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| RESEARCH ARTICLE

Effect of Sodium Lactate on the Safety of Cold-smoked Salmon during Cold Storage

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| ABSTRACT

Effect of sodium lactate (SL) on inhibition growth of *Listeria monocytogenes*, *L. innocua*, *Escherichia coli*, and *Pseudomonas aeruginosa* as compared to ampicillin and chloramphenicol antibiotics in cold-smoked salmon stored at 1.0 ± 4.0 ^{0}C were evaluated in this study. The sliced smoked salmon were coated with different concentrations of sodium lactate (0.5, 0.75 and 1.0 % SL). Sterilized deionized water was used as a negative control. Total bacterial counts (TBC), lactic acid bacteria (LAB), *E. coli* (EC), *Pseudomonas* count (PC) and *Listeria* count (LC) were examined. *In vitro*, the antibacterial agent (SL) exhibited antibacterial activities against all the tested bacteria. The antimicrobial action of 1.0 % of SL was more effective than the control. Therefore, thus coating with 1.0 %, SL prevented *Listeria* spp. and other pathogenic bacteria growth and prolonged the shelf life of cold-smoked salmon.

| KEYWORDS

Antibacterial; Sodium lactate; Cold Smoke salmon; Safety; *Listeria monocytogenes*

| ARTICLE INFORMATION

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1. Introduction

Listeria monocytogenes is an important human foodborne pathogen that causes febrile gastroenteritis in healthy individuals and life-threatening invasive infections in susceptible individuals (Mead *et al.,* 2006), such as the young, elderly, pregnant, and immune-compromised (De Cesare *et al.,* 2007). *L. monocytogenes* is widespread in the environment and can grow over a wide range of temperatures, including at refrigeration temperatures (Graves and Swaminathan 2001), in high concentrations of sodium chloride and low concentrations of oxygen (Farber 2000). These properties, along with the severity of human listeriosis, make *L. monocytogenes* of particular concern for manufacturers of ready-to-eat foods (Romanova *et al.,* 2002; Shen and Higgins, 2006). Cold smoked salmon and sushi salmon have been implicated in outbreaks of listeriosis (Eicher *et al.,* 2020). *L. monocytogenes* infection has been associated with consuming unpasteurized milk, soft cheese, ice cream, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, raw meats (all types), cold cuts, and raw and smoked fish (Czuprynski 2005; Hamon *et al.,* 2006). The prevalence of these organisms in freshly produced cold-smoked fish is relatively high and is typically between 10 to 40% (Rorvik *et al.,* 1991; Fonnesbech Vogel *et al.,* 2001; Autio *et al.,* 2004; Azevedo *et al.,* 2005; Miettinen and Wirtanen 2006). This high prevalence could be due to the low smoking temperature involved during the cold-salmon processing, as these conditions would be ideal for the proliferation of *L. monocytogenes* if the raw salmon harbored the pathogen or acquired the pathogen from the processing environment. Under favorable conditions of storage time and temperature, *L. monocytogenes* may exceed the legal limit of 100 cfu/g (FSAI 2008). *L. monocytogenes* contamination in cold-smoked salmon depends on several factors, such as raw materials, working habits and the presence of surface persistent *L. monocytogenes*. Contamination, survival, and growth of *L. monocytogenes* in cold-smoked salmon represent serious health hazards to consumers and major challenges for salmon processors (Heir *et al.,* 2019). Natural bioactive molecules work as antibacterial agents. Although successful applications of sodium lactate in beef shelf-life extension are available (Sallama and Samejima, 2004; Ercolini *et al.,* 2015), few reports have been concentrated on salmon fish quality enhancement by sodium lactate. Therefore, the aims of the present study were to control Gram-positive and Gram-negative bacteria in cold smoked salmon stored at cold temperature by sodium lactate.

2. Materials and Methods

2.1. Antimicrobial agents

Distilled water and sodium lactate (SL: Sigma-Aldrich) were used for film production. Cold-smoked salmon (CSS) were bought from a local market in Egypt. All these materials were tested for their antimicrobial action against Gram-positive and Gram-negative bacteria (i.e., *L. monocytogenes*, *L. innocua*, and *Pseudomonas aeruginosa*).

2.2. Bacterial Strains

Listeria monocytogenes, *Listeria innocua*, and *Pseudomonas aeruginosa* were obtained from the Microbiology Department, Faculty of Agriculture, Zagazig University. The strains were aseptically sub-cultured in Tryptic Soy broth (TSB: Merck 1.05459, Darmstadt, Germany) and checked for purity onto Tryptic Soy agar plates (TSA: Merck 1.05458, Darmstadt, Germany), incubated for 24 h at 37 °C.

