Zagazig Veterinary Journal, ©Faculty of Veterinary Medicine,
Zagazig University, 44511, Egypt.
Volume 51, Number 4, p344354 December 2023
DOI: 10.21608/zvjz.2023.231448.1214

RESEARCH ARTICLE

Exploitation of Probiotic a Lactobacillus rhamnosus Strain for Removal of Heavy Metal Residues from Milk

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Abstract

The ability of Lactobacillus rhamnosus to remove some heavy and trace metals from milk through fermentation was investigated. For this purpose, one hundred samples of raw milk, fermented milk, kareish cheese and ice cream were initially screened fordetection of cupper, zinc, lead and cadmium. Different concentrations were obtained and compared with some national and international findings. Upon milk fermentation with probiotic strain Lactobacillus rhamnosus, reduction was achived in the examined metals with the following percentages, Cupper 6.8%, Zinc 79.65%, Cadmium 21.62% and Lead 22.58%. The reduction in heavy metal residues declared the health benfits from the use of Lactobacillus rahmnosus and protection of Egyptian population from the risk of heavy metals exposure from milk and milk product consumption. Probiotic strain Lactobacillus rahmnosus could be a promising solution for lowering the milk content of toxic metals and decrease the exposure to heavy metals from milk consumption.

Keywords: Heavy metals, Lactobacillus rhamnosus, chelation, metals toxicity.

Introduction

Milk and dairy products are very numerous and they contain different trace elements, such as copper, zinc, manganese and iron [1]. These metals have a vital role in a lot of physiological mechanisms and their deficiencies may disrupt these mechanisms [2]. Contamination of milk and dairy products with heavy metals is a prominent food safety hazard, and this is based upon the adverse toxic effects caused by cadmium (Cd), lead (Pb) and mercury (Hg) [3-5]. Copper (Cu) and zinc (Zn) are essential elements to life, but they may be detrimental if ingested in high concentrations [6].

Oxidative stress and cellular damages are among the consequences of heavy metals food contamination, specially chromium, iron and arsenic. These metals leads to generation of hydroxyl radical. Carcinogenicity is the main fate of heavy metals chronic toxicity.One of the worst consequences of food heavy metals is the liberation of free radicals. Cellular lipid membrane get attacked by the released radials and lipid/dioxygene reaction was happened. Later, a stable lipid molecule is being produced from consequitive reaction.Animal received heavy metals from ingestion of contaminated water and feeds. These sources have been considered as the main source of metal residues in produced milk [7]. Moreover, faulty processing may expose raw milk to heavy metal contamination and leaching from containers may lead to increase their levels [8].

Heavy metal residues in milk or milk products, even in low levels, can lead to significant concentration in human body. Cd, Pb and mercury can persist in the body and exert their toxic effect in form of cellular disturbance or clinical manifestation [9,10]. Also, Cu and Zn when given in excess are toxic to man and animal [11].

Scientists have proposed a lot of methods to decontaminate water, milk and dairy products from heavy metals. Each method has its own pros and cons. Recently, some microorganisms were found to have the ability to remove heavy metals. Probiotics are among those benficial microorganisms. Probiotics are easily supplemented in different fermented foods. It is reported that dead and living lactic acid bacteria are able to remove toxins [12]. It is believed that this process is through the physical binding to cell wall components rather than covalent binding. Probiotic's metals chelation mechanism is accomplished through binding of metallic ions to bacterial cell wall followed by accumulation inside the bacteria. Therefore, this study was carried out to investigate the ability of probiotics microorganisms in reduction of some heavy and essential metals from milk.

Materials and Methods

Collection of samples

One hundred samples of raw milk, fermented milk, kareish cheese and ice cream (25 each, mostly of small manufacturer scale) were randomly collected from markets at Zagazig, Sharkia governorate, Egypt. Samples were transferred in their original conditions to the Central Laboratory, Faculty of Veterinary Medicine, Zagazig University. The samples were submitted for detection and quantification of heavy metals.

Detection of heavy metal residues

Wet digestion technique

All containers were initially soaked in 10% HNO_3 then adequately rinsed with distilled water [13]. Before conducting the experiments, glasswares were washed with washing solution (6.26:5:2 of de-ionized water, concentrated HCl and H_2O_2) then with 10% HNO_3, with a final rinse with de-ionized water, and left to dry [14].

Digestion of tissue samples

Perkin Elmer model (spectra-AA 10, USA) flame atomic absorption spectrometer (AAS) with computer system was used. One gram of milk or milk product sample was homognized and placed in a tightly capped tube. Five milliliters of digestion solution (3 mL HNO_3: 2 mL $HClO_4$) were poured to each tube [15]. The tubes were adequately shaken and left overnight. Thereafter, the tubes were warmed to 70ºC for 3 hours with sporadic shaking. After cooling, the tubes were diluted using 20 mL de-ionized water, and filtered. The tubes were kept at room temperature until analysis for heavy metals content. Analysis procedures were following those stated in the operator's manual of the atomic absorption spectrophotometer, Per Kin Elmer model (spectra-AA10, USA).

