EFFICACY OF DISINFECTANTS AGAINST *LISTERIA MONOCYTOGNES*

Aidar Sambetbayev
MSc Thesis
Green Biotechnology and Food Security
Faculty of Science and Forestry
University of Eastern Finland
23rd November 2016

UNIVERSITY OF EASTERN FINLAND, Faculty of Science and Forestry

Green Biotechnology and Food Security program
Aidar Sambetbayev: Efficacy of disinfectants against *Listeria monocytogenes*
MSc thesis 36 pages
Supervisors: Jenni Korhonen, PhD; Roseanna Avento, MSc
23rd November, 2016

Keywords: *Listeria monocytogenes*, listeriosis, disinfectant.

ABSTRACT

Listeria monocytogenes is a human pathogenic bacterium which can cause serious disease, and nowadays the regulation of the bacterium in the food processing environment has become a primary issue in the assurance of food safety. Listeriosis is mostly related to the ingestion of contaminated food, with 99% of human cases of listeriosis connected to food sources. However, the epidemic and sporadic incidences of listeriosis are relatively low compared to other foodborne diseases, for instance campylobacteriosis and salmonellosis, but the mortality from listeriosis is significantly higher. This makes listeria one of the most important pathogenic bacteria.

Various studies substantiate the ability of *L. monocytogenes* to contaminate food processing environments. The main target for food enterprises is to achieve a high level of hygiene, which can be achieved by adequate sanitation, especially disinfection practices with disinfection agents that are effective.

The aim of this study was to determine the efficacy of five commercial disinfectants against five *L. monocytogenes* strains. Disinfectants were tested in accordance with BS-EN1276 quantitative suspension test for the evaluation of the bactericidal activity of chemical disinfectants. The experimental part was undertaken during the period of May/June 2015.

According to European Standard BS-EN 1276:2009 disinfectants must achieve five log reduction for assessing the ability of disinfectant to eliminate or inhibit bacterial activity. The reduction was calculated by subtracting the log of control from sample (after disinfection) group. In this study, during laboratory tests all five disinfectants achieved at least 6.96 log reduction, the majority of results exceed 7 log reduction. Based on the results, it can be assumed that disinfectants used in this study are effective against *L.monocytogenes* bacterium.

УНИВЕРСИТЕТ ВОСТОЧНОЙ ФИНЛЯНДИИ, Факультет естественных наук и лесного хозяйства
Программа Зеленая Биотехнология и Пищевая Безопасность
Самбетбаев Айдар: Эффективность дезинфектантов против листерии (*Listeria monocytogenes)*.
Магистерская диссертация, 36 страниц
Руководители: Енни Корхонен, Phd; Розанна Авенто, MSc.
23 Ноября, 2016

Ключевые слова: листерии, листериоз, дезинфектанты

L.monocytogenes - это бактерии патогенные для человека, которые могут вызвать опасное заболевание, в настоящее время контроль листериями в производственной среде пищевой промышленности стал основным вопросом в обеспечении пищевой безопасности. Листериоз в основном может быть связан с употреблением контаминированной пищи, 99% установленных случаев листериоза связаны с пищевыми продуктами. Однако, эпидемические и спорадические случаи листериоза сравнительно низки при сопоставлении с другими заболеваниями пищевого происхождения, как например кампилобактериоз и сальмонеллез, но смертность от листериоза значительно выше. Все это делает листерии одними из основных бактерии. Различные исследования подтверждают способность листерии загрязнять производственную среду пищевой промышленности. Основной задачей предприятии пищевых продуктов заключается в достижении высокого уровня гигиены, который может быть достигнут надлежащей санитарией, особенно дезинфекционной практикой с применением эффективных дезинфицирующих средств.

Цель данного исследования заключалась в определении эффективности пяти коммерческих дезинфицирующих средств, против пяти штаммов *L.monocytogenes*. Дезинфектанты были испытаны в соответствии с Европейским Стандартом BS-EN1276 «Количественный суспензионный тест для оценки бактерицидной активности химических дезинфицирующих средств». Экспериментальная часть исследований была проведена в период май/июнь 2015г.

В соответствии с Европейским Стандартом BS-EN 1276: 2009 дезинфектанты должны достичь пятикратного логарифмического снижения общего числа микроорганизмов, для подтверждения способности ингибировать активность бактерий. Снижение рассчитывали путем вычитания логарифмов контрольной группы от опытной группы (после дезинфекции). В данном исследовании, в ходе лабораторных испытаний, все пять дезинфицирующих средств достигли по меньшей мере снижения на 6,96 Log, большая часть достигла свыше 7,0 Log понижения. На основании полученных результатов, можно предположить, что дезинфицирующие средства, используемые в данном исследовании, являются эффективными против *L.monocytogenes*.

ШЫҒЫС ФИНЛЯНДИЯ УНИВЕРСИТЕТІ, Жаратылыстану ғылымдары және орман шаруашылығы факультеті
Жасыл Биотехнология және Тағам Қауіпсіздігі бағдарламасы
Самбетбаев Айдар: Листерияга (*Listeria monocytogenes)* қарсы дезинфектанттарың тиімділігі.
Магистрлік диссертация, 36 бет
Ғылыми жетекшілер: Йенни Корхонен, Phd; Розанна Авенто, MSc.
23 Қараша, 2016

Түйін сөздер: Кілт сөздер: листерия, листериоз, дезинфектанттар

Listeria monocytogenes-пен байланысты әр түрлі аспектілерін зерттеу жалғасуда. *L.monocytogenes* даму сипаты қауіпті және асқынулары ауыр ауруын тудыруы мүмкін адамдарға патогенді бактериялар болып табылады. Адамдардың листериозды жұқтырудың негізгі жолы - азық-түлік арқылы, листериозды жұқтырудың 99% жағдайы *L.monocytogenes*-пен ластанған азық-түлік өнімдерін тұтынумен байланысты. Тамақ өнеркәсіптері үшін негізгі мәселе тиімді дезинфекциялау құралдарын қолдану арқылы дезинфекция тәжірибесімен тазалық пен санитарияның жоғары деңгейін қамтамасыз ету болып табылады. Кәсіпорын азық-түлік негізгі мақсаты тиісті санитария арқылы қол жеткізуге болады гигиена жоғары деңгейін, тиімді пайдалана отырып, дезинфекциялау әсіресе тәжірибесін қол жеткізу болып табылады.

Бұл зерттеу жұмысының мақсаты *L.moncytogenes*-тің бес штаммына қарсы бес коммерциялық дезинфекциялаушы құралдардың тиімділігін анықтау болды. Барлық дезинфекциялаушы құралдар «Химиялық дезинфекциялау құралдарының бактерицидтік қызметін бағалаудың сандық суспензиялық сынағы» BS-EN1276 Еуропалық стандартына сәйкес сыналған. Зерттеу жұмысының эксперименталды бөлігі 2015 жылдың мамыр/маусым айлары аралығында жүргізілді.

Еуропалық BS-EN 1276:2009 стандартына сәйкес дезинфекциялаушы құралдар бактериялардың белсенділігін тежейтін қабілетін растау үшін олардың микроорганизмдердің жалпы санының бес есе логарифмдік азаю деңгейіне жету керек. Азаю деңгейі эксперименттік топтан бақылау тобының логарифмін шегеру жолымен есептелген (залалсыздандырудан кейін). Бұл зерттеу жұмысында зертханалық сынақтар барысында, барлық бес дезинфекциялаушы құралдардың азаю деңгейі 6,96 log дейін жетті, барлық көрсеткіші -7,0 log. Алынған нәтижелерге сүйене отырып, осы зерттеуде қолданылған дезинфекциялаушы құралдар *L.monocytogenes*-ке қарсы тиімді болып табылады деп болжауға болады.

CONTENTS

AKNOWLEDGEMENTS

The study was conducted at the faculty of Institute of Public Health and Clinical Nutrition of the University of the Eastern Finland. I would like to acknowledge Professor Atte von Wright for putting his trust in me and allowing me to do the research study on the effectiveness of disinfectants against *Listeria monocytogenes*. I appreciate my supervisors Dr. Jenni Korhonen and Roseanna Avento who constantly assisted me in conducting my study. Being supportive, they gave me invaluable feedback and substantial criticism.

