



## Growth of *Listeria monocytogenes* as Affected by Thermal Treatments of Rainbow Trout Fillets Prepared with Liquid Smoke

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Received 13 January 2012  
Accepted 19 March 2012

### Abstract

Dipping rainbow trout slices into liquid smoke flavoring and applying one of two heat treatments was effective in reducing the initial inoculation levels of *Listeria monocytogenes*. The liquid smoke treatment against *L. monocytogenes* was dipping for 10-30 min with longest duration being most favorable for inactivation. A thermal treatment of 65°C for 20 min had eliminated by 2- or 4 logs the two levels of inocula. The lower temperature, longer time treatment (30°C, 3 h) was not efficient to control *L. monocytogenes*, did not eliminate the bacterium and survivors multiplied at 4°C in package bags.

**Keywords:** *Listeria monocytogenes*, liquid smoke flavoring, antimicrobial effects, rainbow trout.

### Gökkuşluğu Alabalık Filetolarındaki *Listeria monocytogenes* Gelişimine Sıvı Tütsü ve Isıl İşlemlerin Etkileri

#### Özet

Gökkuşluğu alabalık filetolarına uygulanan sıvı tütsü ve iki farklı ısıl işlem başlangıçtaki *L. monocytogenes* sayılarının azalmasında etkili olmuştur. Çalışmada alabalık filetolarının sıvı tütsü içerisinde farklı bekletilme süreleri incelenmiştir. Alabalık filetolarının sıvı tütsü içerisinde bekletilme süreleri arttıkça, *L. monocytogenes* sayılarında azalma saptanmıştır. Sıvı tütsü içerisinde 30 dakika bekletmenin, diğer bekletme sürelerine göre daha etkili olduğu bulgulanmıştır. Alabalık filetolarına 65°C'de 20 dakika uygulanan ısıl işlem, *L. monocytogenes*'in iki farklı inokülasyon değerlerinde de 2 ve 4 log cfu/g eliminasyonunda etkili olmuştur. Düşük derecede uzun süre uygulanan (30°C, 3 saat) ısıl işlemin *L. monocytogenes*'in eliminasyonunda etkili olmadığı ve alabalık filetolarının streç film ile kaplandığında 4°C'de depolandığında çoğaldığı saptanmıştır.

**Anahtar Kelimeler:** *Listeria monocytogenes*, sıvı tütsü, antimikrobiyal etki, gökkuşluğu alabalık.

### Introduction

*Listeria monocytogenes* can grow under anaerobic or microaerophilic conditions and under a wide temperatures range (0-45°C) with an optimum range of 30-37°C. Because it can grow at low temperatures it is considered as psychrotrophic organism that can easily adapt and grow under the conditions of most foods. Its capacity to grow at refrigeration temperatures can be one of the most important factors that make them present at the end of the shelf life of non-sterile refrigerated products (Beumer, 1997). *L. monocytogenes* shows a higher growth rate on fish and shrimp tissue than in beef or chicken at 4°C and under anaerobic conditions (Shineman and Harrison, 1994).

*Listeria* is an environmental contaminant which

has been isolated from marine and fresh waters, as well as various seafoods. Furthermore, *Listeria* including *L. monocytogenes* has been isolated from processed seafood products. Cold smoking at 25-30°C is not sufficient heat to kill the microorganisms. Consumption of these products without additional heating or cooking may present a public health risk (Huss, 1997; Kilinc, 2001). Smoked fish products are often contaminated with *L. monocytogenes* and under refrigeration this organism may be able to multiply to high levels and represent a hazard even with low inoculum doses (Rorvik, 2000).

Smoke extracts could be a factor to control its presence and proliferation in smoked fish products (Poysky *et al.*, 1997). While both plant essential oils and wood smoke can serve as natural antimicrobial agents for a variety of foods, it is unlikely that a

single natural antimicrobial from each group can be used at one concentration in all food products and be effective against all kinds of organisms. Different concentrations of even the most potent compounds are required in different food products to show desirable antimicrobial activity. The optimum concentration will depend on the type and number of problematic bacteria, as well as the type of food and food storage temperature (Holley and Patel, 2005).

