

ABSTRACT

Mona Sabry Ali: Biocontrol of Pathogens in Soft Cheese Using Bacteriophage. Unpublished M.Sc. Thesis, Department of Food Science, Faculty of Agriculture, Ain Shams University, 2018.

The study aims to throw some light on the recent health safety situation of some local white soft cheese varieties towards certain pathogenic species and identification them and some of their specific phages to practically assess the potential biocontrol of any pathogenic microbe that may be found in a certain market white soft cheese variety.

To achieve this study, a total of eighty different white soft cheese samples, 20 from each type namely, Baramilli, Domiati, Kariesh and Tallaga were randomly during the period of May - June 2014 from various markets located at Cairo. Samples were transported under aseptic conditions to the laboratory. Moreover, five sewage water samples were obtained from Fac. of Agric., Ain Shams Univ., and Shoubra EL-Kheima station of drinking and sewage water. The external surfaces of sampling were scrubbed with alcohol, flaming and taps were flushed. The obtained samples were taken in sterile amber glass bottle of 250 ml capacity and directly transferred to the Virology Lab., Agric. Microbiol. Dept., Fac. of Agric., Ain Shams Univ. in refrigerated container and then maintained at 4-8 °C. The microbiological analysis was carried out within 12 h of sampling, including bacteriophage isolation. Furthermore, Kariesh cheese was made from pasteurized skimmed buffalo's milk warmed at 40° C, inoculated with 2% of freshly activated bacterial yoghurt starter culture and then divided into 5 equal portions. The 1st portion was the control. The 2nd, 3rd, 4th and 5th portions were contaminated with equal levels (1%) *Salmonella typhimurium* suspension containing 10⁵ colony forming unit (CFU)/ml followed by adding phage suspension 10⁸ plaque forming unit (PFU)/ml at the levels of nil, 1, 2 and 3% respectively. All portions were separately incubated at the same temperature up to curdling. The curds were cut and individually filled into stainless steel moulds lined with

cheese cloth and consolidated by a slight pressure for 24 h. The blocks of curd were then cut, dry salted using 2% NaCl (w/w), packaged into plastic containers and at refrigerator $5\pm 2^{\circ}\text{C}$ for periodically analyses along 14 days.

The surveying results indicated that, Baramilli cheese possessed the highest infection level (70%) with pathogens followed by Domiati or Kariesh (50% for each), while Tallaga cheese contained the lowest level of contamination with pathogens (40%). Twenty % of infected Baramilli cheeses were contaminated with one pathogenic specie, 20% with two species and 30% with three or four pathogenic species (30% for each). On the other hand, 30% of positive Domiati cheese samples were infected with one pathogen specie, while the rest infected samples (20%) were found to be contaminated with two pathogenic species. Nevertheless, all infected Kariesh cheese was observed to be contaminated with two pathogenic species. Moreover all infected Tallaga cheese was found to be contaminated with either one (20%) or two pathogenic strains (20%). Most cheese infected with *E.coli* was found in Baramilli cheese (60% with a log count ranged from 1.49 to 2.60 CFU/g) followed by Kariesh cheese (50% with a log count fluctuated from 1.66 to 2.62 CFU/g), Tallaga cheese (30% with a log count fluctuated from 1.55 to 1.75 CFU/g) and Domiati cheese (20% with a log count ranged from 1.11 to 1.32 CFU/g). Likewise, most of staphylococci were detected in Baramili cheese (50% with a log count ranged from 2.65 to 3.71 CFU/g) followed by Domiati cheese (40% with a log count ranged from 1.59 to 2.80 CFU/g), Kariesh cheese or Tallaga cheese (20% for each of them with a log count fluctuated from 2.06 to 2.88 CFU/g for the former and 2.81 to 4.01 CFU/g for the latter). While, *Salmonella* sp. has been found to possess of 30% of infected Kariesh cheese followed by Baramili cheese (20%). Whilst, any of Domiati cheese or Tallaga cheese came in the last order (10% for each). Baramilli cheese was the sole cheese which was contaminated with *Listeria monocytogenes* in all surveyed white soft cheese studied.

Identificationally, detected pathogen species of SWSC using either biochemical methods or polymerase chain reaction (PCR) technique confirmed that, the detected *E.coli* was two strains namely *E. coli* O121 and *E. coli* O104:H21, *Salmonella* sp. was defined to strain of *S. typhimurium*. *Staphylococcus*. was found to be *Staph. aureus*. Moreover the strain of *L. monocytogenes* was stated.

Virologically, electron microscopy of *Salmonella* bacteriophage particles revealed that, the virus is long, curled contractile tail. The phage particle has an isometric head with diameter of about 91.11 nm and the tail has 23.07 nm in length. The phage assigned to family Myoviridae as indicated by the presence of long contractile tail. The *Salmonella* phage had highly stability at different temperature ranged from -20 °C to 42 °C for 7 days. The phage was gradually harmed as the thermal temperature increased, so that, full phage damage was occurred at 80°C or more. The viral infectivity was determined qualitatively by the spot test technique. The virus lost ability to lyses *Salmonella* cells at pH values of 9, 10, 11 or 12. It was active only at pH values of 4, 5, 6, 7 or 8. Spot test showing the bacterial lysis caused by virulent bacteriophage specific for *Salmonella typhimurium*.

The contamination of experimental Kariesh cheese with *S. typhimurium* led to weaken the ability of cheese curd to drain whey as explained from the dry matter (DM) content which decreased due to the presence of pathogen and increased by the pathogen elimination with bacteriophage, which resulted also to increase the protein /DM content. The ash content reduced by both reasons, namely the contamination with *S. typhimurium* and/or the spiking level of phage suspension. The presence of *S. typhimurium* slowed the LAB population and acid production by them.

In terms of health safety, although the number of pathogen microbe added was gradually reduced due to the acid developed by prolonging the cold storage period in the absence of phage, but it still present until the

end of experimental period. While, the pathogen was completely eliminated within 7 days of cheese age when the phage suspension (10^8 PFU/mL) has been spiked at the level of 1% at least.

Finally, as a conclusion, the spiking of Kariesh cheese milk with 1% *Salmonella typhimurium* phage suspension (10^8 PFU/ml) is quite enough to eliminate this microorganism when it present at the level of 1% suspension containing 10^5 CFU /ml.

Key words: *Salmonella typhimurium* identification by polymerase chain reaction (PCR), Morphology particles of *Salmonella* bacteriophage examination by transmission electron microscope, cheese properties, Kariesh cheese properties.

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LIST OF ABBREVIATION

%	Percentage
/	Per
APC	Aerobic Plate Count
AOAC	Association of Official Analytical Chemists
°C	Degree Celsius.
Co.	Company
CSP	Cold storage period
DM	Dry Matter
e.g.	Exempli gratia (for example)
<i>E.</i>	<i>Escherichia</i>
EOSQ	Egyptian organization for Standardization and Quality
<i>et al.</i>	And others
Fac.	Faculty
FDA	Food and Drug Administration ration
Fig.	Figure
GRAS	Generally Recognized As Safe
g	gram
<i>L.</i>	<i>Listeria</i>
LAB	Lactic acid bacteria
NaCl	Sodium chloride
NC	Not counted
ND	Not detected
nm	nanometer
No.	Number
PCR	polymerase chain reaction
PFU	Plaque forming unit
<i>S.</i>	<i>Salmonella</i>
sp.	Species
<i>Staph.</i>	<i>Staphylococcus</i>
SWSC	Surveyed white soft cheese

VIII

TA	Titratable acidity
w/w	Weight per weight