

## Utilization of Calcium Carbonate to Improve the Nutritional Quality of Vegetable and Fruit Juices via Reducing Oxalic Acid

,Abdulrahman H. Laftah<sup>1,\*</sup>, Nawfal Alhelfi<sup>1</sup>, Sadeq K. Al. Salait<sup>2</sup>

Department of Food Science, College of Agriculture, University of Basra, Basra, Iraq<sup>1</sup>

Hematologist, Oncology unit, Al-Sadr Hospital, Basra, Iraq<sup>2</sup>

Corresponding author: uneabdo@yahoo.com\*

### Abstract:

Oxalic acid in vegetable and fruit juices had a significant health concern due to its potential to form insoluble calcium oxalate, which can lead to kidney stones. This study investigates the effect of calcium carbonate in reducing oxalic acid levels in various vegetable and fruit juices. High-Performance Liquid Chromatography (HPLC) was employed to measure oxalic acid, water- and fat-soluble vitamins, and mineral content before and after calcium carbonate was added. The results demonstrated a significant reduction in oxalic acid levels post-treatment from (41.965, 89.77, and 79.59 ppm) to (3.92, 4.83, and 5.76 ppm) in VFD1, VFD2, and VFD3, respectively. Additionally, there was an observed increase in the overall vitamins, and minerals. Moreover, the results of pH showed there was a significant increase between formulas, where VFD1 was increased from 3.81 to 4.3, VFD2 from 3.84 to 4.35, and 3.7 to 4.25 in VFD3. These findings suggest that calcium carbonate effectively reduces oxalic acid and enhances the nutritional profile of the juices. This method could be a viable approach for improving nutritional quality and safety of vegetable and fruit juices

**Keywords:** Calcium carbonate, fruits and vegetables, minerals, oxalic acid, and vitamins

### Introduction

Culturally, fruits and vegetables are vital food that contain the necessary nutrients and bioactive components for nutrition and chronic diseases. Regular intake has therefore become desirable, especially with the emerging trend of lifestyle diseases including obesity, diabetes, and cardiovascular diseases (1). Fruits and vegetables are important as an antioxidant, due to its reduce the oxidative stress, which is a major factor in many chronic diseases. The natural C and E vitamin form an antioxidant defense system that counteracts free radicals in the body, and then lowering cellular damage and inflammation (2). For example, the previous studies revealed, and for many times that the fruits and vegetables consumption increased have an inverse

relationship with the risk of coronary heart as well as specific types of cancer disease, those explained the fruits and vegetables could be as normal antioxidant (1). In addition, their intake help the body obtained necessary nutrients, and improves the natural immunity of the human body.

Fruits and vegetables are commonly recommended for their health-promoting properties; but, they contain oxalate and phytic acid, which is considered anti-nutritional factors when consumed in large, amounts. Oxalic acid mainly presents as calcium oxalate, that its a role for raising urine oxalate levels (hyperoxaluria), thus cause those stones in kidney. This risk especially with the juice produced from spinach, beet greens, rhubarb,

and some berries (3, 4). The over intake of fruit juices, particularly, which high in oxalates could increase the kidney stones and other diseases. For instance, in a case study described a man who experienced severe acute oxalate nephropathy (AON) and end-stage renal disease (ESRD) after consuming a high-spinach juice diet, and resulted intake everyday around 1500 mg, the average diet and for ten times (5). Hyperoxaluria can worsen nephrocalcinosis and chronic kidney disease (CKD) (6,7). The preparation of these juices are also play a significant role in oxalate level of juice detected. Juices are prepared using the masticating juicer have higher concentrations in oxalate than using a high-speed blender, which results in smoothie-like juice in low concentration of oxalate (8). The boiled vegetables tend to reduce soluble oxalates, while those cooked in wok could increase the soluble oxalates (9). In order to manage the negative effects for high consumption of oxalate, it is recommended to consume semi-ripe fruits and added the calcium salts to juices for lower the soluble oxalate content without flavor alterations (9, 10).

Therefore, the calcium carbonate to vegetable-fruit juices could be as an effective way to influence oxalate absorption and the related risks. Calcium is necessary for several biological processes such as maintenance of the bone health; it is a greatly inhibits the absorption of oxalate in gastrointestinal tract. (11). This helps for preventing kidney stones formation, the soluble oxalates are converted

into insoluble forms which are not absorbed in the digestive system (12). The selection of the calcium salt is crucial. Although calcium citrate has higher soluble calcium content and better absorption, calcium carbonate is more effective in decreasing oxalate absorption; thus, it is suitable for supplementation (13).

The aim of this study to reduce the formula concentration of oxalic acid in fruit and vegetables juice by adding calcium carbonate, as well as study the water and fat- soluble vitamins and minerals before and after adding calcium carbonate.

#### Materials and Methods

##### Fruits and Vegetables

Fresh vegetables and fruits were bought from a market in Basra, Iraq, such as green apple, carrot, beet rot, lemon, ginger, spinach, and peppermint. Soil, dead leaves, and stalks ends were removed using a stainless-steel knife. And then processed using a masticating juicer (Oscar 9000), Dongah Industrial Co., Ltd, Gyeongsangnam Do, South Korea). The juicer produced two fractions, a clear juice fraction, and a pulpy fiber fraction, which was normally discarded. Both fractions were subsequently either analyzed immediately or stored at -20°C until analysis.

Preparation of the Vegetable – Fruit juice formula

Formulations juice have been prepared from vegetables and a fruit as clarified in Table 1 and VFD is a code to descript the vegetables – fruits juice formulation.

**Table (1): Vegetables- Fruits content in VFD1, VFD2, and VFD3**

No.	Apple	Carrot	Beet Root	Ginger	Lemon	Mint	Spinach	Total
VFD1	25 mL	50 mL	-	1.5 mL	8 mL	15.5 mL	-	100 mL
VFD2	25 mL	10 mL	35 mL	1.5 mL	8 mL	15 mL	5.5 mL	100 mL
VFD3	32 mL	-	45 mL	-	8 mL	15 mL	-	100 mL

Whereas, Table1 included, VFD1: Apple, carrot, ginger, lemon, and mints (V:V); VFD2: Apple, carrot, ginger, lemon, mints, spinach, and beetroot (V:V); VFD3: Apple, lemon, mints, and beetroot (V:V).

