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# Disinfection of Fruits with Activated Water

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## Abstract

The effect of ionized aqueous solutions (anolytes and catholytes) in the processing of fruits (cherries, morellos and strawberries) for decontamination has been tested. Freshly prepared anolytes and catholytes without the addition of salts were used, as well as stored for 7 months anolytes, prepared with 0.5% NaCl and a combination of 0.5% NaCl and 0.5% $Na_2CO_3$. The anolyte prepared with a combination of 0.5% NaCl and 0.5% $Na_2CO_3$, as well as the anolyte obtained with 0.5% NaCl, exhibit high antimicrobial activity against the surface microflora of strawberries, cherries and sour cherries. They inactivate *E. coli* for 15 minutes. The other species of fam. *Enterobacteriaceae* were also affected to the maximum extent, as is the total number of microorganisms, especially in cherries and sour cherries. Even stored for 7 months, they largely retain their antimicrobial properties.Anolyte and catholyte, obtained without the addition of salts, showed a lower effect on the total number of microorganisms, but had a significant effect on Gram-negative bacteria, and especially with regard to the sanitary indicative *E. coli*.

**Keywords:** Anolyte, Catholyte, Cherries, Sour Cherries, Strawberries, Decontamination

## Introduction

Fruits and vegetables are the most useful food for humans, especially when consumed raw. Fruits are a preferred breakfast for children in schools and are also part of a number of desserts offered at catering establishments and commercial network. However, they carry different microorganisms on their surface, some of which are pathogenic to humans. Their microbial composition depends on a number of factors, such as the type of fruit or vegetable, region of cultivation, air pollution, rainfall, fertilization with natural fertilizers, post-harvest contamination, etc. Most of the microorganisms are representatives of the epiphytic non-pathogenic microflora, as well as soil bacilli, actinomycetes and fungi.

The natural fertilization or application of irrigation water and sewage sludge may result in contamination with enterobacteria and other microorganisms causing infection. For example, strawberries grow close to the soil and become contaminated by it, as well as by natural fertilizers. On the other hand, in the case of moist and warm storage of the fruits, conditions for the propagation of the microorganisms are created. Therefore, their reliable removal before consumption is particularly important for human health. Disinfection of fresh fruits and vegetables is an essential first step in their post-harvest processing. The minimum requirement for the disinfection procedures is to destroy fungi and bacterial pathogens after harvest and thus to improve food safety. Some of the major disinfectants available for use today include chlorine, chlorine dioxide, ozone, ethanol, hydrogen peroxide or dioxide, organic acids, peroxyacetic acid or a combination of hydrogen peroxide and peroxyacetic acid, as well as electro-activated aqueous solutions (Feliziani et al., 2016; Adhikari et al., 2019).

In recent years, due to the increasing number of outbreaks of gastrointestinal disease after consumption of raw products, including peeled, more attention has been paid to the interventions that kill or eliminate pathogens on the fresh products. Washing techniques and water quality can affect product safety. Carriage of putrefactive microorganisms, yeast, molds and foodborne pathogens such as *E. coli* and species of the genera *Cyclospora, Listeria* и *Salmonella* is potentially dangerous (Adhikari et al., 2019). The range of microorganisms associated with fresh fruits and vegetables includes bacteria, viruses and parasites. However, most of the reported outbreaks of infections are related to bacterial contamination, in particular with members of the *Enterobacteriaceae* family. Among them, of particular importance are *Escherichia coli* O157 and *Salmonella enterica* (Beuchat, 1996; European Commission, 2002). To prevent such infections, pre-washing of vegetables in solutions of chemical, mainly chlorine-containing disinfectants was introduced in some places (Ivanov, 2016). Chlorination has been widely used by producers, processors and traders of fresh fruits and vegetables around the world for decades. However, chlorine-containing agents are not safe for consumers because of the residues of the preparations used in the products being processed.

