



Received: 26-06-2025
Accepted: 06-08-2025

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

Effects of Artificial Ripening on Nutritional Value of Orange Fruits in Bayelsa State, Nigeria

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Abstract

This study investigates the effects of artificial ripening of orange fruit using calcium carbide (CaC_2). This was done to gauge the impact of this chemical on the nutritional quality of the orange fruit and the wider implication on human health. The orange fruit were washed with clean water and put into ten litres plastic bucket with a perforated plastic sieve place at the bottom of the bucket with five (5g) grams of calcium carbide (CaC_2) under it and kept in a dark cupboard. Evaluation of proximate composition, anti-nutritional factors, mineral content and vitamin profile of oranges (citrus) was done using standard procedures. Result showed that a decrease in crude protein from $1.00 \pm 0.24\%$ to $0.40 \pm 0.02\%$ Lipid from $0.20 \pm 0.01\%$ to $0.10 \pm 0.01\%$ while carbohydrates content increased from $8.00 \pm 0.27\%$ to $18.90 \pm 0.43\%$ and moisture content increased from $88.00 \pm 0.26\%$ to $79.00 \pm 0.31\%$. Ash content declined from $0.80 \pm 0.05\%$ to $0.20 \pm 0.01\%$, indicating a general reduction in dry matter. Energy values increased from 33.67 ± 0.49 kcal to 69.69 ± 0.55 kcal. Anti-nutritional factors showed

significant reductions because tannins declined from $0.12 \pm 0.03\%$ to $0.01 \pm 0.00\%$, phytates from $0.35 \pm 0.05\%$ to $0.10 \pm 0.01\%$, and oxalates from $0.23 \pm 0.03\%$ to $0.03 \pm 0.00\%$. These reductions suggest improved nutrient bioavailability post-fermentation of orange. Mineral content increased in potassium from $0.09 \pm 0.01\%$ to $2.40 \pm 0.11\%$, iron from $2.60 \pm 0.11\%$ to $9.44 \pm 0.13\%$, and zinc from $0.70 \pm 0.05\%$ to $5.31 \pm 0.30\%$. Calcium and sodium increased slightly, while toxic metals (Pb, Cd, As) were not detected. Vitamin levels decreased. Vitamin C dropped from 37.8 ± 0.35 mg/100g to 8.40 ± 0.12 mg/100g, Vitamin A from 16.0 ± 0.22 mg/100g to 2.10 ± 0.04 mg/100g, and Vitamin B3 from 0.37 ± 0.02 mg/100g to 0.04 ± 0.01 mg/100g. All values are from control to treatment levels. Vitamin D was not detected in all samples. These findings indicate the use of Calcium Carbide (CaC_2) to induce fruit ripening caused alteration in nutrient content of the fruit and may cause adverse health outcomes.

Keywords: Artificial, Ripening, Nutritional, Orange, Bayelsa State, Nigeria

1. Introduction

Vitamins are important nutrient required in small quantity for the sustenance of life and are obtainable from fruits and vegetables. Fruits are the seed producing structures in higher plants, they are formed from the ovary after fertilization. Fruits constitute the route by which higher plants disperse seeds. Consequently, fruits account for a substantial fraction of the world's agricultural output, and the commonly used fruits such as orange have acquired extensive cultural and symbolic meanings ^[1]. Fruits are excellent source of vitamins and minerals, and are symbolic in improving immunity and contributes in preventing vitamin C and vitamin A deficiencies ^[2].

Individuals who eat fruits as part of an overall healthy diet generally have a reduced risk of chronic diseases ^[3]. The World Health Organization (WHO) recommends five servings of fruits and vegetables every day for a healthy living ^[3]. However, the palatability of fruits depends on the ripening process. Fruit ripening is a natural process in which the fruit goes through various chemical changes and gradually becomes sweet, colored, and gets soft and palatable for human consumption ^[4]. It is the process by which fruits attain their desirable flavour, quality, colour, palatable nature and other textural properties. The need of artificial fruit ripening is often encountered when the fruit-sellers offer fruits to the customers before due season. Fruit-sellers artificially ripen green fruits even during the due season to meet the high demand and make high profit of seasonal fruits. They also ripen fruits artificially to deal with the transportation and distribution issues. Transporting and distributing fruits from the farmers' orchards to consumers' baskets can take several days.

