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The impact of bacterial cultures on changes in contents of PCB congeners in yoghurt and bioyoghurt - alternative methods for PCB reduction in dairy products

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Abstract

Persistent organic pollutants, including polychlorinated biphenyls, may pose serious health hazard to consumers due to their lipophilic character as well as their high stability and toxicity. They are common in milk and also in dairy products. Therefore, to provide consumers with food of the lowest possible level of pollutants, it is important to estimate the influence of technological processes in milk and dairy products manufacture on changes in the contents of toxic PCB congeners. The PCB congeners content was determined using gas chromatography with mass spectrometry. Our study showed that yoghurt starter cultures turned out to be an effective tool in decreasing the toxicity equivalent of yoghurts. The presence of additional two starter cultures of bacteria *Lactobacillus acidophilus* and *Bifidobacterium* sp. in the A.B.T. bioyoghurt starter culture was most likely the reason of the highest efficiency of this culture to reduce the value of toxicity equivalent (TEQ_{PCB}) in bioyoghurts (reduction by nearly 50 %). However, none of the four tested starter cultures of yoghurts and bioyoghurts ensured complete biodegradation of any of the tested PCB congeners. These cultures contributed to a distinct reduction in contents of the PCB congeners in the finished products and, simultaneously, to a significant increase in PCB 28 and PCB 77, which may result from the degradation of more chlorinated congeners. In consequence it can improve the quality of fermented dairy products.

Key words: **reduction of polychlorinated biphenyls, starter cultures of yoghurt and bioyoghurt**

Introduction

The capability of microorganisms (bacteria and lower fungi in particular) to degrade toxic contaminants is exploited in practice do develop technologies for bio-reclamation of chemically-polluted soils and bottom deposits which were demonstrated to the colonized by many microorganisms capable of chlorinated biphenyls degradation (Beurskens and Stortelder 1995; Yadav et al. 1995).

Some bacterial species are also capable to produce necessary energy by degradation of organochlorine xenobiotics (Singleton, 1994; Abramowicz and Olson, 1995). Usually, these microorganisms which use biphenyl as a source of energy additionally metabolize other congeners of polychlorinated biphenyls (PCBs) (Bedard et al., 1987; Brown et al., 1987; Safe, 1994). These compounds are degraded mainly by bacteria from *Pseudomonas*, *Rhodococcus* and *Alcaligenes* genera, and by *Phanerochaete chrysosporium* fungi. Products of bacterial degradation of PCBs include water-soluble compounds which are ultimately transformed into CO_2 and H_2O (Safe, 1994).

The extent and rate of microbiological degradation are determined by such factors as composition and activity of bacterial microflora, temperature, pH value, access of oxygen, presence of other compounds, content of nutrients, and physicochemical properties of the environment which the process occurs in (Gotvajn and Zagorc-Končan, 1999; Prince and Drake, 1999).

The efficiency of microbiological degradation is considerably higher in the case of poorly chlorinated congeners, whereas that of congeners having more than 2 atoms of chlorine is much more difficult (Borja et al., 2005). Biodegradation of polychlorinated biphenyls may proceed under both, aerobic and anaerobic conditions.

During PCBs degradation by microorganisms under anaerobic conditions, more strongly chlorinated PCBs may be dechlorinated in bottom deposits, which results in the synthesis of congeners with a lower chlorine content in a molecule. Afterwards, these compounds are biodegraded by microorganisms under aerobic conditions (Abraham et al., 2002). However, PCBs degradation by aerobes is usually limited to congeners with 5 or less atoms of chlorine in a molecule (Harkness et al., 1993).

The presence of polychlorinated biphenyls in milk, especially in processed milk intended for the manufacture of many food products, may pose a serious risk to human health. This risk is aggravated by the stability and lipophilic character of these compounds. We found no data in the available literature on the potential influence of microorganisms on changes in PCB contents in food products, dairy products in particular. However, some reports have appeared concerning the impact of microorganisms on reducing contents of other compounds also classified to the group of persistent organic pollutants, namely some organochlorine pesticides (DDT, lindane), during the storage period (Abou-Arab, 1997, 1999). This author suggested that a decrease in the total DDT content in cheese was caused by streptococci, lactic acid bacteria, and yeast (Abou-Arab, 1997, 1999).

The objective of this study was to evaluate the effect of selected bacterial starter cultures on changes in contents of polychlorinated biphenyl (PCB) congeners in yoghurt and A.B.T. bioyoghurt, as well as in processed milk from the aspect of the possibility of PCBs degradation in food products.

