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Research Article
Biocontrol of Processed Cheese by Incorporation of Probiotic Bacteria and its Metabolites

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Abstract

Background: Processed cheese could be estimated as microbiologically safe, nevertheless microorganisms may gain entrance during all stages of processing especially spore forming bacteria, one way to limit the growth of undesirable microorganisms is incorporation of probiotics which show high antimicrobial activity against these pathogens. **Materials and Methods:** The antibacterial activity of six different lactic acid bacteria against *Bacillus cereus* and *Clostridium perfringes* were studied. **Results:** *Lactobacillus curvatus* and *Lactobacillus acidophilus* were the most effective cultures against *B. cereus*, while *Leuconostoc mesenteroides* against *C. perfringes*. Supplementation of processed cheese with *L. curvatus* and the mixed culture of the three other probiotics effectively suppressed the growth and survival of *B. cereus* and it could not be recovered after 14 days of storage and room temperature. While, supplementation of the cheese by metabolites of *L. curvatus*, *L. acidophilus* and mixed culture of *L. curvatus*, *L. acidophilus* and *L. mesenteroides* reduce the viable counts of *B. cereus* and the reduction reached 100% at 14 days using metabolites of *L. curvatus* and the mixed culture of *L. curvatus*, *L. acidophilus* and *L. mesenteroides* and at 21 days using *L. acidophilus* when the cheese stored at 25°C. But, cold storage delayed the reduction of *B. cereus*. Also, a pronounced reduction in viability of *C. perfringes* was observed with *L. mesenterials* culture, as *C. perfinges* could not be recovered after 30 and 21 days at room and refrigerator temperature, respectively. **Conclusion:** Supplementation of probiotic metabolites of *L. mesenteroides* and the mixed culture (*L. curvatus*, *L. acidophilus* and *L. mesenteroides*) led to decrease the viability and survival of *C. perfringens* as it could not be recovered after 30 days at refrigerator temperature for 45 and 30 days respectively, at room temperature.

Key words: Processed cheese, probiotic, *Bacillus cereus*, *Clostridium perfringes*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Processed cheese is made by heating a mixture of natural cheeses of different types and different ages with emulsifying salts and various ingredients under lower pressure and constant stirring until a homogenous mass of desired properties is formed[1-3].

The growing demand for high-value health promoting foods such as dairy products has encouraged the industries and the research field, not only to explore new emerging food processes and formulations in which they are able to assure functional but also safe products[4-6]. Probiotics are recently defined by Krishna[7] as a live microbial food ingredient. When probiotics are ingested in sufficient quantities, extra health benefits to the consumer will be added such as enhanced immune response, alleviation of symptoms of lactose intolerance, treatment of diarrhea, reduction of serum cholesterol, vitamin synthesis and anti-carcinogenic and anti-microbial activities[8-12]. There are different types of Lactic Acid Bacteria (LAB) they are used as probiotic which include *Lactobacillus* and *Bifidobacterium*[13]. Highly undesirable microbial contaminates of processed cheese are rod-shaped endospore-forming bacteria of the genera *Bacillus* and *Clostridium*, formation of the spores allows it to be resistance of heat, chemicals and other adverse environments that undergoes during processing preparation of the product. *Bacillus cereus* might be the most common cause of food borne illnesses worldwide, but barely reported to health agencies because its symptoms tend to be short-lived and self-limited[14].

Moreover, *Clostridium perfringens* causes a toxin mediated disease represents as the 3rd most commonly reported food-borne illness[15]. Some *C. perfringens* strains produce important toxin named *C. perfringens* enterotoxin (CPE), which is responsible for several human gastrointestinal diseases[16]. In addition, *B. ceraus* reported to be capable of producing 2 types of enterotoxin, first diarrheal disease which occurs when ingested food contains 10^5 cells, second enteric disease which occurs when food contains preformed toxin[17].

Lactic Acid Bacteria (LAB) are a group of Gram-positive bacteria including the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Streptococcus*. It produces a wide range of antimicrobial metabolite comprising organic acids, carbon dioxide, ethanol, hydrogen peroxide, diacetyl and bacteriocins[18]. Bacteriocins of lactic acid bacteria have attracted the attention of many investigators especially in the recent years because of their use as a natural food preservative[19]. So, this study aimed to validate the effectiveness of antibacterial activity of some LAB against these pathogens for supplementation of processed cheese with suitable strains to suppress the growth of *B. cereus* and *C. perfringens* and subsequently to increase the safety and quality of the cheese.

MATERIALS AND METHODS

Bacterial strains

Lactic acid bacterial strains: *Leuconostoc mesenteroides* B-118 and *Lactobacillus acidophilus* were provided by Chr. Hansen's Laboratory, Denmark. *Streptococcus thermophilus* and *Lactococcus lactis* were obtained from the Dairy Department, National Research Center (NRC). *Lactobacillus curvatus* B-4562 and *Lactobacillus rhamnosus* B-445 were supplied by the Northern Regional Research Laboratory, Illinois, USA (NRRL). *Leuconostoc mesenteroides* B-118, *Lactobacilluc acidophilus*, *Lactobacillus curvatus* B-4562 and *Lactobacillus rhamnosus* B-445 were previously studied in (NRC) and proved to be probiotics[20,21] by testing for probiotic characteristics (acid tolerance, survivability in different bile salt concentration and antibiotic susceptibility as well as antimicrobial activity to some pathogens according to FAO. and WHO.[22].