*L. monocytogenes* is a problem for cold smoked and hot smoked fishery products. For this reason in this study cold smoking temperature (30°C for 3 hours) and hot smoking temperature (65°C for 20 minutes) were chosen. Rainbow trout slices were inoculated by two inoculums (10<sup>2</sup> cfu/g is the maximum level for acceptability) or high contamination level (10<sup>4</sup> cfu/g) of *L. monocytogenes* were chosen whether or not these *L. monocytogenes* concentrations were eliminated after cold or hot smoking by using liquid smoke. And also examined two different doses of *L. monocytogenes* contamination is a risk for consumption during storage period for cold and hot smoked rainbow trout.

In this study, the antimicrobial effect of commercial liquid smoke flavoring plus applying two heat treatments against two inoculated loads of *L. monocytogenes* in rainbow trout slices was evaluated.

## Material and Methods

### Bacteria Preparation

*Listeria monocytogenes* strain (Scott A) obtained from Egean University Food Engineering Department in Turkey was used. Cultures were taken from Trypticase soy agar (bioMerieux S.A., 51044, France) stored at 5°C. Before use, *L. monocytogenes* strains were precultured in Trypticase soy broth (bioMerieux S.A., 51019, France) with 0.6% yeast extract (Merck, 1.03753, Germany) at 30°C for 18 h. (Sunen *et al.*, 2003).

### Sample preparation

Fresh-farmed rainbow trout fillets (6 kg) (approximately 200 g per piece) were obtained from a fish processing factory. The fish fillets were washed with tap water. Fillets were brined in a 10% NaCl solution for 30 minutes. The ratio of fillets to brine was (1:1). After air drying for 10 min., fillets were cut aseptically into 25 g pieces. Then each portion was surface inoculated with *L. monocytogenes* giving final inocula of 2 log or 4 log cfu per gram of rainbow trout. Rainbow trout pieces (25 g) inoculated by using dropping method with 15 µl of *L. monocytogenes*. The cell suspension was randomly dropwise on the surface of the trout pieces distributed using a sterile spatule. After being air dried for 10 min., trout pieces were dipped into the liquid smoke preparations. Trout pieces inoculated with 2 log cfu/g of *L.*

*monocytogenes* dipped into the liquid smoke for 10 min. (Group B), 20 min (Group C) and 30 min. (Group D). Group A (trout pieces inoculated with 2 log cfu/g of *L. monocytogenes*) was indicated as control. Trout pieces inoculated with 4 log cfu/g of *L. monocytogenes* were dipped into the liquid smoke for 10 min (Group F), 20 min. (Group G), 30 min (Group H). Group E (trout pieces inoculated with 4 log-cfu/g of *L. monocytogenes*) was indicated as control group. The liquid smoke flavoring (Aromatech, 7.27531, Maltepe, İstanbul, Türkiye) was used as a 0.5% solution in distilled steril water. The ratio of trout : smoke flavoring was (1:1). Trout pieces were air dried for 10 min. In this study two different heat treatments 65°C for 20 minutes and 30°C for 3 hours were applied in the oven. Group I was indicated as control group (liquid smoke was not used). Trout pieces inoculated with 2 log cfu/g of *L. monocytogenes* were dipped into the liquid smoke for 10 min and then heat treatment was applied at 30°C (Group J). Trout pieces inoculated with 2 log cfu/g of *L. monocytogenes* were dipped into the liquid smoke for 20 min and then heat treatment was applied at 30°C (Group K), Trout pieces inoculated with 2 log cfu/g of *L. monocytogenes* were dipped into the liquid smoke for 30 min and then heat treatment was applied at 30°C (Group L). Trout pieces inoculated with 4log cfu/g of *L. monocytogenes* were dipped into the liquid smoke for 10 min (Group N), 20 min (Group O), 30 min. (Group P). Heat treatment of 30°C were also applied for Group N, O and P. Group M indicated as control group. Control group was not dipped into the liquid smoke.

Trout pieces were put into styrofoam plates stretched by film and stored at 4°C. Inoculated trout pieces without liquid smoke flavoring indicated as controls. Samples were taken for microbiological analyses initially and at 5 and 10 days of storage.

### Microbiological Analyses

#### Methods

The Horizontal Method (ISO 11290-2: 1998) for the enumeration of *Listeria monocytogenes* was used. Two replicates for each dilution were tested. Each 25 g of sample was put in stomacher bag and added 225 ml of buffered peptone water. Samples were homogenised by using stomacher (IUL, Barcelona, Spain) for 30 s at low speed and incubated at 20°C for 1 h. Inoculum (0.1 ml) was spreaded on Ottaviani-Agosti Agar (bioMerieux S.A, 43641, France). Plates were incubated at 37°C for 24 hours. Characteristic blue-green colonies with halos were enumerated as *Listeria monocytogenes*.

