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Review Article

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Effect of Probiotics and Natural Extracts on Hygienic Quality of Some Dairy Products

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Abstract

Dairy products are responsible of contamination with multiple microorganisms by different ways during handling, processing, and production, thus it would be unfit for consumption and form a public health hazard. Food borne diseases become a great trouble containing an extensive range of disorders caused by viral, bacterial, parasitic, or even chemical contamination of food. That reflected a problem due to the massive use of traditional antibiotics in human and animal diseases treatment and from this time, the persisted development of new programs of antimicrobial agents has become of rising importance to medicine. Probiotics not only used in treating gastrointestinal disease, but also used in food industry as natural antimicrobial substances which have a lot of bacteriostatic or bactericidal effect against different food borne pathogens. Contamination by foodborne pathogen in foods represents a serious challenge that may lead to severe disorders as toxic infection, food poisoning and intoxication. These pathogens caused illness, mortality and product withdraws. In fact, attention the using of herbal products has increased during the recent decades. Plants play an significant role in human health, it is likely that 25% of modern medicine were originated directly or indirectly from herbs. Many compounds present in plants have been reported to be antimicrobial, allopathic antioxidants, biologically active and have bioregulatory properties that have been proven real anti-bacterial, insecticidal, antifungal, antiparasitic, anti-viral and antioxidants. Aromatic essential oils are used as flavouring and prevent the growth of microbial contaminants and mould in food industry. Recently, probiotic bacteria have been used as substitutes for antibiotics to treat or different prevent intestinal infections. Studies of using natural preservatives either alone or in combination with other alternative have been verified (as probiotic) not only to evaluate synergism but also to produce effective combinations. Finally, we will discuss the effect of probiotic and natural extract on hygienic quality of some dairy products.

KEYWORDS
Antimicrobial activity, Foodborne pathogens, Natural extract, Essential oil, Antibiotic resistance, Probiotic.

INTRODUCTION

Dairy products as milk, cheese and yoghurt are considered as healthy food due to its great value, but it can easily contaminate causing foodborne disease. Due to multidrug resistance, medicinal plants and probiotics have been used in wide range to overcome this problem.

Hygienic quality of raw milk and its dairy products

Although milk is recognized as one of the accepted environments of lactic acid bacteria that regarded generally as safe but can easily contaminated by other milk decomposition organisms which found in the milking equipment, the wash water, the atmosphere or on the staffs during milking and handling process (Sansanwal *et al*., 2017).

Various bacteria may contaminate raw milk that effect on both their safety and quality (Kable *et al*., 2016). Due to little awareness of food micro-organism, people consume raw milk with no caution (Jayarao *et al*., 2006) or even utilizing dairy products manufactured from this un heat-treated milk. In Egypt, processing of raw milk would occur around 80% of the dairy farmers (Holt *et al*., 2011). Till 5% of food-borne infectivity were correlated to the intake of milk and its products in developed countries (Ahmed and Shimamoto, 2014). For these developing countries, it could be worse where high ratios of milk contamination associated with lack of efficient preservation and unhygienic milk production (Garedew *et al*., 2012).

Milk and its products are wide media of many organisms, involving *Bacillus cereus* which affect badly on both market and public situation (Velázquez-Ordoñez *et al*., 2019).

The ability of *Yersinia enterocolitica* growth in raw milk and capability at low temperatures for a prolonged period characterised as a special public health significance. Therefore, it was important not to drink raw milk or consume its dairy products to avoid their hazard on human (Rahimi *et al*., 2014).

Public health importance of some foodborne disease

Dairy foodstuff is implicated in the submission of several foodborne disease-causing bacteria as *B. cereus* (Liu *et al*., 2020). Foodborne diseases (FBD) are progressing to be universal pub-

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lic-health disorder that has major effects on human health. On standard 600 million individuals, almost 1 in 10 humans in the world, fall sick annually after consuming contaminated food (WHO, 2019).