Pathogenic strains: Three strains of *B. cereus* (tested as mixed strain) and also, three strains of *C. perfringens* which were isolated and identified previously[23] were used for inoculation of the resultant cheese.

Media: The used media are tryptone soya broth (Oxoid) for antagonism, MRS broth media for culture of lactic acid. Bacteria strains, polymyxin-pyruvate-eggyolk-mannitol-bromothymol blue agar (PEMBA) (Oxoid) for *B. cereus*, *perfringens* agar (TSC) (Oxoid) for *C. perfringens*, M17 was used for *Streptococcus thermophilus* and *Lactococcus lactis*, (MRS agar medium) (Oxoid, UK) for *Lactobacillus rhamnosus*, *Leuconostoc* Medium (MRS with sucrose and sodium azide)[24] for *Leuconostoc mesenteroides*, MRS medium with replacing glucose with trehalose and addition of bile salt for *Lactobacillus acidophilus* and MRS media with addition of sodium chloride for *Lactobacillus curvatus*.

Media used throughout the current investigation were in a dehydrated form and prepared according to the manufacturer's instructions.

Experimental procedures

Growth and preservation LAB strains: Each lactic acid bacteria culture was maintained individually in 11% (w/v) sterile Reconstituted Skim Milk (RSM) powder at 37°C for 24 h, except *L. lactis*, *L. mesenteroides* incubated at 30°C for 18 h and stored at 4°C between transfers, each culture was sub cultured twice in MRS medium (Oxoid) before use.

Screening for antibacterial activity of the tested LAB metabolites towards *B. cereus* and *C. perfringens*

Cell-free supernatant: The cell-free supernatant fluids were obtained by centrifuging the overnight cultures of the tested LAB strains (grown at 37°C for 24 h in MRS broth media) at 4000 rpm for 15 min at 4°C. Supernatant was filter-sterilized through 0.22 µm Millex-GV membranes (Millipore) and pH was adjusted to 6 to exclude the organic acid effects.

Antibacterial activity: The activity of the resulting metabolites of the tested LAB strains were tested against *B. cereus* and *C. perfringens* using the well diffusion agar assay as described by Lyon and Glatz[25]. Aerobically for *B. cereus* and anaerobically for *C. perfringens* in gas pack anaerobic jar using kits of anaerobic Gas Generating Kits (Oxoid). Each strain was tested in triplicate per plate and plates were incubated for 24 h at 37°C. The ability of each probiotic strain metabolite to inhibit the growth of *B. cereus* and *C. perfringens* was determined by measuring the diameter in millimeter of the clear inhibition zone formed around discs.

Antagonism activity of the tested LAB strains against pathogenic strains: *Bacillus cereus* and *C. perfringens* strains were transferred into tryptone soya broth (Oxoid) and each tested LAB strains were cultured in MRS broth media and incubated at 37°C for 24 h. The cultures individually were diluted in saline solutions to an appropriate inoculum size (10^5 CFU mL^{-1} for *B. cereus* and *C. perfringens* and 10^7 CFU mL^{-1} for lacic acid bacteria tested strains) and were ready for inoculation together into 100 mL tryptone soya broth. Each of six LAB strains in tryptone soya broth was inoculated with *Bacillus cereus* and *C. perfringens* with two controls for pathogenic. All incubated at 37°C with gas pack anaerobic jar using anaerobic kits.

The counts of *B. cereus* and *C. perfringens* in the mixed culture were measured after incubation at intervals (0, 24 and 48 h).

Table 1: Ingredients used in the preparation of blends for processed cheese

Ingredients	Percentage
Shortening oil	21.50
Skim milk powder	11.00
Chedder cheese	3.00
Milk protein concentrate	2.40
Modified starch	2.00
Maltodextrin	1.50
Salt	1.20
Sodium di and polyphosphate (corino 75)	1.00
Sodium ortho and polyphosphate (corino 73)	0.70
Citric acid	0.30
Stabilizer	0.25
Chedder cheese flavor	0.15
Water	47.00

Manufacturing of processed cheese (triangles): Processed cheese was manufactured as described by Mayer[26] and Schar and Bosset[27]. The composition and percentage of ingredients used are given in Table 1. The mixture of ingredients was heated in a batch cooker with constant agitation, until a homogeneous mass is obtained. The melting temperature of processed cheese was 85±1°C and the total melting time was 15 min from the beginning of heating to the beginning of discharge. The resultant was manually filled into sterilized glass jar and covered with aluminum foil and their covers.

All the used ingredients were tested and were found free from aerobic and anaerobic spore forming bacteria. The batches were performed in the laboratories of Faculty of Agriculture, Ain Shams University.

The first batch was divided into five equal portions and were inoculated with LAB strains, *B. cereus* and *C. perfringens* strains at 45°C and divided according to antimicrobial results as following:

- Inoculation with 2% *L. curvatus*
- Inoculation with 2% *L. acidophilus*
- Inoculation with 2% mixed culture (*L. curvatus*, *L. acidophilus* and *L. mesenteroides*)
- Inoculation with 2% *L. mesenteroides*
- As control without inoculated with LAB strains

All these treatments were obtained with addition of citric acid to adjust pH. The first and second treatments were inoculated separately with 10^5 CFU mL^{-1} of *B. cereus* strain.

