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Cabbage Inhibits Nitrite Formation in other Vegetable Juices during Storage

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Abstract

Although it is well known that green vegetables contain high content of nitrate, reported results regarding nitrite accumulation in fresh green vegetables are controversial. Recent studies suggest that nitrate from food has health benefits including reduce blood pressure and increase blood flow. Intake excess amount of nitrite is believed to cause increased risk of some cancers and methemoglobinemia in infants. In this study, we investigated the dynamics of nitrite and nitrate contents in spinach juice, iceberg lettuce juice, celery juice, green cabbage juice, and red cabbage juice. Nitrite concentration increased significantly in home-made spinach juice, iceberg lettuce juice, and celery juice after only two days of cold storage at 4°C; while nitrate concentrations in these vegetable juices decreased significantly with time during the storage, suggesting a conversion from nitrate to nitrite. However, no significant change in nitrite and nitrate concentrations observed in green cabbage juice and red cabbage juice during the storage. We further discovered that both green cabbage juice and red cabbage juice can completely inhibit the formation of nitrite in spinach juice, iceberg lettuce juice, and celery juice during cold storage at 4°C. Sodium tungstate, an inhibitor of nitrate reductase, was effective in inhibiting nitrite formation in celery juice during storage. The nitrite formation in these vegetable juices was much faster during storage at ambient temperature. The ability of cabbage to inhibit the formation of nitrite in other vegetable juice was also observed during storage at ambient temperature. However, if cabbage juice was boiled for five minutes prior to mixing with other vegetable juice then no inhibitory effect was observed. Cabbage had an inhibitory effect on nitrite formation when mixed with other vegetable juice. This effect was lost upon boiling, suggesting that cabbage may contain some compounds that can inhibit nitrate reductase that was decomposed by boiling.

Keywords: Nitrate; Nitrite; Cabbage; Vegetable juice; Inhibition; Chemiluminescence analysis

Introduction

The highest sources of nitrate in human diet are found in certain vegetables, such as spinach, lettuce, and celery. It was estimated that vegetables contribute about 80% of human dietary nitrate intake [1,2]. Although nitrate is not an essential nutrient for humans, recent studies have suggested that nitrate from food has health benefits [2,3]. It was reported that drinking nitrate rich beetroot juice could significantly reduce blood pressure [4] and increase regional blood flow in the brain of older adults [5].

Pure inorganic nitrate is an inert anion. However, nitrate in vegetables can be reduced to nitrite by some enzymes or bacteria during storage. The intake of excess amount of nitrite is believed to cause increased risk of some cancers and methemoglobinemia in infants [6,7]. There are many reports on the nitrate and nitrite contents in retail leave vegetables [2,8,9]. In respect to nitrate and nitrite contents change in leave vegetables during storage, there are inconsistent and controversial results; some reports indicated that nitrate levels dropped and nitrite levels increased dramatically in leave vegetables during storage in a few days at ambient temperature, but nitrate and nitrite levels did not change significantly over 7 days of storage in a refrigerator [10,11]; some researchers reported that the nitrite content in spinach increased about 27% after being frozen for 6 months at -25°C while no significant change in nitrate content [12]. However, other researchers reported that the nitrate content in canned vegetable- based infant foods increased 15% and 29% after 48 hours of refrigerated storage and room temperature storage respectively, while no significant change in nitrite content was observed [13]; it was also reported that both nitrate and nitrite contents in lettuce increased significantly during storage of 48 hours at refrigerated and room temperature [14]. Tamme et al. reported that the nitrite concentrations in raw home-made carrot juice, beetroot juice, and radish juice increased 1870, 275, and 518 fold respectively after 48 hours of storage at room temperature, while the nitrate concentrations in these juices decreased significantly [15]. The increase in nitrite concentration and decrease in nitrate concentration in these juices were much smaller after 48 hours of storage at refrigerated temperature. The study also showed no significant increase in nitrite concentration in raw home-made cabbage juice after 48 hours of storage at room temperature or refrigerated temperature [15].

