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Effect of Increasing Zearalenone Levels on the Coagulation Properties of Milk and the Viability of Yogurt Bacteria

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Abstract

Hanuš O., Křížová L., Hajšlová J., Lojza J., Klimešová M., Janů L., Roubal P., Kopecký J., Jedelská R. (2018): Effect of increasing zearalenone levels on the coagulation properties of milk and the viability of yogurt bacteria. Czech J. Food Sci., 36: 277–283.

The effect of increasing levels of zearalenone (ZEA) artificially supplemented to milk on the coagulation characteristics and the viability of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* was examinated. Cow milk was inoculated with the yogurt culture YC-180 – YO-Flex and divided into 72, 25-ml flasks. Two samples were collected before fermentation (0 h) and remaining 70 flasks were divided into 7 groups – control (C), Z0 with 0.5 ml of ethanol and Z10, Z100, Z250, Z500, and Z1000 that were spiked with ZEA to reach the final ZEA concentrations of 10, 100, 250, 500, and 1000 µg/l, respectively. Samples were fermented at 43 ± 2°C for 5 hours. Two samples per group were collected at 1-h intervals and analysed on pH, titratable acidity, ZEA and count of *Lb. bulgaricus* and *Str. thermophilus*. The addition of ZEA resulted in slower acidification in Z100, Z250, Z500, and Z1000. The highest ZEA binding capacity (25%) was observed in Z10 and the lowest (3.1%) was found in Z1000.

Keywords: detoxifying capability; fermentation process; *Lactobacillus delbrueckii*; mycotoxin; *Streptococcus thermophilus*

The contamination of milk and dairy products with mycotoxins is directly related to the type and quality of diet ingested by lactating animals following the metabolism of mycotoxins and their subsequent excretion in milk (Flores-Flores *et al.* 2015). In general, the levels of mycotoxins excreted in milk are low (Flores-Flores *et al.* 2015) and the rate of excretion varies in dependence on many factors, including nutritional (e.g. diet, rate of ingestion and digestion) and physiological ones (e. g. biotransformation capacity of liver, animal production and health) (Duarte *et al.* 2013; Becker-Algeri *et al.* 2016). Except of aflatoxin (AFL) other mycotoxins such as zearalenone (ZEA) (Huang *et al.* 2014), T-2 toxin and deoxynivalenol (Sorensen & Elbaek 2005), fumonisins (Gazzotti *et al.* 2009) or ochratoxin A (Pattono *et al.* 2011) have also been detected in milk and dairy products. Recently, occurrence of ZEA up to 12.5 µg/kg in milk was reported from Argentina, Egypt, USA, UK and China (Huang *et al.* 2014;

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Flores-Flores *et al.* 2015) and ZEA and its metabolites were also detected in cow's milk-based infant formulas (Meucci *et al.* 2011).

In many fermented dairy products, natural inactivation of mycotoxins was observed as a side effect of fermentation. Recent studies focus on detoxification activities of several lactic acid bacteria strains toward other mycotoxins that may occur in milk, such as ZEA (El-Nezami *et al.* 2002; Sangsila *et al.* 2016) or ochratoxin A (Fuchs *et al.* 2008).

The aim of the study was to examine the effect of increasing levels of zearalenone artificially supplemented to milk on the coagulation characteristics of milk and the growth and viability of *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus.*

MATERIAL AND METHODS

Preparation of ZEA solutions. A 50 mg/l stock solution of ZEA was prepared from 50 mg of ZEA (Z2125 Sigma; Sigma-Aldrich, Germany) that was dissolved in 68.6% ethanol solution. From the stock solution dilutions were prepared at the following concentrations: 0.5 mg/l, 5 mg/l, 12.5 mg/l and 25 mg/l. These working solutions were used to spike milk inoculated with yogurt culture to obtain final ZEA concentrations of 10, 100, 250, 500 and 1000 µg/l.

