



ISOLATION, IDENTIFICATION AND BIOCONTROL OF *SALMONELLA* *TYPHIMURIUM* IN KARIESH CHEESE BY BACTEIOPHAGE

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ABSTRACT

The study aims to assess the possibility of biological control on one of the most serious pathogenic microbes that found to infect Kariesh cheese, namely *Salmonella typhimurium*.

To achieve this object, firstly a total of 20 Kariesh cheese samples were collected randomly from various markets located at Cairo and exposed to microbiological isolation and identification of *S. typhimurium*. The obtained results revealed that, *S. typhimurium* was detected in 30% of surveyed market Kariesh cheese according to the strain identified by polymerase chain reaction (PCR) technique. Secondary, five sewage water samples were obtained from Fac. of Agric., Ain Shams Univ., and Shoubra EL-Kheima station of drinking and sewage water for specific bacteriophage isolation and morphology particles of *Salmonella* bacteriophage was examined by transmission electron microscope. Third, pasteurized skimmed buffalo's milk was converted into experimental Kariesh cheese at 40°C by milk inoculation with 2% of freshly activated yoghurt bacterial starter culture and then milk was divided into 5 equal portions. The 1st portion considered as control. The 2nd, 3rd, 4th and 5th portions were contaminated with equal level (1%) of *S. typhimurium* suspension containing 10⁵ colony forming units (CFU)/mL, previously isolated from foregoing surveyed Kariesh cheese samples, followed by adding phage suspension,

from which isolated from sewage water, containing 10⁸ plaque forming units (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) and packaged into plastic containers.

Experimentally, there were proportional reductions in lactic acid bacteria (LAB) population as the level of phage spiked into cheese milk increased, as which the reduction rate of LAB count during cold storage period (CSP) prolonging was however declined.

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 stilled present until the end of experimental period. While, the pathogen was completely eliminated within 7 days of cheese age when the phage suspension (10⁸ PFU/mL) has been spiked at the level of 1% at least.

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.

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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.

INTRODUCTION

White soft cheese is one of the common delicious cheeses consumed in Egypt. There are many varieties of white soft cheese depend on the technique of manufacture, salt percentage and many other factors. White soft cheese is conventionally manufactured from cow or buffalo milk or a mixture of them according to the Egyptian cheese-making technology (Abd El-Salam et al 1976 and Abou-Donia, 1991). Production may be artisanal or industrial, depending on whether the cheeses are manufactured with raw thermized (heated below pasteurization level) or pasteurized milk (Abd-El Salam and Benkerroum, 2006 and Ibrahim and Sobeih, 2010).

Skimmed milk (Kariesh) cheese is one of the indigenous white soft cheese types in Egypt. Kariesh as well as Domiati chesses are the most popular varieties of soft cheese in Egypt, but the former is the oldest type manufactured since 3000 B.C. Kariesh cheese occupies a significant space in the consume cheese market. It composes of about 50% of white soft cheeses produced in Egypt (Abou-Donia, 1991; Hegazy et al 2012 and Fayed et al 2013 and 2014). Kariesh cheese is usually produced from skimmed milk using acidification coagulants containing about 70% moisture and not more than 10% fat (El Gendy, 1983; Fayed, 1986 and Fayed et al 2013 and 2014).

In the past traditional Kariesh cheese had been made on the farms from "Rayeb" milk. The latter is a naturally developed acidity coagulant during gravity creaming in earthenware containers. After skimming the sour cream, the "Rayeb" milk is drained in folded mats. Draining of the whey takes two or three days, or until the desired texture of cheese is obtained. Finally, cheese is cut into suitable pieces, then dry salted to taste. The salted cheese is left for a few more hours in the mat until no more whey drains out and is then ready to be consumed as fresh cheese. Resultant cheese is either consumed fresh or after pickling in available farm-house milk by-products, e.g. butter milk, "Murta", whey ... etc. (Fayed, 1986).

When, centrifugal separators are used, this cheese is conventionally produced by acid coagulation of mechanically skimmed milk by culturing with lactic acid bacteria. Skimmed milk results infe-

rior Kariesh cheese type due to lower fat content in separator's skimmed milk. The quality and composition of Kariesh cheese may vary considerably due to such factors affecting the quality and composition of the clotted skimmed milk, the method of manufacture, the time required to complete the drainage of whey, the quality of salt added and the method of handling the finished cheese (Fahmi, 1950; El-Gendy, 1983; Abou-Donia, 1984, 1991, 1999 a, b and 2008).

In spite of cheese was previously classified under "safe foods" but across ages, many reports of infections and the intoxications related to the consumption of contaminated cheese which poses a threat to public health and also quality defects in cheese leads to big economic losses (Lindqvist et al 2002).

