Vol.13,n.2,pp.10-14 (Dec 2015 – Feb 2016) Brazilian Journal of Surgery and Clinical Research- BJSCR

MICROBIOLOGICAL RESEARCH IN APPLES; EFFECTIVENESS EVALUATION OF HYGIENE METHODS WITH WATER AND SANITIZING WITH VINEGAR AND CHLORINE

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Received: 10/02/2015; **Accepted:** 12/23/2015

ABSTRACT

The search for healthy habits have become increasingly common among the population fruit consumption is part of a healthier diet since fruits are sources of vitamins and have low calorie. However, the intake of fruits without proper cleaning can be a vehicle for the transmission of micro-organisms that may be harmful to health, which cause food poisoning. The bacteria *Staphylococcus aureus* and *Escherichia coli* are commonly involved in food poisoning outbreaks. In the domestic environment disinfecting fresh fruits is normally done with water solution and addition products such as chlorine or vinegar. The aim of our study was to evaluate the presence of *S. aureus* and *E. coli* in apples and effectiveness of water, vinegar and chlorine in sanitizing fruit. 9 apples were acquired in a city trade Timoteo - MG. Apples in all microbiological analysis were performed without cleaning after they were divided into three groups: immersed in water, immersed in a vinegar solution and the chlorine solution. The result was that the chlorine was more effective in eliminating the bacteria observed in the samples before cleaning.

KEYWORDS: *S. aureus. E. coli*. Apple. Sanitation.

1. INTRODUCTION

The cultivation of apple has agricultural and economic global importance; Brazil is the tenth leading producer of apples in the world. It is cultivated in the country mainly in the southern states, where the climatic conditions are more favorable. The states of Rio Grande do Sul and Santa Catarina are the largest Brazilian producers, with annual production of 1.1 million tons of apples.

In recent years the production chain of apple in the state of Minas Gerais developed significantly, which allowed a more competitive position of the State in the national market[1,2].

Importantly the high nutritional value of apple, especially for its high content of vitamin B complex, vitamin C and E and the mineral potassium. Compound of fibers, particularly of pectin, provides approximately 10% of the daily requirements fibers. The daily intake of pectin is showing efficacy in controlling blood glucose levels, helping diabetics to have better health. Pectin also helps in reducing bad cholesterol, fiber adheres to the intestinal wall and prevents the absorption of cholesterol and other fats in this way also helps in losing weight[3].

The choice and the proper application of chemical sanitizer in fruits is essential for food. As apples are products ready for consumption must be free of pathogens. Thus, it becomes necessary to sanitization step of this fruit, to obtain a product microbiologically safer[4,5].

The washing of a food only with good water, can reduce about 90% of the microbiota present in fresh food, however, there is still the risk of having a contaminant in the food; therefore, it is important to perform sanitization[6].

Sodium hypochlorite is the group of sanitizers compounds most widely used due to the low cost, product availability and increased antibacterial activity. This sanitizing interferes with the transfer of nutrients, reacts with membrane proteins from microbial cells and thus causes reduction of microbiological cell constituents[4,5,7].

The vinegar, comprising an acetic acid solution has antibacterial characteristics due to reduced cell internal pH which hinders the transport through the cell membrane and inhibit the glycolytic pathway[8].

The foodborne illnesses are mostly related to the quality of food which can be contaminated by pathogenic and opportunistic micro-organisms or toxins produced by them, that produce symptoms such as vomiting, diarrhea and abdominal pain[9].

The bacterium *Escherichia coli* is an important microorganism associated with cases of food poisoning, apart from being a marker of faecal contamination, because the bacteria inhabiting the intestine of warm-blooded animals

such as man. *E. coli* has the ability to ferment sugar into carbon dioxide, acids and alcohol, is aerobic and facultative anaerobic. His ideal temperature for growth is between 30 and 37 °C and optimum pH between 7.2 and 7.5. The presence of *E. coli* suggests contamination by sewage or feces, also indicates the failure in handling or packaging, which in turn compromises the quality of food and consumer health[9,10,11].