### Statistical Analyses

SPSS 9.0 program (SPSS version 9.0 Chicago, IL, USA) was used to search for significant

differences between mean values of the different results. Differences between means were analysed by one-way analysis of variance (ANOVA) followed by Tukey and Duncan tests. When a significant differences ( $P < 0.05$ ) were determined between the treatments, either the Tukey or Duncan multiple comparison test was done to a certain more conservative differences using multiple comparison.

## Results and Discussion

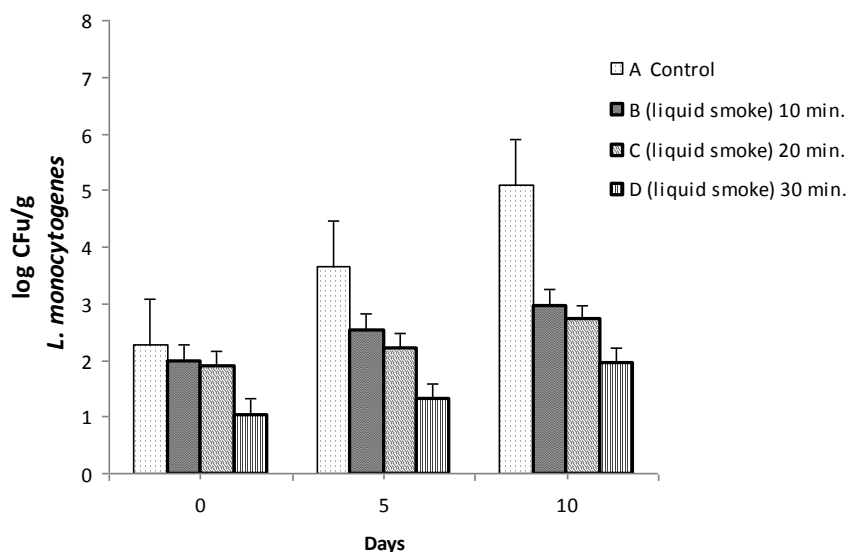
Figure 1 shows *L. monocytogenes* detection on trout slices inoculated with 2 log cfu/g. After dipping into liquid smoke flavoring *L. monocytogenes* values decreased to 2.00, 1.91, 1.05 log cfu/g for the groups B, C and D. After 10 days of storage *L. monocytogenes* population increased to 2.97, 2.73 and 1.96 log cfu/g for the groups B, C and D, respectively. In control group (A); *L. monocytogenes* concentration increased from 2.28 log cfu/g initially to 5.09 log cfu/g on day 10. The exposure time was determined very important for reducing *L. monocytogenes* concentration. The most effective *L. monocytogenes* reduction on trout slices was determined for the Group D (exposure time was 30 min.). Significant ( $P < 0.05$ ) differences were observed between the group A and the other groups for *L. monocytogenes* counts according to time of storage. There were no significant differences ( $P > 0.05$ ) between the treatments B and C, but significant differences ( $P < 0.05$ ) were observed between the treatments B and D according to time of storage.

Sunen *et al.* (2003) were used three different commercial liquid smoke extracts (L1, L2 and L3) and one dried smoke (S) in their study but they were not indicated the formulation of the commercial liquid smoke extracts. The solid extract was used as a 10% solution in distilled sterile water. They studied the effects of different commercial liquid smoke extract

against elimination of *L. monocytogenes*. They showed that only smoke extracts L1 and L2 were effective against *L. monocytogenes*. They caused a reduction in viable cell counts of *L. monocytogenes* below the detectable level by surface plating procedure during the experimental period. However, the qualitative estimation of *L. monocytogenes* showed that *L. monocytogenes* cells were still present in most of the samples. The commercial liquid smoke extract L3 did not show any inhibitory effect against *L. monocytogenes*. Finally, no significant differences were observed in cell counts between control samples and samples treated with the extract S (dried smoke) until day 21 when *L. monocytogenes* was only detected by the qualitative method.