Materials and methods

Analyses were conducted with 3 types of yoghurts and bioyoghurt produced under laboratory conditions by the thermostat method.

The yoghurt samples were produced using pasteurized and homogenized cow milk with fat content of 3.2 %, lyophilized yoghurt starter cultures, and an additional starter culture, originating from two producers (from Canada and Denmark) (Table 1).

The yoghurt was produced according to the standard method using traditional yoghurt starter cultures containing two genera of bacteria: *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. In turn, the bioyoghurt contained also additional strains, i.e. apart from *Streptococcus thermophilus* bacteria, the A.B.T. culture starter contained also strains *Lactobacillus acidophilus* and *Bifidobacterium* sp. (Table 1).

TABLE 1. Characteristics of starter cultures used in the production of yoghurt and bioyoghurt

Symbol	Production country	Composition
A.B.T.	Canada	*Lactobacillus acidophilus, Bifidobacterium* sp. *Streptococcus thermophilus*
Super yoghurt	Canada	*Streptococcus thermophilus* *Lactobacillus delbrueckii* subsp. *bulgaricus*
YC-X11	Denmark	*Streptococcus thermophilus* *Lactobacillus delbrueckii* subsp. *bulgaricus*
YC-X16	Denmark	*Streptococcus thermophilus* *Lactobacillus delbrueckii* subsp. *bulgaricus*

Technology of yoghurt production with the thermostat method

Yoghurts and bioyoghurts, being the experimental material, were produced from a total of 2 L of processed milk. This milk was enriched with skimmed milk powder to a dry matter content of 16 % and divided into 4 portions. Each portion of the processed milk (0.5 L) was inoculated by an appropriate starter culture (Table 1) in the amount corresponding to 5 % addition of the bulk starter (0.025 L of bulk starter/0.5 L of processed milk). After thorough mixing of processed milk with added starter cultures, the samples were poured into containers (50 mL into each).

Then, standard solutions containing indicatory PCB congeners: PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, PCB 180; dioxin-like PCBs: non-*ortho* PCBs (PCB 77, PCB 81, PCB 126, PCB 169) and mono-*ortho* PCBs (PCB 105, PCB 114, PCB 118, PCB 156, PCB 157); and 200 µL of the mixture of standard solutions (NEN 0813, LGC Promochem GmbH D-46 485 Wesel and PCB Mix-8 CERTAN, LGC Promochem, NE 90152, Germany), in which concentration of each congener was 160 ppb (ng/g), were added to the containers to enable identification and determination of changes in the contents of the analysed compounds.

Once the containers had been hermetically closed, the prepared yoghurts were subjected to fermentation process in an incubator at 42 °C until a uniform curd with pH 4.5-4.6 has been obtained. After incubation, the yoghurts were cooled to 5±1 °C and frozen (-20 °C) until analysed. Analyses were conducted for yoghurts and bioyoghurts being the experiment material as well as for processed milk (for comparative purposes).

The accuracy of analysis was checked through the addition of an internal standard containing decachlorobiphenyl (Pesticides Surrogate Spike Mix, Supelco 4-8460, Bellefonte, USA) to all samples. Analyses of the research material were carried out in accordance to the guidelines for the preparation of samples and determination of PCBs provided in the following Polish Standards: PN-EN 1528-1:2000, PN-EN 15282-2:2000, PNEN 1528-3:2000, and PN-EN 1528-4:2000.

The content of fat was determined with the gravimetric method using 4-hour extraction with an acetone/*n*-hexane mixture, (1/1, v/v), in a Soxhlet apparatus.

To prepare the samples for analyses of PCB congeners, the material was lyophilized in a LyoLab 3000 apparatus for 3 days, and then weighted portions were sampled in 5 replications from a collective sample of each assortment. Extraction was conducted in a Soxhlet apparatus for 12 hours with 120-150 mL of an *n*hexane/acetone mixture (3/1, v/v).