Listeria, *Campylobacter* spp., *Staphylococcus*, *Salmonella*, *Bacillus* spp., and *Yersinia* spp. act as etiological agents for food-borne diseases. Among all strains, two strains including *Yersinia* spp. and *Bacillus* spp. revealed higher antibiotic resistance (Koluman and Dikici, 2013). These food-borne pathogens influence principally gastrointestinal tract; therefore, the most notable defensive immune response is the actual immunity of the digestive tract. Certain symptoms for some disorders (i.e., intoxication) appear within 30 min to an hour.

The main factor of foodborne pathogens to subsist the acidic conditions (pH 3) of stomach for up to 2 hours before passage to the intestinal tract of the individual host is infectious dose of pathogenesis (Heredia and García, 2018). So, they threaten public health all over the world, especially in developing countries. Direction to use bacteriocin and probiotics as biological supplements have been provided (Mahmoudi *et al*., 2012).

Worries over the safety of biochemical preservers and undesirable customer feedbacks to either artificial or chemical preservatives have been increased. Further natural and safe options such as Eos approaches were provided to prolong the shelf life (Smith-Palmer *et al*., 2001).

Yersinia species

Yersiniosis is defined as a zoonotic illness with an expansive spreading and an important to public health which ranked as third-largest food-borne illness in the European Union produced by *Y. enterocolitica* (EFSA, 2015).

The European Authority of Food Safety (EFSA) recorded *Y. enterocolitica* as an important foodborne pathogen in Europe (Fois *et al*., 2018) which causes different human illnesses, involving critical gastroenteritis and persistent disorders as appendicitis, mesentery lymphadenitis and sepsis (Mastrodonato *et al*., 2018).

As member of the family Enterobacteriaceae, it characterised by e Gram-mutable or Gram negative, coccoid cells or rod-like, non-bacterium-forming rods, catalase positive and oxidase negative (Cabello, 2007). Other *Yersinia* spp. is movable on 22– 30℃ not on 37℃ which are permanently whipped except Y. pestis. It's able to stay alive in both aerobic and anaerobic cultivation conditions. Also, can grow up in atmosphere range between 0 to 45℃, being Optimal on 25–28℃ (Dekker and Frank, 2015).

Yersinia spp. were categorized as a vehicle for food-poisoning in humans (Soltan-Dallal *et al*., 2004). It was known to be spreaded to humans via drinking of infected milk (Thoerner *et al*., 2003) where enter the milk from faeces, bedlinen inappropriately cleaned teats and milk processing equipments infected with water or soil (Sharma *et al*., 2003).

Y. enterocolitica is linked with many immunologic and clinical symptoms which involve inflammatory bowel disease with bloody diarrhoea in serious cases. The extraintestinal signs are rarely erythema, mycotic aneurysm, pneumonia, reactive arthritis, axillary inflammations and endocardium infections (Menzies, 2010).

Bacillus cereus

B. cereus are rod shape, motile, spore-forming, Gram-positive bacteria that correlate with genus *Bacillus* (Montville and Matthews, 2005). It shaped like an oval endospore to below the undesirable surrounding conditions, so it may stay for longer periods even among maximum situations (Delbrassinne *et al*., 2012).

Under European Food Safety Authority, *B. cereus* recorded as pathogens in 77 epidemics and 17.1 % of cases with the report on food-borne outbreaks due to bacterial toxins (EFSA 2007).

B. cereus documented as a significant spoilage microorganism (Lücking *et al*., 2013). It caused two modes of food poisoning, emetic form which characterized by nausea, vomiting, short incubation period 1-6 hours and abdominal cramps and diarrheal form which characterized by long incubation period 8-16 hours, abdominal cramps and diarrhea. In both types of symptoms commonly lasted less than 24 hours after onset but in little patient's symptoms might last longer (Kenneth, 2011).