2.3. Antibacterial activity of the preservatives used

The antibacterial activity of the preservative agent (SL) was assessed against three bacterial strains. Gram positive bacteria, i.e. *Listeria monocytogenes*, and *Listeria innocua,* as well as Gram negative bacteria, i.e. *Pseudomonas aeruginosa,* using agar well diffusion assay (Magaldi *et al.,* 2004; Nanda and Saravanan, 2009) to determine antimicrobial activity of sodium lactate (0.5, 0.75 and 1.0 % SL). Sterilized deionized water was used as a negative control, and antibiotics were used as a positive control. All the pathogenic microbes were grown overnight in Tryptic Soy broth (TSB). 100 µl culture of each strain was spread evenly on a Tryptic Soy agar (TSA) plate. Then the wells were made into agar at 8 mm diameter using a sterile cork-borer with a distance between the well and another more than 22 mm. 100 µL of each concentration of antibacterial was placed over the TSA plates. The plates were incubated at 37°C for 24 h. Similarly, the antibacterial effect of commercial antibiotics like AMP (10µg), Chloramphenicol (5µg) has been tested. Antibiotics were treated as a positive control. After 24 h., the zones of inhibition were measured. The diameters of the inhibition zone against the tested bacteria were recorded in millimetres using a metric ruler.

The minimum inhibitory concentration (MIC) was determined by a microdilution method in 96 well-round bottomed sterilized microtitre plates (Kartell S.p.a., Italy). Each microorganism was prepared in Tryptic Soy broth media. Portions of 80 µL of each of the diluted antimicrobials were pipetted into the wells of the microtitre plates, together with 20 µL of a 10^6 CFU mL^{-1} culture of each microorganism, once at a time. The range of concentrations tested were sodium lactate (0.5, 0.75 and 1.0 % SL). Microtitre plates were incubated at 37 ± 1ºC for 24 h. The visual detection of turbidity in the wells, as compared with the negative and positive controls, was considered as the absence of inhibition. Negative and positive controls were tested in parallel, being the former no inoculated Tryptic Soy broth and the latter inoculated Tryptic Soy broth free of antimicrobials. The MIC was defined as the highest dilution showing inhibition after 24 h. of incubation according to the NCCLS (2004) recommendations.

2.4. Film-forming liquid and inoculum of pathogenic preparation

The film-forming solution was prepared by the method of Ahmad et al. (2012), sodium lactate solution (0.5, 0.75 and 1.0 % SL). Each concentration solution was put on the sliced smoked salmon, then left to dry for 10 min, within a Laminar flow at room temperature. Each strain of pathogenic bacteria was maintained on tryptic soy agar and stored at 4˚C. The strain was grown separately in tryptic soy broth (TSB) for 24 h. at 37 ˚C, and 100 µl of overnight culture was transferred to 10 ml of fresh TSB broth and incubated at 37˚c for another 24 h. The culture was centrifuged at 3000 rpm for 10 min; the pellet was washed in sterile saline solution (8.5g/l) and then centrifuged and resuspended in the same solution to reach a cell density of 5.5 Log CFU /ml, which served as the inoculum. Serial dilutions were plated into tryptic soy agar (TSA) plates and incubated at 37 ˚C for 24 h. to determine cell numbers.

2.5. Cold-smoked salmon storage under refrigeration conditions

Cold smoked salmon (CSS) was immediately obtained during their shelf-life; they were kept frozen at -20 ˚C and thawed at 2 ± 2 ˚C (< 4 ˚C) for 1 day immediately before use. Slices of smoked salmon have punched aseptically into 5.8 cm diameter round pieces weighing 10 ± 1 g. Salmon samples were aseptically subdivided into portions of about 10 g and subjected to the following treatments: CSS was distributed into 16 portions (100 g), divided into 4 groups of 4 packs each and transferred in sterile packs. The first group was not treated and served as negative control. The second group was inoculated with a cocktail of pathogenic bacteria and served as a positive control. The third and fourth groups received 0.5% (T1)and 1.0% (T2) sodium lactate. Appropriate dilutions of each strain were then surface-inoculated on one side of the slices, spread evenly using a hockey stick, and allowed to absorb for 30 minutes in laminar-flow, then re-air packed. The slices of CSS treatments were inoculated with 0.1 mL of mixed cultures and then air-dried by leaving them in a laminar-flow hood under ventilation for 20 minutes. The second, third and fourth were equally inoculated with 0.1 mL of a mixed culture of *L. monocytogenes*, *L. innocua* and *P. aeruginosa* so that the final count of each becomes ~ 4.5 log CFU/g (4.54, 4.70, and 4.34 CFU/g, respectively). All packs were kept at ≤ 4 °C for 30 days, where samples were taken under aseptic conditions every 2 days for the microbiological assay. About 10 g of the sliced salmon samples were mixed with sterile Ringer's solution (225 ml) for 1 minute for evaluation using a stomacher machine (Lab. Blender 400; Seward