The resultant extracts were concentrated in a rotating vacuum evaporator to the volume of 2 mL, purified with a fuming sulphuric acid (7 % SO_3 in concentrated H_2SO_4), and re-purified in columns on the bed (1 g) of basic Al_2O_3, by subsequent elution of: 14 mL of 2% DCM solution in *n*-hexane followed by 17 mL of 50 % DCM solution in *n*-hexane. The samples were concentrated in a flux of nitrogen to the volume of 0.1 mL. Each sample was analysed

in 3 replications with the method of capillary gas chromatography coupled with mass spectrometry, using an MS HP 6890/5973 gas chromatograph with an HP-5 MS column (60 m × 250 µm × 0.25 µm). Analyses were carried out under the following conditions of apparatus work in the mode of Selective Ion Monitoring (SIM) (Table 2):

- program of column furnace: 130 ˚C (0.5 min) → increase by 7 ˚C/min → 200 ˚C (5 min) → 4 ˚C/min → 280 ˚C (10 min) → 290 ˚C (5 min) (post run),
- injection dose: 2 µL,
- carrier gas: helium,
- flow rate: 1.1 mL/min,
- duration of analysis: 50.5 min.

TABLE 2. SIM mode parameters for chromatographic analysis

PCB*	Molecular ion	Confirmation ions	PCB	Molecular ion	Confirmation ions
28	256	258; 186	77	292	290; 220
52	256	290; 220	126	326	254; 324
101	326	254; 328	169	360	362; 290
118	326	328; 254	81	292	220; 290
153	360	290; 362	105	326	328; 254
138	360	362; 290	156	360	290; 362
180	394	396; 324	157	360	290; 362
			114	326	328; 254

*PCB number according to IUPAC

Identification of compounds (based on their retention times) was confirmed by additional separation of the fortified samples in the SCAN mode, based on the complete mass spectrum.

Recoveries of compounds were determined based on the addition of a standard solution (with the concentration of 120 ppb, added in portions of 50 µL each) containing PCBs labelled with isotope ^{13}C ($^{13}C_{12}$-labelled PCB Mixture-A in nonane, CIL - Cambridge Isotope Laboratorie, Inc. EC-4938, Cambridge, USA; (3,3',4,4'TetraCB; 3,4,4',5-TetraCB; 2',3,4,4',5-PentaCB;3,3',4,4',5-PentaCB; 3,3',4,4',5,5'-HexaCB; 2,2',3,4,4',5,5'HeptaCB) and a Pesticides Surrogate Spike Mix solution (4-8460, SUPELCO, USA), being an acetone solution of decachlorobiphenyl and 2,4,5,6-tetrachloro-*m*-xylene as the internal standard (all samples were prepared with the addition of the surrogate: 100 µL of surrogate with the concentration of 80 ppb added to each sample, and each time one analytical replication with the addition of the isotope solution) (table 3).

Recoveries of the compounds for which no isotope-labelled standards were available, were estimated based on the recovery of the samples fortified with the analysed PCB congeners with an identical number of chlorine atoms in a molecule.

The mean recoveries of the non- and mono-*ortho* PCB congeners were at: PCB 77 - 77.79 % PCB 126 - 79.59 %, PCB 169 - 87.40 %, PCB 114 - 78.0 %; PCB 156 - 79.0 %, PCB 157 - 82.90 %, and PCB 81 - 94.15 %. In turn, the recovery of the indicatory congeners ranged from 77.19 to 88.15 %.

The recovery of decachlorobiphenyl (PCB 209) contained in the applied internal standard (Pesticides Surrogate Spike Mix) fluctuated between 84.10 and 98.32 %.

To control method accuracy, also reference material (Reference Material BCR 450-PCBs in natural milk powder, Community Bureau of Reference, Geel, Belgium) was subjected to analyses with each batch of the assayed samples.

The recovery of PCB congeners contained in the reference material (BCR 450 - PCBs in natural milk powder), determined against the mean declared value, ranged from 87.93 to 98.15 % (Table 4).

TABLE 3. The recovery of PCB congeners labelled with ^{13}C

Congeners	The number of chlorine atoms in the molecule	Recovery[a], %	Min., %	Max., %	CV[b], %
PCB 77	4	77.79	76.20	79.02	4.26
PCB 81	4	69.45	67.15	70.12	5.99
PCB 126	5	79.59	77.62	82.19	4.57
PCB 169	6	87.40	85.63	89.11	6.36
PCB 180	7	98.15	97.40	100.09	3.04

[a]the arithmetic mean of the analytical repeats; [b]CV - coefficient of variation

TABLE 4. Analysis of the reference material BCR 450 (PCBs in natural milk powder)

