Effect of sanitizing disinfectants on onion puree chemical and microbial properties

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Abstract: There is a growing demand for fresh cut, value-added and ready-to-use food in households, as well as for widespread use in retail, food services and the various food industries. Amongst vegetables, onions have many medicinal properties and can lead to health. This study evaluated the effect of disinfection methods including; T1: Nisin (50μg mL^{-1}) + Citric Acid (1%), T2: Sodium Hypochlorite (100 mg/L) + Citric Acids (0.25 M) + Sulphuric Acid (eq/L), T3: Calcium Chloride (0.5%) + Ascorbic Acid(1%)+ Citric Acid(1%) +hot water 50°C, T4: Control (without disinfectant) during 21 days storage at 4°C on onion pure. Each week, pH, soluble solids, acidity, weight loss, total phenolic compounds antioxidant activity and microbial tests (total microbial count, total molds and yeasts) were performed on the samples. Statistical analysis of the data by way of a completely randomized factorial design, demonstrated that in all treatments pH was not significant ($p > 0.05$) while pH increased with increasing of storage days. The lowest TSS (6.44 °Brix) was recorded in T3 (CC+AS+CA) followed by T1 and T4 (control). The lowest weight loss (3.51%) was recorded in T1 followed by (3.77%) in T3, while the highest weight loss (5.89%) was observed for untreated (controlled). The highest TPC (54.01 mg GAE/100 g), lowest total count 0.95 log CFU g^{-1} and total molds and yeasts 1.40 log CFU g^{-1} was recorded in T3.

Keywords: onion puree, microbial contamination, disinfectant

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1 Introduction

Over the past decades, the consumption of minimally-processed ready-to-eat products has risen dramatically in the diet due to the convenience and greater awareness of consumers of the health benefits of vegetables and fruits (Ma et al., 2017). In addition to convenience, new products offer benefits such as higher added value, reduced waste, increased product variety, improved security and quality, as well as less labor in stores (Ayala-Zavala et al., 2008). Onions are widely used as a food seasoning in a wide range of foods, from breakfast to dinner (Sharma et al., 2020). However, cutting or slicing onions, because of their specific properties that bring tears to eyes and leave a distinct odor on the skin, is bothersome for many consumers (Berno et al., 2014). In spite of these benefits, fresh onion products face significant challenges due to tissue damage that results in chemical and physiological reactions that limit storage period (Liu and Li, 2006). The cold chain is not well developed in most developing countries, in particular during marketing. The disruption of the cooling chain contributes to substantial reduction of the

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product's consistency and shelf-life, which is most definitely the biggest obstacle to expanded promotion and use of fresh-cut goods in these countries (Berno et al., 2014). These products are then required to have optimum hygienic, sensory and nutritional properties. Washing is one of the most important methods of operation that, in the form of physical and chemical treatments, eliminates or minimizes the population to pathogenic microbes (Silveira et al., 2008). Tap water cleaning is usually ineffective for controlling microorganisms unless it is combined with appropriate sanitizers (Wang et al., 2019). Specific sanitizers have different degrees of antimicrobial activity on fresh food. Chlorine is now widely used as a disinfectant in the fresh produce industries. Nonetheless, the use of chlorine-based compounds, especially with regard to the health of the community as well as problems related to the impact of chlorine on the environment, has been highly controversial (Gil et al., 2009). It is proposed that organic acids such as (propionic, acetic, lactic, malic, and citric acid) can be used as a strong replacement sanitizer to chemical sanitizers for the hygiene of organic fresh fruits and vegetables (Park et al., 2011). Nisin and citric acid are in the category of additives that are generally recognized as safe (GRAS) reliable and healthy compounds (Mostafidi et al., 2020; Park et al., 2011) and treatment with Nisin and citric acid decreased the microbial counts and total viable counts (TVC) of fresh-cut onions (Chen et al., 2016). Citric acid is used as an anti-browning compound in the food industry and can halt the activity of polyphenol oxidases (Liu and Li, 2006; Pace et al., 2020). Nisin is a bacteriocin that has been licensed for use in many products and is a perfect candidate for fruit survival, because it exhibits strong antibacterial activity, acid pH tolerance, unwillingness to cause modifications in the food component and fairly low toxicity to humans (Chen et al., 2016)

Barbosa et al. (2013) reported that use of nisin leads to inhibition of the growth of *Staphylococcus aureus* and *Listeria monocytogenes* in mango fruit and it should be noted that it did not adversely affect its appearance, texture, and nutrients. At present, the results of various disinfected approaches on the physicochemical attributes and microbial contents of onions puree have not yet been examined. Therefore, a study of this fresh vegetable is necessary to understand the main changes in product quality and the resulting consequences for the consumer.

