

Original article

A practical and inexpensive alginate-based tool for semiquantitative detection of vitamin C in juices and beverages

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Abstract

Background: Vitamin C is an essential nutrient for maintaining good health. However, vitamin C cannot be synthesized in the human body, and its bioavailability depends solely on dietary intake. In Thailand, 9.9% of adults are deficient in vitamin C. Therefore, development of a simple and user-friendly test kit for determining vitamin C levels in foods and beverages is needed.

Objectives: This study aimed to develop a rapid, practical, and low-cost semiquantitative test kit for measuring vitamin C content in juices and commercial beverages.

Methods: Calcium alginate beads loaded with a starch–iodine complex (blue-colored beads) were used for vitamin C detection. In the presence of vitamin C, iodine molecules in the beads were reduced to iodide ions, and the blue beads became colorless. The reaction rate was proportional to the vitamin C concentration. A total of 88 juice and beverage samples were analyzed, and vitamin C contents in those samples were categorized into four levels: very high, high, medium, and low.

Results: Vitamin C levels were very high in juices from Indian gooseberry, Chinese jujube, red guava, chili, and bell pepper; high in strawberry and guava juices; medium in kiwi and lemon juices; and low in orange and melon juices. Among commercial beverages, vitamin C levels were high in fortified products but low in diluted fruit/vegetable juices and functional drinks. Importantly, the entire testing procedure required only 5 minutes.

Conclusion: A quick, practical, and inexpensive test kit for semiquantitative detection of vitamin C in juices and commercial beverages was successfully developed. This innovative tool could be beneficial for health-conscious consumers who want to measure the content of vitamin C in their foods.

Keywords: Beverage, juice, test kit, vitamin C, z-thru, simple.

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Almost a century ago, vitamin C or ascorbic acid, one of the most essential vitamins for humans, was discovered. In 1973, Norman Haworth received the Nobel prize for chemistry for the discovery of vitamin C molecular structure, and Albert von Szent-Györgyi Nagyrápolt received the Nobel prize for physiology or medicine for the elucidation of the biological functions of ascorbic acid. ⁽¹⁾ Vitamin C plays vital roles in maintaining good health. ⁽²⁾ It is required for collagen biosynthesis, acts as antioxidants, improves iron absorption and maintains a proper immune function. ^(3, 4) Evidently, vitamin C is effective for prophylaxis and reducing the severity of common cold. ^(5, 6) In humans, vitamin C cannot be synthesized endogenously. Bioavailability of vitamin C in the body is exclusively dependent on diet consumption. The primary dietary sources of vitamin C are fruits and vegetables, especially citrus fruits, bell peppers and berries, while vitamin C contents in animal sources are low. ^(7 - 9)

In Thailand, vitamin C deficiency is reported at 9.9% in adults. ⁽¹⁰⁾ The recommended dietary allowance (RDA), an average daily level of intake to sufficiently meet the nutrient requirement of healthy individuals, for vitamin C varies by age and gender. In adult males and females, the RDA values for vitamin C are 90 and 75 mg/day, respectively. ⁽¹¹⁾ Although vitamin C is relatively safe even at high dose consumption, some individuals may experience mild adverse effects of taking too much vitamin C, mostly related to digestive discomfort. Thus, the tolerable upper intake level (UL) of vitamin C, which indicates the highest level of regular daily intake of vitamin C that produces no adverse effects to most people, is established at 2000 mg/day in adults. ⁽¹²⁾ To be able to monitor and control daily intake of vitamin C, it is important to know the levels of vitamin C in foods and beverages. This would allow individuals to select food items to consume to meet the RDA and not to exceed the UL for vitamin C consumption. Hitherto, several methods have been reported for determination of vitamin C in food samples, but the simple and widely used one is still a method relied on redox titration with iodine using starch as an indicator. ⁽¹³⁾

In this study, we aimed to develop a quick and practical test kit for semiquantitative visual detection of ascorbic acid in juices and beverages using the blue-colored alginate beads containing starch-iodine complex. Fundamentally, the blue starch-iodine complex is formed through the trapping of iodine molecules inside the helix structure of amylose.

Ascorbic acid is a strong reducing agent that readily donates electrons. In the presence of ascorbic acid iodine (I_2) was reduced to iodide ions (I^-), resulting in less available iodine molecules to react with starch. Therefore, the blue color of the beads is bleached and becomes colorless. The rate of color disappearance is semiquantitatively proportional to the amount of vitamin C existed in samples. After establishing the testing procedure, we measured vitamin C levels in 88 samples of fresh juices (from fruits and vegetables) and commercial beverages. The vitamin C levels in samples were categorized into 4 groups, i.e., very high (equivalent to > 1000 mg/100 g), high (equivalent to 150 - 1000 mg/100 g), medium (equivalent to 50 - 150 mg/100 g) and low (equivalent to < 50 mg/100 g).