Food poisoning is the greatest known health problem caused by *B. cereus*; its toxicity controlled by pathogen doses, the host variable and their strain that explains why a natural occurrence of *B. cereus* is not determining factor to the pathogenesis (Kamar *et al*., 2013). Etiology is varying from 2 to 22% of overall gastroenteritis instances (Dodd *et al*., 2017). Nonetheless, its occurrence is regard as misjudged because of progressive reasons; i) contamination of samples could be occurred by the existence of this bacteria in tests; ii) thermostable toxins which persevered during food storage by microbes that die in the gastrointestinal tract produce symptoms making hard to define the cause; iii) *B. cereus* symptoms frequently are low-slight symptoms in cases that do not need medicinal aid and stay unobserved in data;; and iv) in hospitals, from 45% to 60% of digestive tract diseases are not crystal clear (Glasset *et al*., 2016).

B. cereus are capable to elaborate multi-drug resistance (MDR) which improves the difficulty of infections treatment (Sadekuzzaman *et al*., 2015). Yet, the occurrence of multidrug resistance caused by the huge misuse of antibiotics (Tule and Hassani, 2017).

Other approach to MDR

The spread of antibiotic-resistant bacterium establishes a fundamental public hygiene interest in foodstuff as different pathogens can be transmitted by food chain (Lupo, *et al*., 2014). Antibiotics have multiple uses as animal growth promoters, prophylactic and therapeutic functions. Multi-drug resistance is progressed due to the massive abuse of antibiotics (Tule and Hassani, 2017; Jans *et al*., 2018). So safer, released of artificial preservatives and healthier food have been inquired by customers as these synthetic additives are frequently believed to be dangerous and carcinogenic (Moro *et al*., 2015). Intended for that, there are influences all over the world for eliminating the antibiotic therapy in animal diets and providing environmental choices as an alternate to drugs used in different diseases. So that, scientist searched for different approach to overcome the multidrug resistance by using of essential oils and probiotics as natural antimicrobial alternatives.

Aromatic plants and essential oils

Aromatic plants have been labelled as plant varieties with specific tastes and/or aromas. This significance due to their volatile components in designated essential oils with distinctive characteristics. So, they could be used in various purposes. For example, in domestic and pharmaceutical manufacturing. These composites are secondary metabolites accumulated in leaves, roots, seeds and flowers (Forlin, 2012). These biological functions related to defence processes against extreme ultraviolet (UV), infections and herbivorous (Costa, 2015).

There are several subsections of the plant as herbs and spices, which are used in food stuff (Librán *et al*., 2013). Recently, these compounds were discovered to have antimicrobial activities with potential function in food preservation (Calo *et al*., 2015).

Black seeds (Nigella sativa Linn.) constitutes one of the therapeutic herbs which can be used as innate feed substitutes to enhance performance and moderate the prevalence of improving antibiotic-resistant bacteria in human beings (Doyle, 2001). They also have antiviral and anti-bacterial effects (Abdel–Shafi, 2013).

Cinnamon essential oil (CEO) was declared as a proper source of antifungal and antimicrobial activity (Paudel et al., 2019). Its action is mainly related to cinnamic acid, eugenol and cinnamaldehyde (Siripatrawan, 2016). The ability of CEO against various food-borne pathogens in dairy product have been explained in several studies (Cava-Roda et al., 2010 and Aliakbarlu et al., 2013)

Black cumin EO can cause reduction of growth of *Salmonella* enteritidis, *L. mononocytogenes*, *E. coli* and *S. aureus* by concentration of 0.1% in a culture medium (Hassanien *et al*., 2014)

It has been noticed antibacterial action of the clove oil effect against a varied series of bacteria especially those associated with foodborne illness and having antifungal, antioxidant, antimutagenic, antiallergic and anticarcinogenic properties (Cordery *et al*., 2018).

Pomegranate and its by-products are rich with ellagitannins, which accumulates in the large intestines and relates with intestinal microflora causing inhibition of pathogenic microorganisms as *Staphylococcus aureus* and clostridia and does not affect probiotic lactobacilli and bifidobacteria. (Bialonska *et al*., 2009). It have antimicrobial effect against various bacterial species like *B. cereus*, *E. coli*, *C. perfringens*, *S.* Typhimurium, *S. aureus* and *L. monocytogenes* (Aal-Tay, 2015) .