Medical, London, UK) at ambient temperature. The serial dilution volume in Ringer's solution was prepared and duplicated 1 ml or 0.1 ml samples of proper dilutions and was spread on selective or non-selective media agar plates. The total bacterial count (TBC) was counted on Plate Count Agar (PCA; Merck, 1.05463) incubated for 72 h at 25 ºC, and lactic acid bacteria (LAB) on de Man, Rogosa, Sharpe (MRS Biolife) overlain with the same medium (5 ml) and incubated for 72 h at 25 ºC; *Listeria* spp. were determined by an overlay method to enhance recovery of injured cells (Kang and Fung 1999) and counted on polymyxin–acriflavin–lithium chloride–ceftazidime–aesculin–mannitol agar (PALCAM agar, Biolife, 401604, Italy) after incubation for 24 h at 37 °C and confirmed according to ISO 11290. *Pseudomonas* was counted *Pseudomonas* Agar Base (PAB), PAB with CN supplement X107, and PAB with cetrimide, and this media supplemented with magnesium and potassium salts to enhance the production of the pigment pyocyanin, then the plates were incubated for 48 h at 32 °C. For experimental purposes, the detection limit of these techniques was 2 log CFU/g except for LAB, for which the limit was 1 Log CFU/g. The bacterial populations shown are the mean of three replicates transferred to log10 CFU/g.

3. Results and discussion

3.1. Antibacterial and anti-biofilm activity in vitro

One of the standards applied by most researchers to quantity the antimicrobial activity of the agents is to determine the minimum inhibitory concentration and the minimum concentration of the antibacterial agent. This study evaluated the sodium lactate (SL) on inhibition growth of *Listeria monocytogenes*, *L. innocua*, *E. coli* and *Pseudomonas aeruginosa* as compared to ampicillin and chloramphenicol antibiotics using agar well diffusion assay and microdilution method. Using agar well diffusion assay (Table 1), the tested antimicrobial agent induced inhibition zones against four tested pathogenic bacteria (*L. monocytogenes*, *L. innocua*, *E. coli* and *P. aeruginosa*). The diameter of the inhibition zones increased proportionally with the increase in the agent concentration**.** The minimum inhibitory concentration (MIC) of SL was 1.0 g/L against the four bacteria. According to the results (Table 1), the highest MBC values were observed in *L. monocytogenes* and *L. innocua,* and the lowest values were found in Gram negative bacteria ($p < 0.5$). Several studies have reported that Gram-positive bacteria are more susceptible to antibacterial agents than Gram-negative bacteria. The resistance of Gram-negative bacteria against antibacterial agents with the hydrophilic surface of the outer membrane of bacteria that is rich in lipopolysaccharide molecules and creates a buffer against the penetration of different antibiotic molecules, as well as with perivascular. However, in this current study, SL was more effective against Gram positive and Gram-negative bacteria.

Table 1: *In vitro* antibacterial activity of sodium lactate and lactic acid against some pathogenic bacterial strains (Inhibition zone, mm).

		Inhibitory zone (mm)			
Antimicrobial agent	**g/L**	***L. monocytogenes***	***L. innocua***	***E. coli***	***P. aeruginosa***
Control	**0**	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Sodium lactate	**0.5**	21±0.18	19±0.22	18±0.15	19±0.13
	0.75	28±0.19	26±0.22	27±0.19	23±0.15
	1.0	32±0.28	29±0.21	35±0.25	30±0.23
Ampicillin	**0.1**	35±0.13	32±0.13	11±0.23	10±0.21
Chloramphenicol	**0.2**	33±0.15	31±0.22	26±0.27	29±0.32