Especially I would like to express my sincere gratitude to the staff of the Kazakh National Agrarian University for giving me the opportunity to study abroad and acquire new knowledge in the area of food security under the Master's degree programme Green Biotechnology and Food Security, that was organised by both Universities.

Finally, I am thankful to my parents and my wife for believing in me, their thoughts and caring.

1 INTRODUCTION

Listeria monocytogenes is considered to be one of the leading causative agent of food borne illnesses (Lundén *et al.,* 2005). *L. monocytegenes* is a ubiquitously intra-cellular gram-positive, pathogenic bacterium which can cause listeriosis, a dangerous infection with severe manifestations such as septicaemia, meningitis and foetal death (Garrido *et al.,* 2009; Farber and Peterkin, 1991). The morbidity of human listeriosis is low, but it has a high case-fatality rate of around 30% (Vazquez-Boland *et al.,* 2001). In the European Union, about 2,161 human cases were reported in 2014, with a mortality rate of 15% (European Food Safety Authority, 2015). *L. monocytogenes* is primarily transmitted through lightly preserved or ready-to-eat food (RTE) products in which it can survive and grow. Listeriosis is associated with RTE products and recent recalls of contaminated batches has caused major economic losses in the food industry (Teratanavat and Hooker, 2004). The incidences of food borne listeriosis are rising in the EU for example there was an incidence increase of 30% in 2014 compared to 2013 (European Food Safety Authority, 2015).

A number of characteristics help the microorganism to persist and multiply in various types of refrigerated or frozen RTE products. It can tolerate high concentrations of sodium chloride, low temperatures and low oxygen content (Yousef and Lado, 2007). Modern lifestyle and urbanisation lead to the rise in consumption of RTE (Garrido *et al.,* 2009).

Today, food enterprises are facing problems connected with bacterial contamination of food processing environment with pathogenic bacteria (Autio *et al.,* 2004). Pathogenic bacteria are spread ubiquitously, they can cause serious consequences and threats to public health (Nyachuba, 2010). Pathogenic microorganisms and particularly *Listeria monocytogenes* have the ability to contaminate different food and food contact surfaces (Kusumaningrum *et al.,* 2003). In European RTE products, the highest prevalence of *L.monocytogenes* (10.6%) was determined in ready-to-eat fish and fishery products (EFSA, 2015).

Preventing contamination by *L. monocytogenes* can be mainly achieved through efficient cleaning of surfaces and especially disinfection measures (Yousef and Lado, 2007), which is considered to be a critical step in reducing the level of pathogenic bacteria in the food processing environment. Food enterprises and related industries adopt different cleaning and disinfection measures for reduction of pathogens to acceptable levels, or for their elimination by using different chemical

agents (Dvorak, 2008). Disinfection in the food processing environment can be done using chemical or physical methods. Physical factors include heat treatment such as pasteurization, dry heat sterilization, moist heat sterilization, nonionizing radiation with ultra-violet (UV) rays, and filtration with membrane filters (Otto *et al.,* 2011). Chemical treatments include sterilization with addition of antimicrobial agents such as disinfectants and antiseptics (Mc Donnell, 2010). The success of disinfection measures depends on certain conditions, such as concentration of chemical substance, exposition time, and treated surface (Yousef and Lado, 2007). Disinfectants used in the food industry should be able to eliminate or inhibit the growth of pathogenic bacterium (reference),

The aim of this study was to determine the efficacy of five commercial disinfection agents against five *L. monocytogenes* strains. It is vital to analyse the effectiveness of the disinfectants in the food processing environment (Breidt *et al.,* 2006; Tompkin, 2002) as the cases of the human listeriosis are likely caused by unsafe products for human consumption.

This study was done in the Institute of Public Health and Clinical Nutrition of the University of Eastern Finland in May-June of the academic year 2015-2016.

2 LITERATURE REVIEW

2.1 *LISTERIA MONOCYTOGENES*

The *Listeria* genus is a group of gram-positive bacteria with low content of guanine-cytosine, similar to *Staphylococci* and *Bacilli* (Sallen *et al*., 1996). The *Listeria* genus consists of seven species: *L. monocytogenes, L. ivanovii, L. seeligeri, L.innocua, L. welshimeri, L. grayi* and recently discovered *L. marthii* (Graves *et al*., 2009; Sallen *et al*., 1996). Two members of the *Listeria* genus are pathogenic, *L. monocytogenes* and *L. ivanovii* (Seeliger and Jones, 1986). *L. monocytogenes* is pathogenic to humans, domestic animals and wild animals, whereas *L. ivanovii* is the major pathogen for animals (Seeliger and Jones, 1986). *L. seeligeri* is generally recognized as avirulent, even though it was isolated from a case of human listeriosis (Rocourt *et al*., 1986).

Morphologically *L. monocytogenes* are rods of 0.5-2 μm in length and 0.4 μm in diameter found in short chains or as single cells and they can not form capsules or produce spores (Garrido *et al*., 2009). Peritrichous flagella make the motility of *L. monocytogenes* possible when it is cultured under +25ºC (Seeliger and Jones, 1986). In the temperature range between 0.5ºC to 45ºC *L. monocytogenes* has the ability to persist and multiply, with optimal temperature for growth from 30-37ºC (Seeliger and Jones, 1986). Nevertheless, the growth of *L. monocytogenes* at the temperatures lower than 4ºC is very slow (Garrido *et al*., 2009).

Depending on the type of acid and temperature *L. monocytogenes* can grow in the pH range 4.3-9.8 and can grow in acidic pH down to 4.0 (Yousef and Lado, 2007; Martin and Fisher, 1999). *L. monocytogenes* can multiply in complex media with sodium chloride content up to 10 % and different strains of *L. monocytogenes* can resist even 20% sodium chloride content (Yousef and Lado, 2007; Seeliger and Jones, 1986). Consequently, *L. monocytogenes* can tolerate low water activity, as low as 0.91 (Yousef and Lado, 2007). Furthermore, *L. monocytogenes* has the ability to multiply with competitive microbes in the modified atmosphere (vacuum) (Wimpfheimer *et al*., 1990).

The prevalence of *L. monocytogenes* in the outdoor environment is relatively low despite the ubiquitous nature of the microorganism (Porsby *et al*., 2008). It could be isolated from different sources in outdoor environment such as water, soil, sewage, decaying organic matter, human and animal feces (Mohhamad *et al*., 2010; Rocourt, 1994).

L. monocytogenes is divided in 13 serotypes (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4c, 4d, 4e, 7) due to existence of somatic and flagellar antigens (Seeliger and Jones, 1986). Serotypes 1/2a, 1/2b, 1/2c and 4b are responsible for at least 95% human cases of listeriosis (Swaminathan and Gerner-Smidt, 2007; Farber and Peterkin, 1991). The majority of human invasive listeriosis cases are caused by serotype 4b, while non- invasive forms are caused by serotype 1/2 (Swaminathan and Gerner-Smidt, 2007) However, serotype 1/2 is commonly found in isolates obtained from food and food processing environment (Jacquet *et al*., 2002).

The morbidity of human listeriosis is low, but it has a high case-fatality rate of around 30% (Vazquez-Boland *et al.,* 2001). In the European Union, about 1,476 human cases were reported in 2011, with a mortality rate of 12.7% (European Food Safety Authority, 2015). *L. monocytegenes* is primarily transmitted through lightly preserved or ready-to-eat food (RTE) products in which it can survive and grow. A number of characteristics help the microorganism to persist and multiply in various types of refrigerated or frozen RTE products. It can tolerate high concentrations of sodium chloride, low temperatures and low oxygen content (Autio *et al*., 2004). Modern lifestyle and urbanisation lead to the rise in consumption of RTE (Garrido *et al*., 2009).

