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Research Article

Effects of Wash Procedures and Storage Time on the Antioxidant Activity of Fresh Vegetables

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ABSTRACT
Vegetables were either retained unwashed or were washed using only water or using various combinations of water with detergent and/or calcium hypochlorite. The first analyses were performed immediately after washing on day one and further analyses were done after three and five days of storage at 4°C. The extent of decrease in antioxidant activity was different for different procedures. In comparison with other washing procedures, washing sequential with water, detergent, and calcium hypochlorite significantly ($P < 0.05$) decreased antioxidant activity. Antioxidant activity decreased of 37.6, 35.0, 35.5, and 27.0% in basil, lettuce, tomato, and capsicum, respectively, after storage for 5 days. Based on the results obtained, it is highly recommended that vegetables be purchased and consumed fresh.

Keywords: Disinfection – Calcium hypochlorite – DPPH (2,-2-diphenyl-1-picrylhydrazyl) – FRAP (Ferric reducing antioxidant power) – Storage

INTRODUCTION

Antioxidants are a group of compounds that facilitate survival in plants and may promote the health in humans who consume a variety of plant foods[1]. According to recent studies, food scientists and nutritionists agree that increasing daily fruit and vegetable consumption may have a significant role in reducing the incidence of many chronic diseases, for example, the major causes of death in industrialized nations, such as cancer, cardio vascular, and cerebro-vascular disease[2-6]. Antioxidants and other phytochemicals in food plants are believed to protect against these chronic diseases[7-9]. Fruits and vegetables contain hundreds of compounds with potential antioxidant activity, including the vitamins C and E, carotenoids, chlorophylls, and a wide variety of phytochemicals, such as simple phenolic compounds and, flavonoid glycosides[10]. Although there is an effective defense system that protects the human body against oxidative attack, it cannot cope with the oxidant load, and therefore requires additional dietary antioxidants[11]. Raw vegetables may be contaminated with different microorganisms and the range of contamination is 10^3 to 10^7 Colony Forming Unit (CFU)/g[12]. Vegetable washing is a common practice to remove soil, pesticide residues, or debris, and is also a good way to reduce microbial contamination. Washing of vegetables at a domestic level generally involves rinsing under a running tap[5]. In addition, using a detergent before the disinfection step may help remove microorganisms from the surface of a fresh product[13]. Disinfectants can play an important role in reducing the microbial load of fresh fruits and vegetables. Calcium hypochlorite ($CaCl_2O_2$) is the most common source of chlorine used for disinfection of processed water. Disinfection of fruits and vegetables using different chlorine compounds can reduce their disease – causing potential.

In Iran, raw vegetable decontamination is done using either a produce disinfectant or a mild detergent, or a combination of both. Very less information is available about the effect of washing procedures on the antioxidant activity of fresh vegetables. Our aim in this study was to investigate the effect of different washing procedures on the quantitative antioxidant activity of fresh vegetables. The effects of different washing procedures (to determine the efficacy of a commercial detergent and disinfectant) and storage times on the antioxidant activity of fresh vegetables were also studied.

