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Binding and removal of polycyclic aromatic hydrocarbons in cold smoked sausage and beef using probiotic strains

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ABSTRACT

The aim of this study is to bio-monitor the levels of 16 polycyclic aromatic hydrocarbons (PAHs) in cold smoked beef and sausages. The ability of probiotics to remove PAHs was also investigated as function of the cell viability (viable, non-viable and acid-treated cells), bacterial counts (10^7, 10^8, and 10^9 CFU/mL), pH (3, 5, and 7), and incubation time (6, 12, and 24 h). The results indicated a significant difference ($p < 0.05$) among the analyzed sausages and beef samples for the PAHs concentration. Non-viable probiotics achieved the highest PAHs reduction rates. *Limosilactobacillus fermentum* EMCC 1346 presented the lowest binding activity value (*i.e.* 41.10–56.80 %) for all PAHs, followed by *Lacticaseibacillus rhamnosus* EMCC 1105 with binding percentage of 50.40–65.80 %. On the other hand, the highest removal for all PAHs was achieved by *Lactobacillus bulgaricus* EMCC 1102 with binding rate of 60.50–76.80 %, at 10^9 CFU/mL, pH 7, after incubation for 24 h. The fortified sausages results revealed that *L. bulgaricus* EMCC 1102 cultures exhibited the maximum and significant reduction ($p < 0.05$) of PAHs with values of 44.71 μg/kg for the center part, compared to control non treated sausages (82.65 μg/kg). Regarding the sensorial profile, treated samples with probiotics led to a preference from the panelists, compared to control. Consequently, the results confirm that fermented probiotic suspension is a feasible future strategy to control PAHs levels in cold smoked meat stuffs.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are defined as tertiary tar components formed by pyrolysis and biomass gasification (Stumpe-Vīksna, Bartkevičs, Kukāre, & Morozovs, 2008). The International Agency for Research on Cancer (IARC) has reported sixteen PAHs as possible human and animal carcinogenic agents (IARC, 2010). PAHs can cause toxic, mutagenic and/or carcinogenic effects in laboratory animals through various exposure forms, such as inhalation and dermal contact (IARC, 2010). Due to the higher lipid solubility of the PAHs, they are readily absorbed from the gastrointestinal tract of mammals, rapidly distributed in a wide range of tissues and accumulated in body fat. Thus, not only the laboratory mice, but all the mammals are highly susceptible to the PAHs (Abdel-Shafy, & Mansour, 2016).

In food industry, PAH compounds are developed during the processing stages, such as drying, grilling, roasting, and smoking, on the basis of the employed temperature and time (Singh, Varshney, & Agarwal, 2016). Other minor components of condensed smokes, such as water, tar, activated charcoal, ketones, aldehydes, and formaldehyde (Woods, 2003), can be formed, and some of them furnish organoleptic

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and antimicrobial properties to the smoked foods. Thus, this treatment allows to provide the final smoked product with the desired sensory attributes (Ledesma, Rendueles, & Díaz, 2015a). However, some other compounds present in the smoked products have non-desirable impacts on products, and among them, PAHs are the most significant, especially in meats. The harmful PAHs released in smoked particles can contaminate the smoked food products (Rose et al., 2015). Moreover, the oral exposure to PAHs can take place by consuming specific foods, such as smoked meat products (US EPA, 2017). Thus, taking into account the PAHs adverse effects on hematological and immune systems, their levels in food matrices should be as low as possible (Hokkanen et al., 2018).

Recently, microbial-assisted degradation (bioremediation), bio-adsorption, and microbial binding ability have been reported as efficient methods for the detoxification or removal of chemical and organic pollutants, such as PAHs (Cruz et al., 2021). Bioremediation has emerged as a green and promising technology for restoring PAHs-contaminated sites. In comparison to physicochemical methods and other remediation technologies, bioremediation techniques involve minor energy expenditure, relatively lower cost, and an improved probability of ecologically safe consequences (Mandree et al., 2021; Imam, Suman, Kanaujia, & Ray, 2022; Bisht et al., 2015). Over the years, there is a growing interest to isolate and characterize xenobiotic microbes which can utilize chemical contaminants, including PAHs. The potential of these biological systems has been thoroughly studied to maximize the PAHs degradation, involving microorganisms able to convert specific pollutants into inactive substances, as a result of the biochemical and physical processes associated to their microbial activity (Imam et al., 2022; Patel, Trivedi, Bhatt, Nath, & Butani, 2021; Rathore, Varshney, Mohan, & Dahiya, 2022). In addition, several studies have been conducted to investigate the possible elimination of carcinogenic contaminants in food *via* stable physical binding by probiotic e.g., lactic acid bacteria (LAB) and Bifidobacteria species (Zoghi et al., 2021; Cuevas-González et al., 2022).The probiotics have the ability to eliminate PAHs through many mechanisms such as co-metabolic transformations, whole mineralization, and non-specific oxidation (Rodríguez-Morgado et al., 2015; Bartkiene et al., 2017; Lawal, 2017).

On the basis of above considerations and the binding ability of toxic compounds in meat stuffs, *i.e.* PAHs, to the bacterial cell wall as one of the most effective bio-detoxification mechanisms by probiotics. Based on this context, this study aims to: **I)** bio-monitor the PAHs levels in cold smoked sausages and beef samples and quantify the uppermost levels of total PAHs in these products, **II)** assess the binding ability of selected probiotic bacteria on removing sixteen PAHs from contaminated phosphate buffer saline (PBS), **III)** evaluate the influence of external and internal treatments of cold smoked sausages fortified with probiotic bacteria (previously propagated in an alternate substratum) on the PAHs production and removal before and after the smoking.

2. Materials and methods

2.1. Chemicals and media

A mixture of 16 PAH compounds standards, containing naphthalene (Nap), acenaphthene (Ace), acenaphthylene (Acy), anthracene (Ant), benzo (a) pyrene (BaP), benzo (a) anthracene (BaA), benzo (g, h, i) perylene (BgP), benzo (b) fluoranthene (BbF), chrysene (Chr), dibenz (a, h) anthracene (DhA), fluoranthene (Flu), fluorene (Fl), indeno (1,2,3,-cd) pyrene (IcP), phenanthrene (Phe), pyrene (Pyr) and benzo (k) fluoranthene (BkF), was obtained from Supelco company (Supleco Park, Bellefonte, PA, US). De Man-Rogosa-Sharpe (MRS) media, and chemicals were purchased from Merck, Germany. Deionized water was obtained from a Milli Q water purification system (Siemems, Ultra Clear UV UF TM, Germany).

2.2. Probiotic strains and growth conditions

Strains of *Bifidobacterium bifidium* EMCC 1334, *Lactobacillus bulgaricus* EMCC 1102, *Lacticaseibacillus rhamnosus* EMCC 1105, *Limosilactobacillus fermentum* EMCC 1346 were obtained from Cairo Microbiological Research Center, Cairo MIRCEN, Faculty of Agriculture, Ain-Shams University, Egypt. The strains were stored at −18 °C until their use. For the activation step, all strains were cultured in MRS broth for 24 h at 37 °C, except in the case of *B. bifidium* EMCC 1334, which needed a MRS broth supplemented with 0.05 % of L-cysteine. Afterwards, 1 mL of 24-hours-old culture was mixed with 100 mL MRS broth and then incubated for 20–24 h at 37 °C. Microbial concentration of 2×10^9 CFU per mL was determined by measuring the optical density at 600 nm with a spectrophotometer (T80 + uv/vis spectrophotometer - PG Instruments ltd, UK) (Zhu, Yang, Luo, Zhou, & Liu, 2017). The cells were harvested from MRS media through the centrifugation at 5000*g* for 15 min at 4 °C (Microcentrifuge, J.P SELECTA, Spain). Subsequently, the cells were washed twice with phosphate buffer saline (PBS;0.1 mol /L, pH 7.2, 0.85 % NaCl), and then suspended in a phosphate buffer.

2.3. Samples' collection

Fifty cold smoked sausages and beef samples (250 g for every kind) were purchased from the local supermarkets and restaurants in Alexandria governorate and Borg El-Arab city, Egypt, and kept at −20 °C for further PAHs detection.

