

**ANTIBACTERIAL ACTIVITY OF GARLIC EXTRACT AND
DEHYDRATED GARLIC POWDER AGAINST *LISTERIA*
monocytogenes IN SYNTHETIC MEDIUM AND
SOFT CHEESE**

[43]

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ABSTRACT

The antibacterial effect and minimal inhibitory concentration (MIC) of garlic extract and MIC of dehydrated garlic powder (DGP) against *Listeria monocytogenes* were studied. Its use as an additional barrier in the hurdle technology concept against cross contamination by such pathogen during soft cheese processing was investigated. The agar well diffusion method (**Benkerroum and Sandine 1998**) and the method of **Hogg et al (1987)** were used to determine the antilisterial activity and minimal inhibitory concentration (MIC) of garlic extract and its powder respectively. Garlic extract showed antilisterial effect on plates of tryptose soy agar as revealed by inhibition zones ranged from 10 to 19mm diameter, depending on the strain used. MIC for garlic extract was 2% while it was 3% for DGP₁, DGP₂, DGP₄, DGP₅ and >5% for DGP₃. The potency of garlic powder against the pathogen depends on the temperature used in its processing. Addition of 3% garlic extract to milk intended for soft cheese processing artificially contaminated with 10⁴cfu/ml of the pathogen resulted in producing safe soft cheese. The pathogen could not be detected in soft cheese after 24h from its addition to cheese milk.

Key words: *Listeria monocytogenes*, Garlic, Soft cheese

INTRODUCTION

Listeria monocytogenes is a Gram positive nonsporing rods pathogenic bacteria which causes listeriosis in human subjects. Since 1980s a number of major

outbreaks of human listeriosis were shown to be food- related (**Harvey and Gilmour 1992**), of which dairy products shared high degree of probability such as Mexican style soft cheese (**James et al 1985**) and Swiss regional- type soft

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cheese (**Bille and Glauser 1988**). Also sporadic listeriosis was attributed to consumption of soft cheese in England (**Brannister, 1987**) and camembert cheese in Belgium (**Gilot et al 1997**). Soft cheese are particularly sensitive to colonization with *L.monocytogenes* (**Ennahar et al 1994 and 1998**).

In general, soft cheese owes its safety against pathogenic bacteria to particular environmental barriers such as low pH, salt content and cold storage of the product (**Farber and Petrkin 1991**). However, these barriers may be challenged by the ability of *L.monocytogenes* to grow and proliferate under these conditions (**Gay and Cerf 1997 and Wang and Johnson 1997**). Therefore, additional barriers against *L.monocytogenes* in soft cheese are of great interest and seem to be indispensable.

The antimicrobial activity of several plants and their essential oils on some food borne and food spoilage microorganisms have been studied (**El-Gayyar et al 2001**). In this concern, the antimicrobial properties of *Allium sativum* (garlic) was documented over the decades (**Harris et al 2001**). Garlic has been used worldwide as a spice, food and folk medicine (**Naganawa et al 1996**).

Recent public health concern about *L.monocytogenes* in soft cheese created a dilemma to dairy industry and the problem ought to be controlled. Therefore, this study was planned first to determine the antilisterial activity of

aqueous garlic extract against 10 strains of *L. monocytogenes*. Second, the minimal inhibitory concentration (MIC) for aqueous garlic extract and five dehydrated garlic powder were defined. Lastly, the survival of *L. monocytogenes* artificially inoculated into cheese milk intended for processing was monitored in the cheese stored at 8-10°C for 12, 24 and 48h.

MATERIAL AND METHODS

Cultures: Ten strains of *L. monocytogenes* were graciously provided by Prof. Tham of the Swedish University of Agricultural Sciences, Uppsals, Sweden. Table (1) shows their identities. Stock cultures were kept in a deep freezer (-20°C). Working strains were transferred to 10 ml tubes of tryptose soy broth (Oxoid) supplemented with 0.6% yeast extract and incubated at 37°C for 16h.

