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ORIGINAL PAPER

Investigation of bioaccessibility of vitamin C in various fruits and vegetables under in vitro gastrointestinal digestion system

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Abstract

This study aimed to investigate the in vitro bioaccessibility of vitamin C from various fruits and vegetables. Vitamin C bioaccessibility of 14 fruits and 8 vegetables was examined under the simulated gastrointestinal digestion method. Vitamin C values were analyzed by reverse-phase High-Performance Liquid Chromatography. The initial vitamin C amounts ranged between 1.9 (yellow apple) and 61.8 mg/100 g (strawberry) in fruits and 3.8 (curly lettuce) to 326.9 mg/100 g (purple cabbage) in vegetables. The bioaccessibility of vitamin C in fruits ranged from 2 to 91%, while the bioaccessibility of vegetables ranged between 4 and 86%. Parsley exhibited the highest bioaccessibility in vegetables, while green paper showed the lowest. It is seen in this study that vegetables and fruits with high vitamin C content have high bioaccessibility values, except for green pepper. For example, parsley, purple cabbage, red pepper, grapefruit, and orange have high bioaccessibility values (53–86%). On the other hand, intestinal pH and temperature, flavanones, minerals, and other vitamins are also thought to affect the bioaccessibility of vitamin C. Considering that vegetables and fruits are the primary vitamin C sources in the diet, it is crucial to investigate the bioaccessibility of this kind of foods vitamin.

Keywords Vitamin C · Bioaccessibility · In vitro · Fruit · Vegetable

Introduction

Vegetables and fruits contain many essential nutrients [1]. It is thought that the antioxidant content of diets rich in fruits and vegetables will be high; therefore, it will be protective against reactive oxygen species, and thus this kind of diet may be protective against cardiovascular diseases and cancer [2]. Because of that, diets rich in fruit and vegetable are encouraged worldwide [1]. The World Health Organization (WHO) has recommended 400 g of fruit/vegetable intake daily [3]. On the other hand, it is reported that the consumption of vegetables and fruits by almost 75% of people around the world is below that recommendation [1].

Vitamin C is an essential micronutrient and is found in two forms, its reduced (ascorbic acid) or oxidized (dehydroascorbic acid) forms [4]. Vitamin C has anti-inflammatory and immune-supporting effects, acts as a cofactor for mono- and di-oxygenases, and is necessary for synthesizing biological molecules such as collagen and catecholamines [5, 6]. Vegetables and fruits, like citrus fruits, strawberries, and green peppers, are excellent sources of vitamin C [7]. Therefore, fruits and vegetables, the primary sources of this molecule, should be included in the diet [8].

Although the recommended daily requirement for vitamin C varies between countries, it is 90 mg and 75 mg/day for men and women in the United States and Canada [8]. In contrast, the nutrition societies in Germany, Austria, and Switzerland think that the recommended intake should be 95 mg and 110 mg/day for women and men. However, relevant figures may vary depending on age, gender, and health [9]. In Turkey, for adults, the vitamin C recommendation is 110 mg/day for men and 95 mg/day for women [10].

Vitamin C is abundant in vegetables and fruits such as rose hips, currants, strawberries, kiwi, parsley, oranges, lemons, grapefruit, papaya, pineapple, mango, quince, cabbage varieties, broccoli, cauliflower, peppers, turnips, and potatoes [9]. Vitamin C, a very delicate vitamin, is susceptible to loss during conventional processes, such as heat treatments and drying [11]. In addition, retention of vitamin C in the food matrix, its degradation, or inhibition by other

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components found in food may reduce its bioavailability [1]. Bioavailability is a term that refers to the portion of the digested nutrient that reaches the systematic circulation and is available for use in normal physiological functions [12]. In contrast, bioaccessibility represents the release of compounds from food matrices, followed by easy accessibility for intestinal absorption [13]. Digestive transformations of foods into materials ready for absorption into the gastrointestinal (GI) tract are major processes that aid in achieving bioaccessibility [13]. Therefore, when calculating daily vitamin intakes, the bioavailability of vitamins cannot be predicted precisely because their bioaccessibility in the gastrointestinal tract is not fully known [14].