2.4. Extraction and clean up

PAHs concentrations in the cold smoked meat samples were detected by using the clean-up method according to Chung et al. (2011) with slight modifications. Firstly, 30 g from each sample were blended and saponified with 2 M KOH solution in 100 mL of 90 % methanol, followed by adding 2 g of sodium sulfate. Then, the sample was refluxed in a water bath at 70 °C for 2 h, and 100 mL of *n*-hexane were added. After 15 min, the blend was cooled and kept in the dark overnight. The samples were concentrated with 60 mL *n*-hexane and the organic phase (upper phase) was extracted. The extraction was repeated twice with 30 mL *n*-hexane, and the *n*-hexane layer was dried under a nitrogen stream in a vacuum evaporator (Zymark Corporation, Turbovap LV, Massachusetts, US), and then filtered. The *n*-hexane layer was concentrated at 35 °C by a rotavapor. The concentrated layer was then purified by column chromatography using silica gel (60–230 mesh, Merck Co., Germany) and organic solvents (*n*-hexane). The silica gel slurry was made by adding 40 mL of *n*-hexane to 20 g of silica gel and pouring the mixture into the column. The sample mixture was added to the column after the silica gel settlement. PAHs elution was done by passing 50 mL of *n*-hexane followed by 8 mL of *n*-hexane-dichloromethane (3:1 v/v). After that, the solvent was concentrated to 1 mL through a rotavapor (underwater vacuum aspirator with a rotation speed of 100 rpm and pressure of 200 mmHg) in a water bath held at 35 °C, and then filtered through a 0.45 μm microporous syringe in vials. The vials were kept at −20 °C for the gas chromatography–mass spectrometry (GC–MS) analysis.

2.5. Quantification analysis of PAHs in samples by GC–MS

The stock solution of PAHs was prepared according to Siddique et al. (2020), by adding 200 μg of each standard in acetonitrile up to 1 mL. Twenty micrograms per millilitre (20 ug/mL) of working standard solution was prepared from 1 mL of each standard solution and diluted up to 10 mL by acetonitrile. The standard solutions of PAHs were then used for GC–MS analysis.

The detection limits for Nap, Acy, Ace, Fl, Phe, Ant, Flu, Pyr, BaA, Chr, BbF, BkF, BaP, IcP, DhA and BgP were 0.90, 0.45, 0.58, 0.50, 0.29, 0.35, 0.27, 0.35, 3.85, 2.24, 4.57, 4.69, 3.33, 5.74, 5.05, and 5.60, μg/

mL, respectively, while the quantification limits were 2.70, 1.35, 1.74, 1.50, 0.87, 1.05, 0.81, 1.05, 11.55, 6.72, 13.71, 14.07, 9.99, 17.22, 15.15, and 16.80 μg/mL, respectively.

The method of Yousefi et al. (2019) was followed in order to quantify the PAHs in the samples through Agilent Technologies 7890A GC (ltd Corporation, Mundelein, Illinois, US), with a triple-axis detector (HP-5 MS capillary column, 30 m × 250 μm ID, 0.25 μm film thickness) attached to a 5975C inert MSD network mass selective detector. Helium gas acted as a carrier, at a constant flow rate of 1 mL for 1 min. 10 μL from the sample were injected into a split mode for analysis. The column's initial temperature was 220 °C for 5 min, then increased to 290 °C. The compound concentrations were determined through selected ion monitoring mode, in which one qualifier ion was selected, and the PAHs percentage was calculated from the following equation:

$$\text{PAHs ratio } (\%) = \left[1 - \frac{\text{Peak area of each PAHs in sample}}{\text{Peak area of each PAHs in positive control}}\right] \times 100$$

2.6. Removal and binding of polycyclic aromatic hydrocarbons by probiotic bacteria

To test the toxin binding, the procedure of Yousefi et al. (2019) was followed. The first mixture of 16 PAHs was dissolved in acetonitrile (200 μg/mL), and an aliquot of this mixture was then diluted in PBS to get a working solution with concentration of 20 μg/mL of each PAH. Finally this solution was employed to test the toxin binding ability of the strains. The ability of probiotics (viable, non-viable, and acid treated) to bind PAHs was evaluated as follows: 1 mL from MRS with defined bacteria concentration (1×10^9 CFU/mL) was centrifuged at 5000*g* for 15 min at 4 °C. Then, the obtained bacterial pellets were washed twice with PBS and suspended into 1 mL phosphate saline buffer which contained 20 μg/mL of each PAH. The bacteria and 16 PAHs containing suspensions were carefully shaken for 12 h at 37 °C and pH 7, in a shaking incubator (Thermo Scientific SHKE4450CC MaxQ 4450 Benchtop Incubator Shaker, South San Francisco, California, US). Moreover, the binding affinity of PAHs to probiotic bacteria was tested at various times (6, 12, and 24 h), pH values (3, 5, and 7) and cell density (nearly 1×10^7, 1×10^8, and 1×10^9 CFU/mL). The pH of solutions containing PAHs was set by using 1 M HCl. For each test, a positive control (PBS + PAHs) and a negative control (PBS + bacteria) for viable and non-viable probiotic bacteria were used. After the incubation period, they were separated using centrifugation at 5000*g* for 15 min at 4 °C (ThermoFisher Scientific Co., Egypt); then the cell-free supernatant was examined using the GC–MS system to determine the bounded PAHs content.

2.7. The cell viability effect on the PAHs binding ability

Numerous experiments were applied to bacterial cells to assess the capability of the viable and nonviable cells to bind with PAHs. Bacterial cells were collected by centrifuging and washing, and then suspended in 1 mL PBS containing 20 μg /mL of PAHs and divided into three groups; (a) untreated cells, (b) autoclaved at 121 °C for 15 min, (c) exposed to acid (2 M HCl) solution for 1 h. These groups of the treated and untreated cells were incubated at 37 °C for 12 h under lenient shaking, to estimate the toxin-binding ability of the treated cells. Lastly, the cells were removed by centrifugation at 5000*g* for 15 min at 4 °C (ThermoFisher Scientific Co., Egypt) and the supernatants were analyzed to quantify the free PAHs through GC system. To extract PAHs from the PBS, 1 mL of cyclohexane was added to 1 mL of cell-free supernatants and carefully mixed for 1 min. Then, the blend was centrifuged at 10.000*g* for 10 min and 1 mL of the upper phase was injected into GC/MS system for PAHs quantitative analysis (Yousefi et al., 2019).

2.8. Production of fortified cold smoked sausages with probiotic bacteria

The production of cold smoked sausages was performed according to Bartkiene et al. (2017). Potato juice was used for the LAB propagation as a substitute substrate. Potatoes were bought from the local supermarket in Borg El-Arab, Egypt, to make the potato juice. Potato pieces were filtered through nylon meshes with pore size of 15 mm, then sterilized at 121 °C for 15 min. Afterwards, the LAB cells suspension (2 % (w/w)) was inoculated in the sterilized juice media and fermented at 37 °C. The LAB multiplied in the potato juice was used for the surface treatment of the prepared sausages, before and after the smoking process.

For the cold smoked sausages preparation, 76.9 % beef, 19.4 % frozen back fat (beef fat), 2.4 % salt, 0.4 % spices, and 0.4 % glucose were utilized. The fat was thoroughly ground with meat, then the batch was mixed for 2 min. The blend was stuffed into a casing (40 mm in diameter, and 240 mm in length). The smoked sausage samples were processed in cold-smoking conditions on a mixture of beech and oak wood chips. The prepared sausages were treated with probiotics in two steps, before **(I)** and after the smoking process **(II)**. **I)** 400 g of sausages were individually placed in a container with 1000 mL of fermented potato juice and 1×10^9 CFU/mL of LAB, and then immersed for 60 min at 18 °C. After immersion, the sausage was drained and covered with plastic film. The samples were stored at 20 °C for 24 h, and ripened at 24 °C for 78 h under 86–93 % humidity. The sausages were smoked at 16 °C for 130 min under 80–82 % humidity. **II)** After smoking, sausages were dried for 8 days at 15 °C under 75 % humidity. After smoking and drying, 400 g of each sausage were placed in a bottle with one liter of the fermented potato juice containing 1×10^9 CFU/mL of probiotics. The sausages were covered with plastic film and stored at room temperature for one day. The control was prepared without LAB. To assess the transition of PAHs into the liquid phase, the control samples before and after smoking process were treated with water.

2.9. Sensory evaluation of the fortified cold smoked sausages

The sensory quality characteristics of the fortified cold smoked sausages with probiotics was carried out according to Varlet et al. (2007) with slight modifications. The organoleptic features were estimated by 15 panelists who were staff members of Department of Food Technology, SRTA City, Egypt. The sausage samples were randomly assigned to each panelist. The panelists were asked to assess the organoleptic descriptors intensity for each sample, in terms of taste, odor, color, texture, and overall acceptability, through nine-point scale: dislike extremely (1), dislike very much (2), dislike moderately (3), dislike slightly (4), neither like nor dislike (5), like slightly (6), like moderately (7), like very much (8) and like extremely (9).

2.10. Statistical analysis

Data were statistically analyzed by General Linear Model's procedures in SAS GLM (SAS, 2004) using independent-samples *t*-test and ANOVA analysis. Duncan's multiple range test was used for multiple comparison between means at $p < 0.05$. Kolmogorov-Smirnov's test was employed to test the normal data distribution.

