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# Evaluation of the Effect of Washing Pretreatment on The Reduction of Mesophyll and Thermophile Spores in the Apple Juice Product

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## ABSTRACT

**Juice is an important part of modern diets and drinks in many countries around the world, especially for the needy, such as the sick, the elderly, pregnant women, and children. This study aimed to investigate and compare the effect of using parasitic acid, distilled water, and 80% Tween as a detergent in removing and reducing the number of spores of mesospheric bacteria and thermophiles' in apple juice products. In this research, a quantitative method and a measuring tool have been used. The statistical population of healthy apple samples from the fields was prepared in such a way that at least 9 apples of equal size and weight are available for each repetition of the experiment. The samples were stored in the refrigerator until the experiment. Each shipment of apples was divided into three equal groups in terms of the number and weight of apples in the group and each group received the appropriate washing treatment. The results showed that the difference in 80% Tween yield in removing spores from apple surface compared to the other two treatments had a confidence level of more than 99% and the effect of peracetic acid treatment in removing mesophyll spores was not significantly different from distilled water treatment. As a result, counting mesospheric and thermophiles' spores in apple washing solution showed a significant difference in yield of 80% Tween with both other treatments and its surface activity caused its good performance in separating spores from the fruit surface compared to the other two treatments.**

***Keywords-*** Apple juice; the effect of peracetic acid; Spore reduction; Mesophyll; Thermophile

## I. INTRODUCTION

Juices are an important part of the diet and beverages. These products, with their minerals and vitamins, meet the significant needs of the human body and are a good source of compensation for lost body water. Juice is still used to maintain good health. Juice is a natural source of good health (Adams M.R.et al.1989). Excessive consumption of juice leads to diabetes with high blood sugar. Failure to follow hygienic principles and the presence of chemicals (toxins) can cause premature corruption and economic losses as well as infectious diseases in the consumer (Semit Y. et al. 2011).

Here, several aspects of fruit microbiology, from the past to the future, are examined. An overview of the prevalence of foodborne illnesses and fruit-related microbes is provided. The results show that Escherichia coli poisoning can lead to risks such as gastroenteritis, gastrointestinal inflammation, urinary tract infections, etc. Becomes severely ill (Oliveira MJ, et al. 2011). Also, some Escherichia coli serotypes, such as: (O157: H7) cause food poisoning and diarrhea. It is mainly found in mammals or the intestines of warm-blooded animals. According to national and international standards, its serotype (O157: H7) is present in food. Should be zero (Arnab, et al, 2019) Due to the acidic environment containing sugar and moisture, pH 70-90 is a suitable percentage of a suitable environment for the growth and multiplication of molds and yeasts, and as a result, it leads to the secretion of Mycotoxins that can cause poisoning in humans and animals (Aguirre C.M et al, 2009 and Pontius A.J.et al, 1998). Special standards are set for health indicators in each community. Due to its importance from the point of view of personal hygiene, serious action can be taken to increase the health level of distributed juices to reach the existing health standards and to reduce the occurrence of secondary contamination primary contamination can be prevented by observing hygienic principles in all stages of production and distribution (Warriner K. A. et al, 2009). Increasing the level of knowledge and awareness of manufacturers and distributors, observing the principles, and the degree of increasing the health level of these products can be fundamental steps (Noel, H. et al, 2010). Therefore, the most effective way to improve the health and safety of fruits and vegetables is to rely on an active system that reduces the risk of contaminants in the production and transportation of the product to improve the production conditions of each product, continuous disinfection of storage and moisture control equipment. Temperature is essential and attention to personal hygiene and the environment should be given serious consideration (CDC, 2007). Great focus has been placed on stopping such diseases, finding the causes, tracing the sources, and ultimately controlling their recurrence. This approach is adopted because common foodborne illnesses have enormous economic and social implications (Simforian E, et al, 2015). These diseases will also come at a high cost. However, in the current globalization, food corruption is not only an economic concern but also a social concern. In the past (Postollec F. et al, 2010 and Olorunjuwon B, et al, 2014), juices were less likely to be associated with common foodborne illnesses, mainly due to their low ph. According to the knowledge available at the time, at such values (pH), the growth of the microbial agent was impossible. However, the occurrence of diseases, mainly since the 1980s, has led to more attention being paid to sour juices (Reddi S. Kumarar, et al, 2015).