Materials and methods

Chemicals

Sodium alginate was purchased from TCI America (Tokyo, Japan). Calcium chloride and iodine were products from Merck Millipore (MA, USA). Starch was purchased from J.T. Baker Chemical (NJ, USA).

Four levels of standard ascorbic acid ($C_6H_8O_6$, Loba Chemie, India) solutions were prepared including 1000 mg/100 g, 150 mg/100 g, 50 mg/100 g and 15 mg/100 g. These levels were interpreted as very high, high, medium and low vitamin C contents, respectively.

Fabrication of blue-colored calcium alginate beads (loaded with starch-iodine complex)

Stock starch solution (1%, w/v) was prepared in distilled water, and diluted to working starch solution (0.6%, w/v). Sodium alginate (2.0%, w/v) and iodine (0.6%, w/v) were also prepared in distilled water. A starch-iodine complex was produced by mixing 100 mL of 1.0% (w/v) starch solution with 5 mL of 0.6% (w/v) iodine solution (a ratio of 20:1). Then, 100 mL of starch-iodine complex was added to 100 mL of 2.0% (w/v) sodium alginate solution (a ratio of 1:1) and mixed well. The mixture of sodium alginate and starch-iodine complex was slowly and constantly dropped into a beaker containing 200 mL of 5% (w/v) calcium chloride solution using a 1-mL syringe with 26G needle. Blue-colored calcium alginate beads with average diameter of 2.09 ± 0.18 mm were obtained by electronic digital caliper. The obtained beads were suspended in the calcium chloride solution for 5 min, then washed 3 times with distilled water (**Figure 1**). After washing, these blue-colored calcium alginate beads were ready for vitamin C testing.

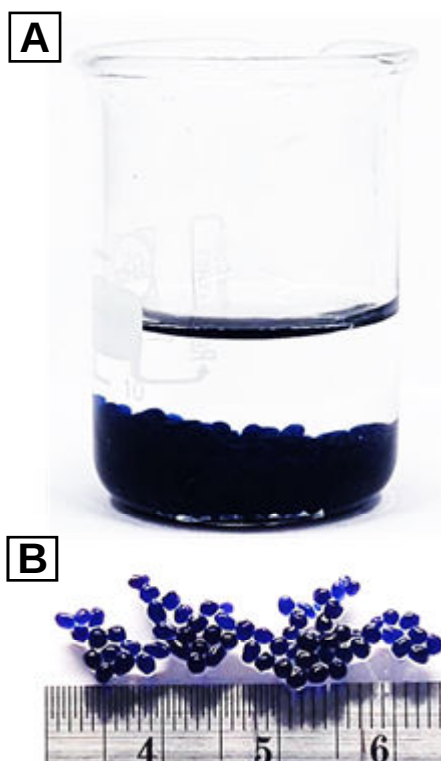


Figure 1. The representative image of fabricated calcium alginate beads containing starch-iodine complex. **A)** The fabricated, blue-colored beads suspended in distilled water; **B)** Size (2.1 ± 0.2 mm), shape (rounded) and color (deep blue) of the fabricated beads. Ruler scales are in cm.

Testing vitamin C in juice and beverages samples (n = 88)

Edible parts of fruits and vegetables (100 g) were chopped, blended and squeezed to obtain fresh juice samples. Each freshly prepared juice (1 mL) was immediately analyzed for vitamin C content using our newly established test kit. Commercial beverage and functional drink samples were purchased from the local convenience stores, e.g., 7-11, Big-C and Lotus's.

In vitamin C testing, blue-colored beads (0.3 g) were placed into the 1.5-mL microcentrifuge tubes. Juice and beverage samples (1 mL) were added and mixed thoroughly. The count-up timer started immediately after adding samples and stopped when blue color disappeared. Total time required for vanishing the color of beads (from blue to colorless) was recorded (**Figure 2**). Samples that could make blue color disappeared within 2 min were reported as

very high vitamin C content (equivalent to > 1000 mg/100 g). Samples with recorded time of 2 - 3 min, 3 - 5 min and > 5 min were reported as high (equivalent to 150-1000 mg/100 g), medium (equivalent to 50-150 mg/100 g) and low (equivalent to 50-150 mg/100 g) vitamin C content, respectively. Each sample was tested twice, and recorded times were averaged.