In recent time, rapid and fast ripening of fruits through artificial factors, has become a norm which should be restricted to preserve the quality and nutritional status of the fruits, during post-harvest period. Climacteric fruit's ripening has been found associated with the changes in fruit's gas composition due to rapid respiration resulting to ethylene production [5, 6]. A variety of literatures have conflicts in the results, about gas production in attached and detached fruits. Pre-harvest and post-harvest factors produce significant differences in ripening duration, physical, chemical and nutritional contents, and shelf life of the mangoes [7, 8]. Orange has been listed among the most cultivated tropical fruits, processed, shipped, preserved and marketed globally. Hence, is very necessary to investigate about the effects of artificial ripening on its nutritional and phyto-chemical content.

The aim of the study therefore is to investigate the impact of artificially induced ripening on the nutritional quality of orange fruits, specifically assessing changes in proximate composition, mineral content, vitamin levels, and anti-nutritional factors, in comparison to naturally ripened (fresh) oranges.

2. Materials and Methods

2.1 Procurement of Chemicals

Industrial grade calcium carbide was purchased from a local welding shop located in Aba, Abia State, Nigeria for use to ripen orange artificially.

Certified ethylene was also purchased from Aba market. Ethylene concentration in the chamber was checked by GC.

2.2 Sample Collection

2.2.1 Collection of Fruits

Mature orange fruits (unripe and naturally ripened fruits) were collected from an orange tree in Ogobiri town, near Amassoma in Bayelsa State. The harvested orange fruits were conveyed to the Pharmaceutical and Medicinal Chemistry Laboratory, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Amassoma Bayelsa State Nigeria.

2.3 Ripening Procedure

The orange fruits were washed with clean water drained and selectively picked into ten liters properly labelled plastic bucket with air tight cover, and kept in a dark cupboard. A

perforated plastic sieve was first placed at the bottom of the bucket with five (5g) grams of Calcium carbide (CaC_2) under it. Then five (5) oranges were placed on the sieve and covered tightly with labels of 3days, 7days, 10days and 14days respectively.

In addition to the test samples, a control experiment was set up for each corresponding time period (3 days, 7 days, 10 days and 14 days).

2.4 Sample Preparation

Fresh orange fruits were extracted using juice extractor and the juice was stored in a bottle tightly covered and stored in the refrigerator pending the analysis. The juice pulp was separated from the epidermal layer, oven dried at 400°C and kept in a black polyethylene bag, then stored in the refrigerator.

2.5 Proximate Analysis

Determination of Moisture Content, Ash Content, Fat content, Crude fiber, Crude protein, Carbohydrates and Total Energy Value were determined using standard procedures.

2.6 Evaluation of Ant-Nutrient in the Oranges

Determination of Tannins, Phytate, Oxalate and Phytic Acid were determined using standard procedures.

2.7 Determination of Selected Metals

Determination of Selected Metals from Orange Samples was done using wet digestion Method.

2.8 Determination of Vitamins in fruits

2.8.1 Data Analysis

The results are presented in simple percentages. The independent sample t-test was used to test for significance difference between the chemical induce and natural ripened fruits. Analysis of variance (ANOVA) was employed to test similarities and variability in levels of ripening between assessment days. Turkey HSD post HOC test was employed to separate means where variability occurred. SPSS® statistical tool kit software version 20.0 was used to aid the analysis.

3. Result

The results of the study are displayed in Tables 1 to 5.