The microbiology of juices depends on whether the target microorganisms are a challenge to stability or safety (Mahale, DP. et al, 2008). The target microorganisms for the preparation of a particular juice depend on the methods used to preserve it (pasteurization, concentration, freezing, etc.), storage conditions (refrigerator, freezer, etc.), and the desired consumption by the consumer. Juices may be infected with various microorganisms such as Listeria monocytogenes and Salmonella (Wells JM. et al, 1997; Ukuku, D. et al, 2002). In addition, microorganisms have distinct characteristics as well as different behaviors against The environmental conditions created in the product show and pose a risk to the consumer or the stability of the juice (Ukuku D. et al, 2002). Given the difficulties in deactivating molds and heat-resistant yeasts, the best solution is to apply appropriate production procedures, proper cleaning and hygienic methods of the fruit surface before preparation and microbiological control of the preparation steps (Tournas VH.et al, 2006) is an alternative. Efficient during the juice preparation phase is the use of soil in the filtration phase, which reduces the number of heat-resistant molds (Vandekinderen I. et al, 2009). Accordingly, the involvement of other spore-forming bacteria in the spoilage problems of juices has been less discussed; other contaminants play an important role in the spoilage of juices. They are mainly present in very low concentrations but cause a bad taste in food (Berger C.N. et al, 2010). These metabolites are produced by various bacteria, which are ubiquitous microorganisms present in the soil (LiaoC.H. et al, 2000). Actinomycetes are ring-shaped, aerobic bacteria that form spores and form long, striated strings. It seems logical that these bacteria enter by poorly washing fruits that are contaminated with soil (Witthuhn R.C. et al, 2012). To preserve fruit juices in the past, present, and future perspectives in search of stability and safety along with nutritional and sensory aspects over the years, juice preservation has relied mainly on low pH, pasteurization, refrigeration, and the addition of decades of preservatives. Mainly two or three of the above methods are needed to maintain the stability of ready-to-drink industrial juices (Abdullah N, et al, 2014). In the past, the consumption of fresh juices was limited to homemade juices that were consumed quickly. Today, changes in consumption habits, increased demand for fresh juices, and a tendency to lack chemical preservatives must also be considered. Hence, the juice industry and researchers are focusing their studies on finding preservation techniques that can maintain the safety aspects of juice (Oita S.et al, 2009).

The purpose of this study is to clarify which of these detergents is more effective and useful in reducing the microbial load of fruit juice. The main question is which of the following is effective in reducing the number of spores on the mesophylls and thermophiles' nature of apple products?

## II. METHODOLOGY

The research method is quantitative and a measuring tool has been used in which the study population of healthy apple samples from the fields was prepared in such a way that at least 9 apples of equal size and weight are available for each repetition of the experiment. The prepared samples were stored in the refrigerator until the experiment. Each prepared shipment of apples was divided into three equal groups in terms of the number and weight of apples in the group and each group received the appropriate washing treatment. Washing with distilled water the apples of this group were washed in a cylindrical glass container containing 400 ml of distilled water for 3 minutes on an orbital shaker at a speed of 150 / rpm. Acid with a concentration of 50 ppm was performed on the orbital shaker for 3 minutes at a speed of 150 / rpm. Washing with Tween 80% solution to wash the apples of this group, Tween 80% solution with a concentration of 100 ppm was used and the apples were washed for 3 minutes by an orbital shaker with a speed of 150 / rpm.

After removing the apples, the peracetic acid washing solution was neutralized by adding alkali to the neutral ph. And the ice-cooled each part of the washing solution of each apple group was filtered separately by a vacuum filtration system using an MCE membrane filter with a pore size of 0.22 microns. The filters were placed on BHI agar plates and the plates related to the two parts of the washing solution were transferred to separate incubators to be incubated under thermophiles' (50) and mesospheric (35) degrees and the number of thermophiles and mesospheric spores, respectively. Wash solution should be counted after the incubation period.