We hypothesize that cabbage may contain some compounds that can resist the degradation of nitrate to nitrite. The aim of this study was to investigate if home-made cabbage juice can inhibit the formation of nitrite and the degradation of nitrate in other home-made vegetable juice during storage. If our hypothesis is correct it will provide useful information in food or vegetable juice storage procedure, since nitrate from vegetables has health benefit while nitrite has potential harmful effect to human, the formation of nitrite in vegetables during storage should be avoid.

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Materials and Methods

All vegetables were purchased from a local supermarket. All chemicals were purchased from Sigma- Aldrich Chemical Company (St. Louis, MO, USA) unless otherwise indicated.

Sample preparation

For spinach (*Spinacia oleracea*), iceberg lettuce (*Lactuca sativa*), green cabbage (*Brassica oleracea var. capitate*), and red cabbage or purple cabbage (*Brassica oleracea var. capitata f. rubra*), all edible parts were used in preparing the juice; for celery (*Apium graveolens*), only edible stalks were used in preparing the juice. Eighty grams of any of the above fresh vegetables were weighed and washed with de-ionized water (DI H_2O) and then homogenized in a blender with 200 ml DI H_2O. The homogenized mixture was then filtered through cheesecloth to remove the solid residue and the juice volume was measured and recorded. All sample juices were immediately stored at different temperature conditions in dark before analysis.

Electrochemical measurement of nitrate

A nitrate ion selective electrode (OAKTON Instruments, Inc., Vernon Hills, IL) was used to determine the nitrate concentration in the vegetable samples using the standard addition method. Since very high concentrations of nitrate were present in all tested vegetables, sample juices were diluted 100 times with DI H_2O before the determination. Briefly, 1.0 ml vegetable juice was added to 99.0 ml DI H_2O while stirring and then the electric potential was measured. Next, a series of small volumes of standard solution of 0.1 M $NaNO_3$ was added to the diluted juice and the electric potential was measured after each addition of standard nitrate solution. After a series of electric potential measurements, the nitrate concentration initially present in the diluted juice was calculated by fitting the series of electric potentials measured and the corresponding standard nitrate concentrations added with the Nernst equation: $E = E^0 - S^{\cdot}\log(X + X^0)$, where X^0 is the nitrate concentration initially present in the diluted juice (μM), X is the standard nitrate concentration added to the diluted juice (μM), E^0 is the reference electric potential which is a constant with a given electrode, E is the electric potential measured (mV), and S is the electrode slope (~ 59 mV at 25°C). The nitrate concentrations in vegetable juices or juice mixtures measured were then converted from μM to $mg.l^{-1}$. The nitrate concentrations in the same type of vegetables or vegetable juices were reported from five different samples (Mean ± SD, n=5).

Griess assay of nitrite

A well-known and widely used method, the Griess assay, was used to determine the nitrite concentration. Briefly, 1.0 ml of a 1% sulfanilamide solution (in 5% H_3PO_4) was added to 1.0 ml of the vegetable juice that had been previously centrifuged and the solid residue was completely removed. This solution was incubated at room temperature in the dark for 10 min and then 1.0 ml of a 0.1% N-1-napthylethylenediamine (NED) solution (in DI H_2O) was added and the mixture solution was again incubated at room temperature in the dark for another 10 min. The absorbance was then measured at 540 nm using a spectrophotometer (Shimadzu PharmaSpec UV-1700). The nitrite concentration (μM) was obtained by comparing the absorbance to a calibration curve. A calibration curve was constructed prior to the measurement of nitrite in the vegetable samples. A blank test using 1.0 ml DI H_2O instead of 1.0 ml vegetable juice with everything else same was used to zero the spectrophotometer. A background test was done by adding 1.0 ml DI H_2O instead of 1.0 ml of 0.1% NED solution with everything else same, the absorbance from this background was subtracted from absorbance of each vegetable samples. The nitrite concentrations in vegetable juices or juice mixtures measured were then converted from μM to $mg.l^{-1}$. The nitrite concentrations in the same type of vegetables or vegetable juices were reported from five different samples (Mean ± SD, n=5).