3.2. Evaluation of antibacterial activity in situ

Cold-smoked salmon (CSS) fillets were surface contaminated with a mix of two Gram positive bacteria, i.e. *L. monocytogenes* and *L. innocua,* as well as Gram negative bacteria, i.e. *P. aeruginosa.* Levels of these pathogenic bacteria were determined during air pack refrigerated storage for 30 days. The use of 1.0 % sodium lactate (SL) and 0.5 % SL resulted in increased lag times and reduced growth rates of *Listeria* and all the tested microorganisms compared to the control. The inhibitory effects were dependent on SL and storage time. The total count of bacteria may be a measure to work out the health quality of a product that expresses the non-usability of the merchandise. With increasing time, total bacterial count (TBC) levels (Table 2) increased in all treatments. The increase in TBC for treatment during the upkeep period depends on the quantity of manipulation, the quantity of health within

the treatments and, therefore, the initial rate of bacteria (Chidanandaiah *et al.,* 2009). So that the highest amount was observed in the control sample and the least in SL 1% ($p < .05$). In fact, the antimicrobial effect of these compounds is due to their reaction with proteins in the cytoplasmic membrane of microorganisms, which changes the permeability of the membrane and the formation of probable pores, and ultimately affects the driving force of the protons (Juven *et al.,* 1994).

Listeria bacteria are a Gram-positive rod and β-hemolytic, which is positive for catalase and coagulase and fermentation of mannitol. This bacterium is commonly the cause of many human infections, and every human being infects the bacterium at least once in his lifetime (Jay et al., 2005). According to the results, over time, the level of *Listeria* (Table 4) increased, and the highest levels of bacteria were observed in the control treatment ($p < .05$), while in treatment containing SL 1.0 g/L, *Listeria* was reduced ($p < .05$), and on the end of experiment its values reduced by 2.47 Log CFU/g. In control, its values have been increasing the time until the 30 days. On sliced salmon, growth inhibition levels of 1.82 and 2.47 Log CFU/g at ≤ 4 °C storage could be obtained using 1.0% SL compared to a 2.47 log increase in *Listeria* counts in the control salmon (Table 4). At cold temperatures (4 °C), corresponding *Listeria* and *Pseudomonas* levels increased by 2.46 log and 1.60 log during 30 days of storage. The injection of sodium lactate at a high concentration (30%) into cold smoked salmon significantly reduced the growth potential of *L. monocytogenes*. In addition to good manufacturing practices, the injection of SL+ nisin may therefore be a useful additional hurdle to prevent the growth of *L. monocytogenes* to high numbers in the tested salmon products. Although successful applications of sodium lactate or nisin alone in beef shelf-life extension are available (Sallama and Samejima, 2004; Ferrocino *et al.,* 2016; Eicher *et al.,* 2020).

Pseudomonas is a Gram-negative bacterium, which is one of the major microorganisms responsible for the spoilage of fish. Gram-negative bacteria (and mainly *Pseudomonas* species) are more probably to increase in growth under aerobic and cold conditions, they are the prevalent microbial population in fish and RTE smoked salmon stored in the refrigerator and exposed to air (Jay *et al.,* 2005). The main group of bacteria responsible for the spoilage of freshly stored fish is gram-negative psychrophilic total count (Ojagh *et al.,* 2010). The important characteristics of these group have a robust proteolytic and lipolytic enzyme and their reproductive rate briefly time (Sallam, 2007). According to the results, on all days, the highest levels of *P. aeruginosa* (Table 5) were observed in the control treatment ($p < .05$) compared to the control.

Table 2. *In situ* effect sodium lactate 0.5 % (T1) and sodium lactate 1.0 % (T2) on inhibition of total bacterial count (TBC) (Log_{10} CFU/g ± SD) in cold-smoked salmon stored at 4 ^{0}C.

Time(day)	Control	T1	T2
0	4.85±0.53	4.85±0.45	4.85±0.57
2	5.13±-.81	5.00±067	5.11±0.67
4	5.33±0.43	4.78±0.36	5.12±0.56
6	5.55±0.81	4.79±0.45	5.14±0.76
8	5.58±0.52	4.78±0.41	5.22±0,87
10	5.69±0.34	4.89±0.32	5.34±0.71
12	5.87±0.67	5.04±0.56	5.45±0.61
14	5.97±0.61	5.16±0.41	5.68±0.51
16	6.27±0.79	5.34±0.34	5.89±0.69
18	6.48±0.65	5.20±0.41	6.22±0.55
20	7.32±0.55	5.66±0.56	6.68±0.34
22	7.56±0.59	6.34±0.67	7.23±0.43
24	8.04±0.68	6.44±0.84	8.04±0.61
26	8.51±0.65	6.57±0.66	8.51±0.45
30	8.55±0.43	6.70±0.59	8.50±0.53

Table 3. *In situ* effect of sodium lactate 0.5 % (T1) and sodium lactate 1.0 % (T2) on inhibition of lactic acid bacteria (LAB) (Log_{10} CFU/g ± SD) in cold-smoked salmon stored at 4 ^{0}C.