The washed apples in each group were placed in a container containing distilled water after being removed from the washing dish. To separate the rest of the detergent apples were placed on disinfected surfaces to dry. Apple juice belonging to each group was used by the juicer by taking the aseptic material for general counting of mesophyll aerobes using the plate technique in the Kant agar plate culture medium. Sterile normal

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saline solution was used to dilute apple juice samples. The plates were incubated at 35 ° C for 48 h and then counted. The normality of data distribution was determined by Kolmogorov Smirnov and Shapiro wick tests using SPSS software. Paired samples T-test was used to evaluate the significance of the difference between the means

## III. FINDINGS

The results were obtained by counting the spores of mesophyll and thermophile bacteria as well as mesophyll aerobic bacteria in apple juice by considering the sample volume and interfering with the surface area of washed apple samples. Normal distribution of data the null hypothesis in the Kolmogorov-Smirnov and Shapiro-Wick experiments is that the distribution of data is normal due to the larger value of 0.05 for the variables:

— The number of spores of mesophyll bacteria isolated from each unit of apple surface.
— The number of thermophile spores isolated from each unit of apple surface.
— Total amount of mesophyll aerobes in apple juice.
— Percentage of relative abundance of mesophyll and thermophile spores isolated from each apple surface unit.
— The null hypothesis was rejected and the data distribution was normal.

Figure 1-4 shows the amount of spore reduction per unit area of apple as a result of different washing treatments.

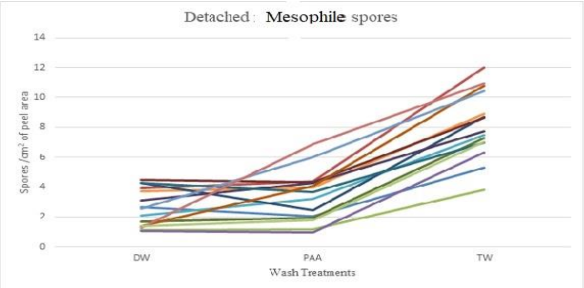


**Figure 4-1: The amount of mesophylls spores isolated from the apple surface unit as a result of washing treatment; DW: distilled water, PAA: peracetic acid solution (50ppm), TW: tween 80% solution (100ppm).**

The difference in twin performance in removing spores from the apple surface compared to the other two treatments was more than 99%. The effect of peracetic acid treatment on the removal of mesophylls spores was not significantly different from distilled water treatment. The comparison of the mean of spores washed from the apple surface as a result of different treatments and the significance of the difference between the treatments are shown in Figures 2-4.

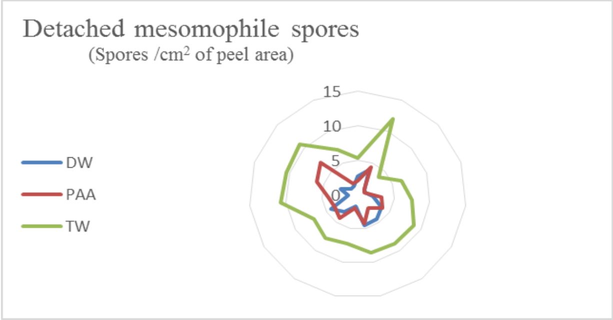


**Figure 4-2: Mean of thermophile spores isolated from apple surface unit as a result of washing treatment; DW: distilled water, PAA: peracetic acid solution (50ppm), TW: tween 80% solution (100ppm); Different letters in the table indicate a significant difference.**

Figure 4-3 shows the decrease in the number of thermophile spores per unit area of apple as a result of different washing treatments

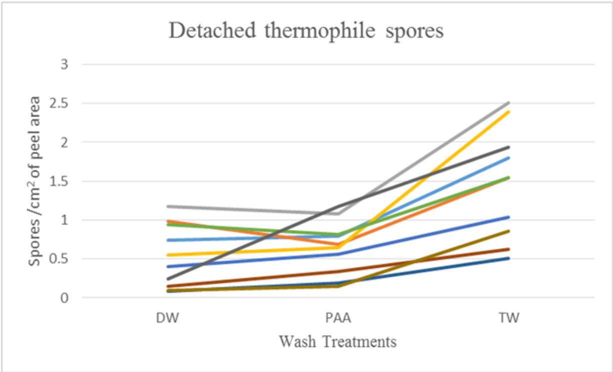


**Figure 4-3: The number of thermophile spores isolated from the apple surface unit as a result of washing treatment; DW: distilled water, PAA: peracetic acid solution (50ppm), TW: tween 80% solution (100ppm).**

