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Experimental Reduction of *Salmonella* in Raw Chicken Breasts

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Contents

Introduction

The world's total poultry consumption was estimated to be 84.6 million tons per year in 2006. Only in Europe about 11 million tons are consumed per year. Increasing consumer demands for food safety put pressure on the food enterprises to produce safe food or to reduce the risks in the food chain. Risks for food safety occur from the contamination with physical, chemical and microbiological hazards. Risk analyses for many kinds of food have been carried out including fruits, vegetables, milk, milk products, meat and meat products. In order to reduce risks, the standards of agricultural and food production such as Good Farming Practice (GFP), Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Point concept (HACCP) etc. have been implemented to control food chains thoroughly. However, human infections from food-borne pathogens still often are reported. Particularly, microbial food-borne diseases have increasingly raised concerns.

In the exporting market, Asia is a big food producing region that annually exports about 1.2 million tons of poultry products (FAO, 2006). Thailand exports frozen cuts and packaged ready-to-cook chicken rather than frozen or chilled whole chickens. Processed chickens are of higher value than whole chickens. The main destinations of Thailand broiler meat exportations are Japan and the European Union (EU). The EU has become a market of increasing importance for Thai chicken meat (FAO, 2004). In order to gain entry into the EU market, the appropriate food safety regulation animal health, animal welfare and environmental standards must be fulfilled. Thailand is challenged to improve its food processing. Not only safe food for exportation is concerned, the government of Thailand has declared national food safety since 2004 a priority for domestic consumption as well as for exportation.

The high poultry consumption leads to concern that the products should be safe and have a low spoilage rate, the right composition, packaging, color, taste and appearances (Río et al., 2007). Poultry products such as frozen and processed chicken meat are important for the export markets but numerous surveillances continue to show high levels of bacterial contamination, especially of *Salmonella*. The European Food Safety Authority (EFSA) as an independent scientific body in risk assessment supplies scientific and technical information to the Community institutions and Member States in order to enable them to take informed and science-based risk management decisions necessary to ensure food and feed safety. In 2005, EFSA reported that campylobacteriosis and salmonellosis were the two most common zoonotic diseases in the European Union (EU).

Infection caused by *Salmonella* is called salmonellosis. It is one of the most common and widely distributed food-borne diseases, constituting a major public health burden and causing significant costs in many countries. Human food-borne pathogen illness cases are reported worldwide every year and annually there are thousands of deaths. Further to known strains, new *Salmonella* strains are emerging that are more resistant to common antimicrobial drugs. Physicians and microbiologists, as well as food producers and the food industry, should be particularly aware of that and take their share in controlling communicable diseases. The numbers of relevant strains in food animals should be reduced and the risk of contamination by resistant *Salmonella* at all stages in the food production chain should be lowered (WHO, 2005b). Unfortunately, *Salmonella* are able to contaminate all steps of food production. Hygienic standard methods are thoroughly embedded in food chains including measures of animal husbandry, slaughtering and in processing plants, but *Salmonella* still are found in end products such as red and poultry meat.

Raw meat, particularly poultry meat, remains an important source of human infection with pathogenic microorganisms. It can easily be contaminated with microorganisms because fresh meat is very suitable for microbial multiplication. Meat has high water activity, is high in nutrients and readily utilizable low molecular weight substances and is a source of carbon and energy by means of glucose, lactic acid, amino acids, creatines, metal and soluble phosphorus. As a result, fresh meat is a suitable substrate for bacterial multiplication (Hinton, 2000).

One approach to control the spread of these pathogens to the human population is to decontaminate the final product. Therefore, decontamination technologies are widely applied in the operation of meat and poultry slaughtering and processing plants under principles of GMP and the hazard analysis critical control point (HACCP) system.

Up to present, more than a decade has passed since many food producers and food researchers have developed decontamination methods and combined them with standard food production. Their purpose was to eliminate food-borne pathogens more effectively during food processing. In principle, there are 2 major decontaminations or antimicrobial treatments. Physical methods on one hand are steam, hot water dip or spray, UV light, high-pressure processing and irradiation. Chemical methods on the other hand cover chlorine and chlorine compounds, trisodium phosphate, organic acids, ozone and electrolyzed oxidizing water. These decontaminations have been applied in processing plants of fruits, vegetables, seafood, and meat carcasses. Nevertheless, some methods are not permitted in some countries. For example, the European Union (EU) does not allow any chemical decontamination on

carcasses and meat cuts. EU only accepts potable or clean water for carcasses and meat washing. On the other hand, most of the chemical decontamination methods have been suggested or authorized as intervention procedures in the US. As a result, the agricultural countries that produce food for exportation have to be concerned not only about production of a safe product but also about the approval of importing countries.

Various decontamination techniques have been used for that purpose including a strong and rapid decontamination action. Ideal decontamination should not leave any residues that may be detrimental to the health of the consumer. Furthermore, the treatment should not adversely affect taste, color, nutrition properties and appearance of the carcasses or meat. Decontamination methods additionally should be cheap, convenient to apply and not harmful to workers and the environment. For chemical methods, all substances should improve the safety and shelf-life of products by inactivating spoilage organisms as well as pathogens. They should be acceptable to the public and compatible with further processing such as using modified atmosphere packaging (Hinton and Corry, 1999a; Van der Marel et al., 1988).

Up to now, regulation (EC) No. 853/2004 of the European Parliament and of the Council does not provide a legal basis permitting the use of antimicrobial treatments to remove surface contamination from poultry (EFSA, 2006). Nevertheless, trisodium phosphate, acidified sodium chlorite and chlorine dioxide as decontaminants are currently under review for final approval by EU authorities. The European Food Safety Authority's (EFSA) former Panel on additives, flavourings, processing aids and materials in contact with food (AFC) has reported that poultry carcass decontamination with trisodium phosphate, acidified sodium chlorite and chlorine dioxide or peroxyacid solutions, under the FDA approved conditions of use, poses no toxicological risk to human health (EFSA, 2005; Río et al., 2007). Organic acids are widely used for the decontamination of meat carcasses.

For the decontaminating application, many variable factors, including temperature, pressure, preservatives, concentration of chemical substances, suitable time for application, types or species of products, and types of microorganisms influence excessively these decontamination techniques. There is no universal reduction option that can eliminate food borne pathogens from the whole food chain. The good hygienic and manufacturing practice and HACCP are mainly operated. The decontamination techniques as an additional method may help to reduce food borne pathogens. Consequently, the implementation of these additional techniques in industries is still not satisfying. Scientific information about the decontamination of meat cuts, particularly raw chicken breasts, is rarely available.

The purpose of this study was to find out an appropriate decontamination method for further processing chicken breasts. Combinations of chemical and/or physical intervention were used and decontamination effects were mainly evaluated by microbiological determination. Adverse effects of these decontaminations were observed by sensory analysis and histological examination.

Objectives

The objectives of this study were to develop a technique for poultry decontamination and to apply it under commercial conditions. The application(s) of decontamination should lead to no or only minor changes in the appearance and taste of the meat tissue. The antimicrobial agents or/and physical methods should be effective and commercially acceptable. Moreover, the application(s) should be suitable to be implemented appropriately in processing.

Demands on effective decontamination technologies are:

o A new invention with definite procedures, point(s) in the processing chain and concentration of substance(s) in the processing line and temperature scales
o Safe for consumers and the environment
o Satisfying to consumers with regard to the sensory quality of the product
o Suitable and practically applicable in food processing plants.

2.   Review of literature

2.1   Situations of salmonellosis

At present, there are more than 2500 known serotypes or serovars of *Salmonella* (WHO, 2005b). Salmonellosis is a most often reported disease in the EU with about 180,000 human cases in 2005 (Hugas and Tsigarida, 2008). With a share of up to 18%, *Salmonella* was the most often detected bacteria in fresh broiler and pig meat and was most frequently found in poultry flocks in EU member states (Hugas and Tsigarida, 2008). From 1998 to 2003, the national reference laboratories participating in an EU Internet surveillance program reported that *Salmonella* Enteritidis and Typhimurium were the predominant identified serovars. They made up more than 80% of all isolates. *Salmonella* Typhimurium and other serotypes slightly increased in 2000 according to statistics, but not *S.* Enteritidis. However, the numbers of *Salmonella* Typhimurium are generally going down. Over the period between 1998-2003, *S.* Enteritidis fell by 36.2 % from 154,928 to 98,915 cases, *S.* Typhimurium by 26.6 % from 25,790 to 18,937 cases and the other serotypes by 35.3% from 39,980 to 25,039 cases (Fisher, 2004).

Salmonellosis is one of the most important food-borne diseases in Thailand too. Isolated *Salmonella* from all regional diagnostic centers are sent for confirmation to the National Salmonella and Shigella Center, which is authorized to publish the annual report. In 2003-2006, the national annual reports showed that the most common cause of salmonellosis was non-typhoidal *Salmonella* rather than typhoidal *Salmonella* (Figure 1). The top-five serotypes which were isolated from patients' blood, were *Salmonella* Enteritidis, *Salmonella* Choleraesuis, *S.*I. ser. 4,5,12: i : -, *S.* Virchow, *S.* Stanley and *Salmonella* Typhimurium (Table 1). *Salmonella* had been isolated from various sources such as frozen chicken, frozen duck, seafood, food products, water, the environment, animals, and raw materials for further food processing, etc (Bangtrakulnonth et al., 2004).
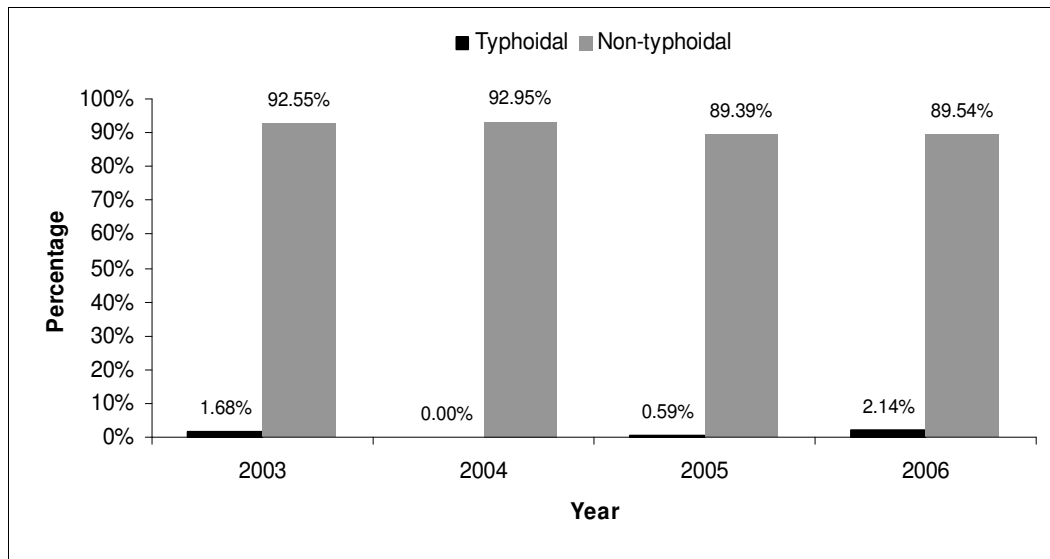
Figure 1 Proportions of salmonellae isolated in Thailand from 2003 to 2006 (modified from Bangtrakulnonth et. al., 2003, 2004, 2005, 2006)

Table 1 Top-ten *Salmonella* serovars in human blood in Thailand from 2003 to 2006 (modified from Bangtrakulnonth et al., 2003, 2004, 2005, 2006)

2003		2004		2005		2006	
S. Enteritidis	46.03%	*S.* Enteritidis	40.94%	*S.* Enteritidis	37.75%	*S.* Enteritidis	48.21%
S. Choleraesuis	22.20%	*S.* Choleraesuis	25.81%	*S.* Choleraesuis	27.45%	*S.* Choleraesuis	28.61%
S.I. ser. 4,12 : i : -	5.84%	*S.* Typhimurium	5.96%	*S*.I 4,5,12:i:-	3.68%	*S*.I. ser. 4,5,12 : i : -	5.03%
S. Typhimurium	3.97%	*S.* Virchow	2.23%	*S*.I 4,12:i:-	3.68%	*S.* Typhimurium	2.38%
S.I. ser. 4,5,12 : i : -	3.50%	*S.* Stanley	1.99%	*S.* Typhimurium	3.19%	*S*.I. ser. 4,12 : i : -	2.38%
S.I. ser. 1,4,5,12 : i : -	2.57%	*S.* Corvallis	1.49%	*S.* Stanley	3.19%	*S.* Stanley	2.25%
S.I. ser. 1,4,12 : i : -	2.34%	*S.* Weltevreden	0.99%	*S.* Typhi	2.45%	*S.* Weltevreden	1.32%
S. Stanley	1.64%	*S.* Bareilly	0.99%	*S.* Corvallis	2.21%	*S.* Typhimurium var. cophenhagen	1.32%
S. Panama	1.40%	*S.* Dublin	0.99%	*S*.I 1,4,5,12:i:-	1.72%	*S.* Panama	0.93%
S. Corvallis	1.17%	*S.* Rissen	0.74%	*S.* Dublin	1.72%	*S.* Rissen	0.79%

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2.2 Salmonella Taxonomy

Salmonellae are members of the *Enterobacteriaceae* family. They are gram-negative, facultative anaerobic, non spore-forming coccobacilli which do not ferment lactose. Salmonellae are usually motile except *S.* Gallinarum and *S.* Pullorum. Up to the present, more than 2500 serotypes are identified by serological identification according to the Kauffmann-White Scheme. The genus *Salmonella* has been divided into two species, *Salmonella enterica* with 6 subgroups (I, II, IIIa, IIIb, IV and VI) and *Salmonella bongori* (formerly subsp. V) (Quinn et al., 1994b; Brenner et al., 2000). *Subspecies* I salmonellae are present in both warm and cold-blooded animals while the other *Salmonella subspecies* are generally associated with cold-blooded animals (Table 2). Approximately 60% of the over 2500 *Salmonella* serotypes belong to *subspecies* I. Salmonellae can be differentiated by biochemical reactions and by serological methods for serotyping. Serotype identification is based on their surface antigens including the O (somatic), H (flagella) and occasionally Vi (capsular) antigens, but most of them do not produce a capsule. All *Salmonella* serotypes can be designated by an antigenic formula, i.e. *subspecies* O antigens: Phase 1 H antigen(s): Phase 2 H antigen(s) e.g. I 8, 20: i: z6. The most significant animal and human pathogens of salmonella belong to *subspecies* I and have been given names (Heyndrickx et al., 2005).

Table 2 Characteristics of *Salmonella* species and subgroups (modified from Quinn et al., 1994b; Bopp et al., 1999; Brenner et al., 2000; Grimont et al., 2000; Andrews and Bäumler, 2005)

Genus	*Salmonella*						
Species	*enterica*						*bongori*
Subspecies	*enterica*	*salamae*	*arizonae*	*diarizonae*	*houtenae*	*indica*	
	(I)	**(II)**	**(IIIa)**	**(IIIb)**	**(IV)**	**(VI)**	**(V)**
Number of serotypes	1,454	489	94	324	70	12	20
Flagella usually monophasic (Mo) or diphasic (Di)	Di	Di	Mo	Di	Mo	Di	Mo
Habitat of majority of strains:							
Warm-blooded animals	+	-	-	-	-	-	-

Genus	Salmonella						
Species	enterica						bongori
Subspecies	enterica (I)	salamae (II)	arizonae (IIIa)	diarizonae (IIIb)	houtenae (IV)	indica (VI)	(V)
Cold-blooded animals and the environment	-	+	+	+	+	+	+
Differential tests:							
Beta-galactosidase (ONPG)	-	-	+	+	-	d	+
Lactose	-	-	(-)	(+)	-	(-)	-
Dulcitol	+	+	-	-	-	d	+
Sorbitol	+	+	+	+	+	+	-
Malonate ultilization (alkalinized)	-	+	+	+	-	-	-
Galacturonate (acid)	-	+	-	+	+	+	+
Gelatin hydrolysis	-	+	+	+	+	+	-
Growth in KCN medium	-	-	-	-	+	-	+
β-Glucuronidase	d	d	-	+	-	d	-
α-Glutamyl transferase	d	+	-	+	+	+	+
L(+) Tartrate[a]	+	-	-	-	-	-	-

+, more than 90% strains positive; (+), 85% strains positive; (-), 15% strains positive; -, less than 10% strains positive; d, different reactions between 10-90% strains positive

ONPG, *ortho*-nitrophenyl-*β*-D-galactopyranoside; [a], Sodium potassium tartrate

2.3   Natural habitat of Salmonella

Salmonella usually lives in the intestinal tract of humans and of warm and cold blooded animals. Reptiles, particularly snakes, are the natural obvious reservoir of *Salmonella enterica* subsp. *arizonae* (Mahajan et al., 2003), as are occasionally insects (Jay, 1978d). Also, they may be found in waste water and contaminated food. Normally, this subspecies rarely causes

infectious diseases in human beings. It may though infect human suffering from immune-deficiency and infants. *Salmonella enterica* subsp. *arizonae* was identified in a fatal gastroenteritis case of an infant who was born into a family of snake charmer in India (Mahajan et al., 2003). Salmonellae are widely spread in the environment such as soil, water, animal production plants and food processing plants by the shedding from intestinal contents of infected humans and animals. They can survive in the environment for several weeks, months or possibly longer depending on favorable conditions of temperature, humidity, pH, etc. *Salmonella* can survive in the feces of infected animals and also remain in the environment of a farm, slaughterhouse, and on vehicles.

2.4   Pathogenesis of salmonellosis

Salmonellae are able to infect many hosts (Table 3). They are ingested via contaminated food or water. Then, they colonize and penetrate in the intestinal cells. The intestine of human beings and animals is covered with a normal flora on the surface of the intestine, so that pathogenic bacteria have developed special mechanisms that help them to become attached on the intestinal mucosa. For example, fimbriae are one of the bacterial structure components (Table 4) that are classified as a virulence factor. It is called adhesion and sticks out from the surface of the bacterial cell. Fimbriae play an important role in the attachment on host cells, but not only on living cells. They also facilitate the bacterial attachment on chicken meat and on connective tissue (Campbell et al., 1987). Another virulence factor is lipopoplysaccharide (LPS). LPS is a structure component of gram-negative bacteria in a part of the outer membrane of the cell wall. It is a harmful substance classified as endotoxin and is released after bacterial cell lysis. Endotoxins help to induce an inflammation response and activate a complementary system by an alternative pathway. In laboratory using animals like mice, monkeys, guinea pigs and rabbits, colonization and invasion of salmonellae occur at the ileum, and penetration at the epithelial layer via specialized M cells, triggering rapid tissue destruction, inflammation and infiltration. Moreover, it is carried by a phagocytic mechanism of macrophages and dendritic cells, then reaches the liver and spleen and colonizes there (Haimovich and Venkatesan, 2006).

In human beings, *Salmonella* Typhimurium usually causes an infection by the fecal-oral route and the symptoms are a self-limiting gastroenteritis with mild fever, diarrhea, abdominal pain, nausea and vomiting. Additionally, in immunocompromised adults it can cause a systemic infection with additional complications. *Salmonella* Typhimurium can be shed in a patient's excretion for several weeks because *Salmonella* Typhimurium often remains in the intestine (Haimovich and Venkatesan, 2006).

Table 3 *Salmonella* serotypes of clinical importance and consequences of infection (Quinn et al., 2003)

Salmonella serotype	Hosts	Consequences of infection
Salmonella Typhimurium	Many animal species and humans	Enterocolitis and septicaemia
Salmonella Dublin	Cattle, sheep, horse and dog	Many disease conditions, enterocolitis and septicaemia
Salmonella Choleraesuis	Pigs	Enterocolitis and septicaemia
Salmonella Pullorum	Chicks	Pullorum disease (Bacillary white diarrhea)
Salmonella Gallinarum	Adult birds	Fowl typhoid
Salmonella Arizonae	Turkeys	Paracolon infection
Salmonella Enteritidis	Poultry, many others species and humans	Often subclinical in poultry, clinical disease in mammals and food poisoning with humans
Salmonella Brandenburg	Sheep	Abortion

Table 4 Structure components of bacterial cells (Quinn et al., 2003)

Structure	Chemical composition	Comments
Capsule	Usually polysaccharide; polypeptide in *Bacillus anthracis*	Often associated with virulence; interferes with phagocytosis; may prolong survival in the environment
Cell wall	Peptidoglycan and teichoic acid in Gram-positive bacteria. Lipopolysaccharide (LPS), protein, phospholipid and peptidoglycan in Gram-negative bacteria	Peptidoglycan is responsible for the shape of the organism. LSP is responsible for endotoxic effects. Porins, protein structures, regulate the passage of small molecules through the phospholipid layer
Cell membrane or cytoplasmic membrane	Phospholipid bilayer	Selectively permeable membrane involved in active transport of nutrients, respiration, excretion and chemoreception
Flagellum (plural, flagella)	Protein called flagellin	Filamentous structure which confers motility
Pilus (plural, pili)	Protein called pilin	Also known as fimbria (plural, fimbriae). Thin, straight, thread-like structures present on many Gram-negative bacteria. Two types exist, attachment pili and conjugation pili

Structure	Chemical composition	Comments
Chromosome	DNA	Single circular structure with no nuclear membrane
Ribosome	RNA and protein	Involved in protein synthesis
Storage granules or inclusions	Chemical composition variable	Present in some bacterial cells; may be composed of polyphosphate (volutin or meta chromatic granules), poly-beta-hydroxybutyrate (reserve energy source), glycogen

2.5 Infective dose

The infective dose of salmonellosis is very variable. Eating food contaminated with approximately 10^5 to 10^6 cells per gram of food causes an illness (Fehlhaber et al., 1992). However, it depends upon the health status, age and immune system of the person. The YOPIS group (Young, Old, Pregnant, Immunocompromised Segments of the public) has the highest risk of getting ill from food-borne diseases. Infants make up one component of the YOPIS group, which are easily infected by *Salmonella* (Mahajan et al., 2003).

A *Salmonella* infection is caused by the consumption of large numbers of *Salmonella* cells. Humphrey et al. (2000) reviewed volunteers that were infected with at least 100,000 cells of *S.* Bareilly and *S.* Newport, and their results showed that over 10 million cells of *S.* Anatum were necessary to cause an infection in the bodies of these volunteers. However, even a low infective dose in contaminated food can infect an organism because many kinds of food materials may protect the bacteria during their passage through the acid regions of the stomach. For instance, foods containing much fat have a good buffering capacity. Hence, lower numbers of *Salmonella* can already initiate an infection (Humphrey et al., 2000).

2.6 Symptoms

Acute gastroenteritis, diarrheal illnesses, bloody diarrhea, nausea, vomiting and bacteremia are caused by non-typhoidal *Salmonella* like *Salmonella* Typhimurium and *Salmonella* Schwarzengrund (MMWR, 2008). The symptoms appear about 5 – 72 hours after eating contaminated food (Baetza et al., 1996), but may be delayed as long as 4 days. The incubation period depends on the state of the host defense mechanism and the amount of ingested *Salmonella*. If *Salmonella* survive and pass through the acidic environment of the stomach, they adhere and penetrate the mucosa of the small intestine.

2.7 Contamination in food processing

Generally, *Salmonella* can be found in intestines of infected humans and many species of animals. Meat and carcasses are mostly contaminated by the leakage of intestinal contents of infected animals during evisceration. In poultry processing, the chilling process by the cool water system is a major source of contamination (Dickson and Anderson, 1992).

2.8 Control of salmonellosis

The approach for the control of *Salmonella* focuses on three lines. The first line concerns the food producing animal by preventing a *Salmonella* infection in herds and by preventing the in-herd transmission. This approach also highlights the strengthening of immunity resistance to infection. The second line concentrates on the prevention and reduction of bacteria during the pre-slaughtering and slaughtering process and carcass dressing, which is followed by a rapid cooling system. The third line refers to the control in the final preparation of a food by the industry and the consumer (WHO, 1980; Hugas and Tsigarida, 2008). Improving consumer education in terms of handling and cooking is very important to control salmonellosis. With regard to the cooking method, the core temperature of meat should hold minimally 75 - 80°C for 10 minutes. This condition will surely kill *Salmonella* (Baetza et al., 1996).

2.9 Bacterial indicators

An indicator of food purity are the *Enterobacteriaceae* family and coliforms. Their general habitats are the gastrointestinal tract of humans and animals. Both may invade the food chain, especially in slaughterhouses at the evisceration process by leakage of intestines. Further processing in the slaughterhouse and the chilling process of poultry carcasses in cooling water immersion is keeping them at this site. Indicators of food spoilage have shown that pseudomonads are normal spoilage bacteria in poultry meat, especially under low storage temperature. They are very susceptible to growth on the surface of poultry meats that are stored at high humidity. Off-odor is produced from spoilage bacteria on the surface at about 7.2-8.0 $\log_{10}$CFU per cm^2. Then they form sliminess on the surface of poultry meat at a bacterial count of about 8.0 $\log_{10}$CFU per cm^2 or above (Jay, 1978a). Moreover, the rate of attachment of *Pseudomonas* onto the meat surface is reportedly higher than with other types of spoilage bacteria (Nychas et al., 2007).

2.10 Antimicrobial treatment used in poultry processing

There are three major types of intervention for the reduction of microbial populations during the processing of poultry carcasses and parts. Physical, chemical and biological treatments have been applied alone or in combination in poultry processing. Hot water and steam with or without vacuuming are known methods of physical decontamination. The combination of decontamination methods, which is known as hurdle technology, has been increasingly considered as an application in food processing.

2.10.1 Physical treatments

Heat

Heat treatment is a very simple, convenient and universal decontamination method including high temperature/short time pasteurization (HTST) or ultra-high temperature (UHT). These methods are commonly used in dairy production. Pasteurization can also be applied to kill pathogenic organisms in beverages, e.g. fruit and vegetable juice, and beer. In principle, high temperature/short time pasteurization (HTST) uses a high temperature within a short time so that the flavor and color of foods are maintained better than with UHT. Huffman (2002) supported the conclusion of the US Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) that hot water above 74°C was suitable for sanitizing carcasses; this temperature is widely used in the beef industry. In poultry products, according to the requirements of the USDA standard and of the FDA (Food and Drug Administration), the end point temperatures of pasteurization are 71°C for commercial cookers and 74°C for retailers, respectively. However, including a safety margin, food processors generally cook at a slightly higher than the recommended temperature, at 75°C to 77°C (Smith et al., 2001). Decontamination based on heat does not only destroy bacterial cells, but it denatures muscle proteins as well. High temperature techniques can cause a coagulation effect on meat. Goeksoy and James (1999) investigated the relationship between the surface temperature of meat and apparent changes on the skin and on chicken breast muscles after dipping them in a hot water bath, followed by immediate cooling in a water bath running at 5°C ± 1°C. The occurrence of irreversible changes in the appearance were observed at 100, 90, 80, 70, 60 and 50 ± 1°C for 1, 2, 6, 9, 60 and 120 seconds, respectively. Their studies showed that treatment with a temperature of 70°C and above caused apparent changes of the skin, which occurred before in the muscles. A previous study with hot water and lactic acid as a chemical substance showed that the two treatments combined produced a 79 % reduction of *E. coli* O157:H7, but this was not better than the treatment with hot water alone. This study suggested that hot

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water would be more beneficial than lactic acid for the decontamination of pre-eviscerated beef carcasses (Bosilevac et al., 2006). Moreover, meat surface decontaminations based on the heat technique are not associated with potential health concerns and with product safety concerns (Sofos and Smith, 1998) because heat treatments do not leave any chemical residues.

Cold

Very low temperature treatments or freezing can inactivate the cells of microorganisms. Freezing injures a living cell by the attribution of ice nucleation and dehydration. Within the initial phase of freezing, microorganisms are destroyed at a high rate. When the temperature cools down rapidly, the inactivation rate highly increases because of the cool shock (Olson et al., 1980). Superchilling as a rapid temperature reduction method, by submersion in liquid nitrogen, affected the inactivation of *C. jejuni* of artificially contaminated chicken wings in Whirl-Pak bags (Zhao et al., 2003). Nevertheless, many microorganisms may be killed after the initial period of freezing, but when they are present in a sufficient number before freezing, they may survive even a long period of storage. For instance, *Salmonella* Typhimurium has been detected after the storage at -25°C for a month (Olson et al., 1980). Therefore, freezing treatment as a decontamination method may not be appropriate if there is a high initial load of microorganisms before the freezing process.

Irradiation

The exposure of ionizing radiant energy, such as gamma or x-ray, has been used to eliminate pathogenic organisms, with the by-effect of extending the shelf-life of a product (Conner et al., 2001). This decontamination method is an expensive method and complicates the food processing management.

2.10.2 Chemical treatments

Acid substances

The pH-value of food plays an important role in maintaining food safety, particularly the microbiological quality. Numerous acids are applied as food preservatives in many kinds of food products (Table 5). Organic acids and esters are frequently used, organic acids to decrease the pH-value of foods and to control the growth of microorganisms. The antimicrobial effect of an acid depends upon the dissociation constant (pK_a) or pH, at which 50% of the total acid is undissociated. The undissociated part of the molecule is related to the antimicrobial effect (Davidson and Taylor, 2007), since the undissociated molecules penetrate into the cells. The activity increases with the chain length, which suggests a direct action of

the organic compound itself. Weak acids have higher undissociated portions than strong acids, and they can effectively penetrate through the cytoplasmic membrane (lipid bilayer) of the microorganism (Davidson and Taylor, 2007). The use of organic acids in foods is usually limited with pH <5.5 because most organic acids, such as acetic acid, citric acid and lactic acid, have pK_as between 3 and 5 (Davidson and Taylor, 2007). Acid solution comes near this value. Its antimicrobial effect is higher (Davis, 1980; Doores, 1983; Davidson and Taylor, 2007). The heat sensitivity of microorganisms increases when the pH-value is lower or higher than the optimal pH-value for bacterial growth (Jay, 1978c; ICMSF, 1988). At low pH levels, the membrane of the microorganism is saturated with hydrogen ions which influence cell permeability and ultimately affect its ability to reproduce. A high concentration of hydrogen ions (anions) induces a high osmolarity which interferes with metabolic processes (Davidson and Taylor, 2007).

