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Efficiency of Multispecies Probiotic Supplements in Bio-Removal of Bisphenol A: An In Vitro Study

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Abstract

Background and Objective: Bisphenol A is a well-known industrial compound which is widely used in producing plastic throughout the world. Containers made with these plastics may expose people to small amounts of bisphenol A in food and water and cause adverse effects on human health. In this study, the effect of commercial probiotic formulations on reduction of bisphenol A in aqueous solution is investigated.

Material and Methods: One dose of six types of commercial mixtures of probiotic strains were added to a certain amount of bisphenol A in saline basal medium at 37°C. During a 24-h treatment with probiotics, samples were taken from the environments at different times and prepared for further analysis with enzyme-linked immunosorbent assay. The experimental framework was set up in a way that compares formulations and determines the most efficient strains for bisphenol A reduction. In addition, the effect of peripheral conditions such as pH and temperature were also studied.

Results and Conclusion: Multi-strain probiotics had an impressively high performance in bio-removal of bisphenol A from aqueous solutions. Up to 80% of bisphenol A concentration was decreased during the first hour of treatment in almost all trials. Among them, the synergy of *Lactobacillus acidophilus* and *Lactobacillus plantarum* strains were the most successful. On the other hand, mixture of probiotics had more persistent effect and robust binding ability than single strains. Finally, it can be expected that regular usage of probiotic supplementation with special mixture of strains can suppress the harmful effects of bisphenol A.

Conflict of interest: The authors declare no conflict of interest.

1. Introduction

Bisphenol A (BPA) is a key monomer in production of polycarbonate plastics and epoxy resins, materials that are utilized in a wide range of applications such as medical equipment, protective coatings in packing industry and linings for food and drinking cans [1,2]. The migration of plastic components or additives from packaging into food may occur and produce a risk for public health. In particular, heat and acidic treatments during sterilization process increase the migration rate of BPA to foods [2,3]. Continuous and prevailing use of BPA-included products brought ubiquitous spreading influences on natural resources of water, sediment/soil, and atmosphere [4,5].

Bioremediation by using microbial organisms is a low cost possible solution to clean up phenol contamination problems. These techniques usually try to stimulate live microorganisms such as bacterium, fungus and virus to consume contaminants as a source of food [6-8]. Up until now, the main aim of BPA degradation studies have been focused on the oxidation reaction including photodegradation [9] and biodegradation. Bacterial organisms can be applied as catalysts for BPA degradation [10]. Using probiotics as a tool for bioremediation provides an option for cleaning up environmental pollutants. Probiotics are helpful live bacteria that can bring many beneficial health effects on their host. Administration of adequate amounts of probiotics maintains the natural balance of microflora in the intestines [11]. The popularity of organic foods and demand for probiotics has continuously been growing and various food products have been marketed. The majority of commercial probiotics are Lactobacillus

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Tel: +98-21-64112809 Fax: +98-21-44765290 E-mail: s_solouki@pasteur.ac.ir and bifidobacteria species used in products such as milk powder, yogurt and frozen desserts [12]. Probiotics have shown many health benefits such as antimicrobial activity, alleviating diarrhea, anti-carcinogenic properties, and ameliorating lactose intolerance and immune system [11,12]. However, those health benefits are strain-specific, and no single strain has all of the proposed health benefits [12,13].

It has been reported that some species like Bifidobacteria and lactic acid bacteria (LAB) have the ability to bind food carcinogens such as heterocyclic amines [14,15], aflatoxin and benzo[a]pyrene [16]. This evidence led to the expectation that probiotics would bind to BPA in the gastrointestinal tract and might be effective in protecting humans from the adverse effects of this compound by preventing its intestinal absorption.

Bacterial cell walls have three main binding mechanisms: (1) ionic exchange reaction with teichoic acid and peptidoglycan, (2) precipitation throughout nucleation reactions, and (3) complexation with nitrogen and oxygen ligands [17,18]. Gram-positive bacteria, especially *Bacillus* spp., has high adsorption capability because of high peptidoglycan and teichoic acid content in their cell walls [19]. In contrast, the membrane of gram-negative cells are lower in these components and is a poorer absorber [20]. As a result, within the human gastrointestinal tract, there are large colonies of bacterial cells with the potential to attach and sequester toxins that enter the body.

Detoxication is the medicinal or physiological ability to prevent entry of damaging compounds into the body [21]. Gut microbiota, and specially probiotic bacteria may have the largest role in binding and neutralizing toxins, preventing their entrance to the body [22]. Recently, the potential role of LAB single strains in detoxification of BPA has been examined [6,26]. However, almost no investigations are available on the binding properties of mixed probiotics toward BPA.