#### Determination of oxalic acid and vitamins

Oxalic acid and vitamins were determined before and after adding calcium carbonate by HPLC. HPLC condition model SYKAM (Germany) it was used for analyses. The mobile phase for oxalic acid determination was an acetonitrile: D.W: (70:30) at flow rate at mL/min, and the detector was UV- 230 nm (14). In contrast, fat and water – soluble vitamins was an isocratic acetonitrile: D.W: (75: 25 ) at flow rate at 0.7 mL/min, and the detector was UV- 280 nm (15). Also, column was C18 – ODS (25 cm \* 4.6 mm).

#### Estimation of minerals:

Minerals in the formulas were determined before and after adding calcium carbonate by Atomic Absorption device, where they were digested using the acid digestion or wet washing method, as per the APHA method (16). Approximately 3 grams of the sample intended for digestion was placed inside a 25 mL Griffin beaker. Subsequently, 3 mL of concentrated perchloric acid solution was added. The beaker was then covered with a watch glass and gently heated on an electric hot plate. The temperature was raised gradually to complete the digestion process. Once the mixture reached near-dryness, the beaker was left to cool. Next, 3 mL of concentrated nitric acid was added. The beaker was covered and the mixture was heated until digestion was completed, resulting in a thin, lightly-colored mixture known as a "light-colored digestate". The mixture was then evaporated until it was near dry. Afterward, 5 mL of hydrochloric acid solution, diluted with water at a 1:1 ratio, was added. The mixture

was heated to dissolve the remaining sample post-digestion. Distilled water was then added, followed by filtration to remove undissolved residues. The solution volume was adjusted based on the anticipated concentration in the samples to 100 mL, 50 mL, or less. Once prepared, the sample was ready for analysis. The absorbance of these digested samples was measured using an Atomic Absorption device, specifically the SHEMADZU AA 7000 model.

#### Measurement of pH

The pH of the formulations was determined before and after adding calcium carbonate by using a pH-meter instrument, with 10 ml of each formula was used for the measurement (17).

#### Calcium Carbonate addition

Calcium carbonate supplementation tablet (Iraq-Bagdad) 100 mg was added to 100 mL of juices in a conical flask for three times in each, then the contents were well stirred for one minute. There was no incubation period and it was carried out at room temperature (25°C), no additional water was added (18).

#### Statistical Analysis

All results were presented as mean  $\pm$  SD. Three pair wise comparisons were made between the three groups using independent t-test to determine the differences between the groups. To address the issue of multiple comparisons, the p-value significance level was corrected using the Bonferroni method. The p-value was less than 0.05 in all the tests conducted in this study hence the results are statistically significant. We defined the p-value less than 0.05 as statistically significant in this study and after adjusting for the variables,  $p < 0.05$  was considered significant. Data analysis was done using SPSS statistical tool and data processing and presentation done using Microsoft excel.

## Results and Discussion

### Determination of oxalic Acid

Figure 1 shows the estimation of oxalic acid of the three formulas before and after adding  $\text{CaCO}_3$ . The analysis of the oxalic acid content in the juice formulations was done by HPLC and resulted in the reduction of the oxalic acid content for all three formulations when calcium carbonate ( $\text{CaCO}_3$ ) was added, standard of oxalic acid and the HPLC of formulas results before abdominal after adding

$\text{CaCO}_3$  are shown in fig. 2,3, and respectively. In VFD1, the oxalic acid content was reduced from 31.965 ppm to 2.920 ppm after the addition of  $\text{CaCO}_3$ , VFD2 also declined from 79.779 ppm to 3.839 ppm. Also in VFD3, the content of oxalic acid reduced from 69.592 ppm to 4.768 ppm when  $\text{CaCO}_3$  was added. The findings of this research therefore support the hypothesis that the inclusion of  $\text{CaCO}_3$  in the juice formulations is useful in minimizing the levels of oxalic acid, as revealed by the

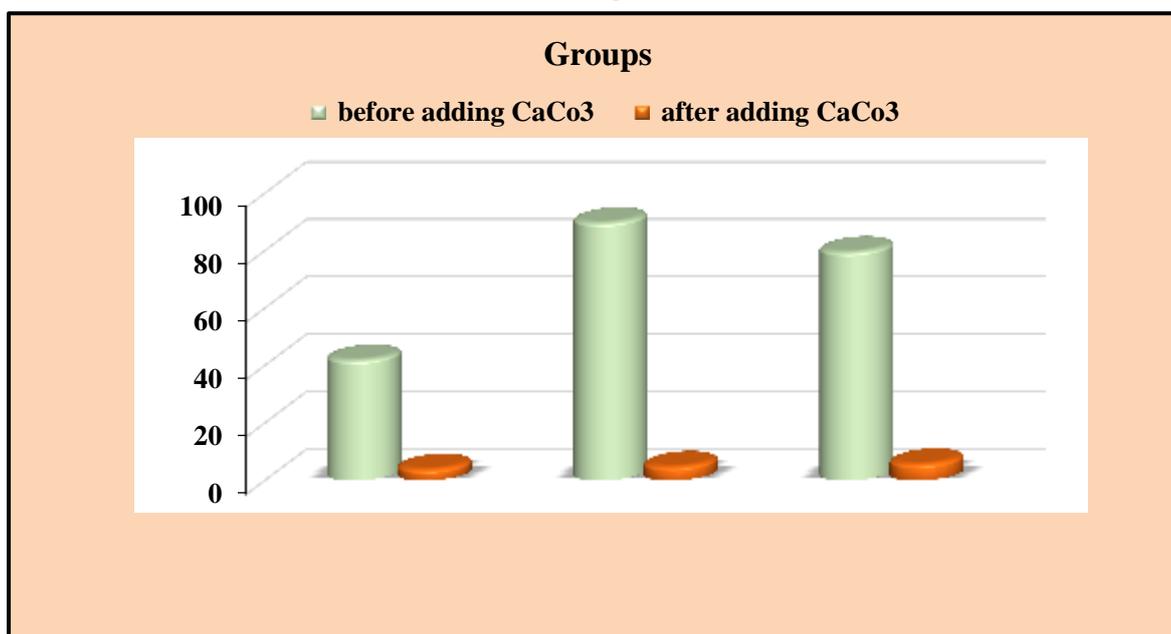


Fig. (1): Estimating the oxalic acid content in three types of juices before and after the addition of calcium carbonate. \*: Significant difference between groups ( $p$  value  $\leq 0.05$ ), NS: Nonsignificant difference between groups ( $p$  value  $> 0.05$ )