Table 1: Proximate Composition of Fresh Orange and CaC_2 Ripened Orange

Sample	Moisture Content%	Ash content%	Protein Content%	Crude Fiber%	Crude Lipids %	CHO %	Energy (kcal)
Control (F. Orange)	88.00±0.26 ^a	0.80±0.05 ^a	1.00±0.24 ^a	2.00±0.04 ^a	0.20±0.01 ^a	8.00±0.27 ^a	33.67±0.49 ^a
Day 3	87.00±0.39 ^b	0.60±0.04 ^b	1.10±0.20 ^a	2.00±0.04 ^a	0.30±0.02 ^b	9.70±0.37 ^b	40.92±0.53 ^b
Day 7	85.00±0.37 ^c	0.40±0.02 ^c	0.80±0.04 ^b	1.80±0.26 ^b	0.20±0.01 ^a	12.20±0.49 ^c	47.98±0.43 ^c
Day 10	83.2±0.28 ^d	0.30±0.02 ^d	0.60±0.02 ^c	1.60±0.24 ^c	0.10±0.01 ^c	14.00±0.49 ^d	52.88±0.43 ^d
Day 14	79.00±0.31 ^e	0.20±0.01 ^e	0.40±0.02 ^d	1.40±0.12 ^d	0.10±0.01 ^c	18.90±0.43 ^e	69.69±0.55 ^e

Control (F. orange) = fresh orange. Means with different letter superscripts (a, b, c, d & e) on the same column indicate statistically significant differences ($P=0.05$)

Table 2 presents the changes in anti-nutritional factors **such as** tannins, phytates, and oxalates in a fresh orange and artificially ripened oranges.

Table 2: Results of Ant-Nutrients in CaC₂ ripened on fresh oranges

Sample	Tannins %	Phytate %	Oxalate %	Range of Tannins %	Range of Phytate %	Range of Oxalate %
Control (F. Orange)	0.12±0.03 ^a	0.35±0.05 ^a	0.23±0.03 ^a	0.05-0.15	0.20-0.50	0.10-0.30
Day 3	0.08±0.03 ^b	0.25±0.03 ^b	0.16±0.02 ^b	0.03-0.10	0.15-0.35	0.08-0.02
Day 7	0.05±0.01 ^c	0.17±0.03 ^c	0.11±0.01 ^c	0.02-0.08	0.10-0.25	0.06-0.15
Day 10	0.03±0.01 ^d	0.13±0.03 ^d	0.31±0.40 ^d	0.01-0.06	0.08-0.20	0.04-0.10
Day 14	0.01±0.00 ^e	0.10±0.01 ^e	0.03±0.00 ^e	0.01-0.05	0.05-0.15	0.02-0.05

Control (F. orange) = fresh orange. Means with different letter superscripts (a, b, c, d & e) on the same column indicate statistically significant differences (p=0.05)

The mineral composition and toxic metal content of the sample (fermented orange or related fruit matrix) are represented in Table 3.

Table 3: Mineral composition of Fresh Orange and CaC₂ ripened substances for days

Sample	Ca %	K %	Na %	Mg %	Mn %	Fe %	Zn %
Control (F. Orange)	0.18±0.19 ^a	0.09±0.01 ^a	0.02±0.01 ^a	0.04±0.02 ^a	0.80±0.03 ^a	2.60±0.11 ^a	0.70±0.05 ^a
Day 3	0.08±0.02 ^b	1.10±0.10 ^b	0.03±0.01 ^a	0.06±0.02 ^a	1.60±0.10 ^b	3.70±0.20 ^b	2.00±0.06 ^b
Day 7	0.11±0.03 ^b	1.40±0.10 ^c	0.60±0.10 ^b	0.09±0.03 ^b	2.20±0.20 ^c	4.80±0.30 ^c	2.60±0.20 ^c
Day 10	0.13±0.02 ^{bc}	1.80±0.21 ^d	0.70±0.12 ^c	0.13±0.02 ^c	3.40±0.12 ^d	7.22±0.20 ^d	3.70±0.21 ^d
Day 14	0.18±0.04 ^c	2.40±0.11 ^e	0.92±0.12 ^d	0.17±0.04 ^d	4.78±0.21 ^e	9.44±0.13 ^e	5.31±0.30 ^e

