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# Occurrence of *Salmonella* in Minimally Processed Vegetables

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## 1. Introduction

Vegetables that have been physically altered from its original state but remain in its fresh state are considered minimally processed. These vegetables are subjected to one or more physical changes (processes of washing, peeling, slicing and cutting), which make them ready for consumption. However, in the dicing step occurs the release of internal cellular fluids, rich in nutrients, which allow microorganisms to multiply rapidly increasing the initial microbial load and thus reducing considerably the shelf life of these products (FARBER, 1999). Therefore, the sanitization step aiming the reduction or destruction of pathogenic and spoilage microorganisms to acceptable levels is critical for these products (BACHELLI, 2010) since food poisoning outbreaks associated with contamination of vegetables continue to exist despite the technological advances. Leafy vegetables have been identified as significant vehicles of pathogens relevant to public health, including enterohaemorrhagic *Escherichia coli* (O157: H7), *Listeria* sp., *Salmonella* sp. and *Shigella* spp. (FRANK & TAKEUSHI, 1999) especially if proper care is not met on the steps of growing, harvesting and processing (GARG et al., 1990). Thus, a minimally processed product should be consistent, to have fresh look, be of acceptable color, free from defects and safe from a microbiological standpoint.

## 2. *Salmonella* sp. as a pontential contamination microorganism of minimally processed vegetables

### 2.1 Taxonomy

*Salmonella* is a genus of Rod-shaped gram negative bacteria that belong to the family *Enterobacteriaceae*. Their species are motile, oxidase-negative, catalase positive and utilize glucose and other carbohydrates with the production of acid and gas.

Officially the genus is composed of a single species, *Salmonella choleraesuis,* divided into seven subspecies, which are also known by Roman numerals: I. *choleraesuis*, II. *salamae,* IIIa. *arizonae,* IIIb. *diarizonae*, IV. *houtenae*, V. *bongori* and VI. *indicates*. In 1987 a proposal was made to change the name *Salmonella choleraesuis* for *Salmonella enterica* and in 1989 the

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proposed elevation of the subspecies to the species category bongori. This proposal received unanimous support of the Subcommittee on *Enterobacteriaceae* of the International Committee on Systematic Bacteriology at the Fourteenth International Congress of Microbiology, but was not made official by the International Committee of Nomenclature of Bacteria. Still, it was adopted and used by the CDC (U.S. Center for Disease Control and Prevention), ASM (American Society for Microbiology) and WHO (World Health Organization). The strains most frequently involved in human disease are *S. enterica* subsp. *enterica*, which is the habitat for warm-blooded animals and are responsible for 99% of human salmonellosis. *S. enterica* subsp. *salamae* subsp. arizonae and subsp. diarizonae, are often isolated from the intestinal contents of cold-blooded animals and rarely humans or warm-blooded animals. *S. enterica* subsp. *houtenae* and *S. bongori* are predominantly isolated from the environment and are rarely pathogenic to humans (SILVA et al., 2010).

More than 50% of the serotypes of *Salmonella* belong to the *Salmonella enterica* subsp. *enterica*, and the most common somatic serogroups are; A, B, C1, C2, D, E1, and E4. Approximately, 99% of *Salmonella* infections in humans and warm-blooded animals, are due these serogroups, including widely known serotypes Parathyphi A (A group), Paratyphi B and Thyphimurium (B group), Paratyphi C and cholerasuis (C group), Typhi, Enteritidis and Gallinarum (D group) (SILVA et al., 2010).

### 2.2 Growth and survival

*Salmonella* sp. has the ability to growth in the temperature range of 2-45ºC, with the optimum at 35-37ºC. The psychotropic attribute of *Salmonellae* and ability to growth slowly at cold temperature raises concerns on cold-induced bacteriostasis as a food safety measure. *Salmonellae* can growth in the pH range with an optimum pH range of 6.5-7.5 for growth. The water activity for this genus is 0.93 or greater (SILVA et al., 2010).

The propensity of *Salmonella* sp. to survive bactericidal food process and to persist for years in frozen foods and in dry foods stores at ambient temperature is a food safety concern. The thermal process in food industry widely used to eliminated bacterial human pathogens is a challenge concerning to *Salmonella* sp., because of its heat resistance in foods with low water activity. The classical study on solute dependent thermal resistance showed that heating of *Salmonella* sp. at 57.2ºC in aqueous solutions of sucrose and glycerol adjust the AW = 0.90 yields D value of 40-55 minutes and 1.8-8.3 min. respectively (GOEPFERT et al., 1970).

### 2.3 Detection methods

The traditional technique for detecting *Salmonella* sp. in food is a classic culture method for presence/absence, developed in order to ensure detection even under extremely unfavorable conditions. This is the case of food microbiology with a competitor microbiota much larger than the population of *Salmonella* and / or food in which the cells of *Salmonella* sp. are very few in number and/or foods in which the cells are injured by the process of preservation (application of heat, freezing, drying) (SILVA et al., 2010).