The supplementation of calcium carbonate ( $\text{CaCO}_3$ ) in the juice formulations was mainly to decrease the amount of oxalic acid as it is a known antigestapoic agent and may lead to the formation of kidney stones if taken in large

quantities. The success of this approach can be seen from the improvement in the parameters of acidity and the pH level in all the formulas (18.)

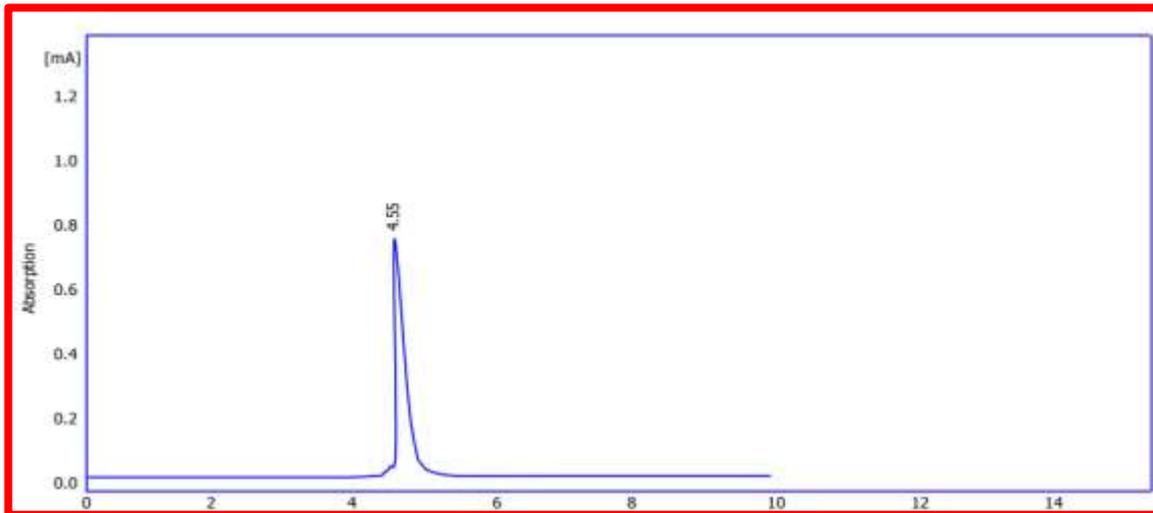


