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Research Article

The Effects Of Washing Practices And Storage On Antioxidant Activity Of Some Selected Fruits

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ABSTRACT
Effects of washing practices using a commercial detergent or disinfectants alone or in combination with each other on the radical-scavenging activity and reducing power of the fresh fruits were examined. The fresh fruits were subjected to a triple wash treatment of washing in tap water for mud removal, washing in water containing a detergent (dishwashing liquid) or disinfectant individually, and rinsing in tap water. The results show that there were significant variations in antioxidant activity across fresh fruits (ranging from 15.02 to 57.15 mmol/L Trolox equivalents per kg of fresh weight). The antioxidant capacities of the all samples were soaked in Kanz Disinfecting Liquid showed lowest antioxidant activity ($P<0.05$). Pretreatment with detergent before washing with calcium hypochlorite or benzalkonium chloride showed significant reductions of antioxidant activity (ranging from 8 to 27%, $P< 0.05$). Levels of total antioxidant activity showed different changes throughout the storage period at 4 ˚C depend on storage duration.
Key words: Total antioxidant activity, washing practices, Storage, Disinfectants, Fresh fruit, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Ferric reducing antioxidant power (FRAP)

INTRODUCTION

Various epidemiological studies have reported that diets rich in fruits, vegetables and grains are associated with a lower risk of several degenerative diseases, such as cancers and cardiovascular diseases [1]. Nutritional recommendations to reduce the risk of coronary heart disease have focused largely on the intake of nutrients that affect the risk factors, including plasma lipid and lipoprotein levels, blood pressure, and body weight, etc. Over the past two decades, considerable evidence has been gathered in support of the hypothesis that the free radicals and free-radical-mediated oxidative processes and specific end products play a key role in atherogenesis [2]. A practical way of fighting against degenerative diseases may be to improve body antioxidant status. Antioxidants constitute a diverse group of compounds with different properties. They operate by inhibiting oxidant formation, intercepting oxidants once they have formed, and repairing oxidant-induced injury. Although there is an effective antioxidant defense mechanism to protect the body against oxidant attacks, sometimes it cannot cope with oxidant load in the body, and additional dietary antioxidants are needed [3-5]. Fresh vegetables and fruits are excellent sources of antioxidant components. Fruits are an important part of our diet and as a source of energy, fiber, vitamins, minerals and antioxidants [6]. Washing of fruits and vegetables is a common method to eliminate soil, pesticide residues, debris and to reduce microbial load [7]. Sanitization treatments can play an important role in reducing the indigenous microflora and pathogens on fresh fruits and vegetables. Application of a detergent before disinfection may help remove microorganisms from the surface of fresh produce [8-9]. Most of the investigations concerning the efficacy of disinfectants for reduction of pathogenic bacteria have been conducted on inoculated fresh fruits such as cantaloupe, berries, and apples [10-13] and vegetables such as lettuce and cabbage [14-17]. But the efficacy of these treatments on the antioxidant activity of the fresh fruits has not been clearly defined. Therefore, this study was carried out to determine the effects of domestic washing practices with detergents and fresh produce disinfectants or their combination, on antioxidant activity of the fresh fruits.

MATERIALS AND METHODS

Fruits: Seven types of fresh fruits including strawberry (*Fragaria ananassa L.*), cherry (*Prunus avium L.*), grape (*Vitis vinifera L., White table grapes*), peach (*Prunus Persica L.*), apricot (*Prunus armeniaca L.*), pear (*Pyrus communis L.*) and apple (*Malus domestica L.*) were purchased during harvest period from the main field of fruits and vegetables in Tehran, Iran, before passing 24 h from havesting, packed in plastic bags, and transported to

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the laboratory within 1 h. Approximately 8 kg of each mature fruit was selected, from which damaged fruits were discarded. The fruits were treated on the day of purchase and used directly for analyses at time zero. Treated fruits were stored at 4 ˚C and analyzed after several storage times (3 and 5 days).

