# Effect of pH, Temperature and Metal Salts in Different Storage Conditions on the Stability of Vitamin C Content of Yellow Bell Pepper Extracted in Aqueous Media

Husni S Farah<sup>1\*</sup>, Jehad F Alhmoud<sup>1</sup>, Atef Al-Othman<sup>1</sup>, Khalid M Alqaisi<sup>1</sup>, Ali M Atoom<sup>1</sup>, Khalid Shadid,<sup>2</sup> Ashok Shakya<sup>2</sup>, Talal AlQaisi<sup>1</sup>

<sup>1</sup>Pharmacological and Diagnostic Research Centre, Department of Medical Laboratory Sciences, Faculty of Allied Medical Sciences, Al-Ahliyya Amman University/ Amman, Jordan.

<sup>2</sup>Pharmacological and Diagnostic Research Centre, Faculty of Pharmacy, Al-Ahliyya Amman University/ Amman, Jordan. h.farah@ammanu.edu.jo; j.alhmoud@ammanu.edu.jo; aalothman@ ammanu.edu.jo; k.alqaisi@ammanu.edu.jo ; a.atoom@ammanu.edu.jo; kshadid@ ammanu.edu.jo; ak\_shakya@ammanu.edu.jo; t.alqaisi@ammanu.edu.jo

#### \*Corresponding author: Dr. Husni S Farah

Department of Medical Laboratory Sciences, Faculty of Allied Medical Sciences, Al-Ahliyya Amman University **Email**: <u>h.farah@ammanu.edu.jo</u>

### ABSTRACT

**Purpose:** Generally, the interest of studying various factors that affect the level of vitamin C in different types of food has been increased due to its great importance to human health. This study aimed to investigate the effect of different incubation factor variables; pH, temperature and metal salts on the amount of vitamin C of yellow bell pepper extracted in aqueous media.

**Methods:** 200 gm of the grinded yellow bell pepper was added to 500 ml of distilled water for 24 hours with continuous shaking. Then the solution of the preparation was filtered, and the clear filtrate was used to obtain the concentration of vitamin C by redox titration. Equal portions of the filtrate (20ml) were used every time to investigate the effect of the following at different incubation periods on the contents of vitamin C: pH ranges from (3 – 8), temperature (-18, 4, 37, 40, 60, 90 C°) and metal salts (ZnCl<sub>2</sub>, MgCl<sub>2</sub>, FeCl<sub>2</sub>) of the same concentration (0.1M). The amount of vitamin C in the treated filtrate was estimated after the end of each incubation time.

**Results:** There was a significant loss in the amount of vitamin C by increasing both the temperature and heating time. Vitamin C was lost about 36.6 % (p< 0.003) of its contents at 90 C° for 1 hour of incubation. Whereas the incubation of vitamin C at 4 C° for 240 hours caused a 11.4 % loss of its contents (p <0.013). It was found that incubation of vitamin C at -18 C° did not cause any significant change to its contents. Vitamin C was more stable at pH 3.4 and less stable at pH 8.1 in which 39.5% of its content was lost at this pH 8.1 (p<0.003). Also, the results showed that FeCL<sub>2</sub> (0.1M) is the only metal salt that significantly influences the vitamin C content (p < 0.045).

**Conclusion:** This study showed the ideal factors that can maintain vitamin C and its stability. The multiple benefits of vitamin C are achieved by preserving the food material that particularly contains this vitamin in appropriate conditions as stated in the current study. Low acidity is an adequate environment for the storage of vitamin C. It is advised not to use vitamin C an additive to any food or beverage containing iron ions (Fe<sup>+2</sup>), as this contributes to the loss of stability of vitamin C and thus has a negative effect on its nutritional value.