Chemiluminescence measurement of nitrite

The chemiluminescence method is the most sensitive and commonly used method in measuring nitric oxide (NO) and its various derivatives including nitrite and nitrate. An aliquot (5 μl) of the vegetable juice was injected into the reaction vessel of a Sievers 280i Nitric Oxide Analyzer chemiluminescence detector. This apparatus directly detects NO and can be used for nitrite and nitrate detection under conditions where these species are converted to NO. For nitrite analysis, the reaction vessel contained 1% w/v KI in glacial acetic acid to reduce nitrite to NO. The concentration of nitrite was determined on the basis of a calibration curve [16]. The nitrite concentrations in the same type of vegetables or vegetable juices were reported from five different samples (Mean ± SD, n=5).

Nitrite and nitrate contents change during storage

For the cold storage experiments (4 ± 1°C) in vegetable juices, the nitrate and nitrite concentrations were determined every two days (48 hours) for up to eight days starting from day zero when the juices were prepared. For the ambient temperature storage experiment (25 ± 1°C) in vegetable juices, the nitrate and nitrite concentrations were determined every 24 hours for up to 72 hours (three days). For the cold storage experiment in whole fresh spinach, iceberg lettuce, celery, and cabbage, nitrate and nitrite concentrations were determined on the day when the vegetable were purchased (day zero) and day eight. For the ambient temperature storage experiment in whole fresh vegetables, nitrate and nitrite concentrations were measured every day over three days.

Cabbage juice inhibits the degradation of nitrate and the formation of nitrite in other vegetable juices

To investigate if cabbage juice can inhibit the degradation of nitrate and the formation of nitrite in other home-made vegetable juices (spinach, iceberg lettuce, or celery) during storage, 50 ml of cabbage juice was mixed with 50 ml of the other home-made vegetable juices (spinach, iceberg lettuce, or celery), in the volume ratio of 1:1 or 50% of cabbage juice. Nitrate and nitrite concentrations were then measured every 48 hours for up to eight days starting from day zero when the juices were prepared and mixed during cold storage in dark at 4°C. Each of these home-made vegetable juices (spinach, iceberg lettuce, or celery) were mixed with DI H_2O instead of cabbage juice in the same volume ratio was set as a control sample. In a similar manner as was done for storage at 4°C, we studied if cabbage can inhibit the formation of nitrite in other home-made vegetable juices during storage at ambient temperature (25°C). In order to determine the minimum concentration of cabbage juice needed to inhibit the formation of nitrite and degradation of nitrate in other home-made vegetable juices, home-made green or red cabbage juices were mixed with home-made celery juice at volume ratios of 1:9, 1:5.7, 1:4, 1:3, and 1:1, or 10%, 15%, 20%, 25%, 50% of cabbage juice respectively; the nitrite concentrations were then measured every 48 hours for up to eight days during cold storage.

Experiments of sterilization and inhibitor

To study the mechanism of cabbage juice inhibiting the degradation of nitrate and the formation of nitrite in other home-made vegetable juice, cabbage juice was boiled at 100°C for five minutes before the incubation with other vegetable juice; the results were compared with those results from the incubation of other vegetable juice mixed with the cabbage juice without boiling. In inhibitor experiment, celery juice was mixed with 1mM sodium tungstate (from a stock solution of 100 mM in DI H_2O), an inhibitor of nitrate reductase enzyme (NR), and stored in a refrigerator at 4°C; celery juice itself was set as a control and store under the same condition. Nitrate and nitrite concentrations were measured in the same manner as described above.

Statistical analysis

All results were obtained from five replications with different samples of the same type of vegetable. The data were analyzed using MS Excel 2010 software (Microsoft Corporation, Redmond, WA, USA). A t-test was used to reveal the statistical significance of differences in measured variables at different time points. Results with $p<0.05$ were considered to be statistically significant.

