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Evaluation of surface decontamination applications in some uncooked chicken meat and fresh cut vegetables.

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Evaluation of surface decontamination applications in some uncooked chicken meat and fresh cut vegetables.

Summary:

Meat and meat products and some fresh vegetables play an important role in healthy nutrition. However this food groups are easily decontaminated with pathogenic micro organisms and suitable media for microbial growth, and therefore they are considered to be risky foods. It has been reported that several pathogenic bacteria, parasites, Hepatitis and some other viruses can be present in fresh vegetables and raw meat. Therefore the surface decontamination of these foods are important with respect to shelf life and food safety applications commonly used in surface decontamination are using disinfectants on the surface of the foods by spraying or dipping the foods in disinfectant solutions for a certain period of a time. Besides these methods, washing with hot water, irradiation, and steam pasteurization can also be applied.

As disinfectants, organic acids, chlorine, hydrogen peroxide, chlorine compounds, Quaternary ammonium compounds, ozone treated water, antibiotics, some other antimicrobials and also acidic electrolyzed water can be used. Important criteria for choosing the suitable disinfectant are the natural micro flora and nature of foodstuffs, the availability and cost of disinfectants.

In this study, the lettuce sample which has 5, 21 log (cfu/g) initial mezophilic aerobic bacteria count dipped in some disinfectant solutions for 15 minutes. Result of the mezophilic aerobic bacteria analysis showed that 5% H_2O_2 solution has the highest disinfectant effect and was followed by respectively % 1 acetic acid (2,76 log(cfu/g)), % 12 trisodiumpHospHate (2,78 log(cfu/g)), % 40 apple vinegar (3.21 log(cfu/g)), % 40 grape vinegar (3.32 log(cfu/g)), 00 ppm sodium hypochlorite (4.07 log(cfu/g)), and

% 1 sodium acetate solution (5.12 log(cfu/g)). The highest value from % 5 hydrogen peroxide is 2.37 log (cfu/g).

The chicken meat sample that has 9.43 log (cfu/g) initial mezophilic aerobic bacteria and 9.12 log (cfu/g) coli form bacteria count dipped in disinfectant solutions for 15 minutes. Results of the aerobic mezophilic bacteria analysis showed that %1 and % 2.5 hydrogen peroxide(5,04 and 4,86 log(cfu/g)) and % 2 lactic acid solutions (4,93 log(cfu/g)) had the highest antimicrobial effect and was followed by respectively % 2 acetic acid (5,61 log(cfu/g)) % 12 TSP (trisodium pHospHate 6,01 log(cfu/g)), 200 ppm sodium hypochlorite (6,55 log(cfu/g)),20 mM EDTA (etilendiamin tetra acetic cid) (6,73 log(cfu/g)) 3% sodium acetate (6,74 log(cfu/g)), and sodium lactate solutions (6,99 log(cfu/g)). Results of the coli form bacteria analysis showed that 2,5 % hydrogen peroxide reduced the bacteria count to 4,50 log (cfu/g) was followed by respectively % 1 H_2O_2 (4,85 log(cfu/g)) % 2 lactic acid (5,03 log (cfu/g)) , % 12 TSP (5,35 log (cfu/g)), %2 acetic acid (5.90 log(cfu/g)), 200 ppm sodium hypochlorite (6,22 log (cfu/g)) ,% 3 sodium acetate.

(6,44 log (cfu/gr)) and sodium lactate (6,99 log(cfu/gr)) and 20 mM EDTA solutions (6,6399 log (cfu/gr)).

AEW (acidic electrolyzed water) prepared from two different sodium chloride solutions (1 and 1.5%) that has 20 and 30 ppm free chlorine concentration compared to 200 ppm sodium hypochlorite solution by 10 minutes surface decontamination using lettuce sample which had 7.66 log (cfu/gr) initial mezophilic aerobic bacteria and 7.40 log(cfu/gr) coli form bacteria count results show that 30 and %20 AEW and 200 ppm sodium hypochlorite reduced mesopHilic aerobic bacteria count to 6.36,6.45,6.44 log (cfu/gr) respectively. For the same solutions the results for coli form bacteria counts were determined as 5.80, 6.10, and 5.97 log (cfu/gr). Using the same solutions the raw chicken meat was decontaminated. The results showed that 30 and 20 ppm AEW and 200 ppm sodium hypochlorite solutions reduced the mesopHilic aerobic bacteria counts to 7.37,7.66,and 7.60 log (cfu/gr) and coli form bacteria count to 7.05,7.21,7.18 log (cfu/gr) respectively.

The lettuce sample inoculated with StapHylococcus aureus (initial bacteria count was 4.81 log (cfu/gr)) dipped in some disinfectant solutions for 15 minutes. Results showed that 12% TSP (2.68 log(cfu/gr)) had the highest antimicrobial effect was followed by 2% lactic acid (3.27 log (cfu/gr)), % 2 acetic acid (3.29 log (cfu/gr)), 200 ppm sodium hypochlorite (3.52 log (cfu/gr)), 20mM EDTA (4.02 log(cfu/gr)), % 3 sodium lactate (4.16 log(cfu/gr)), and % 3 sodium acetate solutions (4.31 log(cfu/gr)) respectively.

In another study lettuce sample inoculated with Salmonella typHimurium (initial bacteria count was 7.06 log (cfu/gr)) decontaminated for 15 minutes.2 % lactic acid and % 8 TSP solutions inhibited the bacteria on the lettuce surface completely. The bacteria reduced to 2.78 and 3.71 and 5.10 log (cfu/gr) by 2 % acetic acid, 5 % hydrogen peroxide and 200 ppm sodium hypochlorite solutions respectively.

The chicken meat sample inoculated with stapHylococcus aureus (initial bacteria count was 5.74 log (cfu/gr)) dipped in disinfectant solutions for 15 minutes. While % 2.5 hydrogen peroxide solution s inhibited the bacteria completely, 12 % TSP, 2 % lactic acid, 2 % acetic acid and 200 ppm sodium hypochlorite reduced bacteria count to 2.54,2.58,2.93,4.46 log(cfu/gr) respectively.

Agar diffusion assay was applied to StapHylococcus aureus and Salmonella TypHimurium to determine the inhibition effects of disinfectant solutions. For StapHylococcus aureus results showed that 200 ppm sodium hypochlorite and 2 % acetic acid had no inhibition effect and 2 % lactic acid 8 and 12 % TSP, 2.5 and 5% hydrogen peroxide solutions caused the inhibitions zone in diameter of 8.8, 10.2, 10.2 , 43.3,and 48 respectively. For Salmonella TypHimurium 2 % acetic acid had no inhibition effect and 2 % acetic acid and lactic acid 8 and 12 % TSP, 2.5 and 5% hydrogen peroxide solutions formed inhibitions zone in diameter of 16.3, 11.8, 10.8, 13.7, 30, 33.5 respectively but 200 ppm sodium hypochlorite solution showed no inhibitory effect.

Introduction:

Some fruits and vegetables which grow very close to the ground can be easily contaminated by diversity of pathogen bacteria. When these micro organisms are not inactivated, they threatened the food safety and cause very serious nutrition diseases. Because of that lettuce that is used to prepare salad is in the risky food groups. at the same time meat products also have same risky group because of their natural micro flora that they have (Robinson and oth.2000). You can decrease the amount of risk in these groups by using good manufacturing techniques but you must also use some sanitation techniques. (Dickson And other., 1994). Surface decontamination applications are very important when you think of product shelf life and product safety (Brackett, 1992).

There are a lot of diseases related to consumption of raw vegetables. In 1981, in Canada Literiosis disease is observed due to consumption of lettuce and found that the cause was the sheep fertilizer in growth area. Similarly in USA and Canada, contaminated fruits and vegetables also cause verositoxide Escherichia coli syndrome. We can show Pathogens that are in fresh cut vegetables are: Salmonella sap., Campylobacter spp., Clostridium Botulinum, Vibrio cholera and hepatitis A virus, as an example (Odumeru and other 1997, Simons and Sanguansri, 1998). Pseudomonas fluorescenes, Erwinia caratovora and Leuconostoc spp. are some micro organisms that cause contamination of fresh cut vegetables (Simons and Sanguansri, 1997).

Chicken meat also one of very sensitive group to microbial growth. It can be included not only bacteria that cause contamination and as a result decrease in shelf life, but also pathogen bacteria such as Clostridium botulinum, Salmonella spp StapHylococcus aureus Bacillus cereus, Listeria monocytogenes, Yersinia enterecoliticia and E. coli 015:H7 (Capita, 2002; Cutter and Siragusa, 1994).

In decontamination of fresh cut vegetables and meat, washing with disinfectant solutions, radiation, freezing, dehydration, high pressure UV energy, UV radiation and heat process methods are used (Capita and other 2002; Robinson and other, 2000).

You can do washing process either by dipping the product inside the disinfectant solution for a while or use this solution as a spray over the product in some amount. If the process is completed successfully, microbial load can be decreased very effectively (Brackett, 1992). Since enzyme dehydration like in some other methods never become as a negative effect, washing of foods in disinfectant solutions is preferred very extensively (Simons and Sanguansri, 1998).

We can count some disinfectant solutions: Hypochlorite (200 ppm), chlorine dioxide (200 ppm), H_2O_2 (% 5), O_3 (1-4 ppm), Br (200 ppm), I_2 (10-100 ppm), peroxiaseticacid (200 ppm), Lactic acid (1%-10) and some organic acids, TSP(8-12 %) Quartered ammonium, $KMnO_4$. (Beuchat and fri.; Cherry 1999; Escudero and other, 1999; Sapers and Simons, 1998; Soriano and oth.2000; Zang and Farber, 1996).

Organic acids are used in food safety for many years because of their low disassociation coefficients and toxicities (Hui,1992). Ascetic acid and lactic acid disinfectant solutions provide an acidic area, where ever they touch on surface of food and so decrease microbial growth (Mermelstein, 2001). In one study, it is observed that by applying 15 minutes of 8-12 % TSP solution, the growth of Salmonella is under control and also TSP decreases the bacteria contamination in red meat because of its alkaline sanitizer property (Dickson and other 1994). Hypochlorite shows a strong disinfectant effect and for low concentrations it is not toxic for human health (Zsang and Farber, 1996).

Also it is known that number of mesopHilic aerobic bacteria and Enterobacteraceae, hygiene and contamination indicator, decreases as a result of decontamination with using disinfectant solutions. (Gilbert and oth.2000).

In this study before making surface decontamination, first products are dipped inside some disinfectant solutions for a while than we provided surface decontamination and then total bacteria and coli form analysis is done. Besides, after injected Salmonella inside some foods, surface decontamination with disinfectant solutions are applied and as a result it is observed that number of pathogen bacteria decreases and the effects of disinfectant solutions are compared.

2) Literature Summary:

2.1) Food Safety and Micro organisms:

Besides their natural microbial flora, foods also can be contaminated with micro organisms by several ways. Especially some fresh cut vegetables from growth area to home; they can be exposured lots of biologic danger. In unsuitable platform, number of these micro organisms can increase or new micro organisms can contaminate the product. This affects shelf life and safety of product. Because of that by decreasing initial micro organism loads of risky groups such as fresh cut vegetables and raw chicken meat, diseases must be decreased (Koseki, and other 1992).

2.1.1) Evaluation of Fresh cut Vegetables and Fruits in terms of Microbiology:

Ready-to-use or fresh-cut vegetables (lettuce, cucumber, carrot, spinach etc.) are always preferred by consumers because of their less waste and their ease to prepare and no need any more process (Odumen and other 1997).

Lettuce is one of the most consumed products; it is used in preparation of salad and has a big risk in terms of bacterial contamination because it is growth very close to ground. It is also used in such fast food nutrition’s hamburger and sandwiches so it is consumed very much (Soriano and other. 2000).

There are some pathogen bacteria in fresh cut vegetables that are consumed such as Shigella spp., Salmonella spp., Listeria monocytogenes. You can also encounter pathogen micro organisms such as Entere, obacter cloacae, Aeromonas hydropHilia and Klebsialla which are isolated from lettuce and salads. In past years 347 ‘shigellosis’ disease is encountered because of the consumption of cut lettuces that are manufactured as commercial. Vegetables are the first food group which are determined the cause of disease called ‘Listeriosisis’. So to prevent food-oriented diseases, these types of products must be safe in terms of hygiene, and their micro organism load must be decreased with suitable methods (Brackett, 1992; Koseki and Hoh, 2001; Soriano and other, 2000).

Bacillus cereus which has a property of forming endospore causes food-oriented diseases in fresh consumed vegetables by forming emetic toxin. Vegetative spoors and cells are generally in ground and can contaminate in several ways to the food from the beginning of harvest to whole process. (Kim and other, 1999).

Esheria colitis infection O157:H7 is ended in 1996 in Japan with 10000 victims who ate white radish bud (Taormia and Beuchat, 1999).

Because of consumption of alpHa buds which are acquired from contaminated seeds, in 1995 in Oregon and British Columbia; there exists Salmonella Newport infection in 133 people. At the same time analysis on contaminated heap, Positive results are acquired for Salmonella Albany and Salmonella Schwarzengrund infections (Weissinger and Beuchatt, 2000).

Studies that are applied to decrease pathogen bacteria count in seeds which can beat alpHa buds, it is shown that solutions included 2000 ppm chlorine, % 6 hydrogen peroxide and 80 % ethanol and TSP can be used for surface decontamination (Weissinger and Beuchatt, 2000; Beuchatt and other, 2001).

Decontamination with chemical solutions is one of the methods that are used for sanitization of vegetables and diversity of chemical materials is used for this purpose.