Determination of examined metals

Instrumental procedures for various analyses were based on those suggested in the operator's manual of the atomic absorption spectrophotometer (Perkin-Elmer Atomic Absorption Spectrophotometer model d 2380, USA, 1998). However, blanks and standards were prepared in the same manner as for wet digestion and using the same chemicals.

Preparation of the blank and standard solution

Blank solution consisted of 3 parts of nitric acid and 2 parts of perchloric acid that was treated similar to wet digestion procedure then diluted with 20 parts of the de-ionized water. Standard solutions using pure certified metal standards (ICV-GFAA-100, ZNOMS, CUMOS, Zeptomatrix, USA) were prepared for each metal.

Quantitative determination of heavy metals

All samples` and experiments` solutions were syphoned by atomic absorption spectrometer and analyzed. Analysis of Cd, Pb, Zn and Cu was conducted by air/ acetylene flow (5.5/1.11/m) flame A.A.S (Buck Scientific Model 210 VGP).

Cd, Pb, Zn and Cu levels were recorded driven from the AAS scale and calculated according to the following equation;

Element, ppm R*D/W

Where;

R= Reading of element concentration, ppm from digital scale of AAS.

D= Dilution of prepared sample.

W= Weight of the sample.

Metals values were expressed as mg/g wet weight (ppm).

Effect of Lactobacillus rahmnosus LMG 23522 on heavy metal residues ppm (mg/ litre) of examined raw milk samples

A 990 milliliter of milk obtained from the same cow, which divided into two parts, 495 mL for each (the first group was considered as control and the second one was inoculated with 5 mL of *Lactobacillus rahmnosus* LMG 23522 (BCCM) suspension previously prepared in a concentration of one mackferland.Both groups were kept at 37 °C for 24 hours followed by homogenization. The samples were collected for detection of heavy metals (copper, zinc, cadmium and lead) from control and inoculated group. The protocol was repeated five times.

Statistical analysis

Statistical analysis was assessed using the SPSS (v.13, SPSS Inc., Chicago, IL). Results were recorded as mean ± standard errors (SE). The value of $P < 0.05$ was used to indicate statistical significance. One-Way ANOVA test was applied to compare differences among means. Duncan's multiple range test was used as post hock test. Paired smples t-test was used to compare samples before and after adding probiotic strain.

Results and discussion

Milk is rich in calcium and magnesium, and it contains lower levels of essential metals such as iron, Zn, and Cu [16]. However, as a result of increased industrial, agricultural, and urban emissions, milk might be contaminated with different amounts of toxic contaminants [17].

Herein, Cu was detected in 100 % of examined samples with mean values of 0.072 ± 0.0081, 0.042 ± 0.0047, 48.5 ± 11.66 and 0.60 ± 0.18 ppm (mg/ litre) in raw milk, fermented milk, kareish cheese and ice cream, respectively (Table 1). Nearly similar values were detected (except for Kariesh cheese samples) from 0.043 to 0.098 ppm in Czech Republic [18], 0.04 ppm in Swiss [19], 0.069 to 0.096 ppm in Czech Republic [20], 0.0518ppm in Navarra, north Spain [21].

Higher Cu values obtained, 0.592 ppm in Assiut governorate, Egypt [13], 0.250 ppm in milk from southern Poland [22] 0.120 ppm in milk from France [23],1.73 ppm in eastern portion of São Paulo State, Brazil [24], 0.290 ppm in milk from Croatia [25], and 0.117 ppm in milk from Serbia [26].The results in Table 1 declared that Zn was detected in 100 % of examined samples with mean values of 0.11 ± 0.0097, 0.08 ± 0.0082, 31.17 ± 11.66 and 1.85 ± 1.690 ppm (mg/ litre-kg) in raw milk, fermented milk, kareish cheese and ice cream, respectively.