Fig. 2 Chromatograph standard of Oxalic acid before adding Calcium Carbonate

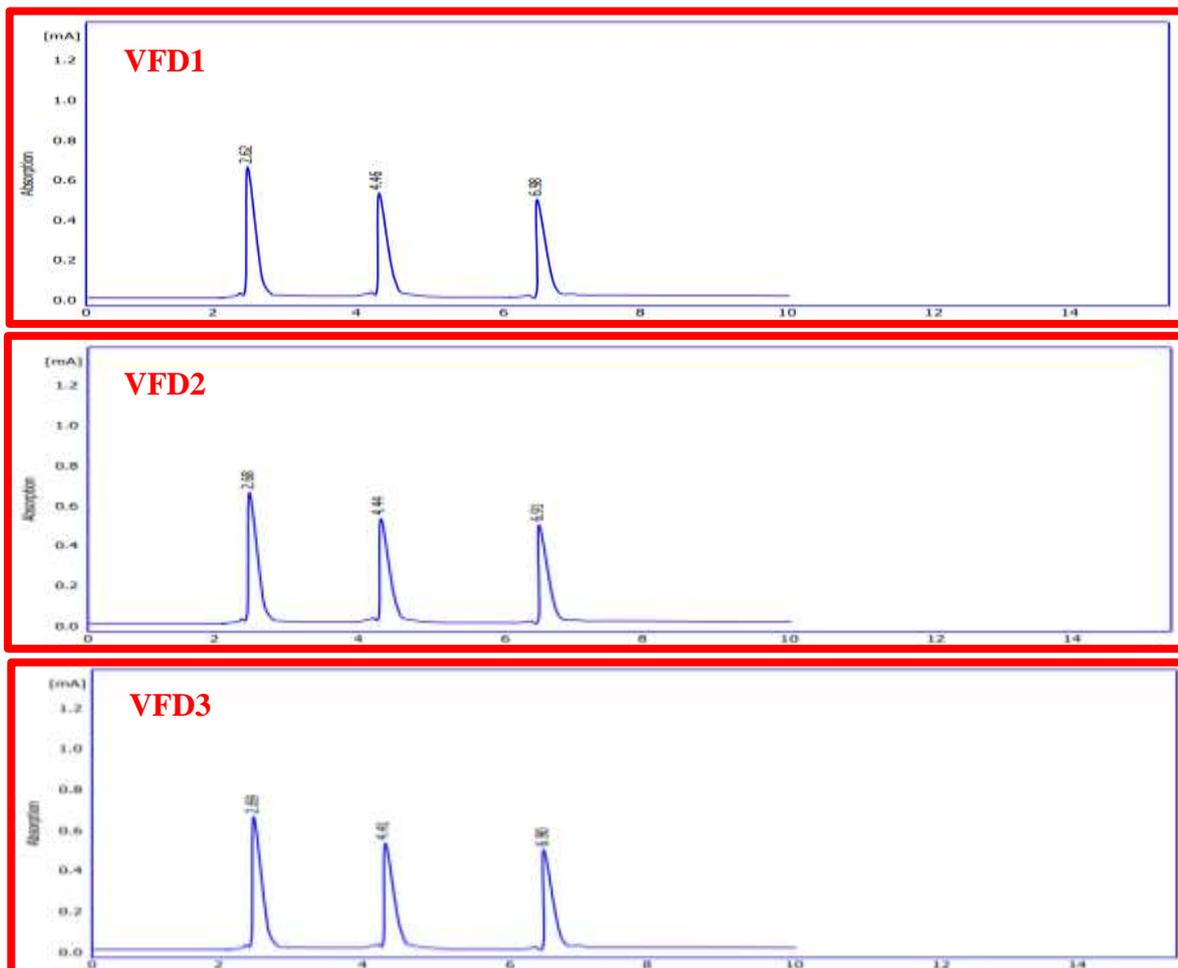


Fig. 3 Chromatograph value of Oxalic acid before adding CaCO<sub>3</sub>

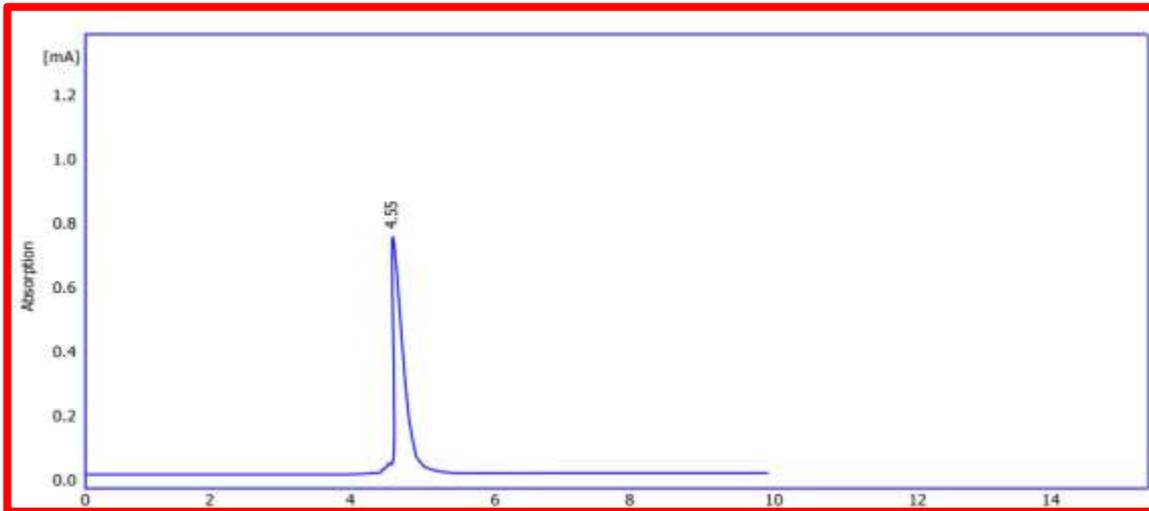


Fig. 2 Chromatograph standard of Oxalic acid after adding Calcium Carbonate

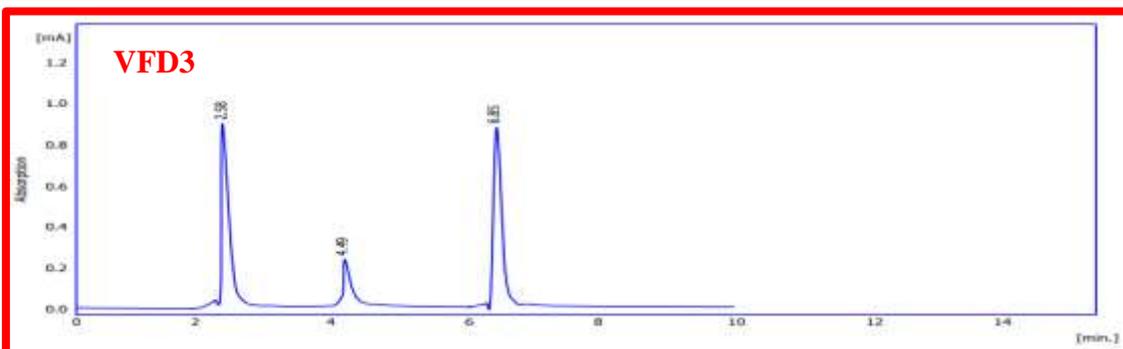
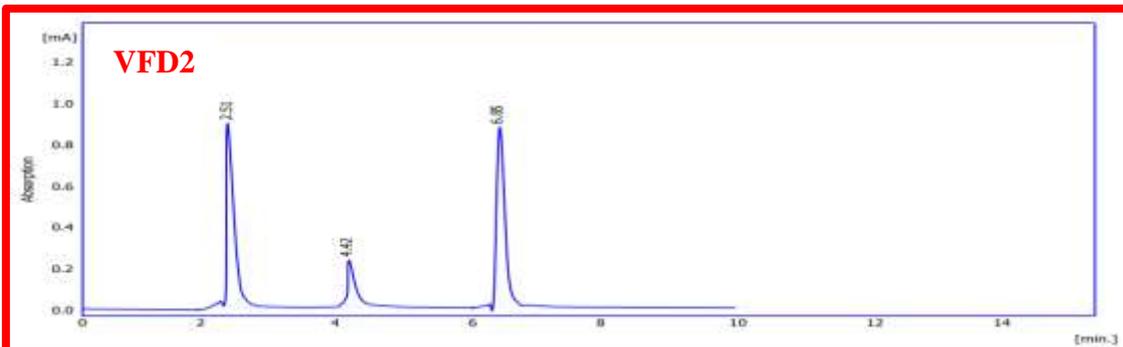
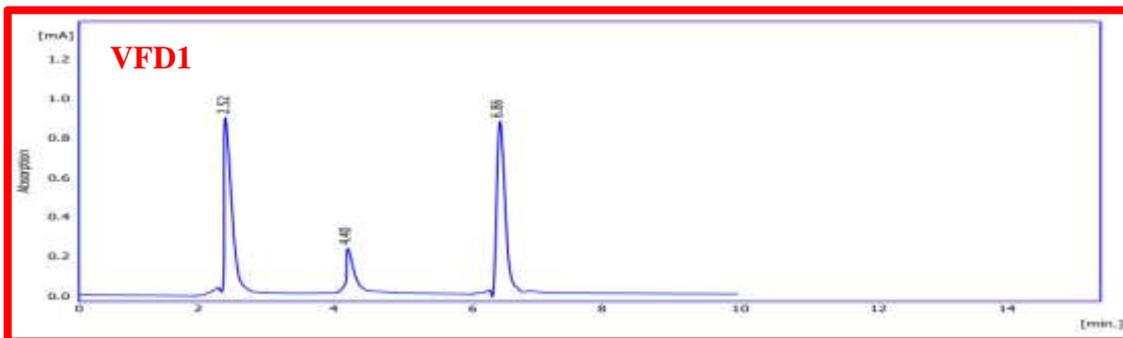