*Preparation and decontamination of fruits :*For each replication of disinfection or washing procedure, about 2 kg of each type of fruits was added to 6 L of tap water with gentle stirring for about 20 s to ensure complete immersion and mud removal. An adequate sample of fruit was separated immediately for antioxidant activity determination before any treatment. Fruits were removed from water, and subjected to the one of the procedures presented in the treatment step in Figure 1 (step 2, procedures 2.1 to 2.4). Each of the treatment procedures was replicated three times.

Preparation of disinfectant solutions: The following disinfectants with their respective concentrations and exposure times (according to the manufacturer's recommendations) were used in the study: benzalkonium chloride (Hygen, Mosmer Co., Tehran, Iran) at 92 ppm for 15 min, calcium hypochlorite (perchlorine, commercial grade) at 300 ppm for 15 min and Kanz Disinfecting Liquid for vegetables & fruits (which is sold in Iran local markets and it's active ingredients are: benzalkonium chloride and cocoamidopropyl betaine) at 110 ppm for 20 min. The concentration of benzalkonium chloride was determined following the method proposed by the U.S. Pharmacopeia [18]. Calcium hypochlorite concentration was determined by titration with sodium thiosulfate [19]. A dishwashing liquid (Golrang Group, Tehran, Iran) containing alkyl benzene sulfonate, sodium lauryl ether sulfate, isothiazolones, and coconate fatty acid diethanol amide was used as the detergent. Kanz Disinfecting Liquid was used alone not in combination with detergent.

Sample preparation: Samples were taken after each processing step and used directly for analyses at time zero. Treated fruits were stored at 4 ˚C and analyzed after 3 and 5 days storage. For the analysis, the extract was