Results and Discussion

Nitrate contents in fresh spinach, iceberg lettuce, celery, green cabbage, and red cabbage on the day purchased were 2.65 ± 0.34 $mg.g^{-1}$, 1.54 ± 0.23 $mg.g^{-1}$, 2.14 ± 1.18 $mg.g^{-1}$, 0.54 ± 0.28 $mg.g^{-1}$, and 0.38 ± 0.29 $mg.g^{-1}$ respectively. Previous reported nitrate contents in fresh spinach, iceberg lettuce, celery, and cabbage varied in a wide range of 0.029 ~ 8.00 $mg.g^{-1}$, 0.079 ~ 4.38 $mg.g^{-1}$, 0.046 ~ 2.67 $mg.g^{-1}$, and 0.008 ~ 0.929 $mg.g^{-1}$ respectively [2,12,17-25]. Nitrite contents were less than 0.1 $mg.kg^{-1}$in all tested fresh vegetables. Previous reported nitrite contents in these vegetables were much lower than that of nitrate and varied in a wide range of 0.01- 200 $mg.kg^{-1}$ [2,12,17,19,20,23-25].

No significant changes in the nitrate and nitrite contents were observed in all fresh vegetables tested during cold storage at 4°C over eight days (data not shown). However, nitrite concentrations increased significantly ($p<0.05$) in home-made spinach juice, iceberg lettuce juice, and celery juice after only two days of cold storage at 4°C. Table 1 shows that the nitrite concentrations in home-made spinach juice, iceberg lettuce juice, and celery juice increased more than 3860 fold (from less than 0.023 $mg.l^{-1}$ to 88.79 $mg.l^{-1}$), 1032 fold (from less than 0.023 $mg.l^{-1}$ to 23.75 $mg.l^{-1}$), and 1845 fold (from less than 0.023 $mg.l^{-1}$ to 42.45 $mg.l^{-1}$) respectively after eight days of cold storage at 4°C; while no significant change in nitrite concentration was observed in home-made green cabbage juice and red cabbage juice during the cold storage over eight days. Interestingly, we found that no significant change in nitrite concentration in mixed juice of green cabbage with spinach, iceberg lettuce, or celery (at the volume ratio of 1:1 or 50% cabbage juice) during eight days of cold storage; the same results were observed in red cabbage juice mixed with any of these vegetable juices during cold storage. However, if green cabbage juice or red cabbage juice was boiled for five minutes and brought to room temperature before mixing with these vegetable juices, the inhibitory effect on the formation of nitrite in these vegetable juices disappeared.

That cabbage juice inhibits nitrite formation in other vegetable juices during cold storage at 4°C was also confirmed by the chemiluminescence method. Figure 1 shows very strong nitrite chemiluminescence signals in celery juice after six days of cold storage, while nitrite signals in the mixed juices of celery and green cabbage (at the volume ratio of 1:1 or 50% cabbage juice) were very weak indicating that green cabbage inhibits nitrite formation in celery juice. The very weak nitrite signals most likely came from cabbage itself as it contains a little nitrite. Figure 1 also shows that red cabbage can inhibit nitrite formation completely in celery juice. Similar to the results in Table 1 using the Griess assay, the chemiluminescence method confirmed that boiled green cabbage juice or red cabbage juice lost their ability in inhibiting nitrite formation in celery juice. This inhibitory effect on nitrite formation in other vegetable juices can be observed at concentrations of green or red cabbage juice as low as 10 ~ 15% (volume ratio of 1:5.7 to 1:9, Table 2).