In table 2.1 some disinfectants and their effective concentrations for disinfection of fresh vegetables and fruits are shown (Cherry, 1999; Soriano and other, 2000):

Disinfection material	**Effects and concentrations that they effective**
Cl	Hypocroz acid, Na and Ca hypochlorite PH: 6.5 Process equipment, process water, all fresh vegetables and fruits. 1-2 log decrease, 200 ppm (20 000 ppm for seeds).
ClO_2	Process equipment, all fresh vegetables and fruits. 1 log decrease, 1-5 ppm (200 ppm for equipments).
H_2O_2	All fresh vegetables and fruits. 3 log decrease , 5% hydrogen peroxide
O_3	In water and fresh vegetables and fruits 1-3 log decrease, 1-4 ppm
Peraceticacid	fresh vegetables and fruits 2 log decrease , 200 ppm
Acids(acetic acid and lactic acid)	Decrease PH Specific antimicrobial activity
TrisodiumpHospHate (TSP)	Lettuce PH 11-12 4 log decrease 1-12 % TSP

2.1.2) Evaluation of some Meat products in terms of Microbiology:

Meat and meat products are also in risky group in terms of microbial contamination. Some methods are developed for their sanitarization. Steam vacuum, steam pasteurization, application of some types of disinfectants and some chemicals such

as organic acids (spraying or by dipping), radiation are some of these methods (Mermelstein, 2001).

Chicken meat is consumed often in our country like in foreign countries. It is also risky because of micro organism's cause's contamination and pathogen bacteria which cause diseases. Bacteria decrease the product quality and shelf life. The contamination of the product is realized because of bad smell and tissue forms over the surface. Contamination firstly begins in surface than observed tissues inside the meat. Micro organism types that are observed in contaminated chicken meat are Pseudomonas, Alteromonas and Acinetobacter. (Mullerat and other, 1994).

In the chicken meat there are also some pathogen bacteria which cause diseases such as Salmonella, Clostridium perfiringes, Stapylococcus aureus, Listeria monocytogenes, Esheria colitis and Bacillus cereus (Mullerat and other, 1994).

In USA and some developed countries determined that, Salmonella species and Campylobacter jejune cause bacterial 'Gastroenteritis' among human. In some researches published in 1998, % 29.1 of 'Gastroenteritis' victims are caused by Salmonella which are encountered in raw chicken and other meat products. Chicken skeletons faces crosswise contamination risk after cutting, and Salmonella which are over skin abundantly can contaminate other skeleton surfaces, equipments used during process, and personnel (Karajan and Seldon, 2000).

In chicken meat products, to increase shelf life and decrease pathojen bacteria count, diversity of methods are applied. Among these methods chemical methods are the most important: Chlorine, TSP and some organic compounds, hAlsogens, hydrogen peroxide, alcohol, ozone and nisin are the disinfectant materials that are used for these purposes. In one study, it is observed that lactic acid and hot water applications in red meat decreases Eshericia colitis, Salmonella, mesopHilic aerobic bacteria count 1.1 1.8 1.5 log cfu/g respectively (Mullerat and other, 1994; Natrajan and Sheldon, 2000; Pohlman and other, 2002).

2.2) Microbiologic Criteria in Nutrition's:

Microbiologic limitations are always taken into account for food safety and product quality. Because of that 'aerobic colony count' or 'aerobic plate count' which is known as mesopHilic aerobic bacteria, count of indicator organism (Enterobacteriaceae, E. colitis, and Listeria spp.), pathogen (Salmonella, Campylobacter, E. colitis O157:H7), vibrio types, Listeria monocytogenes, Clostridium perfringes, Bacillus cereus and other pathogenic Bacillus types are very important in terms of microbial quality in 'ready to eat' foods. Some limitation s for these types of foods is shown below in table 2.2 (Gilbert and other, 2000):

Table 2.2: Limitations for some ready-to-eat products for microbial quality.

Criteria	**Microbial Quality(cfu/g)**			
	Desired	**Suitable**	**Not Suitable**	**Not Acceptable**
MesopHilic Aerobic Bacteria	$< 10^6$	$< 10^7$	$\geq 10^7$	-
Enterabacteriaceae	< 100	$100 < 10^4$	$\geq 10^4$	-
Escherichia colitis (total)	< 20	$20 < 100$	$\geq 10^0$	-
Listeria Spp. (total)	< 20	$20 < 100$	$\geq 10^0$	-
Salmonella Spp.	Negative at 25g	-	-	Negative at 25g
Campylobacter Spp.	Negative at 25g	-	-	Negative at 25g
Escherichia colitis O157: H7	Negative at 25g	-	-	Negative at 25g
Vibrio chlorea	Negative at 25g	-	-	Negative at 25g
Vibrio parahemolyticus	< 20	$20 < 100$	100 <1000	≥ 1000
Listeria Monocytogenes	< 20	$20 < 100$	-	≥ 100
StapHylococcus Aureus	< 20	$20 < 100$	$100 < 10^4$	$\geq 10^4$
Clostridium Perfringens	< 20	$20 < 100$	$100 < 10^4$	$\geq 10^4$
Pathogenic Bacillus types	< 1000	$1000 < 10^4$	$1000 < 10^4$	$\geq 10^5$

MesopHilic Aerobic Bacteria:

If there are lots of mesopHilic bacteria in food material, possible micro organism types must be identified. By means of results taken from here, some information can be got from the entire food sample (Gilbert and other, 2000).

Enterobacteriaceae:

It is evaluated as a hygiene and contamination indicator after processes of food. It can be identified easily as a taxonomic. In Enterobacteriaceae test, Salmonella type micro organisms can be identified this differs it from coli form test. It is not accepted as criteria for fresh vegetables, salads, fruits and sandwiches which include these foods. Because fresh vegetables and fruits includes these organisms because of their natural flora (Gilbert and other, 2000).

Salmonella spp. and E. colitis:

In food materials there must not be Salmonella and E. colitis O157:H7 from gram negative bacteria. If the product is cooked well and rules of hygiene are completely ordered, in last product, expected none of these organisms. (Gilbert and other, 2000; Kim and other, 2000a)

2.2.4) Vibrio Types:

They become very important microbial criteria by means of European Council. Because vibrato cholerea is isolated in some fruits and fresh vegetables that are exported to European countries (Gilbert and other, 2000).

2.2.5) Listeria Monocytogenes:

The ratio 100 cfu/g of Listeria Monocytogenes is very dangerous for human health. It is also the number that shows the violation of the hygiene rules in food preparation and store processes (Gilbert and other, 2000; Kim and other 2000a).

2.2.6) Clostridium perfringes:

Now the number accepted for this micro organism in foods is 20 cfu/g instead of 10 cfu/g (Gilbert and other, 2000).

2.2.7) StapHylococcus Aureus:

It can be included in raw meat products, fish, milk, cheese. It can be poisonous when its amount is about 10^3-10^{10} cfu/g. (Shelton and Shapton, 1991).

Surface Decontamination Applications in Foods:

In fresh consumed vegetables, meat and meat products, there are lot of micro organisms over the surface. By means of decontamination method applications, number of them can be decreased even their growth is decreased too. In recent years for decontamination; washing with disinfectant solutions, dehydration, high pressure radiation, UV heat process methods are used. But using chemicals is mostly used one (Capita and other, 2002; Robinson and other, 2000).

Washing with disinfectant is used for many years in decontamination of foods. It can be used either by dipping the food for a while into the disinfectant solution or by spraying the solution in a certain concentration over the surface of the food (Brackett, 1992).

For surface decontamination in nutrition's; Lactic acid (2-10 %), peroxiaceticacid (200 ppm), and acetic acid (2 %) and chlorine compounds such as chlorine dioxide (200 ppm), Hypo chloride (100-200 ppm) and hAlsogens like $I_{2,}$ Br (200 ppm) Hydrogen peroxide (5 %), ammonium compounds like Quaternary, Alkaline disinfectants like TSP and EDTA and some enzymes and bacteriosine **and acidic ion water** of which studies are still go on are used.

Success of decontamination is related to some factors. Heat of the disinfectant solution, disinfection duration, solution PH, the type of the disinfectant and the structure natural flora of the product (Temiz 2000).

Chemicals used For Decontamination:

Organic acids, disinfectant included free chlorine, EDTA, bacteriosine, some enzymes, ***acidic electrolyzed ion-water***, hAlsogens, hydrogen peroxide and permanganate.

2.3.1.1) Organic acids:

Organic acids are preferred as a disinfectant aside from preservative in food industry. They prevent the intake of the foods that are required for micro organism and it effects as an inhibitor. In food industry the acids that have low toxicity. (Russell, 1992).

Weak acids that are lypopHilic show strong antimicrobial property in low PH values. Because the dissolved acid concentration is high in the area as the PH decreases inactivation, speed of acids increases. For example acid has a pH lower than 3.5 speeds of inactivation increases ten times in every 0.3 decrease in pH. Also the pKa values affected their speed too. Also the pKa values affect their speed too. You can see pKa values of some organic acids which are used for antimicrobial-disinfection purposes in table 2.3 (Shapton and Shapton, 1991).

Table 2.3: pKa values of some organic acids which are used for antimicrobial disinfection purposes:

Acid Ester	**PKa**
Acetic Acid	4,7
Prop ionic Acid	4,8
Sorbic acid	4,8
Lactic acid	3,8
Benzoic acid	4,2
Salicylic acid	3,0
Dehydroaceticacid	5,4
H_2SO_4	1,8

Metile p-hydroxibenzoic acid	8,5
Propil p-hydroxibenzoic acid	8,1

Lactic acid and aseticacid solutions are used for decontaminations of skeletons after cutting. Also lactic acid, citric acid has a property of inhibition of mikotoxine. Also % 1-3 of sodium lactate can increase the shelf life of the meat products by preventing the growth of pathogen bacteria (Russel, 1992).

Acetic acid is an organic acid which prevents growth of bacteria, yeast and mood. Its preservative effect is higher than lactic acid. Some acetic acid salts such as calcium acetate sodium diacetate and oxide acetic acid derivatives such as per acetic acid are the other disinfection materials which are used in food industry. Since acetic acid and some other organic acids are lyopHilising, they cause stronger denaturation than mineral acids that have same hydrogen concentration and as a result provide inactivation. (Luck and Jager; Russel, 1992).
Lactic acid is one of the hydroxiacids that are used for disinfection. It is used in food industry because of cheap, and also it is in GRAS statue. It includes sodium, calcium and potassium salts (Russel, 1992).

2.3.1.2) Chlorine Compounds:

Chlorine compounds are used in food industry extensively. And some of them are HOCl, Cl_2, NaOCl, $Ca(OCl)_2$ and chlordioxide. These are used in decreasing of microbial load of some fresh vegetables, red meat, chicken meat and some sea products. Chlorine can show bactericide effect vastly in a short time (several minutes) (Block and Febiger, 1991; Russell, 1992).
Some chlorine compounds can inactivate bacterial growth in suitable concentrations because they can penetrate easily. They not only used in surface decontamination of food but also disinfection of tap water and sanitarization of nutrition process equipments (Russell 1992).
Chlorine provides inactivation by forming N-chloral compounds using protein connections in cell membrane, by providing diffusion of in-cell compounds out of cell

from cell membrane and by destroying cell membrane. And also it oxidises the SH groups of enzymes of bacteria and since the reaction is not reversible, this process destroys the micro organism (Block and Febiger, 1991).

Fresh vegetables and fruits can be decontaminated by using chlorine in suitable concentrations without decreasing product quality. By applying suitable chlorine to the food, micro organism population can be decreased and the product safety can be provided without decreasing food value(Block and Febiger, 1991). Sensitivity degree of some micro organisms against chlorine shown in table 2.4 (Gardner, 1991).

Table 2.4: Sensitivity degree of some micro organisms against chlorine:

Gram-positive bacteria	Very sensitive
Gram-negative bacteria	Very sensitive
Acid-fast bacteria	Low sensitive
Bacteria spore	Sensitive (PH 7.6 optimum)
LyopHilise viruses	Sensitive
HydropHilic viruses	Sensitive (high concentration)
'Amoebic cysts', algaes	Sensitive
Fungi	Low sensitive
Prion	Low sensitive (in high concentrations)

Effectiveness of chlorine based disinfectants depend on some factors such as PH and concentration, organic material existence, robustness and heat of the water used (Gardner, 1991). The effect of concentration and PH can be evaluated together. NaOCl which has a PH 7.6 and 100 ppm or PH 9 and 1000 ppm can easily inactivate Bacillus subtilis (Gardner, 1991).

Increase in PH can degrade biotical effect of chlorine solution. For example in one study free chlorine concentration with PH 8.2 is enough for inactivation of Bacillus metiens. But when the PH increased up to 11.3, for same amount of bacteria 1000 ppm is used for inactivation (Block and Febiger. 1991).

Chlorine can interact almost all organic compounds (blood and some tissues included) the concentration of chlorine that is used for disinfection must be high (Block and Febiger, 1991; Gardner, 1991).
The alkaline minerals such as calcium magnesium that gives water robustness never affect the activations of disinfectants with chlorine. The chlorine type that is in solution also affects the decontamination. The chlorine in water can be classified in three groups: free, connected and total chlorine. Free chlorine can be in three forms in water: 1) -elemental chlorine 2) -HOCl 3) -hypochlorite ion (Block and Febiger, 1991).
Ammonium that is in water naturally associated with chlorine and forms N-chloral compounds. They are called connected chlorine. Free chlorine and connected chlorine together are called total chlorine (Block and Febiger, 1991).

Disinfection Power of chlorine compounds is depends on free-chlorine concentration included. Free-chlorine is defined as the elemental chlorine concentration which is required to form one molecule sodium hypochlorite. In other definition, free chlorine can be described as a measure of oxidation capacity (Block and Febiger, 1991 and Gardner, 1991).
In a sand mixture, free chlorine concentration can be defined as (w/w) percentage but in solutions it is described as (w/v) percentage, million per ppm. When you want to use a disinfectant in sand form, you should use water in a certain volume (Gardner, 1991).