Using chemical decontamination methods does not only concern the antimicrobial effects but also the acceptable daily intakes (ADI) and the toxicity of the chemical substances. A guideline of generally accepted ADI-levels according to the Codex Alimentarius Commission is represented in Table 6. There is no limitation for adding acetic acid and lactic acid but the levels of adding acids are restricted by consumer acceptance because these sunstances may produce an undesirable flavor and odor; especially acetic acid does (ICMSF, 1988; FAO/WHO, 2001). Two major considerations in the selection of organic acids as meat decontaminants are summarized in Table 7 (Smulders and Greer, 1998).

Limitations of effective organic acids as microbial inhibitors in foods are usually ineffective when the initial levels of microorganisms are high. Many microorganisms use organic acids as metabolizable carbon sources. There is an inherent variability in the resistance of individual cells (Baird-Parker, 1980). According to the suggestion of the European Commission, decontaminating substances are to be regarded as processing aids when they are used in slaughterhouses to reduce the numbers of pathogenic microorganisms on the surfaces of raw foods of animal origin. They should be rinsed off with water because they are unspecified methods of application. On the other hand, the point(s) in the processing chain where decontamination substances can be applied remain unclear as are their most effective concentrations and optimal temperatures for use (EFSA, 2006).

Lactic acid (2-hydroxy propionic acid) or milk acid is the most common carboxylic acid in nature. It is derived from carbohydrate fermentation from dairy and/or non-dairy sources such as cornstarch, potatoes and molasses. Also, it can be created by chemical synthesis. Lactic acid is a weak organic acid. It is the natural preservative of fermentation

products. Its advantages are that it is inexpensive, naturally occurring and environmentally friendly. Lactic acid is a natural metabolite of mammalian muscle tissue and has been generally recognized as safe (GRAS) for consumption by the American FDA. Lactic acid is applied in many food products to control pathogenic and spoilage organisms with minimally influencing sensory properties. For example, the combination of lactic acid and acetic acid has been used in ready-to-eat cooked shrimps (*Litopenaeus vannamei*) under an atmosphere protection packaging (Oceansea®). Lactic acid is often used in chilling water in poultry processing to reduce spoilage and pathogenic bacteria, and to extend the refrigerated shelf life of broiler carcasses. Van der Marel et al. (1988) reported that a 1-2% lactic acid solution (pH-value = 2) decontamination applied to broiler carcasses before chilling was able to immediately reduce about 1 $\log_{10}$ CFU per gram mesophilic and psychrotrophic aerobic bacteria, in particular *Staphylococcus aureus* and *Enterobacteriaceae* on the skin. After 7 days of chilling storage at 0°C, *Enterobacteriaceae* could not be detected on treated broiler carcasses (detection limit was 2 $\log_{10}$ CFU/ g) (Marel et al., 1988). Lactic acid can be applied to reduce not only bacteria, but also to effectively reduce yeasts. Ismail et al. (2001) reported that the number of *Yarrowia lipolytica* significantly decreased by 1.47 $\log_{10}$ CFU /g when chicken wings were treated with 2% lactic acid with or without 0.2% potassium sorbate or sodium benzoate.

Acetic and lactic acids are widely used as preservative substances and pH-adjusting ingredients in various foods. As preservatives, acetic and lactic acids gain their antimicrobial effect by lowering the pH-value below the growth range, and their undissociated forms of acid inhibit the metabolic mechanisms of organisms (Jay, 1978b).

Using other acids for decontamination, like dipping chicken thighs in a solution of 3% w/w phosphoric acid and 4% w/w propyl gallate could also extend the shelf life. *Salmonella* have not been detected for 14 days when stored at 4°C (Jaturasitha et al., 2003), but the scientists did not evaluate the sensory quality of the product after treatment with propyl gallate in combination with phosphoric acid.

Table 5 Some organic acids and esters used as food preservatives (ICMSF, 1988)

Compound	pK_a	Solubility (g/100g)	Typical maximum use concentration (mg/kg)	Examples of use
Acetic acid	4.75	High	No limit	Pickle fish, meat and vegetable products
Sodium diacetate	4.75	High	4000	Bread, bakery products
Sodium benzoate	4.2	50 (25°C)	1-3000	Pickles, acid sauces and salads, semi-preserved fish, fruit juices, soft drinks, jams, margarines
Citric acid	3.1	High	No limit	Soft drinks
Lactic acid	3.1	High	No limit	Salad creams, mayonnaise
Sodium propionate	4.9	High	1-3000	Bread, bakery and cheese products
Sorbic acid	4.8	0.16 (20°C)	1-2000	Fresh and processed cheese, dairy products, bakery products, fruit juices, acid sauces and salads, jams, jellies, soft drinks, margarines

Table 6 Acceptable Daily Intakes (ADI) (FAO/WHO, 2001)

INS[*] No.	Food additive	Maximal level	ADI (mg/ kg body weight)
260	Acetic acid, glacial	Limited by GMP	NOT LIMITED
270	Lactic acid (L-, D- and DL-)	Limited by GMP	NOT LIMITED
296	Malic acid (DL-)	Limited by GMP	NOT SPECIFIED (the group ADI for malic acid and its sodium, potassium and calcium salts; in the case of D(-)-malic acid and its salts, the ADI is not applicable to very young infants)
330	Citric acid	Limited by GMP	NOT LIMITED (Group ADI for citric acid and its calcium, potassium, sodium and ammonium salts)
334	L-Tartaric Acid	1300 mg/kg	0-30 (Group ADI for L-(+)-tartaric acid and its sodium, potassium, potassium sodium salts)

[*]INS = International Numbering System

Table 7 Major considerations in the selection of organic acids as meat decontaminants

(Smulders and Greer, 1998)

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1. Antimicrobial effects of acids are
 (a) Acid-dependent
- pH (acid concentration)
- Intracellular dissociation of the acid (pH-dependent)
- Specific anion effect: determines the ability to penetrate the bacterial cell and relates to targets within the cell and the chemical nature of the attack
- Acid mixtures (affecting extent of dissociation and possible mutual potentiation)
 (b) Tissue-dependent
- Meat species (nature of the meat surface)
- Buffering capacity (lean greater than fat)
 (c) Bacterial-dependent
- Sensitive: e.g., *Campylobacter jejuni*, *Yersinia enterocolitica*, Pseudomonads
- Resistant: e.g., *Escherichia coli*, *Listeria monocytogenes*
- Population of the initial microbial load
 (d) Slaughter technology-dependent
- Degree of initial microbial contamination
- Nature of contaminating material (organic matter content)
 (e) Decontamination technique-dependent
- Time of acid application
- Contact or exposure time with acids
- Temperature of acid sprays
- Sprays pressures, spray angles
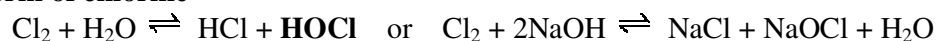- Method of application

2. Sensory effects
(a) Color
- Grey-brown fat discoloration
- Bleaching of the lean
(b) Flavour/odor
- Occasionally vinegar-like off-flavor/off-odor when using acetic acid
- Sensory scores rarely affected by lactic acid at effective concentrations
(c) Water-holding
- Drip loss increase in comminuted meats, particularly after immersion in acids

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Chlorine

Chlorine is a halogen releasing agent that has been used since the early nineteenth century. Chlorinated lime or calcium hypochlorite was used in that century to deodorize sewage. Using chlorine as a disinfectant in most drinking water treatment plants in many countries has practically eliminated waterborne pathogens. In Canada, the free chlorine amount in drinking water distribution systems ranges from 0.04 to 0.80 mg/L (National Consultation, 2007).

For antimicrobial action there are mainly three forms of chlorine, including chlorine gas (Cl_2), sodium hypochlorite (NaOCl) as liquid form and calcium hypochlorite ($Ca(OCl)_2$) as solid form. Sodium hypochlorite is known as hypochlorite salt, chlorine bleach, bleach solution or Javelle water. Commercial solutions are prepared at a concentration of 5-15% (National Consultation, 2007). Sodium hypochlorite or the liquid form of chlorine is the most stable and cheapest form of chlorine available. Sodium hypochlorite combined with water forms hypochlorous acid (HOCl), a strong oxidizing agent, as well as sodium hydroxide (NaOH) (Davis and Dulbecco, 1980). Calcium hypochlorite is available in granular or pellet form and is more expensive than other hypochlorite forms. In the pellet form, the concentration of calcium hypochlorite can be controlled more effectively than other forms of chlorine. Sodium hypochlorite (NaOCl) solution has become the most popular agent for endodontic irrigation (Gomes et al., 2001). All three forms of chlorine, dissolved into water, can produce three chlorine species (Table 8) including hypochlorous acid (HOCl), chlorine (Cl_2) and hypochorite ion (OCl⁻), according to the chemical equations below:
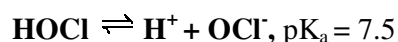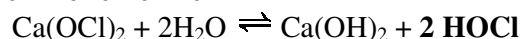
Gas form of chlorine

$$Cl_2 + H_2O \rightleftharpoons HCl + \textbf{HOCl} \quad \text{or} \quad Cl_2 + 2NaOH \rightleftharpoons NaCl + NaOCl + H_2O$$

Liquid form of chlorine

$$NaOCl + H_2O \rightleftharpoons NaOH + \textbf{HOCl}$$

Solid form of chlorine

$$Ca(OCl)_2 + 2H_2O \rightleftharpoons Ca(OH)_2 + \textbf{2 HOCl}$$

$$\textbf{HOCl} \rightleftharpoons \textbf{H}^+ + \textbf{OCl}^-, \text{p}K_a = 7.5$$
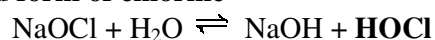
Available chlorine refers to the main active components including Cl_2, HOCl and OCl⁻ (Liu et al., 2006). Hypochlorous acid is the most effective form for microbial inactivation (Kim et al., 2000b; Fenner, 2005) and dominates at a low pH level of an aqueous medium. According to a recommendation of the WHO (2006), the chlorination of drinking water should be conducted at pH levels below 8 for the maximum disinfection efficiency. In high pH solutions, most of the hypochlorous acid disassociates to form hypochlorite ion (OCl⁻), which is a weak sanitizer. The antimicrobial effect of chlorine, used at recommended levels, has a broad spectrum. It can reduce enveloped and non-enveloped viruses and is also effective against fungi, bacteria and algae. Unfortunately, bacterial spores resist the chlorine treatment. The potential antimicrobial action of hypochlorous acid is the following: its molecules penetrate the bacterial cell wall and react with key enzymes to prevent normal respiration (Fabrizio et al., 2002). The bactericidal mechanism of hypochlorous acid is probably the

inhibition of activity of important cytoplasmic enzymes in carbohydrate metabolism (Kim et al., 2000a; Fenner, 2005). HOCl molecules or hypochlorous acid can easily penetrate through the bacterial cell wall and reach the cytoplasm because its suitable properties are a low molecular weight (52.46 g/mol), a water-like structure and its electrical neutrality (Fenner, 2005). Using chlorine and chlorine containing compounds is the most common disinfection method for the contact surface of foods in the US. It is able to kill bacteria in a short time and reduces the exposure period (Kim et al., 2000a).

Concerning organoleptic properties, the limit value of taste and odor of chlorine dioxide compound limits the added amount at 0.4 mg/L (WHO, 2005a; 2006). The guideline value of free chlorine in drinking-water according to the recommendation of the WHO (2006) is 5 mg/L. It is said that most individuals are able to taste the chlorine at this level of concentration.

Free chlorine is defined as chlorine in the form of hypochlorous acid, hypochlorite ion or dissolved elemental chlorine. The fraction of total chlorine exists in the form of chloramines and organic chloramines that is called "combined chlorine". Total chlorine means chlorine present in the form of free chlorine or combined chlorine or both. Chloramines are derivatives of ammonia by substitution of one, two or three hydrogen atoms with chlorine atoms, and include monochloramine (NH_2Cl), dichloramine ($NHCl_2$), nitrogen trichloride (NCl_3) and all chlorinated derivatives of organic nitrogen compounds (Table 8).

With regard to decontamination by chlorine treatment, free chlorine plays an important role in killing microorganisms. The decontaminating efficacy depends on the concentration of free chorine. Therefore, it is necessary to know its concentration in the treated solution, and a determination should be routinely done as quality control for decontamination. The pH value of the solution affects the antimicrobial effect of chlorine. At 20°C and a pH value above 9, the chlorine solution produces a very low free available concentration, and at this pH-level about 96% of the free available chlorine exists in the form of OCl^- ion which is a relatively poor disinfectant. The largest amount of HOCl that has the highest disinfectant properties is produced at a pH below 5 (Fenner, 2005). The interaction between available chlorine and pH value is shown in Figure 2. All the advantages and disadvantages of the three chlorine forms used as antimicrobial agents are summarized in Table 9. Table 10 contains a synopsis of the relevant factors for consideration in the application of decontamination techniques.

Table 8 Terms and synonyms in relation to actual compounds in the solution (ISO7393/2, 1985)

Term	Synonym		Compounds
Free chlorine	Free chlorine	Active free chlorine	Elemental chlorine, hypochlorous acid
		Potential free chlorine	Hypochlorite
Total chlorine	Total residual chlorine		Elemental chlorine, hypochlorous acid, hypochlorite, and chloramines

Table 9 Advantages and disadvantages of three chlorine forms (modified from Russell et al., 2007)

Liquid form or sodium hypochlorite

Advantages	Disadvantages
Low cost	Activity greatly influenced by pH-value
Familiar proven technology	Irritating agent
Relatively non-toxic	Inactivated by organic matter
Wide germicidal activity	Less active at low temperature
Effective at low concentrations	Carcinogenic by-products
Bacteria cannot become resistant	Highly corrosive
Sodium hypochlorite is the most stable and cheapest form of chlorine available	Not accepted in European Union
	Deteriorated by heat, light, ultraviolet

Gas form or chlorine gas

Advantages	Disadvantages
Very effective bacterial disinfectant, even more effective than chlorine for the disinfection of water containing viruses	Extremely irritant to mucous membranes
	Storage and shipping as a pressurized liquefied gas

Solid form or calcium hypochlorite

Advantages	Disadvantages
Easy to handle and transport	Deteriorated by heat, organic materials and humidity
Easy to control the concentration	Expensive

Table 10 Factors for consideration in the application of decontamination techniques

(modified from Smulders and Greer, 1998, Lee, 2004, Hugas and Tsigarida, 2008)

Technical data
- Identity of the chemical substance and specifications e.g., pH, water and fat solubility, mode of action
- Manufacturing process e.g., method of application, concentration of chemical substances, temperature, step of process
- Reactions and residues in the decontaminated product
- Methods of analysis, e.g. monitoring of chemical concentration, temperature and pH value
- The process and its purpose

Exposure assessment
- Estimate of potential daily exposure of the consumer to residues, degradation products and any relevant reaction by-products
- Staff health aspects, including hypersensitization

Toxicological data
- Available toxicological data on each substance, including its potential degradation products and any identified reaction by-products

Data to evaluate efficacy
- All experimental conditions to be performed with the product formulation for authorization
- Comparison of the prevalence and/or numbers of the pathogenic microorganisms between the treated food and the control food
- Measurement of the prevalence and/or numbers of target pathogenic microorganisms before and after application of the product formulation
- Measurement of the prevalence and/or numbers of the target pathogenic microorganisms at the end of the shelf-life of both the treated and control food
- Behavior of non-pathogenic microorganisms, such as indicator microorganisms and total viable counts
- Test on naturally contaminated foods
- Proof that the concentration of the product formulation proposed is justified
- Description of the methods to control and monitor the concentration of the active substance in the processing plant during operational time
- Identification of factors that may influence the efficacy of the active substance
- Recycling, recovery and environmental impact
- Relationship between the number of pathogens and the desired effect: sensory quality, e.g., flavor and texture**,** water holding capacity of meat
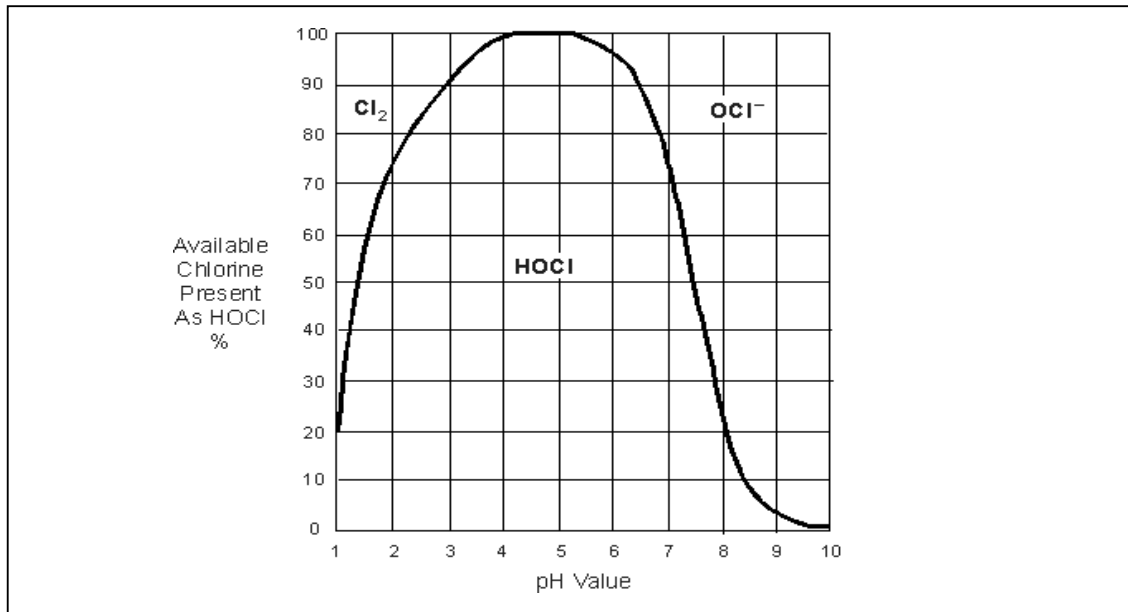- Target shelf-life

Figure 2 Forms of available chlorine at different pH-values (modified from Mahmoud, 2007)

Ozone

Ozone (O_3) is a strong antimicrobial agent with a broad spectrum. Ozone inactivates gram-negative and gram-positive bacteria, spore-forming bacteria, virus, fungi and also protozoa (Rodríguez-Romo and Yousef, 2005). It has been approved by the U.S. Food and Drug Administration in June 2001 to be applied on food, including meat and poultry in aqueous or gaseous phases (U.S. Code of Federal Regulations, 2001). In industry, ozone is usually produced by an ozone generator in a closed system (Kim et al., 1999). Advantage of ozone is that it can be applied in food processing without leaving any residues in the environment because it decomposes spontaneously to oxygen. On the other hand, ozone is very unstable and very quick decomposition occurs in air (Kim et al., 1999; Rodríguez-Romo and Yousef, 2005). For this disadvantage, ozone needs to be produced to the point and time of usage due to its instability and rapid degradation. Since this decontamination method requires an ozone producing machine in the processing plant, food processors have to invest more money to buy machines and to maintain the system. Moreover, there are some limitations. Ozone is harmful to human health. Its toxicity depends on a concentration and a length of exposure. Long-term exposure with 0.1 – 1.0 ppm ozone can cause headaches, dry throat and irritation to the respiratory system and eyes. Short-term exposure at high concentration (1.0 – 100 ppm ozone) can make asthma-like symptoms, throat haemorrhage and pulmonary

congestion. To avoid health risks, the concentration of ozone must be monitored in the working place (Pascual et al., 2007).

Electrolyzed oxidizing water (EOW)

Electrolyzed oxidizing water is an electrolyzed solution which is produced by sodium chloride solution through a low voltage of electric current in an electrolysis chamber. The electrolysis chamber is separated by a diaphragm into two compartments, including the anode and cathode. The anode side produces acidic electrolyzed solution that has a low pH and a high oxidation-reduction potential (ORP), chlorine (Cl_2), hypochlorous acid (HOCl) and hypochorite (OCl^-). The cathode side produces alkaline or basic electrolyzed solution having a high pH and low ORP (Fabrizio and Cutter, 2004; Hsu and Kao, 2004; Fenner, 2005; Huang et al., 2006). The physical and chemical properties depend on concentration of sodium chloride solution, amperage level, water flow rate and time of electrolysis (Fenner, 2005; Huang et al., 2006). The main antimicrobial active component in electrolyzed oxidizing water is available chlorine.

Electrolyzed oxidizing water has been reported to have a strong bactericidal effect against many pathogenic bacteria (Kim et al., 2000a; Kim et al., 2000b; Hsu and Kao, 2004; Fenner, 2005; Liu et al., 2006). It has been commonly used on fruits, vegetables, poultry and the surface of utensils like cutting boards (Liu et al., 2006). Fortunately, electrolyzed oxidizing water is free of strong and unpleasant odors (Fenner, 2005) and antimicrobial properties are decreased when it is stored under the atmosphere due to decreasing of total residual chlorine (Hsu and Kao, 2004).

Trisodium phosphate (Na_3PO_4) or TSP

In artificially contaminated *Salmonella* chicken, dipped in a 10% trisodium phosphate solution, *Salmonella* on the carcass were significantly reduced at about 2 $\log_{10}$ CFU ml^{-1} carcass rinse. The high pH-value (pH 11-12) of it caused lethal or sub-lethal injuries to *Salmonella* cells (Lillard, 1994).

2.11   Basic considerations concerning antimicrobial activities; bactericidal and bacteriostatic effects

In living human-beings and animals, an immunophagocytic response system is in place which attacks bacteria with bacteriostatic agents. Unfortunately, such protective system does not exist in meat or food products, so that the decontamination method in the food process must be carried out by respective bactericidal agents. A bactericidal effect destroys the ability of the organisms to multiply when placed in a suitable environment, or it performs an irreversible lethal action. The mechanism of bactericidal action works in such a way that it damages elements of the cells which cannot replace and repair their organelles, and it also destroys its ribosomes, which can normally regenerate an essential enzyme by protein synthesis. Ribosomes are often the place of the bactericidal action. On the other hand, a bacteriostatic effect is to delay bacterial growth for several generations or to exert irreversible inhibition of growth (Davis and Dulbecco, 1980). The bacteriostatic and bactercidal effects can be measured by the growth of the bacteria by turbidity and bactericidal action also by viability counts or growth cultures.

Up to the present, many food researchers have published numerous studies of new interventions to reduce bacterial contamination and to extend the shelf life of food and food products. For instance, PABA or *p*-aminobenzoic acid showed a greater antimicrobial activity against *L. monocytogenes, S.* Enteritidis and *E. coli* than formic, propionic, citric, acetic or lactic acids. PABA was also capable of reducing these pathogens at higher pH-values than at values at which the other acids showed any bacterial reduction (Richards et al., 1995). Lactic acid and acetic acid not only enhance the flavor in many foods, but also have a bacteriostatic effect owing to the 10-fold increase of bacteriostasis when one pH unit is decreased (Hubbert et al., 1996).

Physical decontamination such as by heat is safe for the consumer since no chemical residues remain in the food. For example, the immersion in hot water at 80°C for 10 seconds gave a 10- to 1000-fold reduction of *E. coli* and *Salmonella* on beef and sheep carcasses, and also the appearance of cooking almost completely disappeared after a few hours of chill-storage at 1- 4°C (Smith and Graham, 1978). Also, the application of steam under vacuum has been investigated and its use was associated with an increase in the shelf-life of poultry meat (Hinton and Corry, 1999b). With regard to the elimination of bacterial contamination, not only temperature and time of the heat treatment are important, but the sensory attributes of the food after treatment have to be considered as well. When temperatures between 70 and 75°C were applied to a *Salmonella* Pullorum suspension, the rupture of cell walls could be observed

by electron microscopy; below that temperature range the rupture of the cell walls did not occur (Salton and Horne, 1951). Castillo and Rodríguez-García (2004) reported that the temperature was critical for achieving a reduction of microorganisms with a high acid tolerance, such as *E. coli* O157:H7. The antimicrobial properties of organic acids are well documented and their effects increase with temperature adjustment. As a result, the combination of physical and chemical decontamination techniques probably enhances the antimicrobial activity.

2.12   Hurdle technology

Hurdle technology is the combination of preservation technologies or different hurdles that aim at inhibiting the multiplication of microorganisms in food products. The preservation technologies establish a series of preservative factors or hurdles so that the microorganisms increasingly lose their ability to overcome hurdles. These hurdles are, for example, temperature, water activity, redox potential, addition of preservatives, and interaction of competitive flora or starter culture, etc (Table 11). The essential action in hurdle technology is known as the disturbance of the homeostasis of microorganisms (Table 12) that causes microorganisms to become inactive or even to die. A combination of different hurdles has been employed in food processing plants in the framework of consumer preferences. Most consumers require a safe, healthy and a more 'natural' food which means less processing and fewer chemical preservatives (Lee, 2004). The ideal hurdle technology should achieve an enhanced level of product safety and stability, produce a pathogen-free food but retain the original nutrition properties of the food.

Table 11 Most important hurdles for food preservation (Lee, 2004)

Symbol	Parameter	Application
F	High temperature	Heating
T	Low temperature	Chilling, freezing
a_w	Reduced water activity	Drying, curing, conserving
pH	Increased acidity	Acid addition or formation
E_h	Reduced redox potential	Removal of oxygen or addition of ascorbate
Pres.	Preservatives	Sorbate, sulfite, nitrite
c.f.	Competitive flora	Microbial fermentations

Table 12 Homeostatic responses to stress by microorganisms (Lee, 2004)

Stress factor	Homeostatic response
Low levels of nutrients	Nutrient scavenging; oligotrophy; 'stationary-phase response'; generation of 'viable non-culturable' forms
Lowered pH	Extrusion of protons across the cell membrane; maintenance of cytoplasmic pH; maintenance of transmembrane pH gradient
Lowered water activity	Osmoregulation; accumulation of 'compatible solutes'; avoidance of water loss; maintenance of membrane turgor
Lowered temperature for growth	'Cold shock' response; changes in membrane lipids to maintain satisfactory fluidity
Raised temperature for growth	'Heat shock' response; membrane lipid changes
Raised levels of oxygen	Enzyme protection (catalase, peroxidase, superoxide dismutase) from H_2O_2 and oxygen-derived free radicals
Presence of biocides	Phenotypic adaptation; reduction in cell wall/membrane permeability
Ionizing radiation	Repair of single-strand breaks in DNA
High hydrostatic pressure	Uncertain; possibly low spore water content
High voltage electric discharge	Low electrical conductivity of the spore protoplast
Competition from other microorganisms	Formation of interacting communities; aggregates of cells showing some degree of symbiosis; biofilms

2.13 Factors influencing the decontamination of the surface

Numerous factors influence the effectiveness of chemical preservation, such as the chemical structure, chemical concentration, microorganisms phase of growth, whether lag or log phase and also the form (either vegetative cell or spore) of microorganisms, the composition of food, the pH-value of food and the treatment condition, the microbial attachment on the surface of meat or food, the temperature and period of storage, etc. The effectiveness of antimicrobial agents depends on the validation and the verification of the application methodology, on the initial microbial load and on treatment conditions. There are many factors concerning the treatment conditions including the type of meat tissue, the microbial load of the natural product, the ability of microbes to attach to the product, biofilm

formation, pH of the solution, time of exposure, and fat and organic matter in the water (EFSA, 2005; Hugas, 2008).

2.13.1 Type of microorganisms

The type of microorganisms is one of the important factors of influence (Jay, 1978c). Gram-positive bacteria are more heat resistant than gram-negative bacteria. Salton and Horne (1951) reported that cells of *Streptococcus faecalis* were incompletely destroyed when its suspension was heated to 100°C for 5 min. On the other hand, *E. coli, Salmonella* Pullorum, *Pseudomonas fluorescens* and *Pseudomonas pyocyanae* were totally eliminated at the same temperature and time. This difference is due to the components of the bacterial cell walls of gram-positive and negative bacteria. The surface of gram-negative bacteria is more complex than that of gram-positive bacteria, but the cell wall of gram-positive bacteria is thicker because it is composed of multiple layers of peptidoglycan and a linear polymer of alternating units of *N*-acetylglucosamine and *N*-acetylmuramic acid. Peptide chains cross-linked with bridges in gram-positive bacteria cell walls are more frequent than in gram-negative bacteria. Moreover, gram-positive bacteria have a compact cell wall with peptidoglycan making up as much as 90% of the thickness, whereas peptidoglycan of gram-negative bacteria only makes up 5-20% of the cell wall thickness. The cell wall of gram-negative bacteria is less compact and thinner than that of gram-positive bacteria (Moat and Foster, 1988). As a result, gram-negative bacteria are probably more susceptible to heat than gram-positive ones. Additionally, psychrophilic microorganisms are the most heat sensitive, followed by mesophiles. The most resistant to heat are the thermophiles. Sporeforming bacteria are more heat-resistant than non-sporeforming bacteria. Cocci bacteria are also more heat-resistant than non-sporeforming rod bacteria (Jay, 1978c).

2.13.2 Attachment of bacteria on surface

The attachment of bacteria is the first step, and an important mechanism is the use of a utensil to become attached to the surface of intestinal cells in living animals and human beings or in animal products such as meat during food processing. When bacteria attach successfully on the surface they then start to multiply and penetrate to the host cells and infect animals and humans. On the surface, after attachment, with the help of a utensil some kinds of bacteria are able to build a biofilm and they cannot be easily removed from the surface by a disinfectant. The biofilm causes the contamination of food and food products with the respective microorganisms. The structures of the bacteria hereby affect the attachment and decon-

tamination. Many structures of bacteria do protect them from an unsuitable environment and in turn increase the chance of an infection. For instance, the bacterial capsule plays an important role to protect the bacterial cell from adverse environmental conditions. The capsules of pathogenic bacteria may help them to become attached to surfaces and to interfere with phagocytosis. More importantly, the cell wall is tough, and the bacteria have a firm structure which protects them from mechanical damages and osmotic lysis.

The cell wall is generally used to divide bacteria into two major groups, gram-negative and gram-positive bacteria, according to the gram stain result. The cell wall of gram-positive bacteria is composed mainly of peptidoglycan and teichoic acids. In contrast, gram-negative bacteria have cell walls with a more complex structure which consists of an outer membrane and a periplasmic space. The outer membrane excludes hydrophobic molecules and causes the gram-negative bacteria to become resistant to some detergents which are lethal to most of the gram-positive bacteria (Quinn et al., 2002). Many studies have indicated that *Salmonella* fimbriae play a role in an additional adhesive and reversible attachment to chicken connective muscle (Thorns et al., 2000).