The result is presented in means ± standard deviation. Where Control (F. orange) = fresh orange, Ca= Calcium %, K=Potassium%, Na=Sodium%, Mg=Magnesium%, Mn = Manganese (ppm), Fe=Iron (ppm), Zn=Zinc (ppm), Means with different letter superscripts (a, b, c, d & e) on the same column indicate statistically significant differences (p=0.05)

Table 4: Heavy Metals Content of Fresh Orange

Sample	Pb %	Cd %	As %
Control (F. Orange)	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Day 3	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Day 7	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Day 10	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Day 14	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

Means ± standard deviation. Where Control (F. orange) = fresh orange, Pb=Lead (ppm), Cd=Cadmium (ppm) and As=Arsenic(ppm). Means with different letter superscripts (a, b, c, d & e) in the same column indicate statistically significant differences (p=0.05)

The provided data illustrates the changes in vitamin composition of a sample—presumably.

Table 5: Results of Vitamins content in Fresh Orange and CaC₂ ripened oranges

Sample	Vit. C mg/100g	Vit. E mg/100g	Vit. A mg/100g	Vit. B1 mg/100g	Vit. B2 mg/100g	Vit. B3 mg/100g	Vit. D mg/100g
Control (F. Orange)	37.8±0.35 ^a	0.40±0.02 ^a	16.0±0.22 ^a	0.04±0.01 ^a	0.03±0.01 ^a	0.37±0.02 ^a	0.00±0.00 ^a
Day 3	33.4±0.33 ^b	0.20±0.02 ^b	12.0±0.20 ^b	0.03±0.01 ^{ab}	0.03±0.01 ^a	0.21±0.02 ^b	0.00±0.00 ^a
Day 7	27.8±0.33 ^c	0.14±0.01 ^c	8.00±0.11 ^c	0.02±0.01 ^b	0.01±0.01 ^b	0.18±0.04 ^c	0.00±0.00 ^a
Day 10	19.6±0.23 ^d	0.10±0.01 ^d	4.00±0.10 ^d	0.01±0.01 ^c	0.01±0.01 ^c	0.01±0.00 ^d	0.00±0.00 ^a
Day 14	8.40±0.12 ^e	0.10±0.01 ^d	2.10±0.04 ^e	0.01±0.01 ^c	0.00±0.00 ^c	0.04±0.01 ^e	0.00±0.00 ^a

Control (F. orange) = fresh orange, Vit. = vitamins. Means with different letter superscripts (a, b, c, d & e) on the same column indicate statistically significant differences (p=0.05)

3.1 Discussion

3.1.1 Proximate Composition of Fresh and Calcium carbide (CaC₂) Ripened Orange

The proximate composition of the natural and artificially ripened oranges using Calcium Carbide revealed significant changes in its nutritional composition, due to spoilage and concentration effects due to moisture loss. These changes have important implications for the sample's shelf life, nutrient stability, and overall food quality. The most important observation was the consistent decrease in moisture content, from 88.00% in the fresh sample to 79.00% by Day 14. Moisture is a critical determinant of food perishability; its reduction over time is typically attributed to evaporation, microbial metabolism, and enzymatic reactions during storage (Fellows, 2009). As moisture content drops, there was a relative increase in dry matter constituents, especially carbohydrates. Consequently, carbohydrate (CHO) content increased from 8.00% to 18.90%, which also contributed to the rise in energy value

from 33.67 kcal to 69.69 kcal. This trend agrees with the findings of Okonkwo *et al.* [9], who noted that drying or extended exposure leads to concentration of energy-yielding nutrients.

Conversely, ash content, which reflects the total mineral composition, decreased markedly from 0.80% to 0.20%. This reduction may result from mineral degradation or leaching, especially in humid environments or when enzymatic activities increased. A similar pattern was seen in the protein content, which declined from 1.00% to 0.40% over the 14-day period. Protein degradation is commonly driven by proteolytic enzymes and microbial action, reducing nutritional value and potentially leading to the formation of undesirable by-products [10]. This suggests that even with increased energy content, the food's quality and nutritive balance declined significantly with time.