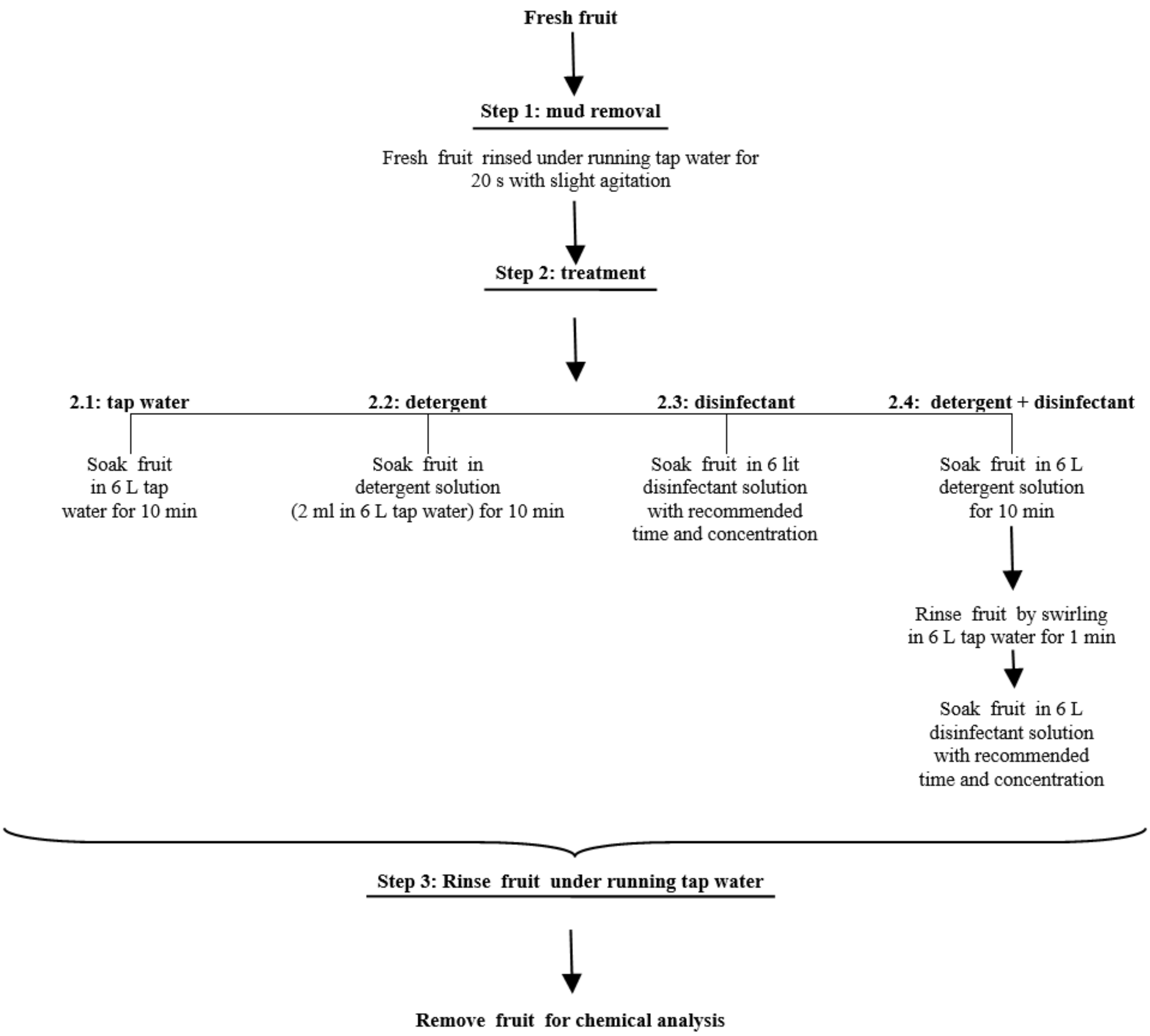

Fig 1. Fresh fruit decontamination protocols.

Table 1. Antioxidant content expressed as mmol L^{-1} Trolox equivalents per kg of fresh weight of antioxidant capacity (DPPH) (mean ±SD) of fresh and treated fruits.

Fruit[a]	Storage time (days)[b]	Treatments[c,d] 1	2	3	4	5	6	7	8
Straw berry	1	51.12±2.17	50.67±1.13	49.61±1.51	49.73±1.19	40.52±1.07	48.99±2.07	41.04±1.18	37.96±1.41
	3	42.73±1.77	41.97±1.10	42.10±1.41	44.08±1.54	35.20±1.19	44.63±2.26	36.05±1.29	33.53±1.92
	5	42.08±1.52	42.05±1.81	41.94±1.66	43.89±1.21	34.71±0.87	45.08±1.19	35.60±1.09	32.92±1.88
Cherr y	1	47.75±1.25	48.06±1.37	46.85±1.86	46.51±1.02	40.10±1.57	47.18±1.11	39.79±2.02	36.10±1.21
	3	42.75±1.07	43.06±1.21	39.25±1.02	38.51±1.79	35.10±1.47	40.18±1.23	36.79±1.12	31.10±1.36
	5	38.83±1.72	38.96±1.51	35.68±0.87	35.18±1.47	30.16±1.17	35.01±0.97	29.59±1.17	27.39±1.27
Peach	1	44.97±2.18	44.81±1.88	45.63±1.95	45.70±1.24	38.88±1.33	44.42±2.41	38.74±1.67	37.70±1.77
	3	39.89±1.80	38.85±1.45	40.30±1.34	40.60±1.18	34.07±0.97	39.74±0.85	33.84±1.33	28.78±1.68
	5	35.03±1.21	35.92±1.35	36.45±1.97	35.89±1.18	30.86±1.88	36.15±1.46	30.06±1.28	24.81±2.21
Pear	1	39.07±1.11	39.23±1.24	38.07±1.31	38.00±1.87	36.21±1.61	37.97±2.09	35.88±1.74	32.84±1.66
	3	38.66±1.47	39.15±1.77	38.45±1.14	37.61±1.57	34.57±2.11	36.88±2.32	35.01±1.49	31.91±1.96
	5	38.98±2.55	38.92±0.96	37.92±1.47	37.91±1.22	34.05±1.18	36.21±1.71	34.17±1.55	31.42±1.41
Apple	1	37.83±1.41	38.40±1.28	38.99±2.57	37.57±1.67	35.93±1.51	38.33±1.63	34.96±1.74	32.68±1.15
	3	36.98±1.49	37.60±1.71	38.33±1.23	36.36±1.49	35.18±1.35	37.50±1.61	34.01±0.82	31.77±1.43
	5	36.13±1.15	36.97±1.94	37.55±1.81	36.96±1.41	34.88±1.58	37.14±2.05	33.42±1.17	31.04±1.51
Grape	1	16.39±1.37	16.32±1.22	16.20±1.02	16.97±0.96	12.02±1.33	15.66±2.07	13.08±1.32	10.81±1.45
	3	15.86±1.23	14.98±1.56	16.83±0.93	16.23±1.41	11.59±1.26	14.96±1.14	12.65±1.57	9.76±1.17
	5	15.78±1.11	15.32±1.49	15.68±1.56	15.96±0.85	12.14±1.34	14.22±1.28	13.16±1.19	9.97±1.05
Apric ot	1	15.02±1.49	14.96±1.13	15.09±0.77	15.19±1.11	12.55±1.32	15.12±2.37	12.75±2.02	8.85±1.95
	3	11.60±1.76	10.71±1.55	12.29±1.37	12.01±1.19	8.60±2.15	11.36±0.89	9.38±1.19	5.46±1.62
	5	8.48±1.44	8.85±1.32	9.02±2.17	8.35±2.03	5.62±0.84	9.41±1.11	6.61±1.21	3.47±1.04