Yogurt preparation. Two litres of bulk tank cow milk (fat 3.89%, crude protein 3.24%, lactose 4.92%, solids non fat 8.86 %, urea 32.5 mg/100 ml, acetone 3.2 mg/l, free fatty acids 0.9 mmol/100 g of milk fat, somatic cell count 212 ths. in 1 ml; pH 6.84) were obtained from commercial Holstein breed herd (milk yield 8932 kg per lactation) for the experiment. Milk was pasteurized in a water bath at 85°C for 5 min with constant stirring, cooled to 43 ± 2°C and inoculated with the commercial thermophilic yogurt culture YC-180 – YO-Flex (Chr. Hansen, Denmark) (Pack size, power 50U for 200 l of milk) containing *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. This basic milk was inoculated in accordance with producer recommendation by lyophilized preparation with weight dose relevant to 2 litres. The start counts of microorganisms were: *Streptococcus* 9.1×10^7 CFU/ ml; *Lactobacillus* 7×10^6 CFU/ml. Inoculated milk was divided into 72 sterile Erlenmeyer flasks (25 ml/flask). Two samples were collected before fermentation (0 h) and were considered as a negative control. Remaining 70 flasks were treated as described in Table 1 and fermented at 43 ± 2°C for 5 hours.

During the fermentation 2 samples from each group were collected at 1-h intervals for 5 hours. Immediately after collection, samples were cooled in the water bath to room temperature and analysed on pH, SH, a count of *Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Str. thermophilus*, and occurrence of inhibition substances (inhibition residues test, IRT). Furthermore, samples were also visually and palpably tested for consistency using 5-point scale according to IDF (1997). Samples taken at 0, 3, and 5 h were also analysed on the ZEA content.

Chemical analyses. The active acidity pH was measured using a CyberScan 510 pH meter (Eutech Instruments, Malaysia) which was calibrated by buffer solutions (pH 4 and 7) at 20°C. Titratable acidity (TA) was measured according to the standard ČSN 57 0530 (1973). The fat, crude protein, lactose monohydrate, solids non fat content, urea and acetone concentration and free fatty acids in experimental milk were determined by infrared spectroscopy with Fourier transformation using instrument Bentley DairySpec FT which was calibrated regularly according to results of relevant reference methods. The somatic cell count was measured by fluoro-opto-electronic

Table 1. Design of the experiment

Group	Final ZEA concentration	Treatment
C	0	control without zearalenone and ethanol
Z0	0	C + 0.5 ml of ethanol (68.6%)
Z10	10 µg/l	C + 0.5 ml of zearalenone (conc. 0.5 mg/l)
Z100	100 µg/l	C + 0.5 ml of zearalenone (conc. 5 mg/l)
Z250	250 µg/l	C + 0.5 ml of zearalenone (conc. 12.5 mg/l)
Z500	500 µg/l	C + 0.5 ml of zearalenone (conc. 25 mg/l)
Z1000	1000 µg/l	C + 0.5 ml of zearalenone (conc. 50 mg/l)

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flow cytometry using apparatus Bentley Somacount 300 (Bentley Instruments, USA), which was regularly calibrated according to the results of direct microscopy.

Extraction and analysis of zearalenone. Zearalenone (purity min. 98%) was purchased from Sigma-Aldrich (Germany). Extraction step: An aliquot of 10 ml of milk sample was mixed with 20 ml of water. Target analytes were extracted using hydrophilic-lipophilic balance (HLB) solid phase extraction (SPE) cartridges (Oasis, Waters). Extraction procedure consisted of following steps: (i) conditioning (10 ml methanol and 10 ml water), (ii) loading of diluted sample (15 ml), (iii) washing out impurities (20 ml water and 5 ml 30% methanol, v/v), and (iv) elution of the analytes (7 ml methanol). Clean-up step: crude extract (SPE fraction iv) was purified in two steps using: (i) liquid-liquid extraction with cyclohexane : ethyl acetate (1 : 1, v/v) and (ii) gel permeation chromatography (GPC, Bio-Beads S-X3, mobile phase cyclohexane : ethyl acetate,1 : 1, v/v). After addition 23 ml of water to 7 ml methanolic SPE eluate (fraction iv) extraction was accomplished with 50 ml of cyclohexane – ethyl acetate mixture. Upper layer was removed and evaporated. The residue was diluted in 3 ml of GPC mobile phase for. 1.5 ml of the sample (corresponds to equivalent 3.5 ml of milk) was injected onto GPC system. Analytes were eluted in 7 ml fraction collected within 13–20 ml. Eluate was evaporated and the residue was dissolved in 0.5 ml of mobile phase prior to high-performance liquid chromatography (HPLC) analysis.