The procedures recommended by different regulatory bodies, although they present some variations in the selection of culture media and method of sample preparation basically follow five steps: pre-enrichment, selective enrichment, plating, biochemical and serological confirmation (DÁOUST, 1994). All samples should be pre-enriched in a non-selective broth medium for 18-24h at 35-37ºC. The aim of this step is to resuscitate the few injured or stressed cells of *Salmonella* sp. By the method ISO 6579 (2007), one of the most recommended

for minimally processed vegetables, a portion of 25 g or 25 mL of the sample, is taken and placed in 225 ml of buffered peptone water (BPW). Incubate for 18 hours at 37°C (SILVA et al., 2010).

Selective enrichment of portion of pre-enriched culture in nine volumes of tetrathionate brilliant green (TBG), selenite cistine (SC) or Rappaport-Vassilads (RV) broth medium for 18-24h represses the growth of competitive microflora and makes easy the recovery in different plating media.

Selective differential plating objectives to promote the preferential development of colonies with typical *Salmonella* sp. It is recommended to be done in one or more culture media. The most common are the Hectoen Enteric Agar (HE), Xylose Lysine Desicolato Agar (XLD) agar and Xylose Lysine tergitol4 (XLT4) (Silva et al., 2010). Each culture purview a streak SVR (depletion) in the differential media recommended. Repeat this procedure with the broth MKTTn. Incubate plates inverted XLD 37°C/24 hours. Follow the incubation plates of the others differential culture media, according to the manufacturer.

Confirmation is a step that aims to verify whether the colonies obtained in the typical differential plating are actually colonies of *Salmonella* sp. is carried out through biochemical and serological tests.

In the XLD medium, typical colonies are dark pink in color with black center and a reddish zone, slightly transparent around. In the second chosen medium, after plating, following the manufacturer's guidelines for evaluating features of typical colonies of *Salmonella* sp. Select at least two colonies of each medium for further confirmation.

Confirmation checks the biochemical profile biochemical characteristics of strains of *Salmonella enterica* subsp. enterica. Miniaturized kits are also recommended for this aim.

Series recommended for biochemical analysis method for *Salmonella* sp.: Incubation of all tests: 37C/24 hours (SILVA et al., 2010).

- Growth test Agar Triple Sugar Iron-TSI: Initial color: orange. Positive test: ramp alkaline (red), background acid (yellow) with production of gas (bubbles) with or without H2S production.
- Urease test: deep pink colour. Negative for *Salmonella* sp. The medium maintains its original color.
- Test Lysine Carboxylase: Most lysine-positive strains. Serotype Parathypi are negative.
- Voges-Proskauer test: tubes with 3 mL of methyl red (VM)-VP, Voges-Proskauer. *Salmonella* sp. are negative.
- Indole: indole-negative: the most of them.
- Beta-galactosidase test. Most strains of *Salmonella enterica* subsp. *enterica* are negative.

A serological confirmation checks for the presence of antigens "O", "Vi" and "H" for tests agglutination polyvalent antisera. The results for confirmed positive *Salmonella* sp. by the ISO 6579 method (2007) are: typical for biochemical tests, no self-agglutination and antibody positive serological test for O, Vi, or H. The methods of analysis of food end up confirming this stage, since the full characterization of *Salmonella* sp. is usually done by reference laboratories in each specific country (SILVA et al., 2010).

Over the past ten years there was a breakthrough in developing new methods, especially immunological methods and to a lesser extent, methods based on nucleic acids. These methods follow the current trend of development kits analytical trademarked defined by AOAC (Association of Official Analytical Chemists) as “a system containing all key components to the analysis of one or more microorganisms, one or more types of food,

according to a particular method" (ANDREWS, 1997). The great advantage of the kits is that the material required for tests (all or part of it) is sold together, eliminating the preparation in the laboratory (SILVA et al., 2010).

The polymerase chain reaction (PCR) detection of *Salmonella* spp is based on the amplification of bacterial DNA sequence that is unique to salmonellae. The PCR assay consists of three different steps: denaturation of duplex bacterial DNA into single strands (94ºC), annealing of synthetic oligonucleotide primers (45-65ºC) that are highly- specific to *Salmonella* sp. DNA sequences that flank the *Salmonella*-specific DNA targed, and a polymerase-dependent extension (72ºC) of the single-stranded DNA starting at the primer site where elongation progress from 3min to 5min end of template DNA strand. The commonly targeted sequence for amplification lies within the inVA gene of *Salmonella* sp. (DÁOUST, 1994).

Related to the minimally processed vegetables, the main method used is the traditional technique described in this chapter together with the use of miniaturized kits for biochemical bacteria identification.

