Original Research Article

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Effect of potassium sorbate and lysozyme on *Bacillus cereus* growth in laboratory Manufactured Domiati cheese

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ABSTRACT: Two of the most commonly used preservatives (potassium sorbate (E202) and lysozyme (E1105)) have been assessed to study their effect on Bacillus cereus growth in Domiati cheese. Domiati cheese with 5% sodium chloride, prepared from laboratory pasteurized milk and infected with approx. 105cells/ml of *B. cereus* culture, containing concentrations of 0.0, 0.3 and 0.6% potassium sorbate and 0.0, 0.025, 0.05% (0.0ppm, 250ppm and 500ppm) lysozyme, were kept at 30°C. Results showed that potassium sorbate reduced the count of *B. cereus* compared to lysozyme and control samples. The reduction percentage of *B. cereus* was higher by using potassium sorbate (0.6%) concentration at the end of second week of storage. On the other hand, 500 ppm of lysozyme has a greater effect than 250 ppm of lysozyme. In conclusion, the addition of potassium sorbate during Domiati cheese manufacture is a better antimicrobial preservative for *B. cereus* count reduction in this type of cheese.

KEYWORDS: *Bacillus cereus*, Domiati cheese, lysozyme, potassium sorbate, preservative.

1. Introduction

Recently, there has been an increasing need for natural and healthier foods, and the food industry has focused on less intense processing to control sustainable processing costs [1, 2]. In Egypt, all socioeconomic classes widely consume Domiati cheese, which is defined by the Egyptian Standards for Domiati cheese 1008 - 3 / 2005 [3], as the fresh or ripened soft cheese obtained after curdling the fresh milk or concentrate or mixture from its fresh or the drier products and pasteurized or treated by any thermal coefficients equivalent to pasteurization[2]. Cheese is a rich food with nutrients, which is deteriorated by microbiological, enzymatic or chemical changes, which creates the need for preservatives to be added to stop or delay nutritional losses by controlling microbial growth and to extend the shelf life of foods. Furthermore, foodborne illness outbreaks have been reported due to making cheese from sub-pasteurized milk [4], as there is an established belief that using raw milk for cheese making allows the cheese to acquire more ripened flavor at a much shorter time with high intensity [5] Among the most frequently spore-forming hazardous food poisoning bacteria *B. cereus* cause the illness (emetic form) by producing emetic toxins (cereulide), which is performed in food [6], and is more likely to survive the gut environment, while enterotoxins are produced after consumption of large amounts of cells, resulting in diarrheal form [7]. *B. cereus* is ubiquitous in nature, and its spores are frequently found in rice, cereals, spices and dairy products [8]. Its capacity to form biofilms presents a challenge for the food industry, which may be initiated by the relatively hydrophobic spores that easily attach to pipelines and surfaces in the processing plant and are difficult to remove [9]. Survival and even germination of spores stimulated by milder heating regimes during processing [10]. It is resulting in premature spoilage of products [11]. In the food industry, evaluation of these preservatives is important for quality assurance purposes as well

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as for human safety [12]. More effective with less intense preservatives needed for product innovation [13]. Natural preservatives are chemical compounds derived from plants, animals, and microorganisms, that are commonly related to the host defense system [14, 15]. and processed meats, cheese and other dairy products[14], which targets mainly Gram-positive bacteria by invading cell-wall component (peptidoglycan) to be easily accessible to the enzyme [16], although some sensitive Gram-negative bacteria have been reviewed [17]. Sorbic acid was also reported to be a potent antimicrobial nucleophilic compound with a hydrophobic nature, providing minimal toxicity due to its rapid metabolism via mechanisms like those of fatty acids [4], provides a rise in pH approximately 0.1 to 0.5pH units depending on the amount, pH and type of product [18]. They are extensively used in the making of cheese, yoghurt and sour cream against bacteria and other spoilage organisms (yeast, mold and selected bacteria) [13]. Ultimately, controlling the growth of *B. cereus* is important, this study was to determine the effect of addition of potassium sorbate and lysozyme as preservatives on the growth of *B. cereus* in manufacture of Domiati cheese.