In another report, antimicrobial compounds like spices, herbs and smoke extracts present in foods can improve taste and enhance the shelf-life of product by reducing microbial growth rate or viability (Kim *et al.*, 2001). The results of our study were very similar to these studies about reducing of bacteria after dipping into smoke extracts.

Figure 2 shows *L. monocytogenes* detection on trout slices inoculated with 4 log cfu/g concentration. Liquid smoke flavoring reduced the populations of *L. monocytogenes*. Dipping into the liquid smoke for 30 minutes was determined the most effective for reducing the population of *L. monocytogenes*. *L. monocytogenes* populations increased according to time of storage. *L. monocytogenes* populations increased from 4.03, 3.23, 2.85, 2.27 log cfu/g initially to 6.97, 4.11, 3.93, 3.08 log cfu/g on day 10 for E, F, G and H, respectively. Significant differences ( $P < 0.05$ ) were determined between the group E and the groups F, G, H according to time of storage. Siskos *et al.* (2007) reported that the storage time at  $4 \pm 1^\circ\text{C}$  of liquid smoked fillets of trout (*Salmo gairdnerii*) produced with a new smoking technique, using a combination of liquid smoke and steaming at



**Figure 1.** *L. monocytogenes* detection on trout slices inoculated with 2log-cfu/g.

2 bar pressure for 30, 45 and 60 min. was studied. Maximum total viable counts (TVC) were reached after 25 days in the samples processed for 30 min and after 48 days in those processed for 45 and 60 min. After processing, the TVC in all samples were at low levels with an average of  $5.3 \times 10^3$  cfu/g, regardless of the processing time. After 25 days of storage at  $4 \pm 1^\circ\text{C}$  a striking increase in microbial count was detected in the samples processed for 30 min and TVC remained stable at high levels ( $7.0 \times 10^6$  cfu/g) after 48 days of storage at  $4 \pm 1^\circ\text{C}$ . The results of this study were very similar to our findings about increasing the bacteria counts according to time of storage.

Figure 3 shows *L. monocytogenes* detection on trout slices inoculated with 2 log cfu/g plus liquid smoke and heat. The mild heat treatment ( $30^\circ\text{C}$ , 3 h) was not sufficient to inhibit the growth of *L. monocytogenes*. After the heat treatment of  $30^\circ\text{C}$ , *L. monocytogenes* populations were 2.13, 1.93, 1.73 ,

1.25 log cfu/g initially and on day 10, *L. monocytogenes* increased to 4.06, 2.85, 2.45 and 2.28 log cfu/g for treatments I, J, K and L, respectively. According to time of storage significant differences ( $P < 0.05$ ) were determined between treatment I and the other groups, but there were no significant differences ( $P > 0.05$ ) were observed between treatments J, K and L.

Porsby *et al.* (2008) reported that *L. monocytogenes* proliferated on salmon blocks that were brined or dipped in liquid smoke and left at  $25^\circ\text{C}$  in a humidity chamber for 24 h. However, combining brining and liquid smoking with a drying ( $25^\circ\text{C}$ ) step reduced 10–100 fold of bacterial proliferation over 24 h period. Non-salted, brine injected or dry salted salmon fillets were surface inoculated with *L. monocytogenes* and cold-smoked in a pilot plant. *L. monocytogenes* was reduced from  $10^3$  to  $10^2$  cfu/cm<sup>2</sup> immediately after cold smoking. The

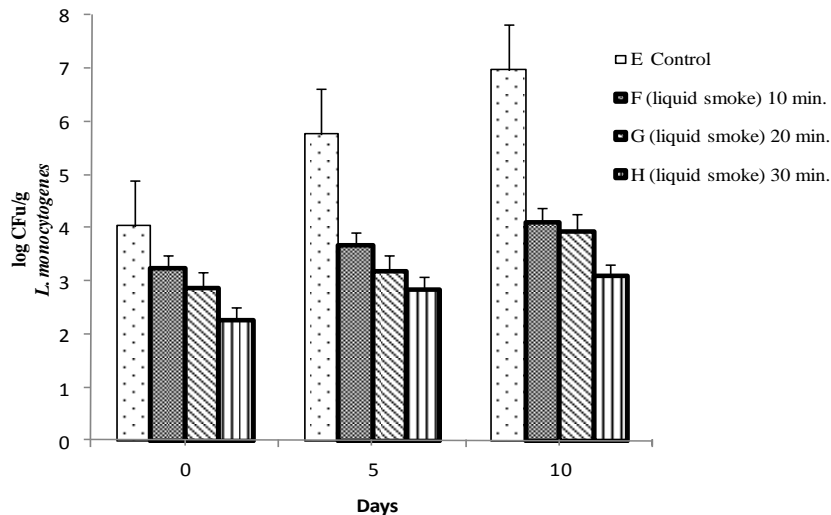


Figure 2. *L. monocytogenes* detection on trout slices inoculated with 4log-cfu/g.

