Review: Trisodium Phosphate (TSP) Treatment for Decontamination of Poultry

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Review: Trisodium Phosphate (TSP) Treatment for Decontamination of Poultry

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Use of trisodium phosphate (TSP) treatment for reducing levels of bacteria in poultry is discussed with reference to: health and economic consequences of poultry contamination, causes or routes of contamination, possibilities for reduction of microbial loads, mechanisms of action of TSP, sensory properties and quality of TSP-treated poultry, antimicrobial effectiveness, influences on shelf-life, and worldwide authorization of this process, with special reference to the situation in the European Union. A summary of the main results of microbial reductions on poultry following TSP treatment is shown for *Salmonella*, coliforms/*Escherichia coli*, *Enterobacteriaceae*, *Campylobacter*, *Pseudomonas*, total counts, *Listeria*, *Staphylococcus aureus* and *Lactobacillus*. The main results on microbial reductions assessed in foodstuffs other than poultry (beef, fruit, fish and shellfish) are also shown.

Key Words: trisodium phosphate, decontamination, foodborne pathogens, poultry, safety, hygiene

En este artículo se discute el uso del tratamiento con fosfato trisódico (TSP) para reducir la contaminación bacteriana de la carne de ave con referencia a: consecuencias económicas y para la salud de la contaminación de la carne de ave, causas o rutas de contaminación, posibilidades de reducir la carga microbiana, mecanismos de acción del TSP, características organolépticas y calidad de la carne de ave tratada con TSP, eficacia antimicrobiana y factores que la afectan, influencia de este tratamiento en el tiempo de vida útil, y autorización de este proceso en el mundo, con especial referencia a la situación dentro de la Unión Europea. Se han resumido los resultados de las reducciones microbianas obtenidas en carne de ave tratada con TSP para *Salmonella*, coliformes/*Escherichia coli*, *Enterobacteriaceae*, *Campylobacter*, *Pseudomonas*, microorganismos totales, *Listeria*, *Staphylococcus aureus* y *Lactobacillus*. También se han contemplado los principales resultados obtenidos en otros alimentos distintos de la carne de ave (carne de vacuno, fruta, pescado y marisco).

Palabras Clave: fosfato trisódico, descontaminación, patógenos, carne de ave, seguridad, higiene

INTRODUCTION

Poultry and processed poultry products have undergone a remarkable increase in consumption since the 1960s because their low price and health implications. At present 27% of the world's total meat consumption is poultry, only exceeded by pork meat (Anonymous, 2001). This high consumption of poultry products makes the concern for marketing a product safe, with a low spoilage rate, and with right composition, packaging, colour, taste and appearance. However, increased consumption of poultry products has been accompanied by an increase in food borne illnesses associated with these foods. Microbiological safety of poultry products is a big concern of the poultry industry, government agencies and consumers. The United States Center for Disease Control and Prevention (CDC) has summarised the surveillance for food borne disease outbreaks for the years 1993–1999 (Olsen et al., 2000). Poultry (chicken or turkey) was the vehicle of transmission in the 3.4–7.1% (depending on the year consulted) of outbreaks and in the 1.4–5.9% of individual cases of food borne disease with known source. In all these outbreaks *Salmonella* was the dominating etiologic agent. *Clostridium perfringens*, *Bacillus cereus*, *Staphylococcus aureus* and *Campylobacter* spp. were identified as responsible of some food borne outbreaks. Poultry may be also the vector of other pathogenic microorganisms, such as *Listeria monocytogenes*, *Escherichia coli*, *Yersinia enterocolitica*, *Vibrio* spp. or *Clostridium botulinum* (Waldroup, 1996).

Contamination of poultry carcasses during slaughtering procedures is undesirable but unavoidable in the

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conversion of live animals to meat for consumption. In the case of poultry the problem starts with the "raw material", because the animals arrive at the slaughterhouse with a huge heterogeneous load of bacteria, both externally and intestinally, including potential pathogens and spoilage microorganisms. During slaughtering and processing, microorganisms may be transferred from carcass to carcass by live-bird crates, lorries, soiled surfaces, equipment, hands of workers or inspectors, airborne dust particles, or water (scalding or immersion chilling).

Poultry carcasses for human consumption are important sources of these infectious agents. Cases of food borne illness caused by the consumption of contaminated poultry meat are due mainly to improper cooking or handling (Panisello et al., 2000).

REDUCTION OF MICROBIAL CONTAMINATION OF POULTRY

According to the World Health Organization (WHO, 1997), illness due to contaminated food is perhaps the most widespread health problem and an important cause of reduced economic productivity. There is a need to reduce the burden of food borne disease by reducing the prevalence of zoonotic pathogens in poultry. The methods for eliminating pathogenic microorganisms from poultry meat have received considerable attention in recent years. The successful control of food borne human pathogens requires a general approach involving producers, processors, distributors, retailers and consumers. The aim must establish a fully integrated control program throughout the poultry supply chain.

The incapacity of traditional food safety assurance methods for combating food borne illness have led Public Health authorities and food industries to implement methods of hazard analysis and critical control points (HACCP). This system is internationally recognized as the most cost-effective for controlling food borne safety hazards from "farm to fork". HACCP may offer the best manners to enhance the safety of raw foods of animal origin, fresh produce, and certain prepared foods by minimizing the incidence and levels of pathogenic microorganisms (Molins et al., 2001). These preventive programs are now being used on a wider scale in poultry manufacturing plants to improve meat quality and safety (USDA-FSIS, 1996).

