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Antibacterial effect of sodium lactate and thymol on *Salmonella Typhimurium* in fish fillet

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ABSTRACT

The aim of this study was to determine the antibacterial effect of sodium lactate and thymol against *Salmonella Typhimurium* in fish fillet. Fish fillet samples were artificially contaminated with *Salmonella Typhimurium*, then sprayed with thymol (0.1%), sodium lactate (1% & 2%) individually and combination of Sodium lactate (1% &2 %)+ thymol (0.1 %).The samples were stored at 4° C and analyzed at zero, third and fifth day of storage for sensory analysis, pH, and count of *Salmonella Typhimurium*. The thymol alone had a little antibacterial effect on Salmonella. The initial count of control was 5.65 ± 0.09 $\log_{10}$ cfu/g on zero day which was declined to 4.50 ± 0.14 $\log_{10}$ cfu/g as a result of combined treatment of sodium lactate (2%) + thymol 0.1%, which showed the best results obtained, followed by the treated samples with sodium lactate 1%+ thymol 0.1%, as it recorded 4.72 ± 0.10 $\log_{10}$ cfu/g, and lastly 4.63±0.11 $\log_{10}$ cfu/g, which obtained by sodium lactate 2% treated samples. The reduction in the Salmonella count in response to the combined treatment of sodium lactate 2% + thymol 0.1% was greater than the effects of the two compounds applied individually.

INTRODUCTION

Food safety and hygienic standard parameters are the most important issues related to human health in the food industry. Food safety concerns each and every food handling facility; therefore many factors that make food unsafe for consumption. The hazards of food are primarily microbiological agents **(Morya et al. 2020).** As microbial food safety is an increasing public health concern worldwide, foodborne pathogens are a major threat to food safety, especially in developing countries where food hygiene and sanitation facilities are often poor **(Ahmed and Shimamoto 2014).** Fish meat is known to be susceptible to undesirable chemical changes and microbial reactions that characterize spoilage **(Raeisi et al. 2020).** During storage the quality of fish degrades due to a complex process in which physical, chemical and microbiological forms of deterioration are implicated **(Gonza´lez et al. 2005).**

Many foodborne outbreaks have been recorded worldwide, and about 30 percent of the population in developed nations is suffering from foodborne disease every year. Foodborne

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diseases are generated due to consumption of contaminated food with the pathogenic micro-organism during the food chain (production, handling, storage, transport and distribution) **(Badui 2015 and Junod et al. 2015).**

Salmonella bacteria have become the major cause of foodborne diseases which have raised a great safety concern to public health (**Rabsch et al. 2001).** *Salmonella typhimurium* is among the most common serovars in the outbreaks of *Salmonella* gastroenteritis. These pathogens are typically associated with infected food and could result in a major economic effect due to illness **(Amagliani et al. 2012).** While most infections cause moderate symptoms to severe death-related infections do occur.

The use of chemical preservatives to inactivate or hinder the growth of pathogenic microorganisms in foodstuffs is growing fast. Massive numbers of people prefer food items which are processed naturally **(Tajkarimi et al. 2010).** To control foodborne pathogens without affecting quality, foods are subjected to combined treatments using "hurdle technology" which focused on natural antimicrobial food additives **(Kim and Rhee 2016, Leistner 2000).**

Sodium lactate is the sodium salt of natural lactic acid, it is approved by the Food and Drug Administration (FDA) as widely accepted as healthy, Generally Recognized As Safe (GRAS) and directly applied to various foods to regulate microbial growth and prolong product shelf life **(Hwang et al. 2011).** Studies have shown that lactates exhibit bacteriostatic effects by affecting the bacteria's metabolism including intercellular acidification and proton transfer across the cell membrane. Further, lactates decrease food water activity **(Seydim et al. 2006).**

Sodium lactate can be added directly to food to control the microbial growth and to extend the shelf life of food products **(Burfoot and Mulvey 2011, Smaoui et al. 2012, Bolton et al. 2014).**

Herbs and spices, and their constituents have been used as flavoring agents in food since the earliest history, and it is well established that many have antibacterial activity (**Bajpai et al. 2012 Jayasena and Jo 2013 Prakash et al. 2015, Seow et al. 2015).** Thymol is one of the phenolic compounds obtained from plants like Origanum vulgare and Thymus vulgaris, and is classified as (GRAS) by (FDA**) (Tajkarimi et al. 2010, Hyldgaard et al. 2012).** Thymol as one of the major constituents of thyme oil presents a wide range of functional possibilities in the food industry **(Placha et al. 2019, Salehi et al. 2018).** It is believed that the mode of antibacterial action of thymol alters the physical and chemical properties of cytoplasmic membrane of acteria, and this may change the permeability of the cell membrane and cause the leakage of ions and other cell contents **(Hyldgaard et al. 2012 Vergis et al. 2015, Calo et al. 2015).** However, their use as preservatives in foods is often limited due to considerations of flavor and aroma and loss of sensory quality. Therefore, combinations of thymol with other preservatives have been used to minimize the concentrations required. There has been conducted much successful research related to the combinations of thymol with the other antimicrobial agents and methods (**Oladunjoye et al. 2013; Ilhak and Guran, 2014, Kim and Rhee, 2016).**