2 Materials and methods

2.1 Chemicals

Yellow onion (*Allium cepa* Linnaeus, *Lilliaceae*) was purchased on September 2019 from a local market and washed with tap water and dried at ambient temperature to remove surface contamination. Ten onion bulbs of 50-60 g mass were selected, cut to small pieces using a steel knife and transformed into a puree using a laboratory electric mixer (Sadoughi et al., 2013). Folin–Ciocalteu reagent, TPTZ, DPPH, methanol, sodium carbonate, iron(II) sulfate, iron(III) chloride hexahydrate, sodium acetate trihydrate, potassium chloride, concentrated hydrochloric acid were purchased from, Sigma-Aldrich (Darmstadt, Germany). All chemicals and reagents were of analytical grade.

2.2 Samples preparation

Combinations of 5 types of chemical disinfectant (chlorin, citric acid, ascorbic acid, nisin, sodium hypochlorite, calcium chloride) were tested. For disinfection treatment, 50 g of onion was immersed in aqueous disinfectant solutions at a ratio of 1 to 4 (W/V) at 4°C for 5 minutes and then the solution was uniformly mixed via the mixer (Berno et al., 2014). Afterwards, the samples were poured into a sieve and rinsed with distilled water for 5 minutes at 4°C.

2.3 Disinfection treatments

T1: Nisin & Citric acid (N+C)

Onion Puree (OP)(50 g) was immersed in 50 $\mu g \cdot mL^{-1}$ of nisin, and 1% ($w\ v^{-1}$) of citric acid (N+C) (Chen et al., 2016).

T2: Sodium hypochlorite (SH+S+C)

Sodium hypochlorite was prepared based on (Pérez-Gregorio et al., 2011) with 100 mg/L concentration and its pH was set at a pH of 4.5 to 5.0 with citric acids (0.25 M) and sulphuric acid (1 eq/L).

T3: Calcium chloride+ Ascorbic acid+ Citric Acid (CC+AS+CA) + hot water 50°C

OP (50 g) was immersed in solution containing 0.5% calcium chloride (CC) + 1% Ascorbic Acid (AS) + 1% Citric Acid (CA) (Ihl et al., 2003).

T4: Control

The control washed with distillated water, without disinfectant, in the same time-temperature conditions.

2.4 Storage conditions

The OP was put in a plastic foam tray, and covered with polyethylene cling film, and stored for 28 days at 4°C.

2.5 Chemical properties of OP

Ten grams of OP with 50 mL of freshly distilled water is extensively combined. Then at room temperature for 10 min, the mixture was centrifuged at 2000 rpm. The supernatant was transferred to a volumetric flask of 100 mL and distilled water was added to it (Ahmed et al., 2001). A pre-calibrated pH meter (Metrohm 691) was used to calculate the pH of the mixture (Ahmed et al., 2001). The handheld refractometer (N-1E, Brix 0-32 per cent, ATAGO Co., Ltd., Tokyo, Japan) was used to assess the minimum soluble solid by testing with purified water accompanied by a reduction of 1–2 drops of sample solution on the clean surface of the refractometer (Ahmed et al., 2001). Weight loss Percent weight loss of the OP was determined after, 0, 7, 14 and 21 days of storage at 7°C for all treatments. The percentage of weight loss was measured and reported using a weighing scale and calculation (initial weight- final weight/initial weight) multiplied by 100 (Page et al., 2016).

2.6 Total phenolics and antioxidants analyses

2.6.1 Samples extraction

After homogenizing the OP in the blender, a sample of 5 g was collected and 40 mL of methanol-80 was added. The substance had been mixed for 1 h on a water-bath shaker and centrifuged for 10 min at 10,000 g. The supernatant was collected and the residues were re-extracted twice using 10 mL of methanol-80 by vortexing (1 min) and centrifuging at 10,000 g for 5 min. The three supernatants were subsequently combined for further analysis (Siddiq et al., 2013).