Crude fiber also decreased gradually from 2.00% to 1.40%, indicating a breakdown of structural polysaccharides such as cellulose and lignin. Fiber loss during storage may alter the

functional properties of the food, such as its water-holding capacity and digestibility ^[11]. Similarly, lipid content showed minor fluctuations but ultimately dropped from 0.20% to 0.10%. Lipids are prone to oxidation, especially under ambient conditions, leading to rancidity and deterioration of flavor and nutritional quality ^[12].

Collectively, these findings demonstrate that while some nutrient concentrations (e.g., CHO and energy) may appear to increase due to moisture reduction, essential nutrients like proteins, minerals, lipids, and fiber deteriorated over time. This highlights the dual nature of food storage: the perceived increase in some components is often offset by a loss in quality and bioavailability of others ^[13]. Therefore, without adequate preservation measures such as refrigeration, drying under controlled conditions, or the use of natural preservatives, food samples like oranges may become nutritionally inferior and microbiologically unsafe. The proximate composition changes observed over 14 days underscore the need for timely consumption or appropriate storage interventions. The degradation of proteins, lipids, minerals, and fiber points to nutrient instability, while increased carbohydrate and energy levels are mostly relative effects of water loss rather than true nutrient enhancement. These results align with existing literature on food spoilage and nutrient dynamics during storage and reinforce the importance of preservation in maintaining food quality.

3.1.2 Anti Nutrient in fresh ripe orange and Calcium carbide induced ripped orange

The observed data reflect the changes in the concentrations of three key anti-nutritional factors tannins, phytates, and oxalates in a food sample (likely fresh orange) stored over a 14-day period. Anti-nutritional factors are naturally occurring compounds in plants that can interfere with the digestion, absorption, or utilization of nutrients. While they often exist in small quantities in fruits and vegetables, their reduction over time can significantly improve the food's nutritional bioavailability and safety ^[14].

Tannin content in the fresh sample was 0.12%, and it declined progressively to 0.01% by Day 14. Tannins are polyphenolic compounds that can form complexes with proteins and digestive enzymes, inhibiting protein digestibility and reducing the bioavailability of essential amino acids ^[15]. The sharp decline over time may be attributed to oxidative degradation or microbial activity during storage, which tends to break down polyphenolic structures. This reduction in tannin levels is beneficial as it improves protein digestibility and enhances micronutrient absorption, particularly of iron and zinc, which are commonly inhibited by high tannin levels. The recorded values fall within the safe consumption range of 0.01–0.15%, ensuring no adverse effects from tannin toxicity ^[10].

Phytate levels also showed a consistent decline from 0.35% in the fresh sample to 0.10% on Day 14. Phytates, or phytic acid, are known for their strong chelating properties, particularly with divalent minerals like calcium, iron, magnesium, and zinc, forming insoluble complexes that reduce mineral bioavailability. Their decline may be the result of enzymatic degradation by endogenous phytases or microbial phytases activated during storage ^[16]. The degradation of phytates is nutritionally advantageous, especially in populations relying heavily on plant-based diets, where mineral deficiencies are prevalent. The values observed lie within acceptable limits of 0.05–0.50%, indicating the food sample remains within safe and

beneficial nutritional thresholds ^[17].

Oxalate content showed a more variable trend. It initially decreased from 0.23% to 0.11% by Day 7, then unexpectedly spiked to 0.31% on Day 10 before sharply falling to 0.03% by Day 14. Oxalates are known to bind calcium, forming calcium oxalate crystals which are insoluble and can lead to kidney stone formation if consumed in excess. The fluctuation in oxalate levels, particularly the spike on Day 10, might be due to metabolic conversion of precursor compounds or inconsistencies in sampling. Nevertheless, the final oxalate value on Day 14 lies well within the safe range (0.02–0.30%), making the food safe for consumption with reduced anti-nutritional impact. The overall downward trend supports the hypothesis that storage time decreases oxalate content, improving calcium availability and overall mineral absorption.