3. Results and discussion

3.1. Biomonitoring of PAHs in cold smoked sausage and beef samples

In Table 1.S, the data related to the calibration curves acquired for all the studied PAHs are summarized. In Fig. 1 the concentrations of 16 PAHs (μg/kg) in the examined cold smoked sausage and beef samples are displayed. It is evident that the total average levels of PAHs were higher in beef samples (81.30 μg/kg) than in sausage samples (77.56 μg/kg), although no significant difference ($p > 0.05$) was noticed. Concerning the concentrations of Nap, Acy, FI, Flu, Pyr, BaP, and BgP,

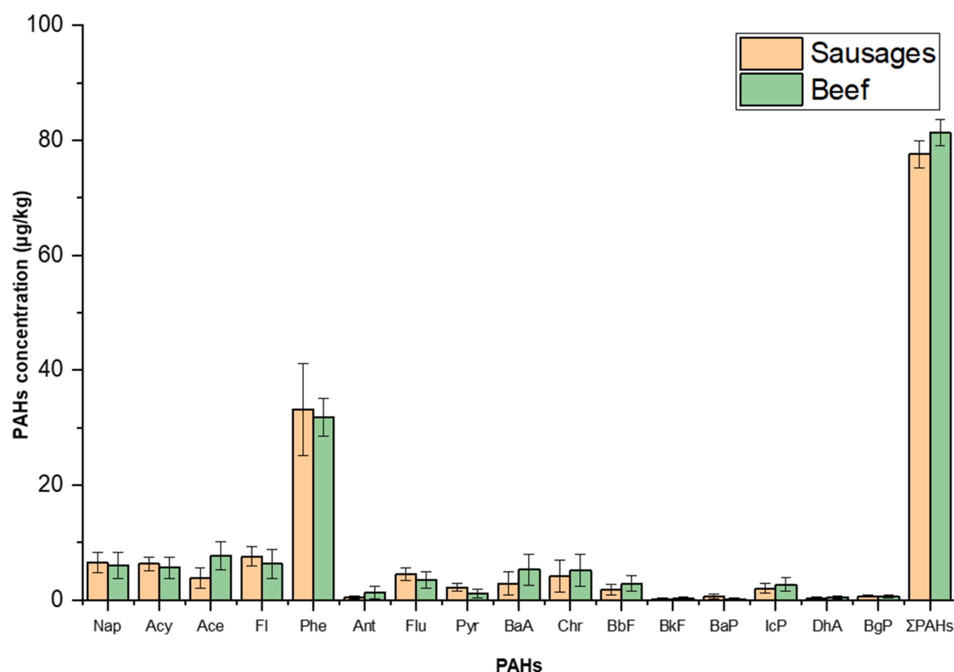

Fig. 1. Occurrence and concentration (µg/kg) of polycyclic aromatic hydrocarbons (PAHs) in examined cold smoked sausages and beef samples.

greater bioaccumulation profiles were revealed in sausages than in beef samples. On the contrary, the Ace, Ant, BaA, Chr, BbF, BkF, IcP, and DhA contents were greater in beef than in sausages. The General Linear Model's showed that PAHs accumulation was significantly different among the analyzed sausage and beef samples for Ace, FI, Ant, Flu, Pyr, BaA, BbF, BaP, IcP, and DhA. No significant differences between sausages and beef were observed for Nap, Acy, Phe, Chr, BkF, and BgP.

Phe content presented the highest level among all tested PAHs in sausage and beef samples with values of 33.18 and 31.80 µg/kg, respectively, taking into account that all smoking processes resulted in elevated concentrations of low molecular weight PAHs like Phe, Nap, Ace, Acy, Ant, and Fl (Varlet, Serot, Monteau, Bizec, Le, & Prost, 2007). Phe was noticed to be the most abundant PAH in both smoked fish and meat products, and ranged in the quantities of 32.2–63.9 µg/kg in smoked common carp (*Cyprinus Carpio*). Futhermore, Phe was reported to be the predominant PAH not only in smoked fish but also in smoked meat products (Babić et al., 2020). This finding is aligned with the Phe contents reported by Lorenzo, Purriños, Fontán, & Franco (2010) in smoked sausages (Chorizo Gallego: 49.78 µg/kg, and Chorizo de cebolla: 53.54 µg/kg). On the contrary, our Phe value is slightly higher than their ratio in the detected smoked salmon, with concentrations of 26.66 µg/kg (Zelinkova, & Wenzl, 2015).

For BaP, values of 0.59 µg/kg in sausages and 0.23 µg/kg in beef were detected. These values are below the value of 2.0 µg/kg accepted by "EC Regulation number 835 of 2011" for smoked meats, being BaP a carcinogen. This experimental finding is in agreement with those obtained in commercial samples in Korea (Chung et al., 2011), smoked meats in Spain (Ledesma, Rendueles, & Díaz, 2015b), smoked meats in Italy (Purcaro, Moret, & Conte, 2009) and conventional smoked products in Portugal (Santos, Gomes, & Roseiro, 2011). In general, the PAHs levels measured in both beef and sausage samples were remarkably lower than those detected by Lorenzo et al. (2011) for Spanish smoked sausages (average value of 101.81 µg/kg). Therefore, this occurred variance may be due to the differences in the origin of meat, and/or feeding practices for the beef cattle.

Furthermore, the European Food Safety Authority (EFSA) proposed that BaP is individualy unsuitable as marker for the occurrence of PAHs in food-stuff. Subsquently, extra three PAHs compounds should be involved, in addition to BaP, namely BaA and Chr, as established by the European Commission Regulation (EC Regulation, 2011), No. 835/2011, to distinguish the toxicity in the meat products in an appropriate way. In addition, the maximum PAH limits for smoked meat are 12.0 µg/kg for the four mentioned PAHs (in total) and 2.0 µg/kg for BaP individually (EFSA, 2008).

3.2. The effect of viable, non-viable and acid treated probiotics on PAHs binding ability and removal

Probiotic binding affinity of viable, non-viable and acid-treated cells towards PAHs is presented in Table 1. All probiotic strains were able to remove PAHs from PBS at various percentages compared with control. It is interesting to evidence that non-viable bacterial cells achieved the maximum binding percentage followed by acid-treated cells, while the viable cells presented the lowest binding affinity. Viable, non-viable, and acid-treated cells of *L. bulgaricus* EMCC 1102 showed the greatest binding affinity for all PAHs (range from 60.50 to 76.80 %) when compared with other bacterial cells, and presented greater binding affinity for IcP > Pyr > Bgp > Ant > Acy > FI than for other PAHs. On the other hand, *L. fermentum* EMCC 1346 was characterised by the minimum binding activity in the range from 41.10 to 56.80 %. Thus, all the collected data indicated high specificity for the stated strains, and showed a probiotic kind-dependent binding ability towards PAHs. In this regard, Zhao et al. (2013) investigated the binding ability of 15 *Lactobacillus* strains to BaP: *L. plantarum* CICC 22135 and *L. pentosus* CICC 23163 exhibited high efficiency in eliminating BaP, and the binding rates were 66.76 % and 64.31 %, respectively. The binding process was affected by several factors *i.e.* incubation time, temperature and pH; however, the cell viability was not essential for the binding ability. The mechanism of the binding ability was a physisorption, and peptidoglycan was the main binding site.

Moreover, the results of the probiotic bacteria binding ability towards PAHs indicated that all the examined bacteria could partially eliminate PAHs from PBS. Moreover, the binding ability depends on the pH, bacterial strain (viability and species), incubation time, availability of nutrients, and type and chemical structure of the targeted PAHs. Numerous investigations assessed the ability of bacteria, such as *Pseudomonas* spp., and *Mycobacterium* spp., in degrading PAHs (Anupama, Rupa, Sunita, Kamlesh, & Pankaj, 2013). However, there are limited

Table 1
Effect of viable, non-viable and acid treated probiotics on PAHs binding ability (%) and removal from PBS buffer at pH: 7, 37 °C for 12 h. PAH concentration: 20 μg/mL.

