undoubtedly persistent organochlorine pesticides, classified as POPs (Persistent Organic Pollutants).

The decomposition of these compounds is primarily biochemical, but can also result from photochemical and chemical reactions (e.g. oxidation, reduction, hydrolysis, interactions with free radicals and nucleophilic substitution with water). It is worrying to the consumer that some pesticide disintegration products may be more toxic than the starting compounds (Ortiz-Hernández et al., 2013).

It is believed that the causes of changes in the content of individual isomers of the test compounds during the production and storage of fermented beverages may be numerous. Under anaerobic conditions, the major products of HCH metabolism are tetrachlorocyclohexene (TCCH), and under aerobic conditions - pentachlorocyclohexene (PCCH). The factor that favours this metabolism is UV radiation (EHC, 1991). Further decomposition leads to the formation of chlorophenols and chlorobenzenes, and in some cases to HCB. The final products are usually: methane, carbon dioxide, hydrogen and chlorine (Phillips et al., 2005).

The insecticide heptachlor belonging to cyclodienes, under UV exposure, can undergo intramolecular photocycling, leading to the formation of photoheptachlor, which is characterized by greater toxicity and stability in the environment. It has also been observed that some bacteria can cause slow conversion of lindane to isomers α, β and δ. The physical properties and stability of each isomer differ due to the different orientation of the chlorine atoms in each molecule (axial or parallel). The most stable isomer is βHCH (Phillips et al., 2005).

The main metabolites of DDT decomposition are DDE and DDD. The decomposition of the DDE metabolite undergoes further transformations leading to the formation of 4,4'-dichlorobiphenyl. The most of the studies investigated the possibility of DDT decomposition under the influence of bacteria living in the soil. It has been shown, inter alia, that *Streptomyces* sp. strain D3 showed a 77 % degradation rate (Wang et al., 2017).

Based on the results of the experiment presented in this work, the changes in pesticides from the OCPs group have been observed during the storage of fermented beverages, both from cow and goat milk. It was noted that, in addition to the changes caused by bacteria contained in YC-X16 yogurt starter (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*), additional changes were probably caused by the addition of probiotic monocultures. In yogurts manufactured with only YC-X16 starters, after 14 days of storage, the losses of analysed pesticides ranged from a few to several dozens per cents, and the maximum reached 96 % for αHCH in cow milk yogurt and 89.4 % for op'DDT in goat milk yogurt.

It was noted that, depending on whether this additive was composed of one monoculture LA-5 (samples A1 and B1) or two at the same time, LA-5 and BB-12 (samples A2 and B2), and depending on the type of raw material used (cow milk or goat milk), changes in pesticide content during cold storage differed significantly ($P<0.05$).

As it was demonstrated by Abou-Arab (1997), during the production of Ras cheese, under the influence of *Streptococci*, lactic acid bacteria and yeast, there was a reduction ∑ DDT in the range of 7.78 to 13.6 % with respect to the content of these contaminants in milk. During the 6-month cold storage period, it was found that the losses ranged from 25.5 to 40.6 %.

The reduction of lindane during the production and the 3-day cold storage of yogurt ranged from 1.4 to 8.9 % (Abou-Arab, 1999). There was also a

36.7 % loss of lindane during the 6-month storage of Domiati cheese.

Based on the results of the experiment, it was concluded that the use of LA-5 monoculture for the production of beverages from cow milk (A1) resulted in significant ($P<0.05$) loss of analysed compounds after 7 days of storage, reaching 30.6 % (αHCH), and after 14 days - 36.6 % (heptachlor) (Table 4).

The changes in goat milk beverages (B1) occurred most likely due to the addition of LA-5 monoculture, which after 7 days of cold storage reached max. 33.3 % (heptachlor epoxide), and after 14 days 54.7 % (pp'DDE) (Table 4).

In the case of simultaneous use of the mixture of the two probiotic monocultures - LA-5 and BB-12, significant ($P<0.05$) changes in the content of tested pesticides during storage were found in both bio-yogurts (cow-A2 and goat-B2). In cow milk beverages (A2), after 7 days of storage, the simultaneous use of two monocultures (Table 1) resulted in maximum losses of -36.3 % for γHCH. A slight increase was observed for βHCH (6.7 %), which increased to 15.6 % after 14 days.