2.6.2 Total phenolic content (TPC)

The amount of total phenolic compounds (TPC) was determined based on the Folin-Ciocalteu method. An amount of 100 µL of the extract (mixture of 100 mg g^{-1} of onion extract with 2000 µL of methanol) was mixed with 2.5 mL of Folin-Ciocalteu reagent and rested for 3 minutes for the reaction to take place. Subsequently, 5 mL of 7.5% sodium carbonate was added to it and then raised to a volume of 50 mL with distilled water. The sample was kept in a dark place for 24 hours and then its absorbance was read at a wavelength of 765 nm against the control. The amount of total phenolic compounds in the sample was determined by standard curves. The standard curve was obtained by plotting the gallic acid uptake data at a wavelength of 765 nm at concentrations ranging from 100 to 950 mg/L. The standard curve was obtained by plotting galic acid adsorption results at 765 nm at a concentration of a 10–100 µg mL^{-1}. Reports have been recorded as mg gallic acid equivalent (GAE)/100 g fresh-weight (FW) (Singh, et al. 2017).

2.6.3 Antioxidant activity

DPPH assay: Total antioxidant activities were performed conducted by DPPH and Ferric reducing-antioxidant power (FRAP) assays. Radical-scavenging assay was carried out by Viera et al. (2017) method by some modifications. The radical-scavenging activity was determined the intensity extract activity in discoloration 2, 2-diphenyl-1-picrylhydrazyl (DPPH) using the Equation 1:

$$\%DPPH = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100 \quad (1)$$

Where A_S is DPPH solution absorbance when the extract has been added at a specified amount and A_{DPPH} is DPPH solution absorbance.

2.6.4 FRAP assay

The Ferric reducing-antioxidant (FRAP) test with the use of 2, 4, 6-Tripyridyl-S-triazine (TPTZ) was conducted using the technique defined by Ouyang et al. (2018) and

Benzie and Strain (1996). The findings were represented as μmol Fe^{2+} per kg at 595 nm relative to the control solution.

2.7 Microbiological assay

Total viable counts, and total yeast and mold count was determined according to according to ISO 4833-1:2013, ISO 5403:1999 respectively. Ten grams of samples were combined with 50 mL of 0.1% peptone and homogenized at high speed for 1 min. The samples were serially diluted with 0.1% peptone in water and surface plated (0.1 mL) in triplicate on plate count agar (PCA, Qingdao Hope Bio-Technology Co., Ltd, Qingdao, China) and sabouraud dextrose agar. To increase the limit of detection to 1 log10 $CFUg^{-1}$, 1 mL of the lowest dilution was placed on the plates. Total viable counts (TVC) were determined by counting the number of colony-forming units after incubation at 37°C for 48 h and total yeast and mold count was calculated after incubation at 25°C for 72 h (Agnihotri, et al., 2018).

2.8 Statistical analysis

The samples were prepared in triplicates, and each measurement was performed on each repetition. The results of chemical and microbial tests were analyzed by a completely randomized factorial design at a 5% probability level using SPSS Statistical Software (IBM SPSS Statistics 19, IBM, NY). The significance levels were used as $p < 0.05$ (*) and $p < 0.01$ (**). Duncan's multiple range test was used to compare the mean values in different storage days.

3 Results and discussion

3.1 The effect of the variables on the chemical properties of OP

3.1.1 pH

The effects of different treatments and storage time on pH changes are shown in Table 1 and Figure 1, respectively. As can be seen from Table 1, the effect of the type of treatment on pH was not significant (p > 0.05). During storage, pH of T3 (CC+AS+CA) onion puree increased with increasing of storage days Figure 1a. Inam-Ur-Raheem et al., (2013) detected the same results on pH of fresh cut guava slices treated with calcium chloride and citric acid. The changes of pH values during the storage period may be associated with growth of microorganisms and subsequent production of organic acids (Heard, 2002). Acidification happened in T1, T2 and control treatment during storage time at different rates depending on the disinfected treatments. (Page et al., 2016) also observed the same results on diced onion in the storage period.