Thyme essential oil (TEO) has antibacterial impact against four bacterial strains as *S.* Typhimurium, *P. aeruginosa*, *E. coli* and *S. aureus* (Hachana *et al*., 2019).

Clove, Thyme, Pomegranate peel extracts have antimicrobial activity against *B. cereus* (Liu *et al*., 2015). Clove Oil has antibacterial activity on *Bacillus cereus* capable of reducing the bacterial count totally almost or less 5000 mg/l. (Siddiqua *et al*.,2015)

Fennel, Cumin, Marjoram, Black seeds, Thyme and Mint showed antibacterial effects against six *Bacillus* spp. including *B. cereus* to prevent the spoilage of food stuff products (Özcan *et al*., 2006). Other studies used Oregano and Thyme in combination are more effective in the antimicrobial activity against pathogenic *Bacillus cereus*, *Pseudomonas aeruginosa* and *Listeria monocytogenes* than when used singly (Gutierrez *et al*., 2008).

Carvacrol and Thymol, respectively, showing activity against the bacteria as *Escherichia coli*, *Pseudomonas*, *Salmonella*, *B. cereus*, *Aeromonas* spp., *Listeria monocytogenes*, *Proteus*, *Shigella*, *Staphylococcus aureus*, the moulds and yeasts (Davidson *et al*., 2012).

Essential oils (EOs) are recognized as sweet-smelling oily fluids established of a mix of volatile composites of minimal molecular-weight groups as terpenoids, phenylpropanoids, and sulfur-nitrogenous mixtures. Addition to the usual procedures, new approach like microwave, super critical extraction and ultrasonic were examined (Dima and Dima, 2015).

Antimicrobial action of essential oils against *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella* serovars and *Shigella dysenteriae* were well-verified in the lab studies (Burt, 2004). Bacteria develop resistance against essential oils less than to antibiotics due to their antimicrobial effects. Nevertheless, essential oils can disturb the sensory characteristics. Thus, the right essential oils must be provided according to the type of food. Additional physical techniques as high hydrostatic pressure, electric fields and mild heat also can be used in conjunction with specified essential oils against infective bacteria (Cherrat *et al*., 2014).

In plant extracts and EOs, phenolic compounds, such as eugenol, thymol, carvacrol, or ingredients such as myrcene, linalool, menthol, camphene and sabinene are the constituents that produced in higher concentrations and related to antifungal and antimicrobial activity (Moro *et al*., 2013; Asensio *et al*., 2015).

Another critical trait in consumption of plants or their ingredients in food stuff are toxicity. Even though, aromatic plants utilized in the food industry were applied for a prolonged time, there is absolutely no usual toxicology data as acceptable daily intake (ADI) or detected side effect (Negi, 2012). In opposition, FDA categorized the EO herbs as materials Generally Recognized as Safe (GRAS) (FDA, 2016).

Probiotics

They are living microorganisms that when given in sufficient quantities, grant health benefits in host (Hill *et al*., 2014). They witnessed a growing interest in research in the last few decades because of its therapeutic effect of dairy products which reduce symptoms associated with chronic diseases (Atwaa *et al*., 2020).

They are functional microorganisms such as lactobacilli and bifidobacteria which help the receiving by improving the intestinal bacterial equilibrium; these live microbial feed additions assistant certainly with the intestinal lining, improving their microbial balance. As a result of their observed health profits, probiotic was gradually involved into a chains of products including milk powders, ice cream, cheeses, yogurts in addition to frozen dairy puddings (Desmond *et al*., 2005)

Probiotics and prebiotics improve gut microbiota balance by improving the growth of useful microbes and preventing harmful bacteria growth (immunostimulant), besides inducing biogenics production such as immunopotentiators and antibacterial substances (Mitsuoka, 2014).

Bifidobacterium and *Lactobacillus* genera are frequently used as probiotics in fermented dairy food (Kirpich and McClain, 2012), while additional bacteria such as *Leuconostoc* spp., *Enterococcus* spp., and some yeasts like *Saccharomyces* spp. were used as probiotics (Didari *et al*., 2014).