2. Material and methods

2.1. Preparation of B. cereus [19]:

The culture of *B. cereus* used in this study was isolated and well identified using molecular identification from strains isolated by [20] in the Faculty of Veterinary Medicine, Assuit University, Egypt. Broth culture of tryptic soya broth (TSB) containing polymyxin β used to enrich the *B. cereus* strain for 24-30 h at 30°C. Serial dilutions followed by enumeration of microorganisms by surface plating technique using mannitol egg yolk phenol red agar (MYP). Inoculated plates were incubated at 30°C for 24 h. The colony forming unit/ ml for each organism was calculated.

2.2. Manufacture of Domiati cheese treated with both potassium sorbate and lysozyme [21]:

Domiati cheese samples were manufactured in the laboratory from laboratory pasteurized milk (63°C/30 min) with added 5% sodium chloride. The broth culture of *B. cereus* was added to the warmed pasteurized milk (40°C) to yield appprox.105cfu/ml. Accordingly, 5 batches of cheese were prepared as follows; a negative control cheese (untreated), two potassium sorbate treated cheese (0.30 and 0.60%potassium sorbate) and two lysozyme-treated cheese (250ppm and 500ppm lysozyme) batches. Potassium sorbate and lysozyme were added just before addition of rennet. Then the salted sorbate and lysozyme-treated samples were thoroughly mixed with rennet 10-15 ml/1 lb milk. The coagulation occurred in 2-3 hours at about 38°C. All treated batches and control were kept at 30°C in their whey and subjected to microbiological examinations at day zero (within 2 hours after curdling), 1, 2, 3 days and then weekly till spoilage.

2.3. Microbiological examinations:

Ten-fold serial dilutions of each sample were prepared using sterile peptone water (0.1%) and examined for:-

Enumeration of B. cereus:- [21]

From the prepared diluted samples, 0.1 ml of each dilution was transferred and evenly spread over a dry surface of Mannitol egg yolk phenol red agar (MYP) using a surface plating technique. Inoculated MYP agar plates were incubated at 30°C for 24 hours. Colonies showing pink color and surrounded by a precipitated zone of lecithinase activity were counted, and then the plates were reincubated for a further 24 hours before being counted.

Results and Discussion

Static agents, commonly found in foods have been identified as preservatives considering their ability to control the growth of microorganisms, and being nontoxic if eaten [22], they are considered adjuncts to good sanitation and hygiene [23]. Also, [23] reviewed that according to the

Table 1: The *B. cereus* count (cfu/g) inoculated in Domiati cheese with different concentrations of potassium sorbate and lysozyme stored in room temperature(30°C).

Time	**Untreated (control) samples**	**Treated with potassium sorbate**		**Treated with lysozyme**	
		0.3%	0.6%	250ppm	500ppm
Zero time	1×10^6	1×10^6	1×10^6	1×10^6	1×10^6
1st day	55×10^6	38×10^6	11×10^6	18×104	$9\times10^2$2
2nd day	12×10^6	3×10^6	2×10^6	9×10^6	7×10^6
3rd day	8×10^6	2×10^6	1×10^6	12×10^7	8×10^7
1st week	3×10^6	3×10^5	1×10^5	8×10^8	1×10^8
2nd week	1×10^6	4×10^4	1×10^4	-*	-*

*The cheese samples become contaminated with mold.