## 3. Foods involved in *Salmonella* sp. outbreaks

*Salmonella* is a bacteria with wide occurrence in animals and in environment, and the main sources are water, soil, animal feces, insects and surfaces of factory's equipment and kitchen utensils. A disease is generally contracted mainly through consumption of contaminated food of animal origin. It is commonly accepted that at between 1 million to 1 billion bacteria are needed to cause infection although some investigators suggest some people may be infected by far fewer bacteria. Other authors mention that the infectious dose is 15 to 20 cells and can reach any age range, with the elderly and children under seven years the more susceptible to get ill (SILVA et al., 2010). Nevertheless, most data suggest food, water, or other sources of contamination contain large amounts of bacteria. Although human stomach acid can reduce and sometimes eliminate *Salmonella* spp., occasionally some bacteria get through to the intestine and then attach and penetrate the cells. Symptoms may include headache, muscle aches, diarrhea, vomiting, abdominal cramping, chills, fever, nausea and dehydration. According to the Illinois Department of Public Health, most persons infected with *Salmonella* bacteria develop diarrhea, fever and abdominal cramps 12 to 72 hours after infection. The illness usually lasts 4 to 7 days, and most persons recover without treatment. However, in some persons, the diarrhea may be so severe that the patient needs to be hospitalized. *Salmonella* sp. infection may spread from the intestines to the bloodstream, and then to other body sites and can cause death unless the person is treated promptly with antibiotics. On the other hand, persons can be infected with the bacteria without having symptoms. Persons with and without symptoms shed the bacteria in their stool, which is why proper handwashing after toileting and before handling food is so important. Children younger than 1 year old, people who have had ulcer surgery or take antacids, the elderly, infants and those with impaired immune systems are more likely to have a severe illness from *Salmonella* sp. which can contaminate a wide variety of foods. These include raw foods derived from animals like eggs and egg products, meat and meat products, unpasteurized milk and other dairy products, and raw poultry. Shell eggs and eggs products figured as prominently in recent years as a human salmonellosis. More recently, *Salmonella* sp. outbreaks have been tied to a variety of fresh produce like lettuce, salad mixes, sprouts, melons, tomatoes and even peanut butter. Minimally processed vegetables have risen, since

the 90"s, as a new source of *Salmonella* sp. in food industry. Fresh cut vegetables are by definition, perishables. The process of cutting, slicing, chopping, breaks the protective skin of fresh vegetables and increases their vulnerability to biological contamination. A poor hygiene in minimally processed vegetables, especially in developing countries are the main cause of food borne disease associated to this product. The minimally processed vegetables are products that have suffered some manipulation, thus, the useful life, compared to fresh produce is much lower (BOONER et al., 2003). Microorganisms that cause disease in humans as bacteria, protozoa, virus, has been the focus of many studies of minimally processed vegetables. *Salmonella* sp. serotypes however are estimated to be responsible for most cases of food poisoning due the consumption of this kind of product worldwide (MEAD et al., 1999).

According to Francys et al. (1999) *Salmonella* is the organism that are relevant to public health more commonly associated with food poisoning outbreaks involving vegetables ready for consumption. An outbreak of salmonellosis occurred in the UK in 1988, involved the consumption of green beans. Epidemiological studies in England and Wales between 1992 and 1996 linked the consumption of coleslaw with *Salmonella* outbreaks that occurred during this period.

Machado et al. (2009), in a research for microbiological evaluation of some minimally processed vegetables in Brazil, evaluated samples of watercress, lettuce, grated carrot, spinach, green cabbage and rocket minimally processed for some pathogens, including *Salmonella* sp. The vegetables were stored at a temperature of 5°C. *Salmonella* sp. was detected in 12.7% of the samples.

Bruno et al. (2005), evaluating the microbiological quality of 15 samples of vegetables including carrots, cabbage, chayote, all minimally processed and marketed in the north part of Brazil verified that *Salmonella* sp. was present in 66% of the samples.

Santana et al. (2011) tested 512 samples of minimally processed vegetables in São Paulo, Brazil, and obtained that *Salmonella* sp. was detected in four samples. The serovars were *Salmonella* Typhimurium (three samples) and *Salmonella enterica* subsp. *enterica* (one sample).

A small outbreak of *Salmonella* sp happened in five states of United States of America, in June, 2011. A total of 21 persons with the outbreak strain of *Salmonella enteritidis* have been reported from 5 states: Idaho (3), Montana (7), North Dakota (1), New Jersey (1) and Washington (9). Among persons for whom information is available, ill persons range in age from 12 years to 77 years old, with a median age of 35 years old. Seventy-one percent are female. Among the 10 ill persons with available information, 3 (30%) persons have been hospitalized. No deaths have been reported. It was announced by Los Angeles Times. The outbreak was linked with the consumption of alfafa sprouts (http:// articles.latimes.com/ 2011). In USA, a total of 99 individuals infected with the outbreak strain of *Salmonella* Agona have been reported from 23 states, late July, 2011. Epidemiologic, traceback, and laboratory investigations have linked this outbreak to eating fresh, whole papayas imported from Mexico (CDC, 2011). According to the Center for Disease Control (CDC), in the United States, food poisoning causes nearly 76 million illness cases with about 325,000 hospitalizations, and approximately 5,000 deaths yearly. The *Salmonellae* organisms are reportedly responsible for as much as $1 billion in medical costs and lost time from work.

Concerning to salmonellosis preventions, it is important to say that *Salmonella* bacteria are killed when food is thoroughly cooked properly. Once cooked, any food held in a buffet should be kept hotter than 55°C. Cross contamination, may be avoided using different utensils, plates, cutting boards and count tops before and after cooking. Cooking food

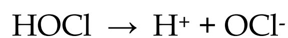
stands at room temperature for a long time, such as two hours, is also at risk. It is important to assent that vegetables, now identified as a source of *Salmonella* sp., must be thoroughly washed in treated or healthy running water before they are eaten, as basic operations of food borne disease. In food industry, internal systems of quality control are essential to prevent occurrence of foodborne illness to consumer. As an example, the HACCP (Hazards Analysis and Control of Critical Points) system, adopted by major international markets, basically ensures that the manufactured products are developed without risk to public health, and also have uniform standards of identity and quality (SILVA, 1999).