Loose and firm bacterial attachment

Studies on bacterial attachment in animal tissues have started in the early 1970's (Schwach and Zottola, 1982). There are two steps of bacterial attachment. The surrounding environment plays an important role in the first step, including pH-value, surface tension, surface charge and other physical factors. These factors lead to physicochemical forces or van der Waals forces (Nychas et al., 2007) which cause bacterial cells to approach the surface closely. In this step, the attachment is reversible, which is called loose attachment. At the second step of bacterial attachment, the bacterial cell produces a permanent adhesive substance as the attachment fibrils firmly link the gaps between the bacterial cells and the surface this is called firm attachment. In this step, the attachment fibrils form small protrusions from the cell to the surface which become longer and more numerous at increasing contact times. Therefore, it is difficult to remove a bacterial contamination on the surface, especially after 12-14 h of contact time (Schwach and Zottola, 1982).

The decontamination of meat with acid substances was more effective when the meat was treated prior to the firm bacterial attachment on the meat surface (Conner et al., 2001). Moreover, the carcass surface was completely contaminated with bacteria even during chilling time (Acuff et al., 1987). Hence, decontamination after the chilling process is probably more difficult and less successful. For a greater effectiveness of bacterial elimina-

tion, the decontamination of cut meat therefore should aim before firm bacterial attachment or the chilling process.

Campbell et al. (1987) reported that attachment could be prevented by addition of physiological levels of sodium chloride to the attachment medium. Laboratory cultures of bacteria were tested for their ability to attach to collagen fibres of intact chicken muscle connective tissue. All salmonellas, fimbrial strains of *Escherichia coli* and a strain of *Campylobacter coli* were able to attach to the tissue only when suspended in distilled water. Prior immersion of tissue in sterile water for 20 min or an extended immersion in these bacterial suspensions was a prerequisite for adhesion. Using the model system developed in the laboratory, the interactions of carrageenans and other polysaccharides with collagen and the *Salmonella* sensor surface were evaluated. The κ-carrageenans blocked 92–100% binding of collagen to the *Salmonella* surface, while sodium alginate and low methoxy pectin blocked 50% and 18% binding, respectively. These biosensor studies revealed the rapid evaluation of compounds that may prevent bacterial attachment to poultry skin and carcasses, thus essentially reducing pathogen contamination of poultry foods (Medina, 2004).

Over 30 years, food researchers who studied and created new decontamination methods had to make use of artificially caused microbial contaminations. Their studies aimed at generating results that were as close as possible to those of a natural contamination. Many such artificial contaminations were performed as shown in Table 13.

Table 13 Review of artificial contaminations with bacteria for antimicrobial tests

Method	Holding time of bacterial attachment	Reference
Dip and drain and hold at 3°C	1 hr	(Klose and Bayne, 1970)
Fine fog spraying, kept at room temperature	45 min	(Klose et al., 1971)
Room temperature	30 – 60 min	(Smith and Graham, 1978)
Spread and allowed to attach at room temperature	30 min	(Lillard, 1994)
Drip on meat and allowed to dry inside a biological hood	Until dry	(Morgan et al., 1996)
Spread and allowed to attach at room temperature	20 min	(Phebus et al., 1997)
Air dry	20 min	(Zhao et al., 2003)
Immersion in culture and allowed to drain	60 s 30 min	(Whyte et al., 2003)
Immersion in culture	10 min	(Gonçalves et al., 2005)
Inoculation of contaminated cecal contents on chicken carcass, kept at room temperature (27°C)	12 min	(Northcutt et al., 2005)

Method	Holding time of bacterial attachment	Reference
Immersion in culture and kept at room temperature	30 min	(Özdemir et al., 2006)
Spread and allowed to attach at room temperature	at least 15 min	(Corry et al., 2007)
Spread and allowed to stand at room temperature	at least 15 min	(James et al., 2007)
Appropriate temperature for growth	30 min	(Kim and Day, 2007)

2.13.3 Initial bacterial load

Olson and Nottingham (1980) showed that a high initial bacterial load to be destructed needs longer heating than a low one. Jay (1978c) further explained that a high population load of organisms makes it harder to kill organisms by heat because a high population of organisms excretes a high amount of protein. These proteins play an important role in protecting cells from heat treatment.

2.13.4 State of bacterial growth

The resistance of bacteria against antimicrobial treatment depends on the state of those bacteria. In the lag phase or early state, bacterial cells are placed in a new environment and they must adapt themselves to this new environment. The logarithmic or exponential phase follows the lag phase (when bacterial cells have already adapted) and describes the multiplication of them. The next state is the stationary phase in which the rate of cell division balances the rate of cell death because the environment provides depleted nutrients and high metabolic wastes from the logarithmic growth phase. The last step is the death or decline phase during which the bacterial cells die as standard bacterial growth curves show (Figure 3). Jay (1978c) reported that *Salmonella* Senftenberg in the stationary phase (old cells) are possibly more heat resistant than in the logarithmic phase. Like bacterial spores, old cells are more thermostable than young cells. Numerous studies concluded that bacterial cells in the logarithmic phase are more sensitive to heat than in any other state of bacterial growth. Overall, the stationary phase has proved to be the phase of maximal heat resistance (Jay, 1978c; Olson and Nottingham, 1980).
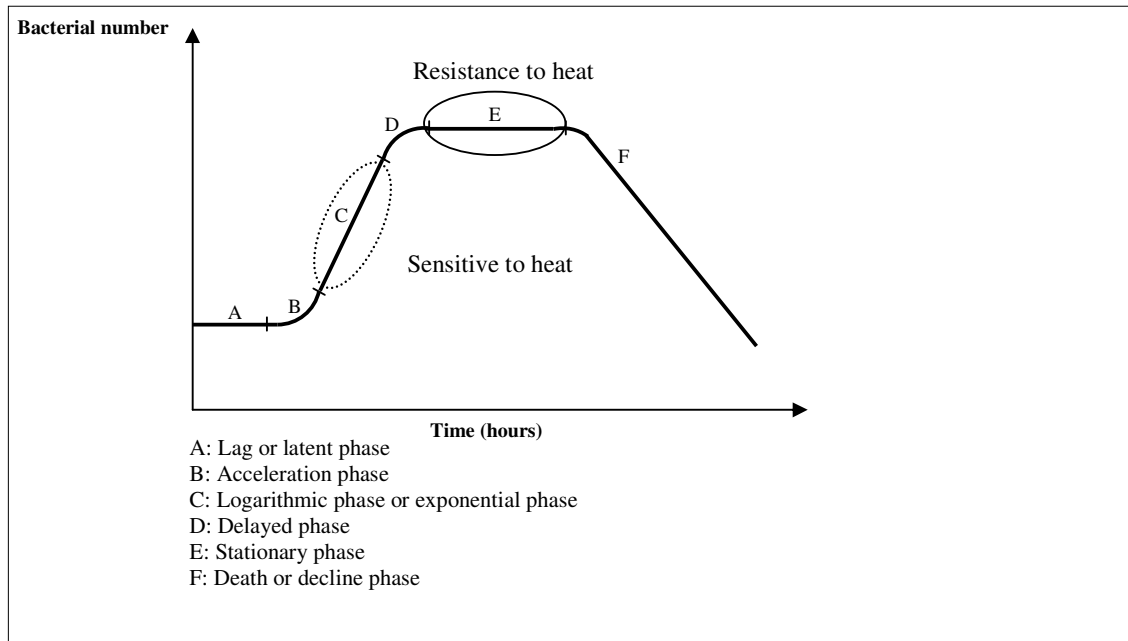
Bacterial number

Resistance to heat

E

D

C

F

Sensitive to heat

A   B

Time (hours)

A: Lag or latent phase
B: Acceleration phase
C: Logarithmic phase or exponential phase
D: Delayed phase
E: Stationary phase
F: Death or decline phase

Figure 3 Standard bacterial growth curve (modified from Fehlhaber and Janetschke, 1992)

Table 14 Grouping of bacteria based on temperature effects on growth (modified from ICMSF, 1988; Conner et al., 2001)

Bacterial group	Temperature ranges that allow growth (℃)		
	Minimum	**Optimum**	**Maximum**
Psychrophilic	-15 to +5	5 to 30	20 to 40
Psychrotrophic	-5 to +8	20 to 30	30 to 43
Mesophilic	5 to 8	25 to 43	40 to 50
Thermophilic	40 to 45	55 to 75	60 to 90

2.13.5 The pH-value of the environment

Obviously, microbial growth can be limited by the pH-value of the growth environment including culture media, food or water, etc. The limits of pH on growth in laboratory media by *Salmonella* Typhi, *Salmonella* Paratyphi, *Escherichia coli, Pseudomonas aeruginosa* were 4.0 - 9.6, 4.5 – 7.8, 4.4 – 9.0, 5.6 – 8.0, respectively as shown in Table 15 (ICMSF, 1988). The optimal pH for the best growth of *Salmonella* is between 6.6 and 8.2 (Jay, 1978d). Under laboratory conditions, salmonellae started to grow in lactic acid solution at pH 4.40 and

in acetic acid solution at pH 5.40 (Jay, 1978d). Izat et al. (1990) reported that the destruction of the organisms begins at a pH below 2.3-2.5.

Table 15 The limits of pH allowing initiation of growth by various microorganisms in laboratory media adjusted with strong acid or alkali (ICMSF, 1988)

	Minimum pH	**Maximum pH**
Gram-negative bacteria		
Escherichia coli	4.4	9.0
Proteus vulgaris	4.4	9.2
Pseudomonas aeruginosa	5.6	8.0
Salmonella Paratyphi	4.5	7.8
Salmonella Typhi	4.0-4.5	8.0-9.6
Vibrio parahaemolyticus	4.8	11.0
Gram-positive bacteria		
Bacillus cereus	4.9	9.3
Bacillus subtilis	4.5	8.5
Bacillus stearothermophilus	5.2	9.2
Clostridium botulinum	4.7	8.5
Clostridium sporogenes	5.0	9.0
Enterococcus spp.	4.8	10.6
Lactobacillus spp.	3.8-4.4	7.2
Micrococcus spp.	5.6	8.1
Staphylococcus aureus	4.0	9.8
Streptococcus faecium	4.4-4.7	9.2
Streptococcus lactis	4.3-4.8	9.2
Streptococcus pyogenes	6.3	9.2
Yeasts		
Candida pseudotropicalis	2.3	8.8
Hansenula canadensis	2.15	8.6
Saccharomyces spp.	2.1-2.4	8.6-9.0
Schizosaccharomyces octosporus	5.4	7.0
Moulds		
Aspergillus oryzae	1.6	9.3
Penicillium italicum	1.9	9.3
Penicillium variabile	1.6	11.1
Fusarium oxysporum	1.8	11.1
Phycomyces blakesleenus	3.0	7.5

2.14   Factors influencing the decontamination of meat

2.14.1 Water holding capacity of meat

The amount of water within the total muscle mass is about 65-80 percent. In the living muscle cell, water plays a major role in the cellular functions. It acts as a solvent or carrier for

substances that must be transported in cells. Water in living muscle cells is bound rather firmly to the various proteins. Denaturation of the proteins causes a loss of solubility, loss of water holding capacity, and a loss in the intensity of the muscle's pigment coloration. Very low water capacity causes a very wet cut surface and dripping fluid from the surface of the muscle. The color of the muscle is pale when the muscle proteins are denatured. Both undesirable effects are caused by a pH-value drop in the muscle. On the other hand, muscles maintain a high pH-value during the conversion of the muscle to meat, and tend to be very dark in color and very dry on the cut surface because the water is tightly bound to the proteins at higher pH-values (Forrest et al., 1975b). Changes of pH and temperature are major causes of muscle fiber denaturation. Therefore, decontamination by heat or by chemical substances which causes a too high or too low pH-value will probably affect the water holding capacity of meat.

2.14.2 The pH value of meat and poultry

In general, the pH of red and poultry meat ranges from 5.6 to 6.4 as shown in Table 16 (ICMSF, 1988). The pH of chicken meat is 6.2-6.7 (Corlett and Brown, 1980). Food processing or decontamination methods are able to change the pH-value of meat. An ideal decontamination method should change the pH-value of poultry meat only slightly or even does not induce any sensory attributes. Especially chemical decontamination does affect the pH-value of meat. The change of the pH-value actually leads to undesirable effects, e.g. water holding capacity, color of meat, and firmness. Therefore, the pH-value is one of the important factors to be considered in the application of meat decontamination. Usually, the pH-value can be determined by the pH meter (Table 17).

Table 16 Approximate pH-values of fresh foods[1] (ICMSF, 1988)

Food	pH range
Meat and poultry	5.6-6.4 (exceptionally 6.4-6.8)
Fish	6.6-6.8
Molluscs	4.8-6.3
Crustaceans	6.8-7.0
Dairy products	
Milk	6.3-6.5
Butter	6.1-6.4
Fruits	
Apples	2.9-3.3
Bananas	4.5-4.7
Oranges	3.6-4.3
Plums	2.8-4.6

Food	pH range
Vegetables	
Beans (string, baked and green)	4.6-5.5
Beans (Lima)	5.4-6.5
Bean (soy)	6.0-6.6
Potatoes	5.6-6.2
Corn (sweet)	7.3
Rhubarb	3.1-3.4
Spinach	5.5-5.6

[1] Various sources, modified.

Table 17 Review of pH determination of meat

Method	Reference
10 g homogenized sausage in 90 ml distilled water, pH meter	(Gounadaki et al., 2008)
Homogenized chicken meat in 10 ml distilled water, pH meter	(Patsias et al., 2008; Chouliara et al., 2007)
5 g homogenized meat in 45 ml distilled water, pH meter	(Jin et al., 2007)
10 g homogenized chicken skin in 10 ml distilled water, pH meter	(González-Fandos and Dominguez, 2007)
5 g homogenized chicken leg skin in 45 ml peptone water, pH meter	(Río et al., 2007)
Modified AOAC (1995)	(Goulas and Kontominas, 2007)
Homogenized carp fillets in distilled water (1:10), pH meter	(Mahmoud et al., 2006)
10 g homogenous fresh pork in 100 ml deionized water and periodic stirring for 30 min, pH meter	(Shrestha and Min, 2006)
10 g homogenized beef in 90 ml of sterile peptone (0.1%) water, pH meter	(Özdemir et al., 2006)
5 g homogenized pork in 25 ml of sterile distilled water, pH meter	(Jensen et al., 2003)
Homogenized chicken legs, pH meter	(Zeitoun and Debevere, 1992)
Homogenized chicken skin, pH meter	(Zeitoun and Debevere , 1990; 1991)
Surface of beef trimmings, surface probe, pH meter	(Ellebracht et al., 2005)
Surface of beef cubes, surface probe, pH meter	(Ariyapitipun et al., 2000)
Surface of broiler breast skin, surface probe, pH meter	(Van der Marel et al., 1988)
Surface of beef loins, surface probe, pH meter	(Acuff et al., 1987)

2.14.3 Color of chicken meat

According to the zoological systematic, chicken belongs to Class *Aves* Subclass *Neornithes* Superorder *Neognathae* Family *Phasianidae* Genus *Gallus* Species *gallus* Subspecies *domesticus*. The chicken's forelimbs are modified wings and the powerful breast muscle has been developed for flying (King, 1975). Therefore, the breast or pectoral muscles

are the biggest part of the chicken muscles (McLelland, 1990; Wilson, 2005). There are two parts of breast muscles, a superficial pectoral muscle and a deep supracoracoid muscle. The pectoral muscle extends from the keel of the sternum, the clavicle and the sternocoraclavicular membrane to the pectoral crest of the humerus. The supracoracoid muscle extends from the keel of the sternum through the triosseal canal to the surface of the humerus. One skeletal muscle is a combination of numerous muscle fiber bundles and a wrap of covering connective tissue. The connective tissue sheath that surrounds and separates the muscle is called epimysium.

The main functions of the superficial pectoral and deep supracoracoid muscle are to raise and lower the wing, respectively. Breast muscles are composed of two types of muscle fibers, known as white and red muscle fibers. The red type of muscle is dark and the white type is lighter in color than the red one. Red fiber or Type I muscle fiber has been characterized by a slow contraction and a high-oxidative metabolism. There is a high density of supply capillaries, mitochondria and myoglobin, and thus, these characteristics cause the red color, known as dark meat in poultry. This muscle fiber type can carry more oxygen and sustain aerobic activity. Red muscle fiber has a higher amount of myoglobin and can use fat rather than glycogen as an energy source. It is more efficient than white fiber because fat yields more energy than carbohydrate per unit weight. As a result, red muscle fibers are more capable of sustained activity than white muscle fibers (McLelland, 1990). Thus, Type I muscle fibers have been found in muscles that require sustained activity such as walking and standing, for example in thighs and drumsticks or in muscle wings of long flying migration birds.

On the other hand, breast muscles are lighter in color than leg muscles because breast muscles mostly contain white muscle fiber (Wilson, 2005). White or type II muscle fiber has a low oxidative demand. This type contains a relatively small amount of myoglobin, fewer mitochondria and capillaries supplying blood and thus appears white in color. Glycolytic metabolism predominates in white fibers and is available in either the presence or absence of oxygen. The white muscle fiber or fast twitch muscle can contract more quickly and more powerfully than the red one because of a more extensively developed sarcoplasmic recticulum and T tubule system in the white fibers (Forrest et al., 1975a). They may be characterized as being more powerful, and are able to contract rapidly in short bursts, but they are not suited for sustained activity (Table 18). They are easily fatigued because of the metabolic waste from anaerobic glycolysis. Consequently, myoglobin is primarily responsible for the color of the meat. Decontamination with an acid solution affects the color of the meat. For example,

treatment of ground beef with 5% acetic acid solution made the beef color a bit darker, and the pH-value of the treated ground beef was 4.41. Acid treatment may cause low oxy-myoglobin and lead to the desired effect of redness of the meat (Stivarius et al., 2002).

Table 18 Characteristics of red, intermediate, and white muscle fibers in domestic meat animals and birds* (Forrest et al., 1975b)

Characteristic	Red Fiber	Intermediate Fiber	White Fiber
Color	red	red	white
Myoglobin content	high	high	low
Fiber diameter	small	small-intermediate	large
Contraction speed	slow	fast	fast
Contractile action	tonic	tonic	phasic
Number of mitochondria	high	intermediate	low
Mitochondrial size	large	intermediate	small
Capillary density	high	intermediate	low
Oxidative metabolism	high	intermediate	low
Glycolytic metabolism	low	intermediate	high
Lipid content	high	intermediate	low
Glycolygen content	low	high	high

*The characteristics are in relation to the other fiber types

2.14.4 Further processing

Marination techniques

Several marination methods exist that can be used on a commercial scale, for instance soaking or still marination, blending, tumbling and mechanical injection (Smith and Acton, 2001). Soaking or still marination is a very common method. The marination increases the water binding of meat, and water binding is related to the swelling of the muscle fibers when the pH-value is reduced below the isoeletric point. The addition of acid below the isoelectric point causes the increasing of positive charges and then leads to an electrostatic repulsion of muscle fiber. This reaction works by an acid solution which protonates the negative charge of carboxyl groups in the protein to break the hydrogen bonding to amino acid groups. As a result, the protein structure is opened up and there is more space for more binding of water. However, strong acids protonate more rapidly than weak acids. Limitation is caused by carboxyl groups which are not available at a pH below 3.5 (Woods and Church, 1999). Using a very low pH value with meat, it should be considered that low pH can cause a white appearance on the surface or precipitation of proteins. The reason for this is that the myo-globin in fresh meat can be oxidized at a lower pH and changes to metmyoglobin which is

brown and has lower color intensity, resulting in a pale appearance. The surface of the meat is then similar to that of cooked meat. Not only the changed surface but also the acidic taste of the meat is recognized by consumers (Woods and Church, 1999). The marination technique influences the microstructure of the muscle, e.g. vacuum tumbling because of pressure.

2.14.5 Meat denaturalization

In order to protect consumers from pathogenic microorganisms and to extend shelf life of foods, high temperature treatment with an optimal duration is a good method for these purposes. Pasteurization, sterilization, boiling or frying can be used to destroy unfavorable microorganisms. The same treatment though cannot be used for all kinds of food because of differences in the composition, structure, color, etc. which all relate to different temperatures. A temperature method consequently must be suitably adjusted for a particular kind of food. Regardless, every application has to ensure that end-products are free from pathogens. Consumer acceptance regarding the sensory quality of the product also must be taken into account.

People eat meat for reasons that not only include saturation and aspects of protein consumption, but also tradition, availability, wholesomeness, variety, and society values as well. The satisfaction derived from meat consumption depends on psychological and sensory responses. Factors such as appearance, aroma during cooking, tenderness, juiciness, flavour and natural taste, etc. all must be considered. Most consumers expect raw meat to have an attractive color. Raw chicken meat is normally grey-white to dull red but cooking affects the meat color to change to a brownish grey caused by a complete conversion of the pigment of the muscle (Forrest et al, 1975c). Using heat effects the appearance of the surface, one has at least to take consumer acceptance into account. Therefore, a suitable temperature of water or a solution should be high enough to eradicate bacteria but low enough to keep the surface in a state so that it still can be compared to the surface of a raw chicken breast.

When muscle proteins are exposed to heat, most meat proteins denature at temperatures below 150°F or 66°C (LaBudde et al., 1996). They lose their native structure and undergo several changes in configuration. Beef samples treated by heat at 75°C for 30 minutes cause the denaturalization of the connective tissue and muscle. From a macroscopic view with the eye, the exterior of the beef surface has a brown, cooked appearance, and microscopically, the connective tissue nuclei are enlarged and distorted (Novak and Yuan, 2004). The denaturation of the protein may be accompanied by an aggregation or clumping of the protein molecules known as coagulation. The coagulated effect is sometimes referred to as protein hardening. Heat can cause both the tenderization and the toughening of meat.

Specifically, myofibrilar protein breaks down upon being heated. At a higher temperature at 72-74°C rapid shrinkage of collagen is followed by protein hardening and toughening. In contrast, continuous heating at this temperature results in a substantial conversion of collagen to gelatin and this change causes meat tenderization. The unnecessary toughening of meat can be avoided by preventing the internal temperature from rising to a level that causes protein hardness. Consequently, temperature level and exposure time of the decontamination application on meat should be considered and controlled.

Since the satisfaction of meat consumers depends on their psychological and sensory responses, the temperature during processing plays an important role for the meat quality. When myofibrilar protein is heated, its aggregation or coagulation can occur. These effects lead to changing of colour, tenderization and toughening of meat. Heat-processed poultry meat and other meats can be scrutinized by careful monitoring of the heat treatment methods and their outcomes. Direct and indirect methods exist to evaluate the destructive effect of heat on microorganisms. The direct methods include bacterial counting that, however, takes some time. In contrast, indirect methods, such as determining the residual enzyme or protein in meat, or temperature monitoring can be easier applied at food processing lines in routine monitoring. Operational monitoring methods are based on the heat-denaturation of protein in meat tissue and such aim at determination of residual enzymes like catalase, phosphatase or leucine aminopeptidase, engage the coagulation test and ELISA (Hsieh et al., 2002) or near infrared spectroscopy (Ellekjaer and Isaksson, 1992). The APIZYM system is based on the presence and level of enzyme activity in the meat tissue which decreases or becomes inactive with an increasing cooking temperature of meat. There are 19 kinds of enzymes in the APIZYM system, including the enzyme leucine aminopeptidase that is a more sensitive indicator enzyme than acid phosphatase (Townsend and Blankenship, 1987; 1988). The catalase test has also been used to estimate the cooking end-point temperature in cooked chicken meat, rare roast and cooked beef (Ang et al., 1994; Liu et al., 1996; Liu et al., 1997). This enzyme is present in all raw meat tissue. It is inactivated at low doses of low-temperature at 62.8°C (Ang et al., 1994). Phosphatase is more tolerant to high-temperature treatment than catalase, and therefore the phosphatase test measuring residual acid phosphatase has been used to estimate maximum cooking temperatures up to 77°C. In terms of food processing, according to USDA-FSIS (1986) the residual acid activity method was used for determining the maximum internal temperature (68.8°C) during the processing of canned hams, canned picnic hams and canned luncheon meat (Liu et al., 1996).

Most of the temperature monitoring methods described in the previous paragraph are based on residual enzymes that remain active in non-completely cooked meat. If high temperatures only have been applied to decontaminate the surface of chicken breasts, the inside of meat still would be raw.

To summarize the present knowledge of decontamination procedures of meat, Table 19 and 20 give a synopsis of the relevant literature.

Table 19 Overview of chemical and physical decontamination techniques

Treatment	Types of sample	Initial population	Reduction effect	Unsatisfactory side effects	Reference
Chemical treatment					
Condensing vapors from 1. 25% acetone (at 70°C), 10 min 2. Isopropyl alcohol (at 82°C), 4 min 3. 93% trichloroethylene (at 73°C), 4 min 4. Water (at 76°C), 8 min	Chicken drumsticks	*Salmonella* Typhimurium of untreated drumstick rinsing solution ($\log_{10}$ CFU /ml) of method no. 1. 7.0-8.0 2. 4.6 3. 5.5 4. 5.3	Reductions* ($\log_{10}$ CFU /ml) method no. 1. 8.0 2. > 4.0 3. 2.7 4. > 5.3	Odor-off by isopropyl alcohol was detected by triangle panels.	(Klose and Bayne, 1970)
Immersion for 10 min in 0.5% and 1.0% lactic acid sol[n] Immersion for 10 min in 0.17%, 0.5% and 1% hydrogen peroxide sol[n]	Broiler carcasses	*S.* Typhimurium 7 $\log_{10}$ CFU /g carcass	1 to 2 $\log_{10}$ CFU /g	Slightly changed colour of the carcasses Bleached and bloated carcasses; these undesirable side effects disappeared after one day storage at 1°C	(Mulder et al., 1987)

Treatment	Types of sample	Initial population	Reduction effect	Unsatisfactory side effects	Reference
Immersion for 15 s in 1-2% lactic acid solution (Purac® 80%) at pH 2	Broiler carcasses	TBC 5.4, ENC 4.2, Psychrotrophic count 4.2 $\log_{10}$ CFU /g skin	About 2 $\log_{10}$ CFU /g	Not determined	(Van der Marel et al., 1988)
Immersion for 15 min in 10% trisodium phosphate solution, ca. 10℃	Chicken carcasses	*S.* Typhimurium 14028 8 and 2 $\log_{10}$CFU /carcass	About 2 $\log_{10}$ CFU / carcass from 8 $\log_{10}$ CFU /carcass of initial bacterial load and not detectable *Salmonella* from 2$\log_{10}$CFU /carcass	Not determined	(Lillard, 1994)
Pre-chill immersion in 0.6% acetic acid ,10 ℃, 10 min	Broiler carcasses	Compared with untreated carcasses	TBC 0.53 ENC 0.71 $\log_{10}$MPN/ml*	Skin of carcasses was dark or yellow and feather follicles appeared to have pucker or protrusion No adverse effects on texture and sensory quality of cooked meat	(Dickens et al.,1994)
Paddle chiller with 0.6% acetic acid, 1 h	Whole chicken carcasses	TBC 4.70, ENC 4.47 $\log_{10}$ MPN/ml of whole carcass rinsing water	TBC 1.16, ENC 1.40 $\log_{10}$ MPN/ml* of whole carcass rinsing water	Not determined	(Dickens and Whittemore, 1995)

Treatment	Types of sample	Initial population	Reduction effect	Unsatisfactory side effects	Reference
		(paddle chiller without acid) *Salmonella* incidence was 87% of 36 carcasses	*Salmonella* incidence was 6.7% of 36 carcasses		
Immersion for 30 min in the combination of 0.5% lactic acid and 0.05% sodium benzoate, pH 2.64	Raw chicken wings	*Salmonella spp., Campylobacter jejuni, Listeria monocytogenes, Escherichia coli* O157: H7 and *Staphylococcus aureus* 7 $\log_{10}$ CFU /ml cell suspension	1 to 2 $\log_{10}$ CFU /ml* of wash solution.	No significant effect on external colour, flavour, texture, moistness or overall acceptability of battered, fried chicken	(Hwang and Beuchat, 1995)
Dip for 15 min in 8,10,12% trisodium phosphate or TSP(w/v) at 20 ℃	Fresh and cooked chicken thighs	Not determined	Not determined	Color, smell1 and overall acceptability of 10%TSP were better than 10%TSP	(Capita et al., 2000)
Electrolyzed oxidizing water (EOW), residual chlorine 10-56 mg/L Chemical modified solutions containing o 2% acetic acid + 1% chlorine o 0.0025 N HCL + 1% chlorine o 2% acetic acid +	Bacterial culture	*E. coli* O157:H7 10 $\log_{10}$ CFU /ml bacterial culture	8.88 $\log_{10}$ CFU /ml bacterial culture for all treatment ca. 0.18 $\log_{10}$ CFU /ml for treatment by deionized water (water effect) EOW, 1% chlorine, 6% chlorine and 1%	Not determined	(Kim et al., 2000b)

Treatment	Types of sample	Initial population	Reduction effect	Unsatisfactory side effects	Reference
6% chlorine o 0.0025 N HCL + 6% chlorine o 2% acetic acid + 1% bromine at 24°C for 30 s exposure time, pH 2.5-2.7			bromine contained 10-56, 13, 60 and 0 mg/l residual chlorine, respectively		
Aerobically tumbled for 3 min (16 rpm) with 5% acetic acid sol[n]	Boneless beef trimming	*Salmonella* Typhimurium and *Escherichia coli* 7 $\log_{10}$ CFU /ml of cocktail suspension	Approximate 1 $\log_{10}$ CFU /g of ground beef	Lost of the desirable red color More off odor of acetic acid	(Stivariusa et al., 2002)
Dipping and 1-1 , 2-1 spray treatment of 1% lactic acid (Purac[®]) 1% glutamal bioactive[®](Lactic acid as activator) 10% trisodium phosphate (TSP)	Chicken carcasses	Natural contamination	Dipping was more effective than spray for *Salmonella* decontamination No significant differences between various treatments in APC, ENC and *Pseudomonas* counts Purac[®] and Glutamal bioactive[®] proved more effective than TSP in *Lactobacillus counts*	Purac[®] treatment showed a very high acceptation level in appearance, smell and taste of raw and grilled chickens Glutamal bioactive[®] and TSP treatments lead to a lower level of acceptance	(Okolocha and Ellerbroek, 2005)