In comparison to sample, additional changes occurred in goat milk beverages (B2), after 7 days of cold storage and ranged from 3.8 % (βHCH) to -38.0 % (pp'DDE), and after 14 days from 0.63 % (βHCH) to -56.7 % (pp'DDE), respectively.

As it has been shown, these changes were probably due to the addition of probiotic monocultures, since the storage conditions and other parameters were the same for all tested products. The importance of the changes was also influenced by the length of the cold storage period.

However, on the first day of storage, these changes were insignificant and minor. Significant changes in the tested compounds in probiotic beverages were observed after 7 days of storage, ranging on average from -9.79 % (A1) to -17.4 % (B2) (Table 4). The arithmetic average of the losses of compounds formed during the 14-day cold storage ranged from -5.39 % (A1) to -20.7 % (B2). These results are partially consistent with those obtained by Abou-Arab (1997, 1999).

Since the compounds from the organochlorine pesticides group are exceptionally durable and have a low susceptibility to degradation, the thermal degradation of these compounds could not have occurred during low-temperature production of yogurt (up to 45 °C), only slight evaporation with steam could have occurred. An important factor influencing the possible changes in the content of OCPs in a fermented beverage in relation to the content in edible milk may be biodegradation under the influence of bacterial cultures.

Taking into account the above considerations, the biodegradation process of lipophilic, durable and relatively resistant to the degradation xenobiotic by some microorganisms deliberately added to dairy products, can be a good way to significantly reduce the exposure of the consumer. This is important because even small doses of these substances can be dangerous due to their accumulation in living organisms, that is to say, in raw materials of animal origin and in the human body.

Conclusion

During cold storage of fermented beverages produced from cow and goat milk, the losses of analysed pesticides content were observed in almost all samples. Studies have shown that, supplementation of traditional yogurt starter by single monoculture or a mixture of two monocultures has resulted in a decrease in the content of analysed OCPs. The importance of the changes of pesticides in the fermented beverages was also influenced by the length of the cold storage period. Extension of the cold storage period up to 14 days resulted in further losses in the content of the analysed compounds. Regardless of the milk type, significantly greater decrease of pesticides was detected in probiotic beverages prepared from the mixture of two monocultures (*Lb. acidophilus* LA-5 and *Bifidobacterium* BB-12), than in beverages containing only LA-5. The losses of content of the analysed compounds in all fermented beverages were higher by several percent in beverages manufactured from goat milk. This was probably due to differences in the properties of cow and goat milk.

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Usporedba utjecaja odabranih probiotičkih sojeva na promjene u koncentraciji rezidua organokloriranih pesticida u fermentiranim napitcima od kravljeg i kozjeg mlijeka tijekom hladnog skladištenja

Sažetak

U ovom je radu ispitivan utjecaj odabranih probiotičkih monokultura na smanjenje koncentracije rezidua organokloriranih pesticida (OCP) tijekom skladištenja fermentiranih napitaka od kravljeg i kozjeg mlijeka. Tijekom hladnog skladištenja dodatak monokultura ili mješovitih kultura probiotičkih sojeva klasično proizvedenim jogurtima rezultirao je snižavanjem koncentracije OCP u ispitivanim uzorcima. Produljenjem perioda skladištenja na 14 dana došlo je do dodatnog snižavanja analiziranih rezidua. Veći pad koncentracije rezidua pesticida zabilježen je u jogurtima koji su sadržavali mješovitu kulturu (*Lb. acidophilus* LA-5 i *Bifidobacterium* BB-12) nego u uzorcima koji su sadržavali samo soj LA-5. Takvi trendovi su utvrđeni u svim uzrocima s mješovitom probiotičkom kulturom, neovisno o vrsti mlijeka korištenoj za njihovu proizvodnju. Najveći pad koncentracije pesticida zabilježen je tijekom 14 dana hladnog skladištenja i kretao se između 39 % to 20,7 %, te je bio nešto viši u uzorcima kozjeg jogurta.

Ključne riječi: **fermentirani napitci, probiotičke bakterije, *Lb. acidophilus*, *Bifidobacterium,* rezidue organokloriranih pesticida (OCP)**

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