HACCP defines critical control point (CCP) as 'a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level' (Anonymous, 1997); within this context, a terminal decontamination step (e.g., trisodium phosphate treatment) would be of great value as a critical control point. However, it must be pointed out that the strict adherence to measures of good hygiene practice (GHP) and good manufacturing practice (GMP) during production and processing should be the basis of food safety strategies. Decontamination steps of whatever nature should serve as only added resources for assuring safety, and must not be allowed to mask bad practices (Smulders, 1987) because most decontamination techniques have only a limited effect in reducing pathogen contamination, and it depends directly on the initial level of contamination. According to the Scientific Committee on Veterinary Measures Relating to Public Health (SCVPH, 1998) there are three important strategies for pathogens reduction in poultry. Firstly, to ensure the lowest possible prevalence of pathogens in the production pyramid. Secondly, to implement hygienic measures which should be applied on the farm, during transport and in the processing plant, and lastly, the use of a decontamination treatment.

From a public health point of view, a decontamination treatment can be sustainable if the efficacy, the safety and the monitoring of that treatment can be achieved using the best available information and data (SCVPH, 1998). Other considerations may refer to the impact of the technique on product quality, worker safety, protection of the environment and consumer acceptance (Figure 1). Numerous decontaminant treatments have been applied so as to reduce carcass contamination including acids, bases, halogens, hydrogen peroxide, alcohols, mannose application, ozonation and irradiation (Figure 2). Although all these treatments have shown some degree of bactericidal efficacy, each one offers at least one significant disadvantage, such as induction of product quality deterioration, lack of stability, a high degree of reactivity, potential user health risks, consumer product safety concerns, high cost, or adverse sensorial changes, which have limited their usage (Bolder, 1997; Farkas, 1998; Smulders and Greer, 1998; Sofos and Smith, 1998; Capita et al., 1999a,b).

Trisodium phosphate (TSP) could contribute to the prevention of food borne illnesses by eliminating pathogens from poultry, minimizing the risk of cross-contamination throughout the food chain, minimizing the risk posed by foods which are consumed raw or processed using non-bactericidal treatments and minimizing the risk of survival or growth of bacterial pathogens as a result of under-cooking or temperature abuse of the food.

USE OF TRISODIUM PHOSPHATE

In August 1992, the Food and Drug Administration (FDA) assigned a generally recognized as safe (GRAS) status for trisodium phosphate (Na_3PO_4) as a food processing aid (with no label declaration needed).

1. It will not change appearance, smell, taste or nutritional properties
2. It will leave no residues
3. It will pose no threat to the environment
4. It will encounter no objections from consumers or legislators
5. It will be cheap and convenient to apply
6. It will improve the shelf-life by inactivating spoilage organisms as well as pathogens

Figure 1. Mean attributes for a ideal decontamination technique.

In October 1992, the United States Department of Agriculture (USDA) added TSP to its list of approved substances for use during the broiler slaughter process. Bender and Brotsky (1991) patented a process designed to reduce the incidence of *Salmonella* during poultry processing using food grade trisodium phosphate dodecahydrate for post-chill poultry treatment (AvGard™). This process began to be used in poultry slaughterhouses in USA (Giese, 1993) and involved dipping or spraying the carcasses with 8–12% solutions of TSP. The contact time was usually 15 s and the temperature of application is 20–30 °C.

The USDA Food Safety and Inspection Service (FSIS) approved TSP on the basis of several facts (Giese, 1993): (1) review of industry test data indicated that TSP could significantly reduce bacterial levels on poultry carcasses; (2) data showed the process left little or no residue on finished poultry products; (3) encourage the development and use of safe, innovative processing techniques to improve the microbiological profile of raw poultry; (4) the FDA's Center for Food Safety and Applied Nutrition notified FSIS that FDA considered that use of TSP as a processing aid for safety when used in accordance with good manufacturing practice. TSP is naturally present in tissues and bones and may thus be considered to be harmless. However, appropriate conditions of application should be designed and controlled to minimize water intake and retention in carcasses and to avoid an increase of effluents from poultry slaughterhouses; (5) FSIS implant studies indicated that TSP could significantly reduce bacteria on poultry.

In 1994 this process was approved for post-chill, pre-chill and air-chill application in Canada, and for pre-chill poultry and pre-chill giblets in USA. In October 1995, TSP treatment was approved for beef decontamination. In May 1997 the process began to be used as "on line reprocessing" (OLR) commercial test in USA, and in November the Canadian government approved the importation of OLR poultry. In July 1999, the AvGard™ technology was approved to treat poultry carcasses in France.

Chemical:
- Organic acids (lactic acid, buffered lactic acid, acetic acid, gluconic acid)
- Inorganic acids
- Chlorine and related compounds
- Carbohydrates (sucrose, mannose)
- Organic preservatives (benzoates, propionates)
- Bacteriocins or (bio) peptides (nisin, magainin)
- Oxidizers (hydrogen peroxide, ozone)
- Inorganic phosphates (**trisodium phosphate –TSP**, polyphosphates)
- Salts (Nitrite, NaCl, $NaHCO_3$)
- Hydroxides (NaOH, KOH)
- Gases (CO_2)
- Lysozyme
- Antibiotics
- EDTA
- Etanol

Physical:
- Water (rinse, spray)
- High presure
- Irradiation (electromagnetic radiation, ionising radiation)
- Electrical methods
- Air ions
- Ultrasonic energy
- UV light
- High temperature (steam, hot water)
- Freeze-thaw cycling

Microbial:
- Lactic acid bacteria
- Bacteriocins
- Microbial parasites

Figure 2. Mean poultry decontamination treatments.

The Situation in the European Union

The use of TSP for poultry decontamination is not widely spread. TSP is in Europe an authorised food additive (E339iii) for use in different food products, including milk and milk products, meat products, drinks and cereals. However, TSP is not authorized as decontaminant of poultry. Within the European Union (EU), antimicrobial treatments are not allowed for treatment of red meat or poultry carcasses, parts or viscera. EU meat hygiene regulations do not allow any method or product decontamination other than washing with tap water. The permission for the adoption of this technology is perceived to hide poor hygienic practices in the slaughterhouse.