MATERIALS AND METHODS

Chemicals and Apparatus

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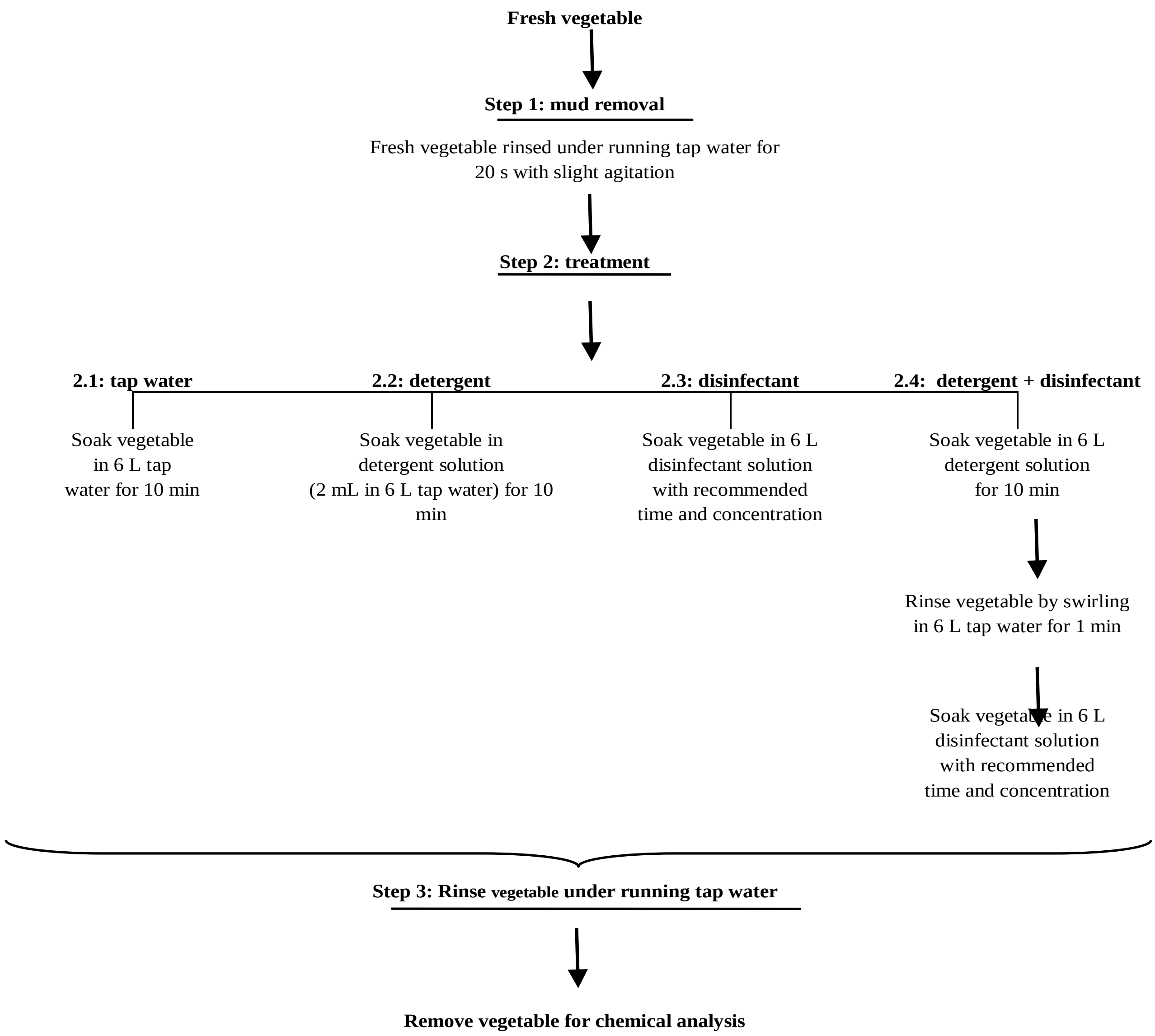

Figure 1: Fresh vegetable decontamination protocols.

DPPH (2,-2-diphenyl-1-picrylhydrazyl) and Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) were purchased from Fluka Chemical Co., USA. TPTZ (2,-4,-6-tripyridyl-s-triazine) and ethanol (Merck no. 1009832500) were purchased from Merck Chemical Co., USA. A Lambda 25 spectrophotometer (Perkin-Elmer, Inc., USA) was used for the determination of antioxidant activity of samples processed using different washing procedures.

Sample collection and extraction

Seven kind of fresh vegetables including tomato (*Solanum lycopersicum*), basil (*Ocimum basilicum*), cucumber (*Cucumis sativus*), carrot (*Dacus carota*), lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea var. capitata*), and capsicum (*Capsicum frutescens var. grossum*) were purchased randomly from public markets and divided into five portions each. Different washing procedures, namely, without washing (mud removal), washing with water, washing with water and detergent, washing with water and calcium hypochlorite, and washing sequential with water, detergent and calcium hypochlorite, were used (Fig 1).