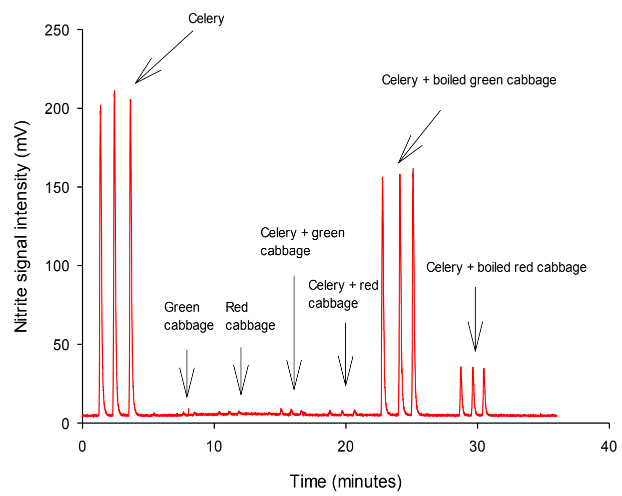

Figure 1: Cabbage inhibits the formation of nitrite in celery juice during storage at 4°C (at volume ratio of 1:1, or 50% cabbage juice). Nitrite concentrations in various vegetable juices or juice mixtures were measured by the chemiluminescence method on day 6 during storage. Each sample analysis was carried out in three injections.

Nitrate concentrations decreased 61.6% (from 343.1 $mg.l^{-1}$ to 131.7 $mg.l^{-1}$), 60.5% (from 203.5 $mg.l^{-1}$ to 80.3 $mg.l^{-1}$), and 34.1% (from 591.4 $mg.l^{-1}$ to 390.0 $mg.l^{-1}$) in home-made spinach juice, iceberg lettuce juice, and celery juice respectively after eight days of cold storage at 4°C (Table 3); while no significant change in nitrate concentration in green or red cabbage juice was observed during eight days of cold storage (Table 3). Green and red cabbage can completely inhibit the degradation of nitrate in home-made spinach juice, iceberg lettuce juice, and celery juice at a volume ratio of 1:1 (or 50% cabbage juice) during cold storage over eight days (Table 3). Again, if green cabbage juice or red cabbage juice was boiled for five minutes and brought to room temperature before mixing with these vegetable juices, no inhibitory effect on the degradation of nitrate in these vegetable juices was observed (Table 3).

The increases in nitrite concentrations in home-made iceberg lettuce juice and celery juice at ambient temperature (25°C) are much faster than that at 4°C. Nitrite concentrations increased more than 1215 fold (from less than 0.023 $mg.l^{-1}$ to 27.98 $mg.l^{-1}$) and 565 fold (from less than 0.023 $mg.l^{-1}$ to 13.04 $mg.l^{-1}$) in home-made iceberg lettuce juice and celery juice respectively after only one day (24 hours) storage at 25°C (Table 4). Again, no significant change in nitrite concentration was observed in green cabbage juice over three days of storage at 25°C. Furthermore, green cabbage juice can inhibit nitrite formation in home-made iceberg lettuce juice and celery juice over two days (at a volume ratio of 1:1) of storage at 25°C (Table 4).

It is well known that nitrate in plants can be reduced to nitrite by nitrate reductase (NR) [26-29]. Spinach and lettuce contain NR [30-33]. The above results suggest that NR initially present in spinach, iceberg lettuce, and celery may be responsible for the reduction of nitrate to nitrite. Our experimental results showed that the nitrate concentration

Vegetable juice	Nitrite concentration (mg.l^{-1})[a]				
	Day 0	Day 2	Day 4	Day 6	Day 8
Spinach juice+ DI H_2O (1:1)[b]	<0.023	1.87[d]	33.37	49.79	88.79
Iceberg juice+ DI H_2O (1:1)[b]	<0.023	0.13[d]	1.83	5.87	23.75
Celery juice+ DI H_2O (1:1)[b]	<0.023	0.19[d]	1.38	10.05	42.45
Green cabbage juice + DI H_2O (1:1)[b]	<0.023	<0.023	<0.023	<0.023	<0.023
Red cabbage juice + DI H_2O (1:1)[b]	<0.023	<0.023	<0.023	<0.023	<0.023
Boiled spinach juice[c]	<0.023	<0.023	<0.023	<0.023	0.37
Boiled iceberg juice[c]	<0.023	<0.023	<0.023	<0.023	<0.023
Boiled celery juice[c]	<0.023	<0.023	<0.023	<0.023	<0.023
Spinach juice + green cabbage juice (1:1)	<0.023	<0.023	<0.023	<0.023	0.33
Iceberg juice + green cabbage juice (1:1)	<0.023	<0.023	<0.023	<0.023	<0.023
Celery juice + green cabbage juice (1:1)	<0.023	<0.023	<0.023	<0.023	<0.023
Celery juice + red cabbage juice (1:1)	<0.023	<0.023	<0.023	<0.023	0.69
Spinach juice + boiled green cabbage juice (1:1)	<0.023	0.63[d]	20.91	59.94	90.85
Iceberg juice + boiled green cabbage juice (1:1)	<0.023	0.33[d]	2.26	7.26	25.89
Celery juice + boiled green cabbage juice (1:1)	<0.023	0.15[d]	2.62	11.82	44.08
Celery juice + boiled red cabbage juice (1:1)	<0.023	0.13[d]	2.37	12.83	45.52