3.1.2 Total Soluble Solids (TSS):

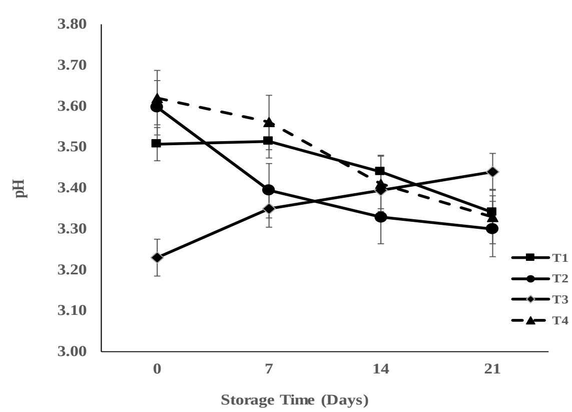
The effects of different treatments on weight loss in different samples are shown in Table 1. As can be seen, the different treatments significantly increased the amount of soluble solids compared to the control sample, but the different treatments did not significantly different (p > 0.05). Demir et al. (2019) reported the same results. The statistical outcomes regarding the TSS of OP showed considerable influence by various disinfected treatments various storage intervals along with their interaction presented in Figure 1b. The lowest TSS (6.44 °Brix) was recorded in T3 (*(CC+AS+CA)*) followed by T1 and T4 (control). Whereas, the highest TSS (6.50 °Brix) was recorded for OP which were T1.

Table 1 Effect of disinfected treatments on the pH, total soluble solids, and weight loss of OP

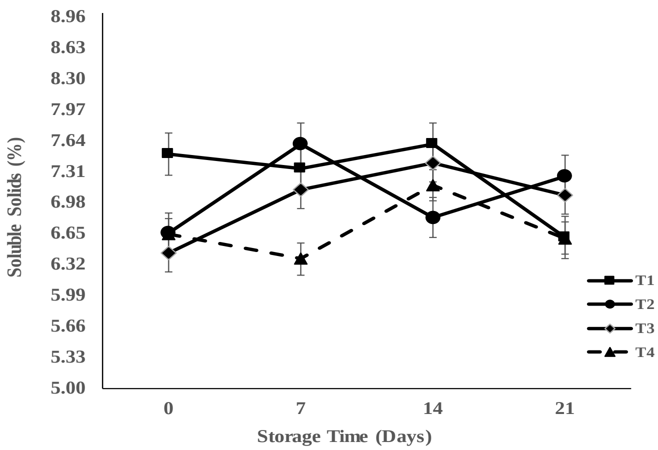
Treatment	pH	TSS (%)	WL (%)
T1	3.53^a±0.05	7.22^a±0.35	4.15^b±1.84
T2	3.45^a±0.05	7.11^a±1.25	4.38^b±0.95
T3	3.43^a±0.05	7.25^a±0.87	4.56^b±1.98
T4	3.57^a±0.05	6.88^b±1.01	5.71^a±1.25

Note: TSS: Total Soluble Solids, WL: Weight Loss,

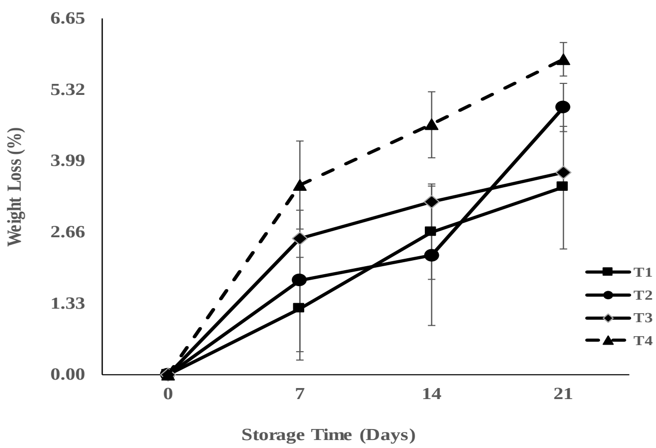
T1: (N+C), T2: (SH+S+C), T3: (CC+AS+CA); T4: Control. Values represent means ± SD of three replicates.



(a) pH



(b) soluble solids



(c) weight loss of OP

Figure 1 Effect of disinfected treatment during 4°C ± 1°C storage

Note: (T1: (N+C), T2: (SH+S+C), T3: (CC+AS+CA); T4: Control); Values represent means ± SD of three replicates.