[a] Results showed that there were significant variation in antioxidant activity across the selected fresh fruits($P<0.05$).
[b] Homogeneous subgroups are discussed in the results and discussion section.
[c] Different treatments: 1) only mud removal, 2) Soak fruits in 6 lit tap water for 10 min, 3) Soak fruits in detergent solution(2 mL in 6 lit tap water) for 10 min, 4) Soak fruits in 6 lit calcium hypochlorite solution (300 ppm) for 15 min, 5) Soak fruits in 6 lit benzalkonium chloride solution (92 ppm) for 15 min, 6) First, soak fruits in detergent solution(2 mL in 6 lit tap water) for 10 min and then in 6 lit calcium hypochlorite solution (300 ppm) for 15 min,7) First, soak fruits in detergent solution(2 mL in 6 lit tap water) for 10 min and then in 6 lit calcium hypochlorite solution (300 ppm) for 15 min, 8) Soak fruits in 6 lit Kanz disinfecting liquid solution (110 ppm) for 20 min.
[d] No significant differences in total antioxidant activity were found on selected fruits after decontamination with treatments 1,2,3,4 and 6 ($P > 0.05$). Decontamination with treatments 5,7and 8 showed significant reductions of antioxidant activity ($P<0.05$).

prepared by a method described by Scalzo et al. [20] and Gao and Mazza [21]. Twenty g of the homogenized sample was extracted with 60 ml of ethanol (Merck no.1009832500) and was placed for 2 h at room temperature in darkness. The mixture was centrifuged, and the clear phase was separated and filled to 100 ml with the extraction solvent. This extract was used for the determination of the antioxidant capacity.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay: A slightly modified method of Alamanni and Cossu [22] strawberry was used in which a stable radical (DPPH) is reduced by the antioxidants of the sample, which leads to a decolonization. Ten ml of a 0.1 mmol/L DPPH (Fluka Chemical Co.) solution in ethanol were mixed with 0.1 ml sample extract. After 30 min, the absorbance at 517 nm was measured with Lambda 25 spectrophotometer (Perkin Elmer co.). Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) was used as reference compound, and the antioxidant capacity is expressed in mmol/L trolox equivalents per kg of fresh weight (mmol/L T.E /kg fw).

Ferric reducing antioxidant power (FRAP) assay: The method described by Benzie and Strain [23] modified by Guo et al. [24], which is based on the reduction of a ferric (III) complex to its colored ferrous (II) form in the presence of antioxidants. The FRAP reagent was prepared freshly before measuring and contained 5 ml of a 10 mmol/L TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) solution (Merck) in 40 mmol/L hydrochloric acid, 5 ml of a 20 mmol/L ferric (III) chloride solution, and 50 ml of 0.3

Table 2. Antioxidant content expressed as mmol L^{-1} Trolox equivalents per kg of fresh weight of antioxidant capacity (FRAP) (mean±SD) of fresh and treated fruits.