Chlorine can cause incomplete oxidation of organic matter and the formation of toxic by-products such as chloramines, chloroform, etc., which at high doses have a carcinogenic effect (Rabin, 1986). Although there is a wide range of different agents for the disinfection of fresh products, their efficacy is variable and it is not possible to guarantee the elimination of the pathogens. Chemical disinfectants are considered to be important for the quality and safety of minimally processed foods, such as fresh and freshly sliced fruits and vegetables. While chlorine remains the most widely used chemical disinfectant in the production of fresh produce, other equally effective and cost-effective alternatives are needed that are more environmentally friendly and harmless to workers. Zoellner et al. (2018) reported that peracetic acid, an equilibrium mixture of hydrogen peroxide and acetic acid, has been approved by the US Food and Drug Administration (FDA) for direct washing of fruits and vegetables, as well as for sanitization of food contact surfaces and equipment. These authors also recommended ultrasound sterilization of fruits and vegetables. Korany et al. (2018) emphasized the importance of timely and complete cleaning of food contact surfaces prior to disinfection and provided practical information and guidance to the food industry in selecting effective disinfectants in sanitization regimens to remove *L. monocytogenes* biofilm.

As disinfectants, they recommended quaternary ammonium compounds, chlorine, chlorine dioxide, peroxyacetic acid, and ozonated water. Although a wide range of different agents are used for the disinfection of fresh fruits and vegetables, none of them is able to completely eliminate pathogens (Ivanov, 2016). Adverse side effects of chemical disinfectants are their residues on the fruit, as well as their environmental impact. In recent years, electrochemically activated aqueous solutions (EAAS) of sodium chloride have been reported to be broad-spectrum and environmentally friendly biocidal products. The anolyte has reduced electronic activity and pronounced oxidizing properties, and catholyte has increased electronic activity and reducing action (reducer). Their properties change depending on their oxidative - reduction potential (ORP) (Gluhchev et al., 2018). In our country Ivanov (2016) for the first time noted the possibility of using electrochemically activated aqueous sodium chloride solutions for the safe disinfection of fresh vegetables intended for direct consumption, but without indicating specific results in this regard.

In our previous studies with leafy vegetables, we found (Popova and Petrova, 2018) that anolytes prepared with the addition of 0.8% NaCl, a combination of 0.4% NaCl and 0.4% $Na_2CO_3$, as well as without the addition of salts, after being treated for 15 minutes, exibited high antimicrobial activity, especially after potentiation by the addition of 96o ethanol at a final concentration of 1%, without viable microorganisms being isolated from the leaves of the lettuce tested. That's why the purpose of this study is to test the effect of electrochemically activated aqueous solutions anolytes and catholytes, which are harmless to the macroorganism and the environment, for disinfection of some fruits.

## Materials and methods

***Anolytes.*** 1) Anolyte 1 neutral, prepared with a combination of 0.5% NaCl and 0.5% $Na_2CO_3$; 2) Anolyte 2 prepared with 0.5% NaCl. Activation time: 12 min. Storage time - 7 months in the dark and at room temperature.

3) Anolyte prepared without the addition of salts, fresh.

***Catholytes.*** • Catholyte prepared without salt, fresh.

***Fruits.*** Fresh strawberries and cherries purchased from commercial establishments on the day of the experiments were used, as well as sour cherries torn from the tree on the day of the tests.

***Nutritient media***. Seedings for isolating microorganisms were made on Mueller-Hinton agar (BULBIO - NCIPD Ltd. - Sofia, Bulgaria) and Chromobio Coliform agar (Biolab Zrt., H 11-14, Budapest Ov utca 43).

***Experimental designs***

Each type of fruit tested was divided into 6 groups of 150 grams, which were placed in the following solutions: 1) anolyte prepared with combination of sodium chloride (0.5%) and sodium carbonate (0.5%); 2) anolyte received with sodium chloride (0.5%); 3) anolyte prepared without salts; 4) catholyte prepared without salts; 5) tap water and 6) untreated control.