Whereas, third treatment divided into two equal portions and inoculated one portion with 10^5 CFU mL^{-1} of *B. cereus* strain and second portion with 10^5 CFU mL^{-1} of *C. perfringens* strain. The fourth treatment was inoculated with 10^5 CFU mL^{-1} of *C. perfringens* strain.

Also, the fifth treatment divided into two equal portions and inoculated one portion with 10^5 CFU mL^{-1} of *B. cereus* strain and second portion with 10^5 CFU mL^{-1} of *C. perfringens* strain as control without inoculated with LAB strains.

The second batch was divided into five equal portions as mentioned previously for the first batch, but used the metabolites of LAB with percent 2% which prepared as mentioned before but the strains grown in sterilized reconstituted skim milk were incubated at 37°C for 24 h and then manufacture processed cheese from these cultures without addition of citric acid. The control made from reconstituted skim milk powder without inoculated with metabolites of LAB.

All the cheese treatments were filled into sterilized glass jars and covered with aluminum foil and their covers. Half of these containers from each treatment were stored at 25°C and the other half at 7±2°C.

All samples including the main control, the contaminated controls and the treated contaminated cheeses were subjected for bacteriological analysis to investigate the behavior of the tested pathogens at zero time and after 1, 2, 3, 7, 14, 21, 30, 45 and 60 days of storage at refrigerator and room temperature. Each pathogenic was recovered on its selective agar media.

Bacteriological analysis: To all cheese samples (25 g) were homogenized for 1 min in 225 mL of sterile solution (2% w/v) of sodium citrate. Analysis was carried out using the following procedures.

***Bacillus cereus* counts:** *Bacillus cereus* counts were carried out by spreading 0.1 mL of the appropriate dilution onto PEMBA medium (Oxoid). The incubation temperature was 37°C for 18-24 h.

***Clostridium perfringens* counts:** *Clostridium perfringens* counts were carried out on *Perfringens* agar (TSC) (Oxoid) according to Varadaraj[28].

RESULTS AND DISCUSSION

Antagonistic activity of some lactic acid bacteria against aerobic spore former (*B. cereus*) and anaerobic spore former (*C. perfringens*): Bacteriocin and bacteriocin like producing species have now been identified among all the genera that comprise the Lactic Acid Bacteria (LAB) including *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Leuconostoc* and *Pedicoccus*[29].

These bacteriocins are extremely important for preventing the growth of spoilage and pathogenic bacteria.

Antibacterial activity of some lactic acid bacteria against *B. cereus* and *C. perfringens*: Using the well diffusion agar assay, the antibacterial activity of six different lactic acid bacteria against *B. cereus* and *C. perfringens* strains is presented in Fig. 1 and 2. The recorded results revealed that all the tested LAB have variable antibacterial activity since supernatants of the six tested LAB strains inhibited the growth of *B. cereus* and this resulted different inhibition zone diameter (9-13 mm). *Lactobacillus curvatus* was the most

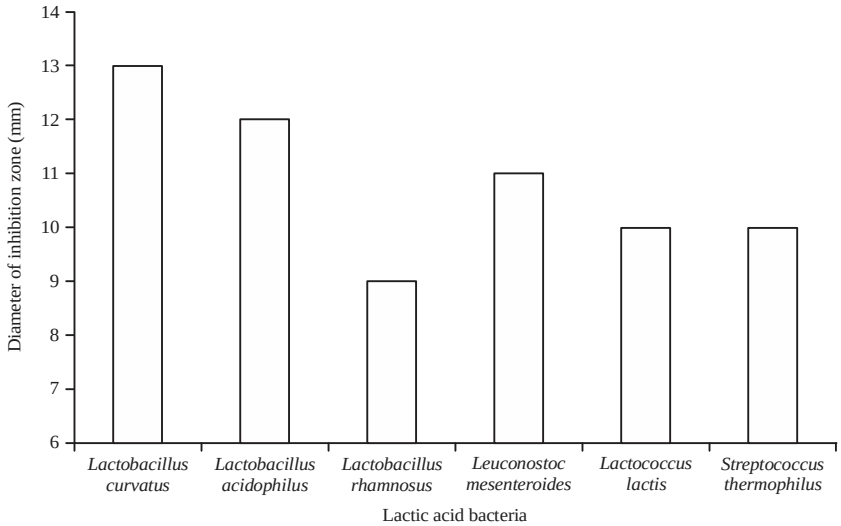

Fig. 1: Antibacterial activity of some lactic acid bacteria against *Bacillus cereus*

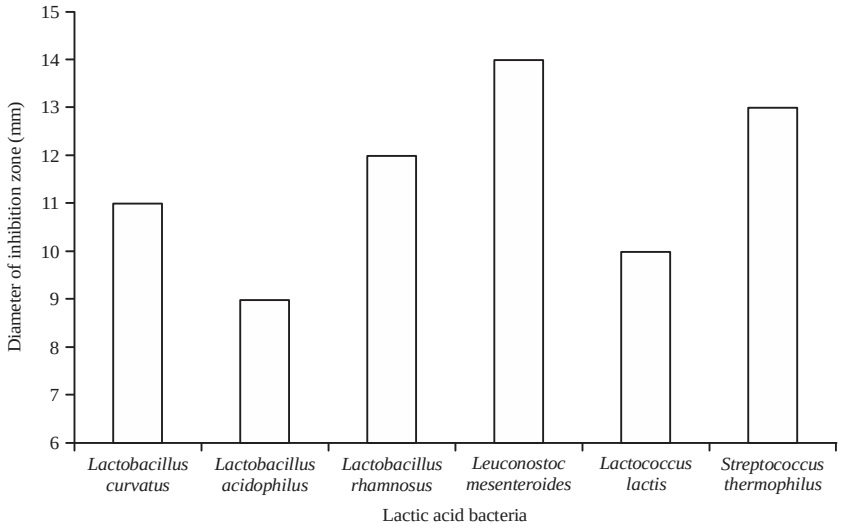

Fig. 2: Antibacterial activity of some lactic acid bacteria against *Clostridium perfringens*