Sample type	Probiotic bacteria 1×10^9 CFU/mL	PAHs conc. μg/mL	PAHs binding ratio (%)															
			Nap	Acy	Ace	Fl	Phe	Ant	Flu	Pyr	BaA	CHR	BbF	BkF	Bap	IcP	DhA	Bgp
−Ve (PBS + bacteria)	**1×10^9 CFU/mL**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
+ Ve control (PBS + PAHs)	**0.00**	20.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
***B. bifidium* PBS + PAHs**	**Viable**	20.00	52.40 ±0.49	43.60 ±0.44	41.70 ±0.40	44.20 ±1.17	42.70 ±0.76	45.70 ±0.61	47.20 ±0.45	49.60 ±0.39	51.60 ±0.37	46.70 ±0.35	47.60 ±0.38	46.50 ±0.63	55.60 ±2.23	48.10 ±0.56	42.50 ±0.69	45.10 ±0.51
	Non-viable	20.00	56.20 ±0.39	49.80 ±0.41	51.60 ±0.26	53.10 ±0.47	49.80 ±0.36	51.70 ±0.25	54.10 ±0.45	56.10 ±0.38	57.70 ±0.52	55.60 ±2.21	53.20 ±0.43	52.80 ±0.46	58.50 ±0.33	54.60 ±0.46	52.80 ±0.51	53.60 ±0.49
	Acid treated	20.00	53.90 ±0.48	46.50 ±1.13	46.20 ±0.51	49.10 ±0.32	46.50 ±0.49	48.60 ±0.48	50.10 ±0.82	53.20 ±0.43	54.40 ±0.46	53.10 ±0.50	48.70 ±0.46	47.10 ±0.31	57.20 ±0.53	51.20 ±0.36	47.40 ±0.36	48.10 ±0.51
***L. fermentum* + PBS + PAHs**	**Viable**	20.00	45.60 ±0.50	41.10 ±0.41	46.30 ±0.45	43.50 ±0.41	47.20 ±0.36	44.70 ±0.22	48.30 ±0.53	45.10 ±0.38	49.10 ±0.40	45.60 ±0.50	46.10 ±0.54	48.20 ±0.51	45.40 ±1.13	49.30 ±0.36	47.60 ±0.30	47.60 ±0.31
	Non-viable	20.00	48.80 ±0.57	49.80 ±1.18	52.10 ±0.46	54.50 ±0.40	50.60 ±0.24	56.80 ±0.40	53.70 ±0.46	49.90 ±0.31	53.20 ±0.48	49.80 ±0.37	50.10 ±0.79	54.20 ±0.42	52.30 ±0.49	56.80 ±0.52	51.40 ±0.35	53.60 ±0.40
	Acid treated	20.00	47.10 ±0.29	49.20 ±0.37	47.50 ±0.30	48.10 ±0.46	50.60 ±0.81	49.10 ±0.46	51.20 ±0.31	48.30 ±0.19	49.10 ±0.40	47.20 ±0.33	49.10 ±0.42	51.20 ±0.37	48.90 ±1.00	51.60 ±0.24	47.50 ±0.25	50.60 ±0.81
***L. bulgaricus* + PBS + PAHs**	**Viable**	20.00	62.60 ±0.40	64.500 ±0.24	62.40 ±0.56	63.70 ±0.49	65.80 ±0.54	62.50 ±0.57	64.20 ±0.86	66.30 ±0.21	60.50 ±0.37	62.60 ±1.00	65.40 ±0.40	63.20 ±0.51	62.80 ±0.39	66.70 ±0.88	64.50 ±0.79	66.10 ±0.54
	Non-viable	20.00	72.90 ±0.38	75.10 ±1.50	72.90 ±0.36	74.50 ±0.27	72.10 ±0.30	76.80 ±0.32	73.20 ±0.21	75.60 ±0.26	73.20 ±0.28	72.90 ±0.36	75.60 ±0.39	74.50 ±0.41	72.60 ±0.36	76.40 ±0.28	73.30 ±0.23	75.60 ±0.53
	Acid treated	20.00	65.40 ±0.14	67.10 ±0.34	65.20 ±1.09	68.30 ±0.57	66.40 ±0.54	69.70 ±0.88	68.30 ±0.71	68.40 ±0.89	63.40 ±0.62	65.40 ±0.55	67.10 ±0.52	65.20 ±0.37	68.50 ±0.44	69.20 ±0.50	66.70 ±0.52	68.30 ±0.81
***L. rhamnosus* + PBS + PAHs**	**Viable**	20.00	57.20 ±0.69	58.20 ±0.61	60.30 ±0.55	59.60 ±0.50	61.50 ±0.27	58.40 ±0.34	57.60 ±1.01	60.10 ±0.58	50.40 ±0.35	57.20 ±0.93	60.80 ±0.51	58.70 ±0.25	59.60 ±0.51	61.30 ±0.37	57.20 ±0.89	60.70 ±0.43
	Non-viable	20.00	60.10 ±0.50	65.80 ±0.44	64.60 ±0.56	61.30 ±0.58	63.50 ±0.40	60.50 ±0.44	62.70 ±0.43	65.10 ±0.51	60.60 ±0.41	60.10 ±0.56	63.10 ±0.49	61.40 ±0.59	64.50 ±0.68	65.60 ±0.50	62.30 ±0.47	64.50 ±0.48
	Acid treated	20.00	58.40 ±1.00	60.40 ±0.34	61.20 ±0.58	60.50 ±0.54	62.70 ±0.38	59.60 ±0.51	61.20 ±0.34	63.30 ±0.52	59.30 ±0.89	58.40 ±0.35	61.50 ±0.62	59.20 ±0.96	60.70 ±0.51	63.40 ±0.62	61.20 ±0.24	60.80 ±0.50

Values denoted as mean ± standard deviation.

studies about the PAHs removal by probiotics. The binding ability of probiotic cells depends on the cell wall structures such as plasma membrane, peptidoglycan, teichoic acids, proteins, and carbohydrates (Sangsila, Faucet-Marquis, Pfohl-Leszkowicz, & Itsaranuwat, 2016). Consequently, it appears that the PAH adsorption rate depends on the PAH type and cell wall composition, along with the hydrophobic interactions which play a crucial role in the adsorption of many food-induced toxicants to microbial cells (Kosztik, Mörtl, Székács, Kukolya, & Bata-Vidács, 2020). The cell wall hydrophobicity is related to the surface proteins and teichoic acids (Wang et al., 2015), which have variable structure even among closely-related probiotic strains (Weidenmaier and Peschel, 2008). Therefore, this study suggests that the variations in the binding ability of the examined probiotics can be related to the structural variations. Exterkate, Otten, Wassenberg, & Veerkamp, (1971) ascribed the variations in the examined probiotics (*i. e. Bifidobacterium* spp. and *Lactobacillus* spp.) binding ability towards PAHs to the differences in their cell wall composition. Indeed, they confirmed that the phospholipid structure of *Bifidobacterium* spp. varies from *Lactobacillus* spp., especially in the polyglycerol phospholipids and phosphatidylglycerol. Moreover, *Bifidobacterium lactis* BI-04, HN019, and *Bifidobacterium infantis* BY12 showed the highest binding rate towards BaP. *Bifidobacterium* is the most important LAB found in the human intestine and owns many important nutritional and therapeutic benefits. *Bifidobacterium* is Generally Recognized as Safe (GRAS), nonpathogenic and not toxic; thus, it can be used as probiotic and is feasible to be employed in detoxification methods (Shoukat, Aslam, Rehman, & Zhang, 2019). Another study by Yousefi et al. (2019) confirmed that *Bifidobacterium lactis* has lower binding activity for all PAHs than *Lactobacillus acidophilus*.

The current findings revealed that the cell viability was not important for the binding ability, and that non-viable and acid-treated bacteria exhibited extra binding activity in eliminating PAHs, in agreement with Zhao et al. (2013). Additionally, Niderkorn et al. (2009) confirmed that the microorganisms physical treatments, such as heat treatments, freezing, and thawing, caused an extra increase in the reduction levels of fumonisin. Consequently, physical treatments can modify the cell wall composition and offer additional binding sites which increase the toxin adsorption ability of the bacterial cells (Zhao et al., 2013). In particular, the physical heating can maximize the binding ability, since this treatment may increase the denatured proteins level and promote the formation of Maillard reaction compounds that elevate the hydrophobic characters of the cell wall. Moreover, the heating can modify the cell wall thickness and increase the pore size (El-Nezami, Hani, Polychronaki, Salminen, & Mykkänen, 2002). In this study, the moderate binding activity of the acid-treated cells may result from the interaction of polysaccharides and teichoic acids with the used acids such as HCl and acetic acid (Niderkorn et al., 2009). The bacterial binding ability in removing PAHs can be affected by the PAHs molecular size of more than three rings (Kanaly and Harayama, 2000). In our study, the maximum binding ability of probiotics was reported for the high molecular-weight PAHs, such as IcP, Pyr, Bgp, Bap, Bkf, Flu, and BaA. Similarly, Yousefi et al. (2019) showed that *Bifidobacterium lactis* and *Lactobacillus acidophilus* removed high molecular-weight PAHs in PBS as follows: BaP > Chr > BaA > BaF. This result contrasts with those of Yu et al. (2014) who reported that *Pseudomonas*, *Bacillus*, and *Rhodococcus* strains had the highest removal ability for low molecular-weight PAHs in the Nap > Fl > Phe order.