Various analytical methods could be applied to determine the bioaccessibility of nutrients and bioactive compounds: in vivo and in vitro studies, and both methods have some strengths and weaknesses [15]. Artificial digestion methods, which simulate gastrointestinal conditions, are widely used to assess the bioaccessibility of bioactive compounds in various food products [16]. Generally, in vitro methods mimic the processes in the human digestive system in the stomach, small intestine, and sometimes in the mouth [16]. The main features of the in vitro methods are temperature, shaking or agitation, and the chemical and enzymatic composition of saliva, gastric juice, duodenal juice, and bile juice [15]. The bioaccessibility in the stomach and intestinal conditions may not be the same as in the in vitro method. Still, this method is emphasized as a valuable model for estimating the preabsorption phase [14]. Vitamin C is an essential vitamin that is not synthesized by the human metabolism, therefore, it must be obtained from the diet [17]. In order to assess the potential role of bioactive compounds in the human body, even if they are provided by fresh foods, changes that take place in the GI system should be taken into account [17]. It is also reported that vitamin C is vulnerable under digestion conditions, especially in the small intestine, due to the alkaline pH, enzyme activity, other nutrients, and temperature [14]. Therefore, evaluating vitamin C bioaccessibility could be important. Vitamin C bioaccessibility of some specific foods have been studied in the literature, like, in fruit-cereal and vegetable-based baby foods [18], in the dietary supplement, infant formula, and fortified foods [19], in orange and orange juices prepared by different processing stages [20], in cashew apple juice [21], etc.

However, to the best of our knowledge, there is no vitamin C bioaccessibility study conducted with a wide variety of samples in the literature. In this study, we aimed to evaluate vitamin C bioaccessibility in various fruits and vegetables.

Materials and methods

Sampling

Twenty-two samples consist of 14 fruits and 8 vegetables, which are commonly consumed daily, obtained from markets in Istanbul, Turkey. Samples were purchased from the nearest market and immediately analyzed, and the same procedure was applied to all samples. We also use insulated cooler bags for fruits and vegetables transport to minimize vitamin loss. Samples were directly analyzed upon arrival to the laboratory. Samples were cleaned, and only the edible parts of fruits and vegetables were analyzed.

This study was conducted in March–April. Depending on the season, the vitamin composition of vegetables and fruits may change. Therefore, although these 14 fruits and 8 vegetables that make up our sample are now produced in all seasons, our results belong to the samples grown and consumed in March–April.

Bioaccessibility determination of vitamin C

Vitamin C bioaccessibility was determined by a simulated gastrointestinal digestion model. This system is composed of mouth, gastric, and small intestine phases [18]. Ascorbic acid is completely protonated at low pH and is slowly oxidized by oxygen [14]. Therefore, there was more loss of vitamin C in the small intestinal phase than the gastric phase due to the high pH [14, 22]. Based on this information, we measured the intestinal phase vitamin C values, where the most losses occur, and the stage for absorption.

The solutions in the human digestive system, such as saliva, gastric, small intestinal, and bile juices, were prepared as shown in Fig. 1. At first, 5 g homogenized sample and 5 mL of saliva solution were mixed and then incubated at water bath with shaking for 5 min at 37 °C. Next, 10 mL of gastric juice was mixed with a solution taken from the previous step and incubated in a shaking water bath for 2 h at 37 °C. After that, 5 mL of bile juice was mixed with a solution from the gastric stage. The pH was adjusted to 7, and then 10 mL of small intestinal juice was added. Then, the solution was incubated at 37 °C for 2 h in a shaking water bath. The final volume of the solution was completed to 50 mL by distilled water and centrifuged at 8000 rpm for 5 min. Finally, 10 mL sample was taken from the collected supernatant was mixed with 10 mL of meta-phosphoric acid (%3) solution. After, the solution was filtered with 0.45 μm cellulose acetate (CA) filter.

Extraction of vitamin C in samples

Sixty mL of meta-phosphoric acid solution (3%) was mixed with 5 g of sample in a vortex for 5 min. Using the