$Cl_2 + 2e^- \longrightarrow 2Cl^-$ (Equation 2.1**)**

In equation 2.2 1 mole hypochlorite interacts with two electron and forms chlorine ion.

$OCl^- + 2e^- \longrightarrow Cl^- + H_2O$ (Equation 2.2)

So it is shown above that 1 mole hypochlorite equals to 1 mole elemental chlorine and 70.91 gr free chlorine is included in it (Block and Febiger, 1991).

Since calcium hypochlorite and sodium hypochlorite include 2 and 1 mole hypochlorite respectively, they also include 141.8 and 70.9 gr free chlorine respectively.
The molecule weight of calcium hypochlorite and sodium hypochlorite are 143 and 74.5 respectively so they include 99.2 % and 95.8 % free chlorine (Block and Febiger, 1991).

The free chlorine measurement techniques are: iodometric, sodium arsenic, ortotolidine, Palin's DPD method (the colour of N, N-dietile-p-fenilen-diamine reactive converts into red from pink in existence of free chlorine), amperometric method, polo grapHic membrane technique (a plastic probe that includes an anode and an electrolyte is inside the membrane which is surrounded by a metal cathode). In the last one, when the probe is immersed into the chlorine solution, Chlorine is reduced to chloral in cathode. And the current that is produced is linear also it can be displayed and read by screen of analysis device. The advantages of this method are: safety, big efficiency acquisition with low price, different types of chlorine types.

Elemental chlorine, water and hypochlorite can form HOCl as shown below: (Block and Febiger, 1991)

$$Cl_2 + H_2O \longrightarrow HOCl + H^+ + Cl^- \quad (2.3)$$

$$Ca(OCl)_2 + H_2O \longrightarrow Ca^{++} + H_2O + 2OCl^- \quad (2.4)$$

$$Ca(OCl)_2 + 2H_2O \longrightarrow HOCl + Ca(OH)_2 \quad (2.5)$$

$$HOCl \rightleftharpoons H^+ + OCl^- \quad (2.6)$$

Chlorine and some chlorine-releasing compounds are formed by HOCl which is in solution with PH 5-8 and they show antimicrobial effect. Hypochlorites ions are generally in high alkaline area and generally do not show activity.

pH is an important criterion for disassociation of HOCl. As the pH increases disinfectant effect of chlorine decreases but at the same time increases parallel related to the concentration increase in dissolved HOCl (Block and Febiger, 1991).

Hypochlorite can diffuse into the bacteria cell than interact with the protoplasm and forms toxic compounds. A 10 degree increase in heat of hypochlorite solution decreases the time by half which is required for the inactivation of bacteria (Block and Febiger, 1991; Gardner, 1991).

In a study, the growth of pathogen bacteria such as Listeria, Yersinia and Campylobacter are limited when the foods are decontaminated by hypochlorite. (Block and Febiger, 1991).

2.3.1.3) Hydrogen Peroxide:

It is naturally found in the honey and milk. And protects the product against contamination. It has a biocide effect on bacteria, yeast, mould viruses and spores (Block and Febiger,1991;Gardner and peel, 1991).

It is a colourless liquid which has a 34.01 molecule weight and also soluble in water. It is disinfectant. Antimicrobial effect depends on: decontamination degree, pH, heat and exposure time. Because of its strong oxidative property it has a stronger effect on bacteria than yeast and mould. It is also preferred in decontamination of materials used in packaging of some fruit juice in aseptic packaging industry (Russell, 1992).

Hydrogen Peroxide forms compounds such as water and oxygen that are not toxic by interact with some enzymes such as catalyze and peroxides. Anaerobic bacteria are more sensitive to Hydrogen peroxide because they do not synthesize the catalyze enzyme which is used to destroy hydrogen peroxide. In some studies it is determined that hydrogen peroxide of 3 % causes inactivation on bacteria fast whereas causes inactivation on yeast, viruses and bacteria spores slowly. Generally gram-negative bacteria are more susceptible to hydrogen peroxide than gram-positive bacteria. The increase in heat and concentration gives successful results on decreasing spores count (Block and Febiger, 1991).

Other than it can be used as a solution for washing purposes, also can be applied as a steam over vegetables and fruits for decontamination. It is not harmful for the food if it issued in suitable concentration. In a study it is determined that, the washing of mushrooms by 5 % hydrogen peroxide, decreases the Pseudomonas count by 90 % (Sapers and Simons, 1998).

Hydrogen peroxide shows a synergy with some chemicals. In a study it is shown that Clostridium bifermentas spores can form 0.028 % colony when 100 μm $CuSO_4$ at 25°C is mixed with 0.28M hydrogen peroxide. But when only with $CuSO_4$ 95 % and with only hydrogen peroxide this ratio is 87 %. There is a 3000 times decrease when they are used together. (Block and Febiger, 1991).

Another example for synergy, the effect of hydrogen peroxide increases with heat. And also it makes spores more sensitive to heat and the heat causes destruction over organism. It can also exhibit synergic effect with UV radiation. When using 0.3 % hydrogen peroxide and UV radiation together over spores decreases the count

2000 times greater than applying only UV radiation and 4000 times greater than applying only hydrogen peroxide (Block and Febiger, 1991).

It can be also used for sterilization of water in low concentrations. In a study it is found that 0.1 % hydrogen peroxide decreases the bacteria count in raw milk 99.99 % also 100 % decrease in count of Salmonella, Clostridium, coli form, StapHylococcus. Also in literature you can encounter some information that proves the 10-25 % concentration of hydrogen peroxide can provide inactivation of spores (Block and Febiger, 1991).

EDTA (Etilendiamine Tetra Acetic Acid)

EDTA is used in disinfection of food Alsone. But since it shows a synergic effect with other antimicrobial material it is preferred to use together. It makes the entrance of antibacterial material easy by affecting the cell membrane of micro organisms. For this purpose sodium and calcium salts are preferred. At high concentrations it is not toxic generally with 300 ppm or 1-20 mM EDTA concentration, decontamination applications are done successfully (Luck and Jager, 1997).

In a study to examine the antibacterial effect of EDTA, 15 bacteria types such as Esheria colitis, Enterobacter cloacae, citrobactere Fruendii, Pseudomonas aeruginosa, salmonella spp., Proteus mirabilis, Proteus vulgaris seratia marcescens, vibrio cholerae NIH41-01, vibrio chlorea-01 ‘methalicin’ resistant StapHylococcus aureus and StapHylococcus auerus ATCC 25293 are used. For this purpose EDTA 10mM solutions at StapHylococcus aureus PH 5,7,9 are interacted with bacteria and as a result it is found that at PH 5, vibrio cholerae StapHylococcus aureus; at PH 9 Pseudomonas aeruginosa and Esheria colitis are sensitive to EDTA solution and at other PH values Proteus mirabilis is sensitive to this solution. As a result of this sensitivity the growth of bacteria is inhibited (Kida and other, 1992).

In other study it is determined that at all concentrations changing between 1-20 mM EDTA solutions, when it is used Alsone or and with nisine the growth of Arcobacter butzleri is inhibited (PHillips and Duggan, 2001).

2.3.1.5) Trisodium PHospHate(TSP)

It is an alkaline sanitizer and by means of effect like detergent it is used to remove the oil over the surface of food like meat. And also shows antibacterial effect because of its high PH. Especially in chicken meat there is acquired successful results by dipping it 15 minutes into the % 8-12 TSP solution causing inhibition of growth of Salmonella, Escherichia colitis, StapHylococcus, Campylobacter. Before process the Salmonella count is 35 % but after process it became 1 %. Also it is possible to encounter some studies in literature that shows by dipping fresh vegetables and fruits into TSP solutions, surface decontamination is made (Dickson and other 1994; Zsang and Farber, 1996; Capita and other 2002).

2.3.1.6) Acidic Electrolyzed Water:

Acidic electrolyzed water is acquired by electrolyze of diluted sodium chlorine solutions by means of electrolyze device which has anode and cathode that are isolated by a membrane. It is also known as super oxide water and it is produced in the anode part of device. It has PH 2.7 or lower, and ORP values more than 1000 mV and also a free chlorine concentration between 10-100 ppm (Al hag and other, 2002, Buck and other, 2003; Kim and other, 2001; Kim and other, 2000a; Kim and other, 2000b; Kiura and other, 2002; Koseki and other, 2001; Len and other, 2002; Morita and other, 2000; Sharma and Demirci, 2002).

After lot of studies the antibacterial and antiviral effects of this water is determined. It is preferred in stabilization of foods which are not sterilized by heating methods. It is used not only for disinfection of fresh vegetables and fruits but also the disinfection of surfaces which the foods are stored or places over where the foods are set. For example it is proved that the acidic electrolyzed water inhibit the growth of L.

monocytogenes bio film over steel (Koseki and other, 2001; Park and other, 2002a; Jung and other, 1996).

Electrolyzed water is made from sodium chlorine as a chemical material. Because of that it is not harmful for the environment. HOCl that is included by it, is weak acid and it is not hydrolyzed easily like OCl^- that is less active (Koseki and other, 2001; Koseki, 2002).

In acidic electrolyzed water ORP is one of the important factors that provide the microbial inactivation. The more ORP value, the more inactivation. Also the free chlorine amount is important in terms of antimicrobial activity. HOCl forms OH radical and OH radical inhibits the micro organisms by means of oxidative property (Koseki and other, 2001).

2.3.1.7. Permanganate

Permanganate is one of the inorganic peroxide compounds which are used in the nutrition industry. It has an antibacterial, antifungal and antiviral effect. It is also used for the disinfection of some fresh vegetables. Because of its dense purple colour, the use of permanganate with other disinfectants is limited. Moreover, it is thought that the pink colour of the washing water is an indicator of the correctness of the procedure (Soriano and others, 2000; Block and Febiger, 1991).

2.3.1.8. Bacteriosines

Bacteriosines, which are produced by the gram-positive and gram-negative bacteria's, are protein complexes having a characteristic of potential bactericide since they cause the growth of bacteria's to be inactive. Recently, they are begun to be used in the nutrition industry for the purpose of decontamination. Their antibacterial effect changes according to the bacteria type by which they are produced. As they are protein originate, it is easy for the digestion enzymes to metabolize them. Therefore, it is thought that bacteriosines are safe. The most frequently used bacteriosine is nisin and it is used for the inhibition of some pathogenic micro organisms such as *Listeria monocytogenes*, *Bacillus and Clostridium* (Luck and Jager, 1997; Russell, 1992).

2.3.1.9. Some Enzymes (Lysosime)

Lysosim is one of the “muramidaz” types in the nutrition industry and used as an antimicrobial substance and disinfectant. Through affecting the permeability of cell membrane, it shows a maximum antimicrobial effect at pH 7. Its usage is limited since it is expensive. It is known that lysosim enzyme prevents especially the growth of *Listeria monocytogenes* in several foods. (Luck and Jager, 1997; Russell, 1992).

3. MATERIAL and METHOD

3.1. Nutrition Samples

The lettuce and chicken samples used in the experiments are obtained from a supermarket in Istanbul and through being kept at cold (<5 ºC), it is taken to be analyzed in 24 hours. In order to equalize the surface of the samples, lettuce leaves are cut at around 2.7 cm diameters, whereas chicken breast meat samples are cut around 5x5 dimension and 0.5 cm thickness.

3.2. Disinfectant Substances and Feeding Areas

The disinfectant substances used in the surface decontamination are shown in Table 3.1 with their prepared concentrations.

Table 3.1. Disinfectant substances used in the surface decontamination and their prepared concentrations

Disinfectant Material	Concentration Prepared
Acetic acid (glacial 100 %), (Riedel-de Haen, Seelze)	1 % and 2 % (v/v)
Lactic acid (90 %) (Merck, Darmstadt, Germany)	2 % (v/v)
Sodium Acetate (sodium acetate anhydrous), (Merck, Darmstadt, Germany).	1 % and 3 % (w/v)
Sodium Lactate (50 % w/w), (Merck, Darmstadt, Germany).	3 % (v/v)
EDTA (extra pure), (Merck, Darmstadt, Germany)	20 mM

TSP ($Na_3PO_4.12H_2O$) (Merck, Darmstadt, Germany).	12 % (w/v)
H_2O_2 (v/v 30 %), (Merck, Darmstadt, Germany).	1 %and 2.5 % (v/v)
Sodium hypochlorite(*)	200 ppm (free chlorine concentration)

*) one of the detergents is used Acidic electrolyzed water is prepared in order to contain 20 and 30 ppm free chlorine through electrolysation of 1 % and 1.5% (w/v) NaCl solutions with 3 ampere current, and IONFARMS `Gold´ HTH5000 machine (GWN Co. Ltd., Korea).

The pH values of the solutions are measured with “Jenway, 3010 pH meter” (Jenway Ltd., England) pH meter machine. While ‘HANNA, HI 98201 ORP meter’ (Hanna Instruments, Mauritius), ORP meter machine is used for the measurement of ORP values, free chlorine concentrations of the solutions are determined by CHEMets Kit Chlorine, K-2500 (CHEMETRICS Inc., USA) kits.

In the studies, as feeding areas, standard APHA PCA (Plate Count Agar) (Oxoid, Hampshire, England) feeding area is used for the analysis of mezophilic aerobic bacteria, whereas URBGA (Violet Red Bile Glucose Agar) (Oxoid, Hampshire, England) is used for coli form bacteria analysis. Baird Parker Agar (bioMerieux, France) is used for the analysis of *StapHylococcus aureus* and BSA (Bismuth SulpHite Agar) (Oxoid, Hampshire, England) feeding area is used for the analysis of *Salmonella typHimurium*.