After 15 minutes imprint seedings from fruits of each group were made in a particular sector of size 1/3 of a 20 $cm^2$ agar plates of both types. After incubation under aerobic conditions at 37° C for 24 - 48 hours the formed colonies were counted and presented as colonies forming units on $cm^2$ ($CFU/cm^2$) from the surface of the fruits studied.

Each experiment was performed in triplicate with by three plants..

***Statistical analysis.*** Data were statistically processed and the results are presented as average ± standard deviation. The differences between two averages were evaluated for statistical significance with Student's t-test, being statistically significant at $P < 0.05$ and high statistical significance at $P < 0.01$ and $P < 0.001$.

**Results and Discussion**

The physical indicators pH, oxidation-reduction potential (ORP) and temperature of the investigated EAAS are presented in Table 1.

**Table 1. Physical parameters of the studied anolytes**

| Source composition | | pH | ORP, mV | $t^0$ C |
|---|---|---|---|---|
| Aqueous sodium chloride (0.5%) and sodium carbonate (0.5%) solution | anolyte 1 – 1 day | 6,63 | 870 | 19,8 |
| | after 1 month | 7,16 | -9 | 21,8 |
| | after 1 months | 7,28 | - 18,7 | 22,7 |
| Aqueous sodium chloride (0.5%) solution | anolyte 2 – 1 day | 2,76 | 1200 | 19,7 |
| | after 1 month | 2,81 | 246 | 19,8 |
| | after 7 months | 2,78 | 247 | 22,8 |
| Water without salts | anolyte – 1 day | 6,29 | 58 | 22,6 |
| | catholyte – 1 day | 9,1 | - 126 | 22,7 |
| Control – water without salts | untreated | 7,07 | 0,67 | 22,8 |

*ORP - oxidation-reduction potential*

As can be seen from the data in the table, the most significant changes were observed in the physicochemical parameters of anolyte 1 obtained with a combination of sodium chloride (0.5%) and sodium carbonate (0.5%), especially during the first month of storage. For anolyte 2, obtained with 0.05% sodium chloride, there was a change in ORP during the first month of storage. After that its indicators are maintained during the entire study period. The results of microbiological studies on the amount of isolated micro-organisms from the fruits after their treatment for 15 min with different solutions, recorded after 24-48 hours incubation of the cuktures at 37 ° C, are presented in Tables 2, 3 and 4.

**Table 2. Quantity of microorganisms after 15 min action of the electrochemically activated aqueous solutions (fresh and stored for 7 months) on strawberries presented in CFU/cm$^2$ of their surface**

| Sampl eNº | Type of activated solution | Quantity of microorganisms (CFU/cm$^2$) | | |
|---|---|---|---|---|
| | | Mueller-Hinton agar | Chromobio Coliform agar | |
| | | | *E. coli* | Others |
| 1 | anolyte of NaCl and $Na_2CO_3$ | 41,3* $\pm$ 6,2** | 0 | 0 |
| 2 | anolyte of NaCl | 2,7 $\pm$ 2,0 | 0 | 0 |
| 3 | salts-free anolyte | 20,3 $\pm$ 8,9 | 3,7 $\pm$ 1,0 | 19,2 $\pm$ 6,9 |
| 4 | salts-free catholyte | 330,2 $\pm$ 16,8 | 2,7 $\pm$ 0,4 | 41,0 $\pm$ 8,3 |
| 5 | tap water | 336,7 $\pm$ 28,5 | 12,0 $\pm$ 1,4 | 44,5 $\pm$ 20,1 |
| 6 | untreated control | 360,0 $\pm$ 32,6 | 13,2 $\pm$ 1,9 | 40,0 $\pm$ 4,0 |

**Average. ** Standard deviation.*

As can be seen from the summarized data in the tables, the microflora on the surface of strawberries is in the highest amount, many times higher than that of the tree fruits. The differences of the total number of microorganisms with that of cherries and sour cherries were significant (P <0.001). The reason for this is obviously the growth of strawberries near the land from which they fall on them, as well as the higher humidity characteristic of their surface. However, the differences between the amounts of *E. coli* on the three types of fruit were negligible (P> 0.05). Despite the expectations, the decrease in the amount of microorganisms from all groups studied (P> 0.05), achieved by washing with water was slight. The treatment of the strawberries with the two anolytes with salts resulted in complete destruction of *E. coli* and of all species of the *Enterobacteriaceae* family. Their use, as well as of that without salts, also significantly reduced the total number of micro-organisms, much higher than the washing of strawberries with tap water (P <0.001). However, the effect of catholyte in this respect was comparable to that of water (P> 0.05).

