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Research Paper

ANTIMICROBIAL PROPERTIES OF SOME BIOACTIVE COMPOUNDS AGAINST PATHOGENIC BACTERIA IN SOFT CHEESE

Ahmed Hassan Ali Saad[1], Ehab Mohamed Salama[2], Amany Mahmoud Shalaby[3], Asmaa Atef Mohammed Abd Ellah[3] and Alaa Abdou Helmy Abd El Gwad[1]

[1]Department of Food Hygiene and Control,
Faculty of Vet. Med., Suez Canal University,
[2]Department of Food Hygiene and Control,
Faculty of Vet. Med., Al-Arish University,
[3]Department of Food Hygiene,
Animal Health Research Institute, Port Said Branch,
Egypt.

Abstract

This study investigated the antibacterial effect of probiotic strain (*bifidobacterium bifidium*) and essential oils (dill and moringa) on some pathogenic bacteria including *Staphylococcus aureus, E. coli* and *Salmonella typhimurium* in soft cheese during the storage period at 4±1°C. Plain cheese and cheese with *bifidobacterium bifidium*, dill oil (1:128) and moringa oil (1:128) were prepared and inoculated with10^3 cfu/ml pathogenic bacteria and stored for 10 days at 4°C. Samples were taken at zero time, 2nd, 4th, 6th, 8th and 10th days of storage for sensory evaluation and bacterial count. The probiotic cheese had the highest score in the sensory evaluation. The microbiological results showed inhibition in the *Staphylococcus aureus* count at the 6th and 8th days of storage in cheese fortified with dill and moringa E.Os and with probiotic, respectively. At the 10th day of storage *E. coli* was not detected in probiotic cheese and *Salmonella typhimurium* was not detected in dill and probiotic cheese samples.

Key words: *Essential oils, Dill, Moringa, Bifidobacterium bifidium., Staphylococcus aureus, E. coli, Salmonella typhimurium, soft cheese*.

INTRODUCTION

Since Ancient Egypt, cheese is considered as one of the most important, economic and palatable food for the Egyptians and other countries. It provides the consumer with essential nutrients, such as proteins, lipid, minerals and vitamins **[1].** Soft cheese is a suitable medium for the spoilage microorganisms due to its high moisture content and nutritional constituents causing off-flavor and visible changes in texture and color **[2].**

Among the pathogens, *Listeria monocytogenes, Staphylococcus aureus,* and *Salmonella typhimurium* are frequently associated with outbreaks occurring in food and specially in cheese **[3].** In general, the use of plants extracts or their EOs in cheeses had a great interest **[4].** Dill (Anathum graveolens) essential oil had been recently used as natural and safe anti pathogenic agent in cheese manufacture **[5].** Moringa oleifera is one of the world most useful plants. It is grown in tropical nations for its leaves, fruits, and root, as well as for food and medical purposes **[6]**. Probiotic bacteria such as *bifidobacterium bifidium* have been shown to possess antagonistic activity against some foodborne pathogens such as *Staphylococcus aureus, E. coli,* and *Salmonella typhimurium,* moreover they have health promoting properties **[7]**.

This study is aimed to investigate the viability of some pathogenic bacteria including *Staphylococcus aureus, E. coli* and *Salmonella typhimurium* in probiotic cheese and cheese with E.Os during 10 days of storage at 4±1^{o}C.

MATERIALS AND METHODS

1. Bacterial strains:

1.1*. Bifidobacterium bifidum* strain (DSM10140) obtained from Cairo-MIRCEN (Microbiological Resource Center), Faculty of Agriculture, Ain Shams University, Cairo Egypt. Strain was prepared according to **[8].**

1.2. Pathogenic strains: *Staphylococcus aureus*, *E. coli* and *Salmonella typhimurium* were reference strains which obtained from animal health research institute, Dokki, Giza, Egypt. Strains were prepared according to **[9].**

2. Essential Oils: Two essential oils; Dill (*Anethum graveolens*) and Moringa (*Moringa oleifera*), were purchased from Medicinal and Aromatic Plants Department, National Research Centre, Giza, Egypt.

3. Preparation of soft cheese: as described by **[10]:** the cheese was divided into 4 equal portions:- plain cheese, cheese with dill (1:128), cheese with moringa oil (1:128) and cheese with B. bifidium (not less than10^{9}cfu/g), then each portion was inoculated with the pathogenic bacteria (10^{3} cfu/ml).

4. Sensory evaluation of cheese: according to **[11].**

5. Microbiological examination: Preparation of cheese samples and Counting inoculated bacteria according to **[12]** at Zero, 2nd, 4th, 6th, 8th and 10th days of storage at 4^{0}C.

6. Statistical Analysis [13]: Data were analyzed by using SPSS software (release 20, IBM CO). Means were separated by Fisher's least significant difference test, and significance was tested at $\alpha = 0.05$.