Treatment	Types of sample	Initial population	Reduction effect	Unsatisfactory side effects	Reference
			APC 0.4-2.5, ENC 0.5-2.7, *Pseudomonas* counts 0.2-2.4, *Lactobacillus counts* 0.2-1.1 $\log_{10}$ CFU /ml of carcass rinse**		
Anode EOW and combined EOW (7:3 anode:cathode, vol/vol; pH 8.3±0.1; ORP, 930 – 950 mV; free chlorine, 271±0.01 mg/L Cl_2	Microbial cultures	7 to 8 $\log_{10}$ CFU /ml mixed culture of *Enterococcus faecium, Mycobacterium avium* subspecies *avium, Proteus mirabilis, Pseudomonas aeroginosa, Staphylococcus aureus*	All bacterial strains were completely inactivated by maximum 10.0% anode EOW (ca.40.0 mg/L) or 50.0% combined EOW (ca.135.5 mg/L) for 30 min	Not determined	(Fenner, 2005)
		8 to 9 $\log_{10}$ cells/ml culture of *Candida albicans*	*C. albicans* was completely inactivated by 5% anode EOW for 5 min (ca.20.0 mg/L Cl_2)		
		6 to 7 $\log_{10}$ CFU /ml culture of endospores of *Bacillus subtilis* subspecies *spizizenii*	*B. subtilis* spores were inactivated by 80% anode EOW for 60 min (ca.217.0 mg/L Cl_2)		
			No antimicrobial		

Treatment	Types of sample	Initial population	Reduction effect	Unsatisfactory side effects	Reference
			activities with anode and combined EOW addition of 20.0% bovine serum into water of standardized hardness		
Dipping for 15 min at room temperature in a. 100 ppm calcium hypochlorite solution, pH 5.0 (adjusted pH value with glacial acetic acid) b. 100 ppm trichloroisocyanuric acid solution, pH 3.5 c. 0.2% sodium acetate, pH 3.1 d. 1.8% sodium lactate, pH 2.4 e. 12% trisodium phosphate, pH 12.8 f. Sterile buffer phosphate as control	Chicken breasts	$7 \log_{10}$ CFU /ml culture of *Listeria monocytogenes*	Reduction ($\log_{10}$ MPN/g)* of method no. a. 4.41 b. 4.41 c. 2.38 d. 2.60 e. 3.88 f. 2.38	Not determined	(Gonçalves et al., 2005)
Dipping in 4.0% L-lactic acid solution, pH 2.6 at 55°C	Chicken breasts	$7 \log_{10}$ CFU /ml culture of *Listeria monocytogenes*	$2.38 \log_{10}$ MPN/g (Comparison with control as dipping in sterile buffer phosphate)	Not determined	(Gonçalves et al., 2005)

Treatment	Types of sample	Initial population	Reduction effect	Unsatisfactory side effects	Reference
Spray washing inside-outside carcass (80 psi) for 5 s by 50 ppm chlorine at 21.1, 43.3 or 54.4°C	Chicken carcasses	Inoculation of contaminated cecal contents of *Campylobacter* and nalidixic acid-resistant *Salmonella* contained 5.3 $\log_{10}$ CFU /carcass	No significant reduction	No effect on carcass skin colour	(Northcutt et al., 2005)
1. Dip for 30 min in 2% lactic acid (v/v) 2. Dip for 30 min in 2% lauricidin (w/w)	Raw chicken breasts	7 to 8 $\log_{10}$ CFU /g of *Listeria monocytogenes* (L55), *Salmonella* Enteritidis (S552), *Escherichia coli* O157:H7 (E19)	1. Lactic acid dipping 1.97, 1.71 and 2.59 $\log_{10}$ CFU /g* , respectively 2. Lauricidin dipping 2.79, 1.31 and 2.27 $\log_{10}$ CFU /g*, respectively	Not determined	(Anang et al., 2007)
Dipping for 15 s at 18±1 °C in 500 ml of - 12% trisodium phosphate or TSP sol[n] - 1200 ppm acidified sodium chlorite or ASC sol[n] - 2% citric acid or CA - 220 ppm peroxyacids or PA sol[n]	Chicken legs with skin	$\log_{10}$ CFU /g skin on day 0 of TBC 5.10 Psychotrophic 4.34 ENC 2.78 Coliforms 2.86 *Micrococcaceae* 4.50 enterococci 2.88 *Brochothrix thermosphacta* 4.06 pseudomonads 4.70	TSP, ASC and CA showed the largest reduction during storage; reduction for all microbial groups was 1 to 2 $\log_{10}$ CFU /g skin*	Sensory attribute including color, smell, general acceptability with hedonic scores were observed. The scores of untreated control samples and those treated with peroxyacids and sterile tap water	(Río et al., 2007)

Treatment	Types of sample	Initial population		Reduction effect	Unsatisfactory side effects	Reference
		lactic acid bacteria	3.50		were lower than those of chickens treated with trisodium phosphate, acidified sodium chlorite and citric acid on day 3 of storage at 3°C±1°C	
		yeasts and moulds count	3.96			
					No differences between sensorial attribute scores were observed between the treatment and sampling day	
Washing by 10, 20 or 200 ppm free chlorine of NaOCl solution for 1 – 10 min	Strawberries, cherry tomatoes, head lettuce	$6 - 7 \log_{10}$ PFU of Hepatitis A virus (HAV) and the indicator virus coliphage MS2		Reduction of ($\log_{10}$ PFU) 10 ppm for 10 min MS2 1.2 – 1.9 HAV 2.2 - ≥2.3 20 ppm for 10 min MS2 1.6 – 2.1 HAV 1.7 - ≥2.4 200 ppm for 5 min MS2 3.2 HAV 2.6	Not determined	(Casteel et al., 2008)

Treatment	Types of sample	Initial population	Reduction effect	Unsatisfactory side effects	Reference
Physical treatment					
Subatmospheric steam treatment 1. 66°C, 8 min 2. 71°C, 4 min	Chicken carcasses	S. Typhimurium 6.7 $\log_{10}$ CFU /ml rinsing solution of untreated carcass	Reduction* of ($\log_{10}$ CFU /m) 1. 4.3 2. 3.3	The cooked breast meat by steam at 71°C, 4 min was almost twice as tough by shear test	(Klose et al., 1971)
Immersion in hot water 80°C, 10 s following chilling at 0-1 °C	Beef and mutton pieces	6.5 $\log_{10}$ cells/cm^2 of $E.$ $coli$ and salmonellae	Hot water $E.$ $coli$ ($\log_{10}$ cells/cm^2) - Beef: 2.64 - Mutton:3.32 salmonellae ($\log_{10}$ cells/cm^2) - Beef: 2.21 - Mutton: 3.33	Discoloured with bleach, cooked fat and grey meat but the normal colour of meat almost completely regenerated within a few hours during refrigeration	(Smith and Graham, 1978)
Immersion in hot water 80 °C 10 s following chilling at 1-4 °C	Sheep carcasses (Artificial contamination) Sheep carcasses (Natural contamination)	$E.$ $coli$ 6.0 $\log_{10}$ cells/cm^2 Coliforms 2.06 $\log_{10}$ cells/cm^2 TBC 3.93 $\log_{10}$ cells/cm^2	Hot water following chilling $E.$ $coli$ ($\log_{10}$ cells/cm^2) - Sheep carcasses 3.61 Coliforms ($\log_{10}$cells/cm^2) - 1.93 TBC ($\log_{10}$ cells/cm^2) - 1.44	No permanent surface discoloration; After a few hours chilled at 1-4°C carcasses almost completely returned to normal appearances.	(Smith and Graham, 1978)

Treatment	Types of sample	Initial population	Reduction effect	Unsatisfactory side effects	Reference
Atmospheric pressure steam at 180-200°C (Jacket and internal chamber temperature), 20 s	Whole carcass and cut-up chicken (Natural contamination)	TBC 5.1 $\log_{10}$ CFU /ml rinsing solution Positive *Salmonella* carcasses 28%	TBC 1.9 $\log_{10}$ CFU /ml* Positive *Salmonella* carcasses 15%	Not determined	(Davidson et al., 1985)
Surface pasteurization by steam at 140°C for 50 miliseconds	Chicken breast	7 $\log_{10}$ CFU *Listeria innocua* SA3-VT per piece	3 $\log_{10}$ CFU per piece*	Not determined	(Morgan et al., 1996a)
Ultra high temperature, ultra short time surface pasteurization at 100 – 162°C of steam temperature for 26 – 1000 milliseconds	Chicken breast, lean beef chuck muscle and lean Boston butt pork	7 $\log_{10}$ CFU of *Listeria innocua* per each meat piece, about 5 g or 10x10x50 mm.	Treatment with 145°C for 25 ± 2 milliseconds killed 4 $\log_{10}$ and with 121°C for 48 ± 2 milliseconds killed 2-4 $\log_{10}$ CFU *	Chicken breasts were cooked at these applications except application at 145°C for 25 milliseconds	(Morgan et al., 1996b)
Steam pasteurization for 8 s	Beef carcasses	2.19 $\log_{10}$ CFU / cm^2 of TBC Positive *Salmonella* carcasses 0.7%	1.35 $\log_{10}$ CFU / cm^2 of TBC Not detectable *Salmonella* on carcasses	Not determined	(Nutsch et al.,1997)
Steam at atmospheric pressure (100°C for 10 s) followed by immersion in cold water at 1± 1°C	Chicken breast (artificial and natural contamination)	5-6 $\log_{10}$ CFU /cm^2 of nalidixic acid resistant strain of *E. coli* serotype 80	*E. coli* 1.90 $\log_{10}$ CFU /cm^2 for artificial contamination TVC 1.65 $\log_{10}$ CFU /cm^2 for natural contamination	Brownish (tanned) color and shrinkage of skin, partially cooked appearance of cut meat surfaces and	(James et al., 2000)

Treatment	Types of sample	Initial population	Reduction effect	Unsatisfactory side effects	Reference
				underside of surface	
Steam- and hot-water post process pasteurization at 88°C for 25 or 35 min (fully cooking) followed by submerging in ice-water bath and vacuum package in thick gas/moisture barrier bags	Chicken breast strips	9 $\log_{10}$ CFU / g of *Listeria innocua*	About 2 $\log_{10}$ CFU /g at 25 min About 7 $\log_{10}$ CFU /g at 35 min	No significant difference in water activity and shear force Significant difference between total moisture and expressible moisture by treatment for 35 min	(Murphy and Berrang, 2002)
Superchill treatment (submersion) with liquid nitrogen at -20 and -30°C for 72 hr	Chicken wings in Whirl-Pak bags	7.0 $\log_{10}$ CFU /g *C. jejuni*	At-20 and -30°C 1.3 and 1.8 $\log_{10}$ CFU /g, respectively	Not determined	(Zhao et al.,2003)
A. Hot water immersion at 1. 75°C 10 s 2. 80°C 10 s 3. 85°C 10 s 4. 75°C 20 s 5. 80°C 20 s 5. 85°C 20 s B. Steam pasteurization at	A: Broiler thighs (Artificial and natural contamination) B: Broiler carcasses	4.48 $\log_{10}$ CFU /ml *C. jejuni* culture (Chickens were immerged in culture.)	A: 80°C and 85°C for 20 s significantly reduced TVC, ENC and *Campylobacter jejuni* ca. 1 $\log_{10}$ CFU /g skin. B: At 90°C, 12 s insignificantly reduced.	A: Hot water immersion at ≥75°C for either 10 or 20 s caused moderate to severe deterioration of skin with cooked coloration. B: Steam pasteurization damaged outer	(Whyte et al., 2003)

Treatment	Types of sample	Initial population	Reduction effect	Unsatisfactory side effects	Reference
1. 90°C 12 s 2. 90°C 24 s	(Artificial and natural contamination)		At 90°C, 24 s significantly reduced all bacteria ca. 1 $\log_{10}$ CFU /g skin	epidermal layers of skin including more yellowed pigmentation, numerous cracks	
Hot water immersion at 1. 80°C, 20 s 2. 75°C, 30 s 3. 70°C, 40 s 4. 65°C, 30 s 5. 65°C, 70 s followed by cold water spray at 12-15°C	Chicken carcasses	APC 5.14, ENC 4.58, *Campylobacter* 1.97 $\log_{10}$ CFU /ml carcass rinse	Mean of APC, ENC, *Campylobacter* $\log_{10}$ CFU /ml carcass rinse* 1. 0.88, 0.69, 0.90 2. 1.01, 0.91, 1.10 3. 1.10, 1.00, 0.00 4. 0.63, 0.50, 0.00 5. 0.92, 0.77, 0.00	The chicken skin tended to break during subsequent trussing at hot-wash treatment	(Purnell et al.,2004)

Steam pasteurisation for 60 s	Beef, chicken meat, chicken skin and pork	*Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT104 7-8 $\log_{10}$ CFU /cm^2	*Escherichia coli* O157:H7 ($\log_{10}$ CFU /cm^2)
			  beef 2.53
			  chicken meat 3.13
			  chicken skin 3.53
			  pork 3.27
			Salmonella Typhimurium DT104 ($\log_{10}$ CFU /cm^2)
			  beef 3.65
			  chicken meat 5.23
			  chicken skin 6.15
			  pork 2.64
				Not determined	(McCann et al.,2006)

Treatment	Types of sample	Initial population	Reduction effect	Unsatisfactory side effects	Reference
1. Steam at atmospheric pressure ca. 100°C for 5, 10, 12, 20 s 2. Hot water immersion at 80°C for 10, 20 s Following crust freezing	Chicken carcasses	*E. coli* K12 ca. 5 $\log_{10}$ CFU / cm^2 *C. jejuni* AR6 ca 6 $\log_{10}$ CFU / cm^2	E. *coli* 3.2 $\log_{10}$ CFU / cm^2 *C. jejuni* ca. 2.9 $\log_{10}$ CFU / cm^2 by hot water immersion at 80°C for 20 s followed by crust freezing without extensive degradation	Steam treatment for over 12 s caused skin shrinkage and change of color. Hot water treatment at 80°C for 20 s caused only minimal carcass changes	(James et al., 2007)
Immersion in hot water at 70°C for 40 s at 75°C for 30 s at 80°C for 20 s	Chicken carcasses	ca. 6 $\log_{10}$ CFU / cm^2 *C. jejuni* AR6 and nalidixic acid resistant strain of *E. coli* serotype K12	*C. jejuni* 1.66 $\log_{10}$ CFU /cm^2 by immersion at 75°C for 30 s *E. coli* 1.31 $\log_{10}$ CFU /cm^2 by immersion at 80°C for 20 s	Not determined	(Corry et al., 2007)

Table 20 Overview of decontamination by combination of chemical and physical methods

Chemical treatment	Physical treatment	Types of sample	Initial population	Reduction effect	Unsatisfactory side effects	Reference
First step: spraying 5 ml. with 1.0% lactic acid, 1.0% acetic acid and a mixture containing 1.0% lactic acid, 2.0% acetic acid, 0.25% citric acid and 0.1% ascorbic acid	Second step: vacuum packaging and storing at 4±1 °C	Beef strip loin	Aerobic plate count of control loin showed 0.67 $\log_{10}$ CFU /cm^2	Bacterial counts did not differ between control and treated loin	Minor differences of appearance scores	(Acuff et al,, 1987)
Second step: spraying with 2% lactic acid solution at 54°C, allowed to drip for 30 s	First step: water washing at 35°C Third step: short-time steam pasteurization for 10 s and cooling down with cold water spray for 20 s	Beef (Cutaneus trunci muscles)	ca. 5 $\log_{10}$ CFU /cm^2 (Inoculation by feces contained *Listeria monocytogenes* Scott A, *E.coli* O157:H7 and *Salmonella* Typhimurium)	3.4 – 4.5 $\log_{10}$ CFU /cm^2 *E. coli* O157:H7 was more resistant than *Listeria monocytogenes*, *Salmonella* Typhimurium	Not determined	(Phebus et al., 1997)
First step: immersion for 5 min and drip-dried 15 min in solution of - 2% low-molecular-weight polylactic acid (LMW-PLA)	Second step: vacuum-packaged and store at 4°C	Lean beef	5.33 $\log_{10}$ CFU /cm^2 of *Listeria monocytogenes* Scott A	Immediate bactericidal effects; remaining viable counts at the end of 42 days were 1.22 and 1.21 $\log_{10}$ CFU /cm^2	Not determined	(Ariyapitipun et al., 2000)

Chemical treatment	Physical treatment	Types of sample	Initial population	Reduction effect	Unsatisfactory side effects	Reference
- 2% lactic acid - 400 IU/ml of nisin and 2% LMW-PLA - 400 IU/ml of nisin and 2% lactic acid - 400 IU/ml of nisin				1.56 and 0.36 $\log_{10}$ CFU /cm^2 1.57 and 2.21 $\log_{10}$ CFU /cm^2 1.94 and 0.84 $\log_{10}$ CFU /cm^2 1.64 and 0.89 $\log_{10}$ CFU /cm^2		
200 ppm of nisin and 1% of lucono-delta-lactone	High-pressure treatment at 450 MPa at 20°C for 15 min	Mechanically recovered poultry meat	APC and ASC ca. 8 $\log_{10}$ CFU /g	APC 5.3 $\log_{10}$ CFU /* ASC above 7.5 $\log_{10}$ CFU /g *	Not determined	(Yuste et al., 2002)
Second step: spraying with 2% lactic acid sol^n (Purac® FCC 80) 1 L per beef carcass and 0.5 L per beef carcass	First step: hot steam with pressure 6 bar 0.013 s of steam velocity	Beef carcasses without skin and pork carcasses with skin	Not determined	Not determined	Pale beef carcasses and minor effects on the color of pork skin	(Pipek et al., 2005)
Second step: immersion in 2% lactic acid at 24-25°C for 15s	First step: immersion in hot water 82°C 15 s Third step: storage at 4°C for 5 days	Beef	7 $\log_{10}$ CFU /ml immersion suspension of *Salmonella* Typhimurium and *Listeria monocytogenes*	*Salmonella* Typhimurium 1.19 to 1.78 $\log_{10}$ CFU /g *Listeria monocytogenes* 1.14 – 3.84 $\log_{10}$ CFU /g	pH of meat was lower than water and only hot water treatment. (no significant difference)	(Özdemir et al., 2006)

Chemical treatment	Physical treatment	Types of sample	Initial population	Reduction effect	Unsatisfactory side effects	Reference
Second step: 2% lactic acid spraying at 44 °C	First step: hot water washing at 74°C for 5.5 s	Beef carcasses (at pre-evisceration wash)	Not determined	Aerobic plate counts were reduced by 2.2 $\log_{10}$ CFU /100 cm^2 and *Enterobacteriaceae* counts were reduced by 2.5 $\log_{10}$ CFU /100 cm^2 (not better than using hot water alone)	Not determined	(Bosilevac, 2006).

*Comparison with untreated sample; **Comparison with control as water treatment; APC: Aerobic Plate Counts or aerobic mesophilic counts (incubated at 30°C 48-72 h); ASC: Aerobic Psychrotroph Counts; TVC: Total Viable Counts; TBC: Total Aerobic Bacterial Counts; ENC: *Enterobacteriaceae* Counts; EOW: Electrolyzed Oxidizing Water; PFU: Plaque Forming Unit

3.   Materials and methods

3.1   Preparation of chicken breasts

In this study, two forms of skinless chicken breasts were investigated, frozen and raw chicken breasts. Frozen chicken breasts in original packages were supplied from the company. Chicken breasts were kept at ≤-18°C prior to the test. Before the decontamination test, frozen chicken breasts were placed in the refrigerator (Snaige®) at 4±2°C overnight until complete thawing. Thawed chicken breasts were only used for experiments at the laboratory at the Institute for Food Hygiene, Freie Universität Berlin. Raw chicken breasts were only used in experiments at the processing line. They were always kept at temperatures below 8°C. The average weight of chicken breasts was 138 gram. There were 6-8 pieces per kilogram in one original package.

3.2   Bacterial preparation

3.2.1   Bacterial strains

Salmonella Typhimurium DSM 5569 was obtained from the Institute for Food Hygiene, Faculty of Veterinary Medicine, Freie Universität Berlin. The strain was stored at -45°C in Cryobank™ (Mast Diagnostics). *Salmonella* culture was grown in Brain Heart Infusion (BHI) broth at 37°C for 18-20 hours and streaked on Columbia blood agar. Then, it was maintained at 4°C for one week and used as a working culture in the study. The purity of the bacterial culture was checked by microscopic examination (Gram-stained smear) and streaked also on Columbia blood agar for isolation. Moreover, biochemical reactions and serological tests were conducted at each sample of working culture.

3.2.2   Bacterial inoculation

Two or three isolated *Salmonella* colonies on Columbia blood agar were picked up and transferred into 10.0 ml Brain Heart Infusion (BHI) broth and incubated at 37°C for 18-20 hours. Bacteria in the stationary phase of growth were used as inocula. *Salmonella* culture in BHI broth contained approximately 10^8 CFU/ml or 0.5 Mcfarland (OD 640 nm, absorbance 0.15). A final inoculum of *Salmonella* Typhimurium was prepared from an overnight incubated *Salmonella* suspension. It was diluted with sterile peptone water to yield approximately 10^4 CFU/ml. This concentration was inoculated on chicken breast for the decontamination experiments. At each final inoculation the number of *Salmonella* Typhimurium was routinely checked by the drop plating method on Plate Count (PC) agar. In this study, the side of the

chicken breast muscle which contacted the sternal bone is called sternal side, and the skin side is the opposite of the sternal side contacting the skin. One milliliter of final inoculum was dropped on both sides of a thawed chicken breast. A sterile glass rod was used to spread the inoculum thoroughly on the sample surface. Based on results of previous studies, presented in the literature review, the inoculated chicken breast allowed *Salmonella* to attach at room temperature (ca. 23-25°C) for 15 min prior to use in the decontamination experiment.

3.3 Microbial decontamination methods

Three decontamination methods were tested, being immersion in lactic acid solution at high temperature, hot water and cold water, and chlorinated water.

3.3.1 Hot lactic acid solution

3.3.1.1 Laboratory experiments

Six conditions of hot lactic acid decontamination were tested (Table 21). Two and three percent of lactic acid solution (LA) were freshly prepared at room temperature in a sterile flask. The pH-value of 2 and 3% lactic acid solution was 2.09 and 2.01, respectively. Lactic acid solution was prepared at an amount of 300 ml for one chicken breast and was heated on a hot plate stirrer until the temperature reached an appropriate level for each method. Moreover, in order to compare the decontamination effect between water and lactic acid, artificially contaminated chicken breasts were immerged in a similar volume (300 ml) of sterile distilled water at room temperature. A microbiological analysis was immediately done after treatment. The treated chicken breasts were further stored at -18°C. Chicken breasts were immediately examined in order to count the number of *Salmonella* by the MPN technique after the decontamination test. *Salmonella* reduction was calculated from the difference of MPN-values between the untreated inoculated chicken breasts (or reference) and the treated inoculated chicken breasts.

Table 21 Conditions of hot lactic acid decontamination on chicken breasts in laboratory experiment

Concentration of lactic acid solution (% w/w)	Temperature (°C)	Contact time (s)
3	75	10
3	75	20
3	70	20
3	70	30
2	75	10
2	75	20

3.3.1.2 Industrial experiments

In the first experiment, raw chicken breasts were treated with (HL1, HL2, HL3, HL4) and without (HW1, HW2, HW3, HW4) 3% lactic acid solution (w/w) at 80°C for 15, 20, 25 and 30 seconds. Chicken breasts were placed on a weave metal conveyor belt at controlled temperature of a water bath which contained 50 l of lactic acid solution.

In the second experiment, chicken breasts were tumbled for 15 minutes with 1% lactic acid solution and left at ambient temperature for 90 minutes. Then they were dipped in 3% lactic acid solution of 80°C for 20 seconds and immediately frozen (TLHL).

In the third experiment, raw chicken breasts were immerged in hot water without lactic acid at 80°C for 20 seconds and then tumbled in vacuum at 0.5 bar (HLT). After that, all treated chicken breasts were frozen to -18°C.

Chicken breasts of each intervention were collected and analyzed (n=5). Samples were kept in a cool box and sent to the laboratory. Microbiological, histological and sensory examinations were done after thawing overnight in a refrigerator. Untreated chicken breasts served as individual references for each experiment. The process parameter of the enterprise experiments are listed in Tables 22 and 23.

Table 22 Conditions of hot water and hot lactic acid decontamination on chicken breasts in enterprise experiment

Treatment	LA	Temperature (°C)	Contact time (s)	Tumbling (min)
HW1	-	80	15	-
HW2	-	80	20	-
HW3	-	80	25	-
HW4	-	80	30	-

Treatment	LA	Temperature (°C)	Contact time (s)	Tumbling (min)
HL1	3%	80	15	-
HL2	3%	80	20	-
HL3	3%	80	25	-
HL4	3%	80	30	-
TLHL	1%	-	-	15
Following step				
	-	ambient temperature	-	90
Following step				
	3%	80	20	-
HLT	-	80	20	20

Table 23 pH values of the acid solutions

Substances	pH value
3% w/w phosphoric acid	1.28
3% w/w acetic acid	2.48
1% w/w lactic acid	2.56
2% w/w lactic acid	2.09
3% w/w lactic acid	2.01

3.3.2 Combined hot and cool water treatment

3.3.2.1 Laboratory experiments: Pure culture

The culture suspension of *Salmonella* Typhimurium DSM 5569 in stationary phase was freshly diluted in a ten-fold series from 10^{-1} to 10^{-7} by sterile peptone water not longer than 20 min before the testing. Ten milliliters of diluted culture suspension were dispensed into a sterile tube with a stopper. For the test, these tubes were submerged in hot and cool water according to the four conditions of treatment in Table 24. Also, they were examined for the precise number of bacteria by the drop plating technique on PC agar. After the treatment, culture suspension in all tubes was immediately transferred into the Brain Heart Infusion (BHI) broth and incubated at 37°C for 20 to 24 hours. After the incubation, it was observed and recorded whether the BHI suspension was clear or turbid. One milliliter of each was dispended and spread on PC agar, XLD agar and BPLS agar to confirm survival of *Salmonella* (Quinn et al., 1994a). Moreover, biochemical tests were conducted by Enterotube™ II (BD BBL™, USA). The death of *Salmonella* was defined as the inability to form a visible colony (Jay, 1978c). The lowest dilution that had no bacterial growth in the

incubated BHI suspension and on all plates was the highest bacterial number that was killed by each treatment. All treatments were performed five times. Positive and negative controls were routinely done.

Table 24 Four conditions of combined hot and cold water decontamination method in pure culture

No.	Treatment	Temperature (°C)	Contact time
1	Hot water	80	20 s
2	Hot water	80	20 s
	followed by cool water	0-4	5 min
3	Cool water	0-4	5 min
	followed by hot water	80	20 s
4	Two-times treatment of hot water	70	15 s
	followed by cool water	1-2	5 min

3.3.2.2 Laboratory experiments: Chicken breasts

As a result of the previous experiment, treatment with hot water and cool water, followed by hot water, reduced the number of *Salmonella* by 1.8 $\log_{10}$ CFU per ml of pure culture. Noticeably, the treatment with or without hot-lactic acid solution at the same temperature and exposure time (at 80°C for 20 seconds) caused surface coagulation of the treated chicken breasts. As a result, a cool dipping bath was added in this experiment after dipping in hot water with the aim of preventing coagulation of the meat tissue. This cool-hot-cool decontamination method was subsequently applied to eliminate *Salmonella* on chicken breast as shown in Table 25.

Table 25 Cool-hot-cool water decontamination method

Condition	Temperature (°C)	Contact time
Cool water	0-4	5 min
Hot water	80	20 s
Cool water	0-4	5 min

3.3.2.3 Industrial experiments

The temperature of the hot water was lowered compared to the previous laboratory experiment because the coagulation effect on chicken breasts had to be avoided. A preliminary test at the processing site was done by hot water immersion at 75°C for 20 seconds, after which the treated chicken breasts showed an unacceptable surface coagulation. Therefore, in this experiment, immersion in hot water at 75 ± 2°C for 15 seconds and cool water for 5 – 10 minutes was tested. Hot water (ca. 50 l) was prepared in the same controlled temperature water bath as in the hot lactic acid experiment and followed by vacuum tumbling at 0.5 bar for 15 minutes (Table 26). All chicken breasts were frozen to -18°C. Moreover, the coagulation effect was compared between placing the sternal and skin sides of chicken breasts on the weave metal conveyor belt of the water bath.

Chicken breast samples were kept at a cool temperature below 4°C and five chicken breasts from each treatment were analysed by microbiological tests, including mesophilic aerobic count, *Enterobacteriaceae* count, coliforms count, and *Pseudomonads* count within 24 hours after treatment.

Table 26 Hot and cold water decontamination method in the industrial experiment

Condition	Temperature (°C)	Contact time	Tumbling
Hot water	75	15 s	
followed by cool water	0-4	≥ 5 min	15 min
Two-time hot and cool water			
Hot water	70	15 s	
followed by cool water	0-4	≥ 5 min	15 min

3.3.3 Chlorinated water

3.3.3.1 Prerequisite experiment: neutralization of sodium hypochlorite solution

Sodium thiosulphate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$) was used to stop the decontamination reaction of sodium hypochlorite solution which was responsible for the effect of decontamination under study. The purpose of this experiment was to confirm the appropriate volume and concentration of sodium thiosulphate pentahydrate solution for complete neutralization of various concentrations of sodium hypochlorite solution and to assure that sodium thiosulphate pentahydrate solution did not affect the growth of *Salmonella* Typhimurium DSM 5569 in the subsequent decontamination test.