1- Garlic preparations

1- Dehydrated garlic powder

Five preparations of dehydrated garlic powder (DG) designated Dg1, 2, 3, 4 and 5 were obtained from prof. S. Imbaby, Department of Pharmaceutical research, National Research Center, Egypt. Samples were stored in tightly sealed containers at room temperature (30°C) until used. Stock solutions were prepared by adding sterilized water to equal amounts of the powder (1: 1 w/w) and the

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reconstituted solution was used in the study.

2- Garlic extract preparation

Fresh garlic bulbs were obtained from the local market. The extract was prepared by the procedure of **Saleem and Al-Delaimy (1982)**. The extract was used within 3h of preparation.

3- Determination of antilisterial activity of aqueous garlic extract

The method described by **Benkerrum and Sandine (1998)** was used. The inhibition zone diameters were measured as mm.

Table 1. Identity of 10 strains of *L. monocytogenes* used

Code No.	Serovar	Origin	Year of isolation
770	1/2a	Cheese	1987
773	1/2b	Cheese	1987
1116	1/2c	Human	1985
1514	3b	Salmon	1993
1877	4b	Salmon	1993
1951	1/2b	Salmon	1993
2112	3b	Cheese	1994
2128	1/2a	Human	1988
2315	46	Coleslaw	1981
2317	46	Human	1989

4- Determination of minimal inhibitory concentration (MIC)

The MIC determination for aqueous garlic extract and five dehydrated garlics were done according to the method of **Hogg *et al* (1987)**. Plates of tryptose soy agar (TSA) containing 0, 1, 2, 3 and 4 percent aqueous garlic or 0, 1, 3 and 5 percent reconstituted garlic (prepared from dehydrated garlic) were seeded with a drop from active culture of *L. monocytogenes* and incubated at 37°C for 24h. The concentration of garlic was considered antilisterial when no growth could be observed after 48h incubation.

5- Experimental soft cheese with garlic

Soft cheese was manufactured using 25l pasteurized cow's milk (63°C/30min) of 3% fat. Cheese milk was tempered to 50°C and 5 percent sodium chloride was added. Prepared milk was distributed into 5 beakers each containing 5 l resembling five treatments (T₁ to T₅). T₁ with no garlic, T₂, T₃ and T₄ with 1, 2 and 3 percent added garlic extract respectively. The remaining T₅ was supplemented with 3 percent garlic extract to process soft cheese for sensory evaluation therefore

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no pathogen was added. The four aforementioned treatments T₁ to T₄ were first inoculated with 1ml of active *L. monocytogenes* (about 10⁴cfu/ml) then rennet was added and the procedure was continued to obtain soft cheese.

Experimental cheeses were kept in plastic containers at 8-10°C and examined after 12, 24 and 48h from adding garlic to cheese milk. Listeria selective agar (Oxford formulation, Oxoid) was used for monitoring the pathogen in cheese sample examined.

6- Sensory evaluation of garlic soft cheese

Soft cheese made from T₅ was subjected for sensory evaluation by staff members of the Dairy Department, NRC.

RESULTS AND DISCUSSION

1- Antilisterial activity of garlic extract

All tested strains of *L. monocytogenes* were inhibited by garlic extract as shown in Table (2) with different degrees. In this regard, the most sensitive strain towards the effect of garlic was the one No. 773. Contrary, strain No. 2315 proved to be the most resistant strain tested. Human listeriosis may be caused by many serovars of *L. monocytogenes* including serovars 1/2a, 1/2b and 4b which were frequently incriminated in most cases (Farber and Peterkin 1991). All these serovars were sensitive to the

antibacterial activity of garlic. It could be concluded that the sensitivity of *L. monocytogenes* to garlic is strain dependent which coincided with other workers (Hefnawy *et al* 1993).