HPLC analyses were performed by HP 1100 Series (Hewlett Packard, USA) liquid chromatograph equipped with DAD (diode array detector HP 1100 Series; Hewlett Packard, USA). C18 Discovery (150 mm × 3 mm, 5 µm) column was used for sample separation. Mobile phase (30% methanol v/v, gradient elution – 20 min 86% methanol) flow rate was 0.7 ml/min; sample injection volume was 20 µl; separation temperature was 45°C. Analytes were monitored at 260 nm.

Enumeration of viable cells. The total count of the bacteria of genus *Streptococcus* and *Lactobacillus* was determined in the period of one hour. For the isolation of *Str. thermophilus* the *Streptococcus Thermophilus* Isolation Agar (HiMedia, India; pH 6.8) was used and the plates were incubated at 36°C for 24–48 h, where the growth control of *Str. thermophilus* was carried out already after 24 hours. Used agar is also suitable for *L. bulgaricus* growth but for the determination and confirmation of our bacterial cultures was used the following medium. For the isolation of *Lb. delbrueckii* subsp. *lactis* and *Lb. delbrueckii* subsp. *bulgaricus* the *Lactobacillus Bulgaricus* Agar Base (HiMedia, India; pH 6.8) was used and the samples were incubated at 36°C for 48–72 h under anaerobic conditions. The method of decimal dilution was performed according to ČSN EN ISO 7218 (2008). Cell number was expressed in $\log_{10}$ CFU/ml. The microbiological counts were determined in duplicates.

Test on inhibition substances. There was used milk inhibition test (IRT) Delvotest T (DSM, Netherland) for antibiotic residues testing with *Geobacillus stearothermophilus* var. *calidolactis* as test microorganisms (100 µl of sample on test position and cultivation period 3 h at 64°C).

Data statistic treatment. The time and growth curves were performed for experimental data statistic evaluation. Also basic statistic parameters and linear regression were used MS Excel (Microsoft, USA). Microbiological indicators were logaritmically transformed (Hanuš *et al*. 2001). The pair t-test was used to determine significant ($P < 0.05$) differences between zero ZEA concentrations and spiked samples for acidity and microbiological indicators (pH, TA, *Lb. bulgaricus*, and *Str. thermophilus*).

RESULTS AND DISCUSSION

The lowest concentration of ZEA (Z10) in our study corresponded to concentration of ZEA either found in milk after experimental feeding of ZEA-contaminated diets (Dänicke *et al.* 2014) or determined in retail milk (Huang *et al.* 2014; Flores-Flores *et al.* 2015). The Z100 and Z250 reflected maximum levels for ZEA in foodstuffs (Commission regulation (EC) No. 1881/2006) and the two highest concentrations (Z500 and Z1000) represented multiples of previous values.