RESULTS

The sensory evaluation of the prepared samples revealed that bifidobifidium cheese had the highest score in the sensory evaluation followed by dill then moringa E.Os (Table 1). **[14]** and **[15]** found that plain cheese without any additives has the highest scores in sensory evaluation. In contrary **[16]** revealed that panelists preferred the cheese preserved with 3 % moringa oil.

Meanwhile the antimicrobial results showed gradual decrease in the bacterial count until complete inhibition of *Staphylococcus aureus* at 6th day of storage for dill and moringa cheese samples and at 8th day for bifidobifidium cheese as demonstrated at Table (2). *Staphylococcus aureus* was more susceptible to essential oils more than probiotics **[17].** This may be due to its cell wall structure and outer membrane that could be destructed by the antibacterial substances resulting in leakage of the cytoplasm **[18].**

Both *E. coli* and *Salmonella typhimurium* were more susceptible to *bifidobacterium bifidium* more than the dill and moringa E.Os as revealed in Table (3) and Table (4). By the end of storage period (10th day of storage) the *E. coli* count was reduced by 99.2% and 94.6% in cheese samples with dill and moringa, respectively., while not detected in the bifidobifidium cheese sample. Whereas *Salmonella typhimurium* count decreased by the end of storage up to 96% in moringa cheese and not detected in both dill and bifidobifidium cheese samples.

Results Obtained by **[19]** showed that dill was able to inhibit *Staphylococcus aureus* by 0.9 and1.5 log cycle after 7 and 14 days of storage and inhibit *E. coli* O157:H7 by1.1 log cycle during 7 days of storage at 7°C ± 2 and it was not detected at 14 days. While he found that *Salmonella typhimurium* was not detected after 7 or 14 days in cheese samples fortified with dill.

Table (1): Sensory evaluation of treated cheese samples:

Cheese types	**Appearance (20)**	**Flavor (45)**	**Body & text. (35)**	**Total (100)**
Plain cheese	17.5	39.5	31	88
Dill	18.6	41.2	30.6	90.4
Moringa	18.2	40	29.8	88
Bifidibifidium	19.2	43.8	34.6	97.6

Table (2): The mean count of *Staphylococcus aureus* and its reduction % in the treated cheese samples throughout the storage period.

Types of cheese	Plain cheese		Dill		Moringa		Bifidobifidium	
Storage period	Mean ±SE	R%	Mean ± SE	R%	Mean ± SE	R%	Mean ± SE	R%
zero time	$2.2 \times 10^3 \pm 1 \times 10^{2\,f}$	-	$3.3 \times 10^2 \pm 1.8 \times 10^{2\,d}$	-	$6.8 \times 10^2 \pm 2 \times 10^{2\,d}$	-	$8.5 \times 10^2 \pm 9 \times 10^{e}$	-
2 day	$1.5 \times 10^3 \pm 2 \times 10^{2\,e}$	33.3%	$1 \times 10^2 \pm 4 \times 10^{c}$	67.6%	$1.9 \times 10^2 \pm 3 \times 10^{c}$	72.2%	$3.1 \times 10^2 \pm 3 \times 10^{d}$	63.2%
4 day	$6.9 \times 10^2 \pm 1 \times 10^{2\,d}$	68.3%	$1.5 \times 10 \pm 0.8 \times 10^{b}$	95.3%	$1.8 \times 10 \pm 1 \times 10^{b}$	97.4%	$1.4 \times 10^2 \pm 2 \times 10^{c}$	84%
6 day	$2.5 \times 10^2 \pm 2.8 \times 10^{a}$	88.5%	N.D ^{a}	100%	N.D ^{a}	100%	$2.8 \times 10 \pm 1 \times 10^{b}$	96.7%
8 day	$1.8 \times 10^2 \pm 3 \times 10^{c}$	91.6%	N.D	100%	N.D	100%	N.D ^{a}	100%
10 day	$6.8 \times 10 \pm 3 \times 10^{b}$	96.8%	N.D	100%	N.D	100%	N.D	100%

*treatments with different letters show significant difference (*P*-value <0.05)
Dill: cheese mixed with (Dill). **Moringa:** cheese mixed with (Moringa). **Bifidobifidium** cheese fortified with probiotic (B.bifidium). **Mean**: average of triplicate. **S E:** standard error of mean. **N. D.:** not detected. **R%:** Reduction % from initial count**.**

Table (3): The mean count of *E. coli* and its reduction % in the treated cheese samples throughout the storage period.