Fruit[a]	Storage time (days)[b]	Treatments[c,d]							
		1	2	3	4	5	6	7	8
Straw berry	1	57.15±1.47	56.77±1.76	56.21±1.37	57.10±2.05	46.72±1.15	55.99±1.93	46.14±1.41	40.86±1.58
	3	48.73±1.31	49.07±1.30	48.50±1.11	49.08±1.29	41.20±2.21	50.03±0.94	40.05±1.51	37.63±1.48
	5	48.48±1.74	48.75±1.58	48.94±1.61	48.89±1.75	40.71±1.23	49.68±1.14	39.60±1.47	38.12±1.56
Cherr y	1	52.75±1.29	52.06±1.37	51.95±1.86	50.95±1.02	48.10±1.57	51.28±1.11	47.79±2.02	40.11±1.21
	3	45.16±1.28	44.15±1.08	43.16±0.85	42.34±1.41	36.16±1.33	44.69±1.57	35.46±1.91	32.77±1.67
	5	40.83±1.55	39.56±2.15	38.68±1.43	38.18±1.29	31.16±1.34	37.81±1.65	30.09±1.11	28.39±0.95
Peach	1	49.27±1.52	48.81±1.24	48.63±1.16	47.98±1.12	39.48±1.03	47.52±1.25	38.54±1.13	36.71±1.29
	3	42.29±1.46	41.65±0.89	40.90±2.25	40.60±2.01	35.77±1.43	38.74±1.23	34.34±1.82	30.74±1.36
	5	37.23±1.02	37.52±1.74	36.95±1.91	35.89±1.35	31.86±1.16	35.05±1.44	30.06±1.49	26.88±2.08
Pear	1	43.67±1.44	43.91±1.21	44.07±1.50	43.25±1.66	41.31±0.94	42.97±1.18	40.88±1.41	33.44±1.50
	3	42.96±1.27	42.85±1.75	43.45±1.45	42.61±1.07	39.77±2.50	43.08±1.58	40.01±1.22	34.01±1.73
	5	43.28±1.36	42.72±1.04	42.92±2.11	42.12±1.75	40.65±1.48	42.64±1.59	39.87±1.12	33.72±1.20
Apple	1	41.83±1.49	41.40±1.06	40.99±1.18	40.27±1.36	37.93±1.65	39.93±1.18	36.96±2.13	32.58±1.35
	3	41.78±1.39	41.60±1.72	40.33±1.52	41.06±1.64	37.98±1.48	39.50±1.67	36.01±1.68	31.77±1.54
	5	40.43±2.25	41.67±1.61	39.59±0.65	39.96±1.26	38.08±1.14	38.94±1.05	36.42±1.25	32.14±1.14
Grape	1	23.19±1.61	22.52±1.26	22.08±1.82	21.97±1.81	17.32±1.70	21.86±1.06	17.58±1.76	11.81±1.32
	3	23.06±1.98	23.13±1.99	21.53±1.70	21.23±1.31	17.59±1.85	21.46±0.87	17.35±1.39	10.96±1.33
	5	22.78±1.68	22.32±1.11	21.18±1.78	20.95±0.99	17.46±1.77	19.82±1.15	16.96±1.25	11.17±1.01
Apric ot	1	18.80±1.75	18.66±1.27	17.82±1.91	17.19±2.25	15.15±1.33	17.52±1.41	14.75±1.19	11.85±1.22
	3	14.32±1.64	14.01±1.76	12.89±1.28	12.31±1.88	10.63±1.91	13.06±2.35	10.88±1.97	8.66±1.77
	5	10.52±1.32	10.15±1.49	9.52±1.54	10.35±1.62	8.72±1.24	9.81±1.05	9.01±0.97	5.77±1.41