Note: [a]Nitrite concentrations were measured by the Griess assay, detection limit was 0.023 mg.l^{-1}.
[b]Vegetable juice mixed with DI H_2O (volume ratio 1:1) was set as a control sample.
[c]Vegetable juice was boiled for five minutes and cooled to room temperature before storage at 4°C was set as another control sample.
[d]Nitrite concentration increased significantly in two days, $p < 0.05$.

Table 1: Changes of mean nitrite concentration in home-made vegetable juices and juice mixtures during storage at 4 ± 1°C (n=5).

Vegetable juice	Nitrite concentration (mg.l^{-1})[a]				
	Day 0	Day 2	Day 4	Day 6	Day 8
Celery+Green Cabbage (1:1)	<0.023	<0.023	<0.023	0.035	0.035
Celery+DI H_2O (1:1)[b]	<0.023	0.19[c]	1.38[d]	10.05[d]	42.45[d]
Celery+Green Cabbage (3:1)	<0.023	<0.023	<0.023	0.036	0.03
Celery+DI H_2O (3:1)[b]	<0.023	0.033[c]	0. 44[d]	38.08[d]	89.71[d]
Celery+Green Cabbage (4:1)	<0.023	<0.023	<0.023	0.043	0.33
Celery+DI H_2O (4:1)[b]	<0.023[c]	0.045[c]	0. 82[d]	44.61[d]	97.03[d]
Celery+Green Cabbage (5.7:1)	<0.023	<0.023	0.026	0.052	0.41
Celery+DI H_2O (5.7:1)[b]	<0.023[c]	0.067[c]	0.89[d]	45.23[d]	99.35[d]
Celery+Green Cabbage (9:1)	<0.023	<0.023	0.033	0.979	16.59
Celery+DI H_2O (9:1)[b]	<0.023[c]	0.12[c]	0.89[d]	48.36[d]	107.52[d]

Note: [a]Nitrite concentrations were measured by the Griess assay, detection limit was 0.023 mg.l^{-1}.
[b]Celery juice mixed with DI H_2O at the same volume ratio as it mixed with green cabbage juice was set as the control sample.
[c]Nitrite concentration in control celery juices increased significantly in two days, $p<0.05$.
[d]Nitrite concentration in control celery juices was significantly higher than it in the mixed juice of celery with green cabbage on the same day, $p<0.05$.

Table 2: The effect of the concentration of green cabbage juice on inhibiting nitrite formation in celery juice during storage at 4 ± 1 °C (n=5)

decreased with time in spinach juice, iceberg lettuce juice, and celery juice (Table 3), while the nitrite concentration increased with time during storage in a refrigerator at 4°C (Table 1), suggesting a conversion from nitrate to nitrite. When enzymes were deactivated by boiling for five minutes, no changes in nitrate concentration or nitrite concentration were observed during the storage (Tables 1 and 3). Our experimental results from these boiled vegetable juices suggest that NR may be responsible for the reduction of nitrate to nitrite during the storage, since boiling for five minutes deactivated only the enzyme initially present in the vegetables. If exogenous bacteria such as bacteria from the air were responsible for the nitrate reduction, we should observe some nitrite formation in these boiled vegetable juices as bacteria can enter juices during the eight days of storage. The experimental results of tungstate, an inhibitor of NR, inhibiting nitrite formation in celery juice during storage at 4°C confirmed that NR was responsible for nitrite formation in celery juice during storage (Table 5). However, these results cannot exclude the role of bacteria that were initially present in these vegetables as they were deactivated as well by boiling. Recent report from Watanabe's lab showed the evidence of microbial NR involved in the formation of nitrite in spinach leaves during storage [8].