The Public Health Section of the Scientific Veterinary Committee of the European Commission – Public

1. The chemical composition and properties, e.g., pH, water and fat solubility, mode of action
2. Safety aspects: is it a European Union approved food additive evaluated by the Joint FAO/OMS Expert Committee on Food Additives or is it a "Generally Recognized As Safe" (GRAS) compound?
3. Residues in the decontaminated product
4. Impact of the product with regard to organoleptic quality (taste, appearance, etc.)
5. Nutritional value
6. Risk of formation of toxic compounds
7. Water retention properties
8. Effect on pathogenic and spoilage organisms; posible implications of flora changes
9. Impact on spoilage and shelf-life
10. Relationship between the number of phatogens and the desired effect
11. Staff health aspects including hypersensitization
12. Recycling, recovery and environmental impact
13. Microbial investigations *in situ* to prove efficacy
14. Method of application and concentrations to be used
15. "Food additive" or "processing aid"?

Figure 3. Factors for consideration in the application of decontaminants (proposed by the Scientific Veterinary Committee of the EU, 1998).

Health Section – (SCVPH, 1998) has expressed its favorable opinion to the use of methods for decontamination (including TSP treatment) in poultry slaughtering. This Commission formulated some criteria that a compound has to be met before considering it for acceptance in poultry processing (Figure 3).

Another negative reason for the European authorities not to authorize TSP might be the enhancement of water retention in the carcasses by its use. However, from that point of view if moisture content does not exceed the levels stipulated in the EU Directives, there would not be objections to its use. In the United States, experiments carried out by USDA-FSIS on water chilled carcasses showed that the water absorption and retention were below acceptable levels.

In June 1999, the French authorities authorized the TSP ($10 \pm 2\%$ TSP treatment for 15 s to pre- or post-chill-water carcasses and to pre-chill-air carcasses) as a "technological auxiliary" (with label declaration needed) for reducing by 2 log units the microbial contamination of poultry carcasses (Anonymous, 1999).

Mechanisms of Action

The mechanisms of bacterial reductions on chicken carcasses by the TSP treatment is still not fully understood. However, it seems to result from a combination of several factors (Anonymous, 1994; Federighi et al., 1995; Hwang and Beuchat, 1995; Rodríguez de Ledesma et al., 1996; Yang et al., 1998). Firstly, the high pH (about 12) of the TSP solutions, for instance, the effectiveness of TSP over *Salmonella* Chester on pepper discs was reduced by 26% when the pH of the solutions was reduced from 12 to 4.5 (Liao and Cooke, 2001). Secondly, the ionic strength, which can cause bacterial cell autolysis. TSP has an activity on bacterial walls, which may account for the greater sensitivity of Gram-negative bacteria due to the structure of the cell membrane, checked by electronic microscopy. The treatment with increase 10% TSP increased the susceptibility of Gram-negative bacteria to nisin and lysozyme (Carneiro et al., 1998). The ability to remove a thin layer of lipids ("detergent" effect) is also an important factor in the bactericidal activity of TSP. During feathering removal, bacteria may find their way into open feather follicles and subsequently be protected by lipid smearing. TSP may remove this protection and also contribute to eliminate bacteria that are not yet firmly adherent to the surface from chicken skin.

Effects on Sensory Properties and Quality of Poultry

Sensory properties of the TSP treated poultry samples seem not to be affected under the conditions proposed for treatment or even at higher time-concentration treatments to those normally applied (Bender and Brotsky, 1991; Giese, 1992, 1993; Hollender et al., 1993; Coppen, 1994; Hathcox et al., 1995; Ellerbroek et al., 1996; Kim and Marshall, 1999; Capita et al., 2000a). The experiments carried out by Rodríguez de Ledesma et al. (1996) showed a preference for the TSP treated wings as opposed to samples dipped in water. This preference might be due to an increased water-holding capacity of the proteins that would improve the appearance of the muscle and skin after dipping the wings in TSP. The increase in yield due to water uptake is around 1% (Rhodia, unpublished data).

Modifications in sensory properties were not found in foodstuffs other than poultry either. Treatment with 12% TSP had a non-significant effect on visual color, texture and odour of pork (Morris et al., 1997). Zhuang and Beuchat (1996) found that the appearance (colour and brightness) of ripened tomatoes was unaffected by 0–5% TSP treatment for 15 s or 2 min.

Antimicrobial Effectiveness of TSP on Poultry

The bactericidal effect of TSP is well documented in scientific literature and confirmed in several industrial tests. TSP is more active on Gram-negative pathogens (e.g., *Salmonella*, *E. coli*, *Campylobacter* spp.) than against Gram-positive ones (e.g., *S. aureus*, *L. monocytogenes*). In general, Gram-negative bacteria are much more sensitive to the effects of alkaline sanitizers than the Gram-positive bacteria (Dickson et al., 1994). Colin and Salvat (1996) found an average of 100-fold reduction for Gram-negative bacteria after treatment with TSP at 10%, while that only a 10-fold reduction is found for total aerobic mesophiles (including Gram-positive and Gram-negative bacteria). However, it is important to point out that TSP can reduce, but not totally eliminate, the incidence of salmonellae and other food borne pathogens on poultry (Tables 1–9).

Apart from the microbial group considered, other factors influence the antimicrobial efficacy of TSP on poultry.