## 4. Sanitizers as a control measure

Minimally processed vegetables are products ready for consumption and must be free of pathogenic microorganisms. Its washing step must be done with good quality water followed by the addition of sanitizer solution aiming to reduce the microbial counting and increasing microbial safety and the product preservation. Thus, the sanitation plays an important role in reducing decay and maintaining quality. Therefore, the types of sanitizers, the forms of application, generally a function of time and concentration, will depend on the accompanying microbiota and characteristics of raw material processing.

Chlorine, in its various forms, is the group of most commonly used compound sanitizers because of its efficiency and low cost. They are compounds of broad-spectrum germicidal action by reacting with membrane proteins of the microorganism. Sodium hypochlorite is the most widely used chemical sanitizer because of its complete dissociation in water, easy application and quick action being effective in reducing populations of bacteria, fungi, viruses and nematodes. In water, produces sodium hydroxide (NaOH) and hypochlorous acid (HOCl), the latter being the germicidal agent, which dissociates into $H^+$ and $OCl^-$ ion according to the following reactions:

$$NaOCl + H_2O \leftrightharpoons NaOH + HOCl$$



$$HOCl \rightarrow H^+ + OCl^-$$

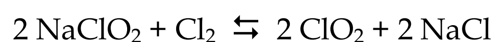
It is proved that the hypochlorous acid (HOCl) has greater disinfecting action (about 80 times more) than the same concentration of hypochlorite ion ($OCl^-$). The amount of HOCl formed depends on the pH of the solution and its concentration is considerably higher at pH 4.0 decreasing as pH increases. Thus, at pH above 5.0 occurs an increase of the hypochlorite ion ($OCl^-$). The sanitizing step is usually performed at pH between 6.5 and 7.0 because in this range there is still considerable amount of hypochlorous acid. The greatest disinfecting power of the hypochlorous acid is explained by the fact that being a small, neutral molecule has a greater ease of penetration through the cell wall. In turn, the hypochlorite ion due to its negative charge is more difficult to cross the cell wall and reach the enzyme system. It is therefore possible that the greatest difficulty in the elimination of sporulated forms is related to the penetration of the disinfecting agent as this may be hampered by the protective mantle of the microorganism.

A study carried out by Berbari et al. (2001) showed that soaking for 15 minutes in a solution containing a chlorine $70mg.L^{-1}$ enables a shelf-life of up to 6 days for minimally processed lettuce stored at 2°C, increasing to 9 days if treated with a solution containing 100 to $130mg.L^{-1}$ of chlorine. On the other hand, a study by Nunes et al. (2010) with Peruvian carrot minimally processed, showed that soaking for 10 minutes in a solution containing $100mg.L^{-1}$ of chlorine

allowed a shelf-life of 6 days when stored at a temperature of 5°C ± 1°C. Nascimento (2002) showed that vegetables washed with a solution containing 50 ppm of free chlorine showed a significant reduction in the total count of aerobic and that fecal coliforms were even more sensitive to chlorine, not being more detected in vegetables after washing. Therefore, chlorine and its salts, especially hypochlorite, are effective and of low cost, and widely applied as a spray for bacteriological control in industries working with vegetables (KIM et al., 1999). However, in recent years there has been some concern in the use of chlorine due to the inconvenience of toxic compounds that can be formed and leave residual taste in food (OLIVEIRA & VALLE, 2000). Among these compounds, there are the trihalomethanes (THM), aldehydes, halocetonas and chloramines, which when hydrolyzed proved related to some types of cancer according to epidemiological studies of Meyer (1994). Depending on the toxicity of these compounds, there is a recognized need to find alternative sanitizers for hygiene and sanitization procedures for vegetables. Thus, chlorine dioxide ($ClO_2$) has received special attention (ARENSTEIN, 2003) for, although it is a derivative of chlorine, generates negligible amount of by-products (trihalomethanes), characterized as a product of low carcinogenic potential (ANDRADE & MACEDO, 1999). In addition, chlorine dioxide is a strong oxidizing agent that reacts mostly through a mechanism of electron transfer by attacking the cell membrane, penetrating, dehydrating, and lastly, oxidizing the internal components of the microbial cell without however causing toxic effects, as most of the chlorine compounds do. It also has the advantage of being effective against gram negative and positive. Still, by the fact that hydrolyzes the phenolic compounds it reduces the possibility of formation of tastes and odors.

Another important aspect of chlorine dioxide is its sharp and sporicidal disinfectant action in lower concentrations than that of chlorine. The explanation of its high bactericidal action is due to the fact that it is soluble in oils, greases and substances of mixed composition, such as cells of virus and bacteria, whose membranes easily penetrates in, as opposed to other disinfectants of polar nature.