The collective decline of tannins, phytates, and oxalates suggests that natural degradation processes during storage, including enzymatic activity and microbial metabolism, can lead to the reduction of anti-nutritional compounds in plant-based foods. This aligns with studies that have shown similar reductions in legumes and fruits over time or through processing methods like soaking and fermentation ^[18, 9]. While the concentration of macronutrients like protein and fiber may decline during storage, the concurrent reduction in anti-nutrients can improve the overall bioavailability of remaining nutrients. The results indicate that extended storage of the food sample up to 14 days led to a notable reduction in anti-nutritional factors, thereby enhancing the sample's nutritional quality and safety. These findings are particularly relevant for food processing and preservation strategies aimed at improving nutrient uptake without the need for chemical treatments.

3.1.3 Mineral Composition Fresh Orange and Calcium Carbide Ripened

The mineral composition and heavy metal content of a food sample, likely fermented orange or similar fruit, were evaluated across a 14-day period, revealing significant changes that highlight both nutritional enhancement and food safety. The parameters assessed included essential macro- and micro-minerals calcium (Ca), potassium (K), sodium (Na), magnesium (Mg), manganese (Mn), iron (Fe), and zinc (Zn) as well as toxic heavy metals lead (Pb), cadmium (Cd), and arsenic (As).

In the fresh (unfermented) orange sample, calcium was recorded at 0.18%, which is typical for many fruits. Calcium is vital for bone health, neuromuscular function, and enzymatic activity. On Day 3, calcium decreased significantly to 0.08%, suggesting possible precipitation or binding to other fermentation byproducts like organic acids (e.g., citric acid or lactic acid). By Day 14, however, calcium content rebounded to 0.18%, indicating the liberation of bound calcium from cellular components through microbial degradation. This pattern implies that while early fermentation may temporarily reduce calcium availability, extended fermentation can restore or even enhance bioavailable calcium levels, possibly making the food more nutritionally valuable over time.

Potassium levels increased steadily from 0.09% in the fresh sample to 2.40% by Day 14. Potassium is essential for maintaining fluid balance, nerve function, and muscle contraction. Its increase over time may be attributed to the breakdown of plant cell walls and membranes, which releases intracellular potassium into the surrounding matrix.

Similar trends were observed with sodium, which rose from 0.02% to 0.92%. Although sodium intake should be moderated, the levels here remain within a range that enhances electrolyte balance without posing health risks.

Magnesium, another crucial element for energy metabolism, nerve function, and bone structure, increased from 0.04% in the fresh sample to 0.17% by Day 14. This gradual increase is beneficial, as magnesium deficiency is prevalent in many populations due to poor dietary intake and food processing losses.

The trace elements manganese (Mn), iron (Fe) and zinc (Zn) also showed notable enhancements. Manganese increased from 0.80% to 4.78%. It functions as a cofactor in enzymatic systems, including those involved in antioxidant defense and metabolism. The dramatic rise in manganese suggests microbial activity breaking down manganese-containing enzymes or releasing it from bound states.

Iron content rose from 2.60% to 9.44%, a significant increase that could improve the iron bioavailability of the product. Iron is fundamental in oxygen transport via hemoglobin and supports immune and cognitive functions. Iron deficiency remains one of the most common nutritional issues globally; hence, foods enhanced through fermentation may serve as better dietary sources.

Zinc, rising from 0.70% to 5.31%, supports immune function, cell growth, wound healing, and many enzymatic processes. The increased zinc content observed here reflects the hydrolysis of zinc-protein complexes during fermentation, making zinc more accessible. The presence of these micronutrients in elevated concentrations suggests that fermentation improved the overall nutritional quality of the product.

One of the most significant and reassuring observations from this analysis is the absence of toxic heavy metals lead (Pb), cadmium (Cd), and arsenic (As) throughout the 14-day period. These elements were consistently reported as 0.00%, indicating no detectable contamination. This absence is essential, especially since fruits can absorb heavy metals from soil or water. Heavy metals pose serious health risks, including neurotoxicity, kidney damage, and carcinogenicity, particularly with long-term exposure. The non-detectable levels in the samples confirm that the source material and processing environment were free from contamination, rendering the food product safe for human consumption.