Table 2: Antagonism activity of some lactic acid bacteria against *B. cereus* in mixed cultures

	Bacillus cereus counts (log_{10} CFU mL^{-1})						
Incubation period (days)	*Lactobacillus curvatus*	*Lactobacillus acidophilus*	*Lactobacillus rhamnosus*	*Leuconostoc mesenteroides*	*Lactococcus lactis*	*Streptococcus thermophilus*	Control
0	5.11	5.11	5.11	5.11	5.11	5.11	5.11
1	3.90	4.04	4.30	4.20	4.28	4.35	7.20
2	2.00	2.30	3.32	3.00	3.20	3.27	8.62

Table 3: Antagonism activity of some lactic acid bacteria against *C. perfringens* in mixed culture

	Clostridium perfringens counts (log_{10} CFU mL^{-1})						
Incubation period (days)	*Lactobacillus curvatus*	*Lactobacillus acidophilus*	*Lactobacillus rhamnosus*	*Leuconostoc mesenteroides*	*Lactococcus lactis*	*Streptococcus thermophilus*	Control
0	5.20	5.17	5.18	5.20	5.15	5.21	5.15
1	3.85	4.26	4.28	4.11	4.40	4.56	7.90
2	3.11	3.34	3.18	2.70	3.38	3.48	9.23

effective culture among all the tested cultures and formed the highest inhibition zone (13 mm diameter). While, *Lactobacillus rhamnosus* exhibited the smallest inhibition zone (9 mm). Regarding the antibacterial activity of the supernatants of LAB tested strain against *C. perfringens*, the largest inhibition activity were deserved by supernatants of *Leuconostoc mesenteroides* followed by *Streptococcus thermophilus* and *Lactobacillus rhamnosus* which inhibitd the growth of *C. perfringens* and this appeared by inhibition zones; 14, 13 and 12 mm, respectively (Fig. 2). Additionally, *L. curvatus* proved to have suppressive effect toward *C. perfringens* with inhibition zone diameter 11 mm, while, *L. acidophilus* show the lowest suppressive effect.

Ahmadova *et al.*[30] reported that the cell free supernatant of culture of *L. curvatus* A6 which exhibited the presence of curvacin A encoding gene inhibited the growth of *Listeria monocytogenes* and *B. cereus* strains.

Saroj[31] reported that the cell-free supernatant bacteriocins produced by strain of *L. acidophilus* showed inhibition zone against *Bacillus* spp.

Morever, the ability of LAB to produce bacteriocins, some of which have a broad spectrum of activity were reported by Gillor *et al.*[32].

Recently, the scientist proved that, leucocin produced by *Leuconostoc* showed antibacterial activity against Gram-positive bacteria[33].

Antagonistic activity of some lactic acid bacteria against *B. cereus* and *C. perfringens*: The antibacterial activity of six LAB strains toward *B. cereus* and *C. perfringens* in tryptone soya broth was evaluated (Table 2, 3).

It is obvious that, *B. cereus* and *C. perfringens* proliferated well in tryptone soya broth and increasing in counts by 3.51 and 4.08 log cycle respectively, in control

after 48 h. While, in mixed culture with the tested LAB bacteria, a pronounced reduction in viability of *B. cereus* and *C. perfringens* was observed. *Bacillus cereus* showed rapid decline in mixed culture with *L. curvatus*, as its numbers were decrease by 3.11 log cycle after 48 h of incubation. *Lactobacillus acidophilus* and *L. mesenteroides* also showed strong antibacterial activity against the pathogen and reduce survival of *B. cereus* by 2.81, 2.11 log cycle, respectively after 48 h of incubation. While, *L. rhamnosus*, *L. lactis* and *S. thermophilus* show slightly weaker inhibitory activity as numbers of *B. cereus* decreased by 1.79, 1.91 and 1.84 log cycles, respectively after 48 h of incubation (Table 1). These results indicated that tested strains of LAB could restrict the activity of *B. cereus* (growth or survival).

Among the tested probiotics *L. mesenteroides* presented a high inhibitory effect against *C. perfringens* resulting of decreasing the counts from 5.20-4.11 $\log_{10}$ CFU mL^{-1} after 24 h of inoculation with reduction percent (21.3%) reaching 2.70 $\log_{10}$ CFU mL^{-1} with reduction percent (48.7%) after 48 h of inoculation. Also, *L. curvatus* and *L. rhamnosus* were effectively inhibited the growth of *C. perfringens* leading to reduce counts by 2.09 and 2 log cycle, respectively after 48 h of incubation. Whereas, *L. acidophilus*, *L. lactis* and *S. thermophilus* were able to inhibit growth and decrease counts by (1.83, 1.77 and 1.73 log cycle) after 48 h.