2- Determination of minimum inhibitory concentration (MIC)

The MIC as percent garlic extract or dehydrated garlic powder (v/v) were shown in Table (3). Two percent garlic extract inhibited all the tested strains of *L. monocytogenes* on TSA at pH 7.3. Lower concentrations of one percent garlic extract was not enough to inhibit all the tested strains. For the different five garlic powder preparations examined, it could be observed that Dg₄ was the most inhibitory while Dg₃ was the least active preparation and Dg₁, Dg₂ and Dg₅ were equally similar. The potency of a given dehydrated garlic as revealed from this study against the pathogen may be attributed to the method used in its preparation and drying. Heat applied in drying of garlic cloves may causes an adverse effect to the naturally occurring antimicrobials as elucidated by Lawson and Hughes (1992).

Table 2. Diameter of inhibition zones (mm) of *L. monocytogenes* (10 strains) caused by garlic aqueous extract using well diffusion method

Diameter of

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1116	1/2c	16
1514	3b	16
1877	4b	12
1951	1/2b	14
2112	3b	16
2128	1/2a	16
2315	4b	10
2317	4b	11

3- Viability loss of *L. monocytogenes* by garlic homogenate in soft cheese

Control soft cheese (T₁) (No added garlic) made from inoculated milk with 10⁴cfu/g of *L. monocytogenes* developed enormous growth after 24 and 48h being 2x10⁷ and 5x10⁹cfu/g respectively (Table 4). Garlic soft cheeses prepared using 1, 2 and 3% garlic extract (T₂, T₃ and T₄ respectively) showed different behaviour.

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Increasing the percentage of the spice was accompanied with decrease in counts of the pathogen (see Table 4).

Table 4. Survival of *L. monocytogenes* as cfu/g in soft cheese

Treatment	Count cfu/g <i>L. monocytogenes</i>	
	24h	48h
Control (T ₁ No garlic)	1 x 10 ⁷	5x10 ⁹
T ₂ (1%)	5x10 ³	4x10 ²
T ₃ (2%)	2x10	<10
T ₄ (3%)	<10	<10

The behaviour of the spice in the food matrix may differ from synthetic medium as observed in the present study and reported by other investigator (**Aureli et al 1992 and Hao et al 1998**).

From the results obtained, it was necessary to add an additional barrier(s) to soft cheese. The garlic is a natural product generally used worldwide with no known adverse effect. Inclusion of garlic in the diet should be considered mandatory to maintain good health (**Thomson and Ali 2003**).

The sensory characteristics of garlic soft cheese were shown by Table (5). The most accepted treatment was that manufactured with 3% garlic homogenate. Not only garlic extract inhibited the growth of *L. monocytogenes* in soft cheese but also it enhanced the growth of lactic acid bacteria normally found in the raw milk in case soft cheese will be processed from unpasteurized milk (data not shown).

In conclusion, garlic extract at 3% (v/v) of cheese milk could be used to control the problem of *L. monocytogenes* in soft cheese whether the pathogen contaminates the product during manufacture or subsequently after opening the packages and kept for consumption.

Table 5. Sensory evaluation of garlic soft cheese with 1, 2 and 3 percentage (v/v) garlic extract

Item	Scores	T ₂	T ₃	T ₄
Colour	5	4	4	5
Texture	40	30	30	38
Flavour	40	20	35	40
Appearance	10	5	7	9
Taste	5	2	2	4
	100	61	78	96

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، 2005

ومدى ل فف ج م لمو ثل اق و ح س موو ثل لى اهل ال خت س م ل ل ا ض م ل ا ث ا ت ل *Isotria monocytogenes* جوت ي س و ا ن و م س ي ا ل ل ا ي ت ك ب ى ر ط ل ل ب خ ل ي ا و ي ت ك ب ق ل ا ي ب ل ل ف

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- 1- 21859 ق د ج 80203 ب ص - ز ي ز ع ل د ب ع ك ل م ل ا ة ع م ا ج - م و ل ع ل ا ة ي ل ك ، ة ا ي ح ل ا م و ل ع م س ق -
ة ي د و ع س ق ل ي ا ب ر ع ل ق ك ل م ل ا
ر ص م ق ز ي ج ل ا - 2311 ق د ل ن و ح ب ل ل م و ق ل ز ك ر م ل ة ي ذ غ ت ل ا و ة ي ذ غ ل ا م و ل ق ب ع ش ن ا ب ل ا ل ا م و ل ع م س ق - 2