3.3. Effect of non-viable probiotics concentrations (CFU/mL) on PAHs binding ability (%) and removal

The results in Table 2 show the binding ability of the non-viable probiotics towards PAHs at different concentrations (CFU/mL). All tested bacterial strains eliminated PAHs from PBS in a concentration-dependent manner when compared to the control. For all tested strains, by increasing the bacterial concentration, a marked reduction of PAHs was achieved. The maximum PAH binding ability was reached by *L. bulgaricus* EMCC 1102 cells (70.6–80.6 %) at 1×10^9 CFU/mL, while other strains displayed a wide-ranging binding ability with PAHs at different concentration. This indicated that the maximum and minimum binding ability was influenced by the PAH-specificity. Regarding the non-viable bacterial concentration effect on the removal of PAHs, higher bacterial concentration can lead to higher PAHs removal from PBS. In our study, a bacterial concentration of 10^9 CFU/mL succeeded to remove most PAHs, in agreement with Yousefi et al. (2019) who achieved the maximum binding activity of *L. bulgaricus* EMCC 1102 in removing PAHs (from 58.16 to 72.28 %) at concentration of 10^9 CFU/mL, with respect to those at 10^7 and 10^8 CFU/mL. Similarly, the previous investigation by El-Nezami, Kankaanpaa, Salminen, and Ahokas (1998) reported that 10^9 CFU/mL of bacteria were needed to get considerable elimination of aflatoxin. Furthermore, the elimination of aflatoxin M1 was significantly maximized by *Lactobacillus helveticus* at a concentration of 10^{10} CFU/mL (Ismail et al., 2017). It suggests that high microbial concentrations facilitate the formation of new binding locations and, hence, additional PAHs are attached to the probiotic.

3.4. Effect of non-viable probiotics pH values on PAHs binding ratio (%) and removal

The binding ratio values of non-viable probiotics towards PAHs (%) as function of different pH *i.e.* 3, 5 and 7 are compared in Table 3, evidencing that all the examined probiotics could partially eliminate PAHs from PBS depending on pH. Indeed, our study showed that PAH binding is pH dependent and there may be a distinct pH range able to promote the ideal contact to binding locations in the peptidoglycan. The lowest binding rates were noticed at pH 3, whereas by pH rising from 5 to 7 the binding percentages increased for all studied strains. The maximum PAHs binding values were achieved by *L. bulgaricus* EMCC 1102 and exceeded above 70 % at all pH values compared with other bacterial strains. Furthermore, BaA was successfully eliminated by all tested bacteria. In this context, the binding ratios of BaA were 85.20, 70.60, 64.10, and 62.10 % for *L. bulgaricus* EMCC 1102, *L. rhamnosus* EMCC 1105, *B. bifidium* EMCC 1334, and *L. fermentum* EMCC 1346, respectively, at pH 7. In contrast, amongst the tested PAHs, the minimum binding level was revealed for Nap, with binding ratios of 75.80, 61.10, 56.90, and 51.60 % for *L. bulgaricus* EMCC 1102, *L. rhamnosus* EMCC 1105, *B. bifidium* EMCC 1334, and *L. fermentum* EMCC 1346, respectively, at pH 7.

Indeed, pH medium-dependency could enhance the accessibility of peptidoglycan, and hence, new binding locations are generated on the cell wall. In addition, the bacterial viability may be lost during their movement in the stomach by the action of low pH. On the other hand, the binding activity of non-viable bacteria can decrease PAHs absorption in the bowel after the consumption of contaminated foods (Topcu, Bulat, Wishah, & Boyaci, 2010). Previous investigation revealed that the probiotics binding activity towards PHAs (Yousefi et al., 2019; Zhao et al., 2013), as well as towards toxins (Hatab, Yue, & Mohamad, 2012), is pH-dependent. In details, Yousefi et al. (2019) reported that the binding activity of *L. bulgaricus* EMCC 1102 was influenced by pH in the range from 3 to 7 with the highest binding level achieved at pH 5. The authors found that the greatest binding ability was towards BaP and BaA with values of 63.13 and 55.66 %, respectively, at pH 7. *Lactobacillus plantarum* presented the greatest binding ability towards BaP at pH 4 and 5, respectively (Zhao et al., 2013). However, on the other hand, El-Nezami et al. (1998) showed that aflatoxin B1 removal by probiotics was not influenced by pH in the ranges 4–6 and 2.5–8.5.

Furthermore, our study revealed that the lowest bacterial binding activity was related to the removal of low molecular-weight PAHs, mainly Nap. This explains their bioavailability because of their sorption on complexes of organic components such as meat products (Crampon et al., 2014). Additionally, some PAHs such as BaP can be partially eliminated in acidified soils, perhaps due to a catabolic collaboration

Table 2
Effect of non-viable probiotics at different concentrations (CFU/mL) on PAHs binding ability (%) and removal from PBS buffer at pH: 7, 37 °C for 12 h. PAH concentration: 20 µg/mL.

Sample type	Probiotic bacterial Conc. CFU/mL	PAHs conc. µg/mL	PAHs binding ratio (%)															
			Nap	Acy	Ace	Fl	Phe	Ant	Flu	Pyr	BaA	CHR	BbF	BkF	Bap	IcP	DhA	Bgp
−Ve (PBS + bacteria)	**1×10^9**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
+ Ve control (PBS + PAHs)	**0.00**	20.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
***B. bifidium* PBS + PAHs**	**1×10^7**	20.00	53.60 ±0.64	56.10 ±0.31	58.20 ±0.77	54.10 ±0.61	55.20 ±0.51	60.30 ±0.41	54.30 ±0.60	59.40 ±0.55	57.60 ±0.62	48.20 ±1.05	53.90 ±0.41	55.30 ±0.45	58.20 ±0.39	60.80 ±0.28	56.90 ±0.96	57.90 ±0.89
	1×10^8	20.00	54.30 ±0.61	60.20 ±0.35	55.40 ±0.63	57.30 ±0.44	58.10 ±0.68	61.10 ±0.51	54.80 ±0.37	56.20 ±0.24	59.30 ±0.51	60.40 ±0.46	54.50 ±0.30	56.10 ±0.32	59.60 ±0.57	61.40 ±0.42	57.10 ±0.33	58.20 ±0.62
	1×10^9	20.00	56.80 ±0.32	62.40 ±0.41	57.10 ±0.29	59.20 ±0.51	61.40 ±0.31	63.20 ±0.39	58.60 ±0.67	60.70 ±0.24	57.10 ±0.61	62.60 ±0.36	56.30 ±0.25	57.90 ±0.52	60.80 ±0.88	63.70 ±0.57	58.40 ±0.77	61.50 ±1.21
***L. fermentum* + PBS + PAHs**	**1×10^7**	20.00	47.60 ±0.29	52.10 ±0.25	48.50 ±0.50	53.40 ±0.61	49.30 ±0.84	54.40 ±0.57	51.20 ±0.21	48.10 ±0.56	53.20 ±0.41	50.30 ±0.39	47.70 ±0.30	48.40 ±0.52	50.10 ±1.02	54.60 ±0.59	49.30 ±0.39	51.10 ±0.40
	1×10^8	20.00	48.40 ±0.50	53.80 ±0.68	49.70 ±1.00	54.20 ±0.43	50.10 ±0.40	55.60 ±0.32	52.40 ±0.44	49.20 ±0.39	54.40 ±0.58	51.10 ±0.24	48.60 ±0.56	50.20 ±0.49	52.10 ±0.29	55.90 ±0.65	51.20 ±0.31	53.30 ±0.60
	1×10^9	20.00	50.80 ±0.48	54.60 ±0.60	50.20 ±0.50	56.30 ±0.31	51.40 ±0.53	57.10 ±0.24	53.80 ±0.56	50.90 ±0.41	55.20 ±0.81	52.20 ±0.49	50.70 ±0.37	51.10 ±0.28	53.60 ±0.47	57.10 ±0.54	52.80 ±0.41	54.70 ±0.62
***L. bulgaricus* + PBS + PAHs**	**1×10^7**	20.00	70.60 ±1.01	75.20 ±0.39	72.10 ±0.35	76.4000 ±0.29	71.90 ±0.30	77.20 ±0.51	73.50 ±0.25	76.60 ±0.14	74.10 ±0.91	70.60 ±0.23	70.60 ±0.19	75.20 ±0.24	72.30 ±0.20	77.50 ±0.51	73.10 ±0.22	74.40 ±0.32
	1×10^8	20.00	71.20 ±0.21	77.10 ±0.54	73.50 ±0.27	78.70 ±0.28	72.10 ±0.32	78.80 ±0.53	74.10 ±1.30	77.30 ±0.50	75.50 ±0.52	76.10 ±0.29	71.20 ±0.36	76.30 ±0.31	73.90 ±0.29	78.20 ±0.25	75.50 ±0.51	76.80 ±0.50
	1×10^9	20.00	73.10 ±0.51	78.40 ±0.29	74.40 ±0.21	79.10 ±0.28	73.80 ±0.23	80.40 ±0.30	75.90 ±0.25	78.80 ±0.31	76.10 ±0.29	77.20 ±0.33	73.60 ±0.50	77.70 ±0.21	75.40 ±0.15	80.60 ±0.26	76.80 ±0.30	78.30 ±0.17
***L. rhamnosus* + PBS + PAHs**	**1×10^7**	20.00	56.90 ±0.51	60.7 0 ± 0.24	57.20 ±0.52	61.90 ±0.46	58.40 ±1.14	63.30 ±0.51	59.10 ±0.54	62.50 ±1.00	57.80 ±0.63	61.40 ±0.50	56.50 ±0.26	61.80 ±0.44	57.50 ±0.51	63.30 ±0.44	62.60 ±0.57	59.60 ±0.57
	1×10^8	20.00	58.50 ±0.59	61.40 ±0.57	59.90 ±0.49	62.70 ±0.81	59.20 ±0.44	65.90 ±0.52	60.80 ±0.47	63.10 ±0.32	58.30 ±0.73	64.70 ±0.62	58.70 ±1.00	62.20 ±0.39	59.90 ±0.37	65.70 ±0.54	64.20 ±0.38	61.30 ±0.49
	1×10^9	20.00	60.70 ±0.81	62.80 ±0.42	65.80 ±0.57	63.20 ±0.38	61.30 ±0.51	67.10 ±0.50	61.20 ±1.08	64.90 ±0.51	60.40 ±0.44	66.80 ±0.52	60.80 ±0.43	64.80 ±0.72	61.10 ±0.40	67.60 ±0.15	66.30 ±0.57	63.70 ±0.33

Values denoted as mean ± standard deviation.