The bacterium Staphylococcus aureus is commonly associated with diseases transmitted by contaminated food. The poisoning occurs due to heat-stable enterotoxins produced by bacteria while it multiplies in foods. It is a bacterium that has no requirements for growth, can grow at a pH above 4.8 and a minimum temperature of 8-9ºC. Most strains are able to produce one or more types of enterotoxins that cause gastrointestinal symptoms during infection, usually occurring between 1 and 6 hours after ingestion of contaminated food. Enterotoxins remain stable in the food surface without compromising the sensory characteristics of the product, making it difficult to identify the contaminated fruit and so the need for disinfection prior to consumption[10,11].

The objective of this study is to assess the antimicrobial efficacy of sanitizers commonly used in the domestic environment: vinegar and sodium hypochlorite and water in eliminating the bacteria *S. aureus* and *E. coli* in apples from a popular market.

2. MATERIAL AND METHODS

They used nine apples, from a commercial setting in the city of Timoteo - MG, all were kept in the establishment without any protection and at room temperature. The samples were placed in a plastic bag and taken to a laboratory located in Timoteo, Minas Gerais, where the analyzes were initiated on the same day. For the selection of apples the following characteristics were observed: color, uniform size, no stains or deterioration.

Research micro-organisms apples before cleaning and sanitization

The apples were submitted to tests for bacteria in a sterile environment. Moistened sterile swabs were used in 0.9% NaCl solution for sample collection. The swabs were passed across the top and sides of each apple, after, were immersed in the enrichment medium thioglycollate. It was then suitably incubated for 24 hours at a temperature of 35 ± 2 °C. The samples which received no growth in 24 hours incubated continued for another 24 hours. After the stipulated time was made a visual analysis of Thioglycolate environment in which it noted the turbidity of the medium. The turbid media were transferred to the culture media Agar MacConkey Agar and Mannitol Salt.

The study also examined water, sodium hypochlorite and the vinegar before being used for disinfection of samples in order to identify possible contaminants in solutions. A 1 mL aliquot of each was sown in thioglycolate medium enrichment and incubated at 35 ± 2 ° C for 24 hours. The samples which received no growth at 24 hours remained incubated for another 24 hours. After the stipulated time was made through a visual examination of thioglycolate was observed in the samples obtained turbidity of the medium or not.

For the research of bacteria were made seeding in liquid and solid media in accordance with the practices script classes Okura MH, Rende JC[12].

Micro-organism research after the sanitization of apples

The samples were separated into groups to be subjected to sanitization process and subsequent evaluation of the effectiveness of each sanitizing agent separately. The samples were divided into 3 groups: Group I was left immersed in 1L of water for 15 minutes; Group II was immersed in vinegar solution 60% for 15 minutes; and Group III was immersed in the sodium hypochlorite solution at 1.8% for 15 minutes. After that stage, it was conducted to collect a new sample with a sterile swab the surface of each apple. It was then suitably incubated for 24 hours at a temperature of 35 ± 2 °C.

The samples which received no growth at 24 hours remained incubated for another 24 hours. After the stipulated time was made a visual analysis of thioglycolate environment in which it noted the turbidity of the medium. The turbid media were transferred to the culture media Agar MacConkey Agar and Mannitol Salt. After the solutions used for sanitizing apples and water were sown at a rate of 1ml of each solution in thioglycolate medium enrichment. It was then suitably incubated for 24 hours at a temperature of 35 ± 2 ° C. The samples which received no growth in 24 hours incubated continued for another 24 hours. Following the stipulated time it was made a visual analysis of Thioglycolate environment in which it noted the turbidity of the medium.

Identification of micro-organisms that obtained growth

To verify the presence of bacteria was carried out the ringing samples grown in thioglycolate medium Mannitol Salt agar for the media, which is a selective medium for growth of Staphylococcus and for the detection of E.coli was used to MacConkey agar; it is a selective medium for Gram Negative.

Furthermore, we performed the positive control of *S. aureus* and *E. coli* with ATCC strains 25923 and 25922 respectively, provided by the National Quality Control Program (PNCQ). These samples were seeded in media used in research time and temperature equal to testing.