PCB congener	Declared value[a], $ng \cdot g^{-1}$ (n^c = 20)	Obtained value[b], $ng \cdot g^{-1}$	Minimum value, $ng \cdot g^{-1}$	Maximum value, $ng \cdot g^{-1}$	CV, %	Recovery[d], %
PCB 52	1.16± 0.17	1.02	0.98	1.23	3.47	87.93
PCB 118	3.30±0.40	2.99	2.90	3.31	3.32	90.61
PCB 153	19.0±0.70	17.91	17.63	19.65	3.95	94.26
PCB 156	1.62±0.20	1.59	1.55	1.63	2.74	98.15
PCB 180	11.0±0.70	9.79	9.44	11.54	4.27	89.00

[a]Material BCR 450 - PCBs in natural milk powder; Community Bureau of Reference; Belgium[b] - arithmetic mean±standard deviation; [C]the number of analytical repetitions; [d]the mean value of recovery

The limit of detection (LOD) and the limit of quantitation (LOQ) were estimated based on the following dependency (Chen and Chen, 2001; Konieczka, 2003):

$$LOD = \frac{3{,}3 \cdot SD}{S} \qquad LOQ = \frac{10 \cdot SD}{S}$$

where:

SD - standard deviation of an absolute term of the calibration curve,

S - slope of the calibration curve.

The relative standard deviation (RSD) was computed for the obtained data and was expressed in per cents (%) and called coefficient of variability (CV). Its value was mostly lower than 10 %, which was indicative of good reproducibility of the method applied.

The limit of quantification (LOQ) of PCB congeners fitted within the range from 0.1 to 0.15 ng/kg wet weight, on average.

Statistical analysis of results

Statistical analysis of the obtained 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 correlation and regression equations were computed as well. The significance of differences between mean values was evaluated with the Tukey test (at<0.05).

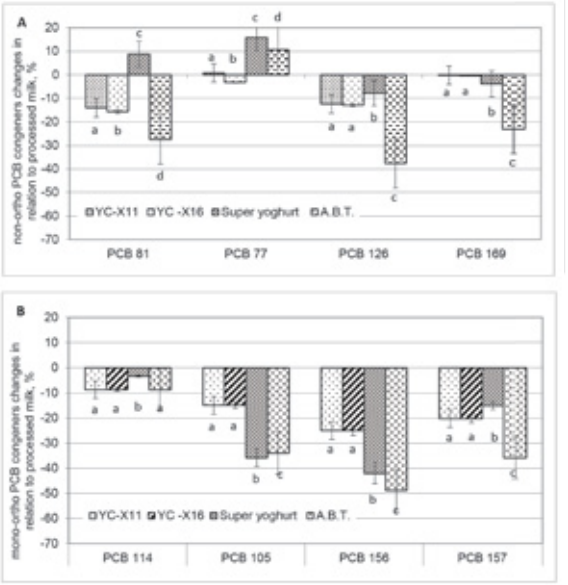
In turn, coefficients of Pearson correlation and regression equations were determined to find correlations between changes in concentrations of individual compounds and changes in contents of lipids, dry matter and pH values observed during manufacture of dairy products.

Results and discussion

Concentrations of PCB congeners in processed milk ranged from 0.52 ng/kg lipids (PCB 169) to 63.6 ng/kg lipids (PCB 105) (Table 5).

Significant ($p<0.05$) changes were observed in the analysed yoghurts in the concentration of polychlorinated biphenyls compared to their concentration in the initial material - processed milk (fig. 1).

Among the non-*ortho* congeners, the greatest loss was observed for PCB 126 in the bioyoghurt with the A.B.T. starter culture (-37.7 %). In contrast, an increase was demonstrated in PCB 77 concentration in three yoghurts, with the greatest increase (by 15.8 %) being noted upon the use of Super yoghurt culture (fig. 1a).



a, b, c, d - different letters indicate statistically significant differences, $p<0.05$

FIGURE 1. Changes in concentrations of non-*ortho* (A) and mono-*ortho* (B) PCB congeners after acidification of yoghurts

Regarding the amounts of mono-*ortho* congeners, the greatest losses were also observed after the use of the A.B.T. starter culture, with a minimal decrease in concentration noted for PCB 114 (3.3-8.8 %), and the maximal one for PCB 156 (25.0-48.8 %) (fig. 1b).

Changes in concentrations of individual dl-PCB resulted in reduced values of TEQ. Compared to processed milk (0.19 pg-TEQ/g lipids), the least reduction in TEQ was observed in the product with Super yoghurt starter and the greatest one in yoghurts with the A.B.T. starter culture (49.5 %) (Fig. 2).