Bacterial Strain

The bacterial strain has a significant influence on the susceptibility to TSP. Capita et al. (2000c) studied the minimum inhibitory concentration (MIC) of TSP for 21 *L. monocytogenes* strains (4 clinical isolates, 12 strains isolated from poultry meat, and 5 ATCC strains) and found that the MICs varied between 1.58 and 1.68% depending on the strain. Fratamico et al. (1996) also found differences in susceptibility of *E. coli* strains to TSP on beef.

Temperature of Application

In general, the higher the temperature of application the greater the activity (Dickson et al., 1994; Kochevar et al., 1997; Xiong et al., 1998b). Slavik et al. (1994) did not find any significant reduction in *Campylobacter* counts at 10 °C, however, marked reductions (1.2–$1.5 \times \log_{10}$ cfu after storage at 4 °C for 1–6 days) were found when the treatment temperature was 50 °C. Kim et al. (1994b) observed that 50 °C TSP-treatment gave 0.4 log greater reduction than 10 °C treatment (although the difference was not statistically significant). However Dickson et al. (1994) did not find any temperature effect in treated beef, and Somers et al. (1994) observed that *Salmonella* Typhimurium cells were more sensitive to TSP treatment at 10 °C than at room temperature.

Concentration of TSP

Concentration of TSP in the range 8–12% was not a significant factor in reducing the *S.* Typhimurium populations on beef (Dickson et al., 1994). However, there was a statistically significant difference between 8 and 12% concentrations with *L. monocytogenes*. There were significant differences in mesophilic, psychrotrophic and *L. monocytogenes* populations on chicken skin treated for 15 min with 8–12% TSP (Capita et al., 2000b, 2001a,b). Other authors also observed a direct relationship between the TSP concentration used and the reductions of bacteria on poultry (Somers et al., 1994; Mu et al., 1997; Kim and Kim, 2000).

Time and Procedure of Application

Duration of application was an important factor controlling the efficacy of TSP treatment against *Salmonella* Enteritidis biofilms (Korber et al., 1997). Pre-chill chicken carcasses inoculated with *S.* Typhimurium with TSP for 30 or 90 s got the greatest microbial reductions in 90 s (Korber et al., 1997).

The application procedure is also an important factor in the antimicrobial effect of TSP. The effect of three 10% TSP treatments on poultry depended on the way

Table 1. Summary of tests for determining the effectiveness of trisodium phosphate against *Salmonella* on poultry.

Sample	Treatment	Reduction	Reference
Chicken carcasses	8–12% TSP, 15 s	35%	Bender and Brotsky (1991)
Chicken carcasses	Dip: 10% TSP, 15 s, 10 °C	24.5%	Charles Felix Associates (1992)
Poultry carcasses	TSP 15 s	35%	Giese (1992)
Chicken carcasses	AvGard™ procedure	17–18.6%	Anonymous (1994)
Chicken drumsticks	Dip: 10% TSP, 15 s, 10 and 50 °C	1.7 and 2.2 $\log_{10}$ for 10 and 50 °C TSP-solutions, respectively	Kim et al. (1994a)[a]
Post-chill chicken carcasses	Dip: 10% TSP, 15 s, 10 and 50 °C	Naturally contaminated carcasses: 12–16%; Artificially inoculated carcasses: 27–46%, 1.6–1.8 $\log_{10}$	Kim et al. (1994b)[a]
Chicken carcasses	Dip: 10% TSP, 15 min	2 $\log_{10}$	Lillard (1994)[a]
Chicken skin	1% TSP, 10 min, 23 °C	73.5 ± 13.1%	Li et al. (1994)[a]
Breast chicken skin	Dip: 1% TSP, 30 min, 25 °C	1.7 $\log_{10}$	Hwang and Beuchat (1995)[a]
Chicken carcasses	Dip: 10% TSP, 15 s	78.6%	Colin and Salvat (1996)
Chicken carcasses	Spray (1 l/carcass): 10% TSP, for 10 s	10%	Ellerbroek et al. (1996)
	Spray (2 l/carcass): 10% TSP, for 20 s	20%	
	Dip: 10% TSP, for 6 s	−10%	
Chicken wings	Dip: 10% TSP, 15 s, 10 °C	93.45 or 62.42% after overnight storage at 10 or 4 °C, respectively	Rodríguez de Ledesma et al. (1996)[a]
	Dip: 10% TSP, 15 s, 10 °C + hot water (95 °C for 5 s)	94.76 or 99.67% after overnight storage at 10 or 4 °C, respectively	
Chicken carcasses (neck skin)	AvGard™ procedure	2 $\log_{10}$	Salvat et al. (1997)[a]
Chicken skin	Spray: 10% TSP, 30 s, 10, 35 or 60 °C, different high pressures	1.6–2.3 $\log_{10}$	Wang et al. (1997)[a]
Pre-chill chicken carcasses	Spray: 5–10% TSP, 90 s, different high pressures	3.7 $\log_{10}$	Yanbin et al. (1997)[a]
Chicken carcasses	AvGard™ procedure	Air-chill process: 57%; water-chill process: 64.6%, >2 $\log_{10}$	Coppen et al. (1998)
Chicken skin	Spray: 5–10% TSP, 30 s, 20 °C, 206 kPa	2.1–2.2 $\log_{10}$	Xiong et al. (1998a)[a]
Pre-chilled chicken carcasses	Spray: 10% TSP, 17 s, 35 °C, 413 kPa	2 $\log_{10}$	Yang et al. (1998)[a]
Chicken carcasses (neck skin)	Dip: 10% TSP, 15 s	From 1.92 $\log_{10}$ cfu/g (in control samples) to undetected levels in TSP-treated samples	Whyte et al. (2001)

[a]Artificially inoculated samples.

Table 2. Summary of tests for determining the effectiveness of trisodium phosphate against coliforms and *Escherichia coli* on poultry.

