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A Study On The Effect of Temperature, pH and *Capsicum annuum* Extract On The Viability Of *Listeria monocytogenes* In Kariesh Cheese

NESREEN ZH ELEIWA^{1*} AND ADEL H EL-GOHARY²

¹Tanta Laboratory, Animal Health Research Institute and ²Department of Hygiene and Zoonoses, Faculty of Veterinary Medicine, Mansoura University, Egypt

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ABSTRACT

A total of three liters of raw milk free from *Listeria monocytogenes* were inoculated with 1×10^7 CFU/ml. *Listeria monocytogenes* strain and used for manufacturing of kariesh cheese, then the cheese samples were classified into 10 groups to study the effect of storage temperature, pH and different concentrations of capsicum annuum extract (2% and 5%) on the viable count of the inoculated strain. The results revealed that there is no significant effect of storage temperature on the viability of the microorganism in control positive groups stored in both room and chilling temperatures. Meanwhile, a high significant difference was recorded in groups treated with capsicum annuum extract in both concentrations (2% and 5%).

The public health importances of *Listeria monocytogenes* and the preventive measures to control the viability of this microorganism in raw and dairy products were discussed.

Keywords: Kariesh cheese, Temperature, pH, *Capsicum annuum* extract and *Listeria monocytogenes*

Introduction

Listeria monocytogenes is a ubiquitous bacterium that causes significant foodborne disease with high mortality rates in immuno-compromised adults. In pregnant women foodborne infection can give rise to infection of the fetus resulting in miscarriage. In addition, the bacterium has recently been demonstrated to cause localized gastrointestinal symptoms, predominantly in immunocompetent individuals [1]. *L. monocytogenes* is a psychotropic microorganism that is capable of growing at refrigeration temperature and at relatively low pH. These characteristics make *L. monocytogenes* particularly difficult to control in food therefore; contamination could lead to high risk factor [2]. Raw-milk cheese has been identified in risk

assessment as a food of greater concern to public health due to listeriosis [3,4]. In recent years, studies have revealed that soft and semi-soft cheeses provide suitable conditions for the survival and development of this pathogen [5,6]. Among the effective means for inhibiting *Listeria* are high temperature, starter culture additives, the addition of lactic acid, bacteriocins, bacteriophages and irradiation [7-12]. The safety and shelf life of food ingredients can also be improved by application of novel technologies to avoid or delay microbial growth like packaging in controlled atmosphere, activated films, non-thermal treatments, irradiation, modified atmosphere packaging, and so on. However, most of these procedures may cause loss of organoleptic properties of foods and reduce consumer acceptability. Therefore, the consumer demands are increasingly focusing on minimally processed food products, with less use of synthetic additives and at the same time without compromising food safety. Although synthetic antimicrobials are approved in many countries, the recent trend has been for use of natural preservatives due to the adverse health effect of synthetic ones.

*Corresponding Author

Dr. Nesreen Zakaria Helmy Eleiwa

Tanta Labarotary, Animal Health Research Institute, Egypt.

Email: nesrnani@yahoo.com



Therefore, alternative sources of safe, effective and acceptable natural preservatives need to be explored [13]. Hot peppers, which belong to the plant genus *Capsicum*, are widely grown for their fruits, which may be eaten fresh (salads, baked dishes, salsa, pizza, etc.) or cooked, used as a dried powder, or processed into oleoresins. Paprika oleoresin, a viscous, dark red liquid, is prepared industrially by solvent extraction (most commonly employed is hexane) of the dried fruit and the subsequent removal of the solvent [14,15]. *Capsicum* oleoresins contain a complex mixture of essential oils, waxes, coloured materials (mainly capsanthin, capsorubin, zeaxanthin, cryptoxanthin and lutein), several capsaicinoids are commonly used as a pungent flavor in food, natural plant colour and pharmaceutical ingredient [16,17]. The reddish colouring matter of oleoresin is due to carotenoids (0.3% to 0.8% in fruit) [18]. Pepper fruits are a rich source of antioxidants [19-21]. They have a high level of vitamins C and E as well as carotenoids and xanthophylls [22].

The aim of this work is to study the effect of different concentrations of *Capsicum annuum* extract on the viable count of *Listeria monocytogenes* inoculated in kariesh cheese and the correlation between pH and storage temperature on the effectiveness of *Capsicum annuum* extract.