3.1.3 Weight Loss

Weight loss of fresh produce is an important parameter because it leads to the economic losses. The rate of weight loss changes in different samples of onion puree is shown in Table 1. As can be seen, treating onion puree with niacin, sodium hypochlorite, citric acid and ascorbic acid has reduced weight loss and there is no statistically significant difference between different treatments ($p > 0.05$). Fresh-cut processing of fruit increases wound-induced C_2H_4, water activity and surface area per unit volume, which may also increase water loss (Sandhya, 2010). OP weight loss increased as a function of storage day (Figure 1c). The statistical evaluation regarding weight loss showed that disinfected treatments and storage intervals have significant ($p < 0.05$) effect on weight loss of OP whereas interaction between treatments and storage intervals, also recorded to be significant. By studying (Bhattarai and Gautam, 2006) observed that weight loss of tomato increases with the increasing of storage intervals. Similarly, Inam-Ur-Raheem et al. (2015) also detected that during storage the weight loss of fresh cut spinach increased in un-treated and treated different varieties stored at different temperatures. At the end of storage, the lowest weight loss (3.51%) was recorded in T1 followed by (3.77%) in T3, while the highest weight loss (5.89%) was observed for untreated (controlled). This is regardless of the sanitizer treatment. It is reported that 5 percent weight losses will reduce the marketability and retail value of entire goods (Ohta et al., 2002). In present study, the OP from all treatment except control remains marketable for 21 days.

3.2 The effects of variables on TPC and Antioxidant activity of OP

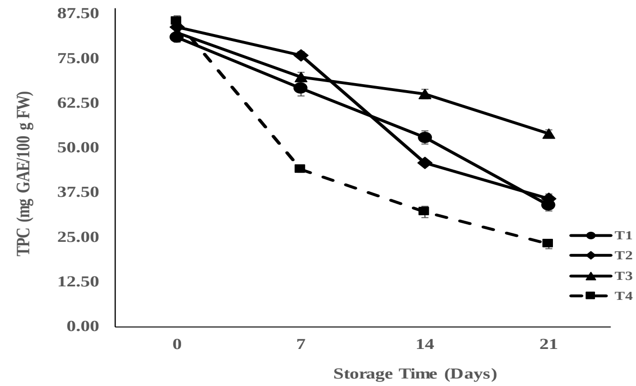
Changes in the amount of total phenolic compounds and the antioxidant activity of treated onion puree compared to the control sample are given in Table 2. As can be seen, treating onion puree with different disinfected has significantly increased the amount of phenolic compounds and subsequently the strength of antioxidant compounds. The total phenolic content of the control sample was 85.32 mg GAE 100 g^{-1} FW (Figure 2a). Phenolic content of onions has been documented to vary greatly in various onion cultivars, from 41.74 to 146.90 mg GAE 100 g^{-1} (Kaur et al., 2009), whereas slightly lower values (9–13 mg GAE 100 g^{-1}) for white onions have been documented by Gökçe et al., (2010). Total phenolic of treated OP decreased significantly during 21 days' storage at 4°C ± 1°C. At the end of storage, the highest TPC (54.01 mg GAE 100 g^{-1}) was recorded in T3 followed by (34 and 35.87 mg GAE 100 g^{-1}) in T1 and T2 respectively, while the lowest TPC (23 mg GAE 100 g^{-1}) was observed for untreated (controlled). (Howard et al., 1994) reported a mixed trend in phenolic content of fresh-cut diced onions. It might be due to the stress (wounding, cutting) which may lead to increased synthesis of phenolic compounds (Siddiq et al., 2013). The antioxidant properties of OP as evaluated by FRAP and DPPH are shown in Figure 2 (b, c). Different disinfected treatments have a significant ($p < 0.05$) impact on the amount of antioxidant properties in the OP relative to the control. During 21 days of 4°C ± 1°C storage, a general decreasing trend in antioxidant assayed was observed for the control and treated samples. The most significant decrease in treated OP occurred from day 14 to 21, as compared to the control. Our results were similar to those reported by Gökçe et al. (2010) for raw white onions. Investigation of Radical scavenging activity (DPPH), free radical scavenging activity is one of the methods to determine antioxidant activity. In this method, the purple color of free DPPH radicals is neutralized and discolored by the antioxidants present in the extract. Thus, the degree of the discoloration of this compound reflects the power of free radical inhibition by the antioxidants present (Ersus and Yurdagel, 2007). The disinfected treatment had a significant effect on the DPPH free radical scavenging activities (Figure 2b), which ranged from 63.26% to 79.46% against 61.73% in the control samples. As shown in Figure 2c, FRAP of the OP decreased throughout storage time. After 21 day of storage, the FRAP of OP was in T3 (56.73 µmol Fe^{2+}/g), T2 (60.03 µmol Fe^{2+}/g) and T1 (66.43 µmol Fe^{2+}/g), which were significantly higher than the control sample (48.20 µmol Fe^{2+}/g). Roldán-Marín et al. (2009) found a significant correlation between the TPC and antioxidant activity as calculated by DPPH and FRAP assay of onion, besides this correlation was reported for many other fresh-cut products (Deng et al., 2013; Supapvanich et al., 2011).