2.1.1 INCIDENCE OF *LISTERIA MONOCYTOGENES* IN FOODS

Today, food enterprises face problems connected with bacterial contamination of food processing environments with pathogenic bacteria (Autio *et al.,* 2004). Pathogenic bacteria are spread ubiquitously and they can cause serious consequences and threats to public health (Nyachuba, 2010). Pathogenic microorganisms and particularly *L. monocytogenes,* have the ability to contaminate different food and food contact surfaces (Nucera *et al*., 2010; Wagner *et al*., 2007; Vitas *et al*., 2004, Kusumaningrum *et al*., 2003). The presence of *L. monocytogenes* in the food processing environment is possible even if food processing process implemented according to hazard analysis and critical control point (HACCP) principles (Autio *et al*., 2004). Specific subtypes of *L. monocytogenes* also have the ability to persist for around ten years in the food processing environment (Wulff *et al*., 2006; Norton *et al*., 2001).

Listeriosis is mostly related to the ingestion of contaminated food with 99% of human cases of listeriosis connected to food sources (Gray *et al*., 2004). However, the epidemic and sporadic incidence of listeriosis is relatively low compared to other foodborne diseases, for instance campylobacteriosis and salmonellosis, but the mortality from listeriosis is significantly higher (Ivanek *et al*., 2004). This makes listeria one of the most important pathogenic bacteria (EFSA 2015; Ivanek *et al*., 2004). Furthermore, listeriosis caused by the ingestion of food contaminated with the *L. monocytogenes* has an extensive effect on economy and public health (Mead *et al*., 1999).

Numerous studies report that the highest numbers of *L. monocytogenes* incidence occur in ready-to-eat food products (Garrido *et al*., 2009). In the United States, the level of prevalence of *L monocytogenes* in RTE food products was detected as 1.82% in 31,705 tested samples (Kastbjerg and Gram, 2009). The percentages of positive samples were highest in smoked seafood 4.3%, and in seafood salads 4.7% (Gombas *et al*., 2003).

In the European Union, *L. monocytogenes* is seldom identified in RTE food products in levels exceeding legal food safety limits. Levels exceeding 100 CFU/g are found more often in smoked fish and RTE fishery products (Gombas *et al*., 2003; Wallace *et al*., 2003). The highest proportion of positive samples have been obtained from the fish based RTE food products in levels exceeding 100 CFU/g and amounted 4.6% (EFSA, 2015).

Nevertheless, despite the amount of positive samples, there are only few reported human cases of listeriosis related with ingestion of contaminated RTE fishery products, and limited numbers of patients were affected (Farber *et al*., 2000; Brett *et al*., 1998). The first mention about *L. monocytogenes* isolated from frozen crab meat was in 1987, human cases of listeriosis linked to ingestion of seafood or fishery products were not reported before 1989 (Facinelli *et al*., 1989; Ibusquiza *et al*., 2011). Furthermore, different fishery food products such as shrimps, steamed mussels, fish salads, salmon and cold smoked rainbow trout were defined as a source of *L. monocytogenes* and have caused human listeriosis (Tham *et al*., 2000; Miettinen *et al*., 1999; Brett *et al*., 1998; Ben Embarek, 1994).

It is also observed that *L.monocytogenes* can develop and reproduce in uncooked meat and vegetables, as well as in unpasteurized raw milk and cheese. It is suggested that *L. monocytogenes* strains can be destroyed through pasteurization and excessive cooking of meat (Cartwright et.al., 2013). However, it is also observed that processed cheese made from unpasteurized milk may also contain *L. monocytogenes*.

The prevalence of *L. monocytogenes* in the food processing environment and its frequent detection has increased the doubts, that the food industry could eliminate contamination of *L. monocytogenes* effectively (Ibusquiza *et al*., 2011). Since complete elimination of *L. monocytogenes* in conditions of food enterprises is impracticable, legal limits for its control are provided by legislation and implemented through effective hygiene practices.

2.2 CONTROL OF *LISTERIA MONOCYTOGENES* IN FOOD PROCESSING

The food legislation regarding the safe limits of *L. monocytogenes* varies in different countries, for instance in the United States, it is zero tolerance for *L. monocytogenes* in RTE. In the European Union, food safety requirements for *L. monocytogenes* are established in accordance with Regulation (EC) No. 2073/2005 which requires the following:

- The level of *L. monocytogenes* should not exceed 100 CFU/g during the shelf-life of the food product.
- *L. monocytogenes* must not be detected in 25 g of samples in food products intended for vulnerable groups of people (YOPI: young, old, pregnant, immunosuppressed).

These levels have been set as acceptable to healthy and vulnerable groups of people (Buchahan *et al*., 1997; Farber *et al*., 1996).

The Eurasian Economic Union (EEU) was established in 2010 and includes five member states (Russia, Kazakhstan, Belarus, Armenia, and Kyrgyzstan) whose food legislation is regulated by the Technical Regulations to Customs Union (TR CU 021/2011 “Food Safety”) which requires the following:

- *L. monocytogenes* should not be detected in 25 g samples in milk and dairy products, meat and meat products, fish and fishery products, frozen vegetables.

For efficient control at the level of food enterprises, potential sources of *L. monocytogenes* should be monitored and cross-contamination should be avoided (Hellström, 2011; Gravani, 1999). The problem of cross-contamination is represented by contamination of the final product from different environmental sources, such as food processing enterprises, retail network, and household kitchen (Soumet *et al*., 2005; Tompkin, 2002). Pathogenic bacteria can stay viable on different food contact surfaces for weeks, and cause consequent contamination of food products (Lappi *et al*., 2004; Lunden *et al*., 2000).

2.2.1 HYGIENE MANAGEMENT

A lack of awareness related to the food hygiene practices among the food manufacturers and suppliers pose a serious threat to entire communities. Currently, in developed countries such as the UK, USA, and the European Union food manufacturers, suppliers and handlers are instructed to follow good manufacturing practices (GMP) at adequate levels (Gall, 2008).

GMP could be defined as a system, which describes practices and conditions for the food industry to produce safe food. GMP includes the basis for determination of the safety of applied techniques during food processing, food handling and food storage. GMP are considered to be a basic of overall HACCP system. Food manufacturers should implement and develop practices in accordance with GMP requirements to protect the end product from contamination with pathogens (Gall, 2008).

In this context, relevant courses for food hygiene management on the governmental level are being designed to create awareness among the food retailers and food handlers (Gall, 2008). It is important to recognize that these hygiene management training are directed to enhance food safety and quality (Early, 2012). Hence, these training sessions shall be designed as such that it may communicate personal health, personal hygiene, and essential kitchen hygiene.

It is suggested that the use of disinfectants shall be deemed compulsory in the good manufacturing practices formulary and the hygiene management practices shall be implemented vigorously (Early, 2012). It is important to prevent microbial accumulation resulting into bio-film formation that is extremely difficult to break (Early, 2012; Seaman and Eves, 2008).

The role of food hygiene training in improving food safety and in the implementation of food safety management systems in the industry has been examined in different research studies (Early, 2012; Seaman and Eves, 2008). It is found that providing awareness about the food safety procedures is not sufficient for changing attitudes prevalent within the food industry (Seaman and Eves, 2008).

Findings of another study (Käferstein *et al.*, 1997) suggested that formally accredited training shall be provided to food handlers providing care to the elderly, children, as the food prepared at care settings are made for a vulnerable population. In care settings, the effect of contaminated food intake could be highly disastrous and could be life-threatening for the diseased population. An appropriate culture of food safety in the working environment of food manufacturing facilities needs to be incorporated for facilitating changes in behaviour and attitude of working staff (Seaman and Eves, 2008). The importance of training shall be emphasized by providing an understanding that the working personnel have the responsibility to protect the well-being of people. Käferstein *et al.* (1997) also stressed on the fact that high-level safety management training shall be provided in care settings for making the workforce competent enough to make decisions about the food safety issues.

The job performance records should also be linked to working procedures and reinforcement of safety practices into work regimes of food handlers. Incentives should be offered to food businesses and personnel working in food industries for maintaining sanitization and use of disinfectants in their food manufacturing and food processing procedures. The study findings (Seaman and Eves, 2008) revealed that the course content designed individually for certain food manufacturing and supplying facilities have shown desirable results and have influenced behavioural changes among the food handlers.