From a health standpoint, Kariesh cheese continues to suffer from many microbial infections, including pathogens across ages. The pathogenic *Escherichia coli* O157H7 has been isolated from 19% out of 15 Kariesh cheese samples collected from Cairo & Giza governorate markets (El-Sayed et al (2011)). The logarithmic (log) mean values of *Staphylococcus aureus* count were 5.0 ± 0.2 CFU/g. Enterobacteriaceae were found in the examined samples with the log mean values of 4.5 ± 0.1 CFU/g. Moreover, *E. coli* was detected in the examined samples with log mean values of 4.3 ± 0.6 CFU/g. The prevalence rate of *Salmonella* spp. was 10% out of 60 samples (Ibrahim et al 2015). In another study, a total of 200 cheese samples (100 Kariesh cheese and 100 Domiati cheese) was collected randomly from different supermarkets and retailer shops in Cairo and Giza governorates. Samples were analyzed for total colony count (TCC), total coliforms, *E. coli*, *Staph. aureus*, *Bacillus cereus* and yeast and mold counts, as well as for the pathogens *E. coli* O157:H7 and *Salmonella* spp. Both Kariesh and Domiati cheese samples were found to be highly contaminated, having bacterial load exceeding the acceptable limit. The microbiological quality of both cheeses was judged as poor. Pathogenic bacteria (*E. coli*, *B. cereus* and *Staph. aureus*) were detected in some of the cheese samples (Hassan and Gomaa, 2016).

That in view is leading to report that hence Kariesh cheeses were contaminated with different types of microorganisms giving an indication of poor sanitary conditions may present a public health hazard to the consumers and emphasizes the need for improved hygienic standard, so strict hygienic measures, efficient heat treatment, and application of HACCP system should be performed.

Consumers dislike the use of chemical preservatives in their food, and with some there is an associated public health risk. This increases the pressure for these chemicals to be removed from food and for the adoption of more "natural" means of preservation. While there are a variety of approaches to using natural preservatives, the most often adopted approach to date has been to use bio-control (McLntyre et al 2007). The use of biological factors against various pathogenic and saprophyte microorganisms, especially pathogenic bacteria, is defined as a bio-control. The use of bacteriophage as a bio-control factor proposed shortly after the discovery of bacteriophage two times independently by d'Herelle (1917). Unfortunately, bacteriophage studies have been eliminated with the discovery antibiotics. Recently, the studies of use of bacteriophages as a bio-control factor (phage therapy) revived because of increasing antibiotic resistances. Usually, these studies are concentrated about food safety, especially food pathogens such as *E. coli* O157:H7, *Campylobacter*, *Salmonella* and *Listeria* causing epidemics of disease (Hages and Loessner, 2007).

Bacteriophages are natural enemies of bacteria and are suitable candidates for the environmentally friendly biocontrol of these pathogens. In recent years, researchers tended to bring new alternative to biological protective systems used in conservation of food and production of safe food. Use of bacteriophage against to pathogen bacteria in food was the most hopeful system in these methods about bio-control. Controls of bacteriophage for each pathogen species and subspecies and determination of phage-host originality are important because efficient bio-control was achieved. Researches concentrated on some food-borne pathogen bacteria such as *E. coli* O157:H7, *Campylobacter*, *Salmonella* and *Listeria*. In a consequence of these studies made as *in vitro* and *in vivo*, first commercial production of phage which will be used in foods was made in Netherlands. Also, it has been informed that use of phage is cost-efficient alternative as compared with other preservatives. This review, discussed application of bacteriophages as bio-control agents in food and advantages and disadvantages about uses of bacteriophages by taking into account antimicrobial characteristics of them (Kalkan et al 2011).

For that in view, the study aims to assess the possibility of biological control on one of the most serious pathogenic microbes that found to infect Kariesh cheese, namely *Salmonella typhimurium*.

MATERIALS AND METHODS

Materials

A total of 20 Kariesh cheese samples were randomly collected during the period of May - June 2014 from various markets located at Cairo. Samples were transported under aseptic condition to the laboratory.

Pasteurized buffalo's skimmed milk (0.5% fat and 8.5% solids not fat) was obtained from the herd of the dairy cattle at faculty of Agriculture, Cairo Univ., Egypt.

Five sewage water samples were obtained from Fac. of Agric., Ain Shams Univ., and Shoubra EL-Kheima station of drinking and sewage water. Before sampling the external surfaces 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.

Concentrated lyophilized yoghurt bacterial culture (YC-183) consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* (1:1) was obtained from Chr. Hansen's Lab A/S Copenhagen, Denmark.

Sodium chloride (NaCl) was obtained from El-Nasr for salt production Co.