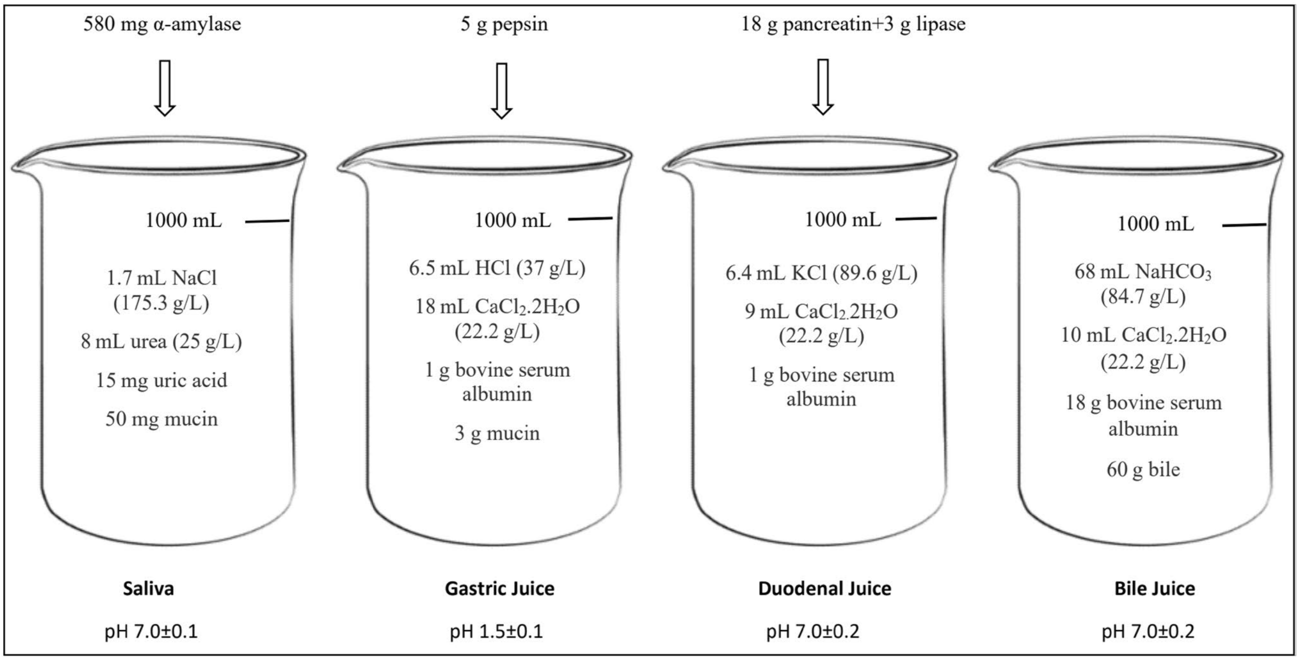

Fig. 1 The solutions in the in vitro human digestive system such as saliva, gastric, small intestinal, and bile juices

meta-phosphoric acid solution, the volume was adjusted to 100 mL. Then, the final solution was filtered using the CA filter. Finally, the filtrate was transferred into vials before the HPLC.

Vitamin C determination by HPLC with UV–visible detection

The vitamin C amounts of samples were analyzed by HPLC. HPLC provisos were selected as the method described by [18]. The HPLC system was integrated with a Shimadzu liquid chromatography 20AT pump by a Shimadzu SPD-20A UV/visible detector (Shimadzu Corporation, Kyoto, Japan). The mobile phase was prepared as 1.24 g KH_2PO_4 was dissolved in 1000 mL of distilled water, and then the ortho-phosphoric acid was used to adjust of pH to 2.4. The reverse-phase C18 HPLC column (5 µm, 250 mm × 4.6) (ACE, Scotland) was used for the separation of vitamin C in samples. The flow rate of the column was 0.5 mL/min. The wavelength was adjusted to 254 nm. The vitamin C amounts were represented as mg/100 g.

Method validation and quantification

Method validation of vitamin C (L-ascorbic acid) was achieved using AOAC guidelines [23]. Range of linearity was selected between 1 and 100 µg/mL using five calibration levels in triplicate. The limit of detection (LOD) and limit of quantitation (LOQ) were determined based on the signal-to-noise (S/N) ratio by 3 and 10, respectively. The Repeatability limit (r) and reproducibility limit (R) were determined by analyzing the tangerine sample ten times on the same day and three times on different 3 days, respectively. Besides, 1 µg/mL of L-ascorbic acid standard was spiked to the tangerine sample to check the method's recovery. Method validation parameters are shown in Table 1. The quantification was done by external standard calibration based on peak area.

Statistical analysis

All analyses were performed in triplicate. Significant differences between the results were shown by the analysis of variance (ANOVA) ($p < 0.05$, Tukey's test).

Table 1 Validation parameters of L-ascorbic acid

Analytical parameters	L-ascorbic acid
Linear range (µg/mL)	1–100
Correlation coefficient (r^2)	0.997
LOD (µg/100 g)	5.60
LOQ (µg/100 g)	16.80
Repeatability limit (r)	0.05
Reproducibility limit (R)	0.12
Recovery (%)	96.20–99.60

LOD limit of detection, *LOQ* limit of quantitation

Results and discussion

Method validation

The method validation results of the vitamin C are shown in Table 1. The linear correlation coefficient (r) for L-ascorbic acid standard curves was 0.997. The calculated limit of detection (LOD) and limit of quantitation (LOQ) were found to be 5.60 µg/ 100 g and 16.80 µg/100 g, respectively. The Repeatability limit (r) and reproducibility limit (R) were 0.05 and 0.12 respectively, and these findings show the good reproducibility for L-ascorbic acid. Recovery values ranged from 96.20 to 99.60%.