Method

Hundred microliters of 10% (w/w) sodium thiosulphate pentahydrate solution were added into 10 ml of various concentrations of sodium hypochlorite study solutions. Neutralization was allowed to take place 5 min at ambient temperature. Then, two milliliters of the mixture were dispensed into 2 ml of double-strength Brain Heart Infusion (BHI) broth. In order to prove complete neutralization, fifty microliters of *Salmonella* Typhimurium DSM 5569 pure culture suspension (approximately 4 $\log_{10}$ CFU per ml) were immediately inoculated into the BHI broth tube and incubated at 37°C for 20-24 hours. Theoretically, if the sodium thiosulphate solution completely neutralizes active chlorine in the sodium hypochlorite solution, growth of bacteria should be observed by the turbidity of the BHI broth after the incubation period. The turbid BHI broth was transferred by sterile wire loop and streaked on XLD and BPLS agars to confirm *Salmonella* characteristics. All tests were performed in duplicates. Positive and negative controls were routinely included.

3.3.3.2 Laboratory experiments: Pure culture

Chlorinated water was freshly prepared from 12% sodium hypochlorite or NaOCl (Roth®, Germany). Several concentrations of sodium hypochlorite solution (5, 10, 20 and 60 ppm) were kept at 3.5 – 4.0°C in dark bottles that protected them from light prior to the test. Nine milliliters of a concentration each then was dispensed into sterile test tubes with a cap. One ml of *Salmonella* culture suspension in stationary phase was added to all tubes of the various sodium hypochlorite solutions. The final concentration of *Salmonella* in a tube was approximately 10^3 CFU/ml. The mixtures were gently mixed for 20 seconds to ensure homogenization and were given contact time for 5, 10, 20, and 60 minutes. After each contact time, 100 μl of 10 % w/w sodium thiosulphate pentahydrate ($Na_2S_2O_3$) solution was added to the test tubes at the end of the exposure time. This volume and concentration of $Na_2S_2O_3$ solution referred to the prerequisite experiment. Sodium thiosulphate is generally used for the microbiological analysis of chlorinated water (Gomes et al., 2001; Young and Setlow, 2003; Gonçalves et al., 2005; Casteel et al., 2008). After neutralization, 2 ml from each tube was transferred to 2 ml of double-strength Brain Heart Infusion (BHI) broth and thoroughly mixed. Then, all BHI tubes were incubated at 37°C for 20-24 hours (Gomes et al., 2001). After the incubation period, the turbidity of the culture broth was indicative for growth of surviving bacteria. For confirmation of the bactericidal effect, the visibly clear tubes of BHI after the incubation period were further transferred in 1 ml and spread on Plate Count (PC) agar and incubated at 37°C for 20-24 hours. After the incubation period, the PC plates were

examined for the growth of bacterial colonies. If growth of colonies was found, they were looped up by a sterile wire loop and streaked on XLD and BPLS agars for confirmation as typical *Salmonella* colonies. All treatments were performed in five replications (n=5). Positive and negative controls were routinely included. The temperature and pH-value of the treatment solution also were determined.

3.3.3.3 Laboratory experiments: Chicken breasts

Frozen chicken breasts were thawed and inoculated with *Salmonella* Typhimurium DSM 5569. Fresh chlorinated water was prepared at 20 and 200 ppm. Then, chicken breasts were immersed in sodium hypochlorite solution under the various conditions shown in Table 27. After the contact period, the chicken breasts were rinsed off by sterile and cool distilled water at $6.0 \pm 0.8°C$ for 10 minutes. Microbiological status, sensory evaluation of flavor, pH-value of the meat and the bleach effect on chicken breast surface were immediately determined. The chlorine concentration of chlorinated water was determined after the immersion on 2 chicken breasts, which were kept for two hours after the preparation at room temperature. The water samples were kept in dark capped bottles in a refrigerator and they were measured within 2 hours for their chlorine concentrations. Moreover, the meat juice was removed after thawing because of the effect of organic matter on the antimicrobial activity of chemical treatments, especially chlorine.

Table 27 Decontamination with chlorinated water

Concentration of NaOCl sol^n (ppm)	pH value	Temperature (°C)	Contact time (min)
20	9.1	5.0 -6.5	40
20	4.0*	4.1-4.4	30
200	6.3*	7.9-9.8	20

* Solutions were adjusted, pH value by lactic acid ca. 90% Ph.Eur (Merck[®], Germany)

3.4 Microbiological analysis*

3.4.1 Most Probable Number (MPN) technique

The number of *Salmonella* was determined by the three tubes MPN technique. 25 g of chicken breast were weighted and cut in a sterile stomacher bag by sterile scissors and forceps. 225 g sterile buffered peptone water or BPW (Merck®, Germany) were poured into the stomacher bag and the material was homogenized by a stomacher for 60 seconds. This homogenized material was the first dilution and used for the further steps of the MPN technique as shown in the annex. After incubation at 37°C for 18-20 hours, 0.1 ml of incubated suspension in BPW from each tube was dispensed to Rappaport Vassiliadis broth (RV) (Oxoid®, UK) which served as selective enrichment medium. Tubes of inoculated RV broth were incubated at 42°C for 22±2 hours. One loop of the incubated RV broth suspension was looped up and streaked on selective media of solid form, including Brilliant-green Phenol-red Lactose Sucrose (BPLS) agar (Merck®, Germany), Xylose Lysine Deoxycholate (XLD) agar (Merck®, Germany), and RAMBACH agar (Merck®, Germany). The media were incubated at 37°C for 22±2 h. The appearance of a typical *Salmonella* colony on XLD agar is a translucent substance with a black center, the colony, and a red-pink color on the area around the colony. On BPLS agar, the pink *Salmonella* colony is surrounded by a red zone. The diameter of the colony approximates 2-3 mm. *Salmonella* colonies on RAMBACH agar are characterized by a red color. The typical colonies were picked up in order to be confirmed by biochemical reactions using Enterotube™ II (BD BBL™, USA).

For MPN determination, the MPN table according to ISO 7402:1993(E) was used for the calculation of the bacterial number.

* List of equipments and list of chemical reagents and culture media are given in Appendix D.

3.4.2 Direct plating count method

Microbiological analysis included Mesophilic aerobic count, *Enterobacteriaceae* count, Coliforms count and *Pseudomonads* count, done by the drop plating technique according to the standard operating procedure of the institute (Table 28). A chicken breast was cut to 25 g and 225 ml of sterile (buffered) peptone water was added in a sterile stomacher bag. The mixture was homogenized by a stomacher for 60 s. The homogenate was diluted in a ten-fold dilution series by peptone water. The drop plating technique was employed in this study.

Table 28 Summary of microbiological analyses

Method	Medium	Atmospheric condition	Incubation
Mesophilic aerobic count	Plate Count (PC) agar	Aerobic	30°C, 72±2 h
Enterobacteriaceae count	Violet Red Bile Glucose (VRBG) agar	Anaerobic*	30°C, 48±2 h
Coliforms count	Violet Red Bile (VRB) agar	Anaerobic*	30°C, 48±2 h
Pseudomonads count	Glutamate Starch Phenol Red (GSP) agar	Aerobic	30°C, 48±2 h
Pre-enrichment for *Salmonella* detection	Buffered peptone water (BPW)	Aerobic	37°C, 18-20 h
Selective enrichment for *Salmonella* detection	Rappaport Vassiliadis (RV) broth	Aerobic	42°C, 22±2 h
Selective plating for *Salmonella* detection	Xylose Lysine Deoxycholate (XLD) agar	Aerobic	37°C, 22±2 h
	RAMBACH agar	Aerobic	37°C, 22±2 h
	Brilliant-green Phenol-red Lactose Sucrose (BPLS) agar	Aerobic	37°C, 22±2 h
Biochemical identification	Enterotube™ II	Aerobic	37°C, 22±2 h

*in anaerobic jar

3.5   pH measurement of meat

10 g of chicken meat was weighted in a sterile stomacher bag containing ten ml of sterile distilled water that was filled and homogenized in the stomacher for 2 minutes. The pH value was measured using an electronic pH-meter (Schott®, type CG 841, no 314171). For calibration, the pH meter was standardized at pH 4 and 7 prior to the measurement.

3.6   Surface temperature of chicken breasts

The objective of this experiment was to observe the changed temperature after immersion in hot-lactic acid solution, and to compare the decreasing temperature rate. The surface temperature of chicken meat was measured before and after the immersion in hot lactic acid solution. There were 4 applications, including immersion in 3% lactic acid solution at 75°C for 10, 20 s and 70°C for 20, 30 seconds. After treatment, chicken breasts were immediately cooled down to ca. 3 to 4°C in the refrigerator. The thermal probe of a digital thermometer (Greisinger®, Germany) was inserted about 1 mm under the surface of the breast muscle (Ellebracht et al., 2005; Nutsch et al., 1997). Temperatures were recorded before and after hot water immersion every five minutes until the temperature reached nearly values before immersion. The average of four temperature measurement points on the surface, including the head, head-middle, middle and the apical part of a chicken breast piece, was calculated and recorded.

3.7   Chemical analysis

3.7.1   Lactic acid concentration

The concentration of lactic acid in the immersion bath was determined by a test kit for the determination of D-Lactic acid/ L-Lactic acid in feed stuffs and other materials (Roche®, Germany). Two bottles of lactic acid solution were collected after treatment of 40 chicken breasts.

3.7.2   Determination of chlorine concentration

The term of free or available and total chlorine were used according to ISO 7393/2. A major active form of chlorine used as a disinfectant is free chlorine which is composed of hypochlorous acid, hypochlorite ion and dissolved elemental chlorine (ISO7393/2, 1985). The international standard procedure consists of three determination methods, being the titrimetric, the colorimetric and the iodometric titration method.

For this study, free and total chlorine of the treated solution was measured and calculated by the method of ISO 7393 part 2 'water quality-determination of free chlorine and total chlorine by colorimetric method using *N,N*-diethyl-1,4-phenylenediamine'. The method can readily be applied to a field test. The principle of the method is as follows: free chlorine is determined through direct reaction with the *N,N*-diethyl-1,4-phenylenediamine (DPD) reagent; a red compound forms at pH 6.2 to 6.5 and is followed by the measurement of color intensity by a visual comparator or spectrometer. For total chlorine, the same method applies,

plus adding an excess of potassium iodide into the test sample solution. The detection limit of this method is between 0.0004 and 0.07 mmol /l or 0.03 to 5 mg /l of total chlorine (Cl_2) concentration.

3.7.2.1 Preparation of water for chlorine determination

Water for this method must be free from oxidizing and reducing substances. Double-distilled water was used in this experiment. Its quality was checked according to ISO 7393/2 (1985):

a) As a first step, 100 ml of a tested water sample is placed into 250 ml chlorine-demand-free conical flasks. About 1 g of potassium iodide is added and mixed for 1 minute. After mixing, 5 ml of buffer solution and 5 ml of DPD reagent are added. The tested water should be colorless.

b) 100 ml of the tested water sample is poured into 250 ml chlorine-demand-free conical flasks and mixed with two drops of 0.01% sodium hypochlorite solution. After 2 minutes, 5 ml of buffer solution and 5 ml of DPD reagent are added and mixed. The tested water should generate a light pink color.

3.7.2.2 Preparation of *N, N*-diethyl-1,4-phenylenediamine sulfate (DPD) [NH_2-$C_6H_4N(C_2H_5)2.H_2SO_4$] solution, 1.1 g/l (Merck® cat. No. 103121)

250 ml water, 2 ml sulfuric acid ($\rho = 1.84$ g/ml) and 25 ml of 8 g/l disodium EDTA dehydrate solution (or 0.2 g of the solid form) were mixed. Dissolved in this mixture was 1.1 g of anhydrous DPD or 1.5 g of the pentahydrate form, diluted to 1000 ml and mixed. The reagent was stored in a dark bottle and protected from heat. The solution was renewed after 1 month or when it became discolored.

3.7.2.3 Preparation of buffered solution, pH 6.5

The buffered solution is composed of anhydrous disodium hydrogen phosphate (Na_2HPO_4) or the dodecahydrate form ($Na_2HPO_4.12H_2O$)**,** potassium dihydrogen phosphate (KH_2PO_4) and disodium dihydrogenethylenedinitrilotetraacetate dihydrate or disodium EDTA dehydrate ($C_{10}H_{14}N_2O_8Na_2.2H_2O$). The pH value of the solution was measured. The buffered solution was kept in a dark glass bottle at refrigerator temperature.

3.7.2.4 Preparation of chlorine-demand-free glassware

Sodium hypochlorite solution was filled into glassware and left for one hour. Then the chlorinated glassware was copiously rinsed with chlorine-free water. Glassware used in the chlorine test must be kept separately from the total chlorine test in order to avoid contamination of the free chlorine set.

This method is based on the measurement of the color intensity by visual comparison of the color with scale standards which are regularly calibrated. Three alternative colorimetric equipments are used consisting of a comparator and a spectrometer. The comparator provides a scale of permanent glass color standards especially made for the DPD technique. Also, a spectrometer with a selector for a continuous or discontinuous wavelength is suitable for use at 510 nm or at its maximum transmission which is as near as possible to 510 nm and rectangular cells with an optical path length of 10 mm or greater. Free chlorine determination was done strictly within 2 minutes, due to its less than 2 min response time (Carlsson et al., 1999).

According to ISO 7393/2-1985, potassium iodate (KIO_3) is recommended for the use as calibration graph because it is more stable than the free chlorine standard solutions and it works equivalently to chlorine standard (Carlsson et al., 1999). Calibration is fundamental for achieving a consistency of measurement. Linear regression is one of the most frequently used statistical methods in calibration. The relation between the input value and response value is established and should be illustrated by a straight line. The calibration model is used to predict a value from the chemically derived data. The Microsoft Excel® program was used to analyze the chemical data and plot the calibration graph. Eight points of standard concentration and an average of three replications were used to develop this calibration graph. This graph shows the chlorine concentration in the treatment solution by sodium hypochlorite in this study (Figure 4). The following equation was found: y = 0.1754x + 0.0486, at alpha < 0.05, whereby y is absorbance and x is the concentration of chlorine (ppm).

Calibration graph

$$y = 0.1754x + 0.0486$$
$$R^2 = 0.9962$$

Absorbance vs. Concentration of chlorine (ppm)
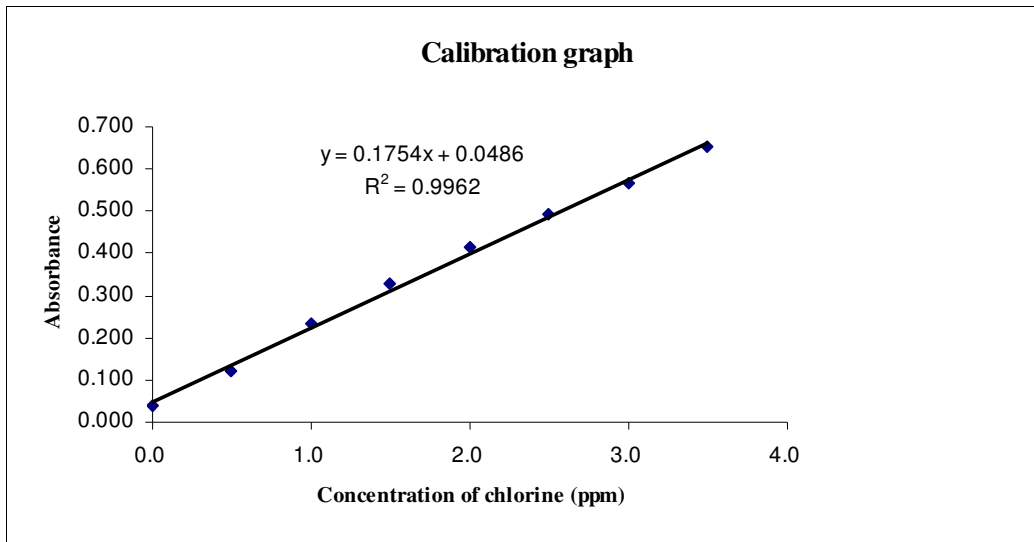
Figure 4 Calibration graph of chlorine concentration

3.8   Histological analysis: collagen swelling by hot water

The histological examination was performed by use of Calleja's staining for the representation of collagen connective tissue according to Amtliche Sammlung von Untersuchungsverfahren nach §64 LMBG, 1989. The anterior and posterior portions of chicken breasts were cut and used for the histological analysis because these portions contain high amounts of collageneous tissue. The denaturation and swelling of collageneous tissue was compared among untreated samples, samples treated with hot water and samples treated with hot-lactic acid.

3.9   Sensory evaluation

The Triangle Test was used according to ISO 4120:2004(E), also the scoring method modified by ISO 6658:2005(E) as well as numerical intensity scales (Bodyfelt et al., 1988). Chicken breasts were kept in the freezer at a temperature below -20˚C after treatment. The frozen chicken breasts were thawed at ambient temperature, whole pieces were fried without oil for ca. 15 minutes until they were completely cooked in the middle of the pieces, and then were served for the sensory test.

In the initial trials, sensory attributes were evaluated by a scoring test using 5-point scales for taste test (Table 29). The control and treated chicken breasts were cooked and served to assessors with blank sensory ballots. The appearance of the external surface of the control and treated chicken breasts were documented by photographs.

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Table 29 Meanings of five-points scale for sour in taste

Scores	Meanings
2.0	very strong
1.5	strong
1.0	moderate
0.5	slight
0.0	absent

Phosphoric, acetic and lactic acid solutions were used in this study at high and ambient temperature. Short time immersion was used with skinless chicken breasts at a high temperature and long time immersion was used at ambient temperature. The conditions of treatments are shown in Table 30. Moreover, sensory evaluation including description of visual appearances (surface coagulation), taste and flavor was identified. Breasts showing the best condition in regards to sensory attributes, those appearing to have the least undesirable effects, were chosen for a subsequent antimicrobial effect test.

Table 30 Conditions of acid treatment in preliminary test

Temperature	Contact time
70°C	10, 15, 20, 25 s
75°C	10, 15, 20, 25 s
80°C	10, 15, 20, 25 s
25°C	3, 5, 8, 10 min

In the enterprise experiment, the triangle test was performed with two lots: the untreated and treated groups with 3% lactic acid solution at 80°C for 30 seconds, being the longest application of lactic acid immersion. Code numbers of samples were randomly labeled. Tested in each case were two samples from the same and one were from a different chicken breast. The two identical chicken samples were presented with different code numbers, being A and B respectively. Each serving code or sample code was a three-digit number, shown in Table 31. The next step was to randomly assign codes and to balance the presentation of samples. Six assessors did receive blank sensory ballots, on which they were asked to write a number. They were provided with drinking water, unsalted crackers, a pen, fork, knife and the randomly coded 3 cooked chicken breast samples on ceramic plates. The ballot presented instructions and the 2 questions to be answered in the test. The test was clearly been explained to all assessors before testing. The assessors had to answer two questions: which sample is different from the others and how is it different from the others. The assessors wrote down their answers without discussion or consultation with the others.

Table 31 Serving code numbers for triangle test

Assessor no.	Samples	Serving code		
1	ABB	108	795	140
2	BAA	189	168	773
3	AAB	718	437	488
4	BBA	535	231	243
5	ABA	893	402	619
6	BAB	145	296	992

3.10   Surface appearance of chicken breasts

Generally, two methods exist for meat color evaluation, including visual color or sensory color and instrumental color. In the same manner surface appearance can be estimated through color instrumental measurement and by visual assessment. Due to the fact that food processors need to determine the efficacy of new processing techniques or products, evaluation methods which are rapid, reliable and economical are preferred for use. Goeksoy et al. (1999) suggested that hot water treatment at a temperature greater than 70°C causes a major change in the visual texture of chicken breast skin and that after treatment at a temperature equal to or over 70°C, instrumental measurements are less useful in assessing the change in appearance. As a consequence for this study slight changes in the appearance through visual assessment were considered unacceptable.   Visual assessment was done because a minor coagulation on the surface of chicken breasts was quite difficult to assess using instrumental color measurement.

3.11   Statistical analysis

Numerical data from the microbiological analyses were transferred to logarithms before statistical analysis. Bacterial counts from the drop plating technique showing no detectable colonies were entered as 100 CFU per gram sample (half of limit of detection) to permit statistical analysis (Nutsch et al., 1997). ANOVA was used to determine significant levels of association between different methods and investigation results. If ANOVA yielded a significant result, pairwise (multiple) comparison between all means was done using the Bonferroni Method. The Rank Sum Test was used for comparing the pH-value of meats with that of the reference. Statistic analysis was performed by use of STATA V 9.2 (College Station, TX).

4. Results

4.1 Microbiological analyses

4.1.1 Initial bacterial population load of chicken breasts

Since natural contamination is interpreted as a bacterial indicator, the initial bacterial population was counted. The mean±SD of mesophilic aerobic count, *Enterobacteriaceae* count, coliforms count and pseudomonads count of the initial background bacterial population of chicken breasts used in the industrial experiments resulted in 4.50 ±0.31, 2.96 ±0.53, 2.82 ±0.48 and 3.29 ±0.44 $\log_{10}$ CFU per gram chicken breast, respectively (n=15). The frozen chicken breasts (n=3) obtained from the company showed an mesophilic aerobic count of 4.98±0.02, an *Enterobacteriaceae* count of 3.29±0.03, a coliforms count of 3.05±0.13, and a pseudomonads count of 4.30±0.09 $\log_{10}$ CFU per gram chicken breast. In one case *Salmonella* was isolated in a 25 gram sample. During the artificial contamination with *Salmonella* Typhimurium DSM 5569, untreated and inoculated *Salmonella* chicken breasts were analyzed by the MPN technique and their mean ±SD was 2.88 ±0.41 $\log_{10}$ MPN *Salmonella* per gram (n=12).

4.1.2 Laboratory experiment: effect of the hot lactic acid solution

In general, the immersion of chicken breasts in hot lactic acid solution resulted in a lower bacterial load compared to the reference or to untreated chicken. *Salmonella* Typhimurium reductions for treated groups are illustrated in Table 32 and Figure 5. In this laboratory experiment, all treatments did reduce the artificial contaminations with *Salmonella* by a range of 0.51 to 2.09 $\log_{10}$ MPN *Salmonella* per gram chicken breast. But only the treatment with 3% lactic acid solution at 75°C for 20 s did reduce the contamination with *Salmonella* significantly more than the water treatment, which showed 2.099 ±1.053 $\log_{10}$ MPN *Salmonella* per gram of chicken breast. Reduction numbers by all other treatments were evident but not significantly different. The water treatment or water effect showed the smallest reduction with 0.519 ±0.665 $\log_{10}$ MPN *Salmonella* per gram of chicken breast. Salmonellae were more effectively reduced by the 3% w/w lactic acid treatment than by the 2% w/w lactic acid treatment at the same temperature and contact time.

The microbiological analysis with the MPN technique was carried out at day 0 and day 7 after storage at -18°C. Results revealed that *Salmonella* reduction between day 0 and 7 was not significantly different (p>0.05) as shown in Table 33.

Table 32 Laboratory experiment: Treatment with hot lactic acid (n=8/treatment) mean±SD of log$_{10}$ MPN *Salmonella* Typhimurium DSM 5569 per gram of chicken breast

No.	Treatment	MPN Salmonella	Reduction*
1	Reference or untreated chicken breast	2.85±0.44	-
2	Sterile distilled water at 20°C	2.33±0.52	0.52±0.67
3	3% LA at 75°C for 10s	1.16±0.72	1.69±0.88
4	3% LA at 75°C for 20s	0.75±1.02	2.10±1.05**
5	3% LA at 70°C for 20s	2.06±0.75	0.79±0.96
6	3% LA at 70°C for 30s	1.33±0.83	1.518±1.06
7	2% LA at 75°C for 10s	1.90±0.81	0.95±0.91
8	2% LA at 75°C for 20s	1.72±0.38	1.13±0.53

* Reduction is the difference of log$_{10}$MPN *Salmonella* between reference and each treatment
** Reduction is significantly different from sterile distilled water (p≤0.05)
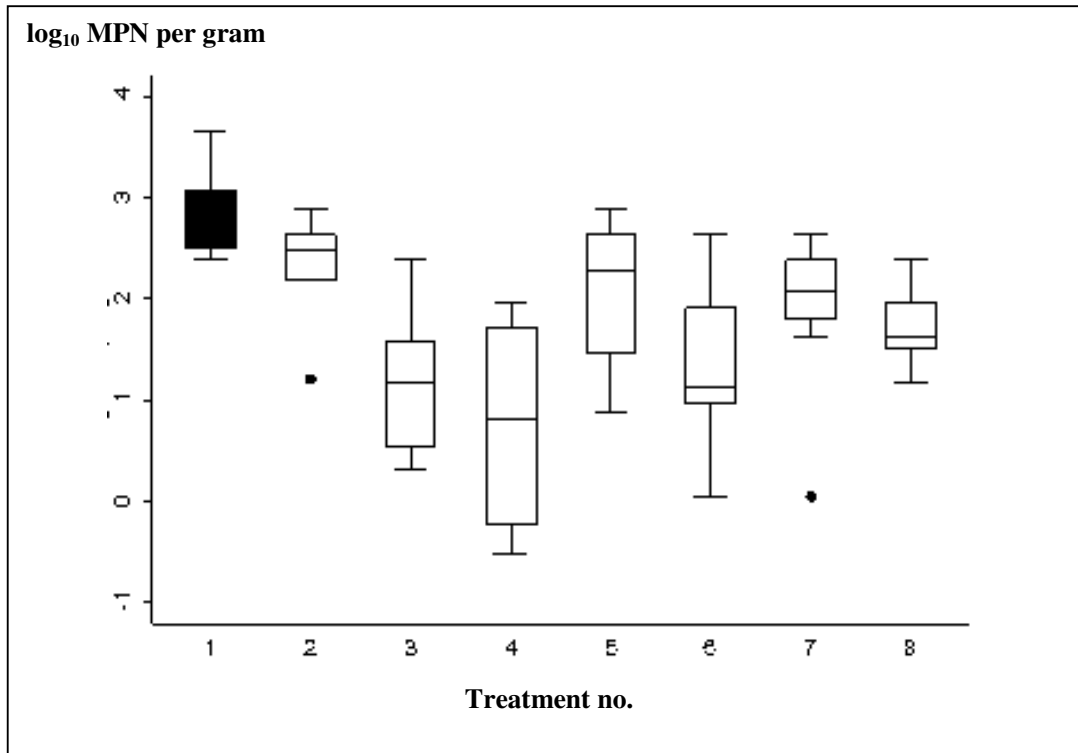
Figure 5 Box plots of $\log_{10}$ MPN per gram of *Salmonella* Typhimurium DSM 5569 in experimental treatment with hot lactic acid solution in various applications (Table 32); (Treatment no. 1 = Reference or untreated chicken breast, no. 2 = Sterile distilled water, no. 3 = 3% LA at 75°C for 10 s, no. 4 = 3% LA at 75°C for 20 s, no. 5 = 3% LA at 70°C for 20 s, no. 6 = 3% LA at 70°C for 30 s, no.7 = 2% LA at 75°C for 10 s, no. 8 = 2% LA at 75°C for 20 s)

Table 33 Laboratory experiment: Reduction (mean $\log_{10}$ reduction of MPN per gram) of

Salmonella Typhimurium DSM 5569 on chicken breasts (n=2/treatment)

Treatment	Day 0	Day 7
3% LA at 75°C for 10 s	2.60	2.66
3% LA at 75°C for 20 s	2.65	2.62
3% LA at 70°C for 20 s	2.55	2.97
3% LA at 70°C for 30 s	2.85	2.97
2% LA at 75°C for 10 s	2.10	2.57
2% LA at 75°C for 20 s	2.00	2.57

4.1.3 Industrial experiments

4.1.3.1 First experiment

The first experiment provided the comparison with regard to bacterial reduction between the hot water and the hot lactic acid solution treatments. Five chicken breasts were taken from each treatment. The microbiological results are summarized in Figure 6a-6e (compare Table 40 HW1-4 and HL1-4). The bacterial reduction caused by the treatment at $80°C$ for 15-30 s with and without lactic solution resulted in a range of mesophilic aerobic bacteria between $0.2 - 0.3$ and $0.1 - 0.3$, *Enterobacteriaceae* between $0.1 - 0.8$ and $0.1 - 0.4$, coliforms between $0.4 - 0.9$ and $0.0 - 0.4$ and, pseudomonads between $0.4 - 0.6$ and $0.0 - 0.4$ $\log_{10}$ CFU per gram, respectively. Treatment by the lactic acid solution seems to be more effective than without using lactic acid. Unfortunately, bacterial reduction between hot water and hot-lactic acid solution at $80°C$ for 15, 20, 25 and 30 s was not significantly different $(p>0.05)$. The surface coagulation of the chicken breasts treated with hot water at $80°C$ for 20 s was less distinctive compared to the treatment with hot lactic acid solution. The maximum contact time for the hot water as well as for the hot lactic acid solution treatment was not longer than 20 seconds, at which time only a minor surface coagulation had occurred.

4.1.3.2 Second experiment

The chicken breasts were tumbled in a vacuum for 15 minutes with 1% lactic acid solution and stored at ambient temperature for 90 minutes. Then they were dipped in 3% lactic acid solution of $80°C$ for 20 seconds and immediately frozen (TLHL). All bacterial counts of this treatment were below the detection limit, except for mesophilic aerobic bacteria. This treatment showed maximal reductions of mesophilic aerobic bacteria and pseudomonads. The results are shown in Figure 7 and Table 40 for TLHL treatment. In contrast, the whole pieces of treated chicken breasts appeared completely coagulated and were very sour in taste. This kind of treatment resulted in noticeable visual and sensory problems.

4.1.3.3 Third experiment

In the last industrial experiment raw chicken breasts were immerged in hot water without lactic acid at $80°C$ for 20 s and then tumbled in a vacuum for 20 min (HLT treatment). This method resulted at same temperature and contact time in a much greater surface coagulation compared to the method of tumbling after treatment with hot water and to hot-lactic acid solution treatment. On the other hand, HLT resulted in significantly reduced mesophilic aerobic counts – more than all other treatments ($p≤0.05$) in the study with the

exception of treatment with TLHL (Figure 8; Table 40 HLT treatment). Concerning *Enterobacteriaceae* counts, HLT appeared to be more effective with regard to its antimicrobial activity than treatment with hot water at 80°C for 15-25 s. Coliforms were further reduced significantly by the HLT method, it was more effective than using hot water at 80°C for 15 s without tumbling (HW1), hot water at 75°C for 15 s followed by cool water and then tumbling for 15 min (HCS2), and hot water at 75°C for 15 s followed by cool water (HCK1) ($p \leq 0.05$).