Experimental procedure

1. Preparation of bacterial starter cultures

Preparation of YC-183 starter culture was carried out by dissolving 15 g of the lyophilized culture in 1 liter UHT skimmed milk and incubation at 40 ± 1° C up to curdling, whereat it usually coagulated throughout 3 h.

2. Isolation and identification of *Salmonella typhimurium*

Salmonella sp. was isolated and enumerated as CFU/g according standardized procedures for microbiological examination of food products as described by (AOAC, 2007). The 1st step of detection *Salmonella* sp. was carried out by inoculation with 25 g from cheese sample to 225 ml of buffered peptone water and incubated at 37°C for 18

h. The 2nd step performed in 10 ml tetra thionate broth medium and 1 ml from 1st step was added and then incubated at 37 °C for 24 ± 2 h. In the 3rd step, 1ml from 2nd step was poured in Petri dish and directly followed by xylose lysine deoxycholate (XLD) or Bismuth Sulphate agar medium (25 mL medium *per* plate) and incubated for 24 ± 3h at 37°C.

Salmonella typhimurium was identified by Polymerase chain reaction (PCR) (Bio rad T100 thermal circular) according to Official method described by Lim et al (2003).

3 .Preparation of *Salmonella typhimurium* suspension

Salmonella typhimurium suspension was prepared according to Fiorentin et al (2005).

4. Phage screening in sewage water in relation to isolated *Salmonella typhimurium*

Qualitatively, the crude lysate of *Salmonella* phages was assayed qualitatively using the spot test according to Borrego et al (1987). Three mL of nutrient broth medium containing 0.7% agar were inoculated with 1 ml of *Salmonella sp.* culture containing 10⁹ CFU/mL and then poured onto nutrient agar petri dishes after gently mixing. After solidification, 100 µL of crude *Salmonella* phage lysate were spotted on surface of media and incubated at 37°C overnight. The clear lysis zone in the sites of spots was recorded as indication the presence of phage.

Quantitatively, the isolation of viruses was carried out according to Othman (1997) by the double-layer technique using NA-Ca²⁺ agar inoculated with a selected propagative culture; the plates were incubated at 37 °C for 16 h. A single plaque was picked up and mixed with a fresh culture (O.D. 600 nm = 0.15) of the indicator strain, after lysis it was centrifuged at 3500 rpm for 15 min and then filtered through a 0.45 µm membrane, this procedure was repeated twice the titer of the phage suspension was defined as PFU/mL.

The crude lysate of *Salmonella* phages were assayed quantitatively according to Othman (1997) by the soft-agar overlay (double ager layer) method using nutrient agar medium inoculated with *Salmonella sp.* and incubated at 37°C for 16 h.

Morphology particles of *Salmonella* bacteriophage was examined using 2% uranyl acetate as negative staining Luftig (1967) and Othman (1997) by transmission electron microscope.

5. Preparation of *Salmonella* bacteriophage stock (single plaque)

Biologically stock phage lysate was obtained by the method reported by Othman et al (2008) using the single plaque isolation method. A large amount of the phage stock was obtained by propagation the phage by the liquid culture propagation method used by Othman et al (2008). To amplify the phages, 10 ml of an overnight culture of bacterial cells (10⁹ CFU/mL) and 1 mL of the phage (10¹¹ PFU/mL) were added to 500 mL of broth media and incubated with shaking at 37 °C for 5 h. After incubation, the bacterium-phage suspension was treated with 10 mL of chloroform to release any progeny phage which might still have been in the host cells, and the suspension was incubated for an additional 10 min with hard shaking. To remove bacterial debris, the suspension was centrifuged at 6000 rpm for 15 min, and the supernatant was withdrawn and filtered through 0.22-µm-pore-size filters. The phage lysates were stored at 4°C as described by Goodridge et al (2001).

6. Preparation of crude *Salmonella* phage suspension

250 mL Erlenmeyer flask containing 100 mL of nutrient broth medium was inoculated with 10 mL of the tested sewage water and 10 mL of *Salmonella* strain was mixed. The flasks were incubated at 37°C for 48 h with (LAB Line instruments Inc. model No.3526-4GMLB. After incubation the cultures were centrifuged at 6000 rpm for 15 min and the supernatant was collected into a clean flask. Chloroform was added with rate of 1:10 followed with vigorously shaking for 5 min. The crude lysate of the phages were obtained and assayed qualitatively and quantitatively according to Borrego et al (1987).

7. Manufacturing procedures of conventional Kariesh cheese

The procedure protocol of conventional Kariesh cheesemaking was applied as described by Fahmi (1950), Fayed (1986) and Fayed et al (2013). Pasteurized skimmed buffalo's milk was heated at 40°C, inoculated with 2% of freshly activated YC 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 units (CFU)/mL fol-

lowed by adding phage suspension 10^8 plaque forming units (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) and packaged into plastic containers (Fig. 1). Three replicates were carried out for each treatment.