Considering the antibacterial effect of thymol, it can be assumed that thymol can facilitate the diffusion of sodium lactate into the cytoplasm of the cells. In this way, when the two are used in combination a synergistic or additional effect can be achieved **(Ilhak and Guran 2014).**

Therefore, the main purpose of the present study was to investigate experimentally the combined antibacterial effect of thymol and sodium lactate on *Salmonella Typhimurium* in fish fillets.

MATERIALS AND METHODS

Collection of samples:

One sample weighted 3000g. of fish fillet was randomly purchased from local markets in Giza governorate in Egypt. The sample was transported to the laboratory of Food Hygiene Department, Animal Health Research Institute (AHRI) as quickly as possible under complete aseptic conditions in ice box. A representative sample was taken to be analyzed for the indigenous *Salmonella* spp. according to (ISO 2002).

Bacterial culture:

Salmonella Typhimurium strain (ATCC® 14028) was provided by food hygiene department, Animal Health Research Institute. The strain was grown in 10 mL of Tryptic soy broth (TSB) (Biomark Laboratories) at 37°C for 18 hrs. One milliliter of the strain was then harvested by centrifugation at 4,192 ×g for 10 min, and washed and serially diluted in 0.1% peptone water (PW) to yield cell suspensions 0f 10^7 CFU/ml. The bacterial population was determined by plating 0.1 ml of appropriately diluted culture on Xylose lysine deoxycholate agar plate (XLD) (Merk) with incubation at 35°C for 24–36 h. Active culture with bacterial density of $1x10^7$ cfu/ml was used in samples inoculations (Health Protection Agency, 2005).

Inoculation of fish fillet, and treatment:

The fish fillets meat sample was prepared for inoculation and dipped in peptone water containing $1x10^7$ cfu/ml *Salmonella Typhimurium* strain for 15 minutes at room temperature (25° C). After the inoculation, the fish fillet samples were kept at refrigerator for 10 min to allow for bacterial cell attachment. The inoculated Fish fillets with known *Salmonella Typhimurium* load was divided into six equal groups (500 g each) for further treatment. The decontamination treatments were as follows; 1st. control group remained without treatment, 2nd .group was treated with thymol 0.1% solution, 3rd .group was treated with sodium lactate 1% solution, 4th. group was treated with sodium lactate 2% solution, 5th. group was treated with sodium lactate 1% + thymol 0.1% solution and 6th . group was treated with sodium lactate 2% + thymol 0.1% solution.

The groups (except control) sprayed separately (rotating all surface) with approximately 30 ml of solutions sodium lactate 1%, 2 % (Sigma -Aldrich /P code 101044503 and thymol (Lanxess SLC2068, colorless crystals, solubility in water 0.1gm/100ml, Germany) using a spray bottles. Bottles of thymol and sodium lactate 1 and 2% were vigorously shaken to be dispersed in the solution before they were used. After spraying, the inoculated fish fillets samples were separately packed in multiple sterile labeled polyethylene bags, heat sealed, and stored at refrigerator of 2 - 4°C for 5 days. Sensory, pH and bacteriological analysis were conducted following 0, 3, and 5 days of storage.

Sensory analysis:

Color, odor, texture and overall acceptability were conducted on days 0, 3, and 5 during storage to determine the best treatment. Sensory analysis was performed by a sensory panel team of seven trained assessors (members of food hygiene department. Their opinion was recorded as acceptable or non-acceptable.

Determination of pH of fish fillets samples:

pH of the rinse solution of the samples was performed after bacteriological analysis on each sampling day. It was measured with pH meter (JENEWAY 3310).

Determination of *Salmonella typhimurium* count:

Bacteriological sampling was performed after the spraying treatment on 0, 3 and 5 day. On each sampling day, 25 g. of the fish meat samples were weighed out under aseptic conditions, placed in sterile "Stomacher" bags with 225ml of 0.1% sterile Buffer peptone water (BP) and homogenized for two minutes. 1 ml solution was taken from the solution and serially diluted in 0.1% sterile peptone water and surface plated on xylose lysine deoxycholate agar for enumeration of *Salmonella.* Characteristic colonies were counted after the plates were incubated at 35°C for 24–36 hrs. (ISO 2002).