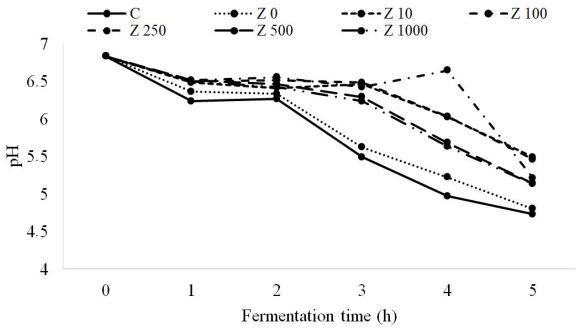

Figure 1. Changes in pH values of fermented milk during manufacture of yogurts in dependence on the amount of spiked zearalenone (for abbreviations see Table 1)

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Table 2. Final values of pH and titratable acidity (SH) after 5 h of fermentation in dependence on the amount of spiked zearalenone

	Concentration of zearalenone (µg/l)*						
	C	Z 0	Z 10	Z 100	Z 250	Z 500	Z 1000
pH	4.73	4.80	5.49	5.46	5.21	5.14	5.13
Titratable acidity (°SH)	32.4	29.2	24.0	21.6	26.4	24.8	26.4

*see Table 1

Fermentation process. The pattern of changes in pH during fermentation of the yogurts is given in Figure 1. The pH of C and Z0 yogurts reached values of 4.73 and 4.8, respectively at the end of fermentation suggesting that the ethanol had no effect on coagulation properties of milk. On the other hand, the final pH of all ZEA-spiked yogurts ranged between 5.1 and 5.5 with the highest values in Z10 and Z100 (Table 2). The pH of both Z500 and Z1000 was almost parallel suggesting that concentrations of ZEA exceeding 500 µg/l could have the same effect on pH values. The addition of ZEA resulted in slower acidification in Z10, Z100, Z250, Z500 and Z1000 but this only become evident after 3 h of fermentation. The pH pair t-test value calculated for zero ZEA concentrations (C and Z0) against spiked (from Z10 to Z1000) sample values was 9.18 (n = 10, P < 0.001) (Table 2). The pattern of TA changes during the fermentation had the opposite trend to pH (Figure 2). The TA pair t-test value calculated for zero ZEA concentrations against spiked sample values was 7.32 (n = 10, P < 0.001) (Table 2). The final TA values measured in our study are comparable with Hejtmánková *et al.* (2000). The above mentioned parameters were considered appropriate after 5 h of fermentation as usual in other studies (Krivorotova *et al.* 2017).

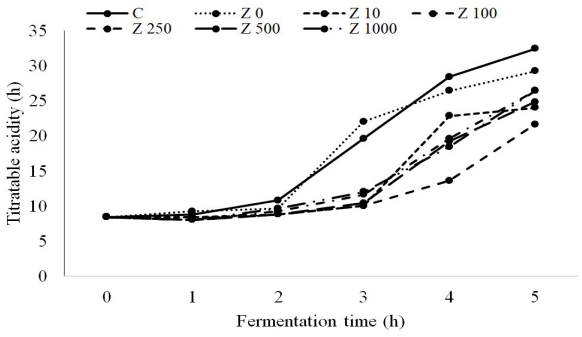

Figure 2. Changes in titratable acidity of fermented milk during manufacture of yogurts in dependence on the amount of spiked zearalenone (for abbreviations see Table 1)

The presence of ZEA appeared to decrease the metabolic activity of yogurt bacteria. This may be related to a negative effect of ZEA on viability of cells as discussed later or to ZEA binding that was the highest in Z10 and Z100. Although the mechanism of mycotoxin binding abilities of *Lactobacillus* strains has not been clearly described, recent findings suggest that more mechanisms such as composition of peptidoglycan layers, specific protein and carbohydrate components of cell wall, hydrophobicity of cell surface, external physicochemical factors or solution chemistry are involved in this process (Sangsila *et al.* 2016). Those machanisms may interact with each other and influence the metabolic activity of yoghurt bacteria. Further studies are needed in this area.