Chlorine dioxide is stable under a wide pH range (6-10) and its decomposition are first formed chlorite ($ClO_3$) and then chlorate ($ClO_2$) which can be seen in the equations:



$$2\ NaClO_2 + Cl_2 \leftrightarrows 2\ ClO_2 + 2\ NaCl$$

$$2\ ClO_2 + H_2O \leftrightarrows ClO^-_2 + ClO^-_3 + 2\ H^+$$

$$4\ ClO_2^- + H_2^+ \leftrightarrows 2\ ClO^-_2 + ClO^-_3 + 2\ H_2O$$

However, the major disadvantages of chlorine dioxide are its cost and its sensitivity to high temperatures.

Currently, several studies are being conducted with chlorine dioxide in different countries. Felkey et al. (2003) and Rash (2003) showed in their studies the efficiency of chlorine dioxide in reducing *Salmonella* on the surface of tomato and melon, respectively. Another sanitizing agent that has been used quite successfully is peracetic acid, also known as peroxide of acetic acid or peroxyacetic acid. It is obtained by the reaction of acetic acid or acetic anhydride with hydrogen peroxide in the presence of sulfuric acid, which has the function of catalyst. The decomposition products are acetic acid, hydrogen peroxide and water.

The peroxyacetic acid has currently one of the largest application as disinfectants in the food industry and its efficiency is similar or superior to sodium hypochlorite (NASCIMENTO, 2002), but more potent than hydrogen peroxide. It is an excellent sanitizer for the great

oxidation capacity of the cellular components of microorganisms having a rapid action at low concentrations and still effective in the presence of organic material and therefore being an effective biocide.
Its biocide action is influenced by the concentration, shape and type of microorganism. It degrades rapidly in biodegradable and harmless substances such as acetic acid and active oxygen, which pose no risk of toxicity and does not affect the taste and odor of food. Do not have mutagenic or carcinogenic effects (COSTA, 2007). However peroxyacetic acid has low stability during storage and handling must be done carefully. A study performed by Hilgren & Salverda (2000) showed a significant reduction in the total count of bacteria and fungi in vegetables treated with peroxyacetic acid. Alvarenga et al. (1991) found that after 1, 3 and 5 minutes of contact with peracetic acid at a concentration of 300mg.L$^{-1}$ reached respectively 0.43, 1.2 and 2.8 decimal reductions in the population of spores of *Bacillus subtilis*.
Also according to Nascimento (2002), there was no significant difference to the performances of the peracetic acid compared to sodium hypochlorite. Similar results were reported by Farrell et al. (1998), Sapers et al. (1999) and Wisniewsky et al. (2000). However other authors have demonstrated the superiority of peracetic acid when compared to the sodium hypochlorite in the presence of organic matter. Jones et al. (1992) got a reduction of 3 log cycle for *Vibrio cholerae* and *E. coli* using peracetic acid (25ppm) when compared to sodium hypochlorite (25 ppm). Thus, although there are a number of studies reported in the international literature, most of the time these were carried out under different conditions not allowing comparisons. Therefore, further studies are needed to know the effectiveness of sanitizers in the real conditions of use, working with vegetables available in the local market, with its natural contaminant microbiota unchanged. It is also interesting the implementation in the food sector, of a rotation between different sanitizers thereby preventing the development of resistance by microorganisms to the active principles of the same.

## 5. Chlorine dioxide and peracetic acid as sanitizers to control microorganisms presents in minimally processed chicory (*cichorium endivia* l.) and rocket (*eruca vesicaria sativa*)

**Combined effect of type, concentration and action time of sanitizer in the microbial control of minimally processed chicory and rocket. An observation.**

Sodium hypochloride has been the sanitizer usually used to reduce the microbial counting in minimally processed vegetables, although its use is questioned due to be precursor in the formation of organic chloramines, compounds of high carcinogenic potential. As a consequence of this fact, other sanitizers have been proposed to replace it, among them chlorine dioxide and peracetic acid. Therefore in this work chlorine dioxide (10, 25 and 50ppm/2, 5 and 10min) and peracetic acid (50, 75 and 100ppm/4, 7 and 10min) were compared with sodium hypochloride (120ppm/15min) in the control of natural microbiota of minimally processed rocket and chicory.
In green leafy vegetables, the physical form of the vegetable being processed is very important because certain types of leaves are difficult to be washed and sanitized requiring greater care. The leafy vegetables, rocket and chicory, present this kind of difficulty, which by being consumed as salad, so fresh, are potentially risk factors, that's why they were chosen for the work associated with their high consumption.

The microbial counts on fresh materials rocket and chicory after washing followed by immersion in water for 15 min. showed high contamination of molds and yeasts (5.90 and 5.62 log CFU.g$^{-1}$), total coliforms (6.22 and 5.59 log CFU.g$^{-1}$) and *Escherichia coli* (2.61 and 2.37 log CFU.g$^{-1}$).

It has also been seen that the samples of rocket showed initial contamination superior to the chicory for the same tests, which may be a consequence of the type of rocket leaf that by being rough ends up retaining contaminants on its surface, unlike the chicory which has the smooth leaf.