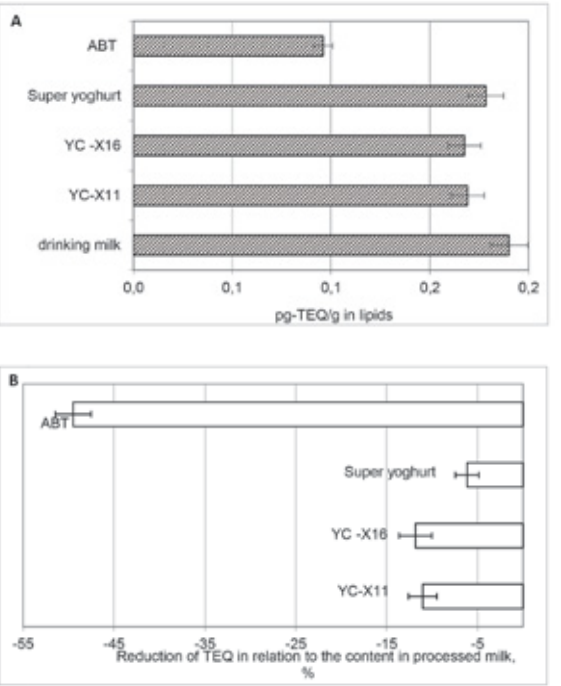
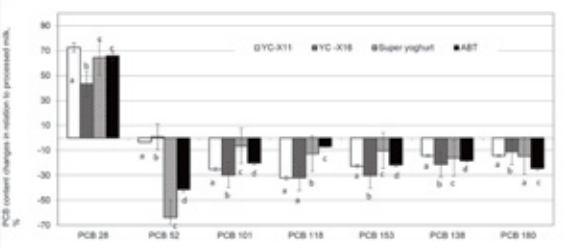


FIGURE 2. Content (A) and changes (B) of TEQ value in processed milk and yoghurts

In addition, depending on the starter culture used, changes were observed after acidification of yoghurts in concentrations of ndl-PCBs (PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, and PCB 180). All analysed yoghurts were characterized by a significant increase in the concentration of PCB 28 congeners, accounting for 43-72 %, compared to processed milk. Concentrations of most of the other studied congeners decreased significantly ($p<0.05$), with the greatest decrease recorded for PCB 52 in yoghurt sample with the Super yoghurt starter culture (Fig. 3, Table 6).



a, b, c, d - different letters indicate statistically significant differences, $p<0.05$

FIG. 3. Changes in concentrations of indicatory PCB congeners in yoghurts

TABLE 5. Content of dl PCB congeners in processed milk and yoghurts

Sample	non-ortho PCB				mono-ortho PCB				TEQ
	PCB 77	PCB 81	PCB 126	PCB 169	PCB 105	PCB 114	PCB 156	PCB 157	
	Content, ng·kg^{-1} lipid								pg-TEQ/g lipids
Processed milk	61.40 ± 5.83[a]	32.20 ± 3.84	1.54 ± 0.12	0.520 ± 0.030	63.60 ± 2.06	45.10 ± 0.61	6.82 ± 0.82	31.80 ± 0.52	0.1901
Yoghurt with YC-X11	61.88 ± 8.29	28.55 ± 4.43	1.35 ± 0.31	0.519 ± 0.020	54.09 ± 2.76	41.15 ± 1.18	5.110 ± 0.40	25.34 ± 1.64	0.1691
Yoghurt with YC -X16	59.40 ± 3.93	28.01 ±3.61	1.34 ± 0.35	0.518 ± 0.030	52.87 ± 2.39	44.96 ± 1.99	3.38 ± 0.35	23.36 ± 2.74	0.1676
Yoghurt with Super yoghurt	71.10 ± 4.65	36.10 ± 2.18	1.42 ± 0.20	0.500 ± 0.030	40.79 ± 3.56	43.61 ± 2.16	3.95 ± 0.73	26.99 ± 3.70	0.1784
Yoghurt with A.B.T.	67.90 ± 3.97	24.08 ±1.57	0.96 ± 0.13	0.400 ± 0.020	41.97 ± 3.32	41.12 ± 2.75	3.49 ± 0.39	20.39 ± 3.50	0.0960