**Table 3. Quantity of microorganisms after 15 min action of the electrochemically activated aqueous solutions (fresh and stored for 7 months) on cherries presented in CFU/cm$^2$ of their surface**

| Sampl e Nº | Type of activated solution | Quantity of microorganisms (CFU/cm$^2$) | | |
|---|---|---|---|---|
| | | Mueller-Hinton agar | Chromobio Coliform agar | |
| | | | *E. coli* | Others |
| 1 | anolyte of NaCl and $Na_2CO_3$ | 2,0* $\pm$ 1,6** | 0 | 0 |
| 2 | anolyte of NaCl | 0 | 0 | 0 |
| 3 | salts-free anolyte | 40,0 $\pm$ 6,7 | 2,3 $\pm$ 0,4 | 6,0 $\pm$ 4,2 |
| 4 | salts-free catholyte | 6,5 $\pm$ 3,0 | 4,0 $\pm$ 2,4 | 5,3 $\pm$ 3,2 |

| | | | | |
|---|---|---|---|---|
| **5** | tap water | 31,3 ± 8,2 | 9,0 ± 3,5 | 8,.0 ± 5,0 |
| **6** | untreated control | 64,0 ± 12,3 | 11,0 ± 1,6 | 20,5 ± 12,3 |

**Average. ** Standard deviation.*

In cherries, washing with water has a better result. More than twofold reduction of bacteria from the *Enterobacteriaceae* family was achieved compared to the untreated control (P <0.05), as well as of the total number of microorganisms (P> 0.05) and to a lesser extent *E. coli* (P> 0.05). However, the treatment of cherries with the two anolytes with salts was very effective and resulted in complete destruction of *E. coli* and of all species of fam. *Enterobacteriaceae*. The catholyte also proved highly effective in significantly reducing the total number of microorganisms (P <0.01), E. coli (P <0.05) and enterobacteria (P> 0.05) compared to untreated controls. The effect of catholyte in reducing the total number of microorganisms in comparison with water-washed cherries was also significant (P <0.05), but the differences in the number of *E. coli* and others species of fam. *Enterobacteriaceae* compared to water-washed cherries were not significant. (P> 0.05). The anolyte without salts was effective in reducing the amounts of *E. coli* (P <0.01 versus untreated controls and P> 0.05 relative to those washed with water) and lower with respect to other enterobacteria and the total number of microorganisms (P> 0.05 against untreated controls). The differences in the results of EAAS without salts in strawberries and cherries are probably due to their different surface microflora. This was more abundant in strawberries, but more sensitive to the action of EAAS without salts compared to that of cherries, which was more sensitive to the action of catholyte and less sensitive to the anolyte obtained without salts.

**Table 4. Quantity of microorganisms after 15 min action of the electrochemically activated aqueous solutions (fresh and stored for 7 months) on sour cherries presented in CFU/cm² of their surface**

| **Sample №** | **Type of activated solution** | **Quantity of microorganisms (CFU/cm²)** | | |
|---|---|---|---|---|
| | | **Mueller-Hinton agar** | **Chromobio Coliform agar** | |
| | | | ***E. coli*** | **Others** |
| **1** | anolyte of NaCl and $Na_2CO_3$ | 0* ± 0** | 0 | 0 |
| **2** | anolyte of NaCl | 4,0 ± 2,2 | 0 | 1,7 ± 1,2 |
| **3** | salts-free anolyte | 31,3 ± 10,2 | 3,0 ± 1,2 | 3,3 ± 1,2 |
| **4** | salts-free catholyte | 29,2 ± 8,6 | 2,0 ± 1,1 | 15,7 ± 1,3 |
| **5** | tap water | 65,3 ± 9,7 | 10,7 ± 4,1 | 23,.0 ± 7,2 |
| **6** | untreated control | 90,8 ± 4,5 | 12,8 ± 1,5 | 22,3 ± 1,6 |