Our experimental results showing no significant increase in nitrite concentration in cabbage juices during cold storage are similar to a report from Tamme's lab except that their storage period was 48 h [15]. Our finding that no significant change in nitrate and nitrite contents in all fresh vegetables tested during cold storage at 4°C over eight days is consistent with the suggestion of nitrite accumulation merely occurs in intact fresh vegetables but only occurs in physically damaged vegetables as discussed by Phillips [34]. More interestingly, we discovered that cabbage (green or red) can inhibit the formation of nitrite and the degradation of nitrate in other vegetable juices during storage. This inhibitory effect can be observed at cabbage juice concentrations as low as 10 ~ 15%. The mechanism of cabbage juice inhibiting the formation of nitrite and degradation of nitrate in other vegetable juices during storage is not clear at present. It is possible that two components were

Vegetable juice	Nitrate concentration (mg.l^{-1})[a]				
	Day 0	Day 2	Day 4	Day 6	Day 8
Spinach juice+ DI H_2O (1:1)[b]	343.1	338	281	208.9	131.7
Iceberg juice+ DI H_2O (1:1)[b]	203.5	197.5	187.2	128.8	80.3
Celery juice+ DI H_2O (1:1)[b]	591.4	568.3	525.5	468.5	390
Green cabbage juice + DI H_2O (1:1)[b]	129.8	132.7	133.3	126.6	130
Red cabbage juice + DI H_2O (1:1)[b]	86.7	85.6	84.9	84.3	84.6
Boiled spinach juice[c]	664.3	651.5	655.5	643.5	615.7
Boiled iceberg juice[c]	410.2	420.1	425.5	414.1	403.6
Boiled celery juice[c]	1265	1267	1199	1189	1156
Spinach juice + green cabbage juice (1:1)	488.4	490.7	491.5	488.9	470.6
Iceberg juice + green cabbage juice (1:1)	331.7	333.6	334. 9	336.9	335.7
Celery juice + green cabbage juice (1:1)	710.5	708.6	720.4	702.7	710.5
Celery juice + red cabbage juice (1:1)	668.4	667.8	666.7	659.1	655.3
Spinach juice + boiled green cabbage juice (1:1)[d]	495.3	479.9	436.9	326.1	250.4
Iceberg juice + boiled green cabbage juice (1:1)[d]	340.4	331.1	312.5	243.6	218.3
Celery juice + boiled green cabbage juice (1:1)[d]	733.2	719.6	677.7	567.3	390.1
Celery juice + boiled red cabbage juice (1:1)[d]	691	687.2	673.4	630.7	504.3

Note: [a]Nitrate concentrations were measured by nitrate ion selective electrode (ISE).
[b]Vegetable juice mixed with DI H_2O (volume ratio 1:1) was set as a control sample.
[c]Vegetable juice boiled for five minutes and cooled to room temperature before storing in a refrigerator was set as another control sample.
[d]Vegetable juice mixed with the boiled cabbage juice (volume ratio 1:1) was set as another control sample.

Table 3: Changes of mean nitrate concentration in home-made vegetable juices and juice mixtures during storage at 4 ± 1°C (n=5).