Table 3
Effect of non-viable probiotics at different pH values on PAHs binding ratio (%) and removal from PBS buffer at 37 °C for 12 h. PAH concentration: 20 μg/mL, bacterial concentration: 1 × 10^9 CFU/mL.

Sample type	pH value	PAHs binding ratio (%)															
		Nap	Acy	Ace	Fl	Phe	Ant	Flu	Pyr	BaA	CHR	BbF	BkF	Bap	IcP	DhA	Bgp
−Ve (PBS + bacteria)	**3**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
+ Ve control (PBS + PAHs)	**3**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
***B. bifidium* PBS + PAHs**	**3**	53.10	60.30	59.20	52.40	55.20	58.80	54.50	56.60	61.70	53.30	57.20	62.10	58.50	61.40	55.60	56.90
		±0.62	±0.22	±0.51	±0.46	±0.22	±0.71	±0.60	±0.39	±0.41	±0.49	±0.29	±0.41	±0.60	±0.44	±0.54	±0.31
	5	54.20	61.40	60.80	53.60	56.70	59.40	55.20	57.10	62.40	54.50	58.90	53.30	60.10	62.40	57.90	58.70
		±0.24	±0.57	±0.71	±0.41	±0.38	±0.52	±0.49	±0.62	±0.40	±0.46	±0.32	±0.50	±0.41	±0.65	±0.37	±1.02
	7	56.90	63.70	61.30	54.70	58.40	60.20	57.30	59.20	64.10	56.30	60.10	54.40	62.90	64.10	59.20	61.30
		±0.33	±0.34	±0.81	±1.23	±0.62	±0.51	±0.50	±0.44	±0.58	±0.52	±0.45	±0.56	±0.84	±0.57	±0.54	±1.12
***L. fermentum* + PBS + PAHs**	**3**	46.20	47.90	49.70	50.30	53.10	48.90	51.80	58.70	55.20	52.10	54.60	51.90	47.80	53.20	49.40	51.50
		±1.00	±0.31	±0.37	±0.43	±0.46	±0.56	±0.61	±0.70	±0.43	±0.44	±0.60	±0.43	±0.29	±0.41	±0.36	±0.42
	5	48.30	48.20	50.80	52.40	54.90	49.80	52.60	60.40	56.90	53.20	56.50	53.20	49.70	54.30	51.20	53.20
		±0.47	±0.61	±0.77	0.43	±1.23	±0.26	±0.22	±0.41	±0.54	±0.50	±0.32	±0.46	±0.37	±0.81	±0.49	±0.27
	7	51.60	51.70	53.90	55.70	56.80	51.10	54.80	61.50	62.10	55.50	57.40	54.30	52.40	56.60	52.10	55.30
		±0.40	±0.40	±0.48	±1.15	±0.71	±0.34	±0.47	±0.84	±0.61	±0.44	±0.41	±0.25	±0.55	±0.31	±0.28	±0.56
***L. bulgaricus* + PBS + PAHs**	**3**	72.50	71.90	74.10	76.10	73.70	75.60	72.50	77.90	80.60	78.20	80.70	79.40	71.80	74.50	76.90	72.10
		±0.25	±0.26	±0.32	±0.51	±0.14	±0.29	±1.04	±0.23	±0.12	±0.18	±0.26	±0.26	±0.21	±0.13	±0.27	±0.30
	5	73.80	73.70	76.40	78.10	75.50	76.20	73.10	78.60	83.80	79.10	82.40	81.60	72.10	76.90	77.50	73.20
		±0.13	±0.20	±0.26	±0.15	±0.51	±0.31	±0.22	±0.25	±0.31	±0.27	±0.17	±0.14	±0.30	±0.34	±0.19	±0.24
	7	75.80	75.10	78.30	79.20	77.30	78.70	75.40	80.30	85.20	81.80	84.20	82.10	74.90	78.80	79.30	74.70
		±0.15	±0.21	±0.24	±0.19	±0.31	±0.20	±0.51	±0.30	±0.25	±0.31	±0.25	±0.14	±0.41	±0.27	±0.22	±0.32
***L. rhamnosus* + PBS + PAHs**	**3**	57.20	58.30	60.60	59.20	61.40	64.40	57.10	62.20	67.50	63.10	59.80	64.20	58.70	65.10	57.90	66.80
		±0.63	±0.44	±0.51	±0.42	±0.58	±0.56	±0.54	±0.41	±0.46	±0.39	±0.41	±0.48	±0.91	±0.57	±0.26	±0.62
	5	59.30	60.40	61.20	60.70	62.30	65.50	59.70	64.10	68.90	65.90	60.10	66.80	69.60	66.30	68.70	67.20
		±0.56	±0.79	±0.47	±0.40	±0.36	±0.52	±0.44	±0.59	±0.82	±0.61	±0.31	±0.47	±0.56	±0.49	±0.24	±0.52
	7	61.10	61.20	62.90	61.40	63.50	66.10	60.90	65.50	70.60	67.40	62.30	68.10	70.70	67.70	69.60	68.10
		±0.48	±0.51	±0.53	±0.58	±0.41	±0.54	±0.96	±0.57	±0.34	±0.52	±0.44	±0.52	±0.29	±0.38	±0.41	±0.46

Values denoted as mean ± standard deviation.

with the bacterial cells (Nekhavhambe, Van Ree, & Fatoki, 2014). Also, the binding ability of toxic substances to bacterial cells can be affected by increasing or decreasing pH (Serrano-Niño et al., 2015), and, thus, the extreme binding occurs at a certain pH level for each toxic compound.

3.5. Effect of non-viable probiotics incubation times on PAHs binding ability (%) and removal

The data in Table 4 describe the effect of non-viable probiotic bacteria incubation times (*i.e.* 6, 12, and 24 h) on PAHs binding ability and removal. Although all the bacterial strains could eliminate PAHs from PBS, this reduction mainly depends on the incubation time. The minimum reduction rates of PAHs were observed at the minimum incubation time (6 h), whereas the maximum reduction levels were achieved at 24 h, for all probiotics. *L. fermentum* EMCC 1346 had the poorest binding activity for all PAHs in the range from 47.30 % (6 h) to 70.60 % (24 h). On the contrary, the highest binding ability was associated with *L. bulgaricus* EMCC 1102 which ranged from 61.10 (6 h) to 95.80 % (24 h). Among the examined PAHs, the greatest binding capability of probiotics incubated for 24 h was related to Chr and Pyr, with binding levels of 95.80 and 94.30 %, obtained for *L. bulgaricus* EMCC 1102, and 80.30 and 79.30 %, for *L. rhamnosus* EMCC 1105, respectively. On the other hand, the poorest binding rate was reported for Nap with the following values: *L. bulgaricus* EMCC 1102, 87.60 % > *L. rhamnosus* EMCC 1105, 72.10 % > *B. bifidium* EMCC 1334, 67.40 % > *L. fermentum* EMCC 1346, 62.40 %. Similarly, Crampon et al. (2014) studied the incubation time impact on the probiotics binding ability in PAHs removal, confirming that low molecular-weight PAHs, particularly Nap and Phe, were removed in less than 2–3 months and were greatly affected by the bacteria ratio in soils. However, the values detected in our work were higher than those obtained by Yousefi et al. (2019), who found that the PAHs binding ratios of *L. bulgaricus* EMCC 1102 and *Bifidobacterium lactis* for 10 h ranged from 54.66 to 63.40 %, and from 43.37 to 58.38 %, respectively. The authors showed the following trend BaP > Chr > BaA > BaF for the PAHs removal. On the contrary, Bisht et al. (2014) conveyed that the Ant and Nap low molecular-weight PAHs binding levels of *Bacillus* sp. SBER3 were 83.4 % and 75.1 %, respectively, after 6 days of incubation. Our study showed that a time of 24 h is sufficient for the bacteria to bind with each PAH, achieving the maximum values. Indeed, even if increasing the incubation time is crucial for the binding sites accessibility in peptidoglycan for PAHs, a prolonged incubation time can result in loss of PAH attachment to the bacteria, owing to the limited binding locations number on the cell wall (Daane, Harjono, Zylstra, & Haggblom, 2001; Haritash, and Kaushik, 2009). Thus, the binding activity decreases concerning low molecular-weight PAHs, particularly Nap.