## **INTRODUCTION**

Vitamin C known as ascorbic acid (AA) is one of the most essential vitamins for the human body in most of life stages. Vitamin C is mainly present in several types of fruits and vegetables such as citrus fruits, strawberries, green peppers, red peppers, tomatoes, broccoli, brussels sprouts, turnip and Indian gooseberry. The body absorb vitamin C into two different ways; the buccal cavity via passive diffusion or in the gastrointestinal tract via active sodium dependent vitamin C transporters (SVCT) (1). The importance of this vitamin is that it is responsible for several vital functions, starting from building tissues, maintaining skin vitality, and raising immunity (2). Different human immune system components are affected by the Vitamin C concentration. Vitamin C seems to be involved in several neutrophil functions including releasing chemotaxis, improved particulate ingestion, stimulated lysozyme-mediated non-oxidative killing (3). A study mentioned that the effect of Vitamin C is more

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#### Correspondence:

Husni S Farah Department of Medical Laboratory Sciences, Faculty of Allied Medical Sciences, Al-Ahliyya Amman University Email: <u>h.farah@ammanu.edu.jo</u>

obvious in cell mediated response rather than humoral immunity. The reason is that the detected T-cell hyporesponsiveness was reversed in the patients with Crohn's disease receiving oral supplementation of vitamin C. In addition, humoral immunity did not influence by vitamin C (4).

However, Vitamin C provides a protection strategy against toxins impacts of superoxide anion radical, reduction of the halide-peroxide-myeloperoxidase system and stimulation of the hexose monophosphate shunt. Vitamin C is one of the most important antioxidants due to its ability to easily donate electrons, leading to protect the body from free radicals that increase certain diseases such as different types of allergies and cancers (5). In addition, biomolecules such as proteins, lipids, carbohydrates, and nucleic acid are protected against the damage which cause from oxidants generated during normal cell metabolism and due to exposure to toxins and air pollutants. (6,7). Also, Vitamin C has other benefits in regulating cholesterol levels and protects against diabetes complications (8). Vitamin C is viewed to be a crucial antioxidant molecule in the brain. Intracellular Vitamin C facilitates to preserve the integrity and function of numerous processes such as; neuronal maturation and differentiation, myelin formation, production of catecholamine, modulation of neurotransmission and antioxidant protection in the central nervous system (9). Also, vitamin C plays a role in several non-oxidant processes such as biosynthesis of collagen, carnitine, tyrosine and peptide hormones in addition to myelin (10). However, it plays the vital role in neurotransmission and neuronal maturation and functions as it was verified that vitamin C has the ability to decrease the damage stimulated by seizure (11,12). Furthermore, Vitamin C is a cofactor for different enzyme families, including biosynthetic and gene regulatory monooxygenase and dioxygenase (13). It has several functions as a cofactor for the lysyl and prolyl hydroxylases essential for stabilization of the tertiary structure of collagen. Also, it's considered as a cofactor for the two hydroxylases included in carnitine biosynthesis, a molecule important for transport of fatty acids into mitochondria for producing of metabolic energy. As the number of patients with diabetes are getting incredibly increase, controlling the development of this disease become highly essential nowadays (14). However, uncontrolled hyperglycaemia leads to long-term damage, dysfunction, and failure of various organs such as eyes, kidneys, nerves, heart, and blood vessels (15,16). Both types of diabetes stimulate oxidative stress by ROS, which indicated the disease onset (17, 18). Vitamin C plays a role in reducing the risk of diabetes mellitus (DM) progression. Dietary iron absorption was induced after AA intake and the level of iron was decreased due to vitamin C that induce of dietary absorption of non-heme iron (19).

This vitamin found in fruits in several concentrations depending on many factors such as the type of fruits, exposure to the sun, Soil contents and climate conditions and. Mostly, the fruits before ripeness contain more vitamin C compared with the fruits after ripeness (20). The vitamin is found in two biologically important forms, one of which is known as L- Ascorbic acid and is the most active form, whereas the other form is Dehydroascorbic acid which is also of biological importance. The vitamin is converted into diketogulanic acid by oxidation, which has no essential functions (21). A study clearly showed that increasing the temperature and pH affects the stability of ascorbic acid (22).