The compounds of TSB (Trip tic Soy Broth) and PBS (PhospHate Buffered Saline) which are prepared by us and used in the studies, are given below. The prepared solutions are sterilized at 121 ºC in 15 minutes (Anon, 2000).

TSB (Trip tic Soy Broth), (pH 7.3)

Compound	G/l
Peptone from casein (Oxoid, Hampshire, England)	17 g
Peptone from soybean meal (Oxoid, Hampshire, England)	3 g
NaCl (Merck, Darmstadt, Germany)	5 g
K_2HPO_4 (Merck, Darmstadt, Germany)	2,5 g
D + glucose (Merck, Darmstadt, Germany)	2,5 g

Compound	G/l
NaCl (Merck, Darmstadt, Germany)	7,650 g
K_2HPO_4 (Merck, Darmstadt, Germany)	0,210 g
Na_2 HPO_4 (Merck, Darmstadt, Germany)	0,724 g

In the preparation of delusion solutions, NaCl (Merck, Darmstadt, Germany) and peptone (Oxoid, Hampshire, England) are used.

3.3. Bacteria Cultivations and Inoculation Preparation

In this study, *StapHylococcus aureus* ATCC 25923, *StapHylococcus aureus* ATCC 29213 and *Salmonella typHimurium* ATCC 14028 such are used.

In order to prepare inoculation, 1 sample eye is taken from the bacteria cultivations which are kept in PCA at 4 ºC, and it is incubated for 24 hours at 35 ºC through transferring into 10 ml TSB two times with 24 hours interval. 0.5 ml is taken from the mixture of equal proportions (1:1) of *StapHylococcus aureus* such and for an active homogenization, one by one, with 10 ml, 240 ml and 250 ml PBS it was diluted gradually. At the end, totally 500 ml PBS and 1:1000 proportioned bacteria

suspension is prepared. The same procedure is repeated for *Salmonella typHimurium* suspension.

3.4. Equalization of the Micro organism Load of Nutrition Samples

As it is explained in part 3.1, without being waited in incubator the prepared lettuce and bacteria included uncooked chicken meat samples and after making the prepared chicken meat for the other analysis at 35 ºC for 6 hours wait in the incubator, in order to equalize the micro organism loads, they are mixed with distilled water for 15 minutes 2-3 times at 1:1 proportion. Then they are kept and waited in sterilized “stomacher” bags and are taken for analysis after their juice is strained.

3.5. The Inoculation of Lettuce and Uncooked Chicken Meat

The lettuce and bacteria suspension (*Staphylococcus aureus* and *Salmonella typhimurium*) in equal weights are mixed 30 times in a sterile closed glass jar for 15 minutes. After their juice is strained, they are kept closed in a freezer (SANYO Medical Freezer, MDF-U441, SANYO Electric Co., Ltd., Japan) at 4 ºC for 24 hours.

For the inoculation of uncooked chicken meat, 0.1 ml is taken from the prepared bacteria suspension (*StapHylococcus aureus* ATCC 25923 and *StapHylococcus aureus* ATCC 29213) and it is speeded over the meat surface. After it is waited in the sterile Petri boxes at room temperature for 30 minutes, is taken for the analysis.

3.6. Application of the Surface Decontamination to Lettuce and the Analysis of Mezophilic Aerobic Bacteria

10 g from each prepared lettuce samples are put into ”stomacher” bags and are kept for 15 minutes through shaking 2-3 times and through adding 200 ml from each 12% trisodium pHospHate, 1% acetic acid, 1% sodium acetate, 200 ppm sodium hypochlorite, 5% hydrogen peroxide, 40% apple and grape vinegar solutions onto them. As a control, distilled water is used. After disinfectant solutions are strained, samples are homogenized with 90 ml pep toned water in the stomacher (Stomacher

400 Lab Blender, England) for 2 minutes and series delusions (10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) are prepared. With dropping record method and using feeding area PCA, sowing is done and after the feeding area is become solid, bacteria numbers are determined through reversing the Petri boxes and incubating in the incubator (GENLAB, INC/160/CLAD/F/D, England) at 35 ºC for 48 hours (Anon, 2001).

3.7. Application of the Surface Decontamination to Uncooked Chicken Meat, Mezophilic Aerobic Bacteria and Coli form Bacteria Analysis

Prepared chicken meat pieces which have equal surface area (approximately 25 cm^2) are put into stomacher bags, 200 ml from each 2% acetic acid and lactic acid, 3% sodium acetate and sodium lactate, 12% TSP, 1% and 2.5% hydrogen peroxide, 200 ppm sodium hypochlorite and 20 mM EDTA solutions are added and for 15 minutes through shaking 2-3 times, they are waited. Disinfectant solutions are removed through straining and then at 1:1 proportions they are homogenized in the stomacher with 0.1 % pep toned water and from these mixture series delusions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} ,10^{-5} and 10^{-6}) are prepared. As a control distilled water is used. With dropping record method and using PCA feeding area, sowing is made and after feeding area is become solid, the number of mezophilic bacteria is determined through reversing the Petri boxes and incubating in the incubator at 35 ºC for 48 hours (Anon, 2001).

For the coli form bacteria analysis, the same procedures are repeated and then with dropping record method and using VRBGA feeding area, double sowing is done. After feeding area is become solid, through reversing the Petri boxes and incubating in the incubator at 35 ºC for 24 hours, the number of coli form bacteria is obtained (Anon, 2001).

3.8. Applications of Surface Decontamination in the Lettuce and Uncooked Chicken Meat with Acidic Electrolyzed Water

The lettuce and uncooked chicken meat samples, which are prepared as explained in part 3.5, are strained after being waited through being shacked 2-3 times for 10 minutes with acidic electrolyzed water (AEW) containing 200 ml 1% and 1.5% NaCl,

and 200 ppm sodium hypochlorite solution. Then mezophilic aerobic bacteria analysis and coli form bacteria analysis are made and micro organism numbers are determined (Anon, 2001).

3.9. Applications of Surface Decontamination in the Staphylococcus aureus Included Lettuce and Uncooked Chicken Meat

Lettuce samples in which *StapHylococcus aureus* is included previously, are waited in the 200 ml (1:20 proportion) 2% acetic acid and lactic acid, 200 ml 3% sodium acetate and sodium lactate, 200 ml 12% TSP, 200 ml 5% hydrogen peroxide, 200 ppm sodium hypochlorite and 20 mM EDTA solutions for 15 minutes through being shacked 2-3 times. After they are strained, incubation is made at 35 ºC for 48 hours through sowing from each delusion (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) into the Petri boxes including Baird Parker Agar feeding area with spreading record method. Then bacteria number is obtained (Anon, 2001).

Uncooked chicken meat pieces in which *StapHylococcus aureus* is included, are waited through being shacked for 15 minutes 2-3 times with 1:10 proportioned 2% acetic acid and lactic acid, 200 ppm sodium hypochlorite, 12% TSP and 2.5% hydrogen peroxide solutions. After they are strained, incubation is made at 35 ºC for 48 hours through sowing from each delusion (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) into the Petri boxes including Baird Parker Agar feeding area with spreading record method. Then bacteria number is obtained (Anon, 2001).

3.10. Applications of Surface Decontamination in the Sample of Salmonella Included Lettuce

To the *Salmonella* included lettuce samples, 200 ml from each 2% acetic acid and lactic acid, 8% TSP, 5% hydrogen peroxide and 200 ppm sodium hypochlorite solutions are applied for 15 minutes through being shacked 2-3 times. After they are strained, incubation is made at 35 ºC for 24 hours through sowing from each delusion (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) into the Petri boxes including Bismuth SulpHite Agar feeding area with spreading record method. Then bacteria number is obtained (Anon, 2001).

3.11. The Determination of the Inhibition affects of the Disinfectant Solutions on the Staphylococcus aureus and Salmonella typhimurium such By Agar Diffusion Method

In order to examine the inhibition effects of the disinfectant solutions, agar diffusion method is used. Through using PCA feeding area with dropping record method and determining the number of micro organisms as approximately 10^6 kob/ml in every Petri, 1 ml sowing is done from the mixture of *StapHylococcus aureus* ATCC 25923 and 29213 sush (1:1) and from *Salmonella typHimurium* ATCC 14028 suspension. After the feeding area is become solid in the cold, through making 6 mm diameter holes in the agar, 0.05 ml is added from the solutions of 200 ppm sodium hypochlorite, 2% acetic acid and lactic acid, 8% and 12% TSP, 2.5% and 5% H_2O_2.

For control purpose, distilled water is used. In order for the agars to absorb the solutions, Petri boxes are waited for 30 minutes without being moved and are incubated at 35 ºC for 24 hours without being reversed. After the incubation, evaluation is made through measuring the zones (mm) occurred around the holes.

3.12. Statistical Analyses

All analyses are made parallel and two times. In order to test whether the disinfectant solutions and the average of micro organism numbers found in the samples after the application are different from each other statistically, ANOVA test is applied at 0.05 significance level. For the evaluation of different averages, Duncan multiple comparison test is used and comparison is made.

4. FINDINGS and DISCUSSION

4.1. pH and ORP Values of Disinfectant Solutions, Free Chlorine Concentrations

pH values of the disinfectant solutions that are used in the experiments and ORP values of some solutions together with free chlorine concentrations are shown in Table 4.1

Table 4.1. pH and ORP values of disinfectant solutions and free chlorine concentrations

Disinfectant material	PH	ORP(mV)	Free Chlorine
Control (Water)	5.8 ± 0.04	455 ± 3	0
Acetic acid 1 %	2.7 ± 0.01	No measurement	-
Acetic acid 2 %	2.6 ± 0.01	-	-
Lactic acid 2 %	2.1 ± 0.01	-	-
Sodium acetate 1 %	7.5 ± 0.04	-	-
Sodium acetate 3 %	8.5 ± 0.05	-	-
Sodium Lactate 3 %	6.4 ± 0.02	-	-
EDTA 20 mM	2.9 ± 0.14	-	-
TSP 12 %	12.9 ± 0.07	-	-
TSP %	12.1 ± 0.09	-	-
H_2O_2 %1	5.5 ± 0.04	-	-
H_2O_2 2.5 %	4.0 ± 0.02	-	-
H_2O_2 5 %	3.7 ± 0.02	-	-
Sodiumhypochloride	8.8 ± 0.06	-	200 ± 25
Acidic electrolyzed water (A)	2.7 ± 0.02	1099 ± 3	20 ± 5
Acidic electrolyzed Water (B)	2.7 ± 0.04	1100 ± 3	30 ± 5

(a): Figures are found with ± standard deviations through finding the means of each two measurements.

(b): Have not been measured.

(A): Acidic electrolyzed water prepared from 1% NaCl solution (free chlorine concentration 30 ppm)

4.2. Changes in the Number of the Mezophilic Aerobic Bacteria of the Lettuce Samples after the Applied Surface Decontamination

After the 15 minute applied surface decontamination to the lettuce sample who's beginning micro organism load is on average 5.21% $\log_{10}$ kob/g, the mezophilic aerobic bacteria numbers in the sample and their reduction amounts are shown in Table 4.2.1 and Figure 4.2.1. With the applied one way ANOVA test, it is obtained that there is statistically significant difference between different solutions in terms of means of micro organism numbers after the application ($p<0.05$). At the end of the analysis it is found that the strongest disinfectant effect is shown by the 5% H_2O_2 solution. Then come sequentially 1% acetic acid, 12% TSP, 40% apple vinegar, 40% grape vinegar, 200 ppm sodium hypochlorite and 1% sodium acetate solution.

Table 4.2. The number of mezophilic aerobic bacteria of the lettuce after the surface decontamination and reduction amounts

Disinfectant material	Bacteria count[i] after decontamination	Decrease in bacteria count [i](log)
Control water	$4.81^{a} \pm 0.11$	0,4
TSP 12 %	$2.78^{cd} \pm 0.11$	2,42
Acetic acid 1 %	$2.76^{cd} \pm 0.19$	2,44
Sodium acetate 1 %	$5.12^{a} \pm 0.12$	0,08
Sodium hypo chloride (200 ppm)	$4.07^{b} \pm 0.01$	1,14
Hydrogen peroxide 5 %	$2.37^{d} \pm 0.28$	2,84
Apple (40 %)	$3.21^{c} \pm 0.1$	2,00
Grape (40 %)	$3.32^{c} \pm 0.35$	1,88

i: Shows the average of two repetitions and ± standard deviations.

a-d: At 0.05 significance level there is no statistically significant difference between the means carrying the same letter Alsong the same column.