Microbial Group	Sample	Treatment	Reduction	Reference
E. coli	Chicken carcasses	Dip: 10% TSP, 15 s	0.3–1.8 $\log_{10}$	Colin and Salvat (1996)
Thermotolerant coliforms	Chicken carcasses (neck skin)	AvGard™ procedure	2 $\log_{10}$	Salvat et al. (1997)
Coliforms	Chicken carcasses	AvGard™ procedure	2.7 $\log_{10}$	Coppen et al. (1998)
E. coli	Chicken carcasses (neck skin)	Dip: 10% TSP, 15 s	1.95 $\log_{10}$	Whyte et al. (2001)

of application (Ellerbroek et al., 1996). These authors studied the effect of three treatments: spray (1 l/carcass for 10 s), spray (2 l/carcass for 20 s) and dip for 6 s, in total aerobic counts at 0, 3 and 6 days of refrigerated storage, and reached the greatest mean reduction by dipping. Wang et al. (1997) found that the greatest reductions in the numbers of *S. typhimurium* on poultry were obtained in TSP spraying treatments in the high pressure range (620.5–1034.2 kPa) at 60 °C. Reduction of salmonellae counts increased linearly with pressure in chicken skin (Li et al., 1996). Antimicrobial effect by spray washing increased in breast area adipose tissue of lamb carcasses at higher pressure (Kochevar et al., 1997). However there have been also results in which

Table 3. Summary of tests for determining the effectiveness of trisodium phosphate against *Enterobacteriaceae* on poultry.

Sample	Treatment	Reduction	Reference
Chicken carcasses	Dip: 10% TSP, 15 s	0.3–2 $\log_{10}$	Colin and Salvat (1996)
Chicken carcasses	Spray (1 l/carcass): 10% TSP, 10 s	Approx. 1–1.4 $\log_{10}$ after 0–6 days of storage at 4 °C	Ellerbroek et al. (1996)
	Spray (2 l/carcass): 10% TSP, 20 s	Approx. 0.4–1.5 $\log_{10}$ after 0–6 days of storage at 4 °C	
	Dip: 10% TSP, 6 s	Approx. 1.1–1.8 $\log_{10}$ after 0–6 days of storage at 4 °C	
Chicken carcasses	AvGard™ procedure	2.5 $\log_{10}$	Coppen et al. (1998)
Chicken carcasses (neck skin)	Dip: 10% TSP, 15 s	1.86 $\log_{10}$	Whyte et al. (2001)

Table 4. Summary of tests for determining the effectiveness of trisodium phosphate against *Campylobacter jejuni*/*coli* on poultry.

Sample	Treatment	Reduction	Reference
Chicken carcasses	Dip: 10% TSP, 15 s, 50 °C	<limit of detection in 72 or 4–36% samples (depending on a nitrocellulose membrane lift or a conventional culture method are used for determination)	Slavik et al. (1994)
	Dip: 10% TSP, 15 s, 10 °C	0.16 $\log_{10}$ ($P > 0.1$)	
	Dip: 10% TSP, 15 s, 50 °C	1.2–1.5 $\log_{10}$ after 1–6 days of storage at 4 °C	
Poultry carcasses (thermophilic *Campylobacter*)	Dip: 10% TSP, 15 s, 16–20 °C	1.3 $\log_{10}$	Federighi et al. (1995)
Chicken carcasses	Dip: 10% TSP, 15 s	100%	Colin and Salvat (1996)
Chicken carcasses (neck skin)	AvGard™ procedure	<limit of detection in 100% of samples	Salvat et al. (1997)
Chicken carcasses (neck skin)	Dip: 10% TSP, 15 s	1.71 $\log_{10}$	Whyte et al. (2001)

Table 5. Summary of tests for determining the effectiveness of trisodium phosphate against *Pseudomonas* spp. on poultry.

Sample	Treatment	Reduction	Reference
Chicken carcasses	Dip: 10% TSP, 15 s	>1.8 $\log_{10}$	Colin and Salvat (1996)
Chicken carcasses	Spray (1 l/carcass): 10% TSP, 10 s	Approx. 0.6–1 $\log_{10}$ cfu after 0–6 days of storage at 4 °C	Ellerbroek et al. (1996)
	Spray (2 l/carcass): 10% TSP, 20 s	Approx. 0.7–1.4 $\log_{10}$ cfu after 0–6 days of storage at 4 °C	
	Dip: 10% TSP, 6 s	Approx. 0.7–1.8 $\log_{10}$ cfu after 0–6 days of storage at 4 °C	
Chicken carcasses (neck skin)	AvGard™ procedure	2 $\log_{10}$	Salvat et al. (1997)
Chicken carcasses	AvGard™ procedure	1.5 $\log_{10}$	Coppen et al. (1998)

variations in pressure did not significantly affect the total and coliform counts (Bautista et al., 1997).

Storage

The best benefits of the utilization of TSP as decontaminant are observed after several days of storage. This fact can be understood because populations in control samples increase from the first day of refrigerated storage, while in treated samples bacterial counts remain practically constant or slightly increase, especially psychrotrophic microorganisms (Dorsa et al., 1997; Capita et al., 2000b, 2001a,b). Ellerbroek et al. (1996) found reductions from 0.65 to 2.1 $\log_{10}$ cfu/g in total aerobic counts on chicken carcasses at 0–6 days of refrigerated storage, and observed that the greatest mean reduction was achieved on day 6 of storage for TSP treatments under the same concentrations and storage conditions. However, no differences between 1- and 6-day stored groups were observed in *Campylobacter* counts on poultry carcasses treated with TSP (Slavik et al., 1994).

Table 6. Summary of tests for determining the effectiveness of trisodium phosphate against total counts on poultry.