The first analysis was done immediately after washing on day one, and further analyses were done after three and five days, respectively. The vegetables were stored (at 4 °C) in a refrigerator (home consumer conditions) for three and five days. About 20 g of sample was homogenized using a blender (National; MX-291N, Kuala Lumpur, Malaysia). Sixty mL of ethanol was added to the homogenized sample, which was maintained at room temperature, in darkness, for two hours. The mixture was centrifuged at 1000 rpm for 15 min, filtered through Whatman No. 1440-125 filter paper to obtain a clear extract, and kept at 4 °C until analysis was performed. To assess antioxidant activity, 2,-2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) methods were applied.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity

Table 1. Antioxidant content expressed as mmol/L Trolox equivalents per kg of fresh weight of antioxidant capacity (DPPH) (mean ± SD) of fresh and treated vegetables.

Vegetable[a]	Storage time (days)[b]	Treatments[c,d]				
		1	2	3	4	5
	1	62.45 ± 0.88	62.22 ± 0.57	62.34 ± 0.46	62.04 ± 0.87	55.96 ± 0.61
Cabbage	3	61.74 ± 1.21	61.37 ± 0.82	62.02 ± 0.87	62.02 ± 1.17	49.13 ± 0.75
	5	55.87 ± 0.91	55.41 ± 1.19	54.56 ± 1.16	54.57 ± 0.81	40.24 ± 0.58
	1	48.32 ± 0.86	48.04 ± 0.22	48.102 ± 0.98	48.23 ± 0.93	40.58 ± 0.806
Basil	3	40.43 ± 0.507	40.502 ± 0.82	40.38 ± 0.95	40.55 ± 0.97	34.16 ± 0.71
	5	31.13 ± 1.09	31.18 ± 1.05	30.57 ± 0.91	31.13 ± 0.35	28.55 ± 1.002
	1	42.05 ± 0.84	42.007 ± 1.007	42.08 ± 0.93	42.02 ± 0.41	37.38 ± 0.66
Lettuce	3	37.66 ± 0.64	37.04 ± 0.53	37.39 ± 0.51	37.28 ± 0.79	29.04 ± 0.81
	5	25.88 ± 0.73	26.37 ± 0.48	26.21 ± 0.58	26.78 ± 0.54	19.38 ± 0.85
	1	34.76 ± 1.09	34.03 ± 1.07	34.91 ± 0.94	35.65 ± 0.68	34.905 ± 0.68
Carrot	3	34.33 ± 1.14	34.05 ± 0.98	34.702 ± 0.76	35.02 ± 1.13	34.502 ± 0.59
	5	30.42 ± 0.58	30.13 ± 0.82	30.09 ± 0.904	30.6 ± 0.92	29.07 ± 0.35
	1	27.63 ± 0.56	27.99 ± 0.98	27.39 ± 1.41	27.38 ± 1.009	21.34 ± 0.85
Capsicum	3	27.15 ± 0.84	26.95 ± 0.74	27.58 ± 0.71	26.43 ± 0.71	17.94 ± 0.53
	5	20.74 ± 0.84	20.44 ± 0.94	21.18 ± 1.01	20.46 ± 1.06	13.53 ± 0.48
	1	25.76 ± 1.03	25.902 ± 0.97	26.57 ± 0.47	26.22 ± 0.89	20.47 ± 0.75
Tomato	3	20.84 ± 0.48	20.41 ± 1.09	20.81 ± 0.78	20.65 ± 1.34	17.07 ± 0.38
	5	17.51 ± 0.705	17.43 ± 1.09	17.45 ± 0.52	16.96 ± 0.609	13.66 ± 0.94
	1	17.58 ± 1.15	18.04 ± 1.02	17.38 ± 1.37	16.76 ± 1.29	16.41 ± 1.69
Cucumber	3	18.22 ± 1.15	17.52 ± 1.24	17.46 ± 1.58	17.402 ± 0.96	16.56 ± 1.24
	5	13.77 ± 0.54	12.92 ± 1.49	13.62 ± 0.84	13.39 ± 0.601	13.12 ± 1.31