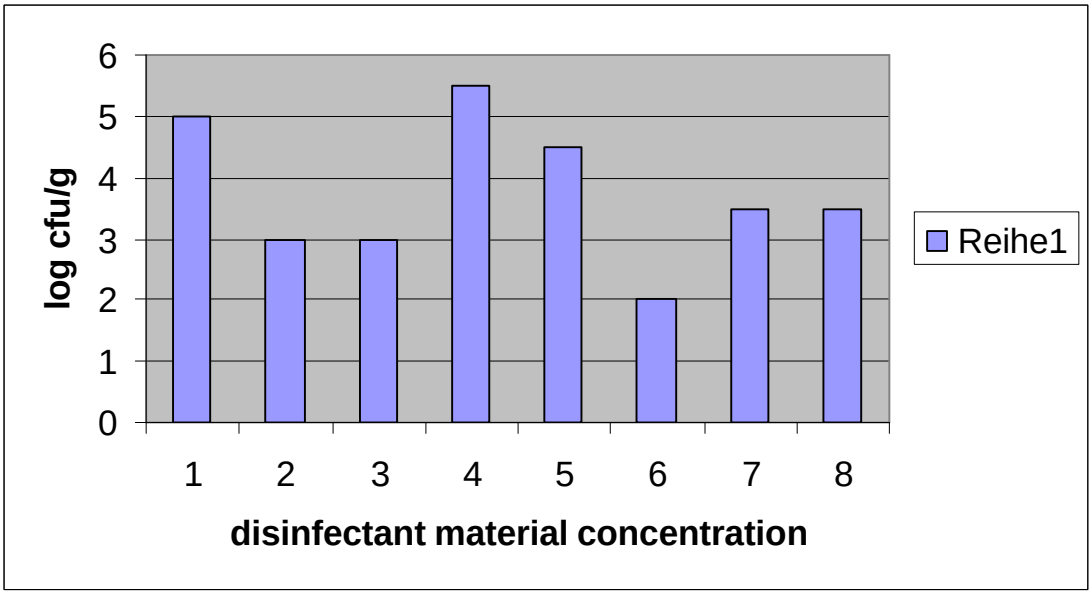

Figure 4.1. The number of mezophilic aerobic bacteria in the lettuce after the surface decontamination and reduction amounts. 1: control (distilled water), 2: trisodium pHospHate (12%), 3: acetic acid (1%), 4: sodium acetate (1%), 5: sodium hypo chloride (200 ppm). 6: hydrogen peroxide (5%), 7: apple vinegar (40%), 8: grape vinegar (40%).

In this study, when sodium hypo chloride solution containing 200 ppm free chlorine and 5% H_2O_2 solution are used, reduction in the micro organism amount is determined as 1.14 and 2.84 log. When these values are compared to the values defined by Cherry (1999) (for sodium hypo chloride and 5% H_2O_2 solution, sequentially 1-2 log and 3 log), it is observed that the results are similar to each other. In another study made by Li and others (2001) related to chlorine, the reduction in the amount of mezophilic aerobic bacteria is obtained as 1.73-1.96 when lettuce samples are decontaminated with chlorine water at 50 ºC containing 20 ppm free chlorine. These reduction amounts are important in terms of nutrition safety. On the other hand, Beuchat (1996) stated that chlorine water reduces the microbial load of fresh vegetables 10 to 100 times and therefore these decontamination methods can be cited as HACCP applications.

Hydrogen peroxide solution is also used for the decontamination of some fresh vegetables. For instance, in a study conducted by Sapers and Simons (1998), it is observed that there is a reduction of 90 % in the number of Pseudomonas after fungi are washed with 5% hydrogen peroxide solution. In this experiment, a change in the colour and characteristic of the lettuce samples after the decontamination with 5% H_2O_2 solution at room temperature. Similarly, in a study conducted by McWatters and others (2002), a negative change is not observed in the sensorial properties of the lettuce samples after they are layered into 2% H_2O_2 solution at 50 ºC.

In the experiment, 2.42 log active reduction is observed when 12% TSP solution is used. However, it is also observed that the colour of the lettuce samples darkened and the characteristic of them softened. In some studies related to this issue, it is stated that 1-10% TSP solution can be effective in surface decontamination of fresh vegetables (Cherry, 1999). Therefore, it may be thought that TSP solutions in concentrations less than 12% are suitable for surface decontamination.

In a study made by Leitao and others (1981), it is stated that there is a 98% reduction in the mezophilic aerobic bacteria amount in the surface of the lettuce that is decontaminated with vinegar containing 2% acetic acid. In our study, it is determined that there is sequentially 99% and 97% reduction in the mezophilic aerobic bacteria amount through the usage of 40% solutions of apple and grape vinegar containing 4-5 % acetic acid.
As a result of the decontamination with acetic acid, 2.42 log active reduction is observed in the number of bacteria and it is found that it is the second strongest disinfectant solution after the 5% H_2O_2 solution.

4.3. Mezophilic aerobic bacteria in the uncooked chicken meat after the surface decontamination and changes in the coliform bacteria number

After the 15 minute surface decontamination applied to uncooked chicken meat whose beginning mezophilic aerobic bacteria load is defined on average as 9.43 $\log_{10}$ kob/g and coli form bacteria load on average as 9.12 $\log_{10}$ kob/g, mezophilic

aerobic bacteria and coli form bacteria number in the sample and their reduction amounts are shown in Table 4.3.1 and Figure 4.3.1. As a result of the applied one-way ANOVA test, it is found that there is statistically significant difference ($p<0.05$) between the means of micro organism numbers after the application with different solutions.

As a result of the mezophilic aerobic bacteria analysis, it is obtained that 1% and 2.5% H_2O_2 and 2% lactic acid solution have the strongest disinfectant effect. Sequentially come 2% acetic acid, 12% TSP, 200 ppm sodium hypo chloride, 20 mM EDTA, 3% sodium acetate and sodium lactate.

As a result of the coli form bacteria analysis, it is obtained that 2.5% H_2O_2 solution has the strongest disinfectant effect. Sequentially come 1% H_2O_2, 2% lactic acid, 12% TSP, 2% acetic acid, 200 ppm sodium hypo chloride, 3% sodium acetate and sodium lactate and 20 mM EDTA solutions.

Table 4.3. The number of mezophilic aerobic bacteria and coli form bacteria in the chicken meat after the surface decontamination and reduction amounts.

Disinfectant material	MesopHilic bacteria count[i] after process	Coli form bacteria count[i] after process	Decrease in mesopHilic bacteria count((log)	Decrease in Coli form bacteria count(log)
Control (Water)	$7.14^{a} \pm 0.04$	$7.11^{a} \pm 0.04$	2.28	2.00
Acetic acid 2 %	$5.61^{bc} \pm 0.38$	$5.90^{abcde} \pm 0.30$	3.77	3.21
Lactic acid 2 %	$4.93^{c} \pm 0.32$	$5.03^{cde} \pm 0.78$	4.49	4.08
Sodium acetate 3 %	$6.74^{ab} \pm 0.01$	$6.44^{abc} \pm 0.02$	2.68	2.67
Sodium Lactate 3 %	$6.99^{ab} \pm 0.04$	$6.46^{abc} \pm 0.25$	2.43	2.65
EDTA 20 mM	$6.73^{ab} \pm 0.34$	$6.63^{ab} \pm 0.13$	2.69	2.48
Sodium hypo chloride (200 ppm)	$6.55^{ab} \pm 0.21$	$6.22^{abcd} \pm 0.24$	2.87	2.89

TSP 12 %	$6.01^{abc} \pm 0.44$	$5.35^{bcde} \pm 0.65$	3.42	3.76
H_2O_2 %1	$5.04^{c} \pm 0.04$	$4.85^{de} \pm 0.20$	4.38	4.26
H_2O_2 2.5 %	$4.86^{c} \pm 0.02$	$4.50^{e} \pm 0.05$	4.56	4.61

i: Shows the average of two repetitions and ± standard deviations.

a-d: At 0.05 significance level there is no statistically significant difference between the means carrying the same letter Alsong the same column.

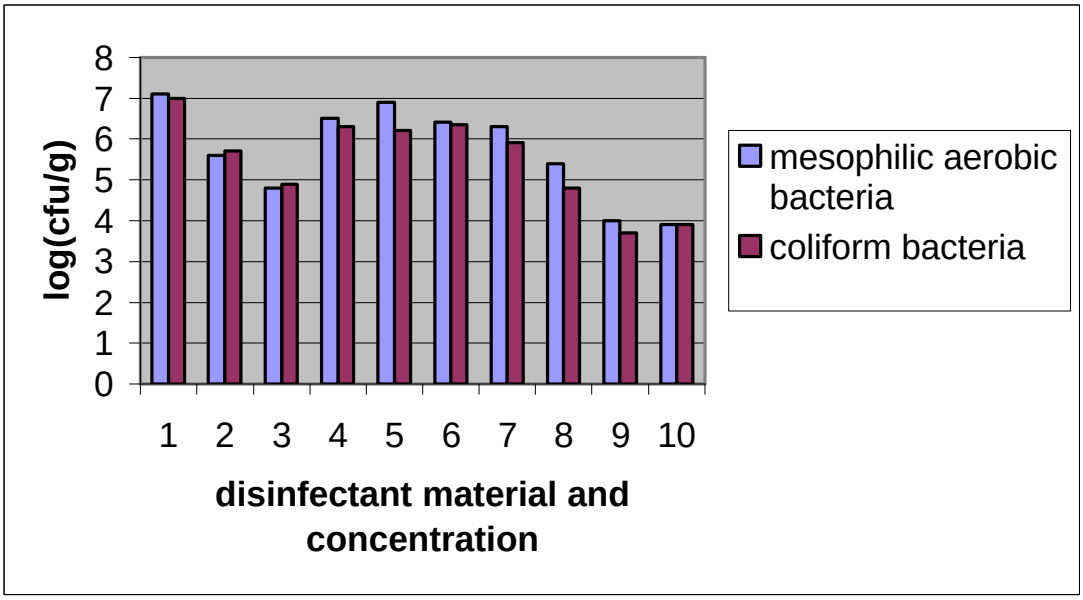

Figure 4.2 The number of mezophilic aerobic bacteria and coli form bacteria in the chicken meat after the surface decontamination and their reduction amounts. 1: control (distilled water), 2: acetic acid (2%), 3: lactic acid (2%), 4: sodium acetate (3%), 5: sodium lactate (3%), 6: EDTA (20 mM),7: sodium hypochlorite (200 ppm), 8: trisodium pHospHate (12%), 9: hydrogen peroxide (2.5%), 10: hydrogen peroxide (1%).

In this study, with the usage of sodium hypochlorite solution containing 200 ppm free chlorine, the reduction in the number of mezophilic aerobic bacteria and coli form bacteria is determined as 2.87 and 2.89 logs. Kenney and others (1995) sprayed water containing 200 ppm chlorine into the cattle skeletons. As a result, 0.4 log reduction in the number of mezophilic aerobic bacteria is observed. However, the

reduction is more in the study conducted with chicken meat. Although it is thought that the reason is the application of plunging method rather than the spraying method, Beuchat and others (1998) stated that in a study they conducted, the results of these two methods are similar.

For the decontamination of the chicken skeletons, chlorine, organic acids, bacteriosines, hydrogen peroxide, ozone, water, high pressure, radiation and UV radiation can be used. However, some of them are preferred less because of their limited applicability and consumer prejudice. In the studies, it is defined that trisodium phospHate does not affect the sensorial characteristic of the chicken meat (Capita and others, 2002). It is also stated that for the purpose of surface decontamination in the uncooked chicken meat, 1- 2.5% lactic acid and acetic acid solutions can be applied (Marel and others, 1989).

In a study conducted by Kenney and others (1995), it is observed that there are 1.8 log reductions in the number of mezophilic aerobic bacteria through plunging cattle meat into the 3% lactic acid.

In another study made by Warren and others (1997) 1.3-2 log reduction is observed in the number of mezophilic aerobic bacteria after spraying 1.5% and 3% lactic acid or acetic acid and 12% TSP solutions to cattle meats.

In a study conducted for cattle meat decontamination, the reduction amounts in the mezophilic aerobic bacteria are obtained as sequentially 1log, 1 log and 0.7 through application of 2% lactic acid and acetic acid and 12% TSP solutions at 50 ºC for 10 seconds whereas the reduction amount in the coli form bacteria for the same solutions are found to be 0.5 log, 0.5 and 0.3 log (Elmore and others, 2000).

Kim and others (1998) have plunged the chicken rumps into 1.5% acetic acid solution for 10 minutes and 1.1 log reductions in the number of mezophilic aerobic bacteria is observed.

Through the application of 10 minute decontamination with 2 % acetic acid and lactic acid and 12% TSP solutions, the reductions in the mezophilic aerobic bacteria numbers are sequentially 3.77 logs, 4.49 logs, and 3.42 logs, whereas the reductions in the coli form bacteria numbers are determined as 3.21 logs, 4.08 log and 3.76 log. It can be said that application period has an important effect on the big amount in the reduction compared to the experiments made by Delmore and others, 2000 and (Kim and others, 1998).

In a study made by Hathcox and others (1995), in a sensorial discussion made after frying of breast and rump parts of chicken skeletons to which 12 % TSP and 0.5 % lactic acid / 0.5 % sodium benzoate solution is applied, consumers made evaluations and it is defined that any negativity is not observed in the organoleptic characteristic of the product.

Similar to the results obtained from the studies conducted by Hathcox and others (1995), it is observed that the solutions we used in our experiment did not cause any negative change in the characteristics of the product visually. However, although micro organism number is reduced significantly as a result of the plunging the chicken meat for 15 minutes into 5% H_2O_2 solution that is experimented before, hydrogen peroxide concentration is applied as 1% and 2.5 % since it is observed that chicken meat tissue is smashed. As a result, it is obtained that there is 4.38 and 4.56 log decrease in the number of mezophilic aerobic bacteria and 4.26 and 4.61 log decrease in the number of coli form bacteria without observing any negative change in the tissue.

4.4. Changes in the Mezophilic Aerobic Bacteria and Coli form Bacteria Number after Surface Decontamination in Lettuce with Acidic Electrolyzed Water

The number of mezophilic aerobic bacteria and coli form bacteria and reduction amounts after the surface decontamination applied to the lettuce whose beginning mezophilic aerobic bacteria load is on average 7.66 $\log_{10}$ kob/g and coli form bacteria load is on average 7.40 $\log_{10}$ kob/g are shown in Table 4.4.1 and Figure 4.4.1.

As a result of the applied one-way ANOVA test, it is found that there is statistically significant difference ($p<0.05$) between the means of micro organism numbers after the application with different solutions.