Microbial Group	Sample	Treatment	Reduction	Reference
Psychrotrops	Chicken skin fragments	Dip: 1% TSP, 30 min, 25 °C	1.8 $\log_{10}$	Hwang and Beuchat (1995)
Aerobic mesophiles	Chicken carcasses	Dip: 10% TSP, 15 s	0.1–1.8 $\log_{10}$	Colin and Salvat (1996)
Total viable counts	Chicken carcasses	Spray (1 l/carcass): 10% TSP, 10 s	Approx. 1.1–1.5 $\log_{10}$ after 0–6 days of storage at 4 °C	Ellerbroek et al. (1996)
		Spray (2 l/carcass): 10% TSP, 20 s	Approx. 0.65–1.25 $\log_{10}$ after 0–6 days of storage at 4 °C	
		Dip: 10% TSP, 6 s	Approx. 1.3–2.1 $\log_{10}$ after 0–6 days of storage at 4 °C	
Spoilage bacteria	Chicken wings	TSP + hot water	3 $\log_{10}$ after 7 d at 4 °C	Rodríguez de Ledesma et al. (1996)
Aerobic mesophiles	Chicken carcasses (neck skin)	AvGard™ procedure	Approx. 1 $\log_{10}$	Salvat et al. (1997)
Total aerobics	Chicken carcasses	AvGard™ procedure	1.1 $\log_{10}$	Coppen et al. (1998)
Total aerobics	Pre-chilled chicken carcasses	Spray: 10% TSP, 17 s, 35 °C, 413 kPa	0.74 $\log_{10}$	Yang et al. (1998)
Spoilage microorganisms	Chicken legs	5% TSP, 10 min	3 $\log_{10}$ cfu after 12 d at 4 °C	Kim and Marshall (1999)
Mesophilic	Chicken skin fragments	Dip: 8–12% TSP, 15 min, 22 °C	0.95–1.78 $\log_{10}$ (day 0) to 2.35–3.08 $\log_{10}$ (day 5 of refrigerated storage)	Capita et al. (2000b)
Psychrotrophic	Chicken skin fragments	Dip: 8–12% TSP, 15 min, 22 °C	0.92–1.94 $\log_{10}$ (day 0) to 2.79–4.09 $\log_{10}$ (day 5 of refrigerated storage)	
Indigenous aerobes microorganisms	Chicken wings	8% TSP	1.21 $\log_{10}$	Ismail et al. (2001)

Table 7. Summary of tests for determining the effectiveness of trisodium phosphate against *Listeria* on poultry.

Sample	Treatment	Reduction	Reference
Breast chicken skin samples	Dip: 1% TSP, 30 min, 25 °C	2.1 $\log_{10}$	Hwang and Beuchat (1995)[a]
Chicken carcasses	Dip: 10% TSP, 15 s	6.67% (day 0 of refrigerated storage); 35% (day 7 of refrigerated storage)	Colin and Salvat (1996)
Chicken wings	Dip: 10% TSP, 15 s, 10 °C	39.04 or 81.41% after overnight storage at 10 or 4 °C, respectively	Rodríguez de Ledesma et al. (1996)[a]
	Dip: 10% TSP, 15 s, 10 °C + hot water (95 °C for 5 s)	79.49 or 94.88% after overnight storage at 10 or 4 °C, respectively	
Chicken carcasses	AvGard™ procedure	2.5 $\log_{10}$ cfu	Coppen et al. (1998)
Chicken skin fragments	Dip: 8–12% TSP, 15 min, 22 °C	1.52–3.63 $\log_{10}$	Capita et al. (2001a)[a]
Chicken legs	Dip: 8–12% TSP, 15 min, 22 °C	1.12–3.34 $\log_{10}$	Capita et al. (2001b)[a]

[a]Artificially inoculated.

Table 8. Summary of tests for determining the effectiveness of trisodium phosphate against *Staphylococcus aureus* on poultry.

Sample	Treatment	Reduction	Reference
Chicken wings	Dip 10% TSP, 15 s, 10 °C	80.33 or 54.45% after overnight storage at 10 or 4 °C, respectively	Rodríguez de Ledesma et al. (1996)[a]
	Dip 10% TSP, 15 s, 10 °C + hot water (95 °C for 5 s)	84.41 or 96.68% after overnight storage at 10 or 4 °C, respectively	

[a]Artificially inoculated.

Table 9. Summary of tests for determining the effectiveness of trisodium phosphate against *Lactobacillus* on poultry.

Sample	Treatment	Reduction	Reference
Chicken carcasses	Spray (1 l): 10% TSP, 10 s	Approx. 0.3–1 $\log_{10}$ cfu after 0–6 days of storage at 4 °C	Ellerbroek et al. (1996)
	Spray (2 l): 10% TSP, 20 s	Approx. 0–0.6 $\log_{10}$ cfu after 0–6 days of storage at 4 °C	
	Dip: 10% TSP, 6 s	Approx. −0.1–0.9 $\log_{10}$ cfu after 0–6 days of storage at 4 °C	

Sample Type

According to Ellerbroek et al. (1996) chicken parts could be more easily affected by decontaminants than whole carcasses because of their smaller size makes contact between the decontaminant and bacteria easier than when the whole carcass is treated. Marked differences in the effect of TSP have also been observed *in vitro* and in *in vivo* tests. Using artificial biofilms or suspension of cells, Somers et al. (1994) found a very high reduction rate: 5 logs in *Campylobacter* counts. Dickson et al. (1994), Kim and Slavik (1994) and Fratamico et al. (1996) found greater reductions in *E. coli*, *S.* Typhimurium and *L. monocytogenes* populations on adipose than in lean beef tissue.