Statistical analysis

Data of recorded time for each sample were presented as mean $\pm$ standard deviation (SD). Categorical data were presented as frequencies and percentages. Coefficient of variation (CV, %) was calculated from $(SD/mean) \times 100$. Infographics were created by Canva® (canva.com). Statistical analysis was performed by GraphPad Prism version 10 (MA, USA). $P < 0.05$ was considered statistically significant.

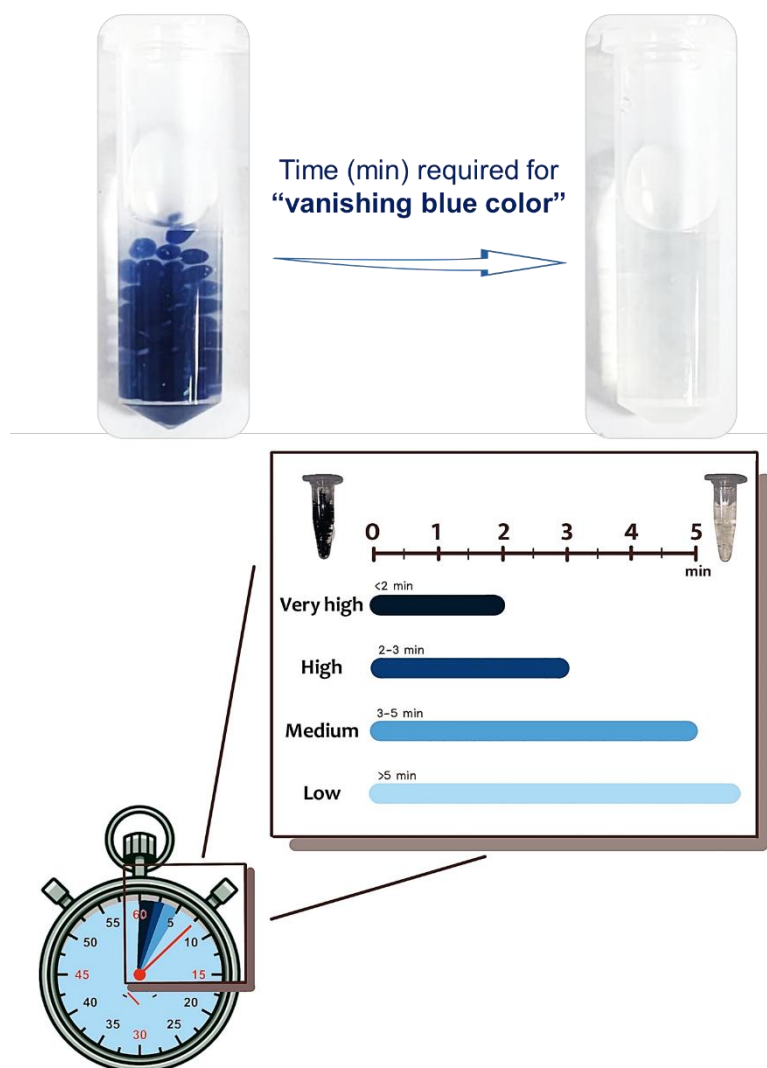


Figure 2. Vitamin C testing and result interpretation. Samples were added to blue-colored beads and mixed well. Time was counted up after adding samples until the blue color completely disappeared. The total time for color vanishing was recorded. Samples with recorded times of < 2, 2 - 3, 3 - 5 and > 5 min were reported as very high, high, medium and low vitamin C contents, respectively.

Results

Time required for color vanishing in vitamin C standards

Four concentrations of vitamin C standards were prepared as mentioned above. Standard concentrations of 1000, 150, 50 and 15 mg/100 g were regarded as very high, high, medium and low vitamin C contents, respectively. We categorized these vitamin C levels (very high, high, medium and low) based on the levels of vitamin C in commercially available vitamin C supplements, guava, lemon and tomato, respectively.

We tested the vitamin C levels in those 4 vitamin C standard solutions using our newly established test kit, at least 17 times for each standard. The times to

completely vanish the blue color of the beads were recorded as shown in **Table 1**. The average time for vanishing beads' color were 1.2 ± 0.1 , 2.3 ± 0.2 , 4.2 ± 0.4 , and > 5 min for very high, high, medium and low vitamin C standards, respectively. The CV (%) of 9.8%, 10.6%, and 10.3% were obtained for very high, high and medium vitamin C standards, respectively. Minimum and maximum recorded times for each standard are also displayed in **Table 1**. We set criteria for reporting and interpreting the vitamin C levels in testing samples according to the results obtained for vitamin C standards, as mentioned in the method section and in **Figure 2**.