3.6. PAHs in traditionally produced cold smoked sausages fortified with probiotics

The PAHs contents in the center and outer layers of the cold smoked sausages bioremediated with probiotic bacterial suspensions are reported in Fig. 2. For all groups, the highest PAHs values were detected for the outer layers. The use of probiotic bacteria for the sausages treatment had significant impact ($p < 0.05$) on PAHs decreasing when compared with control (non treated) groups. In general, all PAHs levels in control groups, both in the center and outer layers, were higher than those in the treated groups, at the end of the smoking period of cold smoked sausages. It is supposed that this is due to the dehydration process (Mastanjević et al., 2019). Accordingly, the mean values of total 16 PAHs for the center and outer layers of control vs water treated sausages were 82.65 and 101.34 vs 72.84 and 86.63 µg/kg, respectively. Sausages treated by bacterial suspension of *L. bulgaricus* EMCC 1102 cultures showed the maximum reduction of PAHs with mean values of 44.71 and 49.01 µg/kg for the center and outer parts of sausages, respectively. The Phe and Chr contents for *L. bulgaricus* EMCC 1102 -treated sausages showed a statistically significant difference ($p < 0.05$) with respect to *L. rhamnosus* EMCC 1105 -treated sausages and all control groups. The other examined PAHs were lower than the quantification limit for all groups and showed a significant difference ($p < 0.05$) for sausages center and outer parts among groups.

Furthermore, PAHs contents in traditionally produced cold smoked sausages with probiotics were lower than those evaluated by Puljić et al. (2019) for the smoked sausages at the end of the traditional smoking (2474 µg/kg in the outer and 145 µg/kg in the center). The revealed differences might be due to several factors, such as the smoking technique, smoking duration, and type of wood. This experimental evidence suggests that all the studied aspects, *i.e.* probiotic bacteria type, sausages layers, and treatment before and after smoking, synergically play a significant impact on PAHs content in sausages.

Our achieved results imply that, during the direct smoking, the highest quantities of PAHs were formed in the outer layer of the sausages in contrast with their inner coat. Similar findings were described by Ledesma et al. (2015b) who found that the highest PAHs contents were accrued in the exterior layer of the meat products. It has been confirmed that the PAHs collected on the exterior of the smoked products during smoking and then moved into the product after smoking maintained their concentration after some time. The migration level of PAHs into the center of sausages is affected by some factors, including the space between the products and the firing product properties like water activity, fat content, and surface/mass ratio. Subsequently, as anticipated, a reduction in PAH concentration can be achieved by light decomposition and interaction with other components in the product (Roseiro, Gomes, & Santos, 2011). Abou-Arab et al. (2010) informed that *Bifidobacterium bifidium*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* decreased the PAHs level by 46.6, 87.7 and 91.5 %, respectively. Bartkiene et al. (2017) showed that the application of *Pediococcus acidilactici*, *Pediococcus pentosaceus*, and *Lactobacillus sakei* for the sausages treatment considerably reduced PAHs ($p < 0.05$) before and after smoking. Furthermore, the application of LAB for the sausages treatment before and after smoking significantly ($p < 0.05$) decreased both BaP and Chr. The results also confirmed that the potatoes juice could be used as an alternative substrate for LAB cultivation, and the obtained fermented bioproducts could be applied for the cold smoked pork sausages surface treatment to decrease the PAHs ratio and microbial contamination in the end product (Bartkiene et al., 2017). Some investigators have proposed that PAHs are inactivated by certain metabolites formed by probiotic cells (Fuchs et al., 2008); hence, the PAHs level after the sausages bioremediation by probiotic bacterial suspensions could be reduced. Previous findings showed that the PAHs decline was due to the attachment of the PAHs to cell wall elements (Haskard, Binnion, & Ahokas, 2000). Tsuda, Hara, & Miyamoto (2008) confirmed that the probiotic bacterial cells exopolysaccharides represented an essential part in eliminating PAHs. In the smoking process, PAHs removal rates depend on the environmental conditions, manufacturing conditions, number and strain of the microorganisms, and chemical structure of PAHs. The results suggest that the fermented suspensions of the tested probiotics could be used as an alternative, cheap, and safe method for the treatment of smoked sausages to control the PAHs levels in the final product, especially in the case of traditional smoking processes.

3.7. Sensory evaluation of the fortified cold smoked sausages quality characteristics

The organoleptic characteristics of the fortified cold smoked sausages are showed in Fig. 3. Sensory properties of the sausages fortified with probiotics were evaluated and the most of panelists accepted and favored the sausages supplemented with probiotics with respect to the control, particularly for texture, taste, and overall acceptability. Regarding the sensorial score profile, the overall acceptability of the sausages fortified with the *L. rhamnosus* EMCC 1105 and L. bulgaricus

Table 4
Effect of non-viable probiotics at several incubation time on PAHs binding ability (%) and removal from PBS buffer at PH: 7, 37 °C. PAH concentration: 20 μg/mL, bacterial concentration: 1×10^9 CFU/mL.

Sample type	Time (hour)	PAHs binding ratio (%)															
		Nap	Acy	Ace	Fl	Phe	Ant	Flu	Pyr	BaA	CHR	BbF	BkF	Bap	IcP	DhA	Bgp
−Ve (PBS + bacteria)	6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
+ Ve control (PBS + PAHs)	6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
***B. bifidium* PBS + PAHs**	6	45.10 ±0.25	47.30 ±0.32	46.20 ±0.30	50.20 ±0.54	52.40 ±0.46	47.50 ±0.31	53.10 ±0.64	48.80 ±0.53	51.90 ±0.41	53.50 ±0.60	49.20 ±0.39	45.30 ±0.51	50.60 ±0.50	53.30 ±0.58	47.70 ±0.33	51.40 ±0.44
	12	56.70 ±0.24	59.70 ±0.50	58.10 ±0.77	61.90 ±0.82	63.30 ±0.24	59.40 ±0.56	63.80 ±0.57	60.20 ±0.41	62.40 ±0.37	64.10 ±0.34	61.40 ±0.44	57.70 ±0.23	62.80 ±0.38	64.10 ±0.37	59.20 ±0.58	63.70 ±0.59
	24	67.40 ±0.50	68.10 ±0.48	72.90 ±0.23	69.50 ±0.54	70.20 ±0.32	74.60 ±0.50	71.50 ±0.28	73.40 ±0.25	68.30 ±0.33	75.30 ±0.49	67.80 ±0.61	72.20 ±0.22	69.40 ±0.41	74.80 ±0.43	75.10 ±0.24	73.80 ±0.36
***L. fermentum* + PBS + PAHs**	6	47.30 ±0.31	49.30 ±0.39	50.10 ±0.41	48.80 ±0.50	51.90 ±0.54	54.30 ±0.60	52.10 ±0.27	47.50 ±0.22	53.80 ±0.51	55.70 ±0.69	48.20 ±0.88	52.10 ±0.43	49.20 ±0.71	53.40 ±0.50	54.30 ±0.22	50.20 ±0.51
	12	50.30 ±0.41	52.80 ±0.50	54.40 ±0.37	51.70 ±0.61	53.60 ±0.53	55.10 ±0.70	50.90 ±0.52	57.30 ±0.63	51.10 ±0.33	58.20 ±0.60	56.70 ±0.31	53.80 ±0.51	55.40 ±0.44	57.60 ±0.38	54.40 ±0.57	52.30 ±0.24
	24	62.40 ±0.53	63.90 ±0.32	65.90 ±0.50	67.10 ±0.62	64.70 ±0.38	68.40 ±0.52	66.80 ±0.24	69.40 ±0.88	62.70 ±0.47	70.60 ±0.23	63.20 ±0.57	67.90 ±0.40	65.30 ± 0.44	68.70 ±0.34	66.40 ±0.31	64.10 ±0.30
***L. bulgaricus* + PBS + PAHs**	6	61.10 ±0.28	65.40 ±0.35	67.70 ±0.51	62.20 ±0.57	68.10 ±0.65	63.70 ±0.50	65.70 ±0.34	64.60 ±0.37	66.30 ±0.80	69.20 ±0.52	62.80 ±0.53	68.70 ±1.00	63.40 ±0.62	67.30 ±0.38	64.50 ±0.24	65.20 ±0.44
	12	73.60 ±0.19	75.50 ±0.25	77.20 ±0.21	64.40 ±0.45	78.50 ±0.32	76.80 ±0.29	80.40 ±0.24	79.20 ±0.22	73.40 ±0.21	81.10 ±0.30	75.90 ±0.41	80.50 ±0.27	74.10 ±0.36	79.10 ±0.29	73.80 ±0.33	76.10 ±0.31
	24	87.60 ±0.27	88.60 ±0.30	87.80 ±0.20	89.20 ±0.14	91.30 ±0.22	90.90 ±0.21	92.10 ±0.17	94.30 ±0.36	93.90 ±0.24	95.80 ±0.31	88.30 ±0.33	95.10 ±0.14	91.30 ±0.39	89.60 ±0.19	94.20 ±0.51	92.40 ±0.27
***L. rhamnosus* + PBS + PAHs**	6	49.30 ±0.45	50.70 ±0.27	52.30 ±0.31	54.10 ±0.66	51.30 ±0.49	49.30 ±1.00	53.90 ±0.89	56.80 ±0.51	51.60 ±0.40	57.90 ±0.25	55.40 ±0.68	52.10 ±0.43	56.10 ±0.32	54.20 ±0.50	53.90 ±0.38	55.40 ±0.44
	12	61.20 ±0.63	62.20 ±0.88	64.70 ±0.71	66.30 ±0.24	65.20 ±0.37	68.10 ±0.34	67.70 ±0.54	63.90 ±0.57	64.80 ±0.38	69.50 ±0.59	62.90 ±0.61	66.40 ±0.79	67.10 ±0.47	65.70 ±0.71	68.80 ±0.61	69.60 ±0.52
	24	72.10 ±0.30	74.10 ±0.22	76.60 ±0.51	73.70 ±0.25	75.80 ±0.50	72.30 ±0.24	77.60 ±0.29	79.30 ±0.28	78.20 ±0.31	80.30 ±0.18	72.80 ±0.26	75.90 ±0.33	78.70 ±0.29	77.90 ±0.51	73.10 ±0.50	76.20 ±0.13