There was a contradiction between the findings obtained from the literature regarding the effect of temperature on vitamin C stability. Ascorbic acid concentrations decreased at a faster rate in rooms temperature than in the refrigerator (23). Another study reported that high storage temperatures are associated with an increase in ascorbic acid breakdown, whereas low temperatures are associated with a decrease in the rate of breakdown (22). However, to alter the stability of vitamin C in the short incubation time, the temperature effect in the 60-80  $C^{\circ}$ range was not important. (24). Ascorbic acid tends to be stable under anerobic conditions at temperatures of up to 70 C° (25). This destruction by oxidation due to high temperature storage is a serious problem because during collection, storage and preparation, a large amount of the vitamin C content of the food is lost (26). Ascorbic acid degradation was seen at pH 4.0 at maximum and at pH 2.5 to 3.0 at minimum (27). Disagreements about the effect of metals salts on vitamin C stability of ascorbic acid were

found. Mn (II) does not have a significant impact on vitamin C stability (10). Oxidation rate of ascorbic acid was significantly increased by the presence of copper (II) ion (28).) It is understood that ascorbic acid autoxidation is influenced by both metal ions such as Cu (II) and Fe (III). Whereas other metal ions, such as zinc (Zn), selenium (Se) and manganese (Mn), do not have a significant influence on vitamin C stability (29). The addition of Se (IV), Mg (II) and Zn (II) induces a substantial decrease in stability of ascorbic acid by approximately 23%. (30).

The objective of this study was to investigate the impact of various temperatures , pH and metal salts conditions on the stability of vitamin C and to verify the appropriate conditions under which vitamin C can be stored without losing its stability and nutritional value.

## **MATERIALS AND METHODS**

### Samples preparations and aqueous extraction

All the chemicals and reagents used in this study were purchased from Sigma Aldricha and were of analytical grade purity. Fresh Yellow bell pepper samples were collected from main vegetables markets in the capital of Jordan, Amman. Samples were thoroughly washed with purified water to avoid any harmful particles and dust particles. The samples were cut into small pieces and grounded to obtain homogenous mixture using a kitchen blender. 200 gm of the homogenous mixture was added to a glass beaker and homogenized with 500 ml of distilled water. The mixture was incubated at 25 C° in a dark place with gentle stirring overnight. To obtain a clear filtrate, the homogenate was filtered with Whatman No. 1 filter paper. The filtrate was transferred to a sealed clean flask.

### Preparation of the iodine solution

5 grams of potassium iodide (KI) and 0.268 grams of potassium iodate (KIO3) was mixed in 200 ml of distilled water after the addition of 2.5 ml of 3 M sulphuric acid. The solution was transferred to a 500 ml volumetric flask and made up to 500 ml of distilled water and was corked properly.

### 2.3 Vitamin C standard solution:

Stock standard solution of vitamin C was prepared by dissolving 0.25 gm of vitamin C in 250 ml of distilled water to obtain 0.1gm /100ml. The solution was shaken gently to ensure complete dissolving of vitamin C.

## Determination of vitamin C in the treated filtrate by oxidation -reduction (Redox) titration method

Redox titration method for the determination of the contents of vitamin C was used (31). 10 ml of each of the freshly prepared filtrates sample solution was taken after it is being treated in different temperature and diluted with 200 ml distilled water. Then 10 ml of each of these solutions were put into conical flask and I ml of starch solution (1%) was added. The mixture was shaken gently to make it homogenous. The solution was then titrated against KIO3 (0.0025 M) from the burette until the appearance of blue -black, color which indicate the end point of the reaction. The titration was repeated three time for each of the treated filtrate. The results were reported, tabulated and calculation of vitamin C concentration. The determination and analysis of ascorbic acid in the citrus fruits will be found by using iodometric titration. Iodometric titration are similar to normal titration but the titrant involves an iodine solution and a starch indicator.