The inhibition of *B. cereus* was due to the antimicrobial metabolites e.g., bacteriocin, organic acids and hydrogen peroxide by the tested strains of *Lactobacillus* and *Lactococcus*[34].

Mkrtchyan *et al.*[35] and Nazzaro *et al.*[36] reported that *L. acidophilus* exhibited broad spectrum activity against *L. monocytogenes* and *B. cereus*.

Hereupon, *L. mesenteroides* gained the highest anticlostridial activity than the other tested strains and appeared to be promising culture.

According to the results of antibacterial and mixed culture assay, three strains of LAB which was previously proved to be probiotics by Mabrouk[20] and Ibrahim[21] (*L. curvatus*, *L. acidophilus* and *L. mesenteroides*) which showed the highest antimicrobial effect toward *B. cereus* and *C. perfringens* were selected for further study.

Biocontrol of processed cheese by incorporation of probiotic bacteria and its metabolites: In recent years, several reports were dealing with antibacterial activity of LAB especially probiotics and their role for liming the growth of undesirable microorganisms.

The processed cheese was manufactured using the chosen probiotic bacteria through the results of antibacterial and antagonistic activity of some LAB towards *B. cereus* and *C. perfringens* in the previous section (*L. curvatus*, *L. acidophilus* and *L. mesenteroides*) or their metabolites with inoculation of *B. cereus* and *C. perfringens* in the processed cheese.

Behavior of *B. cereus* and *C. perfringens* and probiotics during storage of processed cheese at 25°C and at 7±2°C for 60 days: The pattern of selected probiotics and *B. cereus* growth in inoculated processed cheese during storage at 25 and 7°C for 60 days are shown in Fig. 3 and 4.

Generally, *B. cereus* showed a rapid decline in its culturable numbers in cheese manufactured by inoculation with the selected probiotics and *B. cereus* during storage at 25°C as well as 7°C.

It is obvious that *B. cereus* can survive in control cheese till the end of storage period (60 days) reaching maximum counts after 30 days of storage period (Fig. 3). Such counts were nearly the estimated count to be sufficient to produce toxin as detectable enterotoxin concentration considered with colonization[37] level $>10^6$. However, in compromised consumers a much smaller dose of 1.2×10^3 mL^{-1} may cause illness[38].

In contrast, supplementation of processed cheese with *L. curvatus* effectively suppressed the growth and survival of *B. cereus* as it showed higher inhibition effect than inoculation with *L. acidophilus* and the pathogen could not be recovered after 14 days of storage with decreasing of its counts by 1.04 log cycle and the roduction of count was 1.52 log cycle as compared to the control cheese after 7 days of storage at room temperature (Fig. 3), but at cold storage, the pathogen can survive for more than 14 days and no viable cells were detected at 21 days of storage (Fig. 4).

Also, supplementation of cheese with the probiotic *L. acidophilus* led to reduce the viability and survival of *B. cereus* as it could not be recovered after 21 days of storage at room temperature (25°C) as well as at cold storage 7±2°C (Fig. 3, 4).

It could not be recover after 21 days with supplementation of both probiotic strains (*L. curvatus*, *L. acidophilus* and *L. mesenteroides*) at refrigerator temperature (Fig. 4).

Concerning mixed culture, cheese (*L. curavatus*, *L. acidophilus* and *L. mesenteroides*) were found to suppress the survival of *B. cereus* and could not be recovered after 14 days at room temperature (Fig. 3) and 21 days at refrigerator temperature, respectively (Fig. 4).

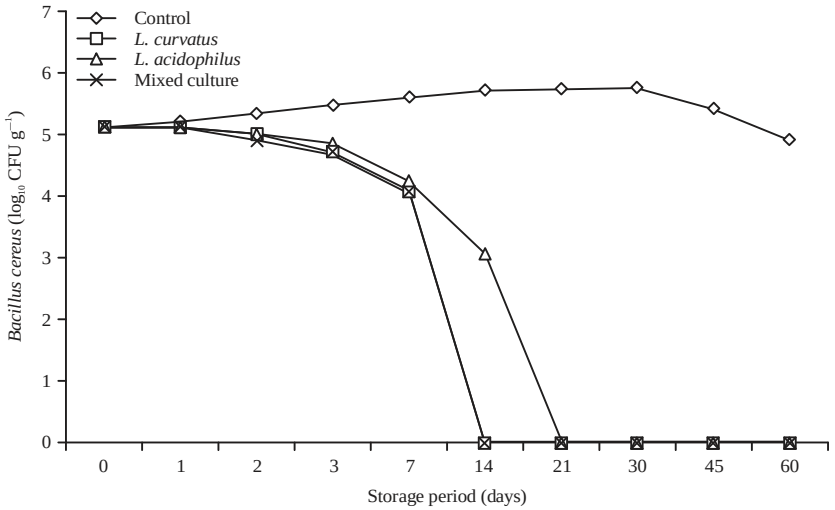

Fig. 3: Behavior of *Bacillus cereus* during storage of processed cheese supplement with probiotic strains at 25°C for 60 days, Mixed culture: *L. curvatus*, *L. acidophilus* and *L. mesenteroides*