Initial vitamin C amount in fruits and vegetables

The HPLC chromatogram of vitamin C in standard, red pepper, and tangerine are shown in Figs. 2, 3, and 4, respectively. The initial vitamin C amount ranged between 1.9–61.8 mg/100 g in fruits and 3.8–326.9 mg/100 g in vegetables (Table 2). According to the Turkish Food Composition Database (TURKOMP), these fruits vitamin C values range between 3.5–75.5 and 6.6–188.9 mg/100 g for vegetables [24] (Table 3). We found that strawberry, kiwi, and pineapple had the highest initial vitamin C values in fruits, respectively, on the other hand, purple cabbage had the highest vitamin C value, and curly lettuce had the lowest vitamin C value among vegetables (Table 2). Our initial vitamin C amounts in fruits were lower than the TURKOMP database. Similar to that, almost all vegetable's vitamin C values were found to be lower than the TURKOMP database, except parsley and purple cabbage. Depending on the season, the vitamin composition of vegetables and fruits may change. Therefore, although these 14 fruits and 8 vegetables that make up our sample are now produced in all seasons, our results belong to the samples grown in March–April.

Many post-harvest processes can affect phytochemical content in foods, such as light exposure, irradiation time, and temperature. Since vegetables and fruits deteriorate quickly after harvest, they are stored in cold storage. However, vitamin C losses were observed in vegetables and fruits during storage in cold weather conditions [25]. Since the products are collected from the markets, this difference with the TURKOMP data may be due to the post-harvest process and storage conditions. In addition, for the TURKOMP study, the products of all products in Turkey were analyzed in various seasons for many years. Still, the vitamin C values in the current study provide a cross-sectional range and are limited to province-based.

Vitamin C bioaccessibility in fruits and vegetables

The amount of bioactive compounds in the bioaccessible fraction is more important than the amounts of these compounds in the relevant food [26]. Bioaccessibility can be investigated using an in vitro digestion model that simulates human physiology and can be considered an indicator of maximum oral bioavailability [19]. Retention of vitamin C in the food matrix, its degradation, or inhibition by other ingredients in the food can reduce its bioavailability [1]. Citrus fruits are rich sources of vitamin C and flavanones