[a] Results showed that there were significant variation in antioxidant activity across the selected fresh fruits(P<0.05).
[b] Homogeneous subgroups are discussed in the results and discussion section.
[c] Different treatments: 1) only mud removal, 2) Soak fruits in 6 lit tap water for 10 min, 3) Soak fruits in detergent solution(2 mL in 6 lit tap water) for 10 min, 4) Soak fruits in 6 lit calcium hypochlorite solution (300 ppm) for 15 min, 5) Soak fruits in 6 lit benzalkonium chloride solution (92 ppm) for 15 min, 6) First, soak fruits in detergent solution(2 mL in 6 lit tap water) for 10 min and then in 6 lit calcium hypochlorite solution (300 ppm) for 15 min,7) First, soak fruits in detergent solution(2 mL in 6 lit tap water) for 10 min and then in 6 lit calcium hypochlorite solution (300 ppm) for 15 min, 8) Soak fruits in 6 lit Kanz disinfecting liquid solution (110 ppm) for 20 min.
[d] No significant differences in total antioxidant activity were found on selected fruits after decontamination with treatments 1,2,3,4 and 6 (P > 0.05). Decontamination with treatments 5,7and 8 showed significant reductions of antioxidant activity (P<0.05).

mol/L acetate buffer (pH 3.6). The extract (0.1 ml) was mixed with 0.3 ml of water and 3 ml of reagent. After 8 min, the absorbance was measured at 593 nm. Trolox was used as the reference compound, and the antioxidant capacity is expressed in mmol/L T.E /kg fw.

Statistical analysis: All statistical analyses were carried out by SPSS for Windows (version 11.5). The data determined were expressed as the mean of three replicate determinations and presented as means ± SD. Data from each treatment or control group were analyzed for differences by using repeated-measures analysis. Tukey's all-pairwise-comparison test was used to identify differences between different groups. P<0.05 was considered statistically significant. Pearson's bivariate correlation coefficients were calculated to compare the DPPH and FRAP assay. Fresh fruits data is expressed on fresh weight basis.

RESULTS

Antioxidant capacity and reducing power of fresh fruits extracts: The DPPH scavenging activity of fresh fruits ranged from 15.02 to 51.12 mmol/L Trolox equivalents per kg of fresh weight (mmol/L T.E /kg fw), with high activity being found in strawberry, cherry, peach, pear and apple, while low activity was observed in grape and apricot (Table 1). Similarly, the FRAP activity of fresh fruits showed a wide range from 18.8 to 57.15 mmol/L T.E /kg fw, the highest activity being in strawberry (57.15 mmol/L

T.E /kg fw) and the lowest in apricot (18.8 mmol/L T.E /kg fw) (Table 2). A significant correlation was found between DPPH and FRAP assays (r = 0.84, r^2 = 70.56%) in fresh fruits. The good correlation between the results from DPPH and FRAP assays in dry fruits has been previously reported [25].

Effects of washing treatment on total antioxidant activity of fruits: The antioxidant capacities of untreated samples were measured with the DPPH and FRAP assay and the antioxidant activity of fresh fruits showed a wide range from 15.02 to 51.12 and 18.8 to 57.15 mmol/L T.E /kg fw, respectively (Table 1 and 2). The differences between the means for fruits washed in tap water, detergent, calcium hypochlorite or benzalkonium chloride solution alone were not significant. The samples which were washed with the combination of detergent and one kind of fresh produce disinfectants in two sequential steps showed significant reductions of antioxidant activity ($P<0.05$). The antioxidant capacities of the all samples were soaked in Kanz Disinfecting Liquid measured with the DPPH and FRAP assay, showed lowest antioxidant activity ranging from 8.85 to 37.96 and 11.85 to 40.86 mmol/L T.E /kg fw, respectively (Table 1 and 2).

Effects of storage time on total antioxidant activity of fruits: In the present study, a significant ($P< 0.05$) decrease in total antioxidant activity were observed for cherry, apricot and peach samples from days 0 to 3 and from days 3 to 5 (Table 1 and 2).