Although there are no comparable data on the effect of ZEA on acidification rate of yogurts, according to Arab *et al.* (2012) the occurrence of AFL in fermented milks was accompanied by longer fermentation time, decreases in the growth, morphology, and activity rate of starter cultures as well as by conversion of homofermentative starter cultures into heterofermentative ones. So we can suppose that ZEA can have at least partly similar detrimental effects on the fermentation process. This suggestion is supported by the results of inhibition test in which C and Z0 samples were negative while all ZEA-spiked yogurts regardless of ZEA concentration showed slightly positive results suggesting certain negative impact on the viability of the test microorganism (data not shown). Furthermore, consistency of all ZEA-spiked yogurts evaluated after 5 h of fermentation ranged between 1 to 3 points and was worse than in C and Z0 samples both receiving 5 points (data not shown).

Concentration of ZEA in model yogurts and detoxifying capability of yogurt culture. Concentration of ZEA in yogurts at the beginning of the trial (0 h) and after 3 and 5 h of fermentation is given in Table 3. Although C yogurts were not analysed, ZEA was not detected in the Z0 yogurts suggesting that also control samples were negative. Recovery of about 76–106% was determined at 0 h after spiking

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Table 3. Concentration of zearalenone (µg/l) in yogurts after 3 and 5 h of fermentation

Fermentation time (h)	Concentration of zearalenone (µg/l)*						
	C	Z 0	Z 10	Z 100	Z 250	Z 500	Z 1000
0	NA	0.0	7.6	89.2	264.3	469.3	897.2
3	NA	0.0	6.9	76.0	243.8	418.5	874.3
5	NA	0.0	5.7	79.7	236.1	429.6	869.7

NA – not analysed; *see Table 1

of the milk and it is in agreements with other studies e.g. Flores-Flores and González-Peñas (2017).

Recently, several *Lb.* strains that are widely used in food industry have been examined for their potential to detoxify ZEA (Sangsila *et al.* 2016). The detoxifying ability of *Lb. bulgaricus* and *Str. thermophilus* that were used in our study is given in Figure 4. The strains were capable of removing ZEA from spiked yogurts but their detoxifying efficiency varied across the initial ZEA concentrations. The highest binding capacity was observed in Z10 after 5 h of incubation and reached 25%. While detoxifying capability of strains in Z100, Z250 and Z500 after 5 h of incubation were closed ranging from 8.5 to 10.7%. The lowest binding capability of only 3.1% was found in Z1000. Similarly, to our study Sangsila *et al.* (2016) found that binding capacity differed across the initial concentrations of ZEA. They observed that binding efficiencies was below 50% with ZEA concentrations bellow 23.08 µg/ml. This is in agreement with our findings and with Fuchs *et al.* (2008). According to Sangsila *et al.* (2016) high availability of ZEA may also contribute to the increase in the detoxifying capabilities of *Lb. pentosus* strains. Furthermore, El-Nezami *et al.* (2002) reported strong binding capabilities of *Lb. rhamnosus* GG and *Lb. rhamnosus* LC-705 not only for ZEA but also for α-zearalenone with detoxifying levels of 38 and 46%, respectively. Furthermore, recent stu dies performed by Vega *et al.* (2017) showed not only the high stability of the complex bacteria–mycotoxin but also the absence of ZEA metabolites in media.

The regression analysis between ZEA concentration in milk and yogurts (Figure 3) showed that the concentration of ZEA in milk could be a good predictor of ZEA concentration in yogurts. Although there are no comparable data concerning to ZEA and yogurts study of Battacone *et al.* (2005) revealed close relationship between AFM1 concentration in milk and curd (R^2 = 0.80) and in milk and whey (R^2 = 0.80). However, we are aware that a small number of samples is a limitation of our study. Thus to confirm our findings more studies on higher number of samples are required.