Statistical Analyses:

Analyses of the microbiological data were carried out using Statistical Package for the Social Sciences (SPSS). One Way ANOVA, The numbers of bacteria were converted to logarithmic values before calculating means and performing statistical analyses. The means were separated using Fisher's least significant differences according to general linear model procedures. Statistical significant level was expressed as ($P \leq 0.05$).

RESULTS

Table (1) Sensory evaluation of fish fillet samples:

Treatment / Items	control	Thymol 0.1%	Sod lact 1%	Sod lact 2%	Sod lact 1% + Thymol 0.1%	Sod lact 2% + Thymol 0.1%
Color	**8**	**8**	**8**	**8**	**8**	**8**
Odor	**7**	**6**	**8**	**8**	**8**	**8**
Texture	**7**	**8**	**7**	**7**	**8**	**8**
Over all acceptability	**7**	**7**	**8**	**8**	**8**	**8**

Ratings: (scale 10= excellent, 1= unacceptable).

Table (2) Effect of sodium lactate and thymol on pH (mean±SD) values of fish fillet

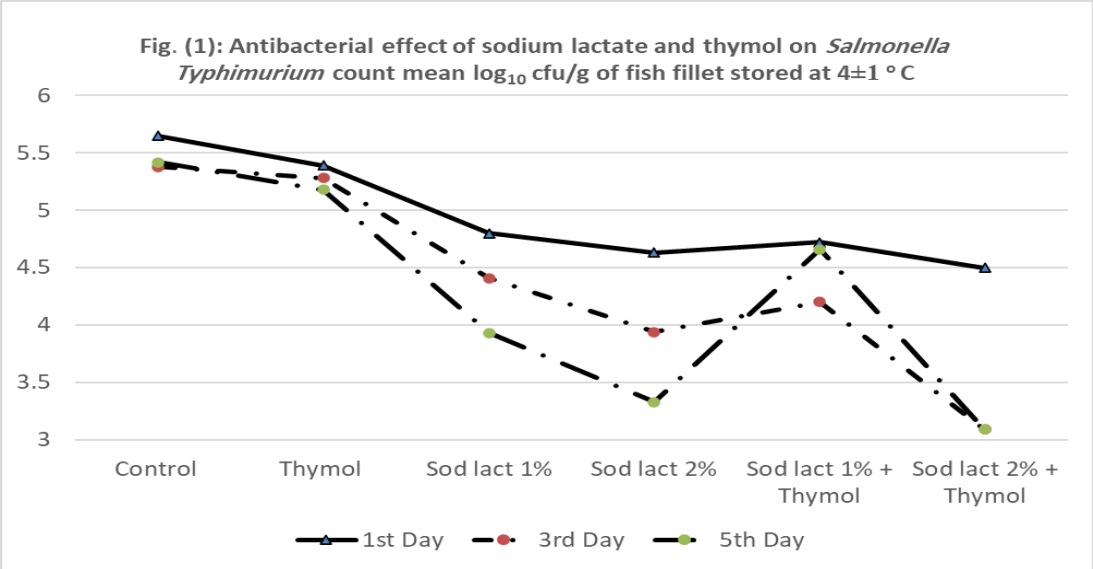
Treatment	Zero Day	3rd Day	5th Day
Control	6.50 ^{a} ±0.05	6.60 ^{a} ±0.02	6.62 ^{a} ±0.01
Thymol 0.1%	6.43 ^{b} ±0.01	6.41 ^{b} ±0.01	6.43 ^{b} ±0.03
Sod lact 1%	6.39 ^{b} ±0.06	6.41 ^{b} ±0.03	6.43 ^{b} ±0.01
Sod lact 2%	6.31 ^{c} ±0.03	6.38 ^{b} ±0.02	6.39 ^{b} ±0.02
Sod lact 1% + Thymol 0.1%	6.28 ^{c} ±0.02	6.31 ^{c} ±0.02	6.32 ^{c} ±0.03
Sod lact 2% + Thymol 0.1%	6.20 ^{c} ±0.00	6.30 ^{c} ±0.01	6.35 ^{c} ±0.02

There are significance differences (P<0.05) between mean having different super scribed letter in the same day of storage for the same column).

Table (3) Antibacterial effect of sodium lactate and thymol on *Salmonella Typhimurium* Count (mean ± SD log_{10} cfu/g) of fish fillet stored at 4 ± 1 °C.

Treatment	zero Day	3rd Day	5th Day
Control	5.65 ^{a} ± 0.09	5.67 ^{a} ± 0.15	5.68 ^{a} ± 0.12
Thymol 0.1%	5.39 ^{b} ± 0.23	5.28 ^{a} ± 0.05	5.18 ^{a} ± 0.16
Sod lact 1%	4.80 ^{c} ± 0.10	4.41 ^{b} ± 0.09	3.93 ^{b} ± 0.05
Sod lact 2%	4.63 ^{cd} ± 0.11	3.94 ^{c} ± 0.05	3.33 ^{c} ± 0.35
Sod lact 1% + Thymol 0.1%	4.72 ^{cd} ± 0.10	4.20 ^{d} ± 0.08	4.16 ^{d} ± 0.13
Sod lact 2% + Thymol 0.1%	4.50 ^{e} ± 0.14	3.09 ^{e} ± 0.16	3.09 ^{e} ± 0.16

There are significant differences (P<0.05) between mean having different super scribed letter in the same day of storage for the same column).