Fig. 4 Chromatograph value of Oxalic Acid After adding CaCO<sub>3</sub> e

### Measurements of pH

Table (1): shows the pH of vegetables – fruits formulations before and after  $\text{CaCO}_3$ . In VFD1, the initial pH was 3.81 and increased to 4.3; however, for VFD2, it increased to 3.84 to 4.35. VFD3 which had an initial pH of 3.7, increased to 4.25 after the addition of  $\text{CaCO}_3$ . These changes are primarily attributed to the .(

**Table (2): Shows the pH of vegetables – fruits formulations before and after adding  $\text{CaCO}_3$**

Formulation	pH before adding $\text{CaCO}_3$	pH after adding $\text{CaCO}_3$
VFD 1	3.81	4.3
VFD 2	3.84	4.35
VFD 3	3.7	4.25

Also,  $\text{CaCO}_3$  reacts with citric acid in fruits such as green apples and lemons to form calcium citrate; also  $\text{CaCO}_3$  reacts with oxalic acid in Beet juice and form calcium oxalate. The decrease in both oxalic and citric acids not only decreases the total acidity of the juices, thus making them less sour tasting but also enhances the bioavailability of calcium as stated by (19). In conclusion, the addition of  $\text{CaCO}_3$  is very significant in altering the chemical composition of these juice formulations positively by decreasing the measures of oxalic acid and increasing the calcium absorption in the body.4.2.3

### Determination of water soluble vitamins

In Table 3 shows the concentration of water soluble vitamins before and after adding  $\text{CaCO}_3$  over all formulas. Whereas, B1 vitamin, the concentrations rose from 92.99 mg/kg to 245.25 mg/kg in VFD1, also from 51.72 mg/kg to 161.23 mg/kg in VFD2, from 54.09 mg/kg to 192.16 mg/kg in VFD3. In same trends were observed for other: B2 Vitamin got increased from 80.54 mg/kg to

interaction between  $\text{CaCO}_3$  and oxalic acid found in fruit and vegetable juices, especially beet juice which is rich in oxalic acid. This reaction leads to the formation of calcium oxalate which is insoluble in the juice and therefore through a process of filtering, the concentration of oxalic acid is reduced (19

284.29 mg/kg in VFD1, and from 63.38 mg/kg to 266.73 mg/kg in VFD2, also from 67.46 mg/kg to 161.52 mg/kg in VFD3. Moreover, Vitamin B3, increased from 53.76 mg/kg to 183.45 mg/kg in VFD1, and from 37.97 mg/kg to 200.03 mg/kg in VFD2, also from 36.02 mg/kg to 217.69 mg/kg in VFD3. The other vitamins followed this pattern, with Vitamin B5, B6, B12 and B9 rising significantly in all the formulas. The enhanced concentrations of water-soluble vitamins in all three juice formulations after the addition of calcium carbonate ( $\text{CaCO}_3$ ) can be associated with the following factors regarding the stability of vitamins. It has been established that  $\text{CaCO}_3$  can neutralize the acidic components of juices, which in turn may help in preventing the degradation of sensitive vitamins. Acidic conditions cause the hydrolysis and oxidation of water-soluble vitamins especially the B group of vitamins due to their solubility in water (20). Thus, raising pH and decreasing acidity,  $\text{CaCO}_3$  probably helped to stabilize these vitamins and increase their concentration

after treatment. For instance, the increase in vitamin B1 (thiamine) content may be attributed to the neutralization of acid conditions that otherwise break down thiamine by  $\text{CaCO}_3$ . It has been established that B12 is easily broken down by acids and its solubility is highly dependent on the Ph level (21).

Similarly, the concentration of vitamins B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), and B6 (pyridoxine) was also seen to increase significantly in concentration which can also be attributed to the protective mechanism of  $\text{CaCO}_3$  (18).

**Table: 3 Water- soluble vitamins content in different formulas before and after adding  $\text{CaCO}_3$**

Vitamins (mg/Kg)	Formulas	Before Adding $\text{CaCO}_3$	After Adding $\text{CaCO}_3$
Vitamin B1	VFD1	92.99	245.25
	VFD2	51.72	161.23
	VFD3	54.09	192.16
Vitamin B2	VFD1	80.54	284.29
	VFD2	63.38	266.73
	VFD3	67.46	161.52
Vitamin B3	VFD1	53.76	183.45
	VFD2	37.97	200.03
	VFD3	36.02	217.69
Vitamin B5	VFD1	92.97	263.42
	VFD2	47.75	232.04
	VFD3	49.87	262.15
Vitamin B6	VFD1	75.51	213.01
	VFD2	53.6	196.24
	VFD3	59.05	241.06
Vitamin B12	VFD1	90.25	243.75
	VFD2	92.77	193.52
	VFD3	101.32	247.61
Vitamin C	VFD1	418.83	502.1
	VFD2	182.78	238.34
	VFD3	528.97	627.37

The ability of  $\text{CaCO}_3$  to increase the solubility of vitamins, particularly its ability to prevent vitamins from binding with oxalates also deserves consideration. Some oxalates can chelate with certain B vitamins and this makes the body to be deprived of the vitamins. Calcium oxalate formation by sequestering oxalic acid would have also led to increased

free concentration of these vitamins in the orange juice since  $\text{CaCO}_3$  was added to the juice and thus improved the nutritional value of the juice (18). In summary, the addition of  $\text{CaCO}_3$  to juice formulations appears to have a dual benefit: It also plays a role in decreasing the oxalic acid content but at the same time enhances the stability and solubility

of water-soluble vitamins. From this finding, it can be deduced that  $\text{CaCO}_3$  may be a useful supplement in juice production especially in products that seek to increase nutritional and vitamin intake and uptake.