Viability of cells. A sum of *Lb. bulgaricus* and *Str. thermophilus* found in all yogurts at the end of fermentation (5 h) ranged between 8.95 and 8.79 log CFU/ml, with no significant variability among the levels of ZEA supplementation (data not shown). The *Lb. delbrueckii* growth in C and Z0 after 5 h of fermentation was higher than in Z10, Z100, Z250, Z500, and Z1000. Similar situation was in *Str. thermophilus* with higher counts in C and Z0 compared to Z10, Z100, Z250, Z500, and Z1000. The longer time will be probably required for the

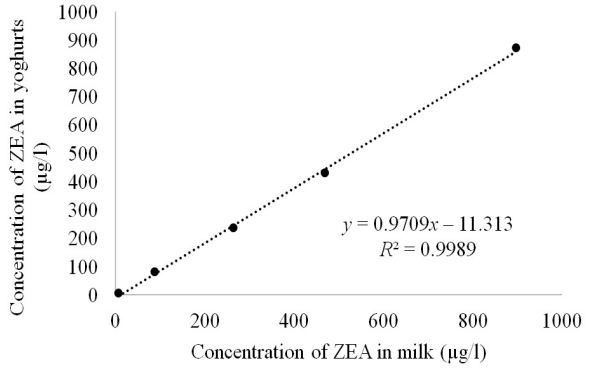

Figure 3. Relationship of zearalenone (ZEA) concentration in yogurts with milk from which they were made..

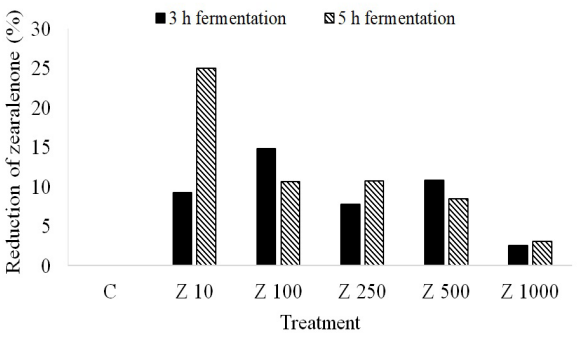

Figure 4. Percent reduction in zearalenone concentration after 3 and 5 h of fermentation.

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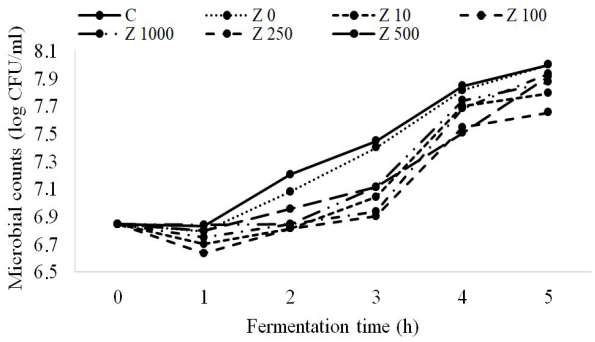

Figure 5. Counts (log CFU/ml) of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *lactis* during fermentation of milk spiked with increasing levels of zearalenone. For abbreviations see Table 1

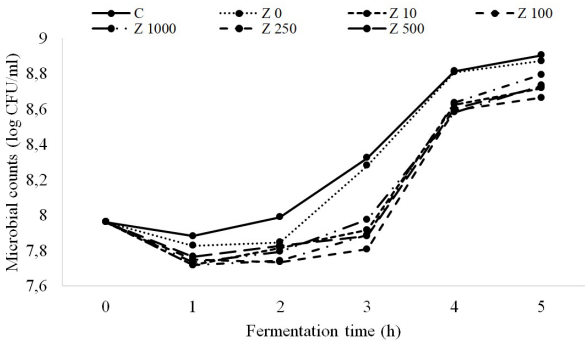

Figure 6. Counts (log CFU/ml) of *Streptococcus thermophilus* during the fermentation of milk spiked with increasing levels of zearalenone. For abbreviations see Table 1

growth of ZEA spiked yogurts to reach the same counts as C and Z0.