Technical knowledge should be supported with documented workplace observations and in-house training sessions to reduce the potential risks associated with food contamination (Seaman and Eves, 2008). It is also observed that on-going supervisions of safety practices and pre-training of the workforce in food industries are the factors that limit the hygiene management and implementation of food safety management systems.

As *Listeria* species are prone to produce infection in meat and dairy products so studies are also conducted on the food safety practices applied in slaughterhouses and meat processing plants. Smigic *et.al* (2016) established that the food safety knowledge score of the meat handlers was not adequate and they do not have the relevant standard operating procedures to reduce the chances of bacterial growth.

2.2.2 Hazard Analysis Critical Control Point (HACCP)

An effective management system for ensuring food safety from raw material production and handling to manufacturing, distribution and consumption of final product is Hazard Analysis Critical Control Point (HACCP) (U.S. Food and Drug Administration, 2003; Khandke and Mayes, 1998). The aim of the HACCP system is the prevention of contamination of final food products from physical, chemical, and biological hazards, which may occur during food processing process (Khandke and Mayes, 1998). HACCP consists of seven basic principles (Khandke and Mayes, 1998).

1) Hazard analysis: food processing plants determine the potential food safety hazards that should be prevented, reduced or eliminated
2) Identification of Critical Control Points (CCP): identification of CCP where control is essential to prevent, reduce or eliminate hazard
3) Establishing critical limits: determination of values of hazards to acceptable levels
4) Monitoring procedures for Critical Control Points: (implementation of effective monitoring procedures at CCP
5) Corrective actions: establishing corrective actions

6) Verification procedures: establishing procedures for verification of efficient working of a program
7) Record keeping and documentation: procedures for verification of successful implementation of a program

In the HACCP system, when deviations appear and control has been lost, the deviations should be identified, and appropriate steps must be taken to re-establish control and ensure food safety (Mortimore and Smith, 1998). For successful implementation of HACCP principles, a number of prerequisite programs should be developed. Basic environment and operating conditions which are essential for the safe production of food are provided by these programs. Prerequisite programs must be documented and regularly reviewed, and they should be supported and defined independently from the HACCP plan.

The aim of the prerequisite programs is to prevent the contamination of the food processing environment and the final product with pathogenic bacteria such as *L. monocytogenes* (Kornacki and Gulter, 2007). In order to achieve this:

1) The microbiological quality of the raw products should be within the legal limits.
2) The employees of the food processing plants should have thorough knowledge about food hygiene management.
3) Food processing plants and equipment should be designed so they could be effectively cleaned and disinfected, in order to avoid possible cross contamination.
4) Critical control points of the processing line where contamination is possible should be identified and controlled.

According to Rocourt and Cossart (1997), *L. monocytogenes* can contaminate processing plants through various media, for example, dirt on work shoe and clothes of the employee, transport equipment, animals which excrete the pathogen in the environment, raw food of animal origin, raw plant tissue, and probably, healthy human carriers.

2.2.3 Codex

In 1963 the Food and Agriculture Organization (FAO) of the United Nations (UN) in collaboration with the World Health Organization (WHO) established the Codex Alimentarius Commission (FAO and WHO, 2005). The Codex Commission provides worldwide harmonization of food safety standards by coordination of the work undertaken between international governments and non-governmental organizations (FAO and WHO, 2005). The Codex Alimentarius, also known as a "Food Code" was created to reduce the number of risks related to food production, trade and consumption. The Codex operations are based on science and focused on risk assessment, management, and risk communication (FAO and WHO, 2005). The Codex Commission promotes to the consumer confidence in food quality, to retailers confidence in food suppliers (FAO and WHO, 2005). Codex aims to develop food safety standards and guidelines intended to protect the health of consumers and to ensure the practice of fair-trade (FAO and WHO, 2005).

The Codex Commission has the jurisdiction in the area of the food safety, on food hygiene, different types of food and food products (raw, semi-processed, and processed), food additives, labeling of food products, residues of veterinary drugs and pesticides, contaminants of food, and methods for assessing the food safety (FAO and WHO, 2005). Today, Codex Alimentarius Commission includes 188 Members, 187 of them are Member Countries and one Member Organization (European Union) (FAO and WHO, 2005).

Recommendations for food manufacturers concerning the prevention *L. monocytogenes* in food processing environment are outlined in CAC/GL 61- 2007 "Guidelines on the application of general principles of food hygiene to the control of *Listeria monocytogenes* in foods".

2.3 DISINFECTANTS USED IN FOOD INDUSTRY

It is has been suggested that common places in the food processing environment, where *L. monocytogenes* might propagate and grow, include food processing equipment and utensils made up of polyvinylchloride, polypropylene, and polystyrene (Møretro *et al.,* 2004) or on the surface of processed or cooked food (Kusumaningrum *et al*., 2003; Autio *et al.,* 1999) which facilitates the growth of *L.monocytogenes* and formation of a biofilm (Møretro *et al.,* 2004).

It has been observed that *L. monocytogenes* has the capacity to survive even after the performance of routine cleaning procedures in food industries (Fouladynezhad *et al.*, 2013) which makes it highly resistant to sanitizing compounds and disinfectants. Therefore, the *L.monocytogenes* strain is considered to be a serious concern and a challenge in order to ensure microbiological safety in food industries.

The cross- contamination of products being manufactured and processed in food manufacturing facilities isalso a serious threat to the food industry (Yousef and Lado, 2007; Møretro *et al.,* 2004). This accounts for the fact that the persistence of bacteria strains due to the formation of unbreakable bio-film is a major issue to be resolved by removing food contamination (Yousef A.H., 2007; Møretro *et al.,* 2004). This pathogenic bacterium on the food surfaces increases the risk of transmission to wide-spread whereabouts because of unlimited reciprocation of its strains (Cabeça, Pizzolitto, and Pizzolitto, 2012).

Studies have indicated (Otto *et al.,* 2011; Yousef and Lado, 2007; Breidt *et al.,* 2006) that *L.monocytogenes* strains are unable to survive chemical treatment. However, Frank (1990) suggested that the resistance of *L.monocytogenes* to heat may increase if they persist on glass and stainless steel. Growth of the bacterium on the utensil surfaces is dependent upon the type and design of the surface (Hellström, 2011). Further, *L.monocytogenes* strains are more resistant to disinfection when observed in practical food processing environment as compared to its development in the laboratory media at standardized conditions(Autio *et al.,* 2004; Farber *et al.,* 2001).

Chmielewski (2006) also supported the previous observations during the study of bacterium resistance through predictive probability models. He also proved that high temperatures may cause total inactivation of the bacterial cells.

The quest for highly efficient disinfectants is extremely important for preventing spoilage and contamination caused by *L. monocytogenes*. The prime goal of disinfection is to remove organic compounds of the food surfaces, and to reduce the microorganisms from the food contact surfaces.

Chemical disinfection techniques are most widely used in the food industry and the factors that may affect the disinfection process may include disinfectant efficacy and strain sensitivity (Hellström, 2011). Therefore, it should be kept in mind that the disinfectant should have the spectrum required for activity so that it could eliminate the contaminants appropriately. It is furthermore suggested that the sessile microbial cells offer more resistance to disinfectants as compared to suspended bacterial cells (Early, 2012). Hence, the presence of *L. monocytogenes* forming a cake on the surface can decrease the efficacy of disinfectant, thus; inactivating its destructive action.

Disinfectants should have a broad spectrum i.e. they should be effective against a wide range of fungi, bacteria, and viruses. A wide range of disinfectants is therefore available in the food industry which could be used on the basis of their appropriateness. They are divided into different classes (Table 1) such as alcohols, biguanides, aldehydes, peroxygens, halogen-releasing agents and QAC (Katsbjerg and Gram, 2009; Asselt and Giffel, 2005; Gilbert and Moore, 2005)

Table 1. Disinfectant agents used in food processing industry (Asselt and Giffel, 2005; Gilbert and Moore, 2005).