ط ب ث و ط ل ل ك ا ت ل ا ة س ا ر م ب ت ح ب ل ا ا ذ و ف
ا ي ر ت س ا ي ل ا ل ا ي ا ت ك ب ل ت ر و ق ي ك ر ت ل ق ا و
د ن ع *Isotria monocytogenes* ج و ت ي س و ن و م
س م خ و ث ل لى اهل ال خت س م ل م ا د خ ت س ا
ف د ه ل ف ج م ل م و ث ل ا ق ق م ل ت خ ت م ا و ن ا
ة ي ن ك م ا س ا ر د و - ط ك ل ذ ن ن ه س ي ي ر ل ا
ا ه ت و ب ت ل ا ل ج ت ا ق ل م و ث ل ا ص ا و خ ة ن ا ف ت س ا ل ا
ة م ي ا ق و ل ف ن ا ت ل ا ا ه ج ا ر د ل ف ب و ر ك ي م ل ا ذ ه ل
ن ب ج ل ة ع ا ن ص ر ي ه ن ت ف ا ض ل ك م ي ت ل ا د ا و م ل ا
ر ك ق ل ل ل ي ب ط ط ي ع ي ب ط ط ف ا ح د ا و م ك ي ر ط ل ا
د ا و م ل ا ل ا ل ج ت ع ا ل ا ب ه ا ذ غ ل ي ا ن ص ت ي ف د ي د ج ل ا
ز ج ا و ح ل ا ن م د ي ز ق ف ا ص ت ر ي ا و ع ي ب ط ل ة ط ف ا ح ل ا
ج ت ن م ل ل ف ب و ر ك ي م ل ا ا ذ ه و م ق ق و ن ت ل ا
ة و ب ع ل ج ت ف د ع م ل ك ا ل ه ت س ق ر ا ت ف ل ا ل ع ل ص و ص خ
ة ج ا ل ت ل ل ف ا ه ط ف ح و

ق ا ب ط ل ل و ف ل ش ت ن ا ل ق ي ر ط م د خ ت س ا
ة ف ل ت خ ت ل ا ي ك ر ت ل ل ي ب ا ن ق ي ر ط و ر ا ج ا ل

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ة س ا ر د ل ل ي ن ح ي ت ت ك ب ل ل

ى اهل ال خت س م ل ا ن ل ج ن ا ت ر ط ل ا خ ل ت ت
و م ن ط ل ب ث و ط ل ل ا ت ل ا م ه ل ا ل ل م و ث ل ا ق و ح س م و
ز ي ك ر ت ل ق ا ن ا ك ت و د خ ت ا ي م ل ك س ل ي ل ل ل ي ت ك ب
3% ق و ي ه ل ل ا ل خت س م ل ل 2% و ط ب ث م
ف ف ج م ل م و ث ل ا ن م 5 ، 4 ، 3 ، 2 ، ت ا ن ي ع ل ل
ا ر ط ي ث ا ت ا ت ل ق ا ي ه 3 م ق ن ي ع ل ت ن ا ل ق ي ب
و م ر ف ق و ق ي ف ا ل و ي غ 5% س ن ت ن ا ك
ب و ر ك ي م ل ا

ة ن ا ف ت س ا ل ق ي ن ا ك م ا و ح ب ل ا ق ص ا ل خ
ة ع ا ن ص ر ي ف و ث ل لى اهل ال خت س م ل ا ص ا و خ
ت ا ل ا ح ت و د ح ي ق س ع ل ي س ل ل ي ر ط ل ن ا ب ج ل ا
ت ت د ج ت ل ل و ب و ر ك ي م ل ا ذ ه ي ا ذ غ ل م س ت ل ا
م ل ا ع ل ا ل و د ن ه ي ت ك ل ف ا ر خ و م

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دم حاي سل اون دأ ليرب نيسوي دم حأ نومي كحت

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