Analytical methods

1. Chemical analyses

Dry matter (DM), fat, protein, ash and titratable acidity (TA) were determined according to AOAC (2007). The NaCl content was determined according to method of Van der Burg, which described by Kotterer and Munch (1978). The pH value was measured electrometrically using Lab. pH meter with a glass electrode, Hanna model 8417 digital pH meter.

2. Enumeration of lactic acid bacteria

Lactic acid bacteria count was determined using MRS agar according to De Man et al (1960). The plates were incubated at 35°C for 48 h.

3. Statistical analysis

The obtained data were exposed to proper statistical analysis according to statistical analyses system user's guide (SPSS, 1998).

RESULTS AND DISCUSSION

1. Safety situation of market conventional Kariesh cheese towards *Salmonella typhimurium*

Data illustrated in Table (1) indicated that *Salmonella typhimurium* was detected in 30% of surveyed market Kariesh cheese according to the strain identified by PCR technique and morphological shape pictured in Fig. (2 A&B).

Moreover, as could be observed in the data given in the Table (1) that, *S. typhimurium* had appeared negative behavior towards the Gram test. While it possessed catalase production. These results are in complete coincidence with those reported by Holt et al (1994).

Table 1. The occurrence percentage and biochemical characteristics of *Salmonella typhimurium* detected in surveyed Kariesh cheese during the period expended from May to July 2014.

Criteria	Result
Percentage of infected samples	30%
Biochemical characteristic	
Gram stain	-
Catalase	+
Growth temperature degree	
30 °C	++++
37 °C	+++
44 °C	+
51 °C	-
58 °C	-
65 °C	-
Sugar fermentation	
Glucose	-
Manitol	-
Arabinose	-
Gelatine liquefaction	-

Concerning the growth ability at different temperatures, *S. typhimurium* could not grow at a temperature higher than 44°C (Table, 1). The forgoing results agree with those reported in Holt et al (1994).

Regarding the possibly of the detected pathogens for sugar fermentation, the obtained results declared that, *Salmonella* sp. could not ferment sugar, namely glucose, mannitol and arabinose. Also it did not able to liquefy gelatin as present in Table (1). These results are in accordance with Holt et al (1994).

2. Characteristics of specialized bacteriophage of *Salmonella typhimurium*

Electron microscopy of *Salmonella* bacteriophage particles revealed that, the virus is long, curled non 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 (Fig. 3). The phage assigned to family Myoviridae as indicated by the presence of along contractile tail.

Furthermore, spot test showing the bacterial lysis caused by virulent bacteriophage specific for *S. typhimurium* (Fig. 4). Likewise, plaque assay showing identical morphological plaques of *S. typhimurium* (Fig. 5).

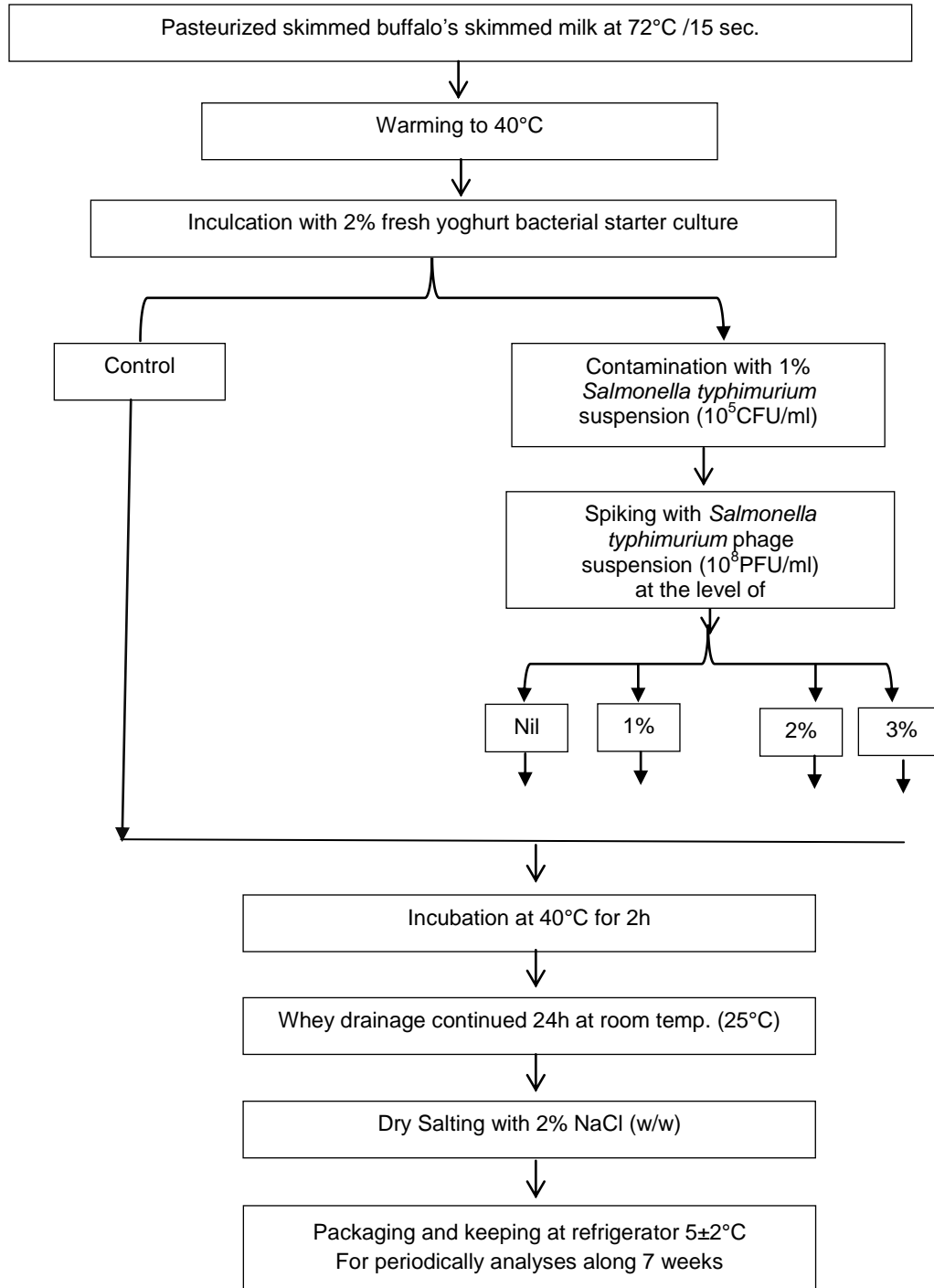


Fig. 1. Flow diagram of conventional Kariesh cheese making contaminated with *Salmonella typhimurium* and spiked with its phage



Fig. 2. Picture of *Salmonella typhimurium* colony by light microscope (A) and by polymerase chain reaction (PCR) technique (B).

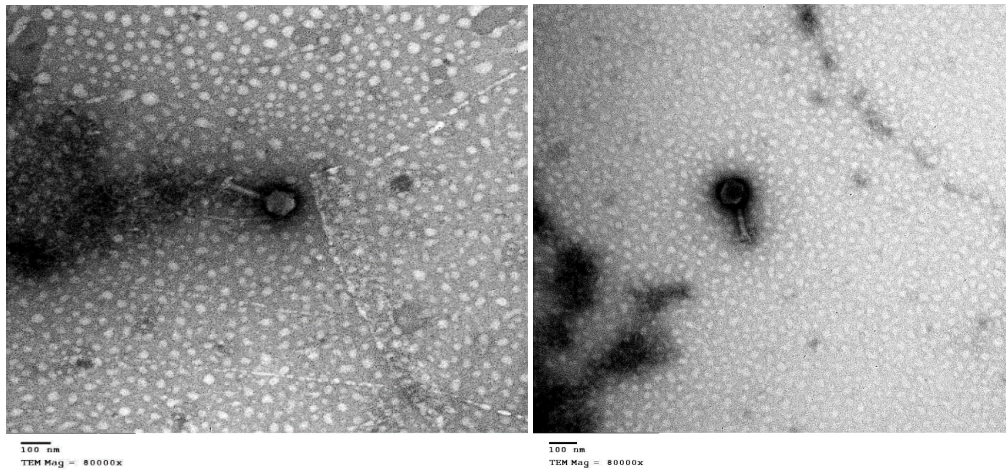


Fig. 3. Electron micrograph of purified *Salmonella* bacteriophage negatively stained with 2% uranyl acetate (Magnification X-80000)



Fig. 4. Spot test showing the bacterial lysis caused by virulent bacteriophage specific for *Salmonella typhimurium*



Fig. 5. Plaque assay showing identical morphological plaques of *Salmonella typhimurium*

Furthermore, the persistence study on the selected phage indicated its resistance ability towards a range of temperature degrees or pH values (**Table, 2**) making it could be suitable for the condition of Kariesh cheese manufacturing.

3. Biocontrol of *Salmonella typhimurium* in Kariesh cheese by bacteriophage

3.1. Microbiological quality of Kariesh cheese

Bacteriologically, there were proportional reductions in LAB population as the level of phage

spiked into cheese milk increased, as which the reduction rate of LAB count during CSP prolonging was however declined (**Table, 3**).

In terms of health safety, although the number of pathogen microbe added was gradually reduced due to the acid developed by prolonging the CSP in the absence of phage, but it stilled 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 (**Table, 3**).

Table 2. Spot test of *Salmonella* bacteriophage particles along different temperature degrees for 7 days and pH values for one day

Post survival (day)	Temperature degree (°C)				
	-20	4	24	37	42
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
4	+	+	+	+	+
5	+	+	+	+	+
6	+	+	+	+	+
7	+	+	+	+	+
	pH value				
Post survival for one day	4	5	6	7	8
	+	+	+	+	+

Table 3. Microbiological quality (log CFU/g) of Kariesh cheese as affected either by the contamination with 1.0% *Salmonella typhimurium* suspension (10^5 CFU/mL) and/or spiking with different levels of its specific phage suspension (10^8 PFU/mL) during cold storage period (CSP) for 14 days

Strain	CSP(Day)	Control	Contamination with 1% <i>Salmonella</i> suspension (10^5 CFU /mL)			
			Spiking level with <i>Salmonella typhimurium</i> phage suspension (10^8 PFU/mL)			
			Nil	1%	2%	3%
LAB	Fresh	5.78 ^{a,a}	5.69 ^{a,a}	4.62 ^{b,a}	4.36 ^{b,a}	3.89 ^{c,a}
	7	4.79 ^{a,b}	2.79 ^{c,b}	2.88 ^{c,b}	3.66 ^{b,b}	3.85 ^{b,b}
	14	1.77 ^{a,c}	1.77 ^{a,c}	1.90 ^{a,c}	1.76 ^{a,c}	1.92 ^{a,c}
<i>Salmonella typhimurium</i>	Fresh	ND	4.04	2.79	2.77	2.74
	7	ND	1.55	ND	ND	ND
	14	ND	1.30	ND	ND	ND

LAB: Lactic acid bacteria ND: Not detected. CFU: Colony forming unit. PFU: Plaque forming unit.

The letters before comma possess the factor of contamination with 1% *Salmonella* suspension. While those after comma possess the factor of spiking level with *Salmonella typhimurium* phage suspension. The means with the same letter at any position did not significantly differ ($P > 0.05$).

3.2. Physicochemical properties of Kariesh cheese

The results present in **Table (4)** indicated that, the dry matter (DM) contents of cheese varied significantly among either the contamination with *S. typhimurium* or the level of bacteriophage suspension spiked. Moreover, the letter of Duncan's test demonstrated that, the presence of *S. typhimurium* weakened the ability of cheese curd to drain whey while, the adequate control of pathogen with its bacteriophage has helped the curd to reject whey to be like the non-infected cheese curd (the control).

While, the prolonging of CSP to 14 days did not lead to any significant changes in the DM% of all cheese samples studied (**Table, 4**).

It is worthy to mention that, DM% of all samples are, indeed, in surrounding on the legal standard of **EOSQ (2005)**, which provided that the DM content of Kariesh cheese should be not less than 25%.

These results are in coincidence with those of Kariesh cheeses whether from street vendors or certain local brands regardless their ages reported by **Bakry et al (2011)** and **Fayed et al (2013 and 2014)**.

Concerning the protein /DM content, date given in **Table (4)** show that, except of the control, there was a significant increment in the protein /DM content associated with the ascending spiking level with bacteriophage suspension. This phenomenon could be ascribed to the fact of the killing action possessed the phage added on the pathogen strain and hence the acidity became better developed leading to more whey drainage followed by increase in the protein portion in the DM. There is another reason, but to a lesser degree, structurally phages consist of a nucleic acid genome enclosed within a protein or lipoprotein coat as described by **Guttman et al (2005)**. While the CSP along 14 days had no significant influence on this criterion.

Table 4. Physicochemical properties of Kariesh cheese as affected either by the contamination with 1.0% *Salmonella typhimurium* suspension (10^5 CFU/mL) and/or spiking with different levels of its specific phage suspension (10^8 PFU/mL) during cold storage period(CSP) for 14 days

Property	CSP(Day)	Control	Contamination with 1% <i>Salmonella</i> suspension (10^5 CFU /mL)			
			Spiking level with <i>Salmonella typhimurium</i> phage suspension (10^8 PFU/mL)			
			Nil	1%	2%	3%
Dry matter (DM)%	fresh	25.63 ^{a,a}	25.09 ^{b,a}	25.09 ^{b,a}	24.88 ^{b,a}	25.48 ^{ab,a}
	7	25.60 ^{a,a}	25.08 ^{b,a}	25.09 ^{b,a}	24.86 ^{b,a}	25.49 ^{ab,a}
	14	25.62 ^{a,a}	25.09 ^{b,a}	25.09 ^{b,a}	24.87 ^{b,a}	25.48 ^{ab,a}
Protein/DM %	Fresh	70.27 ^{d,a}	63.92 ^{c,a}	65.24 ^{c,a}	67.88 ^{b,a}	68.45 ^{a,a}
	7	70.24 ^{d,a}	64.09 ^{c,a}	65.25 ^{c,a}	67.01 ^{b,a}	69.38 ^{a,a}
	14	70.94 ^{d,a}	64.20 ^{c,a}	67.44 ^{c,a}	66.14 ^{b,a}	68.96 ^{a,a}
Fat %	Fresh	0.8 ^{b,b}	0.8 ^{b,b}	0.9 ^{a,b}	0.6 ^{b,b}	1.0 ^{a,b}
	7	0.7 ^{b,ab}	0.7 ^{b,ab}	1.0 ^{a,ab}	0.7 ^{b,ab}	0.8 ^{a,ab}
	14	0.7 ^{b,a}	0.8 ^{b,a}	0.8 ^{a,a}	0.8 ^{b,a}	0.9 ^{a,a}
Ash%	Fresh	1.877 ^{a,a}	1.877 ^{ab,a}	1.866 ^{bc,a}	1.875 ^{bc,a}	1.886 ^{c,a}
	7	1.876 ^{a,a}	1.867 ^{ab,a}	1.865 ^{bc,a}	1.867 ^{bc,a}	1.866 ^{c,a}
	14	1.887 ^{a,a}	1.877 ^{ab,a}	1.880 ^{bc,a}	1.874 ^{bc,a}	1.872 ^{c,a}
Salt %	Fresh	1.357 ^{a,a}	1.367 ^{b,a}	1.159 ^{c,a}	1.340 ^{a,a}	1.330 ^{a,a}
	7	1.324 ^{a,a}	1.324 ^{b,a}	1.148 ^{c,a}	1.368 ^{a,a}	1.387 ^{a,a}
	14	1.346 ^{a,a}	1.135 ^{b,a}	1.135 ^{c,a}	1.327 ^{a,a}	1.264 ^{a,a}
Acidity %	Fresh	0.54 ^{a,a}	0.46 ^{a,a}	0.43 ^{ab,a}	0.36 ^{b,a}	0.40 ^{ab,a}
	7	0.63 ^{a,a}	0.52 ^{a,a}	0.50 ^{ab,a}	0.47 ^{b,a}	0.54 ^{ab,a}
	14	0.70 ^{a,a}	0.56 ^{a,a}	0.72 ^{ab,a}	0.60 ^{b,a}	0.66 ^{ab,a}
pH value	Fresh	5.00 ^{a,a}	5.11 ^{a,a}	5.25 ^{a,a}	5.13 ^{a,a}	4.98 ^{a,a}
	7	4.40 ^{a,a}	4.60 ^{a,a}	4.50 ^{a,a}	4.60 ^{a,a}	4.70 ^{a,a}
	14	4.20 ^{a,a}	4.30 ^{a,a}	4.20 ^{a,a}	4.30 ^{a,a}	4.40 ^{a,a}

CFU: colony forming unit.

PFU: plaque forming unit.

The letters before comma possess the factor of contamination with 1% *Salmonella* suspension. While those after comma possess the factor of spiking level with *Salmonella typhimurium* phage suspension. The means with the same letter at any position did not significantly differ ($P>0.05$).

Regarding the fat content, due to the Kariesh cheese made from skimmed milk, the low fat content was slightly significant affected either by the level phage suspension added or the CSP of resultant product and did not exhibited any obvious trends.

With regard to the ash content of Kariesh cheese as a function of the spiking level with phage suspension data revealed that, similar to that occurred in the DM%, the ash content gradually declined as the phage level raised, nevertheless, the prolonging of CSP did not lead to any significant changes in the ash level of cheese.

While, The salt content in Kariesh cheese appeared different responses towards the spiking level with phage suspension, while the CSP caused nonsignificant differences in the criterion.

Moreover, the statistical analysis declared that the acid development was significantly slowed due to the presence of phage suspension at any level. Nevertheless the differences in the TA% of cheese along the CSP were not significant.

Furthermore, the pH value of cheese did not exhibit any significant changes whether among the spiking level of the phage suspension or along the CSP for 14 days.

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 a count as that gained when contaminated with 1% suspension containing 10^5 CFU *S. typhimurium* /mL. was added.

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عزل و تعريف والتحكم الحيوي لميكروب *Salmonella typhimurium* في الجبن القريش باستخدام البكتيروفاج

[138]

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بمقدار 2% من بادئ اليوجهورت الطازج النشط ثم قسم اللبن الى خمسة أجزاء متساوية. واعتبر الجزء الأول كعينه مقارنة بينما تم تلوين كل من الأجزاء من الثاني وحتى الخامس بنسبة 1% من معلق بكتيريا *S. typhimurium* (المحتوى على 10^5 خليه ميكروبيه/مل) وهو ذلك الميكروب المعزول مسبقا من عينات الجبن القریش المجمع في الدراسة المسحية . كما تم اضافة مستخلص الفاج (10^8 وحدة فاج/مل) المعزول من عينات مياه الصرف الصحي الى أقسام اللبن من الثاني إلى الخامس السابق تلوينها بمعلق بكتيريا *S. typhimurium* وذلك بنسب صفر ، 1 ، 2 و 3% من معلق الفاج على التوالي. ثم تحضين جميع الاقسام كل على حده على نفس درجة الحرارة حتى تمام التجبن. تم تقطيع الخثرة ونقلها كل على حده في القوالب الأستانلس ستيل المثقبة لتصفية الشرش وجرى التملح الجاف بنسبة 2% كلوريد الصوديوم ونقلت كل على حده ووضعت في عبوات بلاستيك لحفظها.

ولقد أوضحت النتائج في العينات التجريبية المصنعة بالمعمل وجود تراجع نسبي في اعداد بكتريا حمض اللاكتيك في العينات وكان ذلك يتناسب طرديا مع زيادة نسبة الفاج المضافة ، كما انخفضت أعداد بكتريا حمض اللاكتيك على مدار فترة التخزين. ومن ناحية السلامة الغذائية فقد حدث انخفاض تدريجي في غياب

الكلمات الدالة: التعريف بواسطة سلسلة تفاعل البوليميريز - الفحص بالميكروسكوب الالكتروني للفاج

الموجز

تهدف هذه الدراسة الى إمكانية التحكم الحيوي لميكروب يعد من أكثر الميكروبات المرضية تواجدا وشيوعا في الجبن القریش ألا وهو بكتريا *S. typhimurium*.

ولتحقيق هذا الهدف تم أولا تجميع 20 عينة من الجبن القریش عشوائيا من مختلف الأسواق المحلية بالقاهرة واجري الفحص الميكروبيولوجي لها بغرض عزل وتعريف بكتريا *S. typhimurium* المحتمل تواجدها بها. اوضحت النتائج المتحصل عليها أن *S. typhimurium* تواجدت في 30% من العينات المجمع من الأسواق وتم إجراء تعريف لها باستخدام تكنيك سلسلة تفاعل البوليميريز (PCR)، ثانيا تم تجميع 5 عينات من مياه الصرف من محطة مياه الشرب والصرف الصحي بكلية الزراعة جامعة عين شمس بغرض عزل البكتيروفاجات المتخصصة في مقاومة هذا الميكروب وفحصها بواسطة الميكروسكوب الالكتروني، ثالثا تم تطبيق ذلك على جبن قریش تجريبي مصنع بالمعمل من لبن فرز جاموسي مبستر حيث لقع اللبن عند درجة حرارة 40 درجة مئوية

السابقين وهما التلويث ببكتيريا *S. typhimurium* والنسبة المضافة من مستخلص الفاج. كما أدى وجود بكتيريا *S. typhimurium* الى بطء تكاثر بكتيريا حمض اللاكتيك وبالتالي انخفاض معدل إنتاج الحامض.

وفى النهاية يستنتج من ذلك إنه يمكن القضاء على بكتيريا *S. typhimurium* بالجبن القريش وذلك بإضافة 1% على الاقل من المستخلص المحتوى على الفاج المتخصص بتركيز 10^8 /مل،، لو كانت ملوثة بأعداد كمثل التي حدثت نتيجة إضافة 1% معلق من بكتيريا *S. typhimurium* المحتوى على 10^5 خليه ميكروبيه/مل.

الفاج لأعداد *S. typhimurium* المضافة كنتيجة للظروف الحامضية السائدة بالجبن على مدار فترة التخزين المبرد ولكنها ظلت موجودة حتى نهاية الفترة التجريبية (14 يوم) من التخزين، بينما اختفت تماما خلال سبعة أيام من التخزين المبرد للعينات المضاف لها معلق الفاج ولو بتركيز 1% وهو أقل تركيز مستخدم في البحث. كما أدى التلويث ببكتيريا *S. typhimurium* الى تقليل قابلية الجبن القريش لتصفية الشرش كما اتضح ذلك من نسبة المادة الجافة التي انخفضت نتيجة وجود الميكروب المرضي وزادت نسبة المادة الجافة نتيجة القضاء عليه بواسطة الفاج والذي أدى ايضا الى زيادة المحتوى البروتيني للمادة الجافة كما انخفض محتوى الجبن من الرماد لنفس السببين



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