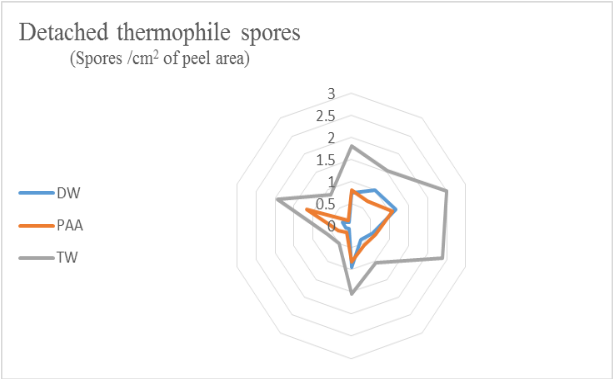


**Figure 4-4: Mean of thermophile spores isolated from apple surface unit as a result of washing treatment; DW: distilled water, PAA: peracetic acid solution (50ppm), TW: tween 80% solution (100ppm); Different letters in the table indicate a significant difference.**

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The comparison of the average number of spores washed by different treatments and the significant difference between the effects of the treatments are shown in Figures 4-4.

The effect of 80% tween in removing thermophile spores from the surface of the apple compared to the other two treatments was significantly different from the confidence level of more than 99%. The effect of peracetic acid treatment on the removal of thermophile spores was not significantly different from distilled water treatment.

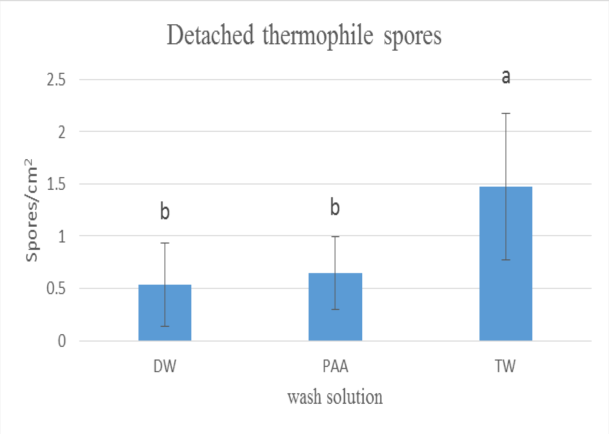


**Figure 4-5: the amount of mesophylls aerobes remaining per unit area of apple as a result of different washing treatments.**

The number of aerobic mesophylls remaining on the surface of apple fruit washed with 80% tween solution was significantly different from the level of more than 99% confidence with other washing treatments. Practical treatment of acid and distilled water did not differ significantly in the amount of mesophylls aerobes in apples.

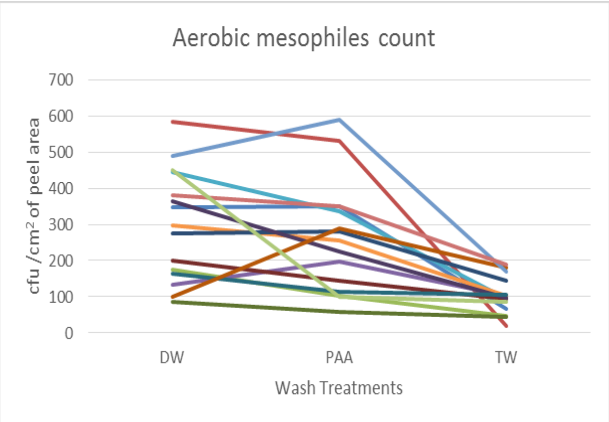


**Figure 4-6: Amount of aerobic Aerobic apple juice per unit area of apple as a result of washing treatment; DW: distilled water, PAA: peracetic acid solution (50ppm), TW: tween 80% solution (100ppm).**

The comparison of the average amount of mesophylls aerobes remaining per unit area of apple skin due to different treatments is shown in Figures 4-7.