Bacteria and yeast are the highest popular microorganisms employed as probiotics. In the main, *Escherichia*, *Bifidobacterium*, *Enterococcus*, *LactoBacillus* and *Saccharomyces*: these four bacterial species and one yeast genre used in whole preparations. Their action via distinct mechanisms which control the function or composition of gut microflora and furthermore modifying immune reactions and host epithelial (Sanders *et al*., 2013).

Organic acids such as lactic acids and acetic which produced by Lactic acid bacteria (LAB) afford acidic pH state and supress the growth of Gram-negative bacteria as *Salmonella*, which are delicate to the acidity conditions also they are considered as the main antibacterial complexes responsible for the repressive action of probiotics against microorganisms (De Keersmaecker *et al*., 2006; Makras *et al*., 2006).

Lactic acid bacteria produce bacteriocins which although have a limited range of action and principally work against further narrowly related bacteria, but certain bacteriocins also they are effectively against foodborne disease-causing bacteria like *L. monocytogenes* (Nielsen *et al*., 2010). Bacteriocins can terminate target cells either by pore advances or by suppression of cell wall combination (Hassan *et al*., 2012).

During food manufacturing, probiotics should not create textures, off-flavours or any abnormal odours (Mattila-Sandholm *et al*., 2002). Microencapsulation for being the one of the latest and highly efficient approaches has extensive performs on probiotic persistence, principally from properties of security against difficult ecological restrictions and regulated release. Main material is compressed in the food category in an array type covering. Proteins, lipids and polysaccharides are the best used extensively materials to encapsulation in food requests. Low manufacturing expenses will remain the challenge facing the upcoming formulation skills and probiotic development (Solanki *et al*., 2013).

Across genetic engineering, we can improve the influences of alive strains (for example, for genetically improved *LactoBacillus* spp., it could resist viruses, strengthen proteolytic activity, enhance metabolism, or digest complex carbohydrates), and also produce totally other probiotics (Cummins and Ho, 2005).

The attention of advanced molecular procedures may guarantee the accurate identification of different genus of probiotics, quality assurance and safety. Even so, in the light of our massive unawareness of gut ecology, natively improved probiotic bacteria cannot be employed in human studies, unless we completely recognize the difficult biological residues that have involved in the human being.

Synergism between Probiotic and EO

Although botanic extracts EOs have been used as a natural antimicrobial food preservative, they must be estimated either alone or in combination with other preservative like probiotic bacteria to create effective compounds and to detect whether there is synergism or not (Pol *et al*., 2002).

In addition to the positive effects of probiotics, scientist did significant efforts in the production and development of fermentative foods which contain probiotic microorganisms. Various species of Lactobacilli and Bifidobacteria were found in over 90% of probiotic products (Hadad Khodaparast *et al*., 2007).

Some researchers declared that using of herbal Eos in fermentative dairy products with different concentrations change the action of starter bacteria (Simsek *et al*., 2007). Low viability of these bacteria was one of the general argues in processing and production of probiotic products due to their sympathy to problematic situation within foodstuffs, effective enzymes of small intestines and stomach. A standard limit of probiotic product must range from10^6 to 10^7 CFU/g active and live probiotic microorganisms while consumption according to FAO (Joint *et al*., 2002).

CONCLUSION

Nowadays, in health care manufacturing, antibiotics are commonly used against bacterial contaminations. Bacterial resistance has been occurred due to repeated use of antibiotic or even misuses, which threats many people with public bacterial infections. So, scientist and researchers made a heroic effort to find a substitute to antibiotics used in bacterial infection for treatment or even prevention. Probiotics, especially *Bifidobacterium* and *Lactobacillus* were one of their legal proceedings which need massive clinical trials to identify dosages, administration, and probable strains regimes for identifiable modes of disorders or infection. Natural preservatives as essential oils and some natural extracts have been demonstrated in food industry due to their major inhibitory activity against foodborne pathogens and spoilage microorganisms.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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