# Testing the validity of the oxidation -reduction (Redox) titration method

Accuracy of the redox titration method was tested by using the standard solution of vitamin C which was prepared as described above. 100 ml of distilled water was added to 10 ml (0.01gm) of standard of vitamin C solution in 250 ml conical flask and 1ml of starch solution (1%) was added to the mixture. Vitamin C solution was titrated against KI03 (0.0025 M). A dark blue color was indicated the end point of the titration. The titration was repeated three times. In this procedure Vitamin C (ascorbic acid) is oxidized to form dehydroascorbic acid as show in the following reactions:

1. 
$$IO_3 + 5I + 6 H + 3 I_2 + 3H_2O$$
  
2.  $C_6H_8O_6 + I_2 C_6H_6O_6 + 2H + 2I_2$ 

Vitamin C was oxidized to dehydroascorbic acid as seen in reaction (1). Ascorbic acid (vitamin C) cannot be titrated directly with iodine due to low solubility of  $I_2$  in water. The iodine required in the titration was obtained from the reaction between KIO3 and KI as indicated in reaction 2. As long as there are any ascorbic acid presents, the  $I_2$ which was generated in the reaction is immediately reduced to I<sup>-</sup>. Once all ascorbic acid has been oxidized, the excess  $I_2$  reacts with starch forming dark blue color indicating the end point of the reaction.

The oxidation-reduction method (Redox) was subjected to test to ensure its accuracy and validity by performing recovery studies via the use of standard ascorbic acid solution (0.1gm/dl). The recovery (%) was calculated as mean value of three trials and found to 93.7 ± 1.3 %.

## The effect of temperature on the stability of vitamin C in yellow bell pepper aqueous extraction

Samples each with 20 ml of freshly prepared filtrates were placed in 100 ml conical flask. The samples were incubated separately for 1 hour in the following temperatures (C°): 37, 40, 60 and 90. In another experiment 20 ml of freshly prepared filtrates samples of aqueous extraction of yellow pepper were incubated separately at 4 C° and -18 C° for 1, 3, 7, 10 days. After the end of the incubation period, vitamin C was determined in each filtrate by redox titration method and compared with the control filtrate sample prepared at room temperature (25 C°). % of loss was obtained from the difference between the vitamin C content in the freshly prepared filtrate (control) and that in the filtrates incubated at different temperatures (4 and -18 C°) for different days.

## The effect of pH on the stability of vitamin C in yellow bell pepper aqueous extraction

Four portions each with 20ml of freshly prepared filtrates samples of aqueous extraction of yellow pepper were placed in 100 ml conical. The pH of the filtrates (5.6) was adjusted to 3.4 and 4.6 separately by using HCl (0.1 N). While other pH values (7.5, 8.1) were achieved by using 0.1 NaOH (0.1N). The achievable pH was determined by using of pH meter model S400 (Seven Excellence. Switzerland). The filtrates with different pH ranges were incubated in dark place for 1 hour at room temperature (25 C°). After the end of the incubation period the concentration of vitamin C were determined in each filtrate sample and the % loss in the concentration of vitamin C was calculated after each treatment.

#### The effect of metal salts ions on the stability of vitamin C in yellow bell pepper aqueous extraction

10 ml of similar concentrations (0.1 M) of metals salts (ZnCl2, MgCl2, FeCl2) were added separately to 20 ml samples of freshly prepared filtrate aqueous extraction of yellow pepper separately at room temperature (25 C°) and the treated filtrates sample were incubated at room temperature for 1 hour. Estimation of vitamin C was

obtained by oxidation -reduction titration (Redox) method and compared with control filtrate sample freshly prepared at 25 C°. The % of loss in the concentration of vitamin C in each treated filtrate was calculated.