Table 1. Vitamin C testing in vitamin C standard samples (n = 4) by the established method showing the recorded times required for completely bleaching the beads' color.

Vitamin C standard/ Recorded time	Very high (1000 mg/100 g)	High (150 mg/100 g)	Medium (50 mg/100 g)	Low (15 mg/100 g)
Mean $\pm$ SD (min)	1.22 $\pm$ 0.12	2.26 $\pm$ 0.24*	4.17 $\pm$ 0.43 [#]	> 5 ^s
Min (min)	1.05	1.43	3.12	nd
Max (min)	1.44	2.58	4.56	nd
Coefficient of variation (CV)	9.84%	10.62%	10.31%	nd

* $P < 0.001$ vs. very high; [#] $P < 0.001$ vs. high; ^s $P < 0.001$ (set value at 6) vs. Medium; nd: not determined.

Table 2. Semiquantitative measurement of vitamin C levels in 39 samples (tested in duplicate) of fresh fruit and vegetable juices using our newly developed method (sorted by recorded time).

No.	Common name	Scientific name	Recorded time (min)	Vitamin C level
1	Chinese jujube	Ziziphus jujube	1.30 $\pm$ 0.08	Very high
2	Indian gooseberry	Phyllanthus emblica	1.25 $\pm$ 0.04	Very high
3	Red guava	Psidium guajava L.	1.32 $\pm$ 0.04	Very high
4	Red chili pepper	Capsicum annuum L. var. acuminatum Fingerh	1.38 $\pm$ 0.01	Very high
5	Red capsicum bell pepper	Capsicum annuum	1.41 $\pm$ 0.35	Very high
6	Yellow bell pepper	Capsicum annuum	1.47 $\pm$ 0.33	Very high
7	Green bell pepper	Capsicum annuum	1.48 $\pm$ 0.35	Very high
8	Common jujube	Ziziphus Mauritians Lam.	2.17 $\pm$ 0.04	High
9	Banana pepper	Capsicum annuum L. var annuum	2.33 $\pm$ 0.12	High
10	Green chili pepper	Capsicum annuum L. var. acuminatum Fingerh	2.46 $\pm$ 0.06	High
11	Guava	Psidium guajava L.	2.40 $\pm$ 0.00	High
12	Strawberry	Fragaria x ananassa Duchesne	2.48 $\pm$ 0.08	High
13	Green Anaheim pepper	Capsicum annuum L. var. acuminatum Fingerh	3.13 $\pm$ 0.09	Medium
14	Papaya	Carica papaya L.	3.26 $\pm$ 0.04	Medium
15	Pomelo	Citrus maxima (Burm.f.) Merr.	3.35 $\pm$ 0.06	Medium
16	Kiwi	Actinidia deliciosa	3.48 $\pm$ 0.04	Medium
17	Red-Green amaranth	Amaranthus tricolor L.	3.50 $\pm$ 0.04	Medium
18	Cape gooseberry	Physalis peruviana	4.29 $\pm$ 0.06	Medium
19	Hog plum	Spondias pinnata (L. f.) Kurz	4.33 $\pm$ 0.06	Medium
20	Lemon	Citrus lemon (L.) Osbeck	4.31 $\pm$ 0.03	Medium
21	Lime	Citrus aurantifolia (Christorante.) Swinngle	4.41 $\pm$ 0.02	Medium
22	Plum tomato	Solanum lycopersicum	4.47 $\pm$ 0.29	Medium
23	Broccoli	Brassica oleracea var. italica	5.11 $\pm$ 0.05	Low
24	Collard	Brassica oleracea var. Alboglabra	5.31 $\pm$ 0.10	Low
25	Pineapple	Ananas comosus (L.) Merr.	5.32 $\pm$ 0.11	Low
26	Orange	Citrus x sinensis	5.33 $\pm$ 0.03	Low
27	Coriander	Coriandrum sati vum L.	6.17 $\pm$ 0.09	Low
28	Cherry	Prunus avium	6.59 $\pm$ 0.23	Low
29	Melon	Cucumis melo L.	7.24 $\pm$ 0.00	Low
30	Roselle (Fresh)	Hibiscus sabdariffa L.	8.48 $\pm$ 0.40	Low

Table 2. (Conts.) Semiquantitative measurement of vitamin C levels in 39 samples (tested in duplicate) of fresh fruit and vegetable juices using our newly developed method (sorted by recorded time).