**Average. ** Standard deviation.*

The results were similar and for the sour cherries. Washing them with water also does not guarantee the removal of microorganisms from the test groups. In them, the total number of microorganisms was higher than that of cherries (P> 0.05) but significantly less than that of strawberries (P <0.01). This is probably due to the fact that cherries and sour cherries were grown in different areas or to the antimicrobial treatment of the fruit commercially available. The two tested anolytes, prepared with salts, had a very good effect in eliminating the surface microflora compared with both the starting amounts of the microorganisms (in untreated controls - P <0.001) and those on the water-washed ones (P <0.01). *E. coli* was also inactivated, as were other bacteria from the *Enterobacteriaceae* family. Minor amounts of the latter were detected after using anolyte 2 prepared by NaCl activation. The effect of this without salts, as well as of catholyte, was also better than that of ordinary washing (P <0.05 with respect to the total number of microorganisms and those of the *Enterobacteriaceae* family) but smaller than that of the anolytes with salts.

Washing with water is the most affordable and widely used approach for removing microorganisms from fruits before consuming them both at home and at catering establishments. The maximum effect in this respect is considered to be achieved after soaking them in water for 15 minutes. The results of this study show that this treatment contributes to reducing the microbial contamination of raw fruits, but it is far from sufficient to decontaminate them before direct consumption, especially strawberries. It does not ensure reliable elimination of the sanitary indicative *E. coli* and, in the presence of a pathogenic strain on the surface of the fruit, can result in infection in consumers. These our results are in line with those of other authors. In 1998, Beuchat reported that the effect of washing for reducing the number of bacteria on the surface of fresh fruits and vegetables was negligible - in the order of 0.1 to 1log10 CFU. Therefore, further processing is required. The studies of Oie et al. (2008) showed that the average microbial contamination level of 10 unpeeled fleshy fruits had been 9.3 x $10^3$ CFU / g after washing with water and 1.3 x $10^3$ CFU / g after washing followed by disinfection. These authors found that the pollution did not decrease significantly even after disinfection with sodium hypochlorite. Samadi et al. (2009) also found significant differences in microbial populations on vegetables after decontamination with tap water, water with detergent (dishwashing liquid) or benzalkonium chloride. According to them, pretreatment with detergent slightly increased the efficiency of all decontamination treatments, but the results did not differ significantly from those obtained after individual application of disinfectants such as peracetic acid (100 ppm for 15 min), hydrogen peroxide (133 ppm for 30 min) , calcium hypochlorite (300 ppm for 15 min) and combined hydrogen peroxide and silver ions (133 ppm for 30 min). According to Sapers (2001), conventional methods of washing and disinfection, even with the use of newer agents for this purpose, are not able to reduce the microbial population by more than 90 or 99%, although greater efficiency is required to ensure the safety of the products. Washing fresh fruits and vegetables with drinking water treated with a hygienic agent may reduce the microorganisms and pathogens on their surface but not reliably eliminate them. Adhikari et al. (2019) recommended chlorine detergents for disinfection and a minimum contact time of one minute to ensure that pathogens will be killed. At comparison of several chemical agents Wei et al. (2017) recommended 0.1% acidic sodium chloride, the treatment with which significantly reduced the natural microbiota and pathogens on strawberries and cherry tomatoes for a relatively short time and resulted in a moderate reduction of microbes on blueberries. According to these authors, at the second position was lactic acid. Although more expensive and more time-consuming, the treatment with these agents is no more effective than the use of anolytes tested by us in this study. Anolytes turn to be cheaper, more affordable and safe for the consumer and the environment.