In the experiment, AEW (acidic electrolyzed water, free chlorine concentration 20ppm) containing 1% NaCl and AEW containing 1.5% NaCl (free chlorine concentration 30 ppm), sodium hypochlorite solution containing 200 ppm free chlorine are compared. It is observed that in the mezophilic aerobic bacteria number, there is no statistically significant difference between AEW containing 20 and 30 ppm free chlorine and 200 ppm sodium hypochlorite solution in terms of reduction amounts they caused. Regarding the reduction amount they caused in the coli form bacteria number, AEW containing 30 ppm free chlorine gives the best result. Second comes the hypochlorite solution and third comes AEW containing 20 ppm free chlorine.

Table 4.4. The number of mezophilic aerobic bacteria and coli form bacteria in the lettuce after surface decontamination with AEW and their reduction amounts.

Disinfectant material	MesopHilic bacteria count after process	Coli form bacteria count after process	Decrease in mesopHilic bacteria count ((log)	Decrease in Coli form bacteria count (log)
Control (Water)	$7.42^{a} \pm 0.01$	$7.32^{a} \pm 0.01$	0.24	0.07
AES with 1 % NaCl(20 ppm)	$6.45^{b} \pm 0.04$	$6.10^{b} \pm 0.02$	1.21	1.29
AES with 1.5 % NaCl(30 ppm)	$6.36^{b} \pm 0.02$	$5.80^{d} \pm 0.02$	1.30	1.59
Sodium Hypochlorite (200 ppm)	$6.44^{b} \pm 0.01$	$5.97^{c} \pm 0.03$	1.22	1.42

i: Shows the average of two repetitions and ± standard deviations.

a-d: at 0.05 significance level there is no statistically significant difference between the means carrying the same letter Alsong the same column.

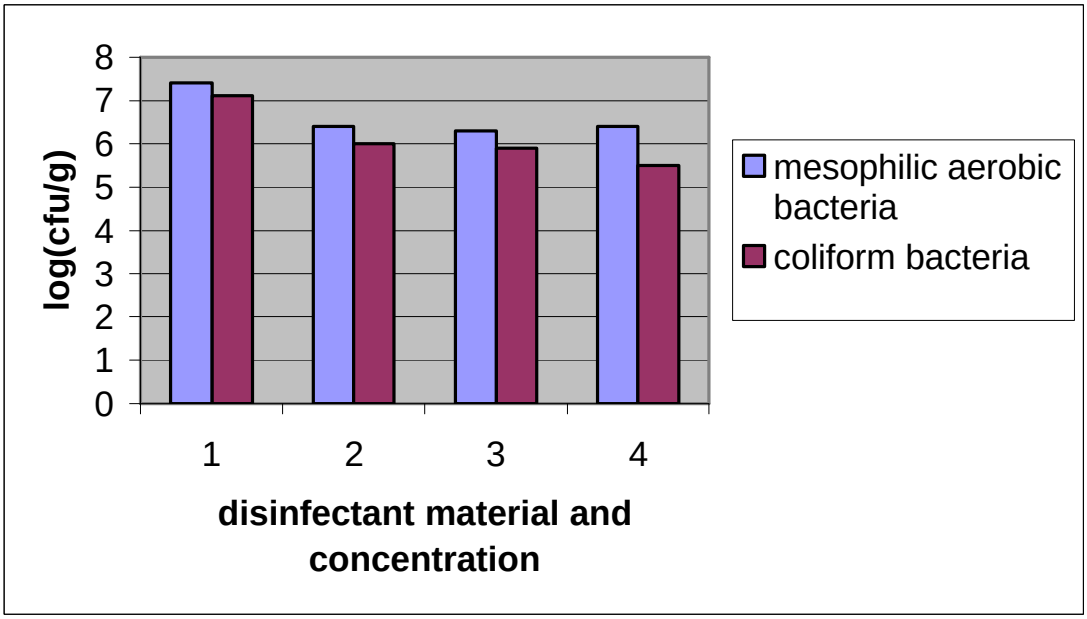

Figure 4.3. The number of mezophilic aerobic bacteria and coli form bacteria in the lettuce after surface decontamination with AEW and their reduction amounts. 1: control (distilled water), 2: AEW containing 1% NaCl, 3: AEW containing 2.5% NaCl, 4: 200 ppm sodium hypochlorite.

In a study conducted by Jung and others (1996) 90% decrease in the mezophilic aerobic bacteria and 2% decrease in the coli form bacteria number are observed when lettuce is plunged into AEW for 20-40 minutes. In our experiment, the reductions in the number of mezophilic aerobic bacteria and coli form bacteria are 95% after the application with AEW containing 20 ppm free chlorine, whereas the reductions are obtained as sequentially 95% and 96% after the application with AEW containing 30 ppm free chlorine. Therefore, it is seen that the reduction amounts in the number of mezophilic aerobic bacteria are similar to the results obtained in the studies of Jung and others (1996).

In another study related to this issue, there observed sequentially 1.7 log and 1.6 log reduction in the number of mezophilic aerobic bacteria and coli form bacteria on the surface of the lettuce which is decontaminated for 10 minutes with AEW which has a pH of 2.5, ORP value of 1140 mV and free chlorine concentration of 40 ppm (Koseki and Itoh, 2001a). In our study, sequentially 1.21 log and 1.30 log reduction in the

mezophilic aerobic bacteria number for 20 and 30 ppm; 1.29 log and 1.42 log reduction in the number of coli form bacteria is determined.

Results that we have obtained can be regarded to be contingent with the datum obtained by Koseki and Itoh (2001a).

In a study conducted by Izumi (1999) with some fresh vegetables (carrot, spinach, pepper, radish, potato and cucumber), AEW which is obtained by the electrolysation of 2.5 % NaCl solution and has a pH of 6.8 and 20 ppm free chlorine concentration, is applied for 4 minutes and as a result it is obtained that microbial load is decreased by 0.6-2.6 log.

In a study, ozone water which has a AEW (pH:2.6, ORP:1140 mV, free chlorine concentration:30 ppm) concentration of 5 ppm and free chlorine concentration of 150 ppm, and NaCl solutions are used for 10 minutes for the lettuce surface decontamination. At the end, it is determined that AEW and NaOCl solutions have similar effects on the number of aerobic bacteria and 2 log reductions with the ozone water are obtained (Koseki and others, 2001).

In a study made by Huang and others (1998), when lettuce samples are plunged into AEW (pH 2.7; ORP:1100 mV) containing 35 ppm free chlorine for 10 minutes, 98% decrease in the number of mezophilic aerobic bacteria is determined, whereas in our experiment this number is obtained as 95% for the AEW containing 20 ppm and 30 ppm free chlorine. Therefore, results are contingent with the results of the mentioned study.

In a study conducted in order to analyze the effect of the electrolyzed water on the quality of fresh vegetables, cabbage, lettuce, cucumber and carrot are processed with AEW, NaCl solution containing 150 ppm free chlorine and tap water for 10 minutes. As a result, it is determined that the loss of quality in the fresh vegetables caused by AEW is the same with the loss of quality caused by NaOCl and tap water (Koseki and Itoh, 2001b). In our experiment, it is observed that acidic electrolyzed water did not cause any negative change in the appearance of lettuce samples.

4.5. Changes in the Number of Mezophilic Aerobic Bacteria and Coli form Bacteria after Surface Decontamination with Acidic Electrolyzed Water in the Uncooked Chicken Meat

The number of mezophilic aerobic bacteria and coli form bacteria in the sample after the surface decontamination applied to the uncooked chicken meat which has a beginning mezophilic aerobic bacteria load of 8.90 $\log_{10}$ kob/g on average and reduction amounts are shown in Table 4.5.1 and Figure 4.5.1. As a result of the applied one-way ANOVA test, it is found that there is statistically significant difference ($p<0.05$) between the means of micro organism numbers after the application with different solutions.

In the experiment, AEW (acidic electrolyzed water, free chlorine concentration 20 ppm) containing 1% NaCl, AEW (free chlorine concentration 30 ppm) containing 1.5 % NaCl and sodium hypochlorite containing 200 ppm free chlorine are compared. Regarding the reduction amount they caused in the number of mezophilic aerobic bacteria, AEW containing 30 ppm free chlorine gives the best result; second comes hypochlorite solution and then comes AEW containing 20 ppm free chlorine. When it is evaluated from the point of view of reduction in coli form bacteria number, 30 ppm AEW is the most effective one. Second most effective is the sodium hypochlorite and then the AEW with 20 ppm.

Table 4.5. The number of mezophilic aerobic bacteria and coli form bacteria in the uncooked chicken meat after surface decontamination with AEW and their reduction amounts.

Disinfectant material	MesopHilic bacteria count after process (log cfu/g)	Coli form bacteria count after process (log cfu/g)	Decrease in mesopHilic bacteria count ((log)	Decrease in Coli form bacteria count (log)
Control (Water)	$8.59^{a} \pm 002$	$8.36^{a} \pm 0.04$	0.31	0.14
AES with 1 % NaCl (20 ppm)	$7.66^{b} \pm 004$	$7.21^{b} \pm 0.01$	1.24	1.29
AES with 1.5 % NaCl (30 ppm)	$7.37^{c} \pm 002$	$7.05^{c} \pm 0.01$	1.53	1.45
Sodium Hypo. (200 ppm)	$7.60^{b} \pm 005$	$7.18^{b} \pm 0.01$	1.30	1.32

i: Shows the average of two repetitions and ± standard deviations.

a-d: At 0.05 significance level there is no statistically significant difference between the means carrying the same letter Alsong the same column.

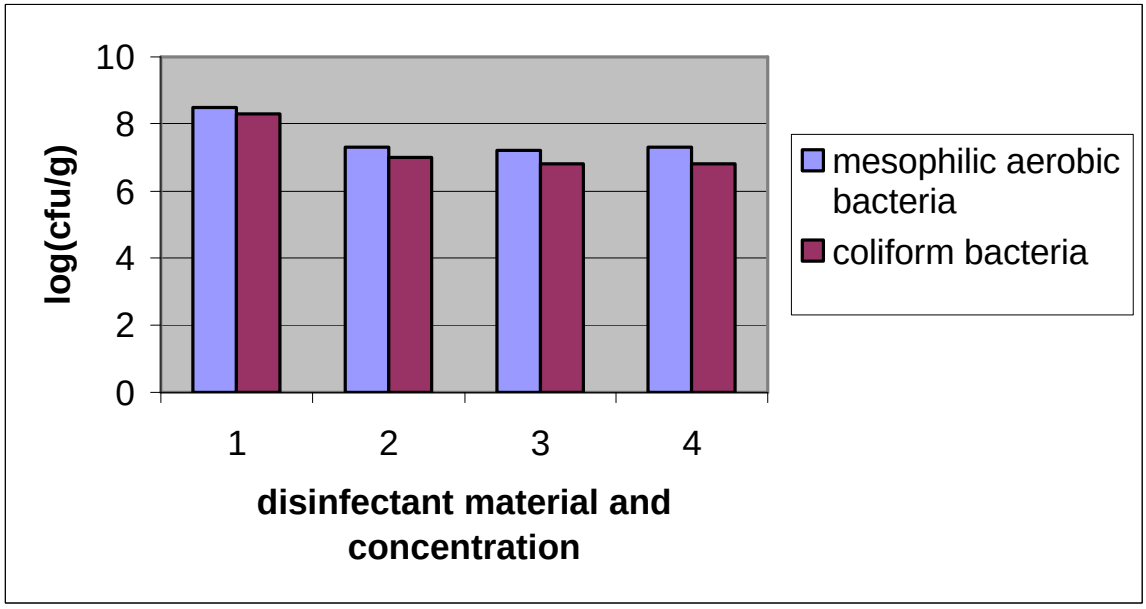

Figure 4.4. The number of mezophilic aerobic bacteria and coli form bacteria in the uncooked chicken meat after surface decontamination with AEW and their reduction amounts. 1: control (distilled water), 2: AEW containing 1% NaCl, 3: AEW containing 2.5% NaCl, 4: 200 ppm sodium hypochlorite.

The number of studies related to the application of AEW to uncooked chicken meat is limited. In a study conducted by Park (2002) *Campylobacter jejuni* is inoculated into chicken meat and for 10 minutes it is used with AEW containing 50 ppm free chlorine and chlorine water. Distilled water is used for control purpose. There seemed a 3 log reduction in the bacteria number because of AEW and chlorine water. As a result, it is stated that AEW decreased the *Campylobacter jejuni* number in the uncooked chicken meat to a great extent and it is a suitable solution for washing chicken meat and prevents the crosswise contamination during the process (Park and others, 2002b).

In our study, it is seen that 20 and 30 ppm AEW reduce the mezophilic aerobic bacteria number sequentially 1.24 and 1.53 log; while it is reducing the coli form bacteria number 1.29 and 1.45 log.

4.6. Change in the Number of StapHylococcus aureus after the Surface Decontamination Applied to Lettuce

Bacteria numbers and their reduction amounts after the surface decontamination applied for 15 minutes to the lettuce samples which has *StapHylococcus aureus* number of 4.81 log kob/g after the inoculation, are shown in Table 4.6.1 and Figure 4.6.1. As a result of the applied one-way ANOVA test, it is found that there is statistically significant difference ($p<0.05$) between the means of micro organism numbers after the application with different solutions.

In the experiment, the most effective one is 12 %TSP. Then come sequentially 5% H_2O_2 , 2% lactic acid and acetic acid, 200 ppm free chlorine sodium hypochlorite solution, 20 mM EDTA, 3% sodium lactate and sodium acetate solutions.