No.	Common name	Scientific name	Recorded time (min)	Vitamin C level
31	Pomegranate	Punica granatum L.	9.08 ± 0.07	Low
32	Salacca	Salacca zalacca	9.17 ± 0.10	Low
33	Spinach	Amaranthus viridis	9.30 ± 0.20	Low
34	Bilimbi	Averrhoa bilimbi L.	> 10	Low
35	Watermelon	Citrullus lanatus (Thung.) Mats. & Nakai	> 10	Low
36	Persimmon	Diospyros kaki	> 10	Low
37	Roselle (Boiled)	Hibiscus sabdariffa L.	> 10	Low
38	Dragon fruit	Hylocereus undatus (Haw.) Britton & Rose	> 10	Low
39	Blueberry	Vaccinium corymbosum	> 10	Low

Table 3. Semiquantitative measurement of vitamin C levels in 49 samples (tested in duplicate) of commercial beverages and functional drinks using our newly developed method (sorted by recorded time).

No.	Name	Brand	Recorded time (min)	Vitamin C level
1	Vitamin lemon (Vitamin C 1000 mg)	C-vitt	1.12 ± 0.02	Very high
2	Vitamin C 1000 mg lemon	Vit a day	1.21 ± 0.03	Very high
3	Vitamin C 1000 mg Honey lemon flavored	Vit a day	1.56 ± 0.02	Very high
4	Pulpy C-boost lemon (Vitamin C 200.0%)	Minute maid	2.06 ± 0.06	High
5	Grapefruit	One day vitamins	2.23 ± 0.11	High
6	Vitamin C 200.0%	Double C	2.26 ± 0.06	High
7	Vitamin C 200%	Hi!	2.33 ± 0.02	High
8	PIYAVATE Yuzu (Vitamin C 200.0%)	PIYAVATE BETTLE	2.40 ± 0.02	High
9	Curcumin	QminC	2.43 ± 0.03	High
10	Vitamin C	Altapharma	2.44 ± 0.00	High
11	Vitamin Orange Vitamin C 1000 mg	C-Vitt	3.23 ± 0.03	Medium
12	Carrot & Orange	Unif	3.23 ± 0.03	Medium
13	Lime with honey	KUKURIN	3.29 ± 0.02	Medium
14	Ener+G	Handy Herb Ener+G	3.45 ± 0.02	Medium
15	Collagen 5,000 mg Vitamin C 100.0%	Takara collagen	4.16 ± 0.04	Medium
16	Vitamin C Plus	eXta	4.30 ± 0.06	Medium
17	Green tea honey lemon	OISHI	4.30 ± 0.04	Medium
18	Splash High vitamin C	Minute Maid	4.31 ± 0.07	Medium
19	Vitamin C 200.0% (Lemon)	B'lue	4.48 ± 0.02	Low

Table 3. (Conts.) Semiquantitative measurement of vitamin C levels in 49 samples (tested in duplicate) of commercial beverages and functional drinks using our newly developed method (sorted by recorded time).

No.	Name	Brand	Recorded time (min)	Vitamin C level
20	Green Tea	SHIZUOKA	5.34 ± 0.09	Low
21	Guava juice	Siripen	6.10 ± 0.01	Low
22	Sponsor active Lemon lime	Sponsor	6.15 ± 0.02	Low
23	Green vegetable	Unif	6.25 ± 0.02	Low
24	Fresh with purple carrot	Unif	7.79 ± 1.22	Low
25	Collagen Tripeptide 5,000mg	Blink	8.48 ± 0.09	Low
26	Lipton lemon	Lipton	9.00 ± 0.00	Low
27	Fruit punch flavor	Fresh doze	9.17 ± 0.16	Low
28	Blackcurrant	Jelé beautie	9.46 ± 0.16	Low
29	Roselle juice	Siripen (fresh market)	> 10	Low
30	Carrot passion fruit juice	Siripen (fresh market)	> 10	Low
31	Beetroot, carrot, and passion fruit juice	Siripen (fresh market)	> 10	Low
32	Centella asiatica leaf juice	Siripen (fresh market)	> 10	Low
33	Passion fruit juice avocado	Siripen (fresh market)	> 10	Low
34	Orange juice	Fresh market	> 10	Low
35	Sparkling	M-150	> 10	Low
36	Collagen 2000+ C 200.0%	Vit a day	> 10	Low
37	Goji berry	Ready	> 10	Low
38	Honey lemon collagen 2000mg	Man Some	> 10	Low
39	Chakrapad lychee	Dee-Do	> 10	Low
40	Orange juice	Siri Shin	> 10	Low
41	All you need	Unif	> 10	Low
42	Doi Kham Lychee	DOI KHAM	> 10	Low
43	Fresh Delight with lychee juice	Unif	> 10	Low
44	Lemon soda	SINGHA	> 10	Low
45	Kyoho flavour	Fresh Doze	> 10	Low
46	Strawberry flavour	Fresh Doze	> 10	Low
47	Krystal collagen	CHAMÉ	> 10	Low
48	White tea	Puríku	> 10	Low
49	Jasmine pandan drink	if	> 10	Low