[a]arithmetic mean±standard deviation

TABLE 6. Content of indicatory PCB congeners (ndl PCB) in processed milk and yoghurts

Sample	Content, ng·g^{-1} lipids							
	PCB 28	PCB 52	PCB 101	PCB 118	PCB 153	PCB 138	PCB 180	∑PCB
Processed milk	0.287 ± 0.051[a]	0.244 ± 0.020	0.585 ± 0.023	0.215 ± 0.008	0.247 ± 0.025	0.218 ± 0.023	0.062 ± 0.005	1.858
Yoghurt with YC-X11	0.495 ± 0.080	0.235 ± 0.031	0.439 ± 0.023	0.146 ± 0.009	0.192 ± 0.013	0.187 ± 0.015	0.053 ± 0.005	1.747
Yoghurt with YC -X16	0.411 ± 0.053	0.246 ± 0.019	0.411 ± 0.033	0.146 ± 0.011	0.173 ± 0.014	0.172 ± 0.009	0.055 ± 0.004	1.614
Yoghurt with Super yoghurt	0.473 ± 0.066	0.088 ± 0.008	0.548 ± 0.067	0.187 ± 0.012	0.222 ± 0.009	0.183 ± 0.011	0.053 ± 0.006	1.754
Yoghurt with A.B.T.	0.476 ± 0.039	0.143 ± 0.012	0.468 ± 0.035	0.199 ± 0.020	0.194 ± 0.022	0.178 ± 0.010	0.047 ± 0.006	1.706

[a]arithmetic mean±standard deviation

Considering the conditions of the production process of experimental yoghurts, correlations between the change in products acidity compared to processed milk and the change in concentrations of the analysed PCB congeners were examined. The pH values decreased from 6.5 (processed milk) to 4.1 (Super yoghurt), 4.3 (YC-X11), 4.4 (YC-X16), and 5.0 (A.B.T.).

Bioyoghurts with the A.B.T. starter culture had the lowest acidity (pH 5.0) and the analyses demonstrated the greatest decrease in toxicity equivalent (by 55 %) which was accompanied by a significant ($p<0.05$) decrease in the concentration of the most toxic PCB conger, i.e. PCB 126 with a concentration decrease by 64 % (Fig. 2). In turn, the yoghurt produced with the Super yoghurt starter culture and having the lowest pH value was characterized by the lowest losses of PCB congeners, which corresponded to a TEQ reduction by cca. 6.2 % (Fig. 2). The statistical analysis of the above-discussed

changes demonstrated a very weak correlation between the pH change in yoghurt and the percentage change in the toxicity equivalent (r=0.123). In turn, a very weak or negative correlations were obtained for individual non-*ortho* congeners (r=-0.302-0.100), as well as significantly stronger positive correlations were demonstrated for mono-*ortho* PCBs (r=0.373-0.722) along with changes in the pH value of particular products (table 7).

Despite the significantly ($p<0.05$) greater decrease in the TEQ value in yoghurts with the A.B.T. starter culture, the sum of concentrations of non-*ortho* congeners decreased only by 3.4 %. The greatest decrease was demonstrated in the product with the YC-X11 starter culture (7.6 %), whereas by using the Super yoghurt culture the concentration of PCB congeners increased by 12.9 % (it referred to lower chlorinated congeners PCB 77 and PCB 81).

TABLE 7. Pearson correlation coefficient r and regression equation between PCB congeners level and pH of yoghurts

Congeners		r_{lip}, $p < 0.05$	Regression equation
ndl-PCB	PCB 28	-0.471	y = 0.8 – 0.068x
	PCB 52	0.524	y = -0.022 + 0.041x
	PCB 101	0.603	y = 0.268 + 0.046x
	PCB 153	0.674	y = 0.107 + 0.020x
	PCB 138	0.491	y = 0.144 + 0.010x
	PCB 180	0.607	y = 0.038 + 0.003x
non-*ortho*	PCB 81	0.082	y = 28.066 + 0.330x
	PCB 77	-0.302	y = 71.768 – 1.529x
	PCB 126	0.100	y = 1.043 + 0.04x
	PCB 169	-0.057	y = 0.506 – 0.0029x
mono-*ortho*	PCB 105	0.665	y = 19.401 + 6.432x
	PCB 114	0.373	y = 39.538 + 0.751x
	PCB 118	0.722	y = 0.066 + 0.023x
	PCB 156	0.620	y = –0.889 + 1.075x
	PCB 157	0.574	y = 13.420 + 2.500x
	TEQ	0.123	y = 0.138 + 0.004x

An increase was also observed in the concentration of PCB 77 in 3 yoghurts, however the maximum increase in its concentration - by 15.8 % - was noted upon the use of Super yoghurt culture.