Table1: Statistical analytical results of metals` residues in examined samples per ppm (mg/ litre or kg) (N = 25 for each)

Metal	Type of sample	Positive samples No	Positive samples %	Minimum	Maximum	Mean ± SE
Cu	**Raw milk**	25	100	0.054	0.099	0.072 ± 0.0081^{b}
	Fermented milk	25	100	0.029	0.055	0.042 ± 0.0047^{b}
	Kareish cheese	25	100	4.500	107.600	48.5 ± 11.66^{a}
	Ice cream	25	100	0.168	2.092	0.60 ± 0.18^{b}
Zn	**Raw milk**	25	100	0.0760	0.1360	0.11 ± 0.0097^{b}
	Fermented milk	25	100	0.0570	0.1040	0.08 ± 0.0082^{b}
	Kareish cheese	25	100	0.1760	104.0000	31.17 ± 11.66^{a}
	Ice cream	25	100	0.0570	17.1000	1.85 ± 1.690^{b}
Cd	**Raw milk**	20	80	0.0000	0.0060	0.0026 ± 0.001^{b}
	Fermented milk	15	60	0.0000	0.0000	0.0018 ± 0.0009^{b}
	Kareish cheese	25	100	9.1000	91.9000	38.34 ± 6.73^{a}
	Ice cream	25	100	.4500	3.4500	1.07 ± 0.28^{b}
Pb	**Raw milk**	25	100	0.0370	0.1410	0.10 ± 0.018^{b}
	Fermented milk	ND	ND	ND	ND	ND
	Kareish cheese	25	100	.5700	4.8260	1.76 ± 0.41^{a}
	Ice cream	25	100	.4510	2.4320	$0.95 \pm 0.\ 18^{ab}$

Mean values of the same column carrying different superscript letters are significant ($p< 0.05$).

Cadmium (Cd), lead (Pb), mercury (Hg), Copper (Cu), zinc (Zn).

Egypt [33] and 0.15 ± 0.01 ppm in milk samples collected from Sharkia Governorate, Egypt [34]. Comparable Cd values in milk (0.001 to 0.011ppm) was detected in Plovdiv region, Bulgaria [35], 0.001- 0.004 ppm in Czech [18], 0.001ppm in Swiss [19], and 0.002 to 0.05 ppm in Czech Republic [20]. Lower values for Cd (0.00004 ppm in Navarra, north Spain [21], and from 0.0021 to 0.0048 ppm in milk from Serbia [26]. In the current study, the Cd residue in fermented milk is 0.0018 ± 0.0009 ppm. Nearly similar finding (0.0063- 0.0241 ppm) in fermented milk from Serbia [26]. Nevertheless, it was 0.218 ppm in samples collected from Zagazig City, Sharkia Governorate, Egypt [36].

The results in Table 1 displayed that lead residues were detected in 100% of raw milk, kareish cheese and ice cream. Meanwhile, lead not detected in all

The level of Zn (0.11 ppm) in milk samples was lower than 3.96 ppm [27], 4.1ppm in Burundi [28], 4.059- 5.878 ppm in Czech [18], 3.6 ppm in Swiss [19], 3.95 to 5.051 ppm in Czech Republic [20], 2.06 ppm in Assiut governorate, Egypt [13], 0.463 ppm in Navarra, north Spain [21] and 4.59 ppm in eastern portion of São Paulo State, Brazil [24].

The mean value of Zn in kareish cheese was 31.17 ± 11.66 ppm. The level was coincide to Rojas *et al.* and Gambelli *et al.* [29, 30]; they detected 37.86 and 34.7 ppm in ripened Manchego-cheese and stirred curd cheese.

The mean level of Cd (0.0026 ppm) in milk samples was lower than values obtained, 0.018 ppm [31], 0.113 ppm in Sharkia Governorate, Egypt [32], 0.017 ppm in Assiut Governorate, Egypt [13], 0.416 ppm in El Dakahlia Governorate, fermented milk, and ice cream. Moreover, kareish cheese introduces hazardous effect on consumer health. Ice cream samples ranked the second in terms of heavy metal contamination, which attributed to raw materials other than milk, equipment used during the production process and packaging materials. Food processing equipment and/or packages materials may be attributed as contamination sources for heavy metals. Migration from the can metal wall into food is a well documented source for some dairy products [43].

Effect of lactobacillus rahmnosus starter culture on heavy metal residues per ppm (mg/ litre) of examined raw milk samples

The effect of *Lactobacillus rahmnosus* on heavy metal concentration in inoculated milk groups in comparison to the control groups as exposed in Table 2. The mean concentrations of Cu, Zn, Cd and Pb in control groups were 0.44, 1.72, 0.074 and 2.17ppm, respectively, meanwhile, in inoculated groups, the mean concentrations reduced to 0.14, 0.35, 0.58 and 1.68 ppm achieving reduction percentages of 6.8%, 79.65%, 21.62% and 22.58%, respectively. examined fermented milk samples. The mean values were 0.10 ± 0.018, ND, 1.76 ± 0.41and 0.95 ± 0. 18 ppm (mg/ litre- kg) in raw milk, fermented milk, kareish cheese and ice cream, respectively.