DISCUSSION

A variety of methods specific to their chemical properties have been used to quantify antioxidant activity in plant foods[26]. Since DPPH and FRAP radical scavenging assays are widely used due to their simplicity, stability, accuracy and reproducibility [25, 27] we conducted these methods to quantify antioxidant activity in the present study.

The selected fruits were subjected to a triple wash treatment: washing in tap water for mud removal, washing in water containing a detergent or disinfectant and rinsing in tap water. The differences between the means for fruits washed in tap water, detergent, calcium hypochlorite or benzalkonium chloride solution alone were not significant. In previous study [7], no significant effects of washing treatments (Tap rinsed, water dipped and chlorine dipped) on levels of total antioxidant activity were observed. The samples which were washed with the combination of detergent and one kind of fresh produce disinfectants in two sequential steps showed significant reductions of antioxidant activity. These reductions were higher in fruits with slim skin and soft flesh such as grape (27%), strawberry (21%), cherry, apricot (17%) and peach (16%). Apple and pear showed less reduction (7 and 8 %, respectively). This observation could possibly be explained by the fact that these fruits have thick skin and fibrous structure, which can reduce the penetration of detergent and disinfectants into the soft tissues.

The antioxidant capacities of the all samples were soaked in Kanz Disinfecting Liquid showed lowest antioxidant activity. The probable cause could be that the using of detergent and disinfectants in one step and at the same time has synergetic effect on the reductions of antioxidant activity. Samadi et al. have reported that application of a detergent at the permitted level before disinfection did not improve antimicrobial activity of the disinfectant significantly [28]. However, the detergent could have other useful biological effects, such as detachment of parasites from vegetables. In summary, we can conclude that the necessity of application of a detergent at the permitted level before disinfection need further studies.

Levels of total antioxidant activity showed different changes throughout the storage period depend on the type of fruits (ranging from 0 to 35%). No significant effects of storage time were observed on activity of apple, grape and pear samples. Similar observations were previously done on some fruits [29-30] or vegetables [31] stored at room temperature or in the refrigerator. The results of these studies indicated that in some fruits and vegetables the storage did not affect negatively the antioxidant capacity. Sacchetti et al. have also shown that the antioxidant capacity of apple derivatives were not negatively affected by storage, and in some cases, an increase of the antioxidant capacity was observed during storage [32]. Kevers and et al. have observed that antioxidant capacity decreased during storage in apricot (25%) and decreased by >50% in spinach, banana, broccoli, and leek [6]. In apricot, Bartolini et al also observed a decrease of the antioxidant capacity during storage at low temperature [33]. Storage also affected the antioxidant activity of strawberry from days 0 to 3, and the decrease observed from days 3 to 5 was not significant ($P > 0.05$). These results also were similar to the ones asserted in the literature [34].

CONCLUSION

Antioxidative capacity is an important fruit quality parameter. The results of this study indicate that each type of fruits had a different antioxidant activity, which may contribute by different antioxidant components, such as α-tocopherol, β-carotene, vitamin C, selenium or phenolic compounds. Washing and sanitizing treatment can play an important role in reducing microbial populations on fresh fruits and vegetables, but the efficacy of these treatments on the antioxidant activity of the fresh fruits has not been clearly defined. The results of this study indicated that washing with chlorine did not negatively affect the total antioxidant activity, but in combination with detergents (in two sequential steps or at the same time) has negative synergistic effects on total antioxidant activity. Application of a detergent at the permitted level before disinfection may help remove microorganisms from the surface of fruits and vegetables but further studies are needed to determine its efficacy. Levels of total antioxidant activity showed different changes throughout the storage period depend on the type of fruits. However this study has some limitations. the current study only examined total antioxidant activity among different fruits without qualification and quantification of antioxidant substances separately,therefore it was impossible to to draw a cause and effect in the observed reduction. Further study of this possible association is warranted. If the results of this research would be confirmed in future prospective studies, May be it is nessecary in order to maximize the contents of antioxidant active substances in fruits, the number of processing steps are minimized as far as possible and long holding times should be avoided.

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