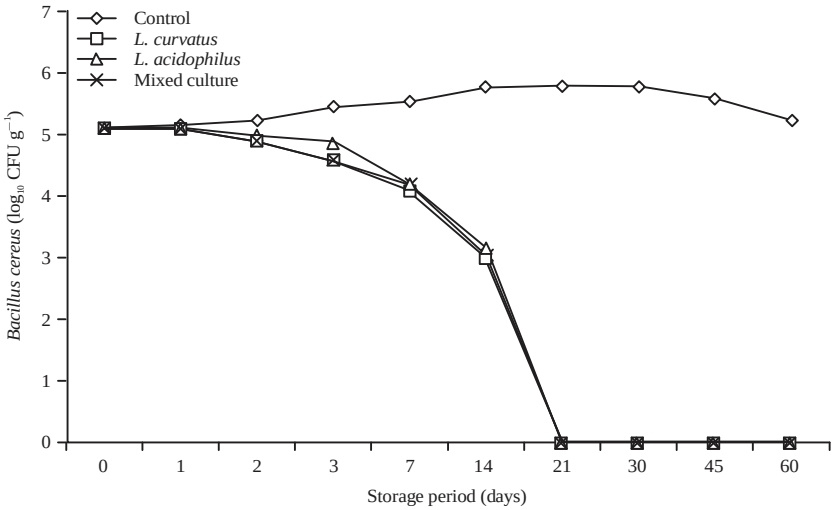

Fig. 4: Behavior of *Bacillus cereus* during storage of processed cheese supplemented with probiotics at 7 ± 2°C for 60 days. Mixed culture: *L. curvatus, L. acidophilus* and *L. mesenteroides*

The reduction of viable counts of *B. cereus* could be mainly attributed to the production of antimicrobial substance produced by the used probiotic cultures.

All probiotics were proliferated with gradual increase in counts during the first weak and then the increase was slight. These results are in line with those reported by Rukure and Bester[39].

Figure 5 and 6 show that *C. perfringens* can be proliferated in control processed cheese without probiotics reached the maximum growth rate at 14 days of the storage period at room temperature. Also, despite storage under refrigerated conditions (7±2°C) there are opportunity for survival and proliferation of the pathogen, as reached the maximum rate in control cheese after 30 days. While, in processed cheese with the selected probiotic bacteria, a pronounced reduction in viability of *C. perfringens* were observed. When processed cheese supplemented with *L. mesenteroides*, *C. perfringens* could not be recovered after 30 and 21 days at room and refrigerator temperature, respectively (Fig. 5 and 6).

Additionally, *C. perfringens* counts were continually decrease and could not be recovered after 21 days in processed cheese made with mixed culture (*L. curvatus*, *L. acidophilus* and *L. mesenteroides*) which appears to have more inhibitory activity than *L. mesenteroides* alone at room and refrigerator temperature. In this respect

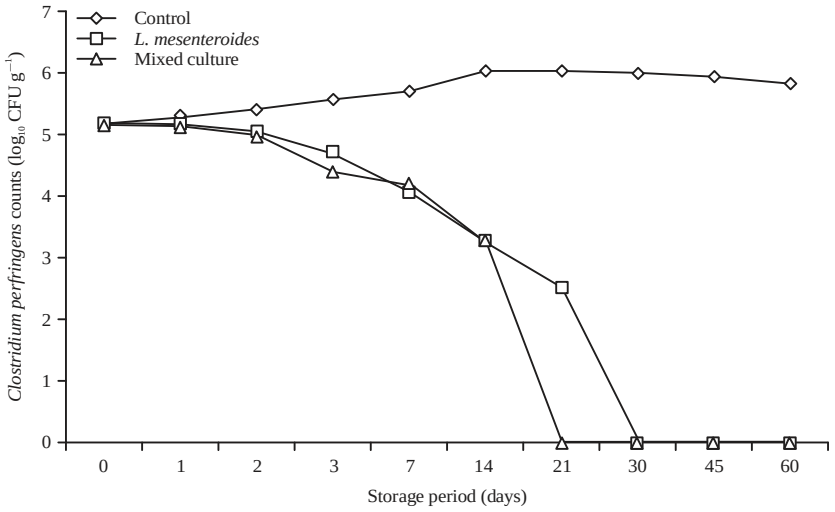

Fig. 5: Behavior of *Clostridium perfringens* during storage of processed cheese supplemented with probiotics at 25°C for 60 days. Mixed culture: *L. curvatus*, *L. acidophilus* and *L. mesenteroides*

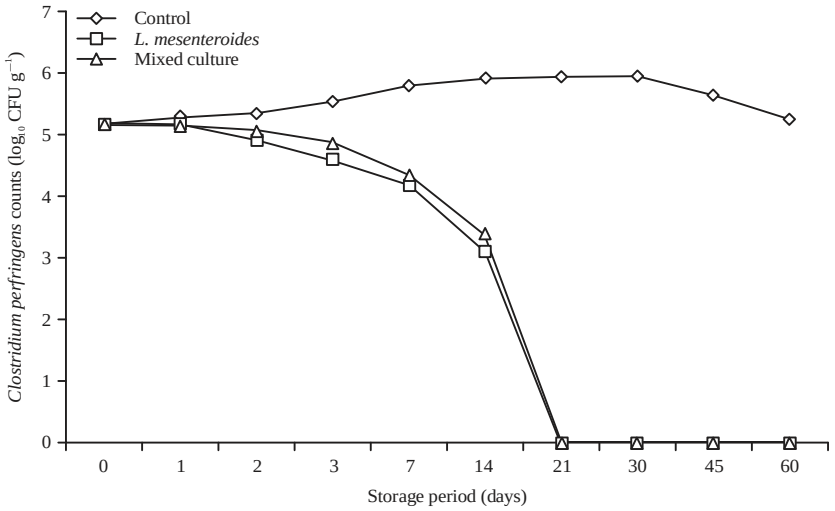

Fig. 6: Behavior of *Clostridium perfringens* during storage of processed cheese supplemented with probiotics at 7±2°C for 60 days. Mixed culture: *L. curvatus*, *L. acidophilus* and *L. mesenteroides*

De Vuyst and Vandamme[40] reported that the bacteriocins e.g., nisin, pediocin and leuconocin have a relatively broad spectrum of activity against Gram-positive bacteria.