The progressive increase in beneficial minerals without concurrent contamination by heavy metals underscores the dual advantage of fermentation: nutritional enrichment and safety assurance. Fermentation is known to enhance nutrient bioavailability by breaking down anti-nutritional factors such as phytates and oxalates, which often bind minerals and reduce their absorption (Hassan *et al.*, 2020). This microbial transformation allows the release of bound minerals, improving their availability for absorption in the human gut [19].

Moreover, fermented foods have long been appreciated not only for improved shelf life and sensory properties but also for their elevated health benefits. Several studies have confirmed that microbial metabolism during fermentation liberates vitamins and minerals while sometimes producing beneficial metabolites like organic acids and peptides [20]. This aligns with the observed rise in mineral content over time in this sample.

In conclusion, the results of this 14-day analysis reveal that

fermentation significantly improved the mineral profile of the food sample. Essential nutrients such as calcium, potassium, magnesium, iron, and zinc increased steadily, with no presence of harmful heavy metals. These findings support the use of fermentation as a safe and effective means to enhance the nutritional value of food products. The increase in trace elements like iron and zinc could help combat common micronutrient deficiencies, especially in populations with limited access to diverse diets. Furthermore, the data validate the product's safety and reinforce the role of fermentation in promoting food security and public health.

3.1.4 Vitamins Determined in Fresh and Calcium Carbide Ripened Orange

The presented data provides a comprehensive insight into the degradation of essential vitamins—namely Vitamins C, E, A, B1, B2, B3, and D during a 14-day fermentation period of fermented orange (F. Orange). Fermentation is known to alter the nutritional and biochemical profile of foods through microbial metabolism, oxidation, and enzymatic transformation. The observed reduction in vitamin content, particularly water-soluble and antioxidant vitamins, suggests that prolonged fermentation significantly compromises the micronutrient quality of the product. These changes have profound implications for the nutritional value of fermented fruits and their application in human and animal diets.

Vitamin C (Ascorbic Acid), a water-soluble antioxidant essential for collagen synthesis, immune function, and iron absorption, showed the most pronounced decline. From an initial concentration of 37.8 mg/100g in the fresh orange, it dropped progressively to 33.4 mg/100g on Day 3, 27.8 mg/100g on Day 7, 19.6 mg/100g on Day 10, and finally 8.40 mg/100g on Day 14. This represents a 77.8% loss. The decline is primarily attributed to the oxidative degradation of ascorbic acid, catalyzed by exposure to oxygen and microbial enzymatic activity. During fermentation, microbial respiration and metabolic heat can increase the oxidative stress on nutrients. Moreover, certain lactic acid bacteria (LAB) are known to utilize Vitamin C as a substrate for growth [21]. The loss of Vitamin C aligns with findings by Nwachukwu *et al.* (2014), who reported significant reductions in Vitamin C content in fermented fruit juices.

Vitamin E (Tocopherol), a lipid-soluble antioxidant that protects cell membranes from oxidative damage, also decreased notably, from 0.40 mg/100g in the fresh sample to 0.10 mg/100g by Day 14—a 75% decline. Although Vitamin E is more stable than Vitamin C, it still undergoes degradation, particularly in the presence of oxygen, light, and heat [22]. Its reduction suggests that the conditions of fermentation (such as exposure to air and light) were not optimal for nutrient preservation. Additionally, the enzymatic activity of fermenting microbes may also play a role in tocopherol breakdown.

Vitamin A (Retinol and Carotenoids) also exhibited a substantial reduction, decreasing from 16.0 mg/100g in the fresh orange to just 2.10 mg/100g by Day 14. This degradation can be linked to the susceptibility of provitamin A carotenoids to oxidative and photolytic breakdown during fermentation. The decline also indicates that the microbial strains involved may lack the enzymatic capacity to synthesize or preserve carotenoids. According to Okoye *et al.* [23], carotenoids in fruits are highly unstable under fermentation and light exposure, which corroborates this

trend.

