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# Sanitizers Effect in Mango Pulp and Peel Antioxidant Compounds

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## Abstract

**Effects of ozonated water as sanitizer method on mango were studied on total phenolics, flavonoids, carotenoids and vitamin C of pulp or peel. Mango cultivar "Palmer" was harvested and subjected to sanitization treatments by immersion in water, chlorinated water (10 minutes sodium hypochlorite 100 mg·L$^{-1}$) or ozonated water for 10 and 20 minutes. After the sanitization process, the mangoes were stored at 15˚C ± 1˚C and 85% ± 5% RH for seven days, followed by 4 days of storage at room temperature (simulating the trading period), totaling 11 days after harvested. Mangoes pulp sanitized with ozonated water for 20 minutes showed the highest values of TA, total soluble carbohydrates, vitamin C, carotenoids and flavonoid content. These data suggest that the use of ozonated water may contribute to inducing increase antioxidants compounds