The possibility of degradation of PCB 169 and PCB 126 congeners and of PCB 153 and PCB 118 congeners resulting in the formation of PCB 77 was mentioned by Gidlund (1995) and Jaysankar et al. (2006). As demonstrated by results obtained in our study, a significant increase was observed in the concentration of PCB 28 congener in all types of yoghurts and bioyoghurts. This compound has the lowest number of chlorine atoms in a molecule and, therefore, may probably be an intermediate product of biodegradation of congeners with a higher number of chlorine atoms. Concentrations of the other indicatory congeners decreased by 3.4-63 %, i.e. 20.5 % on average. Losses of the sum of mono-*ortho* PCB in yoghurts, compared to the processed milk ranged from 14.7 to 27.4 %, with the greatest decrease demonstrated in the yoghurt with A.B.T. starter culture.

Investigations conducted so far have demonstrated losses of PCB congeners as a result of heat treatment of fish (Zabik et al., 1996; Moya et al., 1998; Witczak and Ciereszko, 2006, 2008; Perello et al., 2009). In the case of dairy products, their degradation may be caused by temperature, smoke (smoked cheeses) or bacteria (biodegradation). Because compounds included into the group of polychlorinated biphenyls are characterized by exceptional stability and low susceptibility to degradation, the low-temperature treatment during yoghurts production (to 45 °C) could not induce their thermal degradation, but only minor volatilization with water vapour. In contrast, biodegradation by the bacterial culture could be a significant factor determining potential changes in the concentration

of polychlorinated biphenyls in yoghurts compared to processed milk. It is speculated that this might be affected by a different composition of this starter culture which apart from *Streptococcus thermophilus* bacteria contained also two additional strains: *Lactobacillus acidophilus* and *Bifidobacterium* sp.

According to Abraham et al. (2002), some bacterial species are capable of deriving necessary energy by degradation of organochlorine pollutants. Usually, these bacteria may use biphenyl as a source of energy are may also able to metabolize different PCB congeners. Chlorobiphenyls may be biodegraded under both, aerobic and anaerobic conditions. Enzymatic systems participating in the degradation of chlorobiphenyls are encoded in the chromosomal and plasmid DNA of these microorganisms. Efficiency of this process depends on the saturation of molecules with chlorine (Abraham et al., 2002).

Biodegradation of polychlorinated biphenyls is a complex process which is affected by factors such as the concentration and chemical character of material undergoing biodegradation, genus of bacterial strains, and process conditions (temperature, pH, salinity, access of oxygen). In addition, metabolites produced during PCBs degradation may also be toxic (Hansen, 1987).

A research conducted by Singleton (1994) proved the feasibility of PCBs biodegradation by microorganisms isolated from water and soil and belonging to the following genera: *Alcaligenes*, *Micrococcus*, *Arthrobacter*, *Pseudomonas*, *Candida*, and *Rhodotorula*. Their strains were characterized by a high efficiency of PCBs biodegradation. The highest efficiency of this process (72 %) was achieved by the use of a mixed culture of *Micrococcus*, *Arthrobacter*, and *Pseudomonas* strains. In turn, the efficiency of PCBs degradation by individual isolated strains ranged from 5.4 % (*Micrococcus*) to 60 % (*Arthrobacter*). Other authors also confirmed that this is still a current problem (Passatore et al., 2014).

Another example may the study carried out by Jaysankar et al. (2006) who determined the effect of aerobic degradation by bacteria from the genus *Pseudomonas* CH07 on high-chlorinated PCB congeners from a technical mixture Clophen-50. Their study showed complete degradation of PCB 126 - one of the three most toxic coplanar PCBs (PCB 77, 126 and 169) within 40 hours and over 40 % degradation of PCB 77.

The issue of biodegradation of polychlorinated biphenyls by microorganisms was addressed in many works (Fiebig et al., 1993; Abramowicz and Olson, 1995; Commandeur et al., 1996; Rein et al., 2007), however we found no data describing the effect of bacterial cultures on changes in contents of these compounds in food products, especially in yoghurts.