The results of this study are consistent with those of Abadias et al. (2008), who compared the effect of using neutral electrolyzed water as an alternative to sodium hypochlorite, the most common disinfectant of fresh vegetables. Its bactericidal activity at a content of approximately 50 ppm free chlorine and pH 8.60 against *E. coli* O157: H7, salmonella and others on lettuce was similar to that of the chlorinated water (120 ppm free chlorine). Processing for 1-3 minutes is sufficient. They recommended neutral electrolyzed water for the disinfection of fresh products in the food industry, as it would reduce the amount of free chlorine used for disinfection. This would be a safer and easier way to ensure food safety.

In our previous studies (Popova et al. (2016 a, c), we established high activity of such solutions against Gram-negative and Gram-positive bacteria, and Tasheva et al. (2010) reported an antifungal effect. The antimicrobial activity of EAAS is directly dependent on their redox potential, and it only manifests itself in certain ranges of ORP. Another great advantage of these solutions is that the micro-organisms cannot build resistance against them, as EAAS have a constantly changing dynamic composition that depends on the salts used. These solutions are broad-spectrum and environmentally friendly for humans, animals and the environment biocidal products. The active substances in them are hydrochloric and peroxide compounds, which change their concentration relatively quickly (Ignatov et al., 2015; Gluhchev et al., 2018). The results obtained by us show that the two tested anolytes, prepared with salts, exhibit high antimicrobial activity against the surface microflora of strawberries, cherries and sour cherries. *E. coli* died under their influence in 15 minutes. The other species of fam. *Enterobacteriaceae* were also affected to the maximum extent, as was the total number of microorganisms, especially in cherries and sour cherries. Even stored for 7 months, they largely retained their antimicrobial activity, despite some changes in their physicochemical parameters. The anolyte and catholyte, obtained without the addition of salts, had a lower effect on the total number of microorganisms, but showed a significant activity on the Gram-negative bacteria and especially on *E. coli*. Other advantage of these EAAS (without salts) is that their preparation is easier and more sparing the filter of the apparatus, also it is independent of the availability of salts. In order to increase their antimicrobial activity, it would be appropriate to test their effect after potentiation with 1% 96o ethanol, by which in previous experiments (Popova and Petrova, 2018) we achieved a significant enhancement their effect at lettuce disinfection.

Our current studies strongly indicate that EAAS activated with 0.5% NaCl as well as with the participation of 0.5% $Na_2CO_3$ can be used with considerable success for the disinfection of fruits such as cherries, sour cherries and even strawberries. This disinfection is effective and safe. After storage for 7 months, the solutions retain these properties to a great extent. This is consistent with the results of our previous studies, demonstrating that the antimicrobial activity of these solutions persists even after storage at room temperature for weeks (Popova et al., 2016 b). Apparently a significant role for the antimicrobial activity of the solutions we study, in addition to ORP, has their pH, which is changed very little even after a 7-month storage period. The data from our studies show that the change in the ORP indicator of the solutions tested, which is essential for their antimicrobial activity, is greatest in the first month after receipt and very small - in the next 6 months. Anolytes activated with a combination of 0.5% NaCl and 0.5% $Na_2CO_3$ as well as 0.5% NaCl turn out to be a sure means of safely decontaminating strawberries, cherries and sour cherries, even when the solutions are stored for 7 months.

**Conclusions**

The anolyte prepared with a combination of 0.5% NaCl and 0.5% $Na_2CO_3$, as well as the anolyte obtained with 0.5% NaCl, exhibited high antimicrobial activity against the surface microflora of strawberries, cherries and sour cherries. They inactivated *E. coli* for 15 minutes. The other species of fam. *Enterobacteriaceae* were also affected to the maximum extent, as was the total number of microorganisms, especially in cherries and sour cherries. Even stored for 7 months, they largely retained their antimicrobial properties.

Anolyte and catholyte, obtained without the addition of salts, had a lower effect on the total number of microorganisms, but showed a significant effect on Gram-negative bacteria and especially on the sanitary indicative species *E. coli*.

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