Data regarding to the effects of chlorine dioxide and peracetic acid in the population of yeasts and molds in minimally processed chicory (Table 1) showed that the variables concentration and contact time influenced significantly (at 5%), and both concentrations as the contact times studied was inversely proportional to the population of yeasts and molds naturally present in chicory minimally processed.

| Time* | Treatment with chlorine dioxide ($ClO_2$) | | | |
|---|---|---|---|---|
| (Min) | 10ppm | 25ppm | 50ppm | MSD[1] |
| 2 | $3.312 \pm 0.212^{a, A}$ | $2.871 \pm 0.157^{b, A}$ | $2.436 \pm 0.120^{c, A}$ | 0.419 |
| 5 | $3.026 \pm 0.266^{a, A, B}$ | $2.598 \pm 0.182^{a, b, A}$ | $2.242 \pm 0.084^{b, A}$ | 0.482 |
| 10 | $2.541 \pm 0.278^{a, B}$ | $2.026 \pm 0.046^{b, B}$ | $2.000 \pm 0.000^{b, B}$ | 0.407 |
| DMS[2] | 0.635 | 0.353 | 0.212 | ---- |
| Time* | Treatment with peracetic acid ($CH_3$-COOOH) | | | |
| (Min) | 50ppm | 75ppm | 100ppm | MSD[1] |
| 4 | $3.445 \pm 0.279^{a, A}$ | $3.247 \pm 0.185^{a, A}$ | $2.716 \pm 0.119^{b, A}$ | 0.514 |
| 7 | $3.131 \pm 0.174^{a, A, B}$ | $2.785 \pm 0.094^{b, B}$ | $2.452 \pm 0.119^{b, B}$ | 0.334 |
| 10 | $2.902 \pm 0.139^{a, B}$ | $2.308 \pm 0.166^{b, C}$ | $2.000 \pm 0.000^{b, C}$ | 0.313 |
| MSD[2] | 0.517 | 0.384 | 0.243 | ---- |
| Blank (washing and immersion in tap water for 15 minutes) ** .(log CFU.g$^{-1}$) | | | | 5.616 |
| Standard (washing with water and immersion in a solution of sodium hypochlorite: 120ppm/15min) ** ......................................(log CFU.g$^{-1}$) | | | | <2.000 |

MSD[1] = for the data on the lines; MSD[2] = for the data on the columns; small letter compares averages on the same line, capital letters compare means in the same column, different letters indicate that the data differ significantly at 5% probability; * Time of contact with the sanitizer product; ** reference treatments.

Table 1. Yeast and mold count (log CFU.g$^{-1}$) observed in samples of minimally processed chicory.

In the case of chlorine dioxide, the treatments performed with 25ppm/10min and 50ppm/10min were statistically superior to the others and there wasn't, however, significant differences between the two. Both treatments showed a reduction equivalent to 3 logarithmic cycles in the population of yeasts and molds when compared with the treatment by washing followed by immersion in water for 15 minutes. On the other hand, regarding the effect of peracetic acid in the population of yeasts and molds, the treatments carried out

at concentrations of 75ppm/10min and 100ppm/10min proved to be statically superior to others, but without showing any significant difference between them. Just as in the treatments with chlorine dioxide, peracetic acid treatments had reduced to the equivalent of 3 logarithmic cycles in the population of yeasts and molds when compared with the treatment by washing followed by immersion in water for 15 minutes (blank). Treatment with chlorine dioxide and peracetic acid, described above as having showed the best results in terms of population control of yeasts and molds in chicory, showed the same level of standard treatment (2 log CFU.g$^{-1}$). When the same treatments were performed using minimally processed rocket (Table 2), the counts were higher and showed no significant differences between them, as much for the treatments with chlorine dioxide as for treatment with peracetic acid. However, even with no significant difference between them, the greatest reductions in populations of yeasts and molds were obtained in the case of peracetic acid treatments, with 100ppm/10min and in the case of chlorine dioxide with 50ppm/10min.

| Time* | Treatment with chlorine dioxide ($ClO_2$) | | | |
|---|---|---|---|---|
| (Min) | 10ppm | 25ppm | 50ppm | MSD[1] |
| 2 | 5.149 ± 0.544 $^{a, A}$ | 4.433 ± 0.538 $^{a, A}$ | 4.078 ± 0.479 $^{a, A}$ | 1.305 |
| 5 | 4.839 ± 0.504 $^{a, A}$ | 4.127 ± 0.463 $^{a, A}$ | 3.709 ± 0.387 $^{a, A}$ | 1.138 |
| 10 | 4.327 ± 0.375 $^{a, A}$ | 3.797 ± 0.439 $^{a, A}$ | 3.371 ± 0.370 $^{a, A}$ | 0.992 |
| MSD[2] | 1.202 | 1.207 | 1.039 | ---- |
| Time* | Treatment with peracetic acid ($CH_3$-COOOH) | | | |
| (Min) | 50ppm | 75ppm | 100ppm | MSD[1] |
| 4 | 4.314 ± 0.425 $^{a, A}$ | 3.869 ± 0.577 $^{a, A}$ | 3.400 ± 0.593 $^{a, A}$ | 1.345 |
| 7 | 3.998 ± 0.472 $^{a, A}$ | 3.563 ± 0.640 $^{a, A}$ | 3.020 ± 0.692 $^{a, A}$ | 1.525 |
| 10 | 3.594 ± 0.468 $^{a, A}$ | 3.160 ± 0.690 $^{a, A}$ | 2.644 ± 0.673 $^{a, A}$ | 1.549 |
| MSD[2] | 1.141 | 1.596 | 1.638 | ---- |
| Blank (washing and immersion in tap water for 15 minutes) ** (log CFU.g$^{-1}$) | | | | 5.896 |
| Standard (washing with water and immersion in a solution of sodium hypochlorite: 120ppm/15min) ** ……………………………………(log CFU.g$^{-1}$) | | | | 2.400 |

MSD[1] = for the data on the lines; MSD[2] = for the data on the columns; small letter compares averages on the same line, capital letters compare means in the same column, different letters indicate that the data differ significantly at 5% probability; * Time of contact with the sanitizer product; ** reference treatments.