[a] Results showed that there was significant variation in antioxidant activity across the selected fresh vegetables (P <0.05).
[b] Homogeneous subgroups are discussed in the results and discussion section.
[c] Different treatments: 1) Only mud removal, 2) Soak vegetables in tap water for 10 min, 3) Soak vegetables in detergent solution for 10 min, 4) Soak vegetables in calcium hypochlorite solution for 15 min, 5) First, soak vegetables in detergent solution for 10 min and then in calcium hypochlorite solution for 15 min.
[d] No significant differences in total antioxidant activity were found for selected vegetables after decontamination with treatments 1, 2, 3, and 4 (P >0.05). Decontamination with treatment 5 resulted in significant reduction of antioxidant activity (P <0.05).

A slightly modified method based on that of Alamanni and Cossu was used. Ten mL of a 0.1 mmol/L DPPH solution in ethanol was mixed with 0.1 mL sample extract. After 30 min, the absorbance was measured at 517 nm. Trolox was used as the reference compound, and the antioxidant activity was expressed in mmol/L Trolox equivalents per kg of fresh weight[14].

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to the procedures described in Vijaya Kumar Reddy et al. (2010), and Li, et al. (2009), with some modifications. The stock solutions were 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 mL $C_2H_4O_2$) at pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$. The solutions were prepared daily by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3.6H_2O$ solution. Three mL of FRAP reagent was added to each tube and maintained at 37 °C for 5 min. After 6 min incubation at room temperature, 3 mL of FRAP reagent was gently added to 100 µL of the extracted solution in ethanol. This was used as a blank. The absorbance was measured at 593 nm using a UV-visible spectrophotometer (Perkin-Elmer Inc., New York, USA). Trolox was used as the reference compound, and antioxidant activity was expressed in mmol/L Trolox equivalents per kg of fresh weight [3, 15].

Statistical analysis

SPSS 11.5 for Windows was used for all statistical analyses. The data were obtained from four replicate determinations and presented as means ± SD. Data for each treatment or control group were analyzed for differences by using repeated-measures analysis. To identify differences between different groups, Turkey's test was used. A P-value of less than 0.05 was considered statistically significant. Pearson's bivariate correlation coefficients were calculated to compare the DPPH and FRAP assay results. Fresh vegetable data are expressed per unit of fresh weight.

RESULTS

Antioxidant activity in fresh vegetables

Total antioxidant activity was measured using the DPPH and FRAP methods. As shown in Table 1, DPPH scavenging activity of fresh vegetables ranged from 17.58 to 62.45 mmol/L Trolox equivalents per kg of fresh weight. Cabbage showed the highest activity, while the lowest activities were observed in tomato and cucumber (Table 1).

Table 2. Antioxidant content expressed as mmol/L Trolox equivalents per kg of fresh weight of antioxidant capacity (FRAP) (mean ± SD) of fresh and treated vegetables.