#### Fat - soluble Vitamins

Table (4) shows, the results of fat-soluble vitamins such as D, E, A and C vitamins in the juice formulations with and without the addition of calcium carbonate ( $\text{CaCO}_3$ ). There was a general increasing in the vitamins content for all the three formulations. As for Vitamin D, the concentrations in VFD1 rose from 656.09 mg/kg to 780.74 mg/kg, in the second formula 211.9 mg/kg to 313.23 mg/kg, and in VFD3 from 727.5 mg/kg to 832.49 mg/kg. Same to Vitamin E, VFD1 increased from 341.7 mg/kg to 474.18 mg/kg, in VFD2, it was 365.44 mg/kg to 450.27 mg/kg, and in the third 419.68 mg/kg to 466.77 mg/kg.

Other nutrients such as Vitamin C also rose with VFD1 which increased from 418.83 mg/kg to 512.1 mg/kg, VFD2 from 172.78 mg/kg to 228.34 mg/kg, and for VFD3 it was 528.97 mg/kg to 627.37 mg/kg. Last of all, the Vitamin A content rose in VFD1 to 504.83 mg/kg to 633.74 mg/kg, in Formula 2 from 330.76 mg/kg to 427.5 mg/kg, and in VFD3 from 721.26 mg/kg to 898.34 mg/kg.

The high increases in these fat soluble vitamins indicate that  $\text{CaCO}_3$  is very useful in improving the stability and shelf life of these vitamins in the juice products. Calcium carbonate may neutralize the acidity of the fruit juices and create a more favourable environment for the retention of the water-soluble vitamins, mainly by shielding them from the oxidative breakdown which is favored in acidic mediums (20). Moreover,  $\text{CaCO}_3$  can also function as emulsifier that enhances the solubility of fat soluble vitamins and prevent their aggregation and or rancidity in the juice product (20).

(

**Table (4): Fat- soluble vitamins content in different formulas before and after adding  $\text{CaCO}_3$**

Vitamins (mg/Kg)	Formulas	Before Adding $\text{CaCO}_3$	After Adding $\text{CaCO}_3$
Vitamin D	VFD1	666.09	790.74
	VFD2	221.9	333.23
	VFD3	737.5	842.49
Vitamin E	VFD1	351.7	484.18
	VFD2	375.44	460.27
	VFD3	429.68	476.77
Vitamin A	VFD1	504.83	633.74
	VFD2	330.76	427.5
	VFD3	721.26	898.34

The decreased level of oxalic acid because of the addition of  $\text{CaCO}_3$  may also help in increasing the absorption and utilization of these vitamins since they are less bound or complexed with oxalic acid. According to

these results,  $\text{CaCO}_3$  has also potential for application not only in the reduction of acidity and the stabilization of the juice matrix but also in the preservation or even the enhancement of the nutritional value of the

juices by maintaining higher levels of fat-soluble vitamins, thus it is a useful additive in the processing of fruit juices from the nutritional point of view (18).

#### Elemental Composition

In Table 5 there was significant difference in elemental composition for (Fe, Zn, Cu, Mn) Levels between groups before and after adding CaCO<sub>3</sub> for (VFD1, VFD2, and VFD3) at (p value < 0.05) while there was no significant difference in elemental composition (Se) levels between groups before and after adding CaCO<sub>3</sub> for (VFD1, VFD2, and VFD3) at (p value > 0.05) the data presents the effect of CaCO<sub>3</sub> addition on the elemental composition of three different formulas, with a focus on iron (Fe), zinc (Zn), copper (Cu), selenium (Se), and manganese (Mn) were the highest post-treatment values for each element across the formulas: Iron (Fe): The highest value after CaCO<sub>3</sub> addition is in VFD1, with a concentration of 27.8 mg/kg. Zinc (Zn): The highest value after treatment is also found in VFD1, with a concentration of 20.6 mg/kg. Copper (Cu): The highest value after CaCO<sub>3</sub> addition is in VFD1, with a concentration of 11.5 mg/kg. Selenium (Se): The highest value post-treatment is in VFD1, with a concentration of 5.9 mg/kg, although this change is not statistically significant. Manganese (Mn): The highest value after CaCO<sub>3</sub> addition is in VFD1, with a concentration of 12.6 mg/kg. These values suggest that VFD1 generally exhibits the

highest concentrations of elements after CaCO<sub>3</sub> treatment across most of the tested elements.

The enhanced iron, zinc and manganese contents in all the formulas might be as a result of the decrease in oxalic acid by CaCO<sub>3</sub>. Oxalic acid is known to chelate these minerals and thus decrease their absorption rates (22). Calcium oxalate, for instance, may have been formed from calcium carbonate, CaCO<sub>3</sub>, and this might have led to the release of more of these important minerals into the juice (23)

On the other hand, this decrease in copper levels after the addition of CaCO<sub>3</sub> may be attributed to the formation of copper complexes that are insoluble or reactions between copper and calcium ions that result in precipitation and, therefore, lower concentrations of measurable copper in the juice formulations (24). Although not statistically significant, the changes in selenium levels imply that CaCO<sub>3</sub> might have a stabilizing impact; nevertheless, the lack of substantial changes shows that the variation in selenium content might not be linked to the incorporation of CaCO<sub>3</sub>. In general, it seems that CaCO<sub>3</sub> increases the solubility of some minerals such as iron, zinc and manganese through neutralising oxalates whereas it may reduce copper levels through precipitation. These observations highlight the interrelationships between CaCO<sub>3</sub> and the mineral content of juice concentrates, which may have implications for the nutritional value of the finished product