Time(day)	Control	T1	T2
0	1.48±0.43	1.48±0.45	1.48±0.56
2	2.11±0.34	1.60±0.56	1.60±0.76
4	2.67±0.12	2.48±0.65	2.48±0.34
6	3.23±0.34	2.26±0.46	2.26±0.24
8	3.47±0.32	2.85±0.62	3.00±0.54
10	3.90±0.34	3.34±0.34	3.55±0.52
12	4.11±0.34	3.45±0.45	3.76±0.34
14	5.17±0.45	4.85±0.45	5.17±0.51
16	5.48±0.43	4.93±0.56	5.48±0.34
18	6.83±0.23	4.98±0.77	6.83±0.51
20	8.04±0.45	5.05±0.75	7.00±0.56
22	7.85±0.46	5.35±0.51	7.09±0.76
24	7.48±0.55	5.48±0.41	7.11±0.72
26	7.95±0.45	5.95±0.32	7.23±0.54
30	8.32±0.23	6.00±0.43	7.55±0.81

Table 4. *In situ* effect of sodium lactate 0.5 % (T1) and sodium lactate 1.0 % (T2) on inhibition of *Listeria* spp. (Log_{10} CFU/g ± SD) in cold-smoked salmon stored at 4 ^{0}C

Time(day)	Control	T1	T2
0	4.54±0.34	4.50±0.56	4.49±0.23
2	5.11±0.46	4.30±0.49	4.30±0.25
4	5.45±0.56	4.20±0.28	4.23±0.28
6	5.95±0.46	3.90±0.49	4.21±0.22
8	6.38±0.39	3.30±0.45	4.07±0.31
10	6.32±0.58	3.20±0.47	3.32±0.32
12	6.73±0.56	3.12±0.36	3.29±0.45
14	6.59±0.68	3.10±0.49	3.23±0.43
16	6.69±0.87	2.78±0.39	3.20±0.44
18	7.36±0.79	2.65±0.29	3.11±0.47
20	6.90±0.57	2.45±0.41	3.02±0.49
22	7.18±0.37	2.11±0.49	3.03±0.48
24	7.08±0.56	2.09±0.58	3.02±0.51
26	7.48±0.58	2.06±0.58	2.97±0.39
30	7.00±0.73	2.03±0.57	2.67±0.43

Table 5. *In situ* effect of sodium lactate 0.5 % (T1) and sodium lactate 1.0 % (T2) on inhibition of *P. aeruginosa* (Log_{10} CFU/g ± SD) in cold-smoked salmon stored at 4 ^{0}C

Time(day)	Control	T1	T2
0	4.34±0.48	4.34±0.40	4.34±0.55
2	4.04±0.49	4.04±0.65	4.04±0.65
4	3.95±0.42	3.95±0.55	3.95±0.76
6	3.90±0.41	3.90±0.39	3.90±0.66
8	4.30±0.44	3.20±0.58	3.30±0.45
10	4.70±0.45	3.11±0.59	3.26±0.46
12	4.81±0.57	2.90±0.35	3.05±0.32
14	4.95±0.65	2.70±0.48	3.01±0.43
16	4.98±0.62	2.35±0.41	2.94±0.54

18	5.11±0.66	2.22±0.43	2.91±0.65
20	5.34±0.43	2.11±0.49	2.70±0.76
22	5.47±0.51	2.06±0.58	2.65±0.39
24	5.78±0.33	2.02±0.56	2.61±0.21
26	5.88±0.55	2.01±0.55	2.50±0.44
30	5.94±0.54	2.01±0.59	2.50±0.45

4. Conclusion

In this study, sodium lactate (SL) was used to preserve cold smoked salmon under refrigerated conditions. The results showed that, in general, SL slowed down the level of TBC, LAB and pathogenic bacteria compared to the control treatment, and a better result was observed at level 1.0% SL. The results of microbial studies indicate that altogether treatments, the microbial load increased over time, but this increase was observed in the control compared to the other treatment. However, the best results were observed at 1.0% sodium lactate.

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