The effect of increasing levels of ZEA on the cell counts of *Lb. delbrueckii* and *Str. thermophilus* determined at 5 sampling times during the fermentation are also shown in Figures 5 and 6, respectively. The microbial pair t-test values calculated for zero ZEA concentrations (C and Z0) against spiked (from Z10 to Z1000) sample values for logarithmic forms were: 4.77 (n = 10, P < 0.001) (Figure 5) for *Lb. bulgaricus*; 9.81 (n = 10, P < 0.001) (Figure 6) for *Str. thermophilus*. In general, CFU values of *Str. thermophilus* during and after fermentation were higher than those of *Lb. delbrueckii.* As shown in the figures, supplementation with ZEA affected the growth of both bacteria mainly between 2–4 h of fermentation with the most obvious effect at 3 h of fermentation.

In the literature results of the effect of ZEA on the growth and viability of cells are inconsistent because ZEA exerts different mechanisms of toxicity in different dose and cell types (Zheng *et al.* 2018). While low doses of ZEA can exert the estrogen-like effects and stimulate the proliferation of cells, high doses of ZEA can cause cell death through inducing oxidative stress, DNA damage, mitochondrial damage, cell cycle arrest and apoptosis (Zheng *et al.* 2018). Furthermore, ZEA may exert the cytotoxic effects in the cells from the tissues which have no estrogen receptors and the estrogen-like effects in the cells from tissues with estrogen receptors (Zheng *et al.* 2018). However, it should be noted that the effect of ZEA on viability of various cells was studied after longer exposure periods varying somewhere between 24 h and 7 days (Zheng *et al.* 2018). Nevertheless, decreased viability of cells after 24 h ZEA exposure mentioned in some studies (Benzoni *et al.* 2008) suggest that ZEA could have detrimental effect on the viability of yogurt culture as well. This suggestion is supported by our findings that all ZEA-spiked yogurts showed slightly positive reaction in inhibition test as mentioned above. Furthermore, according to Donkor *et al.* (2006) viability of *Lactobacillus* spp. and *Bifidobacterium* spp. in food are influenced by many factors mainly by the pH, the concentration of some metabolites resulting from fermentation (lactic and acetic acids) and the presence of hydrogen peroxide and dissolved oxygen, the medium buffering capacity, the probiotic strains used or the storage temperature. Further studies are needed on short-time effects of ZEA on cells.

CONCLUSIONS

The present study showed that fermentation process and the viability of *Lb. delbrueckii* and *Str. thermophilus* during the fermentation were negatively influenced by the presence of zearalenone in milk. These effects were the most obvious at 3 h of fermentation. The detoxifying ability of above mentioned strains determined after 5 h of fermentation ranged between 3.1 and 25% and was dependent on the initial zearalenone concentrations.

References

Arab M., Sohrabvandi S., Mortazavian A.M., Mohammadi R., Tavirani M.R. (2012): Reduction of aflatoxin in fermented milks during production and storage. Toxin Reviews, 31: 44–53.

https://doi.org/10.17221/391/2017-CJFS

Battacone G., Nudda A., Palomba M., Pascale M., Nicolussi P., Pulina G. (2005): Transfer of aflatoxin B1 from feed to milk and from milk to curd and whey in dairy sheep fed artificially contaminated concentrates. Journal of Dairy Science, 88: 3063–3069.

Becker-Algeri T.A., Castagnaro D., de Bortoli K., de Souza C., Drunkler D.A., Badiale-Furlong E. (2016): Mycotoxins in bovine milk and dairy products: A review. Journal of Food Science, 81: R544–552.

Benzoni E., Minervini F., Giannoccaro A., Fornelli F., Vigo D., Visconti A. (2008): Influence of *in vitro* exposure to mycotoxin zearalenone and its derivatives on swine sperm quality. Reproductive Toxicology, 25: 461–467.

Dänicke S., Keese Ch., Meyer U., Starke A., Kinoshita A., Rehage J. (2014): Zearalenone (ZEN) metabolism and residue concentrations in physiological specimens of dairy cows exposed long-term to ZEN-contaminated diets differing in concentrate feed proportions. Archives of Animal Nutrition, 68: 492–506.