Stage of Application

TSP treatment has been shown to be more effective when applied promptly after evisceration, when the bacteria are not yet firmly attached to carcass surfaces. According to Fratamico et al. (1996) the attachment of *E. coli* in beef increased with increasing incubation times, with most attachment occurring within 1 min of incubation, and that TSP was efficient for their removal. The effect of exposure time of beef-fat fascia to *E. coli* was significant on the efficiency of removal of bacteria, for instance, in samples TSP-treated after 2–4 h of inoculation reduction was lesser (1.76 or 1.26 logs, respectively) than in samples TSP-treated immediately after inoculation (3.04 logs). However, if decontamination treatment is carried out in a pre-chill stage, care should be taken to avoid recontamination after treatment (airborne contamination or contamination by equipment). Tamblyn et al. (1997) did not find any differences in the antimicrobial effect of TSP depending on the moment of application: during chiller or in a post-chill dip application.

Substratum Topography

Korber et al. (1997) observed that substratum topography affects the susceptibility of *S.* Enteritidis biofilms to TSP, and suggested that the inherent roughness of the substratum on which the biofilm was grown is an important factor controlling the efficacy of TSP treatment. It has been observed that the skin site is an important factor to be considered when decontamination protocols are developed for poultry carcasses using TSP (Capita et al., 2002).

Influence of Microbial Contamination on Shelf-Life of Poultry Products

Microorganism populations slowly increase throughout the refrigeration period, and since the sensory properties of the product do not significant change, the shelf-life of the poultry carcasses increases when exposed to TSP solutions (Salvat et al., 1995; Colin and Salvat, 1996; Ellerbroek et al., 1997). Shelf-life of chicken legs stored at 4 °C could be extended up to 16 days using 7.5 or 10% TSP solution (Kim and Marshall, 1999). Colin and Salvat (1996) also corroborated this finding and explained the increase of shelf-life of TSP-treated poultry because a qualitative change in the psychrotrophic flora.

Pseudomonas spp. are the microorganisms most involved in the spoilage of chicken carcasses refrigerated under aerobic conditions (ICMSF, 1998); when the carcasses are washed with a solution of TSP, the pseudomonas present are severely damaged or destroyed since Gram-negative microorganisms are highly sensitive to this compound. This fact is accompanied by the proliferation of other Gram-positive microorganisms also present, especially *Brochothrix thermosphacta*. In fact, poultry carcasses treated with TSP and refrigerated for 10 days showed that *B. thermosphacta* accounted for 96% of the psychrotrophic bacteria (Coppen et al., 1998). Thus, *B. thermosphacta* are responsible for the spoilage of poultry carcasses treated with TSP and stored in refrigerator. However, those microorganisms grow more slowly than *Pseudomonas* spp., therefore the average shelf-life of the carcasses increases.

Some authors consider that this prolongation of the shelf-life might constitute a hazard for consumers since the pathogenic microorganisms, some of which are psychrotrophic and Gram-positive strains (more resistant to TSP than Gram-negative strains) would have more time to develop. However, Colin and Salvat (1996) and Salvat et al. (1997) reduced substantially Gram-negative bacteria in poultry carcasses without increasing the risk associated with Gram-positive pathogens (particularly *L. monocytogenes*). According to these authors, the growth rate of the

Table 10. Summary of tests for determining the antimicrobial effectiveness of trisodium phosphate in foods other than poulty. Beef.

Sample	Microbial Group	Treatment	Reduction	Reference
Beef	*Salmonella* Typhimurium	Dip: 10% TSP, 15 s, 10 °C	0.91 (fat) or 0.52–0.86 (fascia)	Kim and Slavik (1994)[a]
	Escherichia coli O157:H7		1.35 (fat) or 0.92–1.39 (fascia)	
Beef	*Salmonella* Typhimurium	8, 10 or 12% TSP, ≤ 3 min, 25, 40 or 55 °C	1 (lean) or 1.3–2.5 (fat)	Dickson et al. (1994)[a]
	Escherichia coli O157:H7	8, 10, 12% TSP, ≤ 3 min, 25, 40, 55 °C	0.85–1.5 (lean), 1.3–2.5 (fat)	
	Listeria monocytogenes	8, 10, 12% TSP, ≤ 3 min, 25, 40, 55 °C	0.25–0.8 (lean) or 1–1.5 (fat)	
Beef brisket adipose tissue	Streptomycin-resistant *E. coli*	12% TSP, 12 or 36 s, 16 °C, 1.38 bar + wash with water: 12 or 36 s, 16–74 °C, 20.68 bar	0.17–0.44 (inoculation site), 0.37–0.51 (adjacent to the inoculation site)	Gorman et al. (1995)[a]
		Water wash: 12 or 36 s, 16–74 °C, 20.68 bar, + 12% TSP, 12 or 36 s, 16 °C, 1.38 bar	−0.24–1.94 (inoculation site), −0.34–1.37 (adjacent to the inoculation site)	
	Total aerobics	12% TSP, 12 or 36 s, 16 °C, 1.38 bar + wash with water: 12 or 36 s, 16–74 °C, 20.68 bar	−0.5–0.29 (inoculation site), 0.13–0.48 (adjacent to the inoculation site)	
		Water wash: 12 or 36 s, 16–74 °C, 20.68 bar, +12% TSP, 12 or 36 s, 16 °C, 1.38 bar	−0.31–1.02 (inoculation site), −0.28–0.65 (adjacent to the inoculation site)	
Beef-fat fascia	*Escherichia coli*	Dip: 12% TSP, 12 s	$1.26 \pm 0.25 \log_{10}$ (treatment 4 h after inoculation); $1.76 \pm 1.02 \log_{10}$ (treatment 2 h after inoculation); $3.04 \pm 0.40 \log_{10}$ (treatment immediately after inoculation)	Cabedo et al. (1996)[a]
Beef	*E. coli* O157:H7	Dip: 10% TSP, 25 or 65 °C	0.6–0.8 (tenderloin), 1.8–3.4 (adipose) after 18 h at 4 °C	Fratamico et al. (1996)[a]
Beef (lean tissue)	*E.* O157:H7	Spray: 12% TSP, 15 s, 32 ± 2 °C, 5.5 bar	<1.3 logs	Dorsa et al. (1997)[a]
	Listeria innocua, *Clostridium sporogenes*			
	Total mesophilic aerobic bacteria, pseudomonads, acid lactic bacteria		<1	
Packaged ground beef	*Salmonella* Typhimurium	Spray: 12% TSP, 15 s, 5.5 bar, 32 ± 2 °C	1.6–1.7 before grounding to 0.5–2.2 after grounding and storage at 4 °C	Dorsa et al. (1998)[a]
	Escherichia coli O157:H7		1.8 before grounding to 0–0.6 after grounding and storage at 4 °C	
	Pseudomonads		0.5–1.3 before grounding to 0–0.5 after grounding and storage at 4 °C	
	Mesophilic aerobic bacteria		0.9–1.4 before grounding to 0.4–0.9 after grounding and storage at 4 °C	
	Listeria innocua		1.5–1.9 before grounding to 0.9–1.7 after grounding and storage at 4 °C	
	Lactic acid bacteria		1.4–1.7 before grounding to 0.5–1.5 after grounding and storage at 4 °C	
	Clostridium sporogenes		0.3–1.7 before grounding to 0–0.6 after grounding and storage at 4 °C	
Beef	*Salmonella* Typhimurium and enterohemorrhagic *E. coli*	Spray: 10% TSP, 15 s, 35 ± 2 °C, 125 ± 5 psi	$> 3 \log_{10}$	Cutter et al. (2000)[a]
Beef variety meats	*Escherichia coli*	Dip or spray: 12% TSP, 10 min, 50 °C	-0.3–$2.2 \log_{10}$ (dip), -0.4–2.1 (spray)	Delmore et al. (2000)
	Total coliforms		0.3–$2.1 \log_{10}$ (dip), 0.1–$2.6 \log_{10}$ (spray)	
	Aerobic plate counts		0.1–$1.2 \log_{10}$ (dip), 0.4–$1.2 \log_{10}$ (spray)	