Synopsis of all treatments: TLHL and HLT in summary did significantly reduce the number of mesophilic aerobic bacteria, *Enterobactericeae*, coliforms and pseudomonads. In terms of the visual quality of the surface of chicken breasts, the best quality was achieved by hot water treatment at 80°C for 15 and 20 seconds. The next best methods were treatment with hot-lactic acid at 80°C for 15 s and by HLT. Worst visual quality resulted from treatment with TLHL. Even more, after TLHL-treatment, the outsides and insides of the chicken meats had a pale color.
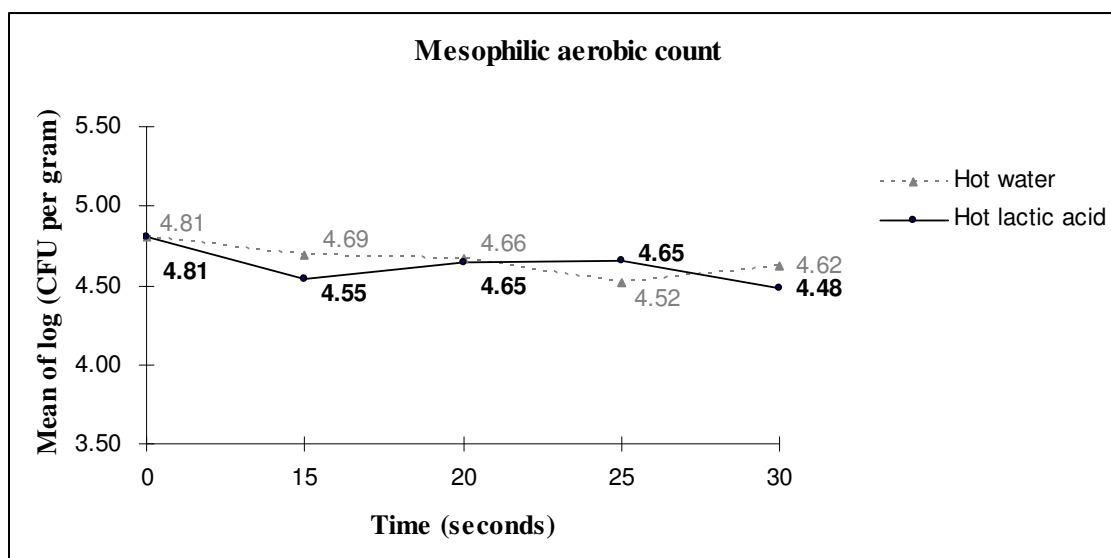
Figure 6a Mean of Mesophilic aerobic counts of treatment with hot water and hot lactic acid
solution (3%) at 80°C for 15 – 30 s (First industrial experiment)

77
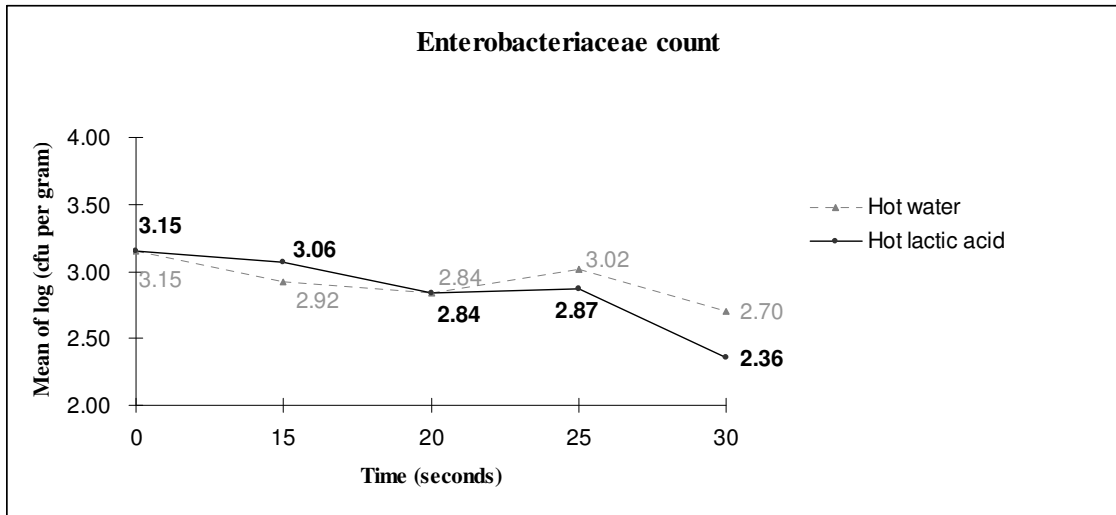
Figure 6b Mean of *Enterobacteriaceae* counts of treatment with hot water and hot lactic acid solution (3%) at 80°C for 15 – 30 s (First industrial experiment)
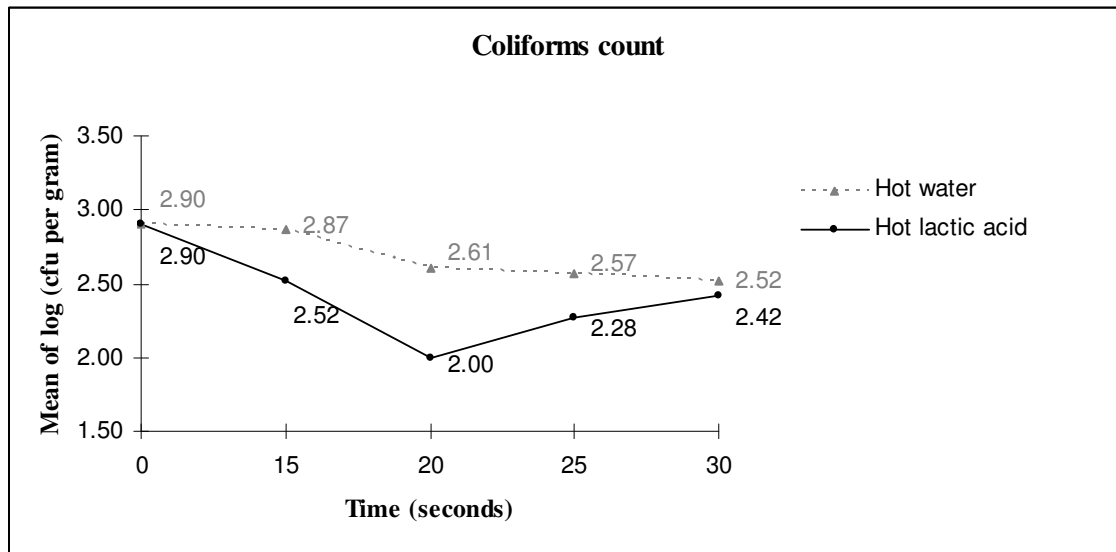
Figure 6c Mean of Coliforms counts of treatment with hot water and hot lactic acid solution (3%) at 80°C for 15 – 30 s (First industrial experiment)
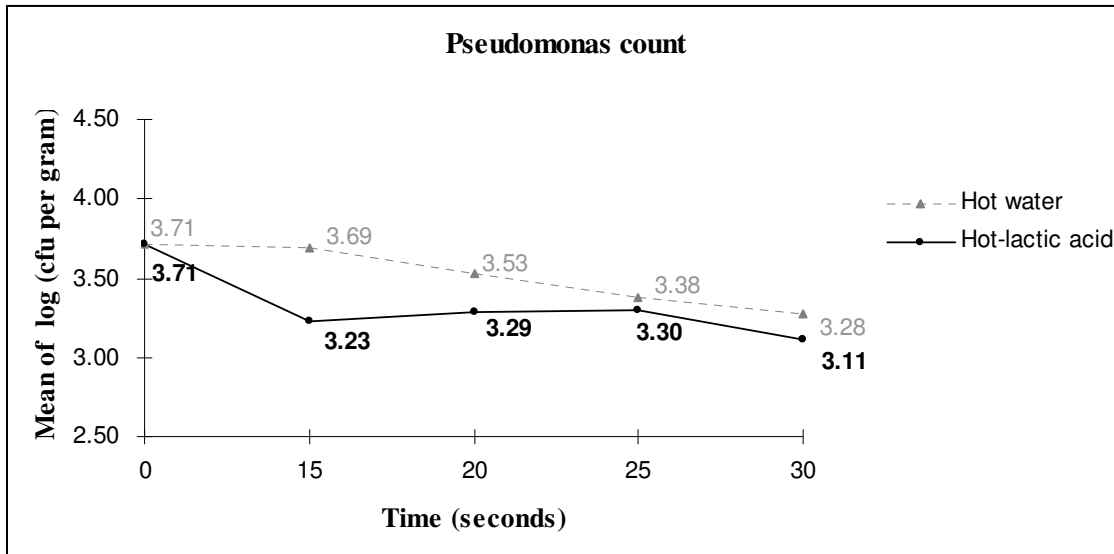
Figure 6d Mean of *Pseudomonas* counts of treatment with hot water and hot lactic acid

solution (3%) at 80°C for 15 – 30 s (First industrial experiment)
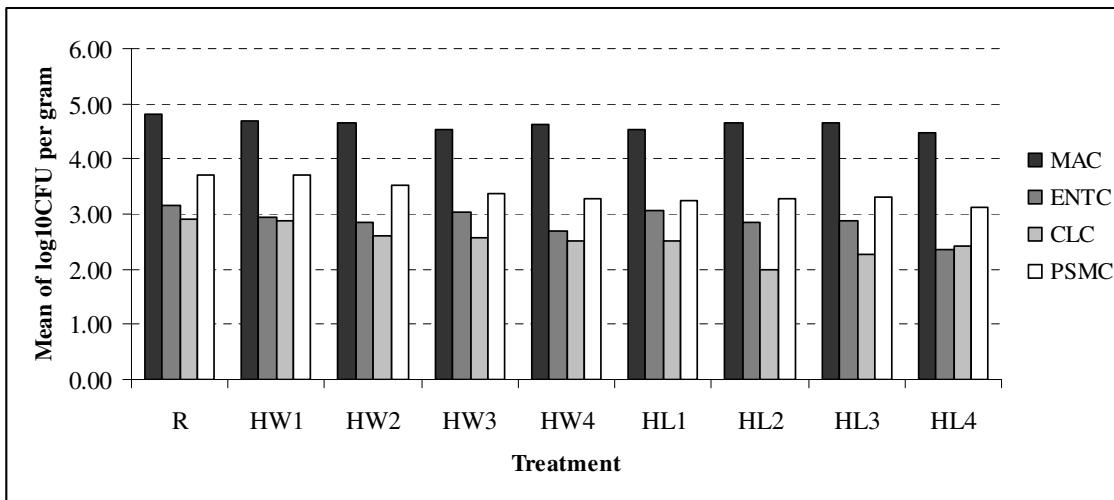
Figure 6e Summary of microbiological analyses of treatment with hot water and hot lactic
acid solution (3%) at 80°C for 15 – 30 s (First industrial experiment);
(MAC = Mesophilic aerobic count, ENTC = *Enterobacteriaceae* count, CLC =
Coliforms count, PSMC = *Pseudomonas* count); (R = Reference or untreated
chicken breast, HW1 = Hot water at 80˚C for 15 s, HW2 = Hot water at 80˚C for
15 s, HW3 = Hot water at 80˚C for 25 s, HW4 = Hot water at 80˚C for 30 s, HL1 =
3% LA at 80˚C for 15 s, HL2 = 3% LA at 80˚C for 20 s, HL3 = 3% LA at 80˚C for
25 s, HL4 = 3% LA at 80˚C for 30 s)
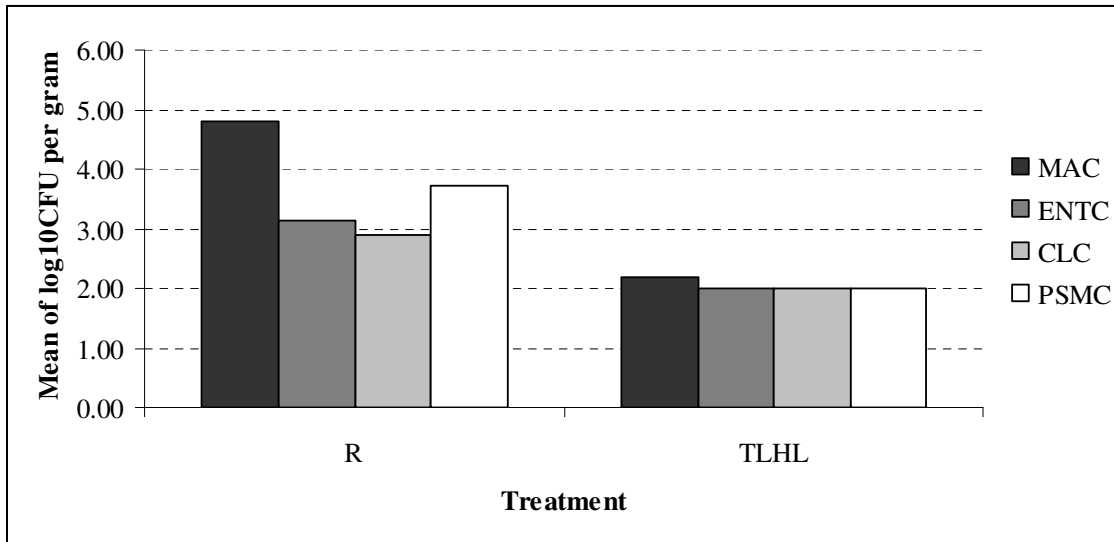
Figure 7 Microbiological analyses of the treatment of tumbling in vacuum for 15 min with 1% lactic acid solution and stored at ambient temperature for 90 min and dipped in 3% lactic acid solution of 80°C for 20 s and immediately frozen (TLHL) (Second industrial experiment);
(R = Reference or untreated chicken breast); (MAC = Mesophilic aerobic count, ENTC = *Enterobacteriaceae* count, CLC = Coliforms count, PSMC = *Pseudomonas* count)
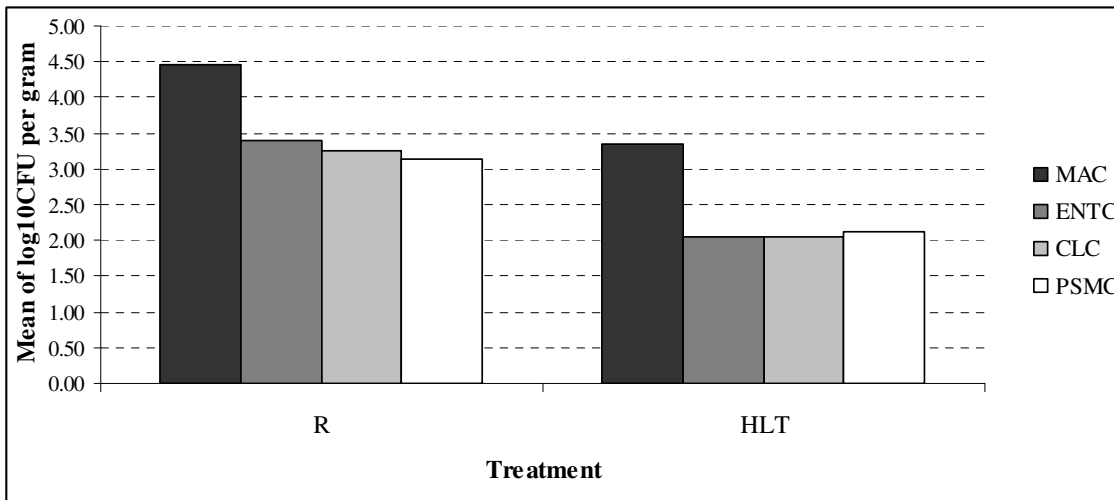
Figure 8 Microbiological analyses of hot water without lactic acid at 80°C for 20 s and tumbling in vacuum for 20 min (HLT) (Third industrial experiment);
(R = Reference or untreated chicken breast); (MAC = Mesophilic aerobic count, ENTC = *Enterobacteriaceae* count, CLC = Coliforms count, PSMC = *Pseudomonas* count)
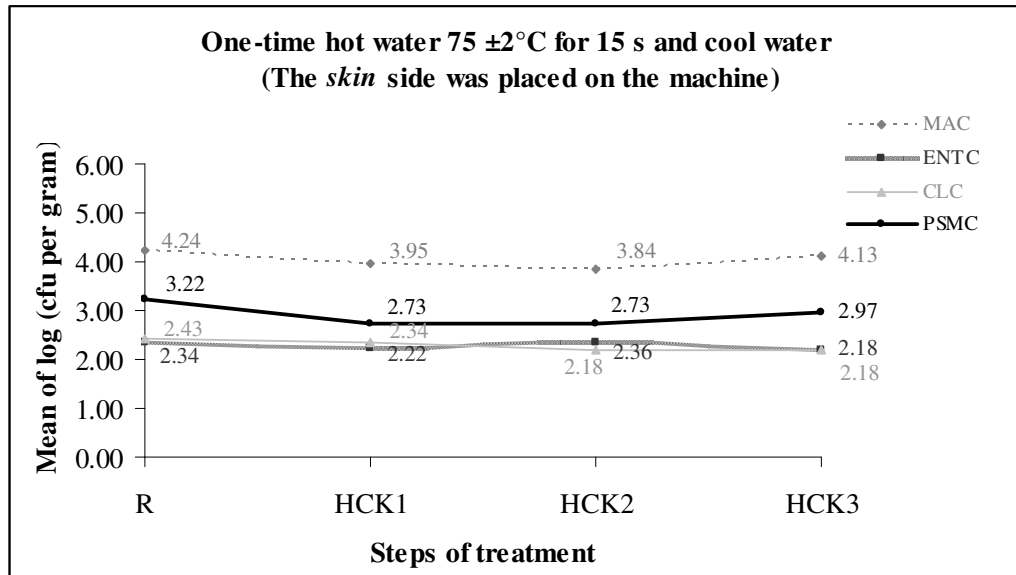
4.1.4   Combined hot and cool water treatment

4.1.4.1 Laboratory experiments: pure culture

All treatments applied in pure culture did not significantly reduce the number of *Salmonella* Typhimurium DSM 5569. However, decontamination with hot water (80±2°C) and cool water (2±2°C) followed by hot water still seemed to be more effective than treatment with hot water followed by cool water. All results of these laboratory experiments are shown in Table 34.

Table 34 *Salmonella* reduction with the hot and cold water decontamination methods in pure culture

No.	Treatment	Mean±SD of $\log_{10}$ CFU per ml
1	Hot water	1.80±0.84
2	Hot water followed by cool water	0.60±0.55
3	Cool water followed by hot water	1.80±0.84
4	Two-times treatment of hot water followed by cool water	1.40±0.89

4.1.4.2 Laboratory experiments: Chicken breast

Treatment of chicken breasts with the cool-hot-cool water immersion method (method in Table 25) resulted in the reduction of mesophilic aerobic bacteria by 1.2 $\log_{10}$ CFU per gram. Moreover, a minor surface coagulation only appeared on the edges and the sternal sides of the chicken breasts. For this reason, this treatment was applied in the further experiments in the industry.

4.1.4.3 One-times hot and cool water immersion (industrial experiment)

Hot water at 75 ±2°C for 15 seconds and cool water (2±1°C) for 5 – 10 minutes were used. After the treatment with hot and cool water the breast have been tumbled for 15 minutes and then frozen. The lot HCS was laid sternal side down during water treatment and lot HCK skin side down. The temperature of the hot water was lower than during the previous experiments in order to avoid coagulation effects. The resulting microbiological analyses are contained in Table 35, Table 40 HCS and HCK and in Figure 9a, b and d. The differences between untreated and treated chicken breasts for mesophilic aerobic counts, *Enterobacteria-*

ceae counts, Coliform counts and *Pseudomonas* counts were 0.02 - 0.53, 0.04 – 0.28, 0.10 – 0.37 and 0.08 – 0.96 $\log_{10}$ CFU per gram of chicken meat, respectively. There was no definite effect of tumbling and freezing. With regard to the visual quality of the surface of chicken breasts, only minor coagulation effects occurred, while the appearance of treated and untreated chicken breasts was not different. It is worth noting that when the skin side, compared to the sternal side, was placed on the metal-belt conveyor, the chicken breasts showed a smaller cooked effect. Concerning microbiological counts, no significant differences were noted.

4.1.4.4 Two-times hot and cool water immersion (industrial experiment)

Chicken breasts were first treated by heat, followed by cold and this procedure was done again. The treatment of hot water was at 70 ±2°C for 15 seconds and was followed by cool water for 5 – 10 minutes. The following steps were 15 minutes tumbling and freezing. The resulting reductions in the counts of mesophilic aerobic bacteria, *Enterobacteriaceae*, Coliforms and Pseudomonas were in the range of 0.33 – 0.37, 0.22 – 0.34, 0.37 – 0.43 and 0.78 - 1.036, respectively (Table 35 HCD1-4, Table 40, Figure 9c and d). The coagulation effect appeared similarly to chicken breasts treated with one-time hot and cool water immersion. The microbiological quality of cool water in this experiment was additionally assessed by the conventional plating technique (n=2). The mean $\log_{10}$ CFU ±SD per ml for mesophilic aerobic bacteria was 2.21 ±0.29; *Enterobacteriaceae*, coliforms and pseudomonads were below detectable levels and *Salmonella* was negative.

With regard to the visual quality of the surface of the chicken breasts, two-time hot and cool water immersion only led to a minimal surface coagulation. Surfaces after treatment looked the most similar to raw chicken meat when compared to all other interventions evaluated in this study. Unfortunately, the antimicrobial effect of the treatment was low.

Table 35 Microbiological analyses of chicken breasts after one-time (4.1.4.3) and two-times (4.1.4.4) treatments with hot and cool water, expressed as mean $\log_{10}$ CFU ±SD per gram (n=5/treatment)

Treatment	Internal temp. of meat (°C)	*Salmonella spp.* (25 g sample)	MAC	ENTC	CLC	PSMC
Raw chicken breast/reference	-0.5	positive	4.24± 0.19	2.34± 0.28	2.43± 0.34	3.22± 0.18
One-time hot water 75°C for15 s and cool water (the **sternal side** was down on the machine)	6.4	positive	3.71± 0.16	2.06± 0.13	2.18± 0.27	2.58± 0.27
After tumbling 15 min	8.8	positive	3.87± 0.12	2.30± 0.30	2.28± 0.32	2.25± 0.42
Frozen chicken breasts	NA	negative	4.22± 0.30	2.12± 0.16	2.06± 0.13	3.14± 0.22
One-time hot water 75°C for 15 s and cool water (the **skin side** was down on the machine)	8.4	positive	3.95± 0.32	2.22± 0.48	2.34± 0.49	2.73± 0.30
After tumbling 15 min	11.3	positive	3.84± 0.09	2.36± 0.39	2.18± 0.16	2.73± 0.29
Frozen chicken breasts	NA	positive	4.13± 0.24	2.18± 0.27	2.18± 0.27	2.97± 0.42
Two-times treatment Step 1: Hot water 70°C for 15 s and cool water	NA	positive	3.90± 0.22	2.12± 0.16	2.06± 0.13	2.18± 0.27
Step 2: Hot water 70°C for 15 s and cool water	4.4	positive	4.25± 0.22	2.06± 0.13	2.00± 0.00	3.34± 0.79
After tumbling 15 min	11.7	positive	3.85± 0.50	2.00± 0.13	2.00± 0.00	2.26± 0.58
Frozen chicken breasts	NA	positive	3.87± 0.06	2.06± 0.13	2.08± 0.13	2.43± 0.42

MAC = Mesophilic aerobic count, ENTC = *Enterobacteriaceae* count, CLC =; Coliforms count, PSMC = *Pseudomonas* count, NA = not available

Figure 9a Survival of bacteria on chicken breasts after treatment with hot and cool
water; (MAC = Mesophilic aerobic count, ENTC = *Enterobacteriaceae*
count, CLC = Coliforms count, PSMC = *Pseudomonas* count); (R =
Reference or untreated chicken breast, HCK1 = Hot water 75°C 15 s and
cool water before tumbling (The **Skin** side was laid down on the machine),
HCK2 = Tumbling 15 min, HCK3 = freezing)
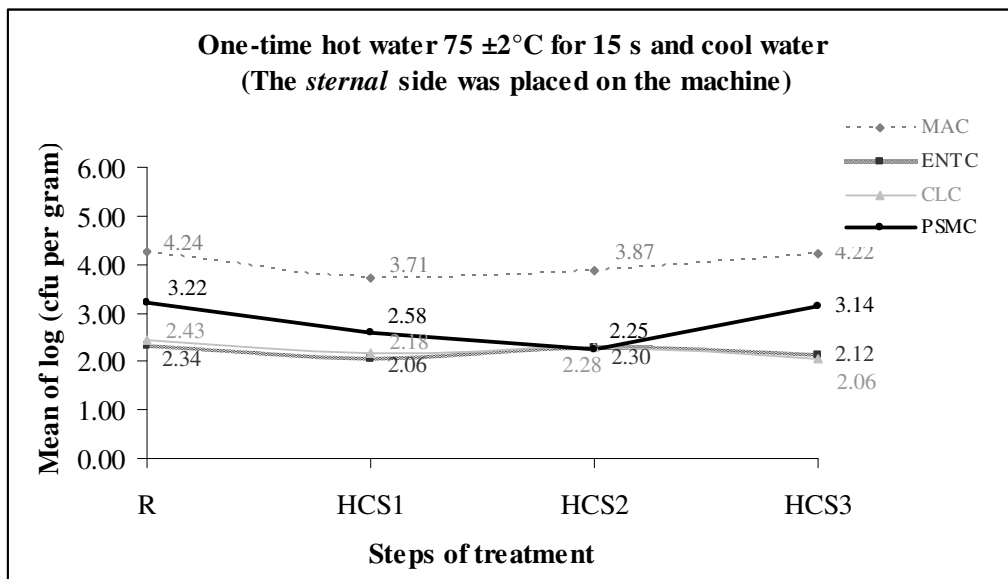
Figure 9b Survival of bacteria on chicken breasts after treatment with hot and cool
water; (MAC = Mesophilic aerobic count, ENTC = *Enterobacteriaceae*
count, CLC = Coliforms count, PSMC = *Pseudomonas* count); (R =
Reference or untreated chicken breast, HCS1 = Hot water 75°C, 15 s and
cool water (The **Sternal** side was laid down on the machine) before
tumbling, HCS2 = Tumbling 15 min, HCS3 = Freezing)
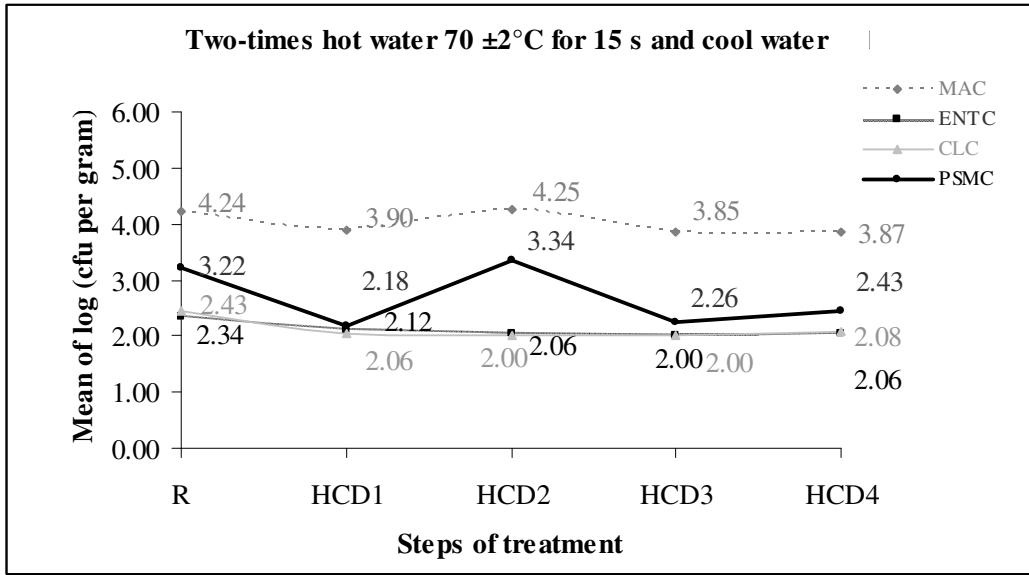
Figure 9c Survival of bacteria on chicken breasts after treatment with hot and cool
water; (MAC = Mesophilic aerobic count, ENTC = *Enterobacteriaceae*
count, CLC = Coliforms count, PSMC = *Pseudomona*s count; R =
Reference or untreated chicken breast; HCD1 = Step 1: Hot water at 70°C
for 15 s and cool water, HCD2 = Step 2: Hot water at 70°C for 15 s and cool
water, HCD3 = Tumbling 15 min, HCD4 = Freezing)
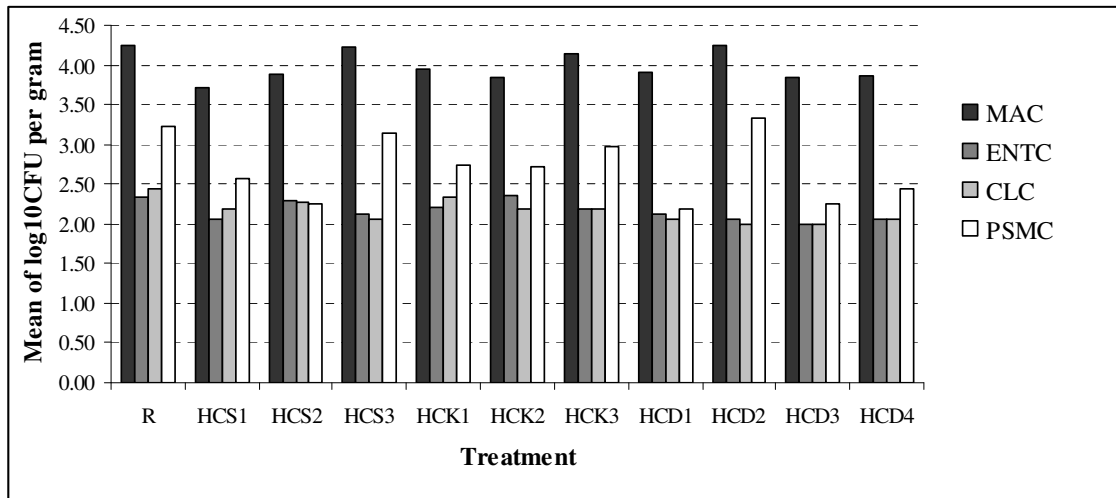
Figure 9d Synopsis of survival of bacteria on chicken breasts after treatment with hot and cool water;
(MAC = Mesophilic aerobic count, ENTC = *Enterobacteriaceae* count, CLC = Coliforms
count, PSMC = *Pseudomonas* count); (R = Reference or untreated chicken breast, HCK1 =
Hot water 75°C for 15 s and cool water before tumbling (the skin side was lying on the
machine), HCK2 = Tumbling 15 min, HCK3 = Freezing, HCS1 = Hot water 75°C for 15 s
and cool water (the sternal side was lying on the machine) before tumbling, HCS2 =
Tumbling 15 min, HCS3 = Freezing, HCD1 = Two-times treatment Step 1: Hot water at
70°C for 15 s and cool water, HCD2 = Step 2: Hot water at 70°C for 15 s and cool water,
HCD3 = Tumbling 15 min, HCD4 = Freezing)

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4.1.5 Chlorinated water

4.1.5.1 Prerequisite experiment: neutralization of sodium hypochlorite solution

During this investigation, *Salmonella* Typhimurium DSM 5569 did survive and grow in BHI broth in all the neutralization experiments (Table 36). Media from the least to the highest concentration of sodium hypochlorite solution were selected, then 100 µl of 10 % w/w sodium thiosulphate pentahydrate was added and allowed to react for 5 min. The results suggest that this exact volume and concentration of 10 % w/w sodium thiosulphate pentahydrate did completely neutralize the potential free chlorine from the sodium hypochlorite solution. Therefore, sodium thiosulphate pentahydrate was found appropriate to be used as a dechlorinating agent in this investigation.