Donkor O.N., Henriksson A., Vasiljevic T., Shah N.P. (2006): Effect of acidification on the activity of probiotics in yoghurt during cold storage. International Dairy Journal, 16: 1181–1189.

Duarte S.C., Almeida A.M., Teixeira A.S., Pereira A.L., Falcao A.C., Pena A., Lino C.M. (2013): Aflatoxin M1 in marketed milk in Portugal: assessment of human and animal exposure. Food Control, 30: 411–417.

El-Nezami H.S., Polychronaki N., Salminen S., Mykkanen H. (2002): Binding rather metabolism may explain the interaction of two food-grade *Lactobacillus* strains with zearalenone and its derivative α-zearalenol. Applied and Environmental Microbiology, 68: 3545–3549.

Flores-Flores M.E., Lizarraga E., López de Cerain A., González-Peñas E. (2015): Presence of mycotoxins in animal milk: A review. Food Control, 53:163–176.

Flores-Flores M.E., González-Peñas E. (2017): An LC-MS/MS method for multi-mycotoxin quantification in cow milk. Food Chemistry, 218: 378–385.

Fuchs S., Sontag G., Stidl R., Ehrlich V., Kundi M., Knasmuller S. (2008): Detoxification of patulin and ochratoxin A, two abundant mycotoxins, by lactic acid bacteria. Food and Chemical Toxicology, 46: 1398–1407.

Gazzotti T., Lugoboni B., Zironi E., Barbarossa A., Serraino A., Pagliuca G. (2009): Determination of fumonisin B1 in bovine milk by LC-MS/MS. Food Control, 20: 1171–1174.

Hanuš O., Bjelka M., Ticháček A., Jedelská R., Kopecký J. (2001): Substantiation and usefulness of transformations in data sets of analysed milk parameters. Sborník VÚCHS Rapotín, 122–137. [in Czech]

Hejtmánková A., Horák V., Dolejšková J., Louda F., Dragounová H. (2000): Influence of yogurt cultures on benzoic acid content in yoghurt. Czech Journal of Food Sciences, 18: 52–54.

Huang L.C., Zheng N., Zheng B.Q., Wen F., Cheng J.B., Han R.W., Xu X.M., Li S.L., Wang J.O. (2014): Simultaneous determination of aflatoxin M1, ochratoxin A, zearalenone and zearalenol in milk by UHPLC–MS/MS. Food Chemistry, 146: 242–249.

Krivorotova T., Sereikaite J., Glibowski P. (2017): Rheological and textural properties of yogurts enriched with Jerusalem artichoke flour. Czech Journal of Food Sciences, 35: 432–439.

Meucci V., Soldani G., Razzuoli E., Saggese G., Massart F. (2011): Mycoestrogen pollution of Italian infant food. Journal of Pediatrics, 159: 278–283.

Pattono D., Gallo P.F., Civera T. (2011): Detection and quantification of ochratoxin A in milk produced in organic farms. Food Chemistry, 127: 374–377.

Sangsila A., Faucet-Marquis V., Pfohl-Leszkowicz A., Itsaranuwat P. (2016): Detoxification of zearalenone by *Lactobacillus pentosus* strains. Food Control, 62: 187–192.

Sorensen L.K., Elbaek T.H. (2005): Determination of mycotoxins in bovine milk by liquid chromatography tandem mass spectrometry. Journal of Chromatography B, 820: 183–196.

Vega M.F., Dieguez S.N., Riccio B., Aranguren S., Giordano A., Denzoin L., Soraci A.L., Tapia M.O., Ross R., Apás A., González S.N. (2017): Zearalenone adsorption capacity of lactic acid bacteria isolated from pigs. Brazilian Journal of Microbiology, 48: 715–723.

Zheng W., Wang B., Li X., Wang T., Zou H., Gu J., Yuan Y., Liu X., Bai J., Bian J., Liu Z. (2018): Zearalenone promotes cell proliferation or causes cell death? Review. Toxins, 10: 184–201.

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