Code of Federal Regulations, when a food preservative is used in a food product, its common name (e.g., potassium sorbate or sorbic acid) should be listed on the product label and its function should be indicated by an explanatory description (e.g., “to maintain freshness,” “to extend shelf life,” or “as a preservative”). [24] showed that sorbates are considered potent preservatives against a wider spectrum of food spoilage microorganisms (yeast, mold and many bacteria) by inhibiting cell growth and multiplication as well as germination of spore-forming bacteria. It is regarded as a naturally occurring unsaturated fatty acid is completely safe concerning health and has the lowest allergenic potential of all food preservatives [23]. [25] stated that Potassium sorbate is a potassium salt version of sorbic acid which is under US FDA regulations generally regarded as safe (GRAS) for use in food, permitted as a food additive (E202) under the European Union, however, it is not listed in Japanese Agricultural Standards, and not explicitly listed as an approved additive in CANDA guidelines. World Health Organization set the acceptable daily intake for sorbate at 25 mg/kg body weight intake per day [23], and they concluded that the relative harmlessness of sorbates and their relative superiority in safety compared to other chemical additives. Results recorded in Table 1 showed that potassium sorbate in general reduced the count of *B. cereus* compared to lysozyme and control samples. Additionally, obtained results (Table 1) demonstrated that a gradual decrease in the numbers of *B. cereus* occurred in the potassium sorbate treated samples along the days of storage until the control sample became unfit for consumption after 14 days (deterioration of the control sample and growth of yeast and mold). Also, it emphasized that the higher concentrations of potassium sorbate 0.6% reduced rapidly than the 0.3%, however obtained results (Table 2) revealed that the reduction rate in samples treated with a concentration of 0.6% potassium sorbate was not very distinct compared to samples treated with 0.3% of potassium sorbate, therefore, economically, using 0.3% potassium sorbate is sufficient to inhibit the growth of *B. cereus* and recommended. In cheese preservation, amounts of sorbic acid permitted and applied range from 0.05 to 0.30%. Close results in sterile milk and ice cream were stated by [26, 27], in sterile milk. The inhibitory effect of the potassium sorbate was reported by [28] to be the greatest against *B. cereus* in raw milk. These results were coincided with [29] which stated that potassium sorbate 0.39% one of the preservatives can inhibit the growth of B. cereus at pH 6.6 utilized. Potassium sorbate is one of the typical preservatives used to control the growth of *B. cereus* within food products [30]. Also, the anti-mycotic effect was noticed in potassium sorbate-treated samples compared to control samples and lysozyme-treated samples. Potassium sorbate is effective against yeast, and mold [18]. Lysozyme has received more attention because of its considerable antimicrobial action against a variety of microorganisms, it has a significant reduction in Total Bacterial count (TBC) recorded by [31] in feta cheese for lysozyme 250 and 500ppm, which supported its role in food preservation and safety in particular for cheese that showed by [32]. For ripened cheeses, lysozyme may be used as a preservative according to [33] and the current European Union standards [34], with an estimated content ranges between 100 and 350 mg per kilogram in cheese as reviewed by [35].

In the case of lysozyme, Table 2 pointed out that a sharp decrease in the reduction rate numbers of *B. cereus*

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Table 2: The reduction percentage of the *B. cereus* inoculated in Domiati cheese with potassium sorbate and lysozyme stored in room temperature (30°C).

Time	Treated with potassium sorbate		Treated with lysozyme	
	0.3%	0.6%	250ppm	500ppm
Zero time	0	0	0	
1st day	30.9	80	99.67	99.99
2nd day	75	83.33	25	41.66
3rd day	75	87.5	-*	-*
1st week	90	96.66	-*	-*
2nd week	96	99	-*	-*

∗There is no reduction percentage due to increase the count of *B. cereus* in treated samples compared to control sample.
∗∗The cheese samples become contaminated with mold

(99.99%) was recorded in the lysozyme-treated samples 500ppm, and (99.67%) for lysozyme 250ppm. Also, Table 1 clarified that the organism achieved the minimum count on the 1st and 2nd day in the lysozyme-treated samples, while after the 2nd day lysozyme (250ppm and 500ppm) had no inhibitory effect on the *B. cereus* count that showed an increase in numbers of bacteria up to 8 x 108 and 1 x 108 cfu/g, respectively by the end of 1st week. Similar results were obtained by [36] that indicated that some Bacillus spp. completely inhibited by lysozyme, but *B. cereus* showed a slightly higher resistance to lysozyme inhibition action. These findings were verified by studies done by [32] who confirmed that the exact mechanism of lysozyme resistance is not fully understood and may vary according to the bacterial strain or species. These results were in line with those reviewed by [37] for lysozyme 200 μg mL^{-1}, which confirmed the resistance of the strain to lysozyme, however, they noticed the rapid breakage of chains of cells as a result of the treatment did indicate some effect of lysozyme on the cell walls. Moreover, the obtained results were confirmed by [38] in Bergey's Manual of Determinative Bacteriology, they stated that *B. cereus* is characterized by its ability to grow in 0·001 % lysozyme, and to be highly resistant to egg-white lysozyme. These results were nearly similar to a study done by [39] for lysozyme 22.4 mg/L, when inoculated model cheeses made of raw milk, and proved that no significant differences were found between *B. cereus* spores counts of controls and lysozyme controls.

Conclusion

B. cereus one of food poisoning bacteria can be transmitted through soft cheese as Domiati cheese. Potassium sorbate and lysozyme are food preservatives that are safe for human health and have a great effect on pathogenic bacteria. By the end of this article, the results showed that adding of potassium sorbate had an inhibitory effect against *B. cereus*.

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