Class of disinfectant	Active compound	Characteristics
Alcohol	Ethanol Isopropanol	Use in high concentrations is needed (60-90%), not for large industrial application.
Biguandines	Polymeric Biguandines Chlorhexidine	Particularly used by food processing industry, biocides due to absence of toxicological properties.
Aldehydes	Formaldehyde Glutaraldehyde	Not frequently used in food processing industry, highly toxic.
Peroxygens	Peracetic Acid Hydrogen Peroxide	Frequently used in food processing industry, effective against organic matters. Disadvantage: corrosive.
Halogen-releasing agents	Hypochlorite Iodine	Majority of available disinfectants are chlorine based, since it is cheap and effective. Disadvantage: corrosive.
Quaternary ammonium compounds		Frequently used in food processing industry, expensive than chlorine. Advantages: non-corrosive.

Chlorine based, peroxygens and QUAC are the most frequently used disinfectants for *L.monocytogenes* in the food industry (Ibusquiza *et al.,* 2011; Katsbjerg and Gram, 2009). The choice among these three disinfectants depends on the site of applying in the food processing plant. Chlorine-based compounds are not a good choice for *L.monocytogenes* as they can be easily deactivated by the presence of organic material contamination (Ibusquiza *et al.,* 2011; Katsbjerg and Gram, 2009). Peroxygens are efficient and are not affected by organic debris to much extent. However, both the compounds have a corrosive nature which limits their efficacy (Ibusquiza *et al.,* 2011; Katsbjerg and Gram, 2009). QAC are the most widely used as they are non-corrosive in nature and have adequate results over *Listeria* species even after coming into contact with the organic material (Cabeça, Pizzolitto, and Pizzolitto, 2012).

The destruction probability of *Listeria* species has been assessed by using different disinfectants. It is assessed that the quaternary ammonium compounds (QAC) are useful in destroying *L. monocytogenes* strains in double concentrations (Carballo, 2012). QAC is also effective against the planktonic *L. monocytogenes* at temperatures higher than 85°C (Carballo, 2012). It is therefore suggested that the combination of both heat and chemical treatment is a reasonable approach in disinfecting food surfaces (Carballo, 2012). Moorman *et al*., (2005) presented similar results that *Listeria* species is more sensitive to QAC at sub-lethal disinfectant concentrations. Furthermore, it is also deduced from experimentation that use of ultra-sonication techniques with quaternary ammonium-based or chlorine disinfectants are highly effective in destroying the resistant bacterium bio-film (Hellström, 2011)

3 OBJECTIVES

The aim of the present study was to determine the efficacy of five commercial disinfection agents against five *L. monocytogenes* strains. In order to substantiate the efficacy, disinfection agents used in this study should ensure the >5 Log_{10} reduction of *L. monocytogenes* to the safe limits (≤100 CFU/g), or inhibit the growth completely.

4 MATERIALS AND METHODS

4.1 MATERIALS

The laboratory studies were conducted in the microbiological laboratory of the Institute of Public Health and Clinical Nutrition at the University of Eastern Finland. The bacterial activity was evaluated using *L. monocytogenes* strains 4030, 4322, 4323, 3144 and 3476 as test organisms. These strains have been isolated from a local fish processing plant, and they have been identified and characterized by Finnish Food Safety Authority (Evira),

Five following commercial disinfectants used in fish industry were obtained to determine antimicrobial efficacy:

1) Mida 165 (Oy Christeyns Nordic AB, Finland), sodium chlorite and sodium hydroxide.
2) Mida Chriox 5 (Oy Christeyns Nordic AB, Finland) peracetic acid, hydrogen peroxide.
3) Mida Flow 109 (Oy Christeyns Nordic AB, Finland), sodium chlorite and sodium hydroxide.
4) Mida Foam 193 (Oy Christeyns Nordic AB, Finland), sodium hydroxide, sodium hypochlorite.
5) F17 Sendes (KiiltoClean Oy), Alkaline foam detergent.

4.2 METHODS

According to European Standard BS-EN 1276:2009 the following are main steps for assessing ability of disinfectant detergent to eliminate or inhibit bacterial activity (Figure 1):

1) Preparing a subculture from the stock culture.
2) Preparing of neutralization solution.
3) Preparing of disinfection solution.
4) Making a serial dilution for control and sample groups.
5) Transferring on TSA-agar plates.
6) Incubation 48h.
7) Counting the CFU/ml

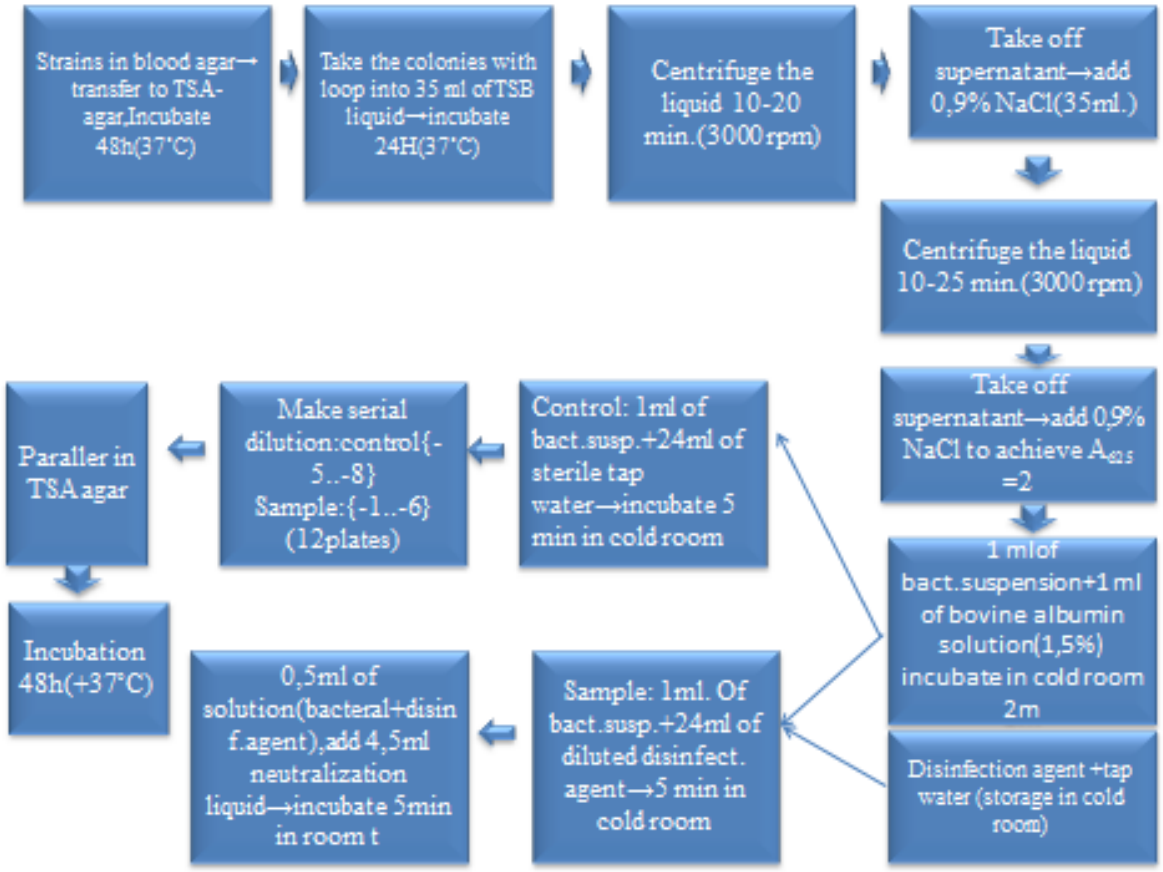

Figure 1. Assessment procedure of disinfectant detergents on the elimination or inhibition of *L. monocytogenes.*

4.2.1 Preparation of suspension culture, growth media used and conditions

Stock cultures were maintained on BAP (Blood agar plates), cultivated 24 hours at 37ºC and kept at 4ºC. Subcultures were inoculated on TSA (Tryptic soy agar) plates and incubated at 37ºC for 48 hours.