## RESULTS

# Effect of temperature on vitamin C content of yellow bell pepper extracted in aqueous media:

The stability of vitamin C found to be decreased significantly (p <0.02) with increasing temperature (more than 37 C°) in 1 hrs incubation time (Fig. 1). Moreover, incubation of filtrate of yellow pepper extracted in aqueous media at 40 °C significantly reduced the amount of vitamin C (Fig. 1; p <0.02) with 19.2% loss compared to control (25 C°) (.(Table A.1). This trend was also found at higher temperatures 60 C° (p <0.008) and 90 C° (p <0.003) with high percent loss of vitamin C content (27.7% and 36.6%, respectively; Table A.1).).



**Fig 1.** The effect of temperature on the stability of vitamin C content of filtrate of yellow bell pepper for 1 hrs. incubation time. SD was obtained from triplicate result. Asterisks represent significant difference compared with control (\* p < 0.05; \*\* p < 0.01), *NS*: no significant difference compared to the control.

**Table A.1.** The effect of temperature on the stability of vitamin C content of yellow bell pepper for 1 hrs incubation time. Percent of loss is the difference between the vitamin C content in the freshly prepared filtrate (control) and that in the filtrates incubated at different temperature.

Temperature °C	% Loss
25 (Control)	-
37	4.4
40	19.2
60	27.7
90	36.6

We also investigated the effect of storage temperature on vitamin C at different storage periods: 24, 72, 168 and 240 hrs (Fig. 2 and Fig. 3). One day storage at 4  $^{\circ}$ C had no significant effect on vitamin C content (Fig. 2). However,

the storage at the same temperature for 72, 168 and 240 hrs significantly reduced vitamin C content compared to the control (p < 0.040; p < 0.014; p < 0.013, respectively). The percent loss of vitamin C found to be very close at 72, 168 and 240 hrs. (Table A.2)



**Fig 2.** Effect of storage at 4 °C on the stability of vitamin C contents in the filtrate of yellow bell pepper at different time periods. SD was obtained from triplicate results. Asterisks represent significant difference compared with control (\* p < 0.05), NS: no significant difference to the control.

**Table A.2.** Effect of storage at 4 °C on the stability of vitamin C contents in yellow bell pepper at different time periods. Percent of loss is the difference between the vitamin C content in the freshly prepared filtrate (control) at 25 °C and that in the filtrates incubated at temperature (4 C°) for different days.

Day	% Loss
Control filtrate at 25	
°C	-
1	3.0
3	7.5
7	10.6
10	11.4

In comparison to the storage temperature 4 °C, storing filtrate of bell yellow pepper extracted in aqueous media at -18 C° for 24, 72, 168 and 240 hrs had no effect on vitamin C content when compared to the control (Fig. 3). The percent of loss is shown in Table A.3.



**Fig 3.** Effect of storage at – 18 C° on the stability of vitamin C contents of bell yellow bell pepper at different time periods. SD was obtained from triplicate results. *NS:* no significant difference compared to the control.

**Table A.3.** Effect of storage at – 18 °C on the stability of vitamin C contents in yellow bell pepper. Percent of loss is the difference between the vitamin C content in the freshly prepared filtrate (control) at 25 °C and that in the filtrates incubated at temperature (-18 °C) for different days.

Day	% Loss
Control filtrate at 25 C°	-
1	2.1
3	3.6
7	3.1
10	2.7

## Effect of pH on vitamin C content of yellow bell pepper extracted in aqueous media:

The effect of pH different values (3.4, 4.6, 7.5 and 8.1) on vitamin C content in aqueous media extracted from yellow bell pepper was investigated in this study (Fig. 4). Low pH with values 3.4 and 4.6 had no significant effect on vitamin C content (Fig. 4) and had low percent loss of vitamin C content compared to the control (Table A.4). In comparison, higher pH values, 7.5 and 8.1, significantly reduced vitamin C content (p <0.032; p <0.003, respectively) compared to the control (pH 5.6).



**Fig 4.** Effect of different pH values on the stability of vitamin C content of yellow bell pepper. SD was obtained from triplicate results. Asterisks represent significant difference compared with control (\*p < 0.05; \*\*p < 0.01), *NS:* no significant difference compared to the control.