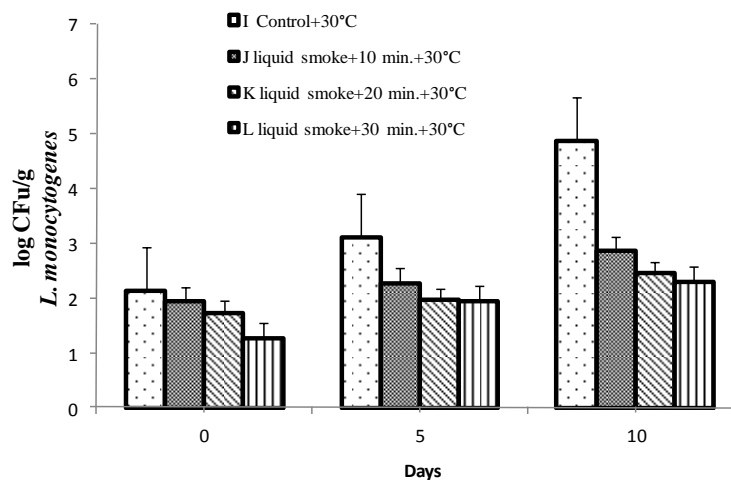


Figure 3. *Listeria monocytogenes* detection on trout slices inoculated with 2 log cfu/g + liquid smoke + 30°C.

greatest reductions were observed in dry salted and brine injected fillets as compared to cold-smoking of non-salted fresh fillets. Levels of *L. monocytogenes* decreased further when the cold-smoked fish was vacuum-packed and stored at 5°C. Dillon and Patel (1993) studied the effects of cold smoking process and storage on *L. monocytogenes* inoculated onto cod (*Gadus morhus*) fillets. Numbers of the organisms remained relatively stable during the smoking process and increased with storage at 4°C. Jorgensen and Huss (1998) reported that *L. monocytogenes* showed moderate growth in naturally contaminated cold-smoked fish while the growth appeared faster in hot smoked fish. Thus *L. monocytogenes* is not under control in these products. Finally, the prevalence and growth of *L. monocytogenes* in naturally contaminated cold-smoked salmon are discussed in relation to controlling this risk. Our results were very similar with these investigations about *L. monocytogenes* populations remaining stable during the cold smoking and had increased according to time of storage.

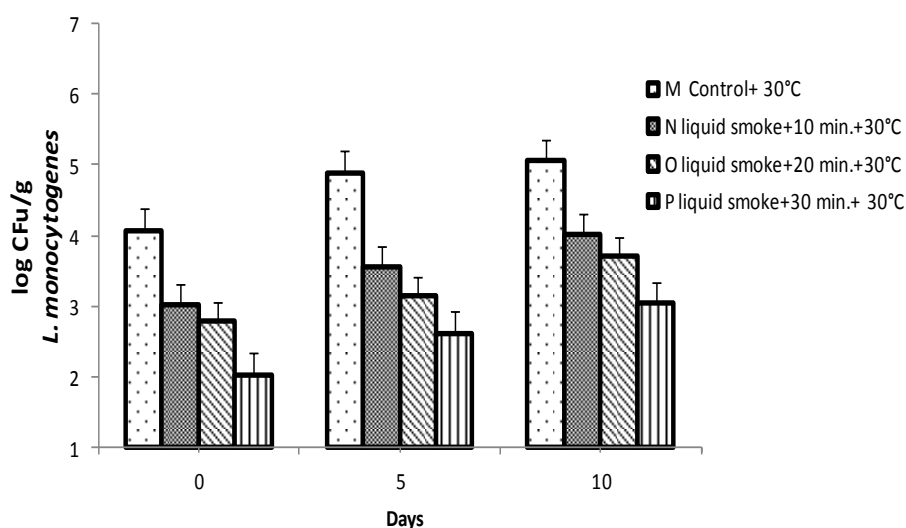
Figure 4 shows *L. monocytogenes* detection on trout slices inoculated with 4 log cfu/g plus liquid smoke plus 30°C heat treatment. After dipping into liquid smoke flavouring, *L. monocytogenes* populations decreased from 4.08 to 3.01, 2.79 and 2.03 log cfu/g initially for treatments N, O and P, respectively. At the end of the storage period, *L. monocytogenes* populations increased to 5.06, 4.02, 3.70 and 3.05 log cfu/g for treatments M, N, O and P, respectively. Significant ( $P < 0.05$ ) differences were observed between the four groups for *L. monocytogenes* counts according to time of storage. Midelet-Bourdin *et al.* (2010) reported that salmon preparations were inoculated with *L. monocytogenes* and were analyzed during storage at 4°C then 8°C. At 8°C, the bacteria growth was of 4.53 log cfu/g in cold-smoked salmon and of 2.06 log cfu/g in the salt-sugar-pepper mixture salmon without background microflora. The growth of *L. monocytogenes* was