Based on the above evidences, the current study is designed to evaluate the effect of different mixture of some gram-positive probiotic strains including Lactobacillus (L.) casei, L. acidophilus, L. rhamnosus, L. bulgaricus, L. plantarum, L. fermentum, L. gasseri, Bifidobacterium (B.) breve, B. longum, B. infantis, Streptococcus (S.) thermophilus on degradation and detoxification of BPA. Six types of supplements, most of them contain a mixture of above probiotic strains plus Fructooligosaccharide (FOS) were exposed to BPA. The amount of BPA was measured during a 24-hour treatment and the obtained result was assessed by statistical paradigms. A metaanalysis was also done to compare the results of previous studies on the bio-removal of BPA both in vivo and in vitro with our work. However, the current study is the first one that exhibits the capability of multi-strains probiotic supplements to remove BPA from aqueous solution.

2. Materials and Methods

Characteristics of supplements. The commercial mixtures of probiotic strains were purchased from the Iranian company of Zist-Takhmir and labeled from p1 to p6: p1=Familact, p2=Gerilact, p3=Kidilact, p4=Kidilact zink, p5=Lactocare and p6=Lactofem. General explanation about the strains and ingredients of each 500mg capsule is presented in Table 1. The supplements contain relatively high amounts of beneficial bacteria and are designed for specific age groups.

BPA powder. BPA (GC grade > 99%) was purchased from Sigma-Aldrich. Its molecular weight and solubility in water are 228.29 g mol⁻¹ and 300 mg l⁻¹, respectively.

BPA Elisa kit. The Elisa kit was purchased from Detroit R&D, Inc., USA. This competitive Elisa test which was used in the current study is based on competition between the BPA-Horseradish peroxidase (HRP) conjugate and the BPA epitope for a limited number of anti-BPA antibody binding sites coated on the bottom of the wells of the Elisa plate. So, the amount of the BPA conjugate which is able to join to each well is inversely correlated with the concentration of BPA in the standard or sample. After the addition of sulfuric acid, the yellow colored product can be read on a plate reader at 450 nm [23].

Table 1. Characteristics of probiotic supplements

Supplement ³	Age group		Probiotic strains ¹									
	(indications)										s	
		casei	acidophilus	rhanmosus	bulgaricus	plantarum	fermentum	gasseri	breve	mngnol	infantis	thermophilu
		L d	L. d	Г	Г	Γ^{\prime}	L J	Γ	В.	В.	В.	S. 1
1. Familact	All the Family	7×10 ⁹	2×109	1.5×109	2×10^{8}	_	_	_	2×10^{10}	7×109	_	1.5×10^{10}
2. Gerilact	Seniors	3×109	3×1010	7×109	5×10^{8}	_	_	_	2×10^{10}	1×109	_	2×10^{8}
Kidilact	Children	3×1010	2×10^{10}	3×1010	2×109	_	_	_	2×10^{10}	_	5×10^{10}	2×109
 Kidilact zink² 	Children	3×10 ¹⁰	2×10^{10}	3×10 ¹⁰	2×109	_	_	_	2×10^{10}	_	5×10^{10}	2×109
Lactocare	Extra Immune	3×109	3×1010	7×109	5×10 ⁸	_	_	_	2×10^{10}	1×109	_	3×10 ⁸
6. Lactofem	Females	_	5×1010	_	_	1.5×10^{10}	7×109	2×10^{10}	_	_	_	_

1. The estimated number of viable bacteria in one dose of supplements is reported in colony-forming unit (CFU).

2. This symbiotic formulation has the elemental Zink sulfate 5mg for infants and children.

3. Other ingredients: FOS as prebiotic, Lactose, Mg stearate, Talc.

Experiment set-up and sample preparation. Normal saline (NS) 0.9% was selected as the basal medium. This solution is referred to as physiological serum because it closely mimics isotonic properties of biological environments. The medium temperature was set at 37°C. Then a certain amount of BPA (5×10^5 pg ml⁻¹) was solved in saline. This concentration was adapted from National Toxicology Program Expert Panel Report [24] regarding to the following considerations: Elisa standard curve limitations, LC₅₀ of BPA in aquatic bacterial mediums [25] and the average daily BPA intake in general adult population. Then saline containing BPA toxin was divided to six separate parts and one dose of each supplement was added to them. During a 24 h treatment with probiotics, samples were taken from the environments at different times of 0, 15 min, 30 min, 1 h, 6 h, and 24 h. After transferring samples to micro tubes, they were centrifuged at 1,000 ×g for 15 min (Sigma 3k30, Germany) to inhibit any damage to bacterial cells. Finally, the supernatant were filtered with 0.22 µm pore size and collected for analysis of residual BPA concentration by Elisa. A cell-free NS containing the same concentration of BPA was considered as the positive control. The percentage of BPA bound to the bacterial cell walls was calculated by Eq. 1:

$$BR = (1 - C/C_0) \times 100 \qquad \text{Eq. 1}$$

Where *BR* is the binding rate of BPA, *C* is the BPA concentration in the supernatant and C_0 is the concentration of BPA in positive control.

Drawing standard curve. The instructions of the kit manufacturer were considered to conduct the assay preparation step by step [23]. All samples were assayed in triplicate. Also, the plates had three blank wells (B_L), three maximum binding wells (B_0), and a six point standard curve (S_1 - S_6). The results were used to plot the %B/B₀ versus the concentration of BPA from the standards in a semi-log scale. Linear regression technique was used for curve fitting. Calibration curve for Elisa analysis of BPA was obtained with a range of 10-10⁶ pg ml⁻¹ and coefficient of determination (R^2) of 0.998.

Data analysis. All experiments were performed in triplicate and to ensure the normal distribution of variables, Histogram and Kolmogorov-Smirnov test were applied. The data were presented as means \pm standard deviation (SD). Repeated measures analysis of variance (using SPSS v.19) was done to evaluate significant difference between each bacteria-treated group and the untreated control group.



Figure 1 (a) Defective cycle of BPA accumulation in nature. (b) Structural similarity between estrogenic hormones and BPA.

3. Results and Discussion

3.1. Binding ability of different mixtures

BPA concentrations were calculated for all multi-strain probiotics in different times, utilizing the standard curve. The trend of alterations is shown in Figure 2. It is clear that multispecies probiotic supplements had a significant impact in reducing environmental BPA. The maximum effect belongs to Lactocare which has the highest number of bacteria and the minimum effect is related to Kidilact zinc. It seems that zinc sulfate -as an external interfering factor- prevents the binding process.

The range of concentration variations during 24 h treatment is depicted in Figure 3. Median which is determined with red line can be a good feature to specify the speed and efficiency of each group in reducing BPA concentration. The lower medians -p2, p5 and p6- had faster reaction time and succeeded to reduce about 80% of BPA during the first thirty minutes. There is another considerable point about p6; in contrast to the other supplements, p6 didn't include bifidobacteria species. It seems that the use of only lactobacillus species was sufficient and could provide an effectively high decreasing performance.

Until now, there are no previous studies about the detoxification of mixture of probiotic strains towards BPA. Therefore, comparisons of the results in this work are done with the efficiency of single strains binding capabilities. Firstly, in the present study, all mixtures tested were able to bind BPA, but in different rates and efficiencies. The existing differences in BPA binding capacity of mixture supplements were hidden behind the superposition of single strains. Therefore, tracing the partial effect of each strain will cue to retrieve what exactly happened. The ability of six strains of lactic acid bacteria (*L. casei, L. acidophilus, L. rhamnosus, L. bulgaricus, L. plantarum, S. thermophiles*) where previously studied by Zhu et al. [26] was used to refer here.



Figure 2. Mean \pm SD values of BPA concentration measured during 24 h



Figure 3. The range of BPA concentration variations during 24 h treatment with 6 different mixtures of probiotic strains. Repeated measures ANOVA was used to evaluate significant difference between each bacteria-treated group and untreated control group, $* p \le 0.05$, $** p \le 0.01$. N=7.