Summing up the above discussion, it would be interesting to investigate changes in contents of non- and mono-*ortho* polychlorinated biphenyls during manufacture of other food products involving bacterial strains. As demonstrated by our study conducted at a laboratory scale and addressing manufacture of yoghurts and bioyoghurts, apart from changes in concentrations of lipids and dry matter and co-distillation with water vapour, the changes in the extent of these products contamination with PCBs may also be affected by the bacterial cultures applied.

The analysis of changes in concentrations of PCB congeners in yoghurts demonstrated that none of the analysed compounds was completely biodegraded by any of the cultures of lactic acid bacteria used in the production of experimental yoghurts, however a significant increase was noted in concentrations of PCB 28 (43-73 %) and PCB 77 (to 17 %) congeners in all products and a decrease in concentrations of all other analysed compounds compared to processed milk. The application of bacterial starter cultures caused also a decrease in the value of toxicity equivalent of the analysed yoghurts compared to processed milk, with the maximal decrease (49.5 %) observed upon the use of A.B.T. starter culture. The greatest reduction in concentrations of the analysed compounds in yoghurts with the A.B.T. starter culture could be due to the presence of two additional strains in this culture: *Lactobacillus acidophilus* and *Bifidobacterium* sp.

Conclusions

Yoghurt starter cultures turned out to be an effective tool in decreasing the toxicity equivalent of yoghurts in comparison to the processed milk. The presence of two additional bacterial strains *Lactobacillus acidophilus* and *Bifidobacterium* sp. in the

A.B.T. bioyoghurt starter culture was the likely the reason of the highest efficiency of this culture in reducing the value of toxicity equivalent (TEQ_{PCB}) in yoghurts (a reduction by nearly 50%). However, the use of any of the four tested starter cultures for the production of yoghurts and bioyoghurts does not ensure a complete biodegradation of any of the tested PCB congeners. These cultures contributed to a distinct reduction in the contents of the tested PCB congeners in the finished products and, simultaneously, to a significant increase in PCB 28 and PCB 77, which may result from the degradation of more chlorinated congeners.

The results related to the influence of yoghurt and bioyoghurt starter cultures on PCB contents during production of yoghurts and bioyoghurt should be treated as a pilot study, indicating a new direction of research, leading to an improvement in the quality of fermented dairy products under the risk of long-lasting exposure to hazardous organic pollutants.

Utjecaj starter kultura na sadržaj polikloriranih bifenila (PCB) u jogurtu i bio-jogurtu - alternativne metode snižavanja koncentracije PCB u mliječnim proizvodima

Sažetak

Postojani organski kontaminanti poput polikloriranih bifenila zbog lipofilnog karaktera, visoke stabilnosti te toksičnosti mogu predstavljati ozbiljan rizik za zdravlje potrošača. Oni se mogu nalaziti u mlijeku pa tako i u mliječnim proizvodima. Kako bi se potrošačima osigurala sigurna hrana s najmanjom mogućom razinom kontaminanata, važno je odrediti utjecaj tehnoloških postupaka koji se primjenjuju u preradi mlijeka i mliječnih proizvoda na promjene koncentracije toksičnih metabolita PBC-a. Koncentracije metabolita PCB-a određene su primjenom plinske kromatografije s masenom spektrometrijom. Ovo istraživanje pokazalo je da su jogurtne starter kulture učinkovite u snižavanju toksičnih metabolita u jogurtima. Prisutnost dvaju dodatnih sojeva - *Lactobacillus acidophilus* i *Bifidobacterium* sp. u starter kulturi A.B.T. bio-jogurta je najvjerojatnije razlog za najvišu određenu učinkovitost u redukciji koncentracije toksičnih metabolita (TEQ_{PCB}) u uzrocima bio-jogurta (redukcija za približno 50 %). Međutim, nijedna od ispitivanih starter kultura jogurta i bio-jogurta nije osigurala potpunu razgradnju ispitivanih metabolita PCB-a. Navedene starter kulture znatno su potpomogle redukciju koncentracije metabolita PBC-a u gotovim proizvodima, no istovremeno je zabilježen porast koncentracije metabolita PCB 28 i PCB 77, što može biti rezultat razgradnje metabolita višeg stupnja klorinacije. Zaključno, primijenjene kulture imaju potencijal u svrhu poboljšanja kvalitete fermentiranih mliječnih proizvoda.

Ključne riječi: **redukcija polikloriranih bifenila, starter kulture za proizvodnju jogurta i bio-jogurta**

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