[a]Artificially inoculated.

Table 11. Summary of tests for determining the antimicrobial effectiveness of trisodium phosphate in foods other than poulty. Vegetables.

Sample	Microbial Group	Treatment	Reduction	Reference
Tomatoes	*Salmonella* Montevideo	Dip: 0–15% TSP, 15 s or 2 min, 37 °C	0.87–4.07 $\log_{10}$ (surface), 0.32–1.97 $\log_{10}$ (core)	Zhuang and Beuchat (1996)[a]
Alfalfa seeds	*E. coli* O157:H7	Dip: 0–15% TSP, 0.5–2 min, 23 °C	1.06 $\log_{10}$–>2.24 $\log_{10}$	Taormina and Beuchat (1999)[a]
Fresh cut apple discs	*Salmonella* Chester	2% TSP	1–2 $\log_{10}$	Liao and Sapers (2000)[a]
Apples	Nonpathogenic *E. coli*	Brush washer: 8% TSP, 20 or 50 °C	Non-significant	Annous et al. (2001)[a]
Fresh-cut green pepper slices	*Salmonella* Chester	3–12% TSP, 5 min	1–2 $\log_{10}$	Liao and Cooke (2001)[a]

[a]Artificially inoculated.

Table 12. Summary of tests for determining the antimicrobial effectiveness of trisodium phosphate in foods other than poulty. Fish products.

Sample	Microbial	Treatment	Reduction	Reference
Blue crab meat	*L. monocytogenes*	Dip: 0.1 M	0.8 $\log_{10}$–>4.6 $\log_{10}$	Degnan et al. (1994)[a]
Sashimi[b]	*Salmonella*	Dip of raw products: 1.3% TSP, 30 min	2 $\log_{10}$	Hiwaki et al. (1995)[a]
Rainbow trout fillets	*L. monocytogenes*	Dip: 20% TSP, 10 min	2 $\log_{10}$	Mu and Huang (1995)[a]
Channel catfish frames	Aerobic plate counts, coliforms	10% TSP, 5 min, 5 °C	1 and 2.5 $\log_{10}$ for APC and coliforms in rinse buffer, respectively; the shelf-life increases by 3 days	Marshall and Jindal (1997)[a]
Rainbow trout and shrimp	*L. monocytogenes*	Dip: 10 or 20% TSP, 10 min, 10 °C	Approx. 0.4–2 $\log_{10}$	Mu et al. (1997)[a]

[a]Artificially inoculated.
[b]Sliced raw meat and edible organs.

Gram-positive spoilage bacteria present in TSP-treated carcasses (mainly *B. thermosphacta*) was greater than that of *L. monocytogenes* at refrigeration temperatures. Thus, it is assumed that spoilage occurs prior to any marked increase of pathogenic microorganisms, and that TSP prevents the risk of the emergence of *L. monocytogenes*. However, concentration of TSP used is crucial, since earlier studies carried out *in vitro* suggested that carcasses washed with very low concentrations of TSP could encourage multiplication of *L. monocytogenes* through reduction of the lag phase and an increased maximum growth rate (Capita et al., 2001c).

Effect of TSP on Other Foodstuffs

Most of studies on the effectiveness of TSP have realized about poultry. However, other foods (fundamentally beef and fruits) have also been tested (Tables 10–12).

The TSP treatment appears to have a potential use also in cleaning operations, especially with respect to reduction of Gram-negative organisms in biofilms (Somers et al., 1994).

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