Table 36 Neutralization by 100 µl of 10 % w/w sodium thiosulphate pentahydrate solution of various concentrations of sodium hypochlorite solution

Concentration (ppm) of sodium hypochlorite (NaOCl)	BHI	XLD	BPLS
200.00	T	G	G
60.00	T	G	G
50.00	T	G	G
25.00	T	G	G
20.00	T	G	G
12.50	T	G	G
6.25	T	G	G
3.13	T	G	G
1.56	T	G	G
0	T	G	G

BHI: Brain Heart Infusion broth; XLD: Xylose Lysine Deoxycholate agar; BPLS: Brilliant-green Phenol-red Lactose Sucrose agar; T: Turbid after incubation 24 hr at 37°C; G: Typical colonies of *Salmonella* growth after an incubation period of 20-24 hours at 37°C

4.1.5.2 Laboratory experiment: Antimicrobial effects of various concentrations of sodium hypochlorite solution on pure culture of *Salmonella* Typhimurium DSM 5569

This treatment principally killed most *Salmonella,* except for the two cases with 5 ppm for 5 min contact time, pH 7.53 at 4.5°C and with 20 ppm for 5 – 10 min contact time, pH 7.64 at 10.4°C. These two treatments rather completely killed approximately 3 $\log_{10}$CFU *Salmonella* Typhimurium, being the final concentration of *Salmonella* in the test tube series.

Table 37 Bactericidal effect (100% inhibited growth) of sodium hypochlorite solution against *Salmonella* Typhimurium DSM 5569 (3 $\log_{10}$ CFU per ml)

NaOCl sol^n (ppm)	Exposure time (min)	pH value	Temperature (°C)	Bactericidal effect (n=5)
5[a]	5	4.60±0.10	4.8±0.10	5 in 5
	10			5 in 5
	20			5 in 5
	30			5 in 5
5	5	7.53±0.06	4.5±0.10	**4 in 5**
	10			5 in 5
	20			5 in 5
	30			5 in 5
10	5	7.55±0.10	9.9±3.10	5 in 5
	10			5 in 5
	20			5 in 5
	30			5 in 5
20	5	7.64±0.43	10.41±2.31	**4 in 5**
	10			5 in 5
	20			**4 in 5**
	30			**4 in 5**
	40			5 in 5
	50			5 in 5
	60			5 in 5
60	30	9.13±0.10	11.3±1.50	5 in 5
	40			5 in 5
	50			5 in 5
	60			5 in 5

Positive control	BHI	PC	XLD	BPLS
10 ml sterile distilled water with sodium thiosulphate pentahydrate (Na$_2$S$_2$O$_3\cdot$5H$_2$O)	Turbid	G	G	G
10 ml sterile distilled water	Turbid	G	G	G

[a] The pH-value of the 5 ppm sodium hypochlorite solution was adjusted by 3 % lactic acid solution; NG: no growth; G: growth; BHI: Brain Heart Infusion broth; XLD: Xylose Lysine Deoxycholate agar; BPLS: Brilliant-green Phenol-red Lactose Sucrose agar

4.1.5.3 Laboratory experiment: Chicken breasts

The volumes and weights of the meat juices from ten pieces of chicken breasts after thawing at 3.5 - 4.0°C overnight were 140 ml and 150.1 g or about 7.5% of the total weight of

the chicken breasts. In the experiment the meat juice was drained as much as possible before treatment in order to avoid the loss of the antimicrobial effect of the chlorinated water. The temperature in the refrigerator during the experiment was between 3.5 and 4.5°C. Further, the temperature of the sodium hypochlorite solution during the treatment was kept at 5.3 ± 0.1°C.

After artificial the contamination with *Salmonella* Typhimurium DSM 5569 chicken breasts were immersed in 20 and 200 ppm sodium hypochlorite solution (at 4 – 10°C) for 30 and 20 minutes, respectively. Immersion with 20 ppm sodium hypochlorite solution at 30 minutes contact time expressed higher *Salmonella* reduction than 200 ppm at a shorter contact time of 20 minutes. The results are shown in Table 38.

Naturally contaminated chicken breasts were immersed in a 20 ppm sodium hypochlorite solution for 40 minutes and rinsed off by sterile cooled distilled water (6.0 ±0.8°C) for 10 min (n=5). The results show that this did not decrease the amounts of bacteria and *Salmonella*, detected in 25 g of the samples after the treatment (Table 39; Figure 10; Table 40 NaOCl). Concerning *Salmonella* Typhimurium DSM 5569, the results suggest that using the 20 ppm sodium hypochlorite solution for 40 minutes did not decrease the numbers of positive *Salmonella* samples which were still detected in 25 g of the chicken breasts. The pH-values of the untreated and treated chicken breasts were 5.98 ±0.05 and 5.96 ±0.08, respectively.

The visual quality of the surfaces, the skin and the sternal side of the chicken breasts was monitored and photographed. All NaOCl-treatments caused a bleaching of the surfaces of the chicken breasts, particular on the sternal side.

Table 38 *Salmonella* reduction (mean $\log_{10}$ MPN per gram) after two applications of chlorinated water (n=2/treatment)

NaOCl sol^n (ppm)	pH value	Temperature (°C)	Contact time (min)	Reduction [a]
20	4.0	4.1-4.4	30	0.75
200	6.3	7.9-9.8	20	0.20

[a] $\log_{10}$ MPN of reference - $\log_{10}$ MPN of treatment

Table 39 Microbiological counts of chicken breasts untreated and treated in 20 ppm sodium hypochlorite for 40 min with subsequent rinsing off with cool water for 10 min (n=5/treatment)

Parameters	Untreated chicken	Treated chicken	Reduction [b]
Mesophilic aerobic count	3.93±0.15[a]	3.96±0.17[a]	-0.04
Enterobacteriaceae count	2.12±0.16[a]	2.12±0.27[a]	0.00
Coliforms count	2.00±0.00[a]	2.00±0.00[a]	0.00
Pseudomonas count	3.21±0.21[a]	3.18±0.24[a]	0.03
Salmonella in 25 g of sample	found 4 in 5	found 5 in 5	ND

[a]mean ±SD $\log_{10}$ CFU/g; [b] $\log_{10}$ count of reference - $\log_{10}$ count of treatment; ND: not determined
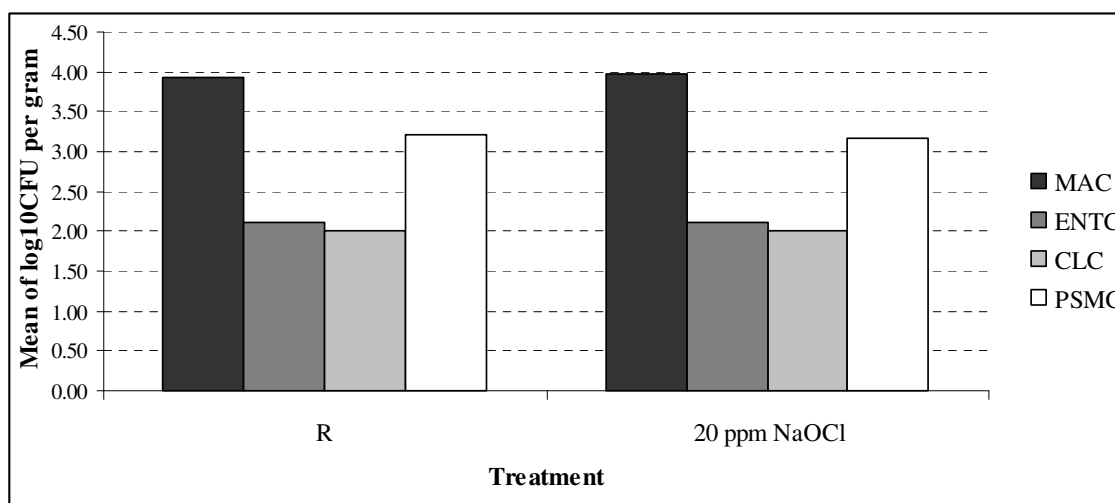
Figure 10 Microbiological counts of chicken breasts untreated and treated in 20 ppm sodium hypochlorite for 40 min with subsequent rinsing off with cool water for 10 min; R = Reference or untreated chicken breast; MAC = Mesophilic aerobic count, ENTC = *Enterobacteriaceae* count, CLC = Coliforms count, PSMC = *Pseudomonas* count

4.1.6 Recapitulation of the experiments

As Table 40 shows none of the decontamination methods evaluated in this study succeeded to completely eliminate surface bacterial contamination of chicken breasts above 4.50 $\log_{10}$ CFU/g. This number corresponds to the natural bacterial contamination of chicken breasts with mesophilic aerobic bacteria, *Enterobacteriaceae,* coliforms and pseudomonads established in this study. The highest reduction in the number of mesophilic aerobic bacteria resulted from treatment with TLHL, the lowest was from treatment with hot water at 80℃ for 15 seconds (HW1). *Enterobacteriaceae* were most effectively reduced by HLT. The lowest antimicrobial activity against *Enterobacteriaceae* was exerted by hot water treatment at 75℃ for 15 seconds following tumbling for 15 minutes (HCS2). HLT lead to the highest reductions of coliforms. In contrast, hot water at 80℃ for 15 seconds (HW1) proved to be the least efficient decontamination method for coliforms. Equally, pseudomonads were reduced at lowest rates by HW1 treatment compared to all other interventions; highest reduction of pseudomonads was achieved by TLHL. Results show that TLHL also caused a significant reduction of mesophilic aerobic bacteria and pseudomonads ($p \leq 0.05$) as well as of *Enterobacteriaceae*. Using hot water at 75℃ for 15 seconds, then cool water followed by tumbling for 15 minutes (HCS2 and HCK2), in contrast, proved to have the lowest reduction efficacy for *Enterobacteriaceae*. Using hot water at 80℃ for 15 seconds alone (HW1) resulted in lowest reduction in mesophilic aerobic bacteria, of coliforms and in pseudomonads. Two-times hot and cool water immersion at 70℃ for 15 seconds without tumbling resulted in no reduction of mesophilic aerobic bacteria and pseudomonads.

Table 40 Summary of bacterial reductions* on chicken breasts by various immersion
treatments

Treatment	Mean± sd $\log_{10}$ CFU per g			
	MAC	ENTC	CLC	PSMC
4.1.3.1 Hot water at 80˚C for 15 s (HW1)	0.129 ± 0.168^{2}	0.223 ± 0.550	0.032 ± 0.375^{2}	0.020 ± 0.577^{2}
Hot water at 80˚C for 20 s (HW2)	0.150 ± 0.150	0.306 ± 0.444	0.295 ± 0.554	0.183 ± 0.340
Hot water at 80˚C for 25 s (HW3)	0.295 ± 0.095	0.127 ± 0.258	0.335 ± 0.441	0.334 ± 0.394
Hot water at 80˚C for 30 s (HW4)	0.190 ± 0.249	0.445 ± 0.818	0.388 ± 0.600	0.430 ± 0.399
4.1.3.1 3% LA at 80˚C for 15 s (HL1)	0.269 ± 0.182	0.082 ± 0.266	0.388 ± 0.334	0.478 ± 0.323
3% LA at 80˚C for 20 s (HL2)	0.165 ± 0.223	0.309 ± 0.380	0.904 ± 0.190	0.420 ± 0.209
3% LA at 80˚C for 25 s (HL3)	0.161 ± 0.120	0.274 ± 0.449	0.628 ± 0.352	0.408 ± 0.261
3% LA at 80˚C for 30 s (HL4)	0.337 ± 0.102	0.785 ± 0.615	0.483 ± 0.462	0.600 ± 0.329
4.1.3.2 15 min tumbling with 1% LA and stored 90 min at ambient temperature and then immersed in 80˚C for 20 s with 3% LA (TLHL)	2.634 ± 0.247^{1}	1.146 ± 0.275	0.904 ± 0.190	1.709 ± 0.375^{1}
4.1.3.3 Hot water at 80˚C for 20 s and then 4x5 min tumbling (HLT)	1.089 ± 0.448	1.336 ± 0.250^{1}	1.205 ± 0.154^{1}	1.020 ± 0.509
4.1.4.3 Hot water 75°C for 15 s and cool water (the sternal side was lying on the machine) before tumbling (HCS1)	0.533 ± 0.289	0.276 ± 0.318	0.251 ± 0.540	0.639 ± 0.374
Tumbling 15 min (HCS2)	0.365 ± 0.117	0.035 ± 0.377^{2}	0.156 ± 0.593	0.964 ± 0.541
Freezing (HCS3)	0.019 ± 0.199	0.216 ± 0.210	0.371 ± 0.285	0.075 ± 0.228
4.1.4.3 Hot water 75°C for 15 s and cool water before tumbling (the skin side was lying on	0.291 ± 0.379	0.120 ± 0.702	0.095 ± 0.546	0.484 ± 0.249

Treatment	Mean± sd $\log_{10}$ CFU per g			
	MAC	ENTC	CLC	PSMC
the machine) (HCK1)				
Tumbling 15 min (HCK2)	0.400±0.226	-0.025±0.556[3]	0.251±0.360	0.491±0.375
Freezing (HCK3)	0.111±0.356	0.156±0.151	0.251±0.515	0.244±0.585
4.1.4.4 Two-times treatment Step 1: Hot water at 70°C for 15 s and cool water (HCD1)	0.334±0.316	0.216±0.299	0.371±0.285	1.036±0.400
Step 2: Hot water at 70°C for 15 s and cool water (HCD2)	-0.007±0.341[3]	0.276±0.318	0.432±0.339	-0.120±0.858[3]
Tumbling 15 min (HCD3)	0.388±0.434	0.336±0.279	0.432±0.339	0.956±0.616
Freezing (HCD4)	0.371±0.163	0.276±0.318	0.371±0.285	0.784±0.517
4.1.5.3 Immersion in 20 ppm NaOCl sol^n pH 9.1 for 40 min, at 5.3±0.1°C and followed by cool water at 6.0±0.8°C for 10 min (NaOCl)	-0.037±0.307[3]	0.000±0.369[3]	-[4]	0.030±0.387

* Bacterial reduction is different between reference and each treatment; [1]Maximal bacterial reduction; [2]Minimal bacterial reduction; [3]No bacterial reduction; [4]Bacterial number of reference was lower than detection limit; MAC: Mesophilic aerobic count; ENTC: *Enterobacericeae* count; CLC: Coliforms count; PSMC: *Pseudomona*s count

4.2 The pH value of meat

Initial trials with the phosphoric and acetic acid treatments lead to undesirable effects in terms of taste and appearance. For these reasons, both acids are not suitable as chemical substances for the decontamination of chicken meat. The treatment with lactic acid resulted in less negative effects.

The average pH-values of treated chicken meat using a 2% lactic solution and immersion for 10, 20 and 30 seconds were 5.86, 5.80 and 5.82, respectively. Immersion for 10 and 20 seconds in 3% lactic acid solution caused pH-values of 5.19 and 5.31, respectively. With 3% lactic acid solution, the pH-value of the meat was slightly lower than with the former two treatments. The application of 20 ppm sodium hypochlorite for up to 40 minutes also caused a change in the pH-values of meat. The results are illustrated in Table 41.

Table 41 pH-values of chicken meat after treatment with various decontamination methods

Treatment	pH-value
Reference or untreated chicken breast	6.02 (n=8)
Inoculated and untreated chicken	6.12 (n=2)
Distilled water	6.09 (n=2)
20 ppm NaOCl sol^n	5.97 (n=5)
3% LA at 75°C for 10s	5.18 (n=3)*
3% LA at 70°C for 20s	5.45 (n=3)*
2% LA at 75°C for 10s	5.86 (n=3)*
2% LA at 75°C for 20s	5.80 (n=2)*
2% LA at 75°C for 30s	5.82 (n=2)

* pH value significantly differs from reference value ($p \leq 0.05$)

4.3 Surface temperature of chicken breasts

Results of the laboratory experiment 4.1.2 showed that after immersion the maximal surface temperature of the chicken breasts was 37.4°C when treated with an immersion of 70°C for 30 seconds and the minimal one was 28.0°C with an immersion temperature of 70°C for 20 seconds. After 35 minutes, the average surface temperature with all applications had fallen to 15.45°C, which is nearly the temperature before treatment (15.5°C). The rates at which temperatures decreased after treatment at 75°C for 10 seconds, 75°C for 20 seconds, 70°C for 20 seconds and 70°C for 30 seconds were between 0.4 and 0.5°C per minute.

Immersion at 70°C for 30 seconds caused the highest temperature of chicken breasts (Table 42, Figure 11).

Table 42 Average surface temperatures of chicken breasts

Temperature of solution (°C)	Duration of immersion (s)	Surface temperature (°C) Before	After
75	10	15.5	30.0
75	20	15.4	30.5
70	20	15.4	28.0
70	30	15.9	37.4
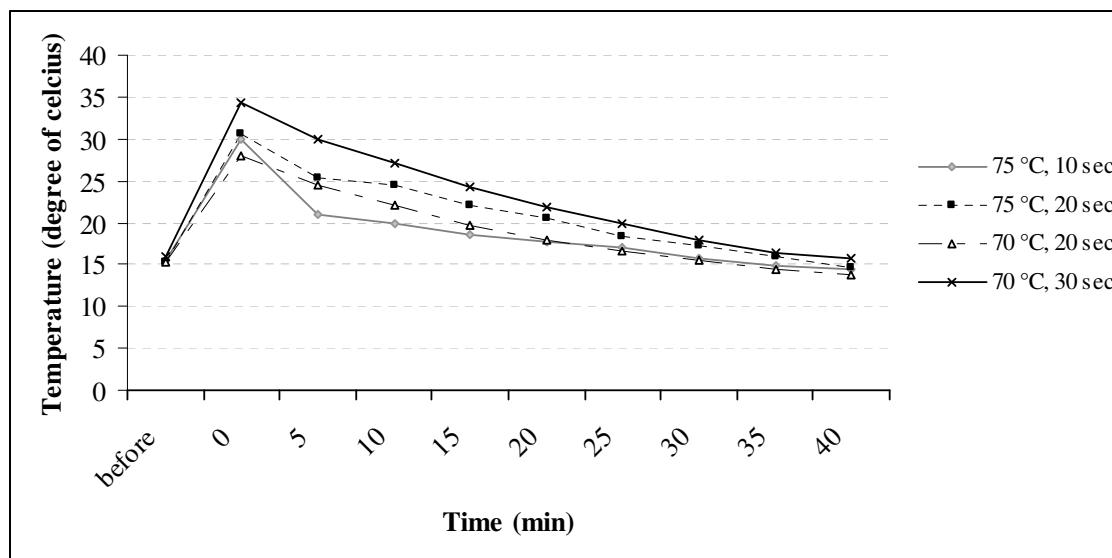
Figure 11 Surface temperatures of chicken breasts before and after immersion in hot lactic acid solution

4.4 Residues of waste water after decontamination

4.4.1 Lactic acid

The 3% LA immersion solution was examined after the treatment process. The concentrations of L-lactic and D-lactic acids in the solution after immersion of ca. 40 chicken breasts were 2.680 and 0.033 % w/w. The pH value was 1.896 at 17.0°C.

4.4.2 Chlorine

Chlorine concentrations, especially in case of 20 ppm chlorine treatment, decreased significantly after dipping two pieces of chicken. The concentration of chlorine in chlorinated water after 2 hours of preparation also decreased (Table 43). Further, chlorine treatment led to

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amounts of meat juice of 140 ml or 150.1 g from ten chicken breasts after overnight thawing in the refrigerator. The dipping solution showed much foam and fragments of denatured protein after single application.

Table 43 Chlorine concentrations of dipping solutions

NaOCl sol^n (ppm)	NaOCl sol^n after 2 hours (ppm)	NaOCl sol^n after dipping (ppm)	
		Filter **	Non filter
20	5.8	< 0.03*	< 0.03*
200	19.6	< 0.03*	10.2

* Lower than detection limit of this method; ** Dipping solution was filtrated by filter paper before the determination of chlorine

4.5   Histological examination: denatured collagen caused by hot water

For the histological investigation, meat sections were stained by Calleja's staining. Results showed that denaturation of collageneous material in form of unfolded collagen fibers could be observed with hot water treatment at 80°C for 20 seconds. Compared to hot water, denaturation began earlier when meat was immersed in hot lactic solution at 80°C. Denaturation of collagen by this method was observed at a contact time of 15 seconds.

4.6   Sensory evaluation

4.6.1 Phosphoric acid

Chicken breasts were immersed with 3% w/w phosphoric acid solution at 70, 75, 80°C, and at ambient temperature. For each temperature, immersion lasted for 10, 15, 20, 25 s and 3, 5, 8, 10 min, respectively.

The visual appearance was observed immediately after treatment. The results showed that the surface of chicken breasts appeared to have produced a gel formation, revealing a superficial coagulation and a shrinking of the surface of the chicken breasts. This finding principally applies to all treatments. Very intense changes were observed with treatments with 70°C for 20 – 25 seconds, 75°C for 15 – 25 seconds, and 80°C for 10 – 25 seconds. A lesser shrinking effect on the surface of the chicken breasts was detected when the samples were treated with 3% w/w phosphoric acid solution at 70°C for 10 - 15 seconds and at 75°C for 10

seconds. A lower heating temperature combined with a longer contact time obviously leads to preferred results.

The taste of the chicken breasts treated with 3% w/w phosphoric acid solution with immersion times of 10, 15, 20 and 25 s at 70°C was described by a panel of 5 assessors. The results showed that all four treatments caused a more sour taste compared to meat of untreated chickens. However, chicken breasts treated only shortly (10 and 15 seconds) at 70°C were only mildly sour in taste and distinctly less sour than when treated for 20 and 25 seconds.

Obviously, treatment of chicken breasts with phosphoric acid led both to a conspicuous flavor and to a visually less appealing surface of the meat. Therefore, phosphoric acid was not used for further experiments.

Table 44 Preliminary sensory evaluation of chicken breasts treated with phosphoric acid solution by a 5-points' test scale for sour taste

Treatment	Assessors					Mean score±SD (n=5)	*Sour taste*
	I	**II**	**III**	**IV**	**VI**		
Untreated chicken	0	1	0	0	0	0.20±0.45	slight
3% PA, 70°C, 10 s	1	2	1	0	0	0.80±0.84	moderate
3% PA, 70°C, 15s	1	2	1	0	0	0.80±0.84	moderate
3% PA, 70°C, 20s	2	2	2	0	0	1.20±1.10	moderate
3% PA, 70°C, 25s	2	2	2	0	0	1.20±1.10	moderate

4.6.2 Acetic acid solution

A superficial coagulation effect was visible after heat treatment at 70 and 75°C for 15, 20 and 25 seconds and after heating at all times at 80°C. By latter treatment for 15 to 25 seconds, surface coagulation on the whole of the chicken breasts did clearly develop. In contrast, the surfaces of the chicken breasts, when treated at ambient temperature for a long immersion period, continued to resemble those of untreated chicken breasts. On the other hand, acetic acid immersion clearly did generate a vinegar flavor during the treatment process. Chicken breasts treated with the acetic acid solution in consequence appeared unacceptable due to impairments of the meat surfaces and their flavor. Consequently, acetic acid was not used for further experiments.

4.6.3 Lactic acid solution

The best sensory quality, including visual appearance and odor, was observed after treatment at 70°C for 10 seconds. Only a minor coagulation effect occurred at the edges of the chicken breasts. The color of the treated chicken breasts was similar to that of untreated ones. Treatment at 75°C for 10 seconds also did not cause unacceptable surface effects. All treatments with lactic acid solution did not produce unacceptable odors of any kind.

The sensory evaluation in the preliminary experiment was carried out by a panel which assessed chicken breasts treated with lactic acid solution. They were more sour than untreated ones (Table 45). Moreover, chicken meat treated with 3 % w/w lactic acid solution at 75°C for 20 seconds showed superficial coagulation, especially at the edges and in the skin side of the chicken breasts.

Table 45 Preliminary sensory evaluation of cooked lactic acid-treated chicken breasts by a 5-points' test scale for sour taste

Treatment	Assessors						Mean score±SD (n=12)	*Sour taste*
	I	II	III	IV	V	VI		
Untreated chicken	0.5	0.0	0.0	0.0	0.0	0.0	0.21±0.39	slight
	1.0	0.0	0.0	1.0	0.0	0.0		
3% LA, 75°C,10 s	2.0	0.0	0.5	1.0	1.0	1.0	0.83±0.68	moderate
	1.0	0.0	0.0	1.0	2.0	0.5		
3% LA, 70°C, 20 s	1.0	0.0	1.0	1.0	2.0	2.0	0.79±0.72	moderate
	0.0	0.0	1.0	0.0	1.0	0.5		
2% LA, 75°C, 10 s	2.0	0.0	0.5	1.0	1.0	0.0	0.75±0.72	moderate
	0.0	0.0	1.0	2.0	1.0	0.5		

For the evaluation of results of the enterprise experiment, the triangle test was used for the sensory analysis. In this test, the minimum number of correct responses required for significance at the stated $\alpha= 0.05$ is five out of six assessors. In this study, four of the six assessors gave correct responses. Untreated and treated chicken meats with 3% lactic acid solution at 80°C in consequence for their sensory quality were different but the difference was not significant ($\alpha= 0.05$). All assessors came to the identical judgement that the taste of the treated chicken meat was not sour. On the other hand, three assessors though noted that the treated chicken meat was juiceless and more salty than the untreated one.

4.6.4 Chlorinated water

The visual quality of the skin and of the sternal sides of the chicken breasts was judged. The results showed that, applying 20 ppm sodium hypochlorite solution for 40 minutes plus subsequent rinsing with sterile cooled distilled water for 10 min, caused a bleach effect on the surfaces of the chicken breasts, especially on the their sternal sides.

The immersion of the chicken breasts in chlorinated water (20 and 200 ppm chlorine concentration) illustrates that the major disadvantages of this treatment are a potential discoloring of the surface of chicken meat, the bleach effect, and particularly a chlorine smell of the breasts (Table 46). Further processing by tumbling and freezing does not eliminate the chlorine smell of the meats. Consequently, decontamination with chlorinated water was tested only in the laboratory, not in the enterprise, because of these resulting unacceptable sensory attributes.

Table 46 Sensory evaluation of chicken breast treated with various sodium hypochlorite solutions

NaOCl sol^n (ppm)	pH of NaOCl sol^n	Temperature (°C)	Contact time (min)	Sensory
20	9.1	5.0 -6.5	40	Bleach effect and chlorine odor
20	4.0	4.1-4.4	30	Same
200	6.3	7.9-9.8	20	Same

5.   Discussion

On the artificially *Salmonella* Typhimurium contaminated meats, none of the evaluated treatment schemes led to a significant reduction of bacteria when compared with decontamination simply with water, except for the treatment with 3% lactic acid solution at 75°C for 20 seconds. This method did reduce numbers of *Salmonella* Typhimurium significantly better than water treatment. The artificial contamination dose rate of *Salmonella* Typhimurium of the chicken meats used in this study was approximately 3 $\log_{10}$ MPN per gram meat. Straver et al. (2007) reported that the number of *Salmonella* on chicken breast filets at five local retail outlets in The Netherlands varied from 1 to 3.81 $\log_{10}$ MPN per gram. This natural contamination was of lower intensity than the artificial contamination used in this study. Treatment with 3% lactic acid solution at 75°C for 20 seconds by immersion did reduce *Salmonella* by 2 $\log_{10}$ MPN per gram meat. It did decontaminate almost all the *Salmonella* on the chicken breasts. This intervention is suitable to significantly eliminate *Salmonella* from meat surfaces.

Treatment with 3% lactic acid solution at 75°C for 20 seconds proved to be more effective than 2% lactic acid solution under the same treatment conditions. Moreover, the efficacy of treatment with 3% lactic acid solution could be optimized when meat is treated at higher temperature and shorter contact time rather than lower temperature and longer contact time. The investigation results support the plausible research hypothesis that a higher concentration of lactic acid likely eliminates bacterial contamination on meat surfaces better than a lower concentration. This finding was also supported by Ockerman et al. (2001) who showed that mean total bacterial counts significantly more decreased when pork carcasses were sprayed with ascending concentrations of lactic acid, from 1, 2, to 3% lactic acid solution. A high concentration of lactic acid is more effective than a low concentration. The reductions of *Salmonella* Typhimurium at contact times of 10 and 20 seconds and 75°C temperature by 3% and 2% lactic acid solution were approximately 2 and 1 $\log_{10}$ MPN per gram chicken meat, respectively. These results are similar to previous studies of Anang et al. (2007) and Özdemir et al. (2006). On the other hand, lactic acid solution in low concentration (0.25%) did need a long contact time (60 min) to activate 5 $\log_{10}$ CFU/ ml of pure culture of *S.* Enteritidis and *S.* Typhimurium (Kanellos and Burriel, 2005).