The level of Pb residues in milk (0.1 ppm) were similar to that in milk samples (0.1ppm) collected from Kenya [37]. Higher Pb residues obtained, 0.2 ppm in milk collected from Northern provinces of Netherlands [38], 20 to 130 ppm in Yugoslavia [39], 0.23 ppm [30], 0.152 to 219 ppm in Malaysia [40], 1.51 ppm in Sharkia Governorate, Egypt [41], 0.24 ppm in Assiut Governorate, Egypt [13], 0.615 ppm in El Dakahlia Governorate, Egypt [33], 0.005 ppm in Navarra, north Spain [21], and 0.23 ppm ineastern portion of São Paulo State, Brazil [24]. Lower Pb residues (0.012- 0.036 ppm) were reported in Czech [18], 0.018 to 0.031 ppm in Czech Republic [20], 0.019 in the Meza valley, Slovenia [41], 0.022 to 0.059 ppm in Turkey [42] and from 0.0543 to 0.0952 ppm in milk from Serbia [26].

The obtained data in Table 1 generally declared that the contents of the all four metals obtained in the kareish cheese product samples were significantly higher than those obtained in raw milk,

Table (2): Effect of *Lactobacillus rahmnosus* starter culture on heavy metal residues ppm (mg/ liter) of examined raw milk samples (N = 20)

Type of heavy metal	Criteria	Mean ± S.E	Reduction %	*P - value*
Cu	Original concentration	0.61 ± 0.17	6.8%	> 0.05
	After adding *Lactobacillus rahmnosus*	0.44 ± 0.1		
Zn	Original concentration	1.72 ± 0.24	79.65%	0.007
	After adding *Lactobacillus rahmnosus*	0.35 ± 0.05		
Cd	Original concentration	0.074 ± 0.03	21.62%	> 0.05
	After adding *Lactobacillus rahmnosus*	0.058 ± 0.01		
Pb	Original concentration	2.17 ± 0.34	22.58%	0.07
	After adding *Lactobacillus rahmnosus*	1.68 ± 0.43		

Cadmium (Cd), lead (Pb), mercury (Hg), Copper (Cu), zinc (Zn).

Different mechanisms elucidated how probiotics can detoxify heavy metals. The metals are binded to the bacterial wall which then accumulated. Additionlly, The toxic forms are transformed into less toxic by probiotics. These process are called metals bioremoval, which is an effective method for removing heavy metals from foods. Many microorganisms are being exolited in these detoxification process (47). Food contaminants bioremoval is a green technology to control many health risks. Some probiotics and potential probiotics are proven for being able to remove mycotoxins and heavy metals from foods. *Lactobacillus rahmnosus* is an interesting model for the bioremoval of heavy metals from milk.

Conflict of interest

Authors declares no conflict of interest.

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The results revealed that insignificant decrease (*P* > 0.05) in all examined metals (Cu, Cd and Pb) and only significant reduction (*P* < 0.05) in Zn concentration.

The reduction in heavy metal residues in milk may be attributed to independent metabolic binding with metal ions with different mechanisms such as adsorption or ion exchange [44]. Halttunen*et al.* [45] showed that LAB are capable to eliminate the Cd and Pb from the water. Elimination was quick, influenced strongly by the pH, pointing out a mechanism of ionic exchange. Optimization of process is dependant on different key factors like pH, temperature and initial concentration of biomass. The best results were obtained at concentration of 1 mg/mL, optimal temperature of 35 ˚C and pH 4-6 [46].

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الملخص العربي

استغلال عترة البروبيوتيك لاكتوباسيلس رامنوسز لإزالة بقايا المعادن الثقيلة من الحليب

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أجريت هذه الدراسة لاستقصاء قدرة اللاكتوباسيليس رامنوسز على إزالة بعض المعادن الثقيلة والنادرة من الحليب خلال التخمر. لهذا الغرض ، تم في البداية فحص مائة عينة من الحليب الخام والحليب المخمر والجبن القريش والآيس كريم لتحديد مستويات النحاس والزنك والرصاص والكادميوم. تم الحصول على تركيزات مختلفة وكانت العديد من العينات أعلى من الحدود المسموح بها وفقًا للمعايير الوطنية والدولية. وعند تخمر الحليب باستخدام اللاكتوباسيليس رامنوسز، تم تحقيق انخفاض في المعادن التي تم فحصها بالنسب المئوية التالية النحاس6.8 ٪ ، الزنك 79.65٪، الكادميوم 21.62٪، والرصاص 22.58٪. هذا وقد أوضح انخفاض مخلفات المعادن الثقيلة عن الفوائد الصحية الناجمة عن استخدام اللاكتوباسيليس رامنوسز وحماية المستهلكين من مخاطر التعرض للمعادن الثقيلة من استهلاك الحليب ومنتجات الألبان.