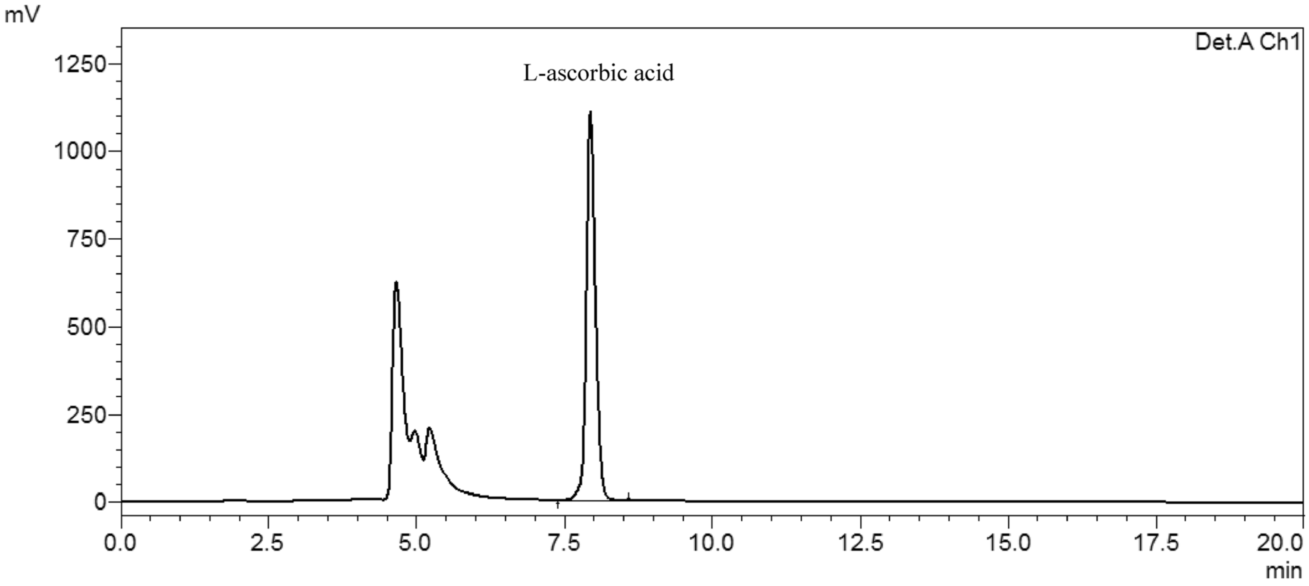

Fig. 2 The HPLC chromatogram of the vitamin C (L-ascorbic acid) standard

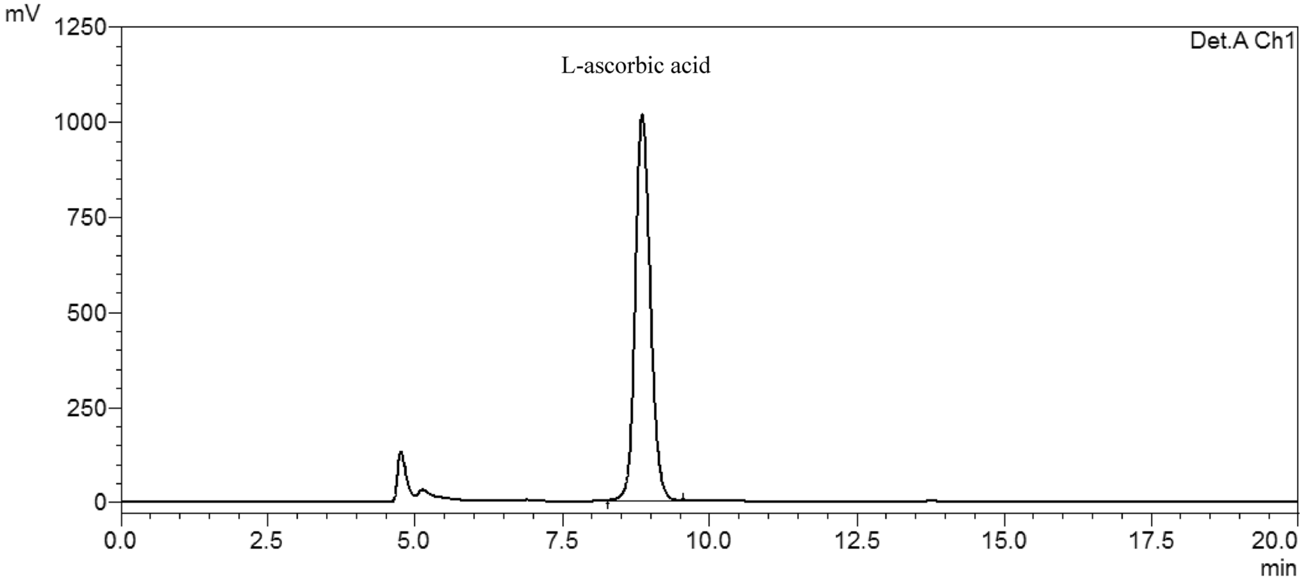

Fig. 3 The HPLC chromatogram of vitamin C in the red pepper sample

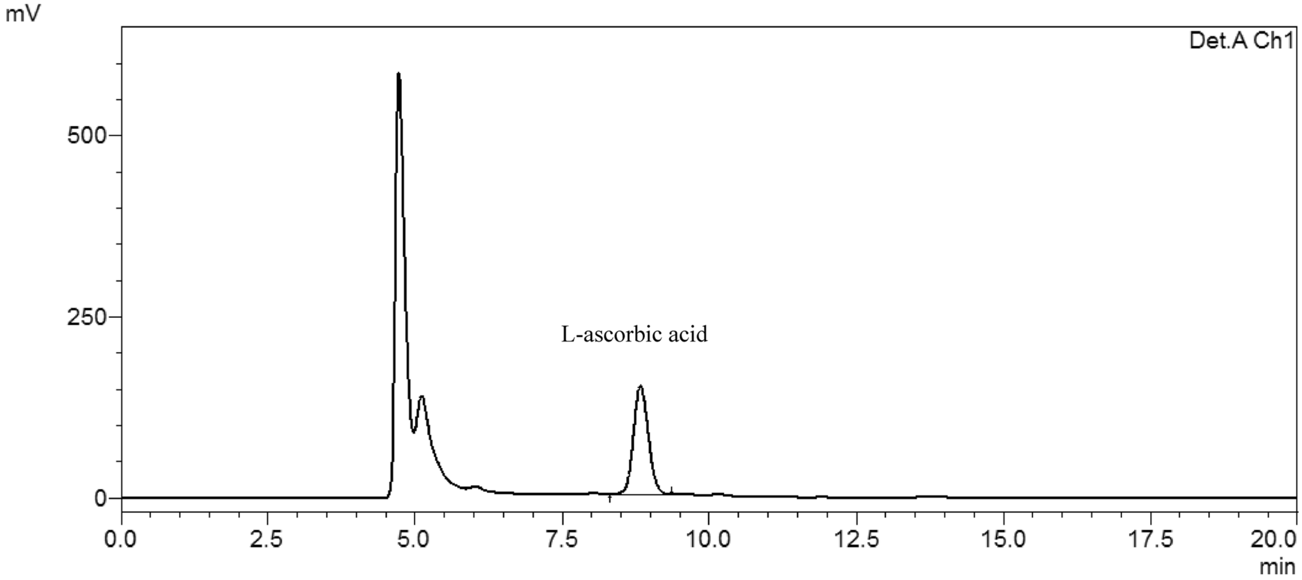

Fig. 4 The HPLC chromatogram of vitamin C in the tangerine sample

[27]. And flavonoids have been shown to inhibit the absorption of vitamin C in vitro [1].

In one study, vitamin C bioaccessibility of the lingonberry jams ranged between 42.8 and 62.9%. The authors claimed that vitamin C amounts decreased in the intestinal phase compared to the gastric phase. The authors concluded that vitamin C is more unstable under intestinal conditions [17]. Uğur et al. investigated vitamin C bioaccessibility in fruit- vegetable and cereal-based baby foodstuffs in both gastric pH 1.5 and pH 4 [18]. The bioaccessibility of vitamin C in cereal-based baby foods was found between 1.3 and 53.8% (in gastric pH 1.5) and between 0.3 and 26.3% (gastric pH 4). Also, they found that in fruit- and vegetable-based baby foods, the bioaccessibility of vitamin C was significantly low in both gastric pH levels ($p < 0.05$) [18]. Vallejo et al. studied the stability of vitamin C in gastric conditions and found that vitamin C was less affected during gastric digestion [28]. Similar to that, Pérez-Vicente et al. also found that loss of vitamin C is fewer in the gastric digestion duration [29]. In accordance with those studies, Rodriguez et al. also found similar results and decreased bioaccessibility percentages after in vitro digestion. And the author claim that alkaline