Table 2. Yeast and mold count (log CFU.g$^{-1}$) observed in samples of minimally processed rocket.

As for the action of these sanitizers in counts of total coliform in chicory (Table 3) and rocket (Table 4), minimally processed, the response was almost linear and inversely proportional, that is, when the concentration of sanitizers or their periods of contact were increased, the population of total coliforms also decreased.
Referring to the action of chlorine dioxide on the total coliform in chicory only the treatment with 50ppm/10min showed the same log cycle (1.34 log CFU.g$^{-1}$) of the standard treatment

(1.48 log CFU.g$^{-1}$) and statistically different from the others. In the case of peracetic acid, 2 treatments were better: 100ppm/10min (1.10 log CFU.g$^{-1}$) and 100ppm/7min (1.44 log CFU.g$^{-1}$) and they were statistically different from the others, however not different from each other. Therefore, as far as the control of total coliform in minimally processed chicory under the conditions of the treatments performed peracetic acid was more effective than chlorine dioxide.
In the case of the action of chlorine dioxide on the total coliform in minimally processed rocket only one treatment (50ppm/10min) provided results (3.85 log CFU.g$^{-1}$) in the same logarithmic cycle of the standard treatment (3.52 log CFU.g$^{-1}$) being statistically different from the others. When peracetic acid was used as sanitizer, only one treatment (100ppm/10min) was able to reduce the count of total coliforms to below the standard, respectively 2.87 x 3.52 log CFU.g$^{-1}$. Other 3 treatments (100ppm/4min, and 100ppm/7min 75ppm/10min) provided counts (3.65 log CFU.g$^{-1}$, 3.33 log CFU.g$^{-1}$ and 3.45 log CFU.g$^{-1}$) similar to the standard (3.52 log CFU.g$^{-1}$) being in the same log cycle.

| Time* | Treatment with chlorine dioxide ($ClO_2$) | | | |
|---|---|---|---|---|
| (Min) | 10ppm | 25ppm | 50ppm | MSD[1] |
| 2 | 3.088 ± 0.647 $^{a, A}$ | 2.944 ± 0.613 $^{a, A}$ | 2.302 ± 0.424 $^{a, A}$ | 1.428 |
| 5 | 2.820 ± 0.535 $^{a, A}$ | 2.578 ± 0.561 $^{a, A}$ | 2.014 ± 0.399 $^{a, A, B}$ | 1.213 |
| 10 | 2.544 ± 0.561 $^{a, A}$ | 2.423 ± 0.515 $^{a,b, A}$ | 1.339 ± 0.308 $^{b, B}$ | 1.883 |
| MSD[2] | 1.460 | 1.415 | 0.953 | ---- |
| Time* | Treatment with peracetic acid ($CH_3$-COOOH) | | | |
| (Min) | 50ppm | 75ppm | 100ppm | MSD[1] |
| 4 | 3.446 ± 0.143 $^{a, A}$ | 3.256 ± 0.194 $^{a, A}$ | 2.344 ± 0.292 $^{b, A}$ | 0.547 |
| 7 | 2.806 ± 0.412 $^{a, A, B}$ | 2.681 ± 0.397 $^{a, A, B}$ | 1.440 ± 0.095 $^{b, B}$ | 0.839 |
| 10 | 2.310 ± 0.544 $^{a, B}$ | 2.170 ± 0.492 $^{a,b, B}$ | 1.100 ± 0.174 $^{b, B}$ | 1.090 |
| MSD[2] | 1.008 | 0.957 | 0.510 | ---- |
| Blank (washing and immersion in tap water for 15 minutes) ** .(log CFU.g$^{-1}$) | | | | 5.587 |
| Standard (washing with water and immersion in a solution of sodium hypochlorite: 120ppm/15min) ** ……………………………….…(log CFU.g$^{-1}$) | | | | 1.480 |

MSD[1] = for the data on the lines; MSD[2] = for the data on the columns; small letter compares averages on the same line, capital letters compare means in the same column, different letters indicate that the data differ significantly at 5% probability; * Time of contact with the sanitizer product; ** reference treatments.

Table 3. Total coliform count (log CFU.g$^{-1}$) observed in samples of minimally processed chicory.

All samples of minimally processed chicory and rocket, treated with chlorine dioxide and peracetic acid were reduced by two logarithmic cycles for *Escherichia coli*, ie, an initial count of 2.86 log CFU.g$^{-1}$ in the treatment by washing and immersion in water to less than 1.00 log CFU.g$^{-1}$. However, in the sample of standard treatment there was a total control, that is, no growth.