Materials and Methods

1- Sample:- Three liters of raw milk.

2- Strain in use: The well morphological, microscopically and biochemical identified strain of *Listeria monocytogenes* (NCTC7973/ATCC®35152) which obtained from the bacteriological laboratory of food hygiene department, Animal Health Research Institute, Dokki, Giza. To obtain a standardization of the inoculums, the bacterium was cultured in trypticase soya broth (TSB) for 18 h. at 35-37 °C. Aliquots were taken and serial decimal dilutions were carried out using fresh broth until an absorbance of 0.05 at 590 nm was reached, to give a standard inoculum of about 10⁷CFU/ml. Bacterial counts were confirmed by culturing on TSA plates, incubating at 37 °C for 24 h.

3- Extract used: *Capsicum annuum* extract with concentrations of 2 % and 5 % obtained from National Research Center. Dokki, Giza. The peppers were washed and their stems cut out. The vegetables were weighed and placed in a blender with an equal amount Of isopropanol (1:1 weight/volume). The mix was blended for 1 minute and then shaken for 15 min. Afterwards it was filtered through a large pore filter paper, and 15% (w/w) of active charcoal was added to the filtrate. Then the mix was gently shaken for 5 min and filtered again through a Whitman filter paper no.1. The separated solids were discarded and the clear filtrate was evaporated under reduced pressure at a temperature of 71 °C, a pressure of 46mbar, and a speed of 28rpm in order to remove the alcohol. Finally, the extracts were

stored at -20 °C until used [23].

The raw milk to be used in cheese making was firstly tested for the presence of *L. monocytogenes* before inoculation. Confirmed milk samples were inoculated with *L. monocytogenes* to obtain a final concentration of 1x10⁷ CFU/ml. Then manufacturing of cheese was done.

The cheese sample was classified into 10 groups:-

1st group: - control negative (kept in room temperature).

2nd group: - control negative, kept in chilling temperature (4±1 °C).

3rd group:- Free cheese sample with 2% *Capsicum annuum* extract, kept in chilling temperature (4±1 °C).

4th group: - Free cheese sample with 5 % *Capsicum annuum* extract (kept in chilling temperature (4±1 °C).

5th group: - control positive (inoculated with 1x10⁷ CFU/ml. *Listeria monocytogenes*) only and kept in room temperature.

6th group: - control positive (inoculated with 1x10⁷ CFU/ml. *Listeria monocytogenes*) only and kept in chilling temperature (4±1 °C).

7th group: - inoculated cheese sample + addition of 2 % *Capsicum annuum* extract and kept in room temperature.

8th group: - inoculated cheese sample + addition of 2 % *Capsicum annuum* extract and kept in chilling temperature (4±1 °C).

9th group: - inoculated cheese sample + addition of 5% *Capsicum annuum* extract and kept in room temperature.

10th group: - inoculated cheese sample + addition of 5 % *Capsicum annuum* extract and kept in chilling temperature (4±1 °C).

All the examined samples were kept in the recommended temperature and subjected to *Listeria monocytogenes* counting and pH recording at the time of investigation (every 48 h).

A. Organoleptic Examination: All Kareish cheese were judged organoleptically when fresh and every two days of storage for flavor (10 points), texture(10 points) and colour (10 points) according to [24].

B) pH determination:- The pH was measured with an Orion pH_meter at 25±1 °C [25].

C) *Listeria monocytogenes* count:- Samples were tested using the official standard ISO procedure 11290-1 for detection and enumeration methods [26].

For enumeration of *L. monocytogenes*: samples (10 g) were homogenized in 90 ml. of 0.1% peptone water using a stomacher and a 0.1ml. Portion of the homogenate was plated on the surface of Agar Listeria according to Ottaviani & Agosti (ALOA) chromogenic agar (AES Chemunex España, S.A., Barcelona, Terrassa). All plates were incubated aerobically at 37 °C for 48 h. and the Typical *L. monocytogenes* colonies (which are blue-green with a surrounding precipitate) were counted.

4. Statistical analysis: - The experiments were set up with 3 replications. All data were subjected to analysis of variance technique (ANOVA) test. The significance of the differences between groups was determined at the 0.01 probability level, by the F-test. The F-protected least significant difference (LSD) was calculated at the 0.01 probability level [27].