Colonies from TSA were suspended in fresh TSB (Tryptic soy broth) medium and incubated at 37ºC for 24 hours. In order to separate supernatant from bacterial cells, overnight cultures in 35 ml tubes with TSB medium were centrifuged for 10 min at 3000 rpm. Supernatant was discarded and 35 ml of 0.9% NaCl (sodium chloride) added to wash bacterial cells. TSB tubes were centrifuged a second time (10 min at 3000 rpm), the supernatant discarded and 35 ml of 0.9% sodium chloride

added. Before further experiments, the bacterial cell density was measured with a spectrophotometer (Genesys, Germany) to achieve 10^8 CFU/ml (A_{625} nm of approximately 2.0). Subsequently, for the experiments, 1 ml of bacterial suspension was mixed with 1 ml of 1.5% BSA solution (Bovine serum albumin) in order to imitate "contaminated conditions" of the food processing environment.

4.2.2 Preparation of disinfection solution

Five commercially available disinfectants Mida Flow 109, Mida 165, Mida Foam 193, Mida 5 Chriox, F17 Sendes were obtained for laboratory experiments. They were used following the manufacturer's instructions to get the solutions with recommended concentrations. Disinfectants were diluted with clean tap water to make an imitation of conditions close to food processing environment.

4.2.3 Suspension test

Control:

1 ml of bacterial suspension was mixed with 24 ml of sterile water and stored for 5 minutes in cold room. After the serial dilution series, 100 µl was pipetted to 12 TSA plates marked in accordance to concentrations and then placed to the incubator at 37ºC for 48 h.

Samples:

1 ml of bacterial suspension was mixed with 24 ml of diluted disinfection agent and stored for 5 minutes in cold room. 0.5 ml of ready solution (disinfectant plus bacteria) was mixed with 4.5 ml of neutralization liquid to reduce the effect of the disinfectant and stored in a cold room for 5 minutes. Subsequently, after the serial dilution series 100 µl was pipetted to 12 TSA plates marked in accordance to concentrations and then placed to the incubator for 48 h at 37ºC.

Laboratory tests conducted in this study were implemented once, not in triplicate, all five bacterial strains were tested with five disinfectants.

4.2.4 Determination of the antibacterial activity

After 48 hours, all TSA plates were removed from the incubator and viable bacterial cells were counted using colony counter. Colony-forming unit (CFU) concentrations were expressed in logarithms. The data obtained was analyzed by the comparison with >5 Log_{10} reduction, to substantiate the efficacy in accordance with European Standard BS-EN 1276:2009. The antibacterial activity (AA) was calculated using equation: $AA = Log\ N_c - Log\ N_d$, where N_c is CFU/ml before disinfection and N_d after disinfection treatment.

5 RESULTS

In this study, the efficacy of five different commercially available disinfectants, namely Mida 165, Mida Chriox 5, Mida Flow 109, Mida Foam 193 and F17 Sendes against five *Listeria monocytogenes* strains was observed, as shown in Table 2.

Table 2. The effect of five disinfectants against five *Listeria monocytogenes* strains in quantitative suspension test according to European Standard BS-EN 1276:2009.

Bacteria	**Strain No.**	**Log_{10} reduction for different disinfectant test solutions[1]**				
		F 17 Sendes	**Mida 165**	**Mida Flow 109**	**Mida 5 Chriox**	**Mida Foam 193**
L. monocytogenes	3144	7.28	7.06	7.57	7.13	7.22
L. monocytogenes	3476	7.29	6.96	7.37	**7.38**	7.03
L. monocytogenes	4030	7.24	7.12	7.39	7.29	7.31
L. monocytogenes	4322	**7.65**	7.37	**8.06**	7.15	7.30
L. monocytogenes	4323	7.56	**7.47**	7.07	7.36	**7.33**

[1] According to EN 1276 standard to determine the efficacy of tested disinfection solutions requires >5 Log $_{10}$ reduction to pass the test.

The highest value of efficacy for all strains of *L. monocytogenes* was demonstrated by Mida Flow 109 disinfectant with log_{10} reduction range equal to 8.06 log units, whereas the lowest value of efficacy was given by Mida Foam 193 with 7.33 units of log10 reduction range respectively. Mida 165 and Mida 5 Chriox had a reduction value of 7.47 and 7.38 log units, respectively.

When compared with the positive controls of *L. monocytogenes* 4322 strain, there were no viable cells after disinfection treatment with sodium chlorite and sodium hydroxide (Mida Flow 109). Treatment with alkaline foam detergent (F17 Sendes) was also effective against *L. monocytogenes* 4322 strain, 7.65 Log units. After disinfection treatment with Mida 165 against *L. monocytogenes* 3476 strain, the Log reduction value was lower in comparison with other results indicated in this study, 6.96 Log units.

Among the five strains of *L. monocytogenes* tested, *L. monocytogenes* 4322 and *L. monocytegenes* 4323 had higher values of Log10 reduction than the other listeria strains with Mida Flow 109 and F17 Sendes. *L. monocytogenes* 3476 showed lower log10 reduction values for Mida 165.

All selected disinfectants demonstrated log10 reduction values significantly higher than 5 log units.

6 DISCUSSION AND CONCLUSION

Low levels of hygiene and insufficient sanitation practices have been determined as a major factor in outbreaks of foodborne illnesses (Cabeça *et al.*, 2012; Chmielewski, 2003). In this study five commercially available disinfectants Mida 165, Mida Chriox 5, Mida Flow 109, Mida Foam 193 and F17 Sendes industrially used in fish processing, were selected to determine the efficacy against five *L. monocytogenes* strains 3144, 3476, 4030, 4322 and 4323. To substantiate bactericidal activity, disinfectants should demonstrate at least 5 Log_{10} reductions in the growth of *L. monocytogenes*.

All selected disinfectants demonstrated log10 reduction values significantly higher than 5 log units implying that selected disinfectants eradicate the growth of *L. monocytogenes* with the concentrations recommended by manufacturers. The results showed that *L. monoctyogenes* 4322 and 4323 had higher log10 reduction rates in growth than other strains, with the disinfectants Mida Flow 109 and Mida 165. This might reflect that these two strains are more sensitive to the tested disinfectants in comparison with other strains. However, based on the data obtained, statistical differences between the bacterial strains and disinfectants cannot be proved.

These data demonstrates that the disinfectant Mida Flow 109 was the most effective against *L. monocytogenes*, whereas, Mida 165 was least effective. Carruthers *et al.,* (2012) reported similar results where four commercial disinfectants tested against *L. monocytogenes* were effective, with the best results demonstrated by sodium hypochlorite (6.46 Log_{10} reduction).Frank, 2003 reported that sodium chlorite and peracetic acid based disinfectant was the most effective against *L. monocytogenes*, >5Log reduction after 1 min exposure.Aarnisalo *et al.,* 2000 reported that disinfectans containing hydrogen peroxide, peracetic acid and acetic acid are considered to be effective against *L. monocytogenes* (more than 4 Log units reduction).

A number of different factors could have an impact on the efficiency of disinfectants, such as concentration, exposure time, water hardness, temperature. Working temperature is critical in the case of *L. monocytogenes*, as it can grow at low temperatures, disinfectants could be less effective or ineffective in cold conditions. Furthermore, laboratory experiments were conducted once, not in triplicate. With this in mind, this data can only be considered preliminary and is thus not conclusive.

REFERENCES

Aarnisalo, K., Salo, S., Miettinen, H., Suihko, M., Wirtanen, G., Autio, T., Lunden, J., Korkeala, H. and Sjoberg, A., 2000. Bacterial efficiencies of commercial disinfectants against *Listeria monocytogenes* on surfaces. *Journal of Food Safety* 20.4, pp 237-250.

Autio, T. J., Lindström, M. K., and Korkeala, H. J., 2004. Research update on major pathogens associated with fish products and processing of fish. *Food safety assurance and veterinary public health Safety assurance during food processing,* pp. 115-134

Autio, T., Hielm, S., Miettinen, M. 1999. Sources of Listeria monocytogenes contamination in a cold-smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis typing. *Appl Environ Microbiol 65,* pp.150–155

Asselt, A. J. v. and Giffel, M. C. t. (2005) Pathogen resistance to sanitisers. In *Handbook of hygiene control in the food industry.* Ed. Lelieveld, H.L.M., Mostert, M.A. and Holah, J. pp. 69-92. Cambridge: Woodhead

Ben Embarek, P.K., 1994. Presence, detection and growth of *Listeria monocytogenes* in seafoods: a review. *International Journal of Food Microbiology 23(1).* pp. 17-34 ·

Buchanan, R.L., Damert, W.G., Whiting, R.C. and van Schothorst, M. 1997. Use of epidemiologic and food survey data to estimate a purposefully conservative dose response relationship for Listeria monocytogenes levels and incidence of listeriosis. J.Food Prot. 60, 918-922.