For the control of possible contaminants, sample collection of handler nasopharynx was performed. The analy-

sis was then likewise that analyzes apples, used the same culture media, supporting procedures and tests.

For identification of *S. aureus*, were made biochemical test catalase and coagulase. Through enzyme catalase bacterium possesses the capability of fermentation and formation of gas evidenced by the presence of bubbles in a glass slide post two drops of hydrogen peroxide and with the aid of a bacteriological loop adds the colony of the bacteria under study if there is the formation of bubbles the result is positive. After the catalase for the positive test is carried out coagulase test, based on the ability of the enzyme to induce coagulation of plasma. On a glass slide blends two drops of 1% rabbit plasma in sodium citrate and the colony of the bacteria under study; observed clot formation, if the result is positive[13].

To perform the identification of *E. coli* were made biochemical tests motility, lysine decarboxylation of glucose fermentation and gas production, hydrolysis of urea, production of hydrogen sulfide (H_2S), fermentation of sucrose, L-Tryptophan deamination (LTD) and Indole production[14].

To conduct these tests was used for person Rugai modified medium and Silva which is used to identify the major species of Enterobacteriaceae, for the identification of *E. coli* bacteria the following expected results: positive motility, lysine-positive decarboxylation, glucose fermentation-positive, positive gas production, hydrolysis of the urea-negative production of hydrogen sulfide (H_2S) -negative, fermentation of sucrose-negative, LTD-negative and production indole-positive[14].

3. RESULTS and DISCUSSION

According to Resolution (RDC) No. 12, January 2001 ANVISA (Brazil), it takes regular action in the sanitary control of food, and regulation of microbiological standards, so that is greater food protection. It is possible to find at the market several trademarks sanitizers chemically consisting of sodium hypochlorite, which due to its low cost and ease of acquisition, are most used in society[4,15]. Vinegar, consisting of acetic acid, has sanitizers features and its use is very common among the population due to its low cost and easy access. Are commonly found both facing commercial food service establishments and in the home environment[16,17].

According to the results of the sample handler, has verified that the handler is a carrier of the bacterium *S. aureus*, but in order to not be any kind of contamination, the practices followed biosecurity rules, with the use of Protective Equipment individual (EPI's) and the laboratory where the analyzes were carried contains all the Collective Protection Equipment (CPE's) required[18].

Analyzing the sanitizing the results show the presence of bacteria in all samples, as shown in Table 1. As can be seen, 66.6% of the samples indicated the presence of *S. aureus* and 44.4% of the samples indicated the presence of *E. coli*. With these results it is clear that the consumption of this fruit without any cleaning or sanitizing is a risk, since these are potentially pathogenic bacteria. Furthermore, *E. coli* is considered a health marker. What may indicate contamination in irrigation water, the presence of warm-blooded animal feces through the steps of planting, transport, storage and commercialization of samples.

Table 1. Microbiological analyzes apples without cleaning, Timoteo-MG, September 2015.

	Presence *S. aureus*	Presence *E. coli*	Presence Bacteria
Sample I	+	+	+
Sample II	+	+	+
Sample III	_	+	+
Sample IV	+	_	+
Sample V	_	_	+
Sample VI	+	_	+
Sample VII	+	_	+
Sample VIII	+	_	+
Sample IX	_	+	+

Although the goal of this work is not quantitatively assess the presence of *E. coli* in apples, the National Commission on Norms and Standards for Foods (CNNPA) regulates the presence of up to 2x102 / g fresh fruit. However, the committee says, "*should be made determinations of other micro-organisms and / or toxic substances of microbial origin, in all kinds of fruit, each time you make it necessary to obtain data on the hygienic and sanitary conditions of this class food, or when there are food toxi-infections*" so whenever there is suspicion of fruit contamination it can be decontaminated if properly used effective sanitizer[19].