3.2. Effect of pretreatment on binding ability

The percentages of BPA removed by viable and nonviable cells presented via acid or heat are described in Table 2. The acidic treatment was performed by suspending the bacterial pellets in 2.0 M HCl for 90 min and the heat-killing treatment was conducted by subjecting bacterial cells to heating at 121°C for 20 min before exposing BPA to the mediums. As shown in Table 2, the six LAB viable strains could remove BPA in the range from 24.48% to 50.80% after 24h incubation. Acidictreated cells of all these six LAB could significantly increase BPA binding (35.77-66.33%) higher than viable cells. Similarly, heat-killed cells could also enhance the binding level (37.87-72.26%) when compared with nontreated viable bacteria. Meanwhile, *L. acidophilus* and *L. plantarum* presented higher binding capacity than those of other four strains. Both acid and heat treatments could significantly enhance the ability of LAB to remove BPA, which clearly indicated that bacterial viability was not a prerequisite for BPA binding. Previous studies about binding of aflatoxin B₁ (AFB₁) and zearalenone by LAB also suggested that treatment of bacterial pellets of LAB strains with hydrochloric acid and heat treatment by either autoclaving or boiling at 100°C in a water bath could significantly enhance the binding ability of the bacteria [27,29]. The binding rates of probiotic supplements are also added to Table 2. As already mentioned p2, p5 and p6 had maximum binding rates and could remove up to 90% of BPA after 24 h incubation.it is obvious that supplements with more doses of L. acidophilus and L. plantarum had better performances which correspond with the results of single strains.

Bacterial species	%Binding rate					
	Viable		Acid-treated		Heat-treated	
L. casei L. acidophilus L. rhamnosus L. bulgaricus L. plantarum S. thermophiles	$\begin{array}{c} 40.28 \pm 0.56^{bA} \\ 48.44 \pm 0.36^{eA} \\ 27.94 \pm 0.29^{cA} \\ 33.17 \pm 0.57^{aA} \\ 50.80 \pm 0.24^{fA} \\ 24.48 \pm 0.80^{dA} \end{array}$		$\begin{array}{c} 62.45 \pm 0.48^{bB} \\ 66.33 \pm 0.20^{dB} \\ 45.49 \pm 0.13^{aB} \\ 47.12 \pm 1.02^{aB} \\ 61.84 \pm 0.41^{bB} \\ 35.77 \pm 0.70^{cB} \end{array}$		$\begin{array}{c} 67.89 \pm 0.64^{\circ C} \\ 70.25 \pm 0.75^{a C} \\ 51.11 \pm 0.51^{d C} \\ 54.78 \pm 0.65^{b C} \\ 72.26 \pm 0.36^{a C} \\ 37.87 \pm 0.67^{e C} \end{array}$	
Mixture supplements	%Binding rate					
	Oh	0.25h	0.5h	1h	6h	24h
P1	1.98 ± 0.36^{aA}	3.021 ± 0.35^{aB}	6.07 ± 0.17^{aC}	79.85 ± 0.80^{aD}	79.62 ± 0.85^{aD}	86.06 ± 0.55^{aE}
P2 P3	0.14 ± 0.49^{aA} 7.78 ± 0.78^{bA}	$25.70 \pm 0.58^{\text{ob}}$ $2.61 \pm 0.55^{\text{aB}}$	$79.86 \pm 0.96^{\text{bC}}$ $22.57 \pm 0.53^{\text{cC}}$	$80.18 \pm 0.52^{\rm ac} \\ 79.85 \pm 0.80^{\rm aD}$	$79.91 \pm 0.42^{aC} \\ 79.73 \pm 0.05^{aD}$	$\begin{array}{l} 87.70 \pm 0.49^{\mathrm{aD}} \\ 85.67 \pm 0.44^{\mathrm{aE}} \end{array}$
P4	7.78 ± 0.80^{bA}	8.70 ± 0.21^{cA}	12.38 ± 0.79^{dB}	15.42 ± 0.48^{bB}	22.84 ± 0.14^{bC}	24.96 ± 0.10^{bC}
P5 P6	$\begin{array}{l} 0.18 \pm 0.41^{aA} \\ 7.78 \pm 0.26^{bA} \end{array}$	$\begin{array}{c} 16.81 \pm 0.74^{dB} \\ 34.06 \pm 0.26^{eB} \end{array}$	$\begin{array}{l} 79.62 \pm 0.81^{eC} \\ 79.86 \pm 0.96^{eC} \end{array}$	$\begin{array}{c} 80.05 \pm 0.84^{aC} \\ 82.88 \pm 0.36^{aD} \end{array}$	$\begin{array}{c} 79.83 \pm 0.26^{aC} \\ 84.69 \pm 0.07^{cD} \end{array}$	$\begin{array}{c} 92.00 \pm 0.82^{cD} \\ 88.11 \pm 0.47^{dE} \end{array}$

Table 2. Binding efficiency of both single and mixed supplements.

The table consists of two parts. (Top) The binding rate of BPA by six LAB strains after incubation adapted from [26]. (Bottom) The binding rate of mixture supplements during 24h incubation.

Values are expressed as mean±SD of samples analyzed in triplicate.

In the same column, means followed by different small letters (a-f) differ significantly (P≤0.05).