There was no *Salmonella* sp./25g in all samples analyzed.
Therefore, when the results of the best treatments were considered, the two sanitizers tested proved to be as effective as treatment with sodium hypochlorite. Thus, both chlorine dioxide and peracetic acid are able to replace the sodium hypochlorite in concentrations and times considered (50ppm/10min chlorine dioxide, peracetic acid and the 100ppm/10min 120ppm/15min sodium hypochlorite). On the other hand, none of the sanitizers caused any kind of physical or unfavorable organoleptic product changes (wilting, darkening, strong odor, color change etc.) at the concentration levels studied.

| Time* | Treatment with chlorine dioxide ($ClO_2$) | | | |
|---|---|---|---|---|
| (Min) | 10ppm | 25ppm | 50ppm | MSD[1] |
| 2 | $5.132 \pm 0.064$ [a, A] | $4.732 \pm 0.047$ [b, A] | $4.487 \pm 0.106$ [c, A] | 0.191 |
| 5 | $4.896 \pm 0.138$ [a, A, B] | $4.530 \pm 0.157$ [b, A] | $4.215 \pm 0.094$ [b, B] | 0.331 |
| 10 | $4.570 \pm 0.177$ [a, B] | $4.136 \pm 0.187$ [b, B] | $3.847 \pm 0.114$ [b, C] | 0.407 |
| MSD[2] | 0.338 | 0.359 | 0.263 | ---- |
| Time* | Treatment with peracetic acid ($CH_3$-COOOH) | | | |
| (Min) | 50ppm | 75ppm | 100ppm | MSD[1] |
| 4 | $5.031 \pm 0.324$ [a, A] | $4.415 \pm 0.341$ [a, A] | $3.652 \pm 0.207$ [b, A] | 0.744 |
| 7 | $4.680 \pm 0.320$ [a, A] | $4.078 \pm 0.283$ [a, A, B] | $3.334 \pm 0.100$ [b, A] | 0.634 |
| 10 | $4.283 \pm 0.353$ [a, A] | $3.451 \pm 0.167$ [b, B] | $2.869 \pm 0.158$ [b, B] | 0.609 |
| MSD[2] | 0.833 | 0.685 | 0.403 | ---- |
| Blank (washing and immersion in tap water for 15 minutes) ** .(log CFU.g$^{-1}$) | | | | 6.224 |
| Standard (washing with water and immersion in a solution of sodium hypochlorite: 120ppm/15min) ** ……………………………….…(log CFU.g$^{-1}$) | | | | 3.517 |

MSD[1] = for the data on the lines; MSD[2] = for the data on the columns; small letter compares averages on the same line, capital letters compare means in the same column, different letters indicate that the data differ significantly at 5% probability; * Time of contact with the sanitizer product; ** reference treatments.

Table 4. Total coliform count (log CFU.g$^{-1}$) observed in samples of minimally processed rocket.

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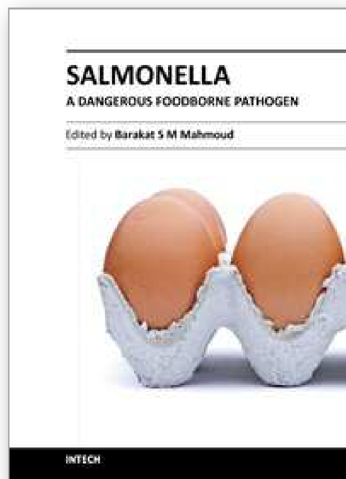


**Salmonella - A Dangerous Foodborne Pathogen**

Edited by Dr. Dr. Barakat S M Mahmoud

ISBN 978-953-307-782-6
Hard cover, 450 pages
**Publisher** InTech
**Published online** 20, January, 2012
**Published in print edition** January, 2012

More than 2,500 serotypes of Salmonella exist. However, only some of these serotypes have been frequently associated with food-borne illnesses. Salmonella is the second most dominant bacterial cause of food-borne gastroenteritis worldwide. Often, most people who suffer from Salmonella infections have temporary gastroenteritis, which usually does not require treatment. However, when infection becomes invasive, antimicrobial treatment is mandatory. Symptoms generally occur 8 to 72 hours after ingestion of the pathogen and can last 3 to 5 days. Children, the elderly, and immunocompromised individuals are the most susceptible to salmonellosis infections. The annual economic cost due to food-borne Salmonella infections in the United States alone is estimated at $2.4 billion, with an estimated 1.4 million cases of salmonellosis and more than 500 deaths annually. This book contains nineteen chapters which cover a range of different topics, such as the role of foods in Salmonella infections, food-borne outbreaks caused by Salmonella, biofilm formation, antimicrobial drug resistance of Salmonella isolates, methods for controlling Salmonella in food, and Salmonella isolation and identification methods.

**How to reference**

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Silvana Mariana Srebernich, Neliane Ferraz de Arruda Silveira and Gisele Mara Silva Gonçalves (2012). Occurrence of Salmonella in Minimally Processed Vegetables, Salmonella - A Dangerous Foodborne Pathogen, Dr. Dr. Barakat S M Mahmoud (Ed.), ISBN: 978-953-307-782-6, InTech, Available from: http://www.intechopen.com/books/salmonella-a-dangerous-foodborne-pathogen/occurrence-of-salmonella-in-minimally-processed-vegetables

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