B-complex vitamins, including Vitamin B1 (Thiamine), Vitamin B2 (Riboflavin), and Vitamin B3 (Niacin), also declined over time. Thiamine decreased from 0.04 mg/100g to 0.01 mg/100g; riboflavin dropped from 0.03 mg/100g to 0.00 mg/100g, and niacin declined from 0.37 mg/100g to 0.04 mg/100g. These water-soluble vitamins are essential cofactors in energy metabolism, and their loss could reduce the bioenergetic value of the fermented product. The reduction is primarily due to microbial consumption and the hydrolytic breakdown under acidic fermentation conditions (Achi & Okere, 2015). While some fermentation processes can enhance B-vitamin content depending on the microbial strain used, this particular fermentation appeared to favor degradation over synthesis, suggesting an absence of vitamin-producing strains like *Lactobacillus plantarum* or *Saccharomyces cerevisiae* [24].

Vitamin D, which plays a key role in calcium absorption and bone health, was undetectable in the fresh and fermented samples across all time points. This is consistent with existing literature, as fruits and vegetables are generally not sources of Vitamin D. Its synthesis primarily occurs in animal tissues through exposure to UVB light, making its absence in fermented orange unsurprising [25].

Overall, the vitamin degradation observed during the 14-day fermentation can be attributed to several interrelated factors: oxidative stress, microbial metabolism, pH changes, light exposure, and enzymatic breakdown. Water-soluble vitamins (C and B-complex) are particularly vulnerable to leaching and enzymatic degradation, while fat-soluble vitamins (A and E) are affected by oxidation and lipid hydrolysis. The fermentation conditions used in this study likely lacked protective factors such as anaerobic control, light shielding, or addition of stabilizers.

The nutritional implications of these findings are significant. Fermented orange may still retain some value as a probiotic-rich or fiber-enhanced food, but its diminished vitamin content limits its utility as a source of micronutrients. If intended for use in human or animal nutrition, strategies such as vitamin fortification, shorter fermentation durations, or use of vitamin-synthesizing microbial strains may be required to enhance or maintain nutritional quality.

Moreover, from a public health perspective, reliance on such fermented products without compensatory dietary sources of vitamins could lead to deficiencies. For instance, loss of Vitamin C may compromise immune function, especially in vulnerable populations, while reduced B-vitamin levels may impair energy metabolism and neurological function. As such, fermentation protocols should be tailored to optimize both microbial activity and nutrient retention.

This study highlights that while fermentation is a valuable food preservation technique, it can result in substantial losses of essential vitamins. These findings emphasize the need for controlled fermentation conditions and possible nutritional interventions to maintain the health benefits of fermented fruit products.

4. Conclusion

Calcium carbonate remain a viable chemical in inducing orange ripening. The fermentation of orange pulp over a 14-day period led to notable changes in its nutritional composition. Proximate composition showed a general decline in moisture, protein, ash, and crude lipids, while carbohydrates and overall caloric value increased. These

changes suggested that fermentation may enhance the energy density of the product but may simultaneously reduce its protein and fat content, potentially affecting its balance as a food source.

Anti-nutrient levels such as tannins, phytates, and oxalates significantly decreased with fermentation time. This reduction implies improved bioavailability of essential minerals and a lower risk of nutrient interference, enhancing the nutritional quality and digestibility of the fermented product. Mineral composition revealed increases in both macro- and micro-elements, indicating that fermentation may liberate bound minerals or enhance their extractability. Importantly, no heavy metals were detected, affirming the safety of the fermented product for consumption. The improved mineral profile supports the potential use of fermented orange pulp in addressing micronutrient deficiencies. However, the artificial fermentation process led to substantial reductions in several vitamins, particularly those sensitive to oxidation and heat, such as vitamins C, A, B1, and B2. This decline suggests that while fermentation enhances some nutritional attributes, it caused reduction in the vitamin content.

Overall, artificial fermentation of orange pulp enhances its energy value, reduces anti-nutrients, and improves mineral availability, but negatively impacts vitamin stability. These findings underscore the need for optimizing fermentation conditions to balance nutrient enhancement with vitamin retention for maximal nutritional benefit.

5. References

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