Table 2 Vitamin C amounts in fruits and vegetables before and after digestion

Sample name	Sample number	Initial value (mg/100 g)	After digestion (mg/100 g)	Bioaccessibility (%)
1. Tangerine	1	22.1 ± 1.0^{a}	5.9 ± 0.3^{b}	27 ± 1
2. Banana	2	7.5 ± 0.3^{a}	0.5 ± 0.0^{b}	6 ± 0
3. Kiwi	3	47.8 ± 2.2^{a}	25.4 ± 1.1^{b}	53 ± 2
4. Strawberry	4	61.8 ± 2.8^{a}	19.9 ± 0.9^{b}	32 ± 1
5. Grapefruit	5	28.9 ± 1.3^{a}	16.9 ± 0.8^{b}	59 ± 3
6. Orange	6	37.8 ± 1.7^{a}	19.9 ± 0.9^{b}	53 ± 2
7. Pineapple	7	46.0 ± 2.1^{a}	23.9 ± 1.1^{b}	52 ± 2
8. Pomegranate	8	6.3 ± 0.3^{a}	2.0 ± 0.1^{b}	32 ± 1
9. Green apple	9	3.1 ± 0.1^{a}	0.1 ± 0.0^{b}	3 ± 0
10. Red apple	10	2.4 ± 0.1^{a}	0.3 ± 0.0^{b}	12 ± 1
11. Yellow apple	11	1.9 ± 0.1^{a}	1.1 ± 0.0^{b}	56 ± 3
12. Quince	12	8.2 ± 0.4^{a}	0.1 ± 0.0^{b}	2 ± 0
13. Pear	13	2.1 ± 0.1^{a}	1.9 ± 0.1^{b}	91 ± 4
14. Pear winter	14	3.4 ± 0.2^{a}	0.3 ± 0.0^{b}	9 ± 0
15. Tomato	15	15.9 ± 0.7^{a}	7.5 ± 0.3^{b}	47 ± 2
16. Cucumber	16	5.8 ± 0.3^{a}	0.3 ± 0.0^{b}	5 ± 0
17. Parsley	17	235.2 ± 10.6^{a}	202.3 ± 9.2^{b}	86 ± 4
18. Purple cabbage	18	326.9 ± 14.8^{a}	230.2 ± 10.4^{b}	71 ± 3
19. Arugula	19	39.5 ± 1.8^{a}	12.6 ± 0.6^{b}	32 ± 1
20. Curly lettuce	20	3.8 ± 0.2^{a}	1.2 ± 0.1^{b}	33 ± 2
21. Red pepper	21	150.6 ± 6.8^{a}	101.7 ± 4.6^{b}	68 ± 3
22. Green pepper	22	57.8 ± 2.6^{a}	2.6 ± 0.1^{b}	4 ± 0