Table 2 Effect of disinfected treatments on TPC, DPPH, and FRAP of OP

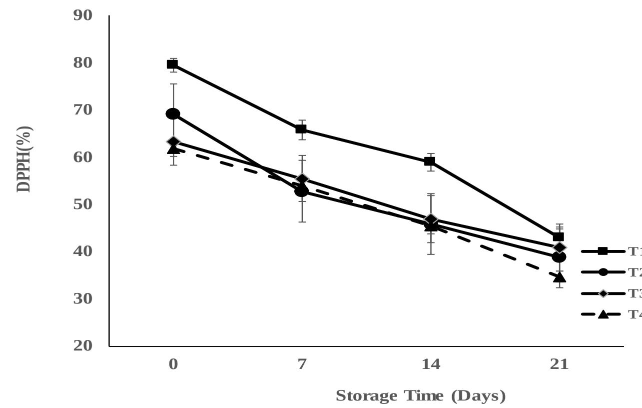
Treatment	TPC (mg GA 100 g^{-1} FW)	DPPH (%)	FRAP (µmol Fe^{2+}/g)
T1	53.98 ^{c}±1.28	48.74 ^{b}±2.35	64.71 ^{b}±12.35
T2	70.24 ^{a}±2.89	58.28 ^{a}±11.28	56.18 ^{c}±9.96
T3	71.34 ^{a}±3.34	52.54 ^{a}±5.74	68.01 ^{a}±5.78
T4	61.91 ^{b}±1.36	51.01 ^{a}±8.54	54.41 ^{c}±4.38

Note: *TPC* Total Phenolic Compound, *FRAP* Ferric Reducing Antioxidant Power

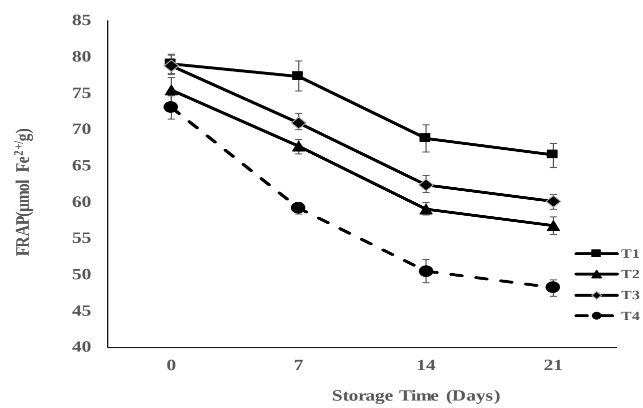
T1: (N+C), T2: (SH+S+C), T3: (CC+AS+CA); T4: Control. Values represent means ± SD of three replicates.



(a) total phenolic contents



(b) DPPH



(c) FRAP of OP

Figure 2 Effect of disinfected treatment during 4 ± 1°C storage

Note: (T1: (N+C), T2: (SH+S+C), T3: (CC+AS+CA); T4: Control; GAE, gallic acid equivalent; Values represent means ± SD of three replicates.

3.3 The effects of variables on microbial properties

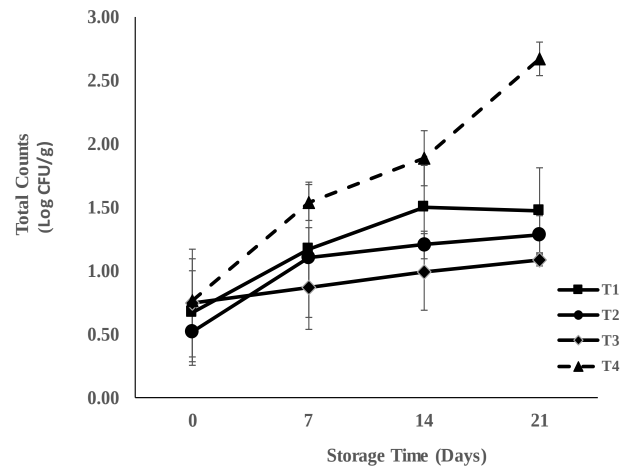
Fresh cut produces are typically infected with foodborne bacteria and spoilage microorganisms. Efficient food protection procedures for fresh-cut onions are essential (Kaczmarek et al., 2019) The amount of microbes in foodstuff is one of the most important indicators of its stability and health. Microbial quality improvement is achieved when microbes are destroyed or deactivated.