Vegetable[a]	Storage time (days)[b]	Treatments[c,d]				
		1	2	3	4	5
Cabbage	1	66.17 ± 0.77	66.24 ± 0.84	65.85 ± 1.07	65.43 ± 0.81	59.23 ± 0.88
	3	64.97 ± 0.25	64.89 ± 0.71	65.64 ± 0.69	65.01 ± 0.805	51.52 ± 0.61
	5	57.38 ± 0.64	57.05 ± 1.06	57.92 ± 0.97	56.87 ± 0.97	41.83 ± 0.93
Basil	1	54.08 ± 0.89	54.87 ± 0.95	55.34 ± 0.62	55 ± 0.68	43.48 ± 1.005
	3	43.28 ± 1.05	43.53 ± 1.27	42.88 ± 1.52	42.98 ± 1.95	35.68 ± 0.84
	5	33.75 ± 1.1002	33.85 ± 1.01	33.09 ± 2.104	32.83 ± 0.95	29.25 ± 1.84
Lettuce	1	45.49 ± 0.99	45.24 ± 0.903	45.39 ± 0.93	45.107 ± 1.45	40.15 ± 1.11
	3	40.26 ± 0.62	40.98 ± 1.208	40.89 ± 1.07	40.48 ± 1.04	33.01 ± 0.79
	5	29.59 ± 0.91	28.91 ± 1.07	29.98 ± 1.92	29.71 ± 1.45	21.65 ± 1.12
Carrot	1	38.57 ± 0.71	38.54 ± 0.69	38.35 ± 0.66	38.13 ± 0.805	37.75 ± 0.41
	3	38.01 ± 0.609	37.77 ± 0.74	37.5 ± 0.909	38.17 ± 0.92	37.69 ± 0.78
	5	32.35 ± 1.62	31.57 ± 1.707	31.73 ± 1.27	31.73 ± 1.11	31.17 ± 0.79
Capsicum	1	30.605 ± 0.69	30.27 ± 0.79	30.27 ± 0.84	29.95 ± 1.07	23.15 ± 1.04
	3	29.35 ± 0.701	29.16 ± 0.95	29.58 ± 0.75	29.12 ± 0.84	20.27 ± 0.68
	5	22.33 ± 0.86	22.5 ± 0.67	22.54 ± 0.57	23.98 ± 0.53	15.88 ± 0.77
Tomato	1	29.87 ± 1.15	29.73 ± 1.38	29.43 ± 1.31	29.507 ± 0.76	22.14 ± 0.93
	3	24.48 ± 0.49	24.76 ± 1.01	25.09 ± 0.77	24.76 ± 0.83	20.01 ± 0.85
	5	19.205 ± 0.67	19.2 ± 1.09	19.28 ± 0.74	19.802 ± 0.89	17.47 ± 0.57
Cucumber	1	20.55 ± 0.71	20.38 ± 0.84	20.81 ± 1.26	20.76 ± 0.76	20.18 ± 0.76
	3	19.66 ± 0.76	19.78 ± 0.52	20.25 ± 0.75	19.62 ± 0.73	19.01 ± 0.44
	5	16.44 ± 0.57	16.307 ± 0.606	16.37 ± 0.53	16.24 ± 0.601	12.91 ± 0.59

[a] Results showed that there was significant variation in antioxidant activity across the selected fresh vegetables (P <0.05).
[b] Homogeneous subgroups are discussed in the results and discussion section.
[c] Different treatments: 1) Only mud removal, 2) Soak vegetables in tap water for 10 min, 3) Soak vegetables in detergent solution for 10 min, 4) Soak vegetables in calcium hypochlorite solution for 15 min, 5) First, soak vegetables in detergent solution for 10 min and then in calcium hypochlorite solution for 15 min.
[d] No significant differences in total antioxidant activity were found for selected vegetables after decontamination with treatments 1, 2, 3, and 4 (P >0.05). Decontamination with treatment 5 resulted in significant reduction of antioxidant activity (P <0.05).

Antioxidant activity in cabbage, basil, lettuce, carrot, capsicum, tomato, and cucumber declined, respectively. Table 2 shows that the FRAP activity of fresh vegetables ranged from 20.55 to 66.17 mmol/L Trolox equivalents per kg of fresh weight. Again, cabbage (66.17 mmol/L Trolox equivalents per kg of fresh weight) showed the highest activity and the lowest activity was found in cucumber (20.55 mmol/L Trolox equivalents per kg of fresh weight) (Table 2).