Breidt Jr., Y., Pan, F., & Kathariou, S. (2006). Resistance of Listeria monocytogenes biofilms to sanitizing agents in a simulated food processing environment. *Applied and Environmental Microbiology,* 72(12), pp. 7711-7717

Brett, M.S.Y., Short, P. and McLauchlin, J., 1998. A small outbreak of listeriosis associated with smoked mussels. *International Journal of Food Microbiology. 43.*pp.223–229

Cabeça, T.K., Pizzolitto, A.C. and Pizzolitto, E.L., 2012. Activity of disinfectants against foodborne pathogens in suspension and adhered to stainless steel surfaces. *Brazilian Journal of Microbiology*, *43*(3), pp.1112-1119.

Carballo, Julia and Ana-Belén Araújo. "Evaluation Of The Efficacy Of Commercial Sanitizers Against Adhered And Planktonic Cells Of Listeria Monocytogenes And Salmonella Spp.". *Food Science and Technology (Campinas)* 32.3 (2012): 606-612. Web.

Carruthers, B., Cross, C., Cutts, T. and Theriault, 2012. Comparative Inactivation Studies Of Listeria Monocytogenes At Room And Refrigeration Temperatures". *Applied Biosafety* 17.2 pp 64-69

Cartwright, E.J., Jackson, K.A., Johnson, S.D., Graves, L.M., Silk, B.J. and Mahon, B.E., 2013. Listeriosis outbreaks and associated food vehicles, United States, 1998–2008. *Emerg Infect Dis*, *19*(1), pp.1-9.

Chmielewski R.A.N., Frank J.F. Biofilm formation and control in food processing facilities. Inst. Food. Technol, v. 2003;2(1):22–32

Dvorak G. 2008. Disinfection 101. Centre for Food Security and Public Health, Iowa State University, IA, USA. Pp.1-22.

Early, R., 2012. Guide to quality management systems for the food industry. Springer Science and Business Media.

EFSA 2015. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2013. *EFSA Journal, 13(1):3991* pp 58-64

FAO/WHO Food and Agriculture Organization, Food Standards Programme. 2005. Codex Alimentarius Commission Procedural Manual, 15th Edition edited by FAO/WHO. Rome/Geneva.

Facinelli, B., Varaldo, P., Toni, M., Casolari, C. and Fabio, U. 1989. Ignorance about listeria. *BMJ, 299(6701),* pp.738

Farber, J.M. and Peterkin, I., 1991. *Listeria monocytogenes*, a food-borne pathogen. *Microbiological Reviews 55,* pp.476-511

Farber, J.M., Kalmokoff, M. L., J. W. Austin, X. Wan, G. Sanders, 1996. Adsorption, attachment and biofilm formation among isolates of *Listeria monocytogenes* using model conditions. *Journal of Applied Microbiology, 91*, pp.725-734

Fouladynezhad, N., Afsah Hejri, L., Rukayadi, Y., Nakaguchi, Y., Nishibuchi, M. and Radu, S., 2013. Efficiency of four Malaysian commercial disinfectants on removing Listeria monocytogenes biofilm. *International Food Research Journal*, *20*(3), pp.1485-1490

Frank, J.F., Ehlers, J. and Wicker, L., 2003. Removal and disinfection of Listeria monocytogenes and poultry soil-containing biofilms using chemical cleaning and sanitizing agents under static conditions. "Center for Food Safety, University of Georgia, Griffin, GA, Highlights

Frank, J. F., and R. A. Koffi. 1990. Surface-adherent growth of *Listeria monocytogenes* is associated with increased resistance to surfactant sanitizers and heat. J. Food Prot. 53**,** pp 550-554

Garrido V, Vitas A.I, Garcia-Jalon I. 2009. Survey of *Listeria monocytogenes* in ready-to-eat products: Prevalence by brands and retail establishments for exposure assessment of listeriosis in Northern Spain. *Food Control, 20.* pp. 986-991

Gilbert, P. and Moore, L.E., 2005. Cationic antiseptics: diversity of action under a common epithet. Journal of Applied Microbiology 99, pp 703-715.

Gillespie, A., Mook, P., Little, C., Grant, K., McLauchlin J. 2010. Human listeriosis in England, 2001-2007: association with neighbourhood deprivation. *Eurosurveillance*, pp. 7–16.

Gombas, D.E., Chen Y., Clavero, R.S. 2003. Survey of *Listeria monocytogenes* in ready-to foods. *Journal of Food Protection 66(4),* pp. 559–56

Gravani, R., 1999. Incidence and control of Listeria in food-processing facilities. In: Ryser, E.T., Marth, E.H. (Eds.), Listeria, Listeriosis and Food Safety, 2nd Edition. Marcel Dekker, New York, pp. 657–709

Graves, L. M. et al. 2009. "*Listeria Marthii Sp.* Nov., Isolated From The Natural Environment, Finger Lakes National Forest". *International Journal of systematic and evolutionary microbiology* pp.1280-1288

Gray, M., Zadoks, R., Fortes, E., Dogan2004. *Listeria monocytogenes* Isolates from Foods and Humans Form Distinct but Overlapping Populations. *Applied and Environmental Microbiology, 70(10),* pp.5833-5841

Harvey, J. and Gilmour, A. 1992. Occurrence of Listeria species in raw milk and dairy products produced in Northern Ireland. *Journal of Applied Microbiology 72,* pp.119–125

Hellström, S., 2011. Contamination routes and control of Listeria monocytogenes in food production

Ibusquiza, P., Herrera, J. and Cabo, M. 2011. Resistance to benzalkonium chloride, peracetic acid and nisin during formation of mature biofilms by *Listeria monocytogenes*. *Food Microbiology, 28(3),* pp.418-425.

Ivanek, R., Y.T Grohn and M. Wiedmann. 2006. *Listeria monocytogenes* in multiple habitats and host populations: review of available data for mathematical modelling *Foodborne Pathogens and Disease 3,*pp.319-336.

Jacquet, C., Gouin, E., Jeannel, D., Cossart, P. and Rocourt, J.2002. Expression of ActA, Ami, InlB, and Listeriolysin O in *Listeria monocytogenes* of human and food origin. *Applied and Environmental Microbiology, 68,* pp 616-622

Kaferstein, F. K., 1997. "Foodborne Disease Control: A Transnational Challenge". *Emerging Infectious Diseases* 4, pp 503-510

Kathariou, S. 2002. *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. *Journal of Food Protection 65,* pp.1811–1829.

Kastbjerg, V.G. and Gram, L. 2009. Model systems allowing quantification of sensitivity to disinfectants and comparison of disinfectant susceptibility of persistent and presumed nonpersistent *Listeria monocytogenes*.. *Journal of Applied Microbiology 106,* pp. 1667-1681.

Khandke, S. and Mayes, T. 1998. HACCP implementation: a practical guide to the implementation of the HACCP plan. *Food Control, 9(2-3),* pp.103-109.

Kornacki, J. L. and Gurtler, J. (2007) Incidence and control of *Listeria* in food processing facilities. In *Listeria, listeriosis, and food safety.* Ed. Ryser, E.T. and Marth, E.H. pp. 681-766. Boca Raton: CRC Press

Kusumaningrum H.D, Riboldi G, Hazeleger W.C, Beumer R.R 2003. Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. *International Journal of Food Microbiology 85,* pp. 227-236.