DISCUSSION

In the present study, the antibacterial effect of sodium lactate and thymol on *Salmonella typhimurium* count was measured to evaluate the most effective compound and also its effect on sensory quality and pH.

In **Table (1)**, the sensory evaluation revealed that only minor differences were noticed for color, odor, texture and overall acceptability of the fish fillet samples. Odor slightly changed in samples which treated with thymol 0.1%. All the treated samples were considered to be accepted by the panelists. **Burt 2004 and Tajkarimi et al. (2010)** mentioned that fish fillet samples which treated with thymol in combination with sodium lactate 1% and 2% were accepted, which agreed with the results of the present study.

Table (2), revealed that the pH mean values of fish fillet samples were increased during storage at 4°C for control and all treated samples. The pH of the control sample was 6.50 and increased to 6.60 and 6.62 on 3rd and 5th days. The results obtained for thymol 0.1%, sodium lactate 1%, sodium lactates 2%, sodium lactate 1% +thymol and for sodium lactate 2%+ thymol were (6.43, 6.39, 6.31, 6.28 and 6.20) on zero day ,(6.41, 6.41,6.38 , 6.31, and 6.30) on 3rd day and finally the 5th day results recorded (6.43,6.43,6.39,6.32, and 6.35), re-

spectively. The results obtained were agreed with Zhou et al. 2007a who mentioned that sodium lactate 2% decreased the pH more than sodium lactate 1%, also it was revealed that the inhibitory effect of sodium lactate increases when the pH of food decreased (Nykänen et al. 2000 and Mani-López et al. 2012). Tiwari et al. 2009 reported that the susceptibility of bacteria to the antibacterial effect of essential oil (thymol) increased with the decrease in pH level of food**.** There was a significant difference ($p<0.05$) between control group and the other treatments as pH was shown to be reduced.

Table (3) and figure (1), showed that the mean value of *Salmonella Typhimurium* count recorded for control samples were (log 5.65 ± 0.09, 5.67±0.15, 5.68±0.12 cfu/g), while thymol results were (log 5.39 ± 0.23, 5.28 ± 0.05, 5.18 ± 0.16cfu/g) 0n zero, 3rd and 5th day, respectively. On finding obtained, the samples which treated with thymol 0.1% showed little decrease in the *Salmonella* count and it was similar to Tiwari et al. 2009**.** There was a significant difference between the control, and thymol 0.1% ($p<0.05$).

Treated samples with sodium lactate 1% showed results of (log 4.80± 0.10, 4.41± 0.09, 3.93 ± 0.05cfu/g) and sodium lactate 2% recorded (log 4.63± 0.11, 3.94± 0.05, 3.33± 0.35cfu/g). Sodium lactate 1% and 2% significantly reduced the count of *Salmonella* than control and thymol ($p<0.05$).

Sodium lactate 1% + thymol 0.1% treatment recorded (log 4.72 ± 0.10, 4.20± 0.08, 4.16 ± 0.13) and finally Sod lactate 2% + thymol 0.1% showed (log 4.50± 0.14, 3.09± 0.16, 3.09± 0.16) on zero,3rd and 5th day, respectively. the obtained results revealed that combination of sodium lactate 1, 2% and thymol gave the best inhibitory effect on *Salmonella* count, while the concentration of sodium lactate 2% combined with thymol 0.1% was the most effective one. The same results were obtained by **(Ilhak and Guran 2014),** who reported that the antibacterial effect of sodium lactate 2% on *Salmonella typhimiurium* could be increased when combined with thymol. Meanwhile, no significance difference between treatments including sodium lactate 2%, sod lactate 1% +thymol and sod lactate 2%+ thymol. Sodium lactate 2%+thymol 0.1% significantly ($p<0.05$) reduced *Salmonella* count than control (from 5.65 to 4.63); the reduction was more than 1log cfu/g.

CONCLUSION

In the current study, antibacterial compounds, sodium lactate in concentration of 1, 2% and thymol 0.1% had an inhibitory effect on *Salmonella* count. The best results were obtained when using sodium lactate 2% in combination with thymol 0.1% as antibacterial on *Salmonella Typhimurium* ,while such used concentration in the current study did not inhibit the organism completely so it is recommended to use a higher concentration and at the same time did not affect the sensory characters of the product.

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