The antimicrobial effects of *Leuconostoc* against pathogenic and spoilage microorganisms have been reported early due to the production of organic acid, peroxide as well as bacteriocin[41].

Behavior of *B. cereus* and *C. perfringens* during storage of processed cheese supplemented with probiotic metabolites at 25°C and at 7±2°C for 60 days: The LAB are considered generally recognized as safe (GRAS) by the US Food and Drug Administration[42]. Thus, the use of their metabolites as biological preservatives has been discussed[43].

Growth and survival of *B. cereus* in processed cheese with and without the chosen probiotics metabolites during storage for 60 days at 25°C and at 7±2°C are shown in Fig. 7 and 8.

Results show that, *B. cereus* grew well in control cheese and reached the maximum growth rate at 30 days of the storage period either at room temperature or refrigerator temperature (Fig. 7 and 8). On the other hand, supplementation of the cheese by metabolites of *L. curvatus*, *L. acidophilus* and mixed culture of *L. curvatus*, *L. acidophilus* and *L. mesenteroides* reduce the viable counts of *B. cereus*

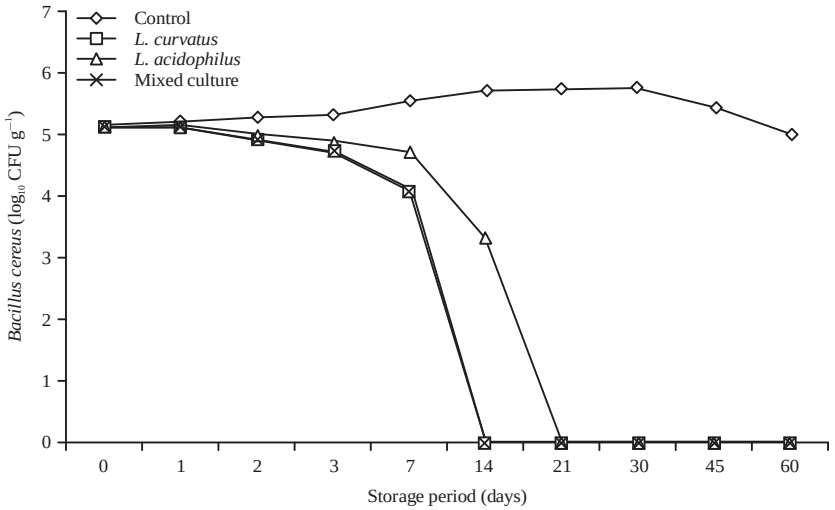

Fig. 7: Behavior of *Bacillus cereus* during storage of processed cheese supplemented with probiotic metabolites at 25°C for 60 days. Mixed culture: *L. curvatus, L. acidophilus* and *L. mesenteroides*

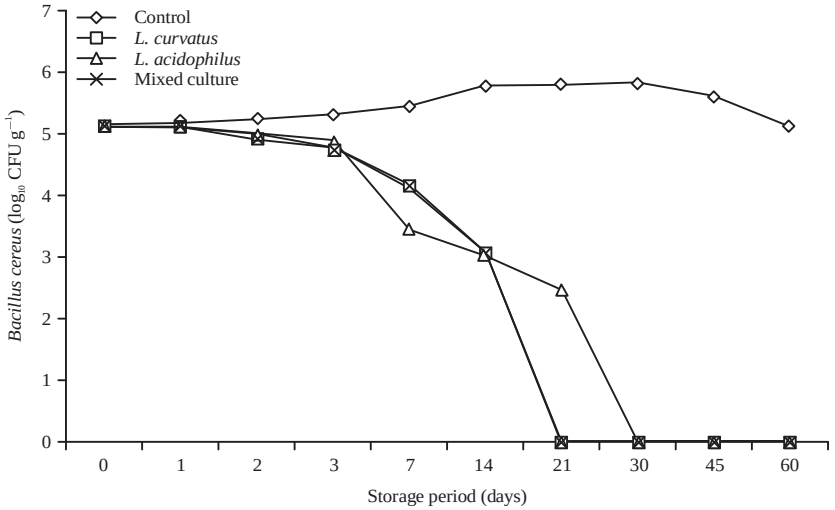

Fig. 8: Behavior of *Bacillus cereus* during storage of processed cheese supplemented with probiotic metabolites at 7±2°C for 60 days. Mixed culture: *L. curavatu, L. acidophilus* and *L. mesenteroides*

and the reduction reached 100% after 14 days by using metabolites of *L. curavatus* and the mixed culture of (*L. curvatus*, *L. acidophilus* and *L. mesenteroides*) metabolites and after 21 days by using *L. acidophilus* when the cheese stored at 25°C (Fig. 7). But, cold storage (7±2°C) of the supplemented cheese delayed the reduction of *B. cereus* (Fig. 7).