Types of cheese	Plain cheese		Dill		Moringa		Bifidobifidium	
Storage period	Mean ±SE	R%	Mean ± SE	R%	Mean ± SE	R%	Mean ± SE	R%
zero time	$2 \times 10^3 \pm 5 \times 10^{f}$	-	$1.4 \times 10^3 \pm 2 \times 10^{2\,f}$	-	$3.6 \times 10^3 \pm 2 \times 10^{2\,f}$	-	$2 \times 10^3 \pm 5 \times 10^{f}$	-
2 day	$1 \times 10^3 \pm 3.9 \times 10^{2\,e}$	47.5%	$7.5 \times 10^2 \pm 2 \times 10^{2\,e}$	44.4%	$1.2 \times 10^3 \pm 3 \times 10^{2\,e}$	68%	$5.9 \times 10^2 \pm 1 \times 10^{2\,e}$	69.8%
4 day	$5.2 \times 10^2 \pm 1 \times 10^{2\,d}$	74.6%	$3.9 \times 10^2 \pm 9 \times 10^{d}$	71.2%	$7.6 \times 10^2 \pm 1.9 \times 10^{2\,d}$	78.9%	$1.9 \times 10^2 \pm 3 \times 10^{d}$	90.3%
6 day	$2.3 \times 10^2 \pm 3 \times 10^{c}$	88.7%	$2.2 \times 10^2 \pm 4.8 \times 10^{c}$	83.8%	$4.9 \times 10^2 \pm 1 \times 10^{2}$ c	86.5%	$7 \times 10 \pm 4 \times 10^{c}$	96.4%
8 day	$1.8 \times 10^2 \pm 2.9 \times 10^{b}$	91.3%	$6.5 \times 10 \pm 2 \times 10^{b}$	95%	$3.1 \times 10^2 \pm 3 \times 10^{a}$	91.5%	$3.3 \times 10 \pm 1 \times 10^{b}$	98.3%
10 day	$5.8 \times 10 \pm 3 \times 10^{a}$	97%	$1 \times 10 \pm 0.5 \times 10^{a}$	99.2%	$2 \times 10^2 \pm 0.5 \times 10^{b}$	94.6%	N.D ^{a}	100%

Table (4): The mean count of *Salmonella typhimurium* and its reduction % in the cheese yoghurt samples throughout the storage period.

Cheese types	Plain cheese		Dill		Moringa		Bifidobifidium	
Storage period	Mean ±SE	R%	Mean ± SE	R%	Mean ± SE	R%	Mean ± SE	R%
zero time	$1.8x10^3$ $\pm 2 x10^{2\ f}$	-	$8.8x10^2$ $\pm 3x10^{2\ f}$	-	$1.2x10^3$ $\pm 1x10^{2\ f}$	-	$1.3x10^3$ $\pm 2x10^{2\ f}$	-
2 day	$8.8x10^2$ $\pm 3x10^{2\ e}$	50%	$4.1x10^2$ $\pm 8x10^{\ e}$	52.8%	$6.6x10^2$ $\pm 1x10^{2\ e}$	44.7%	$3.9x10^2$ $\pm 4x10^{\ e}$	70%
4 day	$4.8x10^2$ $\pm 1x10^{2\ d}$	72.5%	$2.3x10^2$ $\pm 5x10^{\ d}$	73.4%	$5.1x10^2$ $\pm 8x10^{\ d}$	57.7%	$1.2x10^2$ $\pm 1x10^{\ d}$	90.7%
6 day	$1.7x10^2$ $\pm 2x10^{\ c}$	90.2%	$8x10$ $\pm 2x10^{\ c}$	90.8%	$3.1x10^2$ $\pm 3x10^{\ c}$	74.3%	$4.5x10$ $\pm 2x10^{\ c}$	96.5%
8 day	$1.2x10^2$ $\pm 1x10^{\ b}$	93%	$2.2x10$ $\pm 1x10^{\ b}$	97.4%	$1.5x10^2$ $\pm 4x10^{\ b}$	87.9%	$1.5x10$ $\pm 0.5x10^{\ b}$	98.8%
10 day	$3.5x10$ $\pm 2x10^{\ a}$	98%	N.D ^{a}	100%	$4.8x10$ $\pm 3x10^{\ a}$	96%	N.D ^{a}	100%

DISCUSSION

However, their antimicrobials properties, the utilizing of essential oils is limited because of their influence on the cheese`s organoleptic properties **[20].**

Essential oils acts weakly on the Gram positive bacteria such as *Staphylococcus aureus*, while Gram negative bacteria such as *E. coli* and *Salmonella typhimurium* are less sensitive due to the lipopolysaccharide barrier in the outer membrane of Gram negative bacteria **[21].**

The cell wall of Gram negative bacteria is more resistant to the EOs. The *E. coli* cell wall does not allow the hydrophobic molecules to enter as readily as *Staphylococcus aureus*; thus, EOs are less able to affect the cellular growth of the *E. coli* **[22].**

The mechanisms of action of the EOs include cell wall degradation, damaging cytoplasmic membrane, destruction membrane proteins, decreased adenosine triphosphate synthesis and increased permeability resulting in leakage of ions , other cellular contents and death **[23]**.

CONCLUSION

The results demonstrated the capability of *B. bifidum* and E.Os to inhibit the growth of *Staphylococcus aureus, E. coli* and *Salmonella typhimurium* during the storage of soft cheese. Presence of pathogenic bacteria possess a risk for public health. Therefore, It`s a mandatory to ensure the hygienic standard during manufacture and

storage for ensure production of safe and high quality soft cheese. Moreover, It`s necessary to encourage consumers to utilize dairy products preserved by natural substance for their safety and health impacts.

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