Table 4.6. *StapHylococcus aureus* number in the lettuce and reduction amount after surface decontamination

Disinfectant material	bacteria count in a sample after process (log cfu/g)	Decrease in bacteria count (log)
Control (Water)	$4,32^{a}$ ± 0,02	0,48
Acetic acid 2 %	$3,29^{c}$ ± 0,27	1,51
Lactic acid 2 %	$3,27^{c}$ ± 0,02	1,53
Sodium acetate 3 %	$4,31^{a}$ ± 0,07	0,49
Sodium Lactate 3 %	$4,16^{a}$ ± 0,02	0,64
EDTA 20 mM	$4,02^{ab}$ ± 0,11	0,78
Sodium hypochlorite	$3,52^{bc}$ ± 0,03	1,28
TSP 12 %	$2,68^{d}$ ± 0,21	2,12
H_2O_2 %5	$3,01^{cd}$ ± 0,31	1,79

i: Shows the average of two repetitions and ± standard deviations.

a-d: At 0.05 significance level there is no statistically significant difference between the means carrying the same letter Alsong the same column.

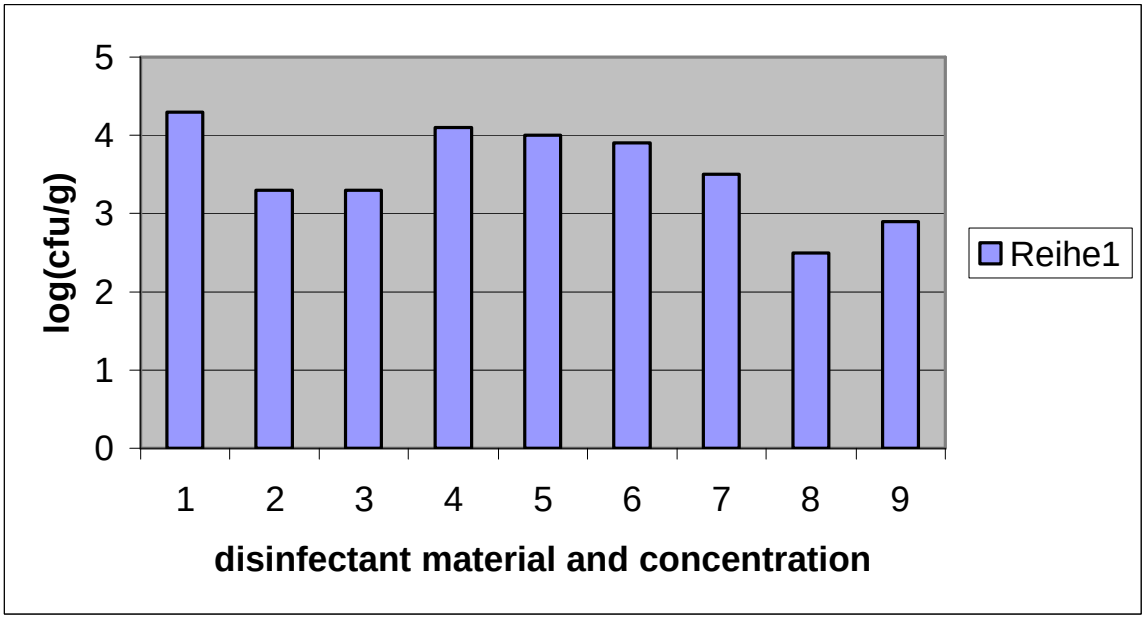

Figure 4.5. The number of *StapHylococcus aureus* in the lettuce after surface decontamination with AEW and their reduction amounts. 1: control (distilled water), 2: acetic acid (2%), 3: lactic acid (2%), 4: sodium acetate (3%),5: sodium lactate (3%), 6: EDTA (20 mM), 7: sodium hypochlorite (200 ppm), 8: TSP (12%), 9: H_2O_2 (5%).

In literature, there are some studies conducted for the pathogen bacteria inhibition in some fresh vegetables. For instance, in a study made by Bracket (1992), *Listeria monocytogenes* is inoculated into Brussels sprouts and as a result of 200 ppm chlorine solution application, 2.5 log reduction is observed. In another study conducted, *Listeria monocytogenes inoculated* lettuce is plunged into 200 ppm chlorine solution at 4 ºC and 22 ºC for 10 minutes, 1.3 and 1.7 log decrease is observed, while 0.5 and 0.2 log decrease is observed after the application of 1% lactic acid and 1% acetic acid (Zhang and Farber, 1996). After Ohsone and others (1999) inoculate *StapHylococcus aureus into* cabbage, as a result of the application of the acetic acid and lactic acid, 1 log reduction is determined in the bacteria number. In our study, as a result of the decontamination of the pathogen bacteria on

lettuce surface with 2 % acetic acid and lactic acid, sodium hypochlorite solution containing 200 ppm free chlorine, sequentially 1.51 log, 1.53 log and 1.28 log decrease is obtained.

In a study, instead of plunging the sample into disinfectant solution, disinfectant solution is applied through spraying and its effect is analyzed. In this study, *Salmonella, Esherichia coli* 0157:H7, *Listeria monocytogenes* are inoculated into apple, tomato and lettuce. After 200-2000 ppm free chlorine solutions are sprayed, for 0,1,3,5 and 10 minutes, as a result of plunging into sterilized water, 0.35 and 2.30 log reduction in the number of pathogens is observed. Apart from these, it is determined that chlorine also causes reduction in the number of total mezophilic aerobic bacteria ferment and mould. As a result, it is determined that the application of spraying the disinfectant solution and, for instance, plunging into disinfectant solution give similar results (Beuchat and others, 1998). In our study as well, food samples are plunged into disinfectant solutions and decontamination is applied. In the number of *StapHylococcus aureus*, there observed a 1.28 log reduction with the sodium hypochlorite solution containing 200 ppm free chlorine.

In another study related to fresh vegetables, after the inoculation of *Salmonella* into green pepper, as a result of the decontamination with 3%-12% TSP solution, 10-100 times (1-2 log) decrease in the bacteria number is obtained (Liao and Cooke, 2001). In our experiment, in the *StapHylococcus aureus* inoculated lettuce sample, 2.12 log reduction is seen with the application of 12%TSP. It is seen that the obtained result is contingent with the mentioned study.

4.7. Change in the Number of Salmonella after the Surface Decontamination Applied To Lettuce

After the 15 minute decontamination applied to lettuce whose *Salmonella typHimurium* number is 7.06 log kob/g after the inoculation, with the application of 2% lactic acid and 8% TSP solution, bacteria on the surface of lettuce is totally inhibit while reductions at different proportions are determined with other disinfectant solutions. Bacteria numbers and reduction amounts are shown in Table 4.7.1 and

Figure 4.7.1. As a result of the applied one-way ANOVA test, it is found that there is statistically significant difference ($p<0.05$) between the means of micro organism numbers after the application with different solutions.

Table 4.7. The number of *Salmonella typHimurium* after the surface decontamination applied to the lettuce

Disinfectant material	bacteria count in a sample after process (log cfu/g)	Decrease in bacteria count (log)
Control (Water)	$6,34^{a} \pm 0,01$	0,72
Acetic acid 2 %	$2,78^{d} \pm 0,17$	4,28
H_2O_2 %5	$3,71^{c} \pm 0,01$	3,35
Sodium hypochlorite (200 ppm)	$5,10^{b} \pm 0,01$	1,96

i: Shows the average of two repetitions and ± standard deviations.

a-d: At 0.05 significance level there is no statistically significant difference between the means carrying the same letter Alsong the same column.

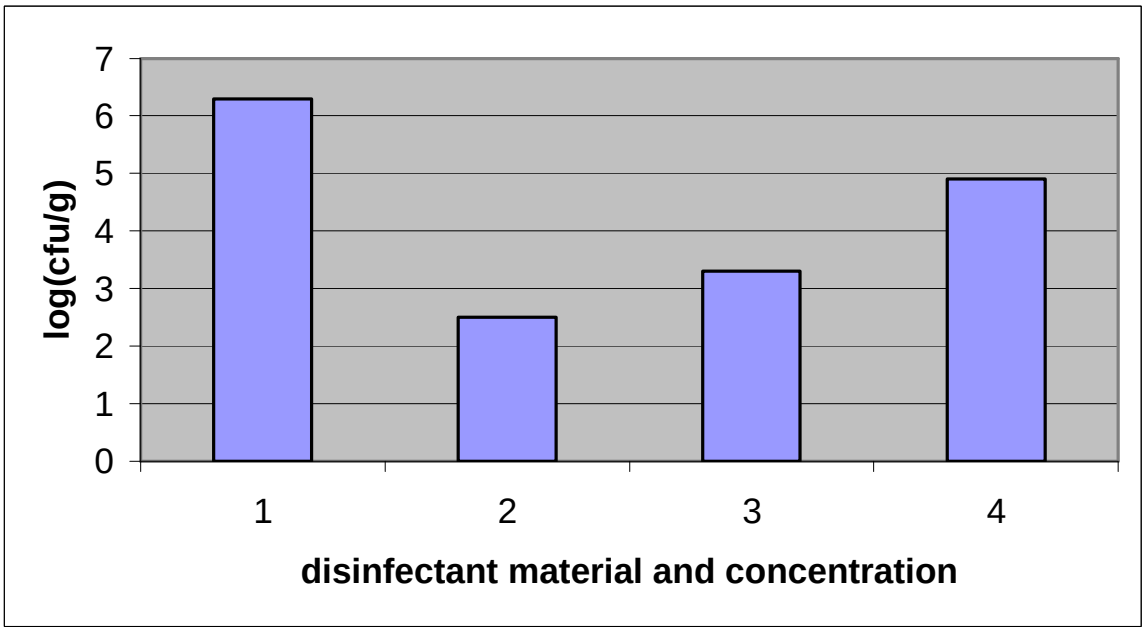

Figure 4.6. The number of *Salmonella typHimurium* in the lettuce after surface decontamination. 1: control (distilled water), 2: acetic acid (2%), 3: H_2O_2 (5%), sodium hypochlorite (200 ppm).

In a study made by Beuchat and others (1998), after 200-2000 ppm free chlorine solutions are sprayed, for 0,1,3,5 and 10 minutes, as a result of plunging into sterilized water, 0.35 and 2.30 log reduction in the number of pathogens is observed.

In our study, with the application of 200 ppm sodium hypochlorite solution, 1.96 log decrease is obtained in the number of *Salmonella typhimurium.* It is obvious that this value is in the interval mentioned above. In another study related to chlorine, the number of *Listeria monocytogenes* in Brussels sprouts is reduced by 2.5 log through the usage of 200 ppm chlorine solution (Bracket, 1992). In a study conducted by Zhang and Farber (1996), with the application of 200 ppm chlorine solution for 10 minutes to lettuce in which *Listeria monocytogenes* is inoculated, 1.7 log decrease is observed.

In a study after *Salmonella* sop. is inoculated into green pepper, it is decontaminated with 3%-12 TSP solution and 1-2 log decrease in the number of bacteria is obtained (Liao and Cooke, 2001). In our study with the usage of 12 % TSP solution, bacteria population is totally inhibit.

In a study made by Lin and others (2002), after lettuce leaves are inoculated by *Eshericia coli* 0157:H7, *Salmonella enterica* serotype Enteritis and *Listeria monocytogenes,* as a result of spraying 2% H_2O_2 solution at 50 ºC for 60-90 seconds, 4 log reduction in *Escherichia coli* 0157:H7 and *Salmonella enterica* serotype Enteritis, approximately 3 log reduction in *Listeria monocytogenes* is observed. In our study, however, through the application of 5% H_2O_2 solution for 15 minutes, *Salmonella typHimurium* is decreased by 3.35 log.

In a study *Yersinia enterocolitica* types [*Yersinia enterocolitica* W1024 O:9 (type A) and *Yersinia enterocolitica* B1 O:5 Lis Xz (type B)] are inoculated into lettuce. As a result of the decontamination of lettuce samples for 10 minutes with 0.5 % acetic acid solution, in type A and type B, sequentially 3.15 log and 2.33 log reduction is

observed. In our study, after 15 minutes 2% lactic acid decontamination, 4.28 log decrease is determined. As a result of the application of 2% lactic acid solution, *Salmonella typHimurium* is totally inhibit.

4.8. Change in the number of staphylococcus aureus after the surface decontamination applied to uncooked chicken meat

As a result of the decontamination applied for 15 minutes to the uncooked chicken meat sample which has StapHylococcus aureus number of 5.74 log kob/g after the inoculation, through the application of 2.5% H_2O_2 solution, bacteria on the chicken meat surface is totally inhibit, while reductions at different proportions are determined through the application of other disinfectant solutions. Numbers of bacteria and reduction amounts are shown in Table 4.8.1 and Figure 4.8.1. As a result of the applied one-way ANOVA test, it is found that there is statistically significant difference ($p<0.05$) between the means of micro organism numbers after the application with different solutions.

Table 4.8. The number of *StapHylococcus aureus* after the surface decontamination applied to uncooked chicken meat

Disinfectant material	bacteria count in a sample after process (log cfu/g)	Decrease in bacteria count (log)
Control (Water)	$5,60^{a}$ ± 0,01	0,14
Acetic acid 2 %	$2,93^{c}$ ± 0,02	2,81
Lactic acid (2 %)	$2,58^{d}$ ± 0,11	3,16
Sodium hypochlorite (200 ppm)	$4,46^{b}$ ± 0,03	1,28
TSP (% 12)	$2,54^{d}$ ± 0,01	3,2

i: Shows the average of two repetitions and ± standard deviations.

a-d: At 0.05 significance level there is no statistically significant difference between the means carrying the same letter Alsong the same column.