Measuring vitamin C levels in fresh juices and commercial beverages

A total of 88 samples were semiquantitatively measured for vitamin C levels using our newly established test kit; 39 samples were fresh fruit and vegetable juices, and 49 samples were commercial

beverages and functional drinks. **Table 2** shows the results of vitamin C testing in 39 fresh fruit and vegetable juice samples, and **Table 3** shows the results obtained from 49 samples of commercial beverages and functional drinks.

Vitamin C levels in fresh fruit and vegetable juices

Very high (>1000mg/100g)

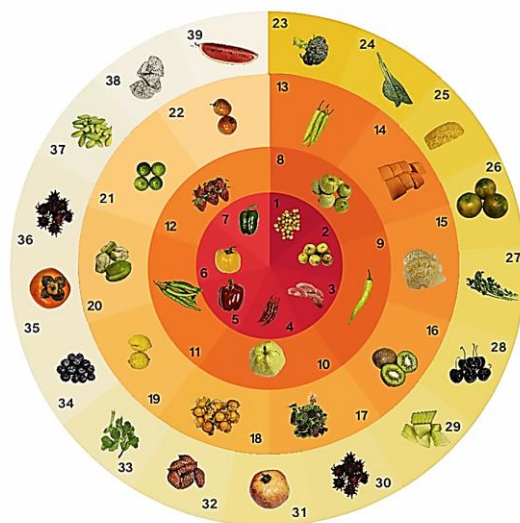
- 1 Indian gooseberry
- 2 Chinese jujube
- 3 Red guava fruit
- 4 Red chilli pepper
- 5 Red capsicum bell pepper
- 6 Yellow bell pepper
- 7 Green bell pepper

High (150-1000mg/100g)

- 8 Common jujube
- 9 Banana pepper
- 10 Guava
- 11 Green chilli pepper
- 12 Strawberry

Medium (50-100mg/100g)

- 13 Green anaheim pepper
- 14 Papaya
- 15 Pomelo
- 16 Kiwi
- 17 Red-Green amaranth
- 18 Cape gooseberry
- 19 Lemon
- 20 Hog plum
- 21 Lime
- 22 Plum tomato



Low (<50mg/100g)

- 23 Broccoli
- 24 Collard
- 25 Pineapple
- 26 Orange
- 27 Coriander
- 28 Cherry
- 29 Melon
- 30 Roselle (Fresh)
- 31 Pomegranate
- 32 Salacca
- 33 Spinach
- 34 Blueberry
- 35 Persimmon
- 36 Roselle (Boil)
- 37 Bilimbi
- 38 Dragon fruit
- 39 Watermelon

Figure 3. Infographic of vitamin C contents in fresh fruit and vegetable juice samples.

Vitamin C levels in commercial beverages and functional drinks

Very high (>1000mg/100g)

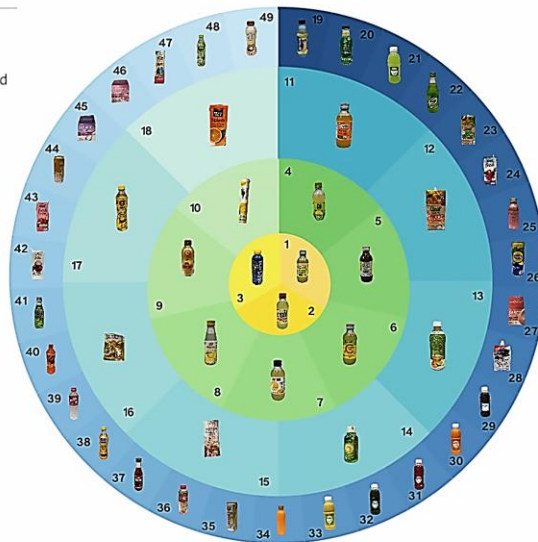
- 1 Vitamin lemon (Vitamin C 1000mg)
- 2 Vitamin C 1000mg lemon
- 3 Vitamin C 1000mg Honey lemon flavored