In the same row, means followed by different capital letters (A-E) differ significantly (P≤0.05).

p1=Familact, p2=Gerilact, p3=Kidilact, p4=Kidilact zink, p5=Lactocare and p6=Lactofem.

Our study was conducted by commercial spray-dried bacteria suspended in low acidic NS medium. Both spraydrying and acidic treatment may inactivate or kill considerable amounts of bacteria [27,28]. However, the enhanced ability of mixtures in removing BPA, confirmed that bacterial viability was not a prerequisite for BPA binding. Alteration in bacterial adsorption via acidic or heat treatment suggests that the binding of BPA to lactic acid bacteria is a physical phenomenon that may be associated with the binding sites and surface structures of cells wall. In addition, hydrophobic interactions may play important role in sequestering BPA and increase this possibility that LAB can bind not only BPA but also to other hydrophobic compounds [6].

3.3. Effect of peripheral conditions on binding ability

The bacterial binding ability may change under different conditions such as initial concentration, incubation temperature and time. The effect of initial bacterial concentration was tested by different concentration from 1×10^8 to 1×10^{10} cfu ml⁻¹. As illustrated in Figure 4.a, the binding capabilities of single strains for BPA relied strongly on the concentration of bacteria. The binding rates of these strains were significantly enhanced by increasing bacteria concentrations. As might be expected, higher amount of bacteria provided broader contact surface by increasing the number of binding sites. Among the six tested mixtures, the highest level of BPA adsorption (92%) was seen at p5 which had the highest bacterial concentration. Although, p1, p2 and p5 had been formulated from similar combination of probiotic strains (see material and methods), they had different doses of bacteria and their binding rates ranked by the level of bacterial dosage, with 92%, 87.7% and 86% respectively for p5, p2, and p1. These results are in agreement with the

previous reports about mycotoxin, which indicated that the zearalenone-binding capabilities of yeasts or bacillus strains decrease with the increased initial concentration of zearalenone [30]. By contrast, some studies have shown that the highest level of detoxifying capability of the tested strains was obtained at a high initial toxin concentration [31].

The effect of incubation temperature on binding ability is depicted in Figure 4.b. It seems that BPA removing was also a temperature-dependent procedure for all tested strains. Almost all strains showed higher binding capability around 37°C. The normal temperature of gastrointestinal tract is also around 37°C which provides an ideal condition for bacterial activation. It has been also reported that the maximum removal of zearalenone by *L. plantarum* occurred at 37°C [32]. Based on this evidence, we adopted 37°C as the optimal temperature for the removal of BPA from NS solution.

The effect of incubation time on BPA detoxification is given in Figure 4.c. All single lactic acid strains showed rapid binding on BPA. They could reach the maximum performance after 15 min of incubation time. Whereas, mixture of strains had incremented and phasic BPA reduction till the end of last incubation hour (Figure 2). According to Figure 3 and Table 2, p6 was the fastest supplement in reducing BPA. It had the largest amounts of L. acidophilus and L. plantarum in its ingredients. So, the type of strains used in mixtures formula, could notably affect the reaction time. Increasing the incubation time from 15 min to 24 h enhanced the binding rates of mixture products significantly but it had almost no effect on single strains. Therefore, it can be inferred that single strains were independent to incubation time while mixture products were strongly dependent on this parameter. The concentration of BPA in the blood of rats were orally

administrated with this toxin was reached to maximum level after 0.5h [6]. Therefore, the golden time for sequestering BPA in the gastrointestinal tract and avoiding its entrance to blood circulation is less than 30 min. In emergency conditions that higher binding velocity is needed, utilizing single strains, specially *L. acidophilus* or *L. plantarum*- will be more efficient than mixture products.

The binding of BPA to single strains showed repeatedly up and down variations during 24 h incubation (Figure 4.c). The results indicated that some BPA was bound weakly by the strains, and could be released back into the solution. These backward variations were compensated by combining single strains. The combinations could make persistent BPA reduction even after 24h (Figure 2). So, it can be expected that regular usage of mixture products will help to remove daily intake of BPA by creating more stable LAB-BPA complexes.