A high correlation was found between the results obtained using the two techniques [DPPH and FRAP ($R2 > 0.96$)].

Effect of washing procedures on the antioxidant activity of fresh vegetables

Decreases in antioxidant activity were not the same following the different washing procedures. The washing sequential with water, detergent, and calcium hypochlorite (the fifth treatment in Tables 1 and 2) produced statistically significant differences compared with other washing procedures (P < 0.05) (Tables 1 and 2).

The fifth treatment, i.e., washing sequential with water, detergent, and calcium hypochlorite led to significant decreases in the antioxidant activity of all the samples, except for carrot and cucumber.

Effect of different storage times on the antioxidant activity of fresh vegetables

Total antioxidant activity of fresh vegetables declined over the different storage times, and the largest decrease was observed on day 5 (P < 0.05) (Tables 1 and 2). The vegetables could be divided into two groups; the first group comprised vegetables that had soft tissue, i.e., basil, lettuce, tomato, and the second group comprised those that which had hard tissue (cabbage, carrot, capsicum, and cucumber). In the former group, antioxidant activity decreased significantly after the first day, and there were significant differences between the results for days 1, 3, and 5, respectively. However, in the latter group, the results were completely different; only the results for day 5 were statistically different from those for days 1 and 3. There was a high correlation between the results of the DPPH and the FRAP methods (P < 0.01).

DISCUSSION

A variety of techniques have been used to quantify antioxidant compounds in plant foods, relevant to their chemical properties[16]. Since DPPH and FRAP radical