High (150-1000mg/100g)

- 4 Pulp C-boost lemon (Vitamin C 200%)
- 5 Grape fruit
- 6 Vitamin C 200% Double C
- 7 Vitamin C 200% Hi!
- 8 PIYAVATE Yuzu (Vitamin C 200%)
- 9 Curcumin
- 10 Vitamin C

Medium (50-100mg/100g)

- 11 Vitamin Orange Vitamin C 1000mg
- 12 Carrot & Orange
- 13 Lime with honey
- 14 Ener+G
- 15 Collagen 5,000mg Vitamin C 100%
- 16 Vitamin C Plus
- 17 Green tea honey lemon
- 18 Splash High vitamin C



Low (<50mg/100g)

- 19 Vitamin C 200% (Lemon)
- 20 Green Tea
- 21 Guava juice
- 22 Sponsor active Lemon lime
- 23 Green vegetable
- 24 Fresh with purple carrot
- 25 Collagen Tripeptide 5,000mg
- 26 Lipton lemon
- 27 Fruit Punch flavour
- 28 Blackcurrant
- 29 Roselle juice
- 30 Carrot passion fruit juice
- 31 Beetroot, carrot, and passion fruit juice
- 32 Centella asiatica leaf juice
- 33 Passion fruit juice avocado
- 34 Orange juice Market
- 35 Sparkling
- 36 Collagen 2000+ C 200%
- 37 Goji berry
- 38 Honey lemon collagen 2000mg
- 39 Chakrapad lychee
- 40 Orange juice Siri shin
- 41 All you need
- 42 Doi Kham Lychee
- 43 Fresh Delight with lychee juice
- 44 Lemon soda
- 45 Kyoho flavour
- 46 Strawberry flavour
- 47 Krystal collagen
- 48 White Tea
- 49 Jasmine Pandan Drink

Figure 4. Infographic of vitamin C contents in commercial beverages and functional drinks.

Our findings showed that the fresh fruit and vegetable juices with the very high vitamin C levels were Chinese jujube, Indian gooseberry, red guava, red chili pepper, red capsicum bell pepper, yellow bell pepper, and green bell pepper. For commercial beverages and functional drinks, the very high levels of vitamin C were observed only in 3 samples, i.e., C-vitt Vitamin lemon (Vitamin C 1000 mg), Vit a day Vitamin C 1000 mg (Lemon) and Vit a day Vitamin C 1000 mg (Honey lemon flavored). Orange and pineapple fresh juices were found to have low vitamin C content like cherry, melon, watermelon and blueberry (**Table 2**).

Based on data in the present study, infographics of vitamin C contents in fruit and vegetable juices and commercial beverages were created as shown in **Figure 3** and **Figure 4**. These infographics could help people to select juices and beverages to consume to meet their vitamin C requirements.

Discussion

Ascorbic acid is an essential vitamin for human life. Our body obtains this vitamin primarily through dietary consumption of fruits and vegetables. The RDA for vitamin C is 90 mg for men and 75 mg for women to maintain the normal physiological function, and for women the requirement is even higher in the pregnant and breastfeeding states. Herein, we successfully developed a rapid and simple test kit for measuring vitamin C in juices and beverages. This user-friendly test kit could help individuals to choose appropriate food items to consume to meet their daily vitamin C requirements. The test principle was based on the redox titration with iodine using starch as an indicator. The test results were obtained within 5 min. Four levels of vitamin C could be semiquantitatively measured by the test including very high, high, medium and low, which were equivalent to > 1000, 150 - 1000, 50 - 150 and < 50 mg/100 g, respectively. We called this test a “Z-thru” vitamin C detection test kit.

The gold standard method for determination of vitamin C in food products remains the high-performance liquid chromatography (HPLC) technique due to its advantages of high specificity, sensitivity and accuracy. ^(14 - 16) However, the redox titration with either iodine (iodometric titration) or 2,6-