Results and Discussion

The trait that makes *Listeria monocytogenes* difficult to control during food processing is that it can multiply over a wide range of temperatures, especially at refrigerator temperatures [28]. Temperature is the most important hurdle encountered by *L. monocytogenes*. It grows in a temperature range of +0.4 to 45 °C, while the optimum growth temperature lies between 30 and 37 °C. *Listeria monocytogenes* is reported to exhibit viability at pH levels between 4.6 and 9.6 with an optimum at 7.1 [29].

The results shown in Table 1, revealed that the group of kariesh cheese treated with capsicum annuum extract 2 % showed a higher organoleptic score (27,24, 22,18 and 14) along the experiment days rather than other groups. *Capsicum annuum* extract 2 % showed a pungent taste and flavor which is palatable to most of consumers. These results were agreed with [30], who found that the ripe fruits of red pepper (*Capsicum annuum* L.) are widely consumed as vegetables and used as food colorants because they are a good source of the red carotenoids capsicum red pigment and capsorubin.

Table 2, showed that temperature did not show any influence on the bacterial behavior in control positive groups kept in both room and chilling temperatures as the mean values of *Listeria monocytogenes* were within 1×10^7 CFU/gm along the experiment days. These results can be explained by the prolonged lag phase of the bacteria in these conditions. Some authors determined that the lag phase for *L. monocytogenes* strains at 35 °C was about 2h [31]. Therefore, growth during the early stages of cheese manufactured would not be expected. The results were in agreement with that reported by [32,33].

The rate of inactivation of the pathogen may also be affected by a combination of factors in cheese such as proteins, lipids, water content, and pH. In addition, lactic flora during fermentation plays an important role in flavor, aroma and texture formation [34,35].

The results illustrated in the same table showed powerful effects of *Capsicum annuum* extract in both concentrations (2 % and 5 %) in days 2, 4 and 6 from the experiment ($4.21 \times 10^5 \pm 0.65 \times 10^5$ and $3.31 \times 10^5 \pm 0.52 \times 10^5$); ($7.33 \times 10^4 \pm 1.85 \times 10^4$ and $2.63 \times 10^4 \pm 0.41 \times 10^4$) and ($3.62 \times 10^5 \pm 0.73 \times 10^5$ and $1.90 \times 10^5 \pm 0.36 \times 10^5$) in groups 7th and 8th respectively. While in groups 9th and 10th in the same days, the recorded results were ($2.13 \times 10^5 \pm 0.40 \times 10^5$ and $2.53 \times 10^5 \pm 0.37 \times 10^5$); ($6.80 \times 10^4 \pm 1.37 \times 10^4$ and $6.03 \times 10^4 \pm 1.29 \times 10^4$) and ($4.01 \times 10^5 \pm 0.57 \times 10^5$ and $1.90 \times 10^5 \pm 0.36 \times 10^5$) respectively. While in the 8th day signs of deterioration was appeared. Such results can probably be due to the inhibitory action of the various hydroxycinnamic acids that are known to be present in the *Capsicum annuum* extract. These acids include the *p*-cinnamic, *o*-cumic, *m*-cumic, ferulic, and caffeic acids, which can be classified as weak lipophilic acids. It is also known that the antimicrobial activity of these compounds is due to their non-dissociated molecules [36]. The finding results agreed with those reported by [33, 37, 38]. When analyzing the effect of pH on the inhibition of *Listeria monocytogenes* as in Table 3, It was observed that a higher

Table 1: Organoleptic properties of examined Kariesh cheese samples.