**Table A.4.** Effect of pH variation on the stability of vitamin C content of yellow bell pepper. Percent of loss is the difference between the vitamin C content in the freshly prepared filtrate (control) at pH 5.6 and that in the filtrates with different pH range.

рН	% Loss
3.4	6.0
4.6	9.9
5.6 (Control)	-
7.5	21.5
8.1	39.5

## Effect of metal salts on vitamin C of yellow bell pepper extracted in aqueous media:

In this study we investigated the effect of metal salts ZnCl<sub>2</sub>, MgCl<sub>2</sub> and FeCl<sub>2</sub> on vitamin C in aqueous extract from yellow bell pepper (Fig. 5). Among the used metal salts in this study, only FeCl<sub>2</sub> reduced vitamin C content significantly (p < 0.045) compared to control. The percent of loss is shown in Table A.5.



Fig 5. Effect of metal salts (ZnCl<sub>2</sub>, MgCl<sub>2</sub> and FeCl<sub>2</sub>) on the stability of vitamin C content of yellow bell pepper. Similar concentration (0.1 M) of metals salts were added to the filtrates of aqueous extraction of vitamin C separately at room temperature (25 C  $\pm$  1.3).

The effect was investigated after mixing the filtrate with 10ml of 0.1M of each separately and estimation of vitamin C was obtained after 1 hour. SD was obtained from triplicate result. Asterisks represent significant difference compared with control (\*p < 0.05), *NS:* no significant difference to the control.

**Table A.5.** Effect of metal salts (ZnCl<sub>2</sub>, MgCl<sub>2</sub> and FeCl<sub>2</sub>) on the stability of vitamin C content of yellow bell pepper for 1 hr incubation time. Percent of loss is the difference between the vitamin C content in the freshly prepared filtrate (control) and that in treated filtrate with metal salts.

Metal salt	% Loss
Control	-
ZnCl <sub>2</sub>	4.7
MgCl <sub>2</sub>	3.0
FeCl <sub>2</sub>	18.2

## DISCUSSION

The objective of this study is to thoroughly investigate the various common factors that may alter the stability of vitamin C, which leading to the loss of its nutritional value. Also, the current study established the proper conditions that would sustain vitamin C stability. As it contains 186.8 mg/100 g of vitamin C, yellow bell pepper has been used as a model for investigating the impact of variable conditions on its vitamin C content. Other study (32) has found 160mg/100g of vitamin c in the yellow pepper this difference may be due to different soil condition, and/or different climatic condition (33). The aqueous extraction method of vitamin C, as can be achieved by using the alcoholic extraction method. A study (34) has shown that the use of ethanolic extraction results in a degradation of