Different letters indicate statistical differences between samples in the same row ($p < 0.05$)

pH, oxygen, light, and enzymatic conditions affect vitamin C stability, and vitamin C is less stable in small intestinal condition [22]. Therefore intestinal environments significantly affect the bioaccessibility of vitamin C (Fig. 5).

According to a study, vitamin C bioaccessibility of the beverages, untreated and treated, which made of the fruit juice plus soy milk, milk, and water, was in the range of 10.9–23.2% [30]. And beverages prepared with soymilk exhibited the highest bioaccessibility. Authors indicate that the beverage made of soymilk is rich in phenols and isoflavones, which may have contributed to stabilizing vitamin C by preventing its oxidation [30]. In another study, vitamin C amounts in dietary supplements and infant formulas were analyzed. It was found that dietary supplements' vitamin C bioaccessibility ranged from 1 to 99%, while infant formulas' bioaccessibility was very low (< 1.0%). Authors thought that the bioaccessibility of vitamin C depends on the product composition and possibly the encapsulation rather than the dietary status [19]. In a study aimed to evaluate the influence of beverage formulation on in vitro bioaccessibility of vitamin C, bioaccessibility of vitamin C was 11.5% in fruit juice plus milk beverage and 14.9% in fruit juice beverage [31]. The processing method significantly influenced the bioaccessibility of vitamins. In the current study, after in vitro digestion treatment, the bioaccessibility of vitamin C in fruits differs from 2 to 91%. After digestion, the pearl had the highest bioaccessibility (91%). The bioaccessibility of vitamin C in vegetables ranges between 4 and 86%, and parsley exhibited the highest bioaccessibility since green paper showed the lowest percentage at 4%. According to the literature, food context could affect the bioaccessibility of vitamin C, such as other vitamins or flavanones or minerals. Since fruits and vegetables are rich sources of vitamins, flavonoids, and minerals, they would be attributed to vitamin C bioaccessibility. Apart from this, pH levels also affect vitamin C concentration, and the acidic gastric phase prevents vitamin C oxidation by chemically and enzymatically. On the other hand, since the pH of the small intestine is closer to neutral value, more vitamin C loss occurs in the small intestinal compared to the gastric medium [12].

Finally, our study has some strengths and weaknesses, which are need to be taken into consideration during interpreting values. First, as this study's aim was not a food composition analysis study, the samples were taken from the nearest market to prevent possible vitamin loss. Depending on the season, the vitamin composition of vegetables and fruits may change. Therefore, although these 14 fruits and 8 vegetables that make up our sample are now produced in all seasons, our results belong to the samples grown in March–April and does not represent seasonal changes. On

Table 3 Vitamin C amount of fruits and vegetables according to the TURKOMP

Sample name	Sample number	Initial value (mg/100 g)
1. Tangerine	1	43.1
2. Banana	2	9.5
3. Kiwi	3	60.1
4. Strawberry	4	75.5
5. Grapefruit	5	36.9
6. Orange	6	45.3
7. Pineapple	7	59.3
8. Pomegranate	8	7
9. Apple, green, and summer	9	4.5–7.3
10. Apple, red, and summer	10	4.5–7.3
11. Yellow apple	11	4.5–7.3
12. Quince	12	15.2
13. Pear	13	3.5
14. Pear winter	14	4.8
15. Tomato	15	19.8
16. Cucumber	16	11
17. Parsley	17	188.9
18. Purple cabbage	18	66.4
19. Arugula	19	109.4
20. Lettuce curly	20	6.6
21. Pepper, red	21	150.7
22. Pepper, green	22	80.0

TURKOMP database, 2022, http://www.turkomp.gov.tr/ [24]

the other hand, we mainly focus on how gastrointestinal conditions affect the bioaccessibility of vitamin C. As seen in recent studies, there is a limited study on vegetables and fruits vitamin C bioaccessibility. Comparing the studies, we have more various samples. As it is an essential vitamin, we thought that information about the bioaccessibility values of fruits and vegetables is important for healthy nutrition and meeting requirements. Seasonal differences also affect vitamin C amounts since future studies focus on the bioaccessibility of the products collected in different seasons, which will be enlightening in this field.