different in new salmon preparation because the mixture salt-sugar-pepper had an anti-*Listeria* activity and its presence could be inhibitory to the growth. It is difficult to generalize findings observed with cold smoked salmon to a new salmon preparation. Rorvik *et al.* (1991) reported that samples of smoked salmon of different hygienic quality were inoculated with low (6 cfu/g) and high (600 cfu/g) levels of a mixture of three strains of *L. monocytogenes* after which they were vacuum-packed and stored at 4°C for up to 5 weeks. *L. monocytogenes* grew well during storage in all the inoculated sample groups. Our results are similar with these studies about increasing *L. monocytogenes* populations during refrigerated storage.

After 65°C for 20 min. *L. monocytogenes* was eliminated in all groups. The combination of liquid smoke flavoring and the treatment of 65°C for 20 min. eliminated *L. monocytogenes*. (data not shown). Liquid smoke flavoring was not efficient to eliminate *L. monocytogenes*. Jemmi and Keusch (1992) reported that raw trout was surface inoculated with *L. monocytogenes* marinated, hot smoked (core temperature 65°C during 20 min.) and stored at 4 and 8-10°C, respectively for up to 20 days. Initial *Listeria* levels in the trout were 10<sup>6</sup> MPN/g. Until smoking the concentrations remained about the same. After the hot smoking process and during storage, however, *L. monocytogenes* could no longer be detected. Rorvik (2000) indicated that hot-smoking seems to eliminate the bacteria when smoke is applied during the whole heating process. The prevention of recontamination of both cold-smoked and hot-smoked salmon is therefore of great importance. *L. monocytogenes* multiply considerably in smoked salmon during refrigerated storage.

## Conclusions

Our results confirmed that dipping trout slices into the liquid smoke flavoring was effective to



**Figure 4 .** *Listeria manocytogenes* detection on trout slices inoculated with 4log-cfu/g +liquid smoke+30°C.

reduce the concentrations of *L. monocytogenes*. Exposure time was very important to control the growth of *L. monocytogenes*. Dipping into liquid smoke flavoring for 30 min. was the most effective against *L. monocytogenes*.

After 65°C for 20 min. *L. monocytogenes* was eliminated in all groups. However the 30°C 3h. treatment was not efficient to control of *L. monocytogenes*. *L. monocytogenes* populations remaining stable during the cold smoking (30°C 3h) and increased according to time of storage. During storage period, the survival of *L. monocytogenes* in cold smoked trout can be risk for human consumption. Dipping of trout slices into smoke flavoring for 10, 20 and 30 min. did not eliminate *L. monocytogenes* completely.

The survival of *L. monocytogenes* in trout slices depended on exposure time with liquid smoke, the contamination level of *L. monocytogenes* and heat treatments. The combination of liquid smoke flavoring and heat treatment of 65°C for 20 min. were efficient against *L. monocytogenes*. Only liquid smoke flavoring was not efficient for elimination of *L. monocytogenes*. After applying hot smoking process, hot smoked trout could be avoided from the contamination of *L. monocytogenes*. Because *L. monocytogenes* can be also risk for hot smoked trout during storage period.. The next study will investigate the increased exposure time of liquid smoke flavoring on the elimination of *L. monocytogenes* in trout slices.

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