As can be seen the sanitized apples with water only obtained the elimination of *S. aureus* and *E. coli*, however, was not good at removing other bacteria that can also be potentially pathogenic. Although the water does not have sanitizers property should take into account that according to Ordinance 36 of January 19, 1990 the Ministry of Health free chlorine value in all drinking water distribution points should be 0, 2 mg / L, and thus may have been an influence in our results[20].

The results of the samples which have passed through the cleaning process with water, Group I, can be observed in Table 2.

Table 2. Microbiological analyzes apples, Group I (sanitized with water), Timoteo-MG, September 2015.

Group I	*S. aureus*	*E. coli*	Bacteria
Sample I	_	_	_
Sample II	_	_	_
Sample III	_	_	+

The results of analysis of samples of Group II, sanitized with vinegar, showed growth of bacteria, as can be seen in Table 3. It is possible to observe the development

of *S. aureus* in two of the three samples, concluding that vinegar notIt was effective in sanitizing fruits. By comparing the results in Tables 2 and 3 it can be seen that both water and vinegar were ineffective in eliminating the bacteria present in the analyzed apples.

Table 3. Microbiological analyzes apples, Group II (sanitized with vinegar), Timoteo-MG, September 2015.

Group II	*S. aureus*	*E. coli*	Bacteria
Sample IV	+	_	_
Sample V	_	_	_
Sample VI	+	_	_

Table 4. Microbiological analyzes apples, Group III (sanitized with sodium hypochlorite), Timoteo-MG, September 2015.

Group III	*S. aureus*	*E. coli*	Bacteria
Sample VII	_	_	_
Sample VIII	_	_	_
Sample IX	_	_	_

The results of the samples passed the sanitization process with sodium hypochlorite, Group III are shown in Table 4. In this group sanitization was performed with sodium hypochlorite and can be observed that in sample there was no bacterial growth. Thus, the sodium hypochlorite was effective in the sanitization, which makes it safe for consumption fruits, microbiological standpoint.

Even with the sensed control possible contaminants, the search was performed on the bacteria used sanitizer solutions before and after use of the solutions. The results of this research can be seen in the Tables 5 and 6.

Table 5. Microbiological analysis of sanitizers before use, Timoteo-MG, September 2015.

Sanitizers	*S. aureus*	*E. coli*	Other Bacteria
Water	_	_	_
Vinegar	_	_	_
Sodium hypochlorite	–	–	–

As can be seen the results in Table 5, the water, sodium hypochlorite and vinegar did not show any contamination with microbial agent, validating the use of these agents in the analysis.

The results showed (Table 6) that there was no bacterial growth in the water after being used in the washing of apples. Thus, the possibility that the absence of bacteria in apples as shown in Table 2 is due to migration of the micro-organisms into the liquid fruit can not be demonstrated. On the other hand, there are two possibilities for elimination of bacteria by water: Water used the city's drinking water distribution system contains sodium hypochlorite that even at very low concentrations, can justify this absence to eliminate bacteria; or cell populations is unsatisfactory for growth in the media used. As with water, it is believed that there was a dilution of bacteria in vinegar solution precluding its growth in culture media, as can be seen in Table 6.

Table 6. Microbiological analysis of sanitizers after use, Timoteo-MG, September 2015.

Sanitizers	*S. aureus*	*E. coli*	Other Bacteria
Water	_	_	_
Vinegar	_	_	_
Sodium hypochlorite	–	–	–

In addition, the sodium hypochlorite also no bacterial growth after its use, but, given that there was no bacteria after sanitizing the apples with this product, it is clear that this is effective in eliminating the bacteria under study.

4. CONCLUSION

The selection of the sanitizing product to be used should be taken into account prior to any food consumed fresh, once the ingestion of contaminated food can lead to a great poisoning the small to the very high degree of severity. The sanitization step of the food being consumed is of utmost importance because it largely eliminates some or all of your microbial load making it safer for consumption. In this study, sodium hypochlorite was more effective in sanitizing the apples than vinegar or cleaning only with water, so only the cleaning with water as and seen widespread in society, is a risk, it should be followed by sanitization process. As an ineffective sanitizing vinegar as shown in this study, the optimal choice is sodium hypochlorite.

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