Vegetable juice	Nitrite concentration (mg.l^{-1})[a]			
	0 hr	24 hr	48 hr	72 hr
Iceberg juice + DI H_2O (1:1)[b]	<0.023	27.98[d]	61.55[e]	47.21
Celery juice + DI H_2O (1:1)[b]	<0.023	13.04[d]	26.52[e]	44.31
Green cabbage juice + DI H_2O (1:1)[b]	<0.023	<0.023	<0.023	0.55
Boiled iceberg juice[c]	<0.023	<0.023	<0.023	2.3
Boiled celery juice[c]	<0.023	<0.023	0.11	45.2
Iceberg juice + green cabbage (1:1)	<0.023	<0.023	4.50[e]	10.3
Celery juice + green cabbage (1:1)	<0.023	<0.023	3.33[e]	22.13

Note: [a]Nitrite concentrations were measured by the Griess assay, detection limit was 0.023 mg.l^{-1}
[b]Vegetable juice mixed with DI H_2O (volume ratio 1:1) was set as a control sample.
[c]Vegetable juice boiled for five minutes and cooled to room temperature before storage was set as another control sample.
[d]Nitrite concentration in iceberg juice and celery juice that mixed with DI H_2O (volume ratio 1:1) increased significantly in 24 hours (p<0.05).
[e]Nitrite concentrations in iceberg juice and celery juice that mixed with DI H_2O were significantly higher than that in iceberg juice and celery juice that mixed with green cabbage juice after 48 hours of storage (p<0.05).

Table 4: Changes of mean nitrite concentrations in home-made vegetable juices and juice mixtures during storage at 25 ± 1°C (n=5).

Vegetable juice	Nitrite concentration (mg.l^{-1})[a]				
	Day 0	Day 2	Day 4	Day 6	Day 8
Celery juice + DI H_2O (1:1, v/v)[b]	<0.023	0.19[c]	1.38	10.05	42.45
Celery juice + DI H_2O (1:1, v/v) + 1mM Na_2WO_4	<0.023	<0.023	<0.023	<0.023	<0.023
Celery juice + green cabbage juice (1:1, v/v)	<0.023	<0.023	<0.023	<0.023	<0.023

Note: [a]Nitrite concentrations were measured by the Griess assay, detection limit was 0.023 mg.l^{-1}.
[b]Celery juice mixed with DI H_2O (volume ratio 1:1) was set as a control sample.
[c]Nitrite concentration in celery juice mixed with DI H_2O increased significantly in two days, p<0.05.

Table 5: The effect of nitrate reductase inhibitor (1 mM Na_2WO_4) on the formation of nitrite in home-made celery juices during storage at 4 ± 1°C (n=5).

involved in the inhibitory effect. It appears that a component contained in green cabbage lost its inhibitory effect upon boiling. On the other hand, another component contained in red cabbage retained some activity even after the treatment as Figure 1 shows. These results suggest that one component is heat sensitive and the other one in red cabbage is heat resistant. Cabbage contains large amount of glucosinolates which can be hydrolyzed to volatile sulfur compounds such as isothiocyanates [35]. These molecules are known as bioactive compounds that show multiple functions such as antimicrobial activity [36]. Red cabbage is rich in a number of bioactive compounds including anthocyanins which also exhibits multiple functions such as antioxidant activity, enzyme inhibition, and antimicrobial activity [37]. It is also possible that plant 14-3-3 proteins involved in the inactivation of NR [38,39]. We will further study the mechanism of cabbage juice inhibiting the formation of nitrite and the degradation of nitrate in other vegetable juices in the future. To the extent that the reduction of nitrate to nitrite during storage has potential harmful effects on health, our finding that cabbage juice inhibits the formation of nitrite in other vegetable juices may provide useful information for storage procedures.

Conclusion

Cabbage can completely inhibit the increasing in nitrite and decreasing in nitrate contents in other home-made vegetable juices (spinach, iceberg lettuce, and celery) during the cold storage at 4°C over eight days or ambient temperature storage over two days. The lowest concentration (volume ratio) that can inhibit nitrite formation in other home-made vegetable juices is about 10 ~ 15% of cabbage juice (or volume ratio 1:5.7 to 1:9). However, cabbage juice lost its inhibitory effect upon boiling for five minutes prior to mix with other home-made vegetable juices.

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