vitamin C and a loss of a substantial proportion of its nutritional value. The Redox titration approach used in this study for vitamin C content determination was validated by the use of a standard vitamin C solution. In addition, this process proved that the recovery rate was almost equal to 93.4%. When exposed to variable conditions such as temperature, pH and long storage periods, vitamin C's nutritional value will degrade. Therefore, maintaining the stability of vitamin C is quite challenging. The heating time has significant impact on the vitamin C content, as the heating time increases, the percentage loss of vitamin C increases too. A substantial decrease in the stability of vitamin C at 40 C° over 1 hour of incubation was observed in the present study and the degradation becomes deeper as the temperature increases. There was no significant effect reported below this temperature, which disagrees with one of the studies (23) which showed a significant loss in the stability of vitamin C stored at 30 C° for a short period. A study (35) reported a substantial loss (64.71 %) of green pepper vitamin C content at 60 C° for 30 minutes, which conflicts with the findings obtained in the current study in which a loss of vitamin C content of 27.7 % at 60 C° for 1 hour of heating occurred. This discrepancy may be due to the atmospheric oxygen exposure of vitamin C, which speeds up the oxidation process and thus increases the rate of its loss, as the vitamin C filtrate in the present study has been treated with limited oxygen exposure. Storing vitamin C at 4 C° (in the refrigerator) leads to a slight loss of its stability, contrary to all predictions, as this loss increases with an increase in the storage time. The current study has shown that the best conditions for preserving vitamin C stability are achieved at a temperature of -18 C°, where the oxidation phase of vitamin C decreases dramatically and the chemical structure remains coherent resulting in its stability being maintained. Ionization of vitamin C has been documented to make it less vulnerable to rapid degradation. Typically, ionization occurs at acidic pH, where vitamin C has retained its efficacy at pH 4.0 (36). The current study found that the stability of vitamin C was preserved at pH 3.4. It is believed that a major ionization of vitamin C has occurred at this pH. The literature showed that there have been conflicting reports of the impact of acidifying on vitamin C stability. A substantial decrease in vitamin C stability was found in extreme acid media, a whereas stability of vitamin C was preserved in mild acidic media (36). This disparity and discrepancy in the impact of pH was due to the degree of purity of the vitamin C, as pure vitamin C is largely stable at extreme acidic media. It is presumed that reducing pH will increase the proportion of undissociated vitamin C structure while retaining its stability. The effect of alkaline media on the stability of this vitamin has not been investigated in previous studies, as was the case in the current study, in which vitamin C lost a significant proportion of stability at pH 8.1. We assume that vitamin C behaves differently at this pH, as it starts to degrade, resulting in a substantial increase in the dissociated portion compared to the undissociated portion. An alkaline pH tended to promote the mechanism of vitamin c dissociation, resulting in a loss of equilibrium therein. One of the studies (37) identified vitamin C as a donor of proton in alkaline media that can be deprotonated on one of its hydroxyls forming ascorbate anion as this may be taken as a substantial change in its structure.

As an essential substance for human health and as an antioxidant, vitamin C is added to certain foods and

beverages as salt forms (commonly sodium ascorbate or calcium ascorbate). Some metal ions are found in these foods, such as zinc, iron, selenium and magnesium. We have decided to investigate the effect of some of the metal ions on vitamin C stability. This will give us a good understanding of the metals that can significantly lower the stability of vitamin C. The results in this study showed that the stability of vitamin C was not reduced when it was stored with zinc and magnesium ions, which disagreed with the report stated that Zn(II) causes a significant decrease in stability by 23 %. (30.). We assume that the iron ion (Fe+2) which was added to vitamin c in the form of FeCl<sub>2</sub> has been oxidized to Fe+3 by air oxygen, which has a major effect on the stability of vitamin C. This finding coincides with the study (38) that the ferric form of iron (Fe+3) has an influence on the decomposition process of vitamin C without giving any explanation for this negative impact of Fe+3 on the stability of vitamin C.

## CONCLUSION

The present study showed the ideal conditions in which vitamin C can be stored without losing its chemical stability and nutritional value, and also showed that even low temperatures have a direct impact on its stability. Less exposure of vitamin C to atmospheric oxygen may reduce the rate of oxidation and preserve the stability. It suggested that low acidity is an adequate environment for the storage of vitamin C and preventing the use of vitamin C as an additive to any food or beverage containing iron ions (Fe<sup>+3</sup>), as this contributes to the loss of stability of vitamin C and thus has a negative impact on its nutritional value.

### DECLARATION

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### **CONFLICT OF INTEREST**

No conflict of interest associated with this work.

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## **AUTHOR PARTICIPATION**

Husni Farah conceptualized and supervised the article. Jehad Alhmoud and Husni Farah wrote the initial draft and revised subsequent drafts. Talal Al-Qaisi, Khalid M. Alqaisi and Ali Atoom analyzed the manuscript. Husni Farah, Jehad Alhmoud, Khalid Shadid, Ashok Shakya reviewed and edited prior to submission. Atef Al-Othman did the practical work in the lab. All authors have read and agreed to the published version of the article.

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