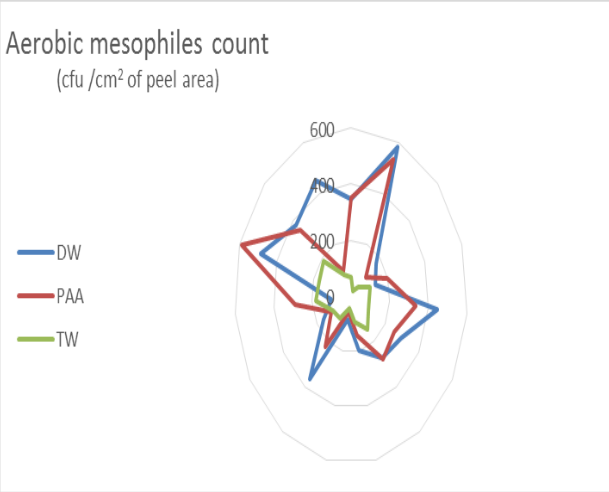


**Figure 4-7: Mean aerobic aerobics of apple juice per unit area of apple as a result of washing treatment; DW: distilled water, PAA: peracetic acid solution (50ppm), TW: tween 80% solution (100ppm); Different letters in the table indicate a significant difference.**

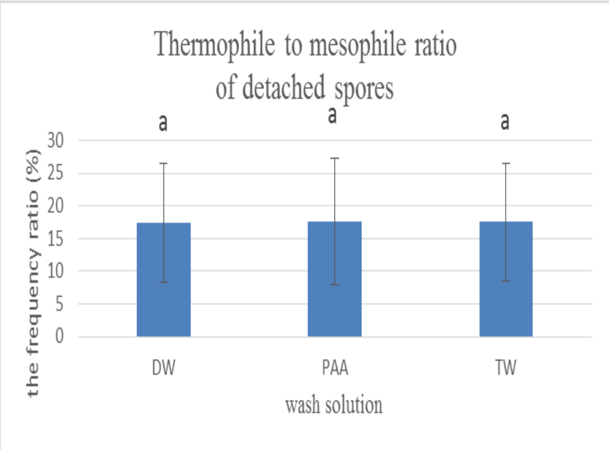


**Figure 4-8: Mean ratio of thermophiles spores to mesophyll isolated from apple water surface unit as a result of washing treatments; DW: distilled water, PAA: peracetic acid solution (50ppm), TW: tween 80% solution (100ppm); Different letters in the table indicate a significant difference.**

A comparison of the average ratio of isolated thermophiles spores to mesophylls spores isolated by different treatments in Figures 4-8 shows a significant difference but indicates a lower frequency of thermophiles spores compared to mesophylls spores. The indices of central tendency and amplitude of the observed ratios of thermophiles spores to mesophylls spores are reflected in Tables 1-4.

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**Table 4-1: Central tendency indices and amplitude relative percentage of the relative frequency of thermophiles to mesophylls spores isolated from the unit area of apple samples.**

| Treatment of Wash | Amplitude of ratio of thermophile to mesophyll spores (%) | | Mean ratio of thermophile to mesophylls spores (%) | Mean ratio of thermophiles to mesophyll spores (%)± Standard deviation |
|---|---|---|---|---|
| | Minimum | Maximum | | |
| DW | 5.74 | 32.50 | 19.34 | 17.44 ± 9.08 [a*] |
| PAA | 4.66 | 33.23 | 18.55 | 17.65 ± 9.74 [a] |
| TW | 4.72 | 32.83 | 17.72 | 17.57 ± 9.05 [a] |

**Different letters in the table indicate a significant difference. DW: distilled water, PAA: peracetic acid solution (50ppm), TW: tween solution 80% (100ppm).*

## IV. DISCUSSION AND CONCLUSION

A study by Noel et al. (2010) found that surfaces exposed to fruit and plant contamination absorb microbial contamination from soil, sewage, air, humans, and animals. Microorganisms cause contamination and poisoning of the consumer in various ways, such as the presence of the pathogen itself, or the secretion of chemicals such as guaiacol and haven't in the juices (Noël H et al, 2010). The most important pathogen-causing bacteria in fruit juices include coliforms, fecal coliforms, Salmonella, Escherichia coli staphylococcus, and mesophyll bacteria (Deinhard G, et al, 1987). Coliform, Escherichia coli, mold, and yeast were used as markers to assess the standard of health, Pseudomonas aeruginosa was indicated for water contamination due to Vestafilococcus aureus and Enterococcus facials, indicating poor health in contact with juice (Lateef A, et a, 2004). The spores are present on the surface of the fruit as resistant forms of life, and if they are not removed during the fruit preparation process for fruit juice, they may not be properly treated to remove the spores and may remain. Spore survival in juice can cause germination (microbial growth) if the right conditions are provided. The result of microbial growth in fruit juice by microbial spoilage is economic losses as well as health problems for the consumer.