**Table: 5 Elemental Composition content in different formulas before and after adding calcium carbonate**

Elements	VFD 1			VFD 2			VFD 3		
	Before adding CaCo3	After adding CaCo3	P value for Paired sample t test	Before adding CaCo3	After adding CaCo3	P value for Paired sample t test	Before adding CaCo3	After adding CaCo3	P value for Paired sample t test
Fe	20.5	27.8	0.033 *	16.5	18.9	0.003 *	17.8	20.5	0.018 *
Zn	14.9	20.6	0.034 *	10.2	13.6	0.034 *	11.5	14.8	0.087 *
Cu	16.5	11.5	0.016 *	8.9	5.2	0.037 *	9.5	6.5	0.0218 *
Se	3.5	5.9	0.104 NS	2.1	3	0.293 NS	2.5	3.4	0.096 NS
Mn	7.8	12.6	0.025 *	5.7	8.9	0.015 *	6	9.4	0.043 *

:\*Significant difference between groups (p value  $\leq$  0.05). NS: Nonsignificant difference between groups (p value  $>$  0.05)

In Table 6: there was significant difference in elemental composition for (Ca, Na, K, P) Levels between groups before and after adding CaCo<sub>3</sub> for (VFD1, VFD2, and VFD3) at (p value  $<$  0.05) while there was nonsignificant difference in elemental composition (Mg) Levels between groups before and after adding CaCo<sub>3</sub> for (VFD1, VFD2, and VFD3) at (p value  $>$  0.05), table 5 presents the effect of CaCO<sub>3</sub> addition on the elemental composition of three formulas, focusing on sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), and phosphorus (P). The highest post-treatment values for each element across the formulas: Sodium (Na): The highest value after CaCO<sub>3</sub> addition was in VFD1, with a concentration of 80.5 mg/kg. Calcium (Ca): The highest post-treatment value was also found in VFD1, at 40.2 mg/kg. Magnesium (Mg): The highest value after treatment was in VFD1, with a concentration

of 74.9 mg/kg, although this change is not statistically significant. Potassium (K): The highest post-treatment value was in VFD1, with a concentration of 960.5 mg/kg. Phosphorus (P): The highest value after CaCO<sub>3</sub> addition was in VFD1, with a concentration of 450 mg/kg. Incorporating calcium carbonate (CaCO<sub>3</sub>) into the juice formulations led to changes in the mineral content of the juices as shown in the sodium (Na), calcium (Ca), magnesium (Mg), potassium (K) and phosphorus (P). The content of sodium and calcium reduced on all formulas after the addition of CaCO<sub>3</sub>. The decline in calcium and sodium concentrations in your juice after the addition of calcium carbonate (CaCO<sub>3</sub>) was most probably due to the formation of precipitates. When CaCO<sub>3</sub> was added, the pH of the juice increases and also calcium ions which can complex with carbonate or some other components of the

juice to form a solid phase and hence remove them from the liquid phase. It could also go into competition with calcium and form less soluble compounds and therefore decrease the quantities recoverable by analysis. The overall consequence is that both calcium and sodium are less likely to exist in the soluble state, which is in agreement with the observed decrease in their concentrations.

However, potassium and phosphorus content rose substantially after the addition of  $\text{CaCO}_3$ , which may indicate that  $\text{CaCO}_3$  could stabilise these minerals and prevent their binding to oxalates or other compounds, and thus increase their free and measurable

concentration in the juice (22). The magnesium concentrations followed the similar trend as calcium and also increased across the formulas; nonetheless, these variations were also insignificant suggesting that the effect of  $\text{CaCO}_3$  on the magnesium levels could be more complex and may be influenced by other factors within the juice matrix (24.)

In general, it is observed that the presence of  $\text{CaCO}_3$  improves the solubility of some minerals for instance potassium and phosphorus but decreases the levels of sodium and calcium.

**Table (6): Elemental Composition content in different formulas before and after adding calcium carbonate**

Elements	VFD 1			VFD 2			VFD 3		
	Before adding $\text{CaCO}_3$	After adding $\text{CaCO}_3$	P value for Paired sample t test	Before adding $\text{CaCO}_3$	After adding $\text{CaCO}_3$	P value for Paired sample t test	Before adding $\text{CaCO}_3$	After adding $\text{CaCO}_3$	P value for Paired sample t test
<b>Na</b>	96.9	80.5	0.028 *	70.9	35.6	0.019 *	74.5	50.2	0.039 *
<b>Ca</b>	184.9	40.2	0.017 *	141.5	28.9	0.004 *	150.6	33.6	0.007 *
<b>Mg</b>	66.9	74.9	0.022 NS	47.9	55.6	0.002 NS	49.8	59.5	0.012NS
<b>K</b>	850.6	960.5	0.038 *	680.9	710.5	0.033 *	700	750.6	0.026 *
<b>P</b>	410	450	0.039 *	311	340	0.034 *	320	360	0.024 *

:\*Significant difference between groups (p value  $\leq 0.05$ ), NS: Nonsignificant difference between groups (p value  $> 0.05$ )

### Conclusion

Adding calcium carbonate is a very efficient way of decreasing the oxalic acid content of fruit and vegetable juices. Calcium ions

combine with oxalate to form an insoluble compound calcium oxalate which can be filtered out from the juice. This process not

only reduces the oxalate level but also enhances the nutritive value by reducing the chances of oxalate induced health conditions including kidney stones. Furthermore, calcium carbonate is approved for use in the food industry, and is considered safe and efficacious for reducing oxalate levels.