Results also showed that an acidic condition develops an antimicrobial activity in regards to bacterial decontamination. Several previous studies are in line with this result (Van der Marel et al., 1988; Özdemir et al., 2006; Anang et al., 2007). Izat et al. in 1990 revealed that organisms generally are destroyed at pH below 2.3-2.5. *Salmonella* could not survive on

the surface of ground beef at a pH below 2.61 and not higher than 3.27 (Waterman and Small, 1998). The permeability of the bacterial cell membrane is interfered by the undissociated acid form which leads to an ionic imbalance between cytoplasm and the exterior. Acidification in the cytoplasm induces energy consumption of bacterial cells so that proton or H^+ across the cell membrane is extruded by the proton motive force (PMF). Also, an increased osmolarity of the cytoplasm interferes with the metabolic processes of the cell, so that it influences the protein synthesis or the genetic mechanism (Davidson et al., 2007). Consequently, organic acids and their esters do possess antimicrobial activity.

Due to the storage period at -18°C, reductions of *Salmonella* did not differ between day 0 and 7 when chicken breasts were previously immersed in 2 and 3% lactic acid solution for 10 to 30 seconds. The fact that the number of *Salmonella* did not decrease during 7 days storage at -18°C is not comparable with the results of previous studies by Hwang and Beuchat (1995), Stivarius et al. (2002) and Özdemir et al. (2006) who observed a higher bacterial reduction during a longer storage period at 4°C. These authors attempted to demonstrate that lactic acid possibly acts at cold temperature.

In the enterprise experiments, hot water and hot-lactic acid at 80°C for up to 30 seconds contact time did show no significantly different results for efficacy on the four studied bacterial groups, being mesophilic aerobic bacteria, *Enterobacteriaceae*, coliforms and *Pseudomonas* spp. In contrast, in the laboratory experiments *Salmonella* were significantly reduced by these treatments. Bacterial reduction on the chicken breasts in the enterprise experiment in any case was lower than in the laboratory experiment when decontamination was applied at a higher temperature. It is suggested that bacterial reduction of meat surfaces which are naturally contaminated is lower than reduction on artificially contaminated surfaces. This discrepancy is probably due to differences in the state of bacterial attachment. It is well possible that the bacteria on the chicken breasts in the enterprise were in a state of firm attachment. If thus bacterial contamination does occur during the slaughter-house or the cutting processes, surface decontamination of chicken breast by any treatment method after overnight storage of the breasts may not be effective due to the already firm attachment of the bacteria on the meat surfaces (Schwach and Zottola, 1982; Acuff et al., 1987; Conner et al., 2001). These decontaminating methods are therefore recommended to be applied as soon as possible after the slaughtering or the cutting process.

Furthermore, organic matter does affect the antimicrobial activity of decontamination methods, especially those using chemical treatment. Salmonellae were found to be protected from low acidic condition when inoculated into boiled eggs, a food high in protein and low in

fat (Waterman and Small, 1998). In the laboratory experiments, one chicken breast was immersed into 300 ml acid solution and this fluid was never reused, but in the enterprise experiments, about 40 chicken breasts were immersed in only 125 ml treatment solution per piece. Subsequent decontamination results suggest that it might be useful when, as in the laboratory experiment, a low load of organic matter is added to the treatment solution. This not being the case in the enterprise experiments, the resulting antimicrobial efficacy of decontamination in the enterprise was lower than in the laboratory.

Hot-water decontamination is an antimicrobial intervention favored by the food industry because it does not require the application of chemicals and does nor lead to the disposal of waste products. Higher temperature water decontamination does eliminate bacterial contamination on meat and carcasses more effective than lower temperature water. Northcutt et al. (2005) reported that washing of contaminated chicken carcasses with warm water (54.4°C) reduced *Salmonella* more effectively than washing with cooler water (43.3°C). Decontamination by physical methods such as hot and cool water do have the advantage of not leaving any chemical residues in the meat and in the environment (Corry et al., 1996). The temperature of the dipping solution is an important factor. Chicken carcasses dipped in 2% lactic acid solution at 37°C showed a lower incidence of *Salmonella* than 2% lactic acid solution at 4.4°C (Izat et al., 1990). In this study, hot and cool water decontaminations did lead to minor surface coagulation on the treated chicken breasts. However, the water applications proved to only have a very low antimicrobial effect regarding decontamination of naturally contaminated chicken breast. Özdemir et al. (2006) also reported that hot water treatment resulted in lower reduction of numbers of *Salmonella* Typhimurium and *Listeria monocytogenes* than the combination of hot water and lactic acid. Thus, using combinations of high temperature and acidic solution does enhance overall antimicrobial efficacy to eliminate bacterial contamination on meat surfaces as much as possible. Klose et al. (1971) reported that steam subatmospheric treatment at 66°C for 8 minutes eliminated artificial contamination with *Salmonella* Typhimurium more effectively than treatment at 71°C for 4 minutes.

Vacuum tumbling has been widely used in poultry to produce ready-to-cook and value-added products. It improves meat juiciness and tenderness due to an increased water holding capacity of the meat. Also, vacuum tumbling does affect the bacterial contamination on meat. In this study, vacuum tumbling seemed to enhance the antimicrobial effect. In fact, vacuum tumbling either causes a decreasing or an increasing risk of bacterial contamination, depending on when and how tumbling is applied in the process. For example, deboned

chicken legs were tumbled under vacuum with 1% lactic acid solution for 1 minute before use for sausage production. The number of *Salmonella*-positive batches of fresh chicken sausages clearly did decrease (François, 2006). On the other hand, when the surface meat has been contaminated by bacteria before vacuum tumbling, tumbling does increase the chances of bacteria to penetrate into the inside of the meat, as shown by the study of Warsow et al. (2008). Turkey breasts were tumbled with a cocktail of *Salmonella* for 5, 10, and 20 minutes; after tumbling for some time, the salmonellae did immigrate into the core of the muscle.

With regard to consumer acceptance, decontamination on carcasses and meat without any chemical substances is preferred by them. In the European Union (EU), chickens treated with chlorinated water subsequently are not been permitted to be imported from e.g. the USA. Surveys of the Germany Society for Consumer Research (Gesellschaft für Konsumforschung) showed that in Germany around 80% of housewives oppose chlorinated chicken. The European Union (EU) only permits potable water to be used for carcass decontamination. Lactic acid, as is chlorinated water, is prohibited for application as decontaminating substance on carcasses and meat cuts. Nevertheless, chemical decontamination methods, including use of lactic acid, are widely used in other countries. Kanellos and Burriel (2005) established that lactic acid between 1% and 3% concentration was optimal in regards to safety and product quality. Treatment with 3% lactic acid solution though already caused a slight sour taste of the meat. In order to avoid the development of a sour taste by an acidic substance, salts of organic acids can be used as antimicrobial agents. However, while using a higher concentration of lactate may cause sour taste problems, use of sodium salts may lead to a slightly bitter taste (Jensen et al., 2003).

Changes on the surface of chicken meat cannot be totally avoided in high temperature solution treatments. Heat treatment at 70°C for 40 seconds could limit the commercial application of this immersion technique because of tearing and epidermis damage of chicken skins during the trussing process (Purnell et al., 2004). Chicken breast muscle though will tolerate treatment with hot water dipping at ca. 70°C better than chicken skin (Goeksoy and James, 1999). In this study, surface temperatures of chicken meat treated with heat treatment at 70°C and 75°C for 10, 20 and 30 seconds were observed. The highest surface temperature measured was 37.4°C. Such temperature cannot denature myofibrils and connective tissues of meat tissue. At such an immersed application, muscle protein and connective tissue protein, such as collagen in chicken breast muscles, did not totally denature. This is due to the fact that myofibrils and collagen of muscles play a role to keep the water holding capacity, and the water holding in meat directly affects the drip loss when chicken breast muscles are heated at

70 to 75°C (Bircan and Barringer, 2002). Higher drip loss means increased meat costs for the meat industry (Huff-Lonergan and Lonergan, 2005). In order to prevent loss of water in treated meat, vacuum tumbling can be used for improvement. In this study, vacuum tumbling for 15 minutes did improve the water holding capacity of chicken meats after heat treatment.

The microscopic structure of meat tissue treated with hot water and hot lactic acid showed that the immersion in hot lactic acid solution at 80°C for 15 seconds caused collagen swelling of chicken meat. Even if this combination therefore does enhance the antimicrobial effect, it also does influence the microstructure of the muscle and affects the texture of the meat. Thermal denaturation of myofibrillar proteins caused an increase in the firmness of the tissue. Myosin and collagen denatures at a lower temperature at 40 to 60°C and 56 to 62°C (Bircan and Barringer, 2002; Martens et al., 2007). Shrinkage of collagen begins at 60 to 70°C and collagen is converted to gelatin at 80°C. The conversion is enhanced by acid. Actin thermal denaturation is at 66 to 73°C (Larick and Turner, 1992). Myofilbrils and collagen denaturation are closely associated with a reduction in juiciness of meat; water and dissolved ions are expelled out of muscle tissue (Bircan and Barringer, 2002). The sensory evaluation of treated chicken with hot lactic acid solution at 80°C in this study brought comparable results: assessors commented on the dry and firm texture and the slightly salty taste.

Concerning the pH-value of meat, the pH-values of chicken meat treated with 2% lactic acid solution and 20 ppm chlorine showed small changes. Their pH-values remained close to general pH-values of poultry meat, in the range of 5.6 to 6.4 (ICMSF, 1988). Results suggested that 2% lactic acid solution and 20 ppm chlorine may be an appropriate decontamination substance for meat surfaces. Treated chicken meat with 3% lactic acid solution had a pH lower than the normal range. However, the meat had only slightly changed in taste.

Chlorinated water washing in this study could not eliminate *Salmonella*. The result is similar to a previous study by Northcutt et al. (2005), who showed that washing with 50 ppm chlorine at 21.1°C, 43.3°C and 54.4°C did not add to the reduction of total bacteria, *E. coli, Campylobacter* and *Salmonella* contamination of the poultry carcasses when compared to water washing at the same temperature. The concentration of available chlorine rapidly dropped after the dipping process of chicken breasts in this study. This process is quite similar to that reported in previous studies by Thomson et al. (1979). They showed that total available chlorine in the chilling water does decline from 20 ppm to 5 ppm at the end of the second pre-chill process of boiler carcasses. Also, James et al. (1992) reported that the residual overflow concentration of a giblet chiller was 3 to 6 ppm from a water intake of 25 ppm. Available

chlorine in its active form kills microorganisms. It is for this reason that it must be maintained along the decontamination process by adding fresh chlorinated water and removing organic matter from the treatment solution; the chlorine concentration constantly has to be monitored. A high load of organic matter obviously interferes with the antimicrobial activity of the available chlorine. Even when these problems are solved, a highly effective concentration of chlorine agent would enhance the antimicrobial effect but it would create difficult environmental conditions for workers, corrode plant equipment and possibly produce toxic-chlorinated by-products. Residues and chemical agents would cause environmental damage; evaporation of sodium hypochlorite into the environment is at a rate of 0.75 g active chlorine per day (http://www.lenntech.com/water-disinfection/disinfectants-sodium-hypochlorite.htm). Chlorinated water is not available for a longer period; after two hours in this study the amount of available chlorine already was lowered. Because of the unstable chlorine, chlorinated water therefore must always be freshly prepared.

Numerous studies on the use of chlorine decontamination did report no or only minor changes on poultry carcasses or cut meat with skin (Thomson et al., 1979; Thiessen et al., 1984; Erickson, 1999; Northcutt et al., 2005). In contrast, cut meat without skin always showed a surface change in color (Lim and Mustapha, 2004). Also in this study discoloring of the entire surface of chicken breast fillets without skin occurred.

The concentration of the lactic acid solution after the immersion process was nearly that of the original solution. In comparison, chlorinated water after immersion had almost no chlorine left. Chlorine is very sensitive to organic matter so that excreted fluid from meat cuts mainly does affect available chlorine. When using chlorine for decontamination or other methods based on available chlorine, such as electrolyze oxidizing water or EOW, one has to monitor its concentration and add fresh chlorinated water to the immersion solution during the decontamination process. Not only does organic matter directly affect the amount of available chlorine, it also affects the pH of the chlorinated water. The hypochlorous form of chlorine which has the highest antimicrobial property is of a high concentration at a pH below 5. This fact is supported by this study. *Salmonella* Typhimurium were completely killed at 5 ppm NaOCl sol[n] at a pH of 4.60 but recovered at 5 ppm NaOCl sol[n] at a pH of 7.53.

6.   Conclusion

Hot lactic acid immersion in a 3% lactic acid solution at high temperature at 75°C for 20 seconds contact time can effectively reduce *Salmonella* contamination on the surface of chicken breasts, with minor visual changes in the surface appearance and in the taste quality of the breasts. However, this application is only recommended before a firm bacterial attachment does occur on the surface of breasts. Hot water immersion at 80°C, 20 seconds without lactic acid and then 4x5 minutes vacuum tumbling effectively reduces mesophilic aerobic bacteria, *Enterobacteriaceae*, coliforms and pseudomonads on the chicken breast surfaces after firm attachment of the bacteria or after the chilling process. This application leads to only minor changes in the sensory quality of the chicken breasts. Two-time treatment with hot and cool water at 70°C for 15 seconds contact time also was determined a most suitable decontamination method; chicken breasts treated in this way almost have the same quality as raw chicken meat. Even more, the method leads to bacterial reduction similar to that of 3% lactic acid solution at 80°C for 30 seconds contact time, except for *Enterobacteriaceae*. This application finally is safe from any chemical residues. Decontamination with such interventions may essentially improve the hygienic quality of chicken meat while maintaining the sensory quality of raw chicken meat. Since contamination of chicken with *Salmonella* seems unavoidable in European poultry farms up to now the European community should reconsider its food safety policy. Mainly the question whether a combined lactate and hot water treatment produces higher health risks for the consumer than higher prevalence of *Salmonella* on chicken meat should be discussed on a scientific level.

7.   Zusammenfassung

<u>Labor- und Praxisversuche zur Reduktion der Salmonellenkontamination von rohen
Hühnerbrustfilets</u>

Die Arbeit diente dem Ziel, ein geeignetes Dekontaminationsverfahren für Geflügelfleisch zu
entwickeln. Hierfür wurden Laboruntersuchungen mit physikalischen und chemischen
Methoden sowie deren Kombination durchgeführt. Die so gewonnenen Ergebnisse wurden
weiterhin mit Hilfe dreier Versuchsreihen in einem Geflügelfleisch verarbeitenden Betrieb
überprüft.

Neben Erhebungen an *Salmonell*a Typhimurium DSM 5569-Reinkulturen wurde bei den
Laborstudien rohe Pectoralis-Muskulatur künstlich kontaminiert und in Test-Flüssigkeiten
eingetaucht, wobei die Art des Mediums (Leitungswasser, Lösungen verschiedener
organischer Säuren, chloriertes Wasser), die Behandlungsdauer und die Temperatur
variierten. Bei den Feldversuchen kamen das Vakuum-Tumbeln und Einfrieren als mögliche
Einflussfaktoren hinzu. Für die einzelnen Ansätze wurden außer der Keimzahlreduktion die
sensorisch erfassbaren Merkmale wie Aussehen, Konsistenz, Geruch und Geschmack und z.
T. mit Hilfe der histologischen Analyse auch der Verleimungsgrad des Kollagens ermittelt.

Gemessen an den mit der kulturellen Technik nachweisbaren Keimzahlen zeigte im
Modellversuch das Eintauchen in heiße Milchsäurelösung eine bessere Inaktivierung als der
reine Abwascheffekt mit kaltem Leitungswasser. Bei den überprüften Varianten handelte es
sich um 70 und 75°C heiße, 2 und 3 %ige Laktatlösung sowie Einwirkungszeiten von 10 – 30
Sekunden. Mit einer Verminderung der Salmonellendichte um 2.01 $\log_{10}$ MPN/g erbrachte
die Kombination 3%ige Milchsäure/75°C/20 sec die stärkste Keimreduktion. Alleinige
Anwendung von 70 bzw. 75°C heißem Wasser eliminierte 1-2 $\log_{10}$ KbE *Salmonella*
Typhimurium/ml Reinkultur und 1 $\log_{10}$ KbE/g kontaminiertes Geflügelfleisch.

Bei den Erhebungen im Verarbeitungsbetrieb fiel der Dekontaminationseffekt vergleichbarer
Vorgehensweisen geringer als im Laborexperiment aus. Die Spannweiten der Keimzahl-
reduktion beliefen sich bei der Prozessparameter-Kombination „80°C/15-30 sec/ ohne und mit
3 %ige Laktatlösung" auf 0,1-0,3 bzw. 0,2-0,3 aerobe mesophile Gesamtkeime, 0,0-0,4 bzw.
0,4-0,6 Pseudomonaden, 0,1-0,4 bzw. 0,1-0,6 Enterobakteriazeen, 0,0-0,4 bzw. 0,4-0,9 für

Coliforme, wobei die letzten beiden Gruppen als Indikatoren für Salmonellen dienten. Außerdem verminderte Vakuum-Tumbeln vor der Milchsäurebehandlung (TLHL) die Gesamtkeimzahl und Pseudomonadendichte signifikant (p<0.05), während Vakuum-Tumbeln nach der Immersion in Heißwasser (HLT) statistisch nachweisbar die Enterobakteriazeen- und Coliformenzahl senkte.

Eine weiterhin im Betrieb überprüfte zweistufige Modifikation, bei der eine Kaltwasser- der 15 sec dauernden 70°C-Heißwassereinwirkung folgte, ergab eine Reduktion der Gesamtkeimzahl von 0,02-0,53, der Enterobakteriazeen von 0,04-0,28, der Coliformen von 0,10-0,37 und der Pseudomonaden von 0,08-0,96 KbE/g Brustfilet. Der inaktivierende Effekt ließ sich verstärken, indem diese Behandlung wiederholt wurde, gefolgt von den Prozessstufen Tumbeln und Einfrieren.

Auf Grund ihrer geringen antimikrobiellen Wirkung empfehlen sich folgende der getesteten Varianten der Immersionstechnik nicht für die Praxis: 80°C heißes Wasser/15 sec (HW1), 75°C heißes Wasser/15 sec mit nachfolgender Kaltwasserbehandlung und 15-minütigem Tumbeln (HCS2 und HCK2), zweimaliges Einwirkeng von 70°C heißem Wasser/15 sec mit anschließender Kühlung ohne Tumbeln (HCD2).

Im Hinblick auf die Mikrostruktur des Fleisches begann die Denaturierung und Quellung der kollagenen Fasern bei 80°C Milchsäurelösung/15 sec bzw. 80°C Trinkwasser/20 sec. Zweimaliges Einwirken von 70°C heißem Wasser und Kaltwasserabkühlung bewirkte die geringsten optischen Veränderungen der rohen Fleischoberfläche. Die Kombination aus Vakuumtumbeln und nachfolgender 3%iger Laktatlösung zeigte allerdings eine deutlich bessere mikrobizide Wirkung, doch wurden Aussehen und Textur zu stark verändert.

Zwar verringerte chloriertes Wasser die Salmonellendichte in Reinkulturen um 3 $\log_{10}$ KbE/ml, doch war dieser Effekt bei kontaminierter Geflügelbrust deutlich abgeschwächt. Darüberhinaus traten merkliche sensorische Abweichungen auf.

Insgesamt muss bei jeglicher Dekontamination von rohem Geflügelfleisch ein Kompromiss zwischen der „erwünschten Denaturierung von mikrobiellem Eiweiß und der unerwünschten Denaturierung von Fleischeiweiß" gefunden werden. Im Gegensatz zu vielen anderen Nationen wären die EG-Länder mit dieser Problematik jedoch erst konfrontiert, wenn die

Gemeinschaft ihre kritische Haltung zur chemischen Dekontamination aufgeben würde. Angesichts weiterhin relevanter *Salmonella*-Prävalenzen bei Schlachtgeflügel scheint ein Umdenken nicht ausgeschlossen zu sein.

8.   Summary

Experimental Reduction of *Salmonella* in Raw Chicken Breasts

The objective of this study was to identify a suitable decontamination method for chicken meat. For this, investigations on a combination of physical and chemical interventions were carried out in the laboratory, and further experiments were done in the process-line of a poultry meat producing enterprise.

Initial trials determined the appropriate temperature, contact time and treatment solution for the immersion treatments which are based on sensory attributes such as the surface appearance, taste, odor and texture of the chicken meat. The antimicrobial effect of bacterial reduction in pure culture and of artificially contaminated chicken meat further was investigated. Trials which imitated natural contamination were designed for and evaluated in an experimental process-line within an enterprise. The applications by immersion were composed of hot water, hot lactic acid, hot and cool water and chlorinated water. Concerning the industrial tests, vacuum tumbling and freezing were further process steps.

In terms of reduction of the microbial counts, immersion in a hot lactic acid solution did enhance the antimicrobial effect. The application of a 2-3% hot lactic acid immersion at 70 and 75°C for 10 - 30 seconds contact time was able to reduce the number of *Salmonella* on artificially contaminated chicken breast surfaces significantly more effectively compared to water immersion. The highest reduction of *Salmonella* (2.01 $\log_{10}$ MPN per gram meat) was achieved by a 3% lactic acid treatment at 75°C for 20 seconds contact time.

Hot water treatment alone eliminated 1 – 2 $\log_{10}$ CFU *Salmonella* Typhimurium per ml pure culture and 1 $\log_{10}$ CFU per gram contaminated meat. Notably, bacterial reductions achieved in the experiments in the enterprise were lower than in the laboratory experiments. The ranges of bacterial reductions by heat treatment at 80°C for 15 - 30 seconds with and without 3% lactic acid solution were for mesophilic aerobic bacteria between 0.2 to 0.3 and 0.1 to 0.3, for *Enterobacteriaceae* between 0.1 to 0.8 and 0.1 to 0.4, for coliforms between 0.4 to 0.9 and 0.0 to 0.4, and for pseudomonads between 0.4 to 0.6 and 0.0 to 0.4 $\log_{10}$ CFU per gram meat for each of the two respective treatments.

Vacuum tumbling employed before the immersion in 3% lactic acid soluion (TLHL) did effectively decontaminate the natural contamination with mesophilic aerobic bacteria and with pseudomonads (p≤ 0.05). Similarly, vacuum tumbling used after the hot water immersion (HLT) also significantly reduced natural contaminations, but for *Enterobacteriaceae* and coliforms in this case. Hot water followed by cool water in the

enterprise experiments resulted in a reduction of bacterial counts of mesophilic aerobic bacteria of 0.02 to 0.53, of *Enterobacteriaceae* of 0.04 to 0.28, of coliforms of 0.10 to 0.37 and of pseudomonads of 0.08 to 0.96 $\log_{10}$CFU per gram chicken meat. Two-time treatment with hot water at 70°C for 15 seconds and cool water, followed by tumbling and freezing, did reduce bacteria more effectively than one-time treatment with hot water followed by cool water.

Based on these low antimicrobial effects, the following immersion methods are not recommended for practical application: hot water at 80°C for 15 seconds (HW1); hot water at 75°C for 15 seconds and then cool water followed by tumbling for 15 minutes (HCS2 and HCK2); two-times treatment with hot water at 70°C, 15 seconds and cool water (HCD2) without tumbling.

In terms of the microstructure of meat, collagen fibers started to denature at 80°C for 15 seconds in a hot lactic acid immersion and at 80°C for 20 seconds in a hot water immersion without lactic acid. With regard to the sensory attributes of treated chicken breast surfaces, aiming at results that almost equal the visual surface of raw chicken meat, the best treatment was two-time hot and cool water immersion. In contrast, TLHL clearly did reduce the natural bacterial contamination of chicken breast. Unfortunately, this method caused unacceptable taste and appearance changes. Treatment with 3% lactic acid did not make the meat sour but led to a change of the meat texture.

In the chemical treatment experiment, chlorinated water inactivated *Salmonella* Typhimurium in culture 3 $\log_{10}$ CFU per ml. However, chlorinated water did not reduce bacteria on chicken breasts very efficiently. Additionally adverse sensory effects occurred.

Concerning the decontamination of raw chicken breasts minimizing a compromise must be found for minimizing tissue damage and sensory alterations on the one hand and maximizing antimicrobial effects on the other hand.

9. References

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10. Appendix

A. Dilution method of MPN technique

D.   List of equipments

Equipment	Manufacturer	Model	Country
Freezer -45°C	TENAK®	LT130	Denmark
Incubator	Memmert®	GTR 0214	Germany
pH meter	Schott®	LG 841	Germany
pH meter	Schott®	CG 841, no 314171	Germany
Spectrophotometer	Shimadsu®	UV-1202 UV-VIS	Japan
Laboratory blender Stomacher	Seward medical	400 BA7021	UK
Digital thermometer	Greisinger®	GTH 175/PT	Germany
Vertex mixer	K Janke & Kunkel IKA®-Labortechnik	VF2	Germany
Water bath	Memmert®	WB29	Germany
Weight	Delta Range®	PE3600	Switzerland

E.   List of chemical reagents and culture media

Reagents/ Media	Company	Country
Buffered peptone water	Merck®	Germany
Rappaport Vassiliadis broth	Oxoid®	UK
Xylose lysine deoxycholate agar	Merck®	Germany
Brilliant-green Phenol-red Lactose Sucrose or BPLS agar	Merck®	Germany
RAMBACH agar	Merck®	Germany
Selenite cystine broth	Sifin®	Germany
Peptone from casein	Merck®	Germany
Agar agar	Merck®	Germany
NaCl	Merck®	Germany
Brain heart infusion broth	Merck®	Germany
Plate count agar	Merck®	Germany
Glutamate Starch Phenol Red or GSP agar	Merck®	Germany
Penicillin G (99.74%)	Calbiochem®	Germany
Violet red bile agar	Merck®	Germany
Violet red bile glucose agar	Merck®	Germany
Nutrient agar	Merck®	Germany
Enterotube™ II	BD BBL™	USA
Gram-color staining set for Gram stain	Merck®	Germany
N,N-diethyl-1,4-phenylenediamine sulfate or DPD (NH_2-$C_6H_4N(C_2H_5)_2 \cdot H_2SO_4$)	Merck®	Germany
di-Sodium hydrogen phosphate dodecahydrate form ($Na_2HPO_4 \cdot 12H_2O$)	Merck®	Germany
Potassium dihydrogen phosphate (KH_2PO_4)	Merck®	Germany
Disodiumdihydrogenethylenedinitrilotetraacetate dihydrate or disodium EDTA dihydrate ($C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O$)	Merck®	Germany
Potassium iodide, crystals	Roth®	Germany
Sulfuric acid 95-97% (H_2SO_4)	Merck®	Germany
Sodium hydroxide (NaOH)	Merck®	Germany
Sodium hypochlorite (NaOCl) 12%	Roth®	Germany
Potassium iodate, standard solution (KIO_3)	Merck®	Germany
Thioacetamide (CH_3CSNH_2)	Merck®	Germany
Phosphoric acid 85%	Merck®	Germany
Lactic acid ca. 90% Ph.Eur.	Merck®	Germany
Sodium thiosulphate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$)	Merck®	Germany
Anaerobic system Anaerobic system™	Oxoid®	UK
Disinfectant	Lysoformin® special	Germany

Reagents/ Media	Company	Country
Oxidase test strip	Merck[®]	Germany
Peptone water* (ingredients for one liter: peptone from casein 1 g, agar agar 0.75 g, sodium chloride 8.5 g	Merck[®]	Germany

* Preparation was done according to the standard operating procedure of the Institute of Food Hygiene, Faculty of Veterinary Medicine, Free University of Berlin.

F.   Blank ballots of the triangle sensory test on chicken breast

Date …………………

Assessor's name……………………………………………………………………No. …………..………….
_____

Intructions

Please take a drink of water before tasting chicken samples and please take a sip of water between samples. **Two samples are alike; one is different.**

Place an "X" under sample code number

Question no. 1   Which one is different?

Sample code   …….   ……   ……
                                      ___   ___   ___

Questions no. 2   How differs from the others?

_____

G.   Abbreviations

A

ADI   acceptable daily intake

B

BHI   Brain Heart infusion

BPW   Buffered peptone water

C

°C   Degree Celcius

C. jejuni   *Campylobacter jejuni*

ca.   approximately

CFU   colonies forming unit

conc.   concentration

D

DNA   Deoxyribonucleic acid

DSM   Deutsche Sammlung von Mikroorganismen und Zellkulturen,

                         Braunschweig, Germany or German Collection of Microorganisms and

                         Cell cultures

E

E. coli   *Escherichia coli*

e.g.   exampli gratia or for example

etc.   et cetera

et al.   et alii or and other people

F

FDA   Food and Drug Administration

G

g   gram

GMP   Good Manufacturing Practice

H

h   hour

HTST   high temperature/ short time

I

i.e.   in other words

L

l   liter

LA   lactic acid solution

log   logarithm

LPS   lipopoplysaccharide

M

mg   milligram

min   minute

ml   milliliter

MPN   Most Probable Number

N

n   number of samples

NA   not available

nm   nanometer

O

OD   optical density

ONPG   *ortho*-nitrophenyl-*β*-D-galactopyranoside

P

PABA   *p*-aminobenzoic acid

PC   Plate count agar

pK_a the negative logarithm of the acid dissociation constant

ppm   parts per million

R

RNA   ribonucleic acid

S

S.   *Salmonella*

s   seconds

SD   standard deviation

sol^n solution

subsp.   subspecies

U

UHT   ultra-high temperature

US   United States

USDA   United States Department of Agriculture

W

w/v   weight by volume

w/w   weight by weight

Y

YOPIS   Young, old, pregnant and immunocompromised segments of the public

11. Publication list

Poster demonstration at 49. Arbeitstagung des Arbeitsgebietes Lebensmittelhygiene der Deutsche Veterinärmedizinische Gesellschaft e.V. (DVG) on 29.09. to 02.10.2008, Garmisch-Partenkirchen, Germany.

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Selbständigkeitserklärung

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschlieβlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.