These results clearly indicate that *L. curvatus* showed strong antibacterial activity against *B. cereus*, weather with supplementation as culture or its metabolites in the processed cheese. In this respect, Kumar and Arumugam[44] reported inhibition zone (9 mm) with curvacin A (produced by *L. curvatus*) towards *B. cereus*.

Recently, Ahmadova[30] found that bacteriocin produced by *L. curvatus* strain was heat stable, resistant to physiological concentrations of bile salts and active in a broad pH range and bacteriostatic against *Bacillus cereus*.

On the other hand, the results were pointed out that supplementation of probiotic metabolites of *L. mesenteroides* and the mixed culture (*L. curvatus*, *L. acidophilus* and *L. mesenteroides*) showed similar results leading to decrease the viability and survival of *C. perfringens* as it could not be recovered after 30 days. While, the counts of the pathogen in the control cheese reached the maximum (5.93 $\log_{10}$ CFU g^{-1}) at the same time at refrigerator temperature (Fig. 9).

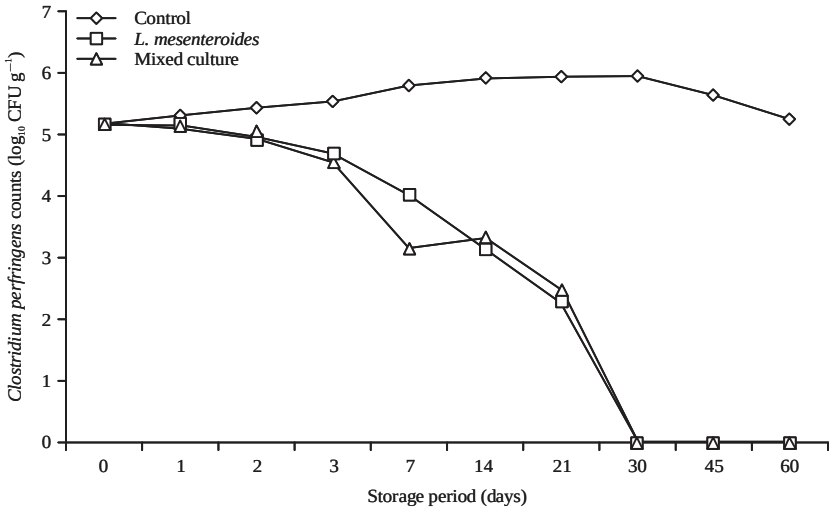

Fig. 9: Behavior of *Clostridium perfringens* during storage of processed cheese supplemented with probiotic metabolites at 7±2°C for 60 days. Mixed culture: *L. curvatus*, *L. acidophilus* and *L. mesenteroides*

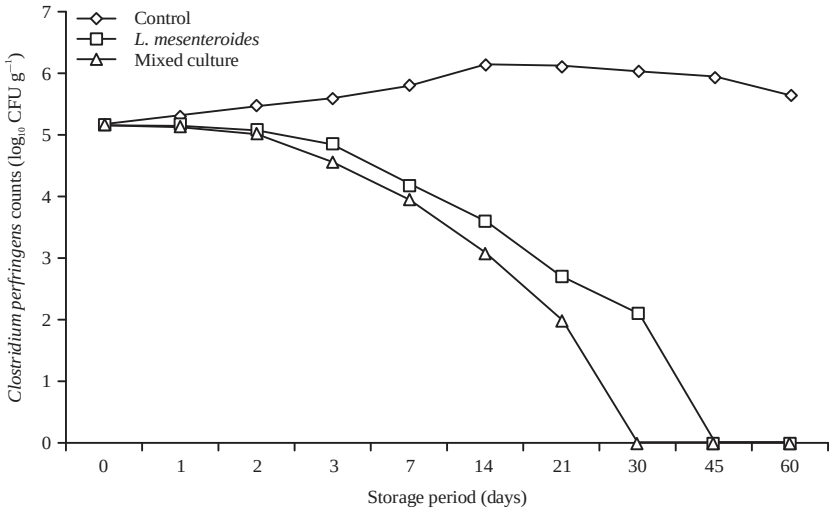

Fig. 10: Behavior of *Clostridium perfringens* during storage of processed cheese supplemented with probiotic metabolites at 25°C for 60 days. Mixed culture: *L. curvatus*, *L. acidophilus* and *L. mesenteroides*

On the contrary, survival of *C. perfringens* could not be recovered after 45 and 30 days for supplementation of *L. mesenteroides* and mixed culture (*L. curvatus*, *L. acidophilus* and *L. mesenteroides*) metabolites when the cheese stored for 60 days at room temperature (Fig. 10).

Ennahar *et al.*[45] found that, the bactericidal activity of leuconocin seems to be targeting primarily *Listeria* strains. In addition *Clostridium* and *Bacillus* have been reported to be sensitive to this bacteriocin.

More recent, Devi and Halami[46] recorded that some pathogenic bacteria being sensitive to the leucocin include *Clostridium*.

CONCLUSION

It is recommended to use improvement of product quality and safety could be applying of Good Hygienic practices (GHP) and Hazard Analysis and Critical Control Point (HACCP) system. The other strategy for reducing or controlling these pathogens can be achieved through incorporation of some probiotics or its metabolites, which suppress or arrest these microorganisms and so to improve the hygienic quality and safety of the product.

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