Time of storage (days)	Flavour (10 points)				Texture (10 points)				Colour (10 points)				Total points (30)			
	1 st	2 nd	3 rd	4 th	1 st	2 nd	3 rd	4 th	1 st	2 nd	3 rd	4 th	1 st	2 nd	3 rd	4 th
	gp	Gp	Gp	gp	gp	gp	Gp	gp	gp	Gp	gp	gp	gp	gp	gp	gp
0	8	8	9	7	7	8	9	9	8	8	9	8	23	24	27	24
2	5	7	8	7	6	6	8	8	6	6	8	6	17	19	24	22
4	3	4	7	7	4	5	8	8	5	6	7	6	12	15	22	21
6	3	3	5	6	3	3	6	7	3	4	6	5	9	10	18	18
8	3	3	5	4	3	3	4	5	3	4	5	4	9	10	14	13

gp:- group

inhibitory effect was obtained when pH values were lower, being the highest effect at pH 3.94 ± 0.02 ; 3.09 ± 0.01 ; 4.09 ± 0.01 and 4.00 ± 0.01 in the 4th day of the experiment specially in groups 7th, 8th, 9th and 10th. The results revealed nearly similar results with those reported by [39, 40]. The pH and polarity are the most prominent factors influencing the effectiveness of a food antimicrobial. Polarity is related to both the ionization of the molecules and the concentration of any alkyl side groups or hydrophobic parent molecules [41]. Table 4, showed the correlation coefficient between pH, temperature and *Capsicum annum* extract versus viability of *Listeria monocytogenes* which showed high significant correlation specially in groups (5) and (8) (+0.58 and + 0.62) respectively. These results agreed with those reported by [42, 43], who reported that the increase of antimicrobial activity in essential oils and plant extract at low

pH values may be due to the increased hydrophobicity of extract constituents at acidic pH levels, and thus are better dissolved in the lipidic phase of the bacterial membrane. The implementation and maintenance of good manufacturing practices (GMPs) together with sanitation standard operating procedures (SSOPs) all along the food chain will contribute to the effective eradication of persistent strains of this pathogenic bacterium [44].

Arrese and Arroye-Izaga [45], reported that the presence of *Listeria spss.* in raw milk cheese suggests that the cheese making process and the hygiene weather at milking or during cheese making could be insufficient. The reduction in counts at the end of the fermentation observed might be attributed to the growth of lactic acid bacteria (especially, during fermentation)

Table 2: Effect of temperature and capsicum annum extract on the viability of *L. monocytogenes* (1×10^7 CFU/ml milk) in the examined Kariesh cheese samples.

Storage time	Zero time	2 nd day	4 th day	6 th day	8 th day
Groups	Mean $\pm$ S.E*	Mean $\pm$ S.E	Mean $\pm$ S.E	Mean $\pm$ S.E	Mean $\pm$ S.E**
5 th group	$1.01 \times 10^7 \pm 0.13 \times 10^7$	$1.05 \times 10^7 \pm 0.19 \times 10^7$	S	S	S
6 th group	$1.00 \times 10^7 \pm 0.15 \times 10^7$	$1.04 \times 10^7 \pm 0.20 \times 10^7$	S	S	S
7 th group	$9.01 \times 10^6 \pm 1.87 \times 10^6$	$4.21 \times 10^5 \pm 0.65 \times 10^5$	$7.33 \times 10^4 \pm 1.85 \times 10^4$	$1.21 \times 10^5 \pm 0.18 \times 10^5$	$1.14 \times 10^6 \pm 0.19 \times 10^6$
8 th group	$1.05 \times 10^6 \pm 0.22 \times 10^6$	$3.31 \times 10^5 \pm 0.52 \times 10^5$	$2.63 \times 10^4 \pm 0.41 \times 10^4$	$3.62 \times 10^5 \pm 0.73 \times 10^5$	$2.26 \times 10^6 \pm 0.39 \times 10^6$
9 th group	$6.11 \times 10^6 \pm 1.42 \times 10^6$	$2.13 \times 10^5 \pm 0.40 \times 10^5$	$6.80 \times 10^4 \pm 1.37 \times 10^4$	$4.01 \times 10^5 \pm 0.57 \times 10^5$	$4.34 \times 10^6 \pm 0.72 \times 10^6$
10 th group	$1.25 \times 10^6 \pm 0.31 \times 10^6$	$2.53 \times 10^5 \pm 0.37 \times 10^5$	$6.03 \times 10^4 \pm 1.29 \times 10^4$	$1.90 \times 10^5 \pm 0.36 \times 10^5$	$2.96 \times 10^6 \pm 0.44 \times 10^6$

S.E * = Standard error

** = High significant differences ($P < 0.01$)

S: Spoiled samples (changes in colour ,odor and texture)

Table 3: Statistical analytical results of pH in the examined Kariesh cheese samples.