dichlorophenolindophenol (DCPIP, dye titration) is a simple and classic method for measurement of ascorbic acid that still has been widely used. ⁽¹⁷⁾ The main concern of ascorbic acid measurement by redox titration is the interference by other reducing agents existing in food samples, and that could compromise the accuracy of the test result. Sugars (such as glucose and fructose) and phenolic compounds can slowly reduce iodine that leads to overestimation of vitamin C. ⁽¹⁸⁾ However, a recent study showed that glucose, sucrose and fructose insignificantly interfered with vitamin C detection by redox titration. ⁽¹⁹⁾ Additionally, the traditional redox titration (titrimetric analysis) has been approved as a method of choice for vitamin C analysis in foods by the Association of Official Analytical Chemist (AOAC). Study by Hernández Y, *et al.* showed that vitamin C contents in tropical fruits compared between measurements by AOAC's redox titration and ion-pair liquid chromatography (LC) were not considerably different. ⁽²⁰⁾ They reported that vitamin C contents in orange measured by redox titration and LC were 68.0 ± 6.0 and 75.0 ± 3.0 mg/100 g, respectively. For pineapple, vitamin C levels measured by redox titration and LC were 26.0 ± 2.7 and 26.0 ± 2.3 mg/100 g, respectively. ⁽²¹⁾ Therefore, the development of redox titration-based test kit, as an on-site, quick, simple and cost-effective tool for screening vitamin C levels in foods, is still worthwhile. To serve this purpose, in this study, we developed the Z-thru test kit for a quick screening of vitamin C levels in juices and beverages.

The sophisticated techniques for vitamin C detection have also been established. Magar HS, *et al.* created an electroactive gelatin grafted with poly sulfonamide derivative for electrochemical detection of ascorbic acid in fresh fruit juices. ⁽²¹⁾ Meng H, *et al.* developed a gold nanoclusters-based turn-on fluorescence detection system for ascorbic acid with an excellent limit of detection (capable of detecting ascorbic acid down to 22 nM). ⁽²²⁾ However, a redox titration-based method is still the most practical and cost-effective one. Recently, Kaewchuay N, *et al.* developed an on-site microfluidic paper-based titration device for a rapid semiquantitative detection of vitamin C in beverages using iodine as titrant and starch solution as indicator. ⁽¹⁹⁾ In this device, iodine solution

was laterally flowed to sample, and the endpoint of titration was detected where all vitamin C molecules in the sample were completely oxidized by iodine, and the excess iodine reacted with starch indicator forming blue starch-iodine complex. In contrast to our established vitamin C test kit, instead of detecting the endpoint of titration, our test worked by adding sample into the blue starch-iodine complex (encapsulated inside calcium alginate beads), and then vitamin C in the sample slowly reduced iodine to iodide ions, and the blue-colored beads progressively become colorless. The higher vitamin C concentration the shorter time required for color bleaching. The between-day precision was assessed, and it revealed that our test was relatively precise (%CV around 10.0%) (**Table 1**).

The database on food chemical composition (<http://www.iao.it/bda2008/homepage.aspx>) reported that vitamin C levels in guava, kiwi and melon were 243, 85 and 22 mg/100 g, respectively.⁽²³⁾ These levels corresponded well with the vitamin C levels measured by our newly established test kit (Z-thru). Our data demonstrated that vitamin C levels in guava, kiwi and melon were high (150 - 1000 mg/100 g), medium (50 - 100 mg/100 g) and low (< 50 mg/100 g), respectively. Although, it was not extensively validated, these findings indicated that the accuracy of our test kit for semiquantitative detection of vitamin C was comparable with the previous studies. Therefore, the accuracy of our in-house test kit was acceptable, and it was practically useful for screening vitamin C levels in juices and beverages.

Limitations of the study should be mentioned. We did not compare the vitamin C levels measured by our test with the standard HPLC technique. The stability of beads was not evaluated. However, according to our experience the blue calcium-alginate beads kept in distilled water for up to 2 weeks still worked properly like the freshly prepared beads. The interfering effects of other reducing compounds existing in juices and beverages were not investigated. The limit of detection was not explored. However, it should be noted that we intended to use our vitamin C test kit for screening the vitamin C levels in juices and beverages, not for absolute quantification.

Conclusion

A practical, rapid and inexpensive test kit for semiquantitative measurement of vitamin C in fresh fruit/vegetable juices and commercial beverages was successfully developed. Only 1 mL of sample was required for the test, and the test result was obtained within 5 min. The detection principle relied on the reduction of iodine by ascorbic acid in the blue-colored starch-iodine complex (encapsulated in the calcium alginate beads). The higher the concentration of ascorbic acid in the sample the shorter time required for a complete reduction of iodine in the starch-iodine complex (the beads become colorless). This innovative test kit was practically useful for health-conscious individuals who want to screen for levels of vitamin C in their foods before consumption. Therefore, the way to choose high vitamin C foods to consume becomes easier.

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Conflict of interest

The authors declare no conflict of interest.

Data sharing statement

The data sets generated or analyzed during the present study are available for non-commercial purposes from the corresponding author upon reasonable request.

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