Lappi, VR, Thimothe, J, Nightingale, KK, Gall, K, Scott, VN, Wiedmann, M., 2004. Longitudinal studies on Listeria in smoked fish plants: impact of intervention strategies on contamination patterns.J Food Prot.67(11), pp 14

Lundén, J., Björkroth, J. and Korkeala, H. 2005. Contamination routes and analysis in food processing environments. pp.539-555

McDonnell, G., Eterpi, M. and Thomas, V. 2010. Disinfectants efficacy against hardy viruses dried onto surfaces. *Journal of Hospital Infection76,* pp. 26

Martin, S. E. and Fisher, C. W. 1999. *Listeria monocytogenes. In Encyclopedia of Food Microbiology 2.* (Eds) Robinson, R.K., Batt, C.A. and Patel, P.D. pp. 1228-1238

Mead, P.S., Slutsker, L., Dietz, V. 1999. Food-related illness and death in the United States. *Emerging Infectious Didease5 5,* pp. 607–625

Miettinen, M.K., A. Siitonen, P. Heiskanen, H. Haajanen, K.J. Bjorkroth., 1999. Molecular epidemiology of an outbreak of febrile gastroenteritis caused by *Listeria monocytogenes* in coldsmoked rainbow trout *Journal of Clinical Microbiology 37*, pp. 2358–2360.

Mohammed, H. O., Atwill, E., Dunbar, L., Ward, T., McDonough, P., Gonzalez, R., and Stipetic, K. 2010. The risk of *Listeria monocytogenes* infection in beef cattle operations. *Journal of Applied Microbiology 108,* pp. 349-356.

Møretrø, T. and S. Langsrud, 2004. "Listeria Monocytogenes: Biofilm Formation And Persistence In Food-Processing Environments". *Biofilms* 1.2, pp. 107-121

Moorman, M., 2005.Altered sensitivity to a quaternary ammonium sanitizer in stressed *Listeria innocua*. *Journal of Food Protection*, v. 68, pp. 1659-1663,

Mortimore, S. and Smith, R. 1998. Standardized HACCP training: assurance for food authorities. *Food Control, 9(2-3),* pp.141-145.

Norton, D.M., McCamey, M.A., Gall, K.L., Scarlett, J.M., Boor, K.J., 2001. Molecular studies on the ecology of Listeria monocytogenes in the smoked fish processing industry *Applied and Environmental Microbiology 67(1),* pp. 198-205.

Nyachuba, David G., 2010 "Foodborne Illness: Is It On The Rise? *Nutrition Reviews 68.5 (2010):* 257-269

Nucera D, Lomonaco S, Bianchi DM, Decastelli L, Grassi MA. 2010. A five year surveillance report on PFGE types of Listeria monocytogenes isolated in Italy from food and food related environments. *International Journal of Food Microbiology.* 140(2-3), pp. 271-276.

Otto, C., Zahn, S., Rost, F., Zahn, P., Jaros, D. and Rohm, H. 2011. Physical Methods for Clea and Disinfection of Surfaces. *Food Engineering Reviews, 3(3-4),* pp.171-188.

Porsby, C.H., Vogel, B.F., Mohr, M., Gram, L., 2008. Influence of processing steps in cold-smoked salmon production on survival and growth of persistent and presumed non-persistent *Listeria monocytogenes*. *International journal of food microbiology 122,* pp. 287-295.

Rocourt, J. and Cossart, P., (1997). Listeria monocytogenes, Food Microbiology — Fundamentals and Frontiers. *American Society for Microbiology (ASM)* press,Washington DC, pp. 337–352.

Rocourt, J. 1994. *Listeria monocytogenes*: the state of the science. *Dairy Food Environ. Sanit.14:* pp 72-82.

Rocourt, J., Hof, H., Schrettenbrunner, A., Malinverni, R. and Bille, J., 1986. Méningite purulente aiguë á Listeria seeligeri chez un adulte immunocompétent. Schweizerische medizinische wochenschrift 116, pp 248-251

Rørvik, L.M., Caugant, D.A. and Yndestad, M. 1995. Contamination pattern of Listeria monocytogenes and other Listeria spp. in a salmon slaugterhouse and smoked salmon processing plant. *International journal of food microbiology 25,* pp. 19-25

Rushdy, A. and Othman, A., 2010. Bactericidal Efficacy Of Some Commercial Disinfectants On Biofilm On Stainless Steel Surfaces Of Food Equipment. *Annals of Microbiology* 61, pp 545-552

Sallen, B., Rajoharison, A., Desvarenne, S., Quinn, F. and Mabilat, C. 1996. Comparative analysis of 16S and 23S rRNA sequences of *Listeria* species. *International Journal of Systematic Biology* 46, pp. 669-674

Seaman, P. and Eves, A., 2008. Food hygiene training in small to medium-sized care settings. *International Journal of Environmental Health Research*, *18*(5), pp.365-374

Seaman, P. and Eves, A., 2006. The management of food safety—the role of food hygiene training in the UK service sector. *International Journal of Hospitality Management*, *25*(2), pp.278-296

Seeliger, H. P. R. and Jones, D., 1986. Listeria. In Bergey's manual of systematic bacteriology volume 2. Ed. Sneath, P.H.A., Mair, N.S., Sharpe, M.E. and Holt, J.G. pp.235-1245. Baltimore: Williams & Wilkins.

Smigic, N., Antic, D., Blagojevic, B., Tomasevic, I. and Djekic, I., 2016. The level of food safety knowledge among meat handlers. *British Food Journal*,*118*(1), pp.9-25

Soumet, C., Ragimbeau, C. and Maris, P., 2005. Screening of benzalkonium chloride resistance in Listeria monocytogenes strains isolated during cold smoked fish production.Letters in Applied Microbiology 41, pp 291-296.

Swaminathan, B. and Gerner-Smidt, P. 2007. The epidemiology of human listeriosis. *Microbes Infection. 9,* pp. 1236-1243

Teratanavat, R. and N. H. Hooker. 2004. Understanding the characteristics of United States-and poultry recalls: 1994-2002. *Food Control 15,* pp. 359-367

Tezel, U. and Pavlostathis, S.G., 2015. Quaternary ammonium disinfectants: microbial adaptation, degradation and ecology. *Current opinion in biotechnology*, *33*, pp.296-304.

Tham, W. 2000. Lessons from an outbreak of listeriosis related to vacuum-packed gravad and cold-smoked fish. *International Journal of Food Microbiology, 62(3),* pp.173-175

Tompkin, R.B. 2002. Control of *Listeria monocytogenes* in the foodprocessing environment. Journal of Food Protection 65, pp. 709–725

Vázquez-Boland, J.A., Kuhn, M., Berche, P., Chakraborty, T., Domínguez-Bernal,G., Goebel, W., González-Zorn, B., Wehland, J. and Kreft, J. 2001. *Listeria* pathogenesis and molecular virulence determinants. *Clinical Microbiology Reviews* 14, pp. 584-640

Vitas A, Aguado V, García-Jalón I.2004. Occurrence of Listeria monocytogenes in fresh and processed foods in Navarra. *International Journal of Food Microbiology90,* pp. 349-356

Yousef, A.E. and Lado B.H. 2007. Characteristics of *Listeria monocytogenes* Important to Food Processors. *Listeria, Listeriosis, and Food Safety. Third Edition.*pp. 157-213

Wagner M, Auer B, Trittremmel C, Hein I, Schoder D. 2007. Survey on the Listeria contamination of ready-to-eat food products and household environments in Vienna, Austria. *Zoonoses Public Health. 54,* pp. 16-22

Wallace, F.M., Call, J.E., Porto, A.C., Cocoma G.J. and Luchansky J.B. 2003. Recovery rate of *Listeria monocytogenes* from commercially prepared frankfurters during extended refrigerated storage. *Journal of Food Protection 66(4),* pp. 584–591

Wimpfheimer, L., Altman, N.S. and Hotchkiss, J.H. 1990. Growth of *Listeria monocytogenes* Scott A, serotype 4 and competitive spoilage organisms in raw chicken packaged under modified atmospheres and in air.. *International Journal of Food Microbiology 11,* pp. 205-214

Wulff, G., Gram, L., Ahrens, P. and Vogel, B.F. 2006. One group of genetically similar Listeria monocytogenes strains frequently dominate and persist in several fish slaughter- and smokehouses. *Applied and Environmental Microbiology 72,* pp. 4313–4322