## References

-1

Callen, C., Bhatia, J., Czerkies, L., Klish, W. J., & Gray, G. M. (2018). Challenges and considerations when balancing the risks of contaminants with the benefits of fruits and vegetables for infants and toddlers. *Nutrients*, 10(11), 1572. <https://doi.org/10.3390/nu10111572>

-2 Alakaam, A. and Lemacks, J. (2015). Fruit and vegetable consumption, fat intake, and physical activity participation in relation to socio-demographic factors among medically underserved adults. *AIMS Public Health*, 2(3), 402-410. <https://doi.org/10.3934/publichealth.2015.3.402>

-3 Garcia, C., Guérin, M., Souidi, K., & Remize, F. (2020). Lactic fermented fruit or vegetable juices: past, present and future. *Beverages*, 6(1), 8. <https://doi.org/10.3390/beverages6010008>

-4 Barghouthy, Y. and Somani, B. K. (2021). Role of citrus fruit juices in prevention of kidney stone disease (ksd): a narrative review. *Nutrients*, 13(11), 4117. <https://doi.org/10.3390/nu13114117>

-5 Harsha, Chaudhari., Jennine, Michaud., Nitya, Srialluri., Smita, Mahendrakar., Christine, Granz., Michael, Yudd. (2022). Acute Oxalate Nephropathy Caused by Excessive Vegetable Juicing and Concomitant Volume Depletion. *Case reports*

Consequently, it is useful for the increase of the safety and quality of fruit and vegetable juices.

in nephrology, 2022:1-4. doi: 10.1155/2022/4349673

-6 Salgado, N., Silva, M. A., Figueira, M. E., Costa, H. S., & Albuquerque, T. G. (2023). Oxalate in foods: extraction conditions, analytical methods, occurrence, and health implications. *Foods*, 12(17), 3201.

-7 Roswitha, Siener., Ana, Seidler., Ruth, Hönow. (2021). Oxalate-rich foods. *Food Science and Technology International*, 41:169-173. doi: 10.1590/FST.10620

-8 Erliana, Novitasari., Geoffrey, P., Savage. (2021). Oxalate contents in green juice prepared using either a high-speed blender or a masticating juicer. 306:04004-. doi: 10.1051/E3SCONF/202130604004

-9 Geoffrey, P., Savage., Warinporn, Klunklin. (2018). Oxalates are Found in Many Different European and Asian Foods - Effects of Cooking and Processing. *Journal of Field Robotics*, 7(3):76-81. doi: 10.5539/JFR.V7N3P76

-10 Kamble, B. S., Acharya, A. P., Girhe, B. E., & Salunkhe, H. J. (2021). Rating of oxalate ions of various fruits in different phases. 765-770. doi: 10.48175/IJARSCT-1039

-11 Bong, W., Vanhanen, L. P., & Savage, G. P. (2017). Addition of calcium compounds to reduce soluble oxalate in a high oxalate food system. *Food Chemistry*, 221, 54-57.

<https://doi.org/10.1016/j.foodchem.2016.10.031>

-12 Savage, G. P. and Vanhanen, L. P. (2018). Oxalate contents of raw, boiled, wok-fried and pesto and juice made from fat hen (*Chenopodium album*) leaves. *Foods*, 8(1), 2. <https://doi.org/10.3390/foods8010002>

-13 Zheng, J., Zhou, Y., Li, S., Zhang, P., Zhou, T., Xu, D. & Li, H. (2017). Effects and Mechanisms Of Fruit And Vegetable Juices On Cardiovascular Diseases. *IJMS*, 3(18), 555. <https://doi.org/10.3390/ijms18030555>

-14 Nozal, M. J., Bernal, J. L., Gómez, L. A., Higes, M., & Meana, A. (2003). Determination of oxalic acid and other organic acids in honey and in some anatomic structures of bees. *Apidologie*, 34(2), 181-188.

-15 Kozhanova, L. A., Fedorova, G. A., & Baram, G. I. (2002). Determination of water- and fat-soluble vitamins in multivitamin preparations by high-performance liquid chromatography. *Journal of Analytical Chemistry*, 57, 40-45.

-16 APHA (American Public Health Association), (2017), Standard Methods for the Examination of Water and Wastewater 23th Edition, 800 I Street, NW, Washington DC, USA

-17 Rezaei, F., Nejati, R., Sayadi, M., & Nematollahi, A. (2021). Diazinon reduction in apple juice using probiotic bacteria during fermentation and storage under refrigeration. *Environmental Science and Pollution Research*, 28(43), 61213-61224.

-18 Vanhanen, L. P. (2018). Oxalate content of green juices and strategies for reduction of soluble oxalate content: A thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy at Lincoln University (Doctoral dissertation, Lincoln University.)

-19 Younes, M., Aquilina, G., Castle, L., Degen, G. H., Engel, K., Fowler, P., & Gundert-Remy, U. (2023). Re-evaluation of calcium carbonate (E 170) as a food additive in foods for infants below 16 weeks of age and follow-up of its re-evaluation as food additive for uses in foods for all population groups. *EFSA Journal*, 21(7). <https://doi.org/10.2903/j.efsa.2023.8106>

-20 Godoy, H. T., Amaya-Farfan, J., & Rodriguez-Amaya, D. B. (2021). Degradation of vitamins. In *Chemical changes during processing and storage of foods* (pp. 329-383). Academic Press.

-21 Rakuša, Ž. T., Roškar, R., Hickey, N., & Geremia, S. (2022). Vitamin B12 in foods, food supplements, and medicines—a review of its role and properties with a focus on its stability. *Molecules*, 28(1), 240. <https://doi.org/10.3390/molecules28010240>

-22 Akter, S., Netzel, M., Tinggi, U., Fletcher, M. T., Osborne, S. A., & Sultanbawa, Y. (2020). Interactions between phytochemicals and minerals in Terminalia ferdinandiana and implications for mineral bioavailability. *Frontiers in Nutrition*, 7. <https://doi.org/10.3389/fnut.2020.598219>

-23 Huang, P., Cheng, Y., Lu, W., Chiang, P., Yeh, J., Wang, C. (.), ... & Li, P. (2024). Changes in nutrient content and physicochemical properties of Cavendish bananas var. 'Pei Chiao' during ripening. *Horticulturae*, 10(4), 384. <https://doi.org/10.3390/horticulturae10040384>

-24 Shen, B., Chen, Z., Mao, H., Yin, J., Ren, Y., Dai, W., ... & Yang, H. (2024). Ctab-induced synthesis of two-dimensional copper oxalate particles: using L-ascorbic acid as the source of oxalate ligand. *RSC Advances*, 14(32), 23225-23231. <https://doi.org/10.1039/d4ra04181j>