scavenging assays are widely utilized due to their simplicity, stability, validity and reproducibility[17]. We performed these techniques to quantify antioxidant compounds in the present study.
Washing procedures and storage can play an important role in preserving the antioxidant activity of fresh vegetables. In comparison with other washing procedures, washing sequential with water, detergent, and calcium hypochlorite produced significantly different results. Washing vegetables with this technique led to 26% up to 37.6% reduction in antioxidant activity.
Under other conditions, i.e., without washing, washing with water, washing with water and detergent, and washing with water and calcium hypochlorite, no significant differences were observed ($P > 0.05$).
Tomato, basil, carrot, lettuce, cabbage, capsicum, and cucumber are sources of antioxidant compounds (Javanmardi et al. 2003; Franke et al. 2004; Kevers et al. 2007). On the first day of analysis and before any treatment, cabbage and cucumber showed the highest the lowest antioxidant activities, respectively. The antioxidant activity of cabbage, basil, lettuce, carrot, capsicum, tomato and cucumber decreased, and the results are in good agreement with the findings of Vijaya Kumar Reddy, C., D. Sreeramulu, et al. (2010) and Kevers, C., M. Falkowski, et al. (2007)[6,15]. There is a lack of information on the effects of washing procedures on the antioxidant activity of fresh vegetables and fruits. Our findings are in good agreement with those of Olive Kenny and David O Beirne, who showed that washing with water and washing with chlorine solution did not reduce the antioxidant activity of lettuce[5].
Washing sequential with water, detergent, and calcium hypochlorite was the procedure that reduced antioxidant activity most significantly. In this procedure, the vegetables were in contact with the disinfectant, i.e., chlorine, for a longer time, while in the other procedures the contact time was shorter. Chlorine and other disinfectants are strong oxidants and are very reactive compounds that combine with oxidizable substances to form secondary compounds. When a disinfectant comes in contact with vegetables, it reacts with the organic matter (such as fruit tissue, cellular juices, soil particles, and microbes). Therefore, in comparison with other procedures, washing sequential vegetables with water, detergent, and calcium hypochlorite for 15 min considerably reduced their antioxidant activity. As a result, the antioxidant activity of vegetables decreased either due to the increased contact time or to the combination of detergent and disinfectant.
Lettuce, cabbage, and basil showed larger decreases in antioxidant activity than carrot, capsicum, tomato, and cucumber. During washing and storage, the differences in preservation of antioxidant activity of different vegetables depends either on the amount of organic matter and inorganic compounds, or on their structure, which reacts with disinfectant.
Lettuce, cabbage, and basil leaves have thin skins and there is no border between tissue and skin. Vegetables with have such a structure are severely affected by disinfectants. Therefore, the reduction in antioxidant activity for these is greater than for the others. Carrot, capsicum, tomato, and cucumber have thick skins in comparison with lettuce, cabbage, and basil. Carrot contains fiber and hard tissue. Capsicum, tomato, and cucumber contain fiber and large amounts of water. Due to their tissue strength, these vegetables are impermeable to disinfectant compounds, and the reduction in their antioxidant activity is less than for the others.
As shown in Tables 1 and 2, storage time clearly had a major effect on the antioxidant activity of vegetables like basil and lettuce; for example, the antioxidant activity of basil on the first day was 54.08 mmol/L Trolox equivalents per kg of fresh weight, but this decreased to 43.28 mmol/L Trolox equivalents per kg of fresh weight (20% decrease) and 33.75 mmol/L Trolox equivalents per kg of fresh weight (37.5% decrease) after three and five days' storage, respectively. Storing lettuce for three and five days led to 11.5 and 35% decreases in antioxidant activity, respectively. However, in the case of cabbage and carrot, the decrease in antioxidant activity was less than 20%; for capsicum and tomato, the decreases were 27 and 35.5%, respectively.
Comparing the different washing treatments, it is clear that treatment 5, i.e., washing sequential with water, detergent, and calcium hypochlorite, was the most effective washing technique. On day one of the experiments, washing carrot and cucumber with this technique led to only a 2% reduction in antioxidant activity, whereas for cabbage, basil, and tomato the reduction was about 20%. The largest reduction (25%) was observed for capsicum. It seems that the type of skin tissue, as well as the type of vegetable, has an important influence on preserving the antioxidant activity.
While the antioxidant activity of basil was almost unchanged, washing after three days' storage led to a greater decrease in antioxidant activity in some samples; for example, in the case of cabbage and lettuce, the rate of decline was doubled. However, on day three, antioxidant activity of carrot, cucumber, and tomato was reduced less than on day one.
After five days' storage, the antioxidant activity had decreased in all of the samples; basil and tomato were the exceptions. Cabbage and lettuce showed the same behavior; after five days, the rate of antioxidant activity reduction increased almost 2.5-fold.
There is significant correlation between antioxidant activity and storage time. Tavarini et al. showed that the antioxidant activity of kiwifruit reduced upon storage at 0 °C, which is in good agreement with the findings of the present study[18]. Antonia Murcia et al. showed that storage of vegetables in the refrigerator for seven days reduced their antioxidant activity, which is consistent with findings of this study[19]. Storage of vegetables in the refrigerator for three and five days led to reduction in antioxidant activity, and the greatest reduction was observed in lettuce, cabbage, and basil.
On the first day of analysis, the washing procedure that involved washing sequential with water, detergent, and calcium hypochlorite resulted in a greater reduction in

antioxidant activity than the other procedures. Using these washing procedures, after vegetables were kept in the refrigerator for three and five days, reduction in antioxidant activity was significantly greater than that observed using other washing procedures under the same conditions.

CONCLUSIONS

Use of DPPH and FRAP methods provide an easy and rapid way to evaluate the antioxidant activity. Washing raw vegetables with only water or detergent or calcium hypochlorite does not reduce the antioxidant activity any more than washing them with a combination of all three. Antioxidant activity decreased with increasing storage periods. However this study has some limitations. The current study only examined total antioxidant activity among different raw vegetables without qualification and quantification of antioxidant substances separately, therefore it was impossible 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 necessary in order to maximize the contents of antioxidant active substances in raw vegetables, the number of processing steps are minimized as far as possible and long holding times should be avoided.
From these results, we strongly recommend that vegetables be purchased and consumed fresh.

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