Conclusion

It is known that diets rich in fruit and vegetables contain many essential nutrients such as C and B group vitamins. The whole world has been struggling with covid-19 for the last 2 years. The importance of vitamins C, which has anti-inflammatory and immune-supporting effects, has been much discussed in the fight against COVID-19. Health professionals strongly recommend taking enough of these vitamins in our daily diet to strengthen our immune system. Most of the vegetables and fruits we examined in this study, such as kiwi, strawberry, parsley, orange, and red pepper, have very high vitamin C values. It is emphasized in some studies that bioaccessibility values are essential in calculating nutrient intake in daily diets. Therefore, when the literature is examined, there are a limited number of bioaccessibility values for vegetables and fruits. It is seen in this study that vegetables and fruits with high vitamin C content

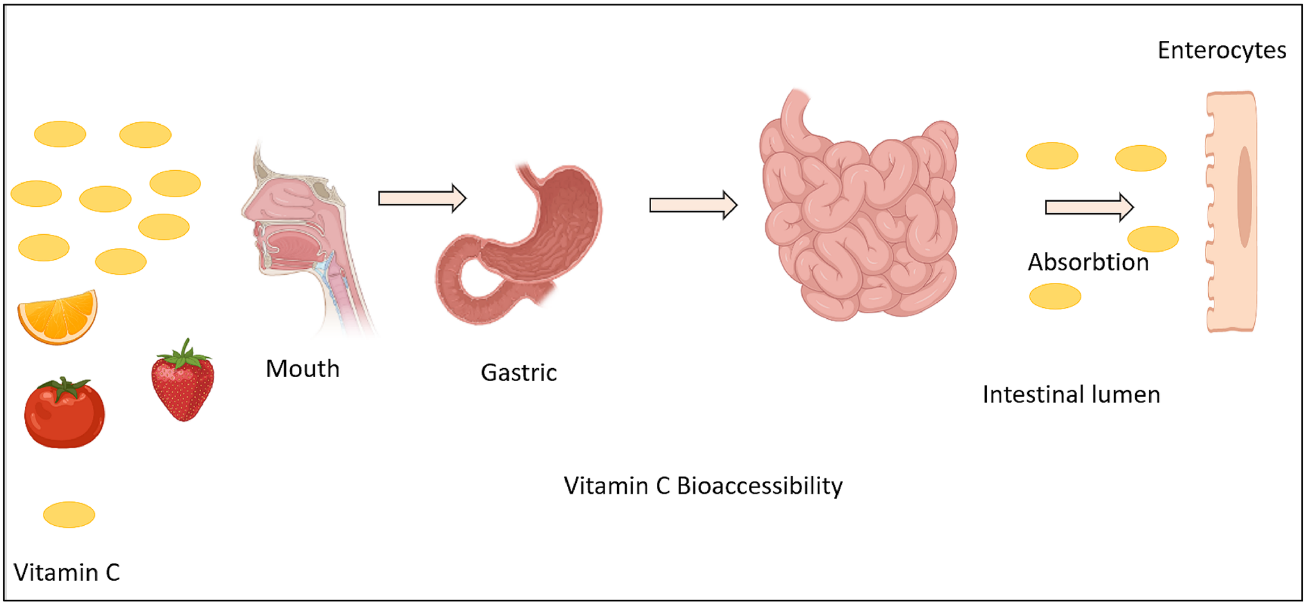

Fig. 5 The schematic description of vitamin C bioaccessibility

have high bioaccessibility values, except for green pepper. For example, parsley, purple cabbage, red pepper, grapefruit, and orange have high bioaccessibility values (53–86%). On the other hand, intestinal pH and temperature, flavanones, minerals, and other vitamins are also thought to affect the bioaccessibility of vitamin C. Considering that vegetables and fruits are the primary vitamin C sources in the diet, it is crucial to investigate this kind of foods vitamin bioaccessibility. Seasonal differences also affect vitamin C amounts since future studies focus on the bioaccessibility of the products collected in different seasons, which will be enlightening in this field.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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