Values denoted as mean ± standard deviation.

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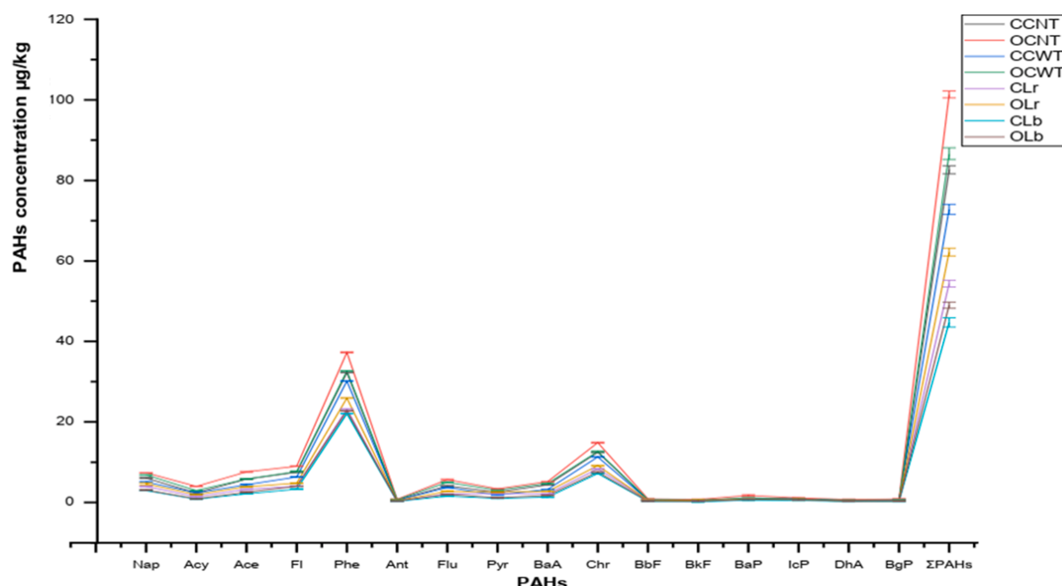

Fig. 2. PAHs contents (µg/kg) in the center and outer layers of traditional-produced smoked sausages with probiotic bacteria (1×10^9 CFU/mL) at pH 7 and initial PAHs content of 20 µg/kg (CCNT: Center layer of control non-treated, OCNT: Outer layer of control non-treated, CCWT: Center layer of control water treated, OCWT: Outer layer of control water treated, CLr: Center layer of *L. rhamnosus* EMCC 1105 -treated sausages, OLr: Outer layer of *L. rhamnosus* EMCC 1105 -treated sausages, CLb: Center layer of *L. bulgaricus* EMCC 1102-treated sausages and OLb: Outer layer of *L. bulgaricus* EMCC 1102-treated sausages).

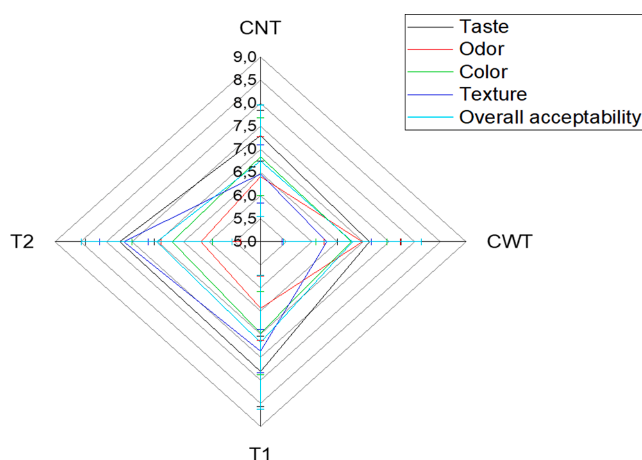

Fig. 3. Sensory characteristics of the fortified cold smoked sausages (CNT: Control non-treated sausages, CWT: Control water treated sausages, T1: *L. rhamnosus* EMCC 1105 -treated sausages and T2: *L. bulgaricus* EMCC 1102-treated sausages).

EMCC 1102 (score of 7.18 and 7.02, respectively) were higher than the control-non treated suasages (score of 6.75), even if accepted organoleptically (like moderately) in both cases. The texture of control-non treated sausages had a lower score than the sausages fortified with probiotics. The excellent texture properties of the fortified samples may be due to the physicosensory properties of LAB, leading to improved features in the end product. The odor and color of sausages fortified with probiotics were similar to those of the control sausages (non treated and water treated), and an overall acceptability of these samples was found to be excellent by the panelists probably due to the low content of PAHs, confirmed in our study. Previous findings by Bartkiene et al. (2017) showed that the cold smoked pork meat sausages treated with *Pediococcus pentosaceus* KTU05-9 were acceptable and comparable with control (non treated) samples, and both of them scored a sensorial attributes of (Happy).

4. Conclusions

The biomonitoring levels of PAHs significantly vary between sausages and beef samples. The cold smoked beef had higher PAHs contamination rates than sausages, showing the highest values for Phe. The non-viable probiotics had the highest PAHs reduction rates, mainly for Icp and Pye. *L. fermentum* EMCC 1346 had the weakest binding activity for all PAHs, whereas the maximum binding aptitude was achieved with *L. bulgaricus* EMCC 1102. The highest PAHs reduction levels were achieved at a bacterial count of 10^9 CFU/mL, pH 7, and incubation time of 24 h. *L. bulgaricus* EMCC 1102 showed higher PAHs reduction rates, both in the center and outer layers of sausages, than *L. rhamnosus* EMCC 1105. The sensory properties of the sausages fortified with probiotics were evaluated and the most of panelists accepted and favored the sausages supplemented with probiotics. Subsequently, the fortified sausages with probiotics could be a promising, cheap, and safe strategy for the treatment of smoked sausages to control PAHs levels in the final product. Therefore, probiotics based binding with PAHs is an effective approach in a lab-scale application, and further studies are still required for the large-scale application of the alternative and promising biological technologies.

CRediT authorship contribution statement

Gamal M. Hamad: Conceptualization, Methodology, Resources, Supervision, Writing – original draft. **Sabrien A. Omar:** Investigation, Validation, Formal analysis. **Aliaa G.M. Mostafa:** Formal analysis, Investigation, Validation. **Ilaria Cacciotti:** Project administration, Writing – review & editing. **Samaa M. Saleh:** Investigation, Validation, Formal analysis. **Marwa G. Allam:** Validation, Investigation, Formal analysis. **Baher El-Nogoumy:** Formal analysis, Investigation, Validation. **Sarah Abd-Elmohsen Abou-Alella:** Formal analysis, Software, Writing – original draft. **Taha Mehany:** Investigation, Formal analysis, Data curation, Software, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2022.111793.

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