4. Conclusion

Regarding overall results, the present study demonstrated the efficacy of combination of probiotic strains on removing environmental BPA toxin. The current study is the first one that exhibits the capability of multistrains probiotic supplements to remove BPA from aqueous solution. We investigated the combination of a wide range of species includes L.casei, L.acidophilus, L.rhamnosus, L.bulgaricus, L.plantarum, L.fermentum, L.gasseri, B.breve, B.longum, B.infantis, S.thermophilus. Up to 80% of BPA was decreased during the first hour in almost all mediums. Influential parameters such as types of strains, incubation time and temperature and dose of each strain were considered and evaluated. Comparing the results with formulation and dose of supplements, we found that L.acidophilus and L.plantarum were the most effective in initially binding and also retaining BPA, suggesting that the complexes formed with these strains were the most successful. Enrichment of probiotic supplements with zinc sulfate had negative effect on the binding ability. It seems that such materials could inhibit the binding process. Acidic and heat treatment could strengthen the binding ability, which clearly indicated that bacterial viability was not prerequisite for BPA binding. The performance of single strains was strongly depended on initial bacteria concentration while the mixtures were more affected by incubation time. Additionally, single strains had faster and at the same time more unstable binding ability and could reach a high level after 15 min, but mixed strains showed an incremental and more robust binding process. Consequently, regular usage of probiotic supplementation with special mixture of strains can be suggested as an efficient way to suppress the harmful effects of BPA. Although a need for further clinical and in vivo trials remains, this research introduces an adjuvant therapeutic tool to improve biosafety level against BPA.



Figure 4. Effect of peripheral conditions on the removal of BPA by six LAB strains. (a) Effect of bacterial initial concentrations ranging from 10^8 to 10^{10} cfu ml⁻¹ (pH 7.0, 30°C, BPA 5 mg l⁻¹). (b) Effect of incubation at different temperature; All strains showed their maximum performance around 37°C (pH 7.0, 24 h, BPA 5 mg l⁻¹, bacteria 10^{10} cfu ml⁻¹). (c) General trend of BPA detoxification during 24 h treatment (pH 7.0, 30°C, BPA 5 mg l⁻¹, bacteria 10^{10} cfu ml⁻¹). The values represents the mean ± SD of duplicates (n=3).

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6. Conflict of Interest

The authors declare that there is no conflict of interests to be declared.

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کارایی مکمل های حاوی چند گونه پروبیوتیکی در حذف زیستی بیس فنول آ: مطالعه آزمایشگاهی

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چکیدہ

سابقه و هدف: بیس فنول آ یک ترکیب صنعتی شناخته شده است که به طور گستره ای در سنتز پلاستیک در سر تا سر جهان کاربرد دارد. ظروفی که با این پلاستیک ها ساخته می شوند مصرف کنندگان را در معرض دریافت بیس فنل آ از غذا و آب قرار می دهند و اثرات مضر بر سلامت افراد دارند. در این مطالعه اثر فرمولهای تجاری پروبیوتیک بر کاهش بیس فنل آ در محلول های آبی مورد بررسی قرار گرفت.

مواد و روشها: یک دوز از شش ترکیب تجاری پروبیوتیک به مقدار مشخصی از بیس فنل آ در یک محلول نمکی در دمای ۳۷ درجه سانتی گراد اضافه شد. در طول ۲۴ ساعت تیمار با پروبیوتیک ها، نمونههایی در زمان های مختلف از مخلوط تهیه شده و جهت بررسی با روش الایزا بکار رفت. در این بررسی فرمول های مختلف پروبیوتیک با یکدیگر مقایسه شدند و موثرترین آنها برای کاهش بیس فنل آ انتخاب شد. به علاوه، تاثیر فاکتورهای دیگر شامل pH و دما نیز مورد مطالعه قرار گرفت.

یافتهها و نتیجه گیری: مکمل های پروبیوتیک به طور موثری در کاهش بیس فنول آ در محلول های آبی موثر واقع شدند. تقریباً در تمام تیمارهای انجام شده بیس فنول آ در یک ساعت اول تیمار به میزان ۸۰ درصد کاهش یافت. در این میان اثر هم افزایی *لاکتوباسیلوس اسیدوفیلوس* و *لاکتوباسیلوس پلانتاروم* موثرتر از سایر عوامل بود. به عبارت دیگر، مخلوط پروبیوتیک ها اثر پایدارتر و قدرت اتصال قوی تری درمقایسه با گونه های منفرد داشتند. بدین ترتیب انتظار می رود استفاده مکرر از مکمل های پروبیوتیک با فرمولاسیون مشخصی از گونه ها می تواند اثرات مضر بیس فنول آ را کاهش دهد.

تعارض منافع: نویسندگان اعلام میکنند که هیچ تعارض منافعی وجود ندارد.

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- بيس فنول آ
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 - پروبيوتيک
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