Numerous studies have studied various factors in preventing juice spoilage, including the effect of hydrostatic spoilage, various heat treatments, irradiation, filtration, use of ultrasonic waves, and preservatives, some of which are used in industry. Are One of the most common methods used in the juice industry is heat treatments, which, although at mild intensities, eliminate a significant part of the problems caused by the presence of microorganisms, in the presence of more resistant forms of microbes, industries will need to use more intense heat treatments. The use of high-intensity thermal processes, in turn, can harm the nutritional and organoleptic properties of the juice product. One of the most important cases of microbial spoilage in the juice industry, and especially in the case of apple juice, is spoilage caused by the genus Allicylobacillus, which is due to the high resistance of spores of this bacterium to acidic conditions of apple juice and heat treatment. The International Food Processing Association stated in 1988 that 35% of the reported spoilage in fruit juices was caused by acidic, thermophile spores of Alicycbacillus, which continue to grow at 25 to 65 ° C. Common in juices and acidic products have lower temperature resistance, more recently this bacterium is referred to as the target bacterium in these products. One of the problems in the juice industry is the bacterium Alicyclobacillus. This is a bacterium found in soil. The thermophile and acid-friendly nature of this bacterium has made it an organism resistant to pasteurization in the juice industry (Groenewald WH et al, 2009).

On the other hand, the initial microbial load of the fruit has a direct effect on determining the microbial quality of the juice product and the adequacy of health treatments, and the focus of some methods is on reducing the microbial load of the fruit before entering the prepared juice line. The main point for applying these processes is the fruit washing step, which can be combined with mechanical methods with physical conditions and the addition of chemicals to reduce the microbial load.

In the present study, the effect of adding peracetic acid as an authorized disinfectant in the food industry and with FDA-approved use of direct contact with food as one of the apple fruit washing treatments was considered. Also, in another treatment, Tween 80 was used as an approved detergent for use in the food industry and with a license to use it in direct contact with it, and the effect of its solution was evaluated with peracetic acid solution and compared with washing with distilled water. In addition to the total microbial count variable, special attention was paid to the count of spores isolated and washed from the fruit surface to evaluate the efficiency of the three substances used in washing apples in addition to reducing the overall microbial count and reducing the number of mesophylls and thermophiles spores.

The results of counting mesophyll and thermophile spores in the washing solution of apples showed a significant difference in the performance of Twin 80 with both other treatments and its surface activity caused its good performance in separating spores from the fruit surface compared to the other two treatments. Washing with peracetic acid solution separated more mesophyll spores and thermophile spores from the fruit surface than distilled water, but this difference was not significant. A slight difference in the number of spores removed by the peracetic acid solution can be due to the acidic pH of the peracetic acid solution, which changes the interaction between the spores and the fruit surface by changing the surface charge of the spores. This could, if necessary, have a positive effect on increasing the number of isolated spores. The microbial load of apples washed with Tween 80% was significantly lower than the apples of the other two groups. This result is in agreement with the results of spore count and shows the good performance of Tween 80% in removing the generality of microbes from the fruit surface.

The results of the count were not significantly different for the other two groups, namely apples washed with distilled water and apples washed with peracetic acid. Although due to the antimicrobial properties of the peracetic acid solution, due to the oxidizing nature and organic acidity, it was expected that the microbial load of apples washed with peracetic acid was less than the distilled water group, but this difference was not significant, and could be due to insufficient practicality. The acid was present during the exposure. Since the apple samples were drained after 3 minutes of exposure to the peracetic acid solution during washing, the change in the microbial population of the fruit surface due to the presence of peracetic acid was probably not significant enough to show a significant difference.

The results of mesophylls and thermophiles spore counting tests of washing solution and aerobic counting, mesophyll of apple juice confirms the very high potential of Tween 80% for use in the process of washing fruits. Since Tween 80% is licensed for use in the food industry and direct contact with food by the Food and Drug Administration (FDA) due to its high efficiency in low concentrations, i.e. 0.01% of low foam and no unpleasant odor and taste in the product. Juice is recommended for use in fruit washing treatment.

## SUGGESTIONS

Investigating the effect of different amounts of solution of peracetic acid and tween 80% on reducing spores and microbial quality of fruit juice To produce healthy fruit juice, tween 80% should be used to reduce the microbial load on the fruit surface.

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