Table 3 Effect of disinfected treatments on total count and total molds and yeasts of OP

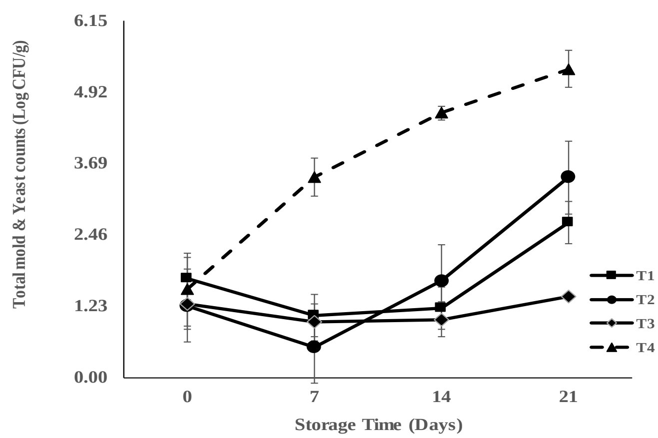
Treatment	Total Count (Log CFU/g)	Total molds and yeasts (Log CFU/g)
T1	1.04 ^{bc}±0.33	1.31 ^{a}±0.46
T2	1.28 ^{a}±0.45	1.01 ^{b}±0.39
T3	0.98 ^{bc}±0.23	1.25 ^{a}±0.38
T4	1.18 ^{ab}±0.53	1.36 ^{a}±0.29

Note: T1: (N+C), T2: (SH+S+C), T3: (CC+AS+CA); T4: Control. Values represent means ± SD of three replicates.

As can be seen from Table 3, treating onion puree with various compounds (niacin, sodium hypochlorite, ascorbic acid, citric acid and calcium chloride) has reduced microbial contamination. The effects of the storage period, pre-treatment method, and the interaction of both variables on the total plate count and of the total mold and yeast counts were significant (p <0.05). Typical growth activity in the total plate count experiment was observed (Figure 3a). A significantly low initial counts TPC (p <0.05) was obtained using T3 (*CC+AS+CA)*, followed by T2 (*Sodium hypochlorite (SH+S+C)*) and T1(*Nisin & Citric acid (N+C)*) compared to the control throughout storage. Microbial counts did not surpass 10^9 and were comparable to those collected from other fresh-cut products (Nguyen-the and Carlin, 1994). By day 21, The highest total counts were in the control followed by T1, T2, and T3. Because of the low pH in the treatment T3, microflora is usually restricted to microbial (Luna-Guzmán and Barrett, 2000). It was often thought that antimicrobial compounds could be extracted from plant cells during cutting operations (Ren et al., 2020; Nguyen-the and Carlin, 1994). Because of the exploratory aspect of our microbial research, more studies should be carried out to explore potential microbial in OP. The total molds and yeasts count of OP was below log CFU g^{-1} in all treatments (Figure 3b). The treatment of OP with (*CC+AS+CA)* reduced the total molds and yeasts below the detection level (1.0 lg CFU g^{-1}) until 7 days of storage. From day 7 onwards, the total molds and yeasts started increasing gradually in the (*CC+AS+CA)* treated OP. At the end of the storage period, the total molds and yeasts values on T3 samples was 1.40 log CFU g^{-1}, which was significantly lower than the control (5.32 log CFU g^{-1}). This indicates that T3 could be applied as an effective preservative for OP.



(a) total Counts



(b) Total mold & Yeast counts of OP

Figure 3 Effect of disinfected treatment during 4°C ± 1°C storage

Note: (T1: (N+C), T2: (SH+S+C), T3: (CC+AS+CA); T4: Control). Values represent means ± SD of three replicates

4 Conclusion

The effect of different disinfected treatments was analyzed for fresh onion puree and compared with untreated (control) sample. Research findings showed that disinfected treatments perform the best for maintenance of pH, TSS, acidity, FRAP antioxidant activity. T3 (CC+AS+CA) were found to be the most useful for extending the shelf life of OP up to 21 days at 4°C.

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