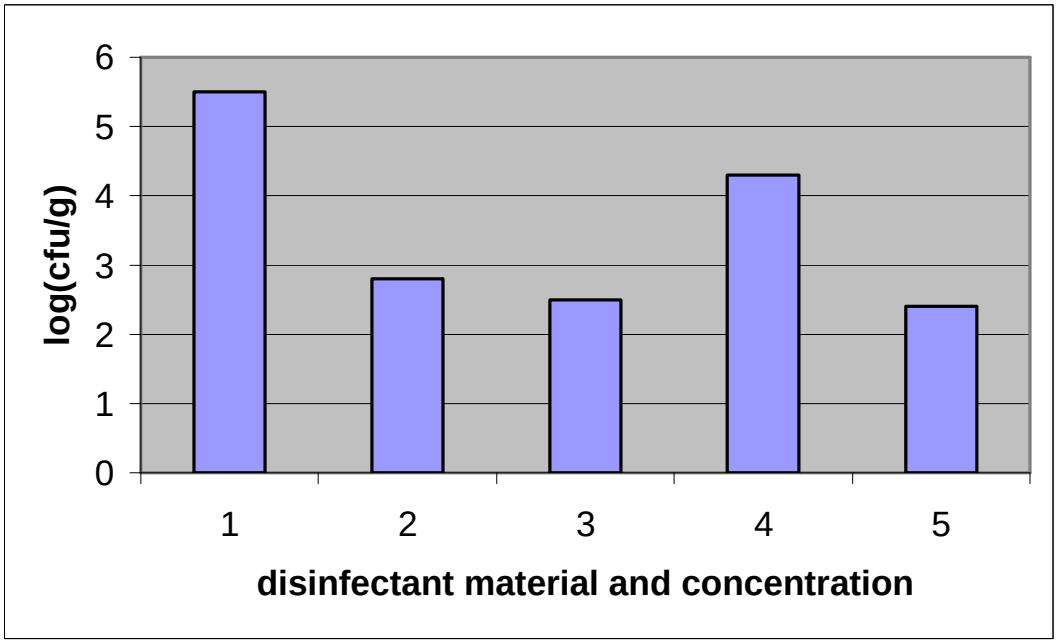

Figure 4.7. The number of *StapHylococcus aureus* in the uncooked chicken meat after surface decontamination. 1: control (distilled water), 2: acetic acid (2%), 3: lactic acid (2%), 4: sodium hypochlorite (200 ppm), 5: TSP (12%).

In a study in which *Salmonella typhimurium, Escherichia coli* 0157:H7 and *Listeria monocytogenes* are inoculated into red meat, as a result of the decontamination with 8-12% TSP solution for 3 minutes, 1-1.5 log reduction in non-fatty tissue and 2-2.5 log reduction in fatty tissue is obtained (Dickson and others,1994). In another study, *Escherichia coli* is inoculated into red meat and 12% TSP solution at 55 ºC is sprayed, therefore 1.8 log decrease in bacteria amount is determined (Ramirez and others, 2001). In a study conducted by Xiong and others(1998), through spraying 5-10 % TSP solution for 30 seconds to the chicken skin into which *Salmonella typHimurium* is inoculated, 2.1 -2.2 log decrease is obtained. *Listeria monocytogenes* is inoculated into chicken skin in another study and after the decontamination with 8-10 % and 12% TSP solution for 15 minutes, 1.12 and 3.34 log decrease is observed (Capita and others, 2002).

In a study conducted by Lillard (1994), after *Salmonella typHimurium* is inoculate into chicken meat, it is plunged into 10 % TSP solution for 10 minutes and as a result, 2 log decrease is observed. In our study, through the application of 12% TSP solution to chicken meat for 15 minutes, *StapHylococcus aureus* is decreased by 3.2 log. It is observed that this value is contingent with the reductions in the pathogen bacteria number in the mentioned studies.

In a study conducted by Morrison and Fleet (1985), as a result of decontamination of *Salmonella* inoculated uncooked chicken meat with 200 ppm chlorine solution, 3 log decrease is obtained in the number of bacteria. However, in our study, through the application of 200 ppm hypochlorite solution, 1.28 log reduction is determined in the number of *StapHylococcus aureus.*

In a study that *Escherichia coli* 0157:H7 is inoculated into cattle meat, through plunging it into 2% lactic acid solution for 30 seconds, 3.3 log decrease is obtained (Ransom and others, 2003). In another study, through spraying 2% lactic acid solution for 9 seconds to red meat in which *Escherichia coli* was inoculated, 1.6 log reduction is obtained (Ramirez and others,2001). In a study conducted by Xiong and others (1998), through spraying 1-2 % lactic acid solution to chicken skin in which *Salmonella typHimurium* is inoculated, 2.2 log decrease is observed. In another study conducted, *Campylobacter jejuni* was inoculated into chicken rump and through decontamination with 10 % lactic acid buffer (pH 3), 1 log decrease in the number of bacteria is determined (Thys and others, 1994). In a study conducted by Zeitoun and others (1994), as a result spraying 10 % lactic acid buffer solution (pH 3), it is determined that in the uncooked chicken meat *Enterobacteriaceae* amount is reduced by 1 log. In our study, however, as a result of the application of 2% lactic acid solution (pH 2.1), *StapHylococcus aureus* is reduced by 3.16 log.

In a study made by Eggenberger-Solorzano and others (2002) 1.8 % acetic acid is sprayed for 3 seconds to pig meat in which *Escherichia coli* is inoculated and 2 log decrease is observed. In a study, cattle meat is inoculated by *Escherichia coli* 0157:H7 and after it is plunged into 2% acetic acid for 30 seconds, 1.6 log decrease is obtained in the number of bacteria (Ransom and others, 2003). In another study

conducted by Graves Delmore and others (1998), cattle meat is inoculated by *Escherichia coli* and for 8 minutes it is plunged into 2% acetic acid solution and therefore 1.3 log decrease is observed. In our study, as a result of the decontamination of chicken meat with 2 % acetic acid solution for 15 minutes, 2.81 log decrease is determined in the number of *StapHylococcus aureus.*

4.9. Inhibition Effects of Disinfectant Solutions on Staphylococcus aureus and Salmonella typhimurium Sush through Agar Diffusion Method

In the study conducted through agar diffusion method, inhibition zones are measured in order to evaluate the effects of disinfectant solutions on the *StapHylococcus aureus* and *Salmonella typHimurium* sush and they are shown in Table 4.9. As a result of the applied one-way ANOVA test, it is found that there is statistically significant difference ($p<0.05$) between the means of micro organism numbers after the application with different solutions.

Table 4.9. Inhibition zones of disinfectant solutions

	Inhibition Zone(mm)*	
Disinfectant Material	**StapHylococcus aureus**	**Salmonella thypHimurium**
Control (Water)	-	-
Sodium hypochlorite (200 ppm)	-	-
Acetic acid (2 %)	-	$16,3^{c}$ ± 0,03
Lactic acid (2 %)	$8,8^{c}$ ± 0,08	$11,8^{c}$ ± 0,03
TSP (8%)	$10,2^{c}$ ± 0,08	$10,8^{c}$ ± 0,16
TSP (12%)	$10,2^{c}$ ± 0,14	$13,7^{d}$ ± 0,1
H_2O_2 (2.5%)	$43,3^{b}$ ± 0,03	30^{b} ± 0,1
H_2O_2 (5%)	48^{a} ± 0,17	$33,5^{a}$ ± 0,09

(*): Figires's are found through finding the means of 3 measurements and are shown with their ± standard deviations.

a-e: At 0.05 significance level there is no statistically significant difference between the means carrying the same letter Alsong the same column.

(-): No inhibition is observed.

In the study, it is observed that distilled water used for control purposes and sodium hypochlorite solution have no inhibition effect. While 2% acetic acid does not create an inhibition zone for *StapHylococcus aureus*, it created 1.63 mm diametric zone for *Salmonella typhimurium.* 5% H_2O_2 is found to be the most inhibitive substance for *Staphylococcus aureus*. Then come sequentially, 2.5% H_2O_2, 12% and 8% TSP and 2% lactic acid solutions. For *Salmonella typhimurium*, this ranking is determined as 5% H_2O_2, 2.5% H_2O_2, 2% acetic acid, 12% TSP, 2% lactic acid and 8% TSP solution. It is observed that, the efficiency of these disinfectant solutions is contingent with the results obtained in the studies of decontamination with nutrition samples.

In a study related to this issue, the effects of various antimicrobial substances on the *Pencillium expansum* through agar diffusion method are analyzed. With the used 1000 ppm chlorine and 1 % acetic acid solution, no inhibition is observed, whereas 2-2.5 mm diametric inhibition zones are observed with 1-10 % H_2O_2 solutions (Venturini and others, 2002).

5. CONCLUSION

In this study, surface decontamination is made through the usage of several chemical substances on the chicken meat. It is observed that through plunging the food samples with their natural micro organism loads into disinfectant solutions at defined concentrations after *StapHylococcus aureus* and *Salmonella typHimurium* is inoculate, bacteria numbers are reduced.

In the scope of the study, lettuce sample is plunged into disinfectant solutions for 15 minute for the purpose of mezophilic aerobic bacteria analysis. As a result, it is determined that 5 % H_2O_2 solution shows the strongest disinfectant effect. Then come sequentially, 1% acetic acid, 12% TSP, 40% apple vinegar, 40 % grape vinegar, 200 ppm sodium hypochlorite and 1 % sodium acetate solution.

As a result of surface decontamination of uncooked chicken meat through plunging into disinfectant solutions for 15 minutes, 1% and 2.5 % H_2O_2 and 2% lactic acid solution shows the strongest disinfectant effect. Then come 2% acetic acid, 12%

TSP, 200 ppm sodium hypochlorite, 20 mM EDTA, 3% sodium acetate and sodium lactate solutions. After the coli form bacteria analysis, it is determined that 2.5 % H_2O_2 solution shows the strongest disinfectant effect . Then come sequentially, 1% H_2O_2 , 2% lactic acid, 12% TSP, 2% acetic acid, 200 ppm sodium hypochlorite, 3% sodium acetate and sodium lactate and 20 mM EDTA solutions.

In a study made with AEW which is among the most attention taking disinfectants, lettuce and chicken samples are used. Lettuce samples are plunged for 10 minutes into 20 and 30 ppm AEW prepared from sodium chlorine solutions at 2 different concentrations and into sodium hypochlorite solution containing 200 ppm free chlorine. After the analysis of mezophilic aerobic bacteria and coli form bacteria, it is obtained that sequentially 30 ppm AEW, 200 ppm sodium hypochlorite and 20 ppm AEW solutions show the strongest disinfectant effect. After the surface decontamination made with uncooked chicken meat for 10 minutes, mezophilic aerobic bacteria and coli form bacteria analysis is made and it is observed that 30 ppm AEW reduces bacteria amount in high proportion. Then come sequentially 200 ppm sodium hypochlorite and 20 ppm AEW solution.

After the surface decontamination applied for 15 minute to lettuce sample in which *Staphylococcus aureus* is inoculated, the most effective result is obtained from 12% TSP solution. Then come sequentially 5% H_2O_2 , 2% lactic acid and acetic acid, 200 ppm sodium hypochlorite solution, 20 mM EDTA, 3% sodium lactate and sodium acetate solutions.

After the decontamination applied for 15 minutes to lettuce sample in which *Salmonella typhimurium* is inoculated, it is obtained that 2% lactic acid and 8% TSP solutions totally inhibit the bacteria on lettuce surface. Moreover, 2% acetic acid, 5% H_2O_2 and 200 ppm sodium hypochlorite solutions show the strongest disinfectant effect.

As a result of the decontamination applied to chicken meat in which *Staphylococcus aureus* is inoculate, bacteria is totally inhibit by 2.5 % H_2O_2. The most effective

decontamination is made sequentially with 12% TSP, 2% lactic acid, 2% acetic acid and 200 ppm sodium hypochlorite solutions.
In a study conducted for the purpose of analyzing the inhibition effects of disinfectant solutions through agar diffusion methods, it is determined that for *Staphylococcus aureus* 200 ppm sodium hypochlorite and 2% acetic acid did not create inhibition zone. The strongest inhibition effect is shown by sequentially 5% and 2.5% H_2O_2, 12% and 8% TSP, 2% lactic acid solutions. For *Salmonella typhimurium*, 200 ppm sodium hypochlorite did not create zone and sequentially 5% and 2.5% H_2O_2, 12% and 8% TSP, 2% lactic acid and acetic acid have the strongest inhibition effect.

Lettuce is contaminated through various ways starting from being sowed in the field. Since it is a vegetable growing near to the soil, it is exposed to various dangers from dung's to insects. It is also carrying risk in terms of several pathogen bacteria's, viruses and parasites from the time of production to the time of consumption. Besides, is its water activity is high and tissue is sensitive, it can be harmed easily and this causes micro organism growth. In terms of product safety, starting from sowing in the field there must be taken some preventions such as not using animal dung, applying insecticide and using accurate irrigation water. The step of washing the fresh vegetables such as lettuce can be evaluated as the point of critical control in the system of HACCP. For the purpose of washing the fresh vegetables previous to consumption, various methods are used containing various disinfectant solutions (especially washing through rinsing) (ICMSF, 1988; Beuchat, 1996).

Chicken meat is sensitive to depravation and risky in terms of pathogen micro organisms. In the production of uncooked chicken meat, washing of chicken meat can be thought as critical control point as well as in the lettuce production (ICMSF, 1988).

Efficient washing procedure applied to fresh vegetables such as lettuce previous to consumption is an important step in terms of product safety both in mass production areas and packaged production. Besides this, it can be a beneficial application to decontaminate the uncooked chicken meat with suitable disinfectant solutions both during the production and previous to selling.

From this aspect, washing fresh vegetables such as lettuce and uncooked chicken meat is an important step. With this purpose, through using various disinfectant substances, beginning micro organism loads can be reduced to a great extent. However, washing procedure is not merely enough for the reduction of micro organism load to not dangerous level. Moreover, the effects of these applications in which disinfectant solutions are also used are limited in terms of the effects on macro parasites and bacteria spores. For this reason, preventions required by HACCP system aiming at 100% food safety must be taken on pre - requirement programs such as working with reliable procurers must be considered (ICMSF, 1998).