Storage time	Zero time	2 nd day	4 th day	6 th day	8 th day
Groups	Mean $\pm$ S.E*	Mean $\pm$ S.E	Mean $\pm$ S.E	Mean $\pm$ S.E	Mean $\pm$ S.E**
1 st group	4.70 $\pm$ 0.01	4.01 $\pm$ 0.01	S	S	S
2 nd group	4.30 $\pm$ 0.01	4.11 $\pm$ 0.01	S	S	S
3 rd group	4.12 $\pm$ 0.01	4.08 $\pm$ 0.02	3.99 $\pm$ 0.01	S	S
4 th group	4.32 $\pm$ 0.01	4.28 $\pm$ 0.01	4.01 $\pm$ 0.01	4.20 $\pm$ 0.01	S
5 th group	4.29 $\pm$ 0.02	4.18 $\pm$ 0.01	S	S	S
6 th group	4.17 $\pm$ 0.01	4.09 $\pm$ 0.01	S	S	S
7 th group	4.05 $\pm$ 0.01	4.08 $\pm$ 0.01	3.94 $\pm$ 0.02	4.23 $\pm$ 0.01	4.28 $\pm$ 0.02
8 th group	4.01 $\pm$ 0.01	4.22 $\pm$ 0.01	3.09 $\pm$ 0.01	4.25 $\pm$ 0.02	4.26 $\pm$ 0.01
9 th group	4.22 $\pm$ 0.01	4.11 $\pm$ 0.01	4.09 $\pm$ 0.01	4.01 $\pm$ 0.01	4.04 $\pm$ 0.01
10 th group	4.32 $\pm$ 0.01	4.21 $\pm$ 0.02	4.00 $\pm$ 0.01	4.14 $\pm$ 0.01	4.27 $\pm$ 0.01

S.E * = Standard error

** = High significant differences ($P < 0.01$)

S: Spoiled samples (changes in colour ,odor and texture)

Table 4: Correlation coefficient (r) between pH, temperature and capsicum annum extract vs. viability of *L. monocytogenes* at room temperature and chilling shelves.

pH at room temperature		pH at chilling shelves	
Groups	R	Groups	R
5 th group	+ 0.58**	6 th group	+ 0.38*
7 th group	+ 0.39*	8 th group	+ 0.62**
9 th group	+ 0.51*	10 th group	+ 0.40*

* = Significant correlation

** = High significant correlation

in turn produces bacteriocins, which led to the reduction in count of *Listeria monocytogenes* in cheese [45]. The increased microbial growth was observed in latent periods of storage, which resulted from recontamination of the product during packaging, handling and failure to maintain refrigerator temperature during storage [45].

Conclusion

Capsicum annum extracts are added at a substantial quantity to produce a characteristic taste of kariesh cheese, which could stand at long period without significant deterioration.

From these results, it may be concluded that it is possible to use this study to provide valuable information on the combined effect of pH and *Capsicum annum* extract concentration on the growth and inactivation of *Listeria monocytogenes*.

The future will see much - needed investigation of food application of naturally occurring antimicrobials, especially the effectiveness of essential oils, individually and in combination with other parts of plant extract, other effective essential oils and other food - processing techniques.

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Authors Column



Dr. Nesreen Zakaria Helmy Eleiwa: Senior Researcher, Food Hygiene Department, Animal Health Research Institute, Tanta Laboratory, Egypt. My Area of research Interest is food safety, microbiology, biotechnology, drug residues and Molecular biology. I am having 10 years of experience in research, I had 12 publications. I am having 11 years of experience in teaching and training the college student. My up-to-date research is the usage of natural antimicrobial essential oils in food control and preservation.



Prof. Dr. Adel Helmy Naguib El-Gohary working as a Professor of Zoonoses and Head of Hygiene and Zoonoses Department at Faculty of Veterinary Medicine Mansoura University – Egypt. My Area of research Interest is Animal Hygiene, Zoonoses, biotechnology, Microbiology. I am having 30 years of experience in teaching and 25 years of experience in research. I had 65 publications. I am a member of editorial board of few national and international research journals and I am a member of scientific committee of high supreme council for Animal Hygiene, Zoonoses and Animal welfare. At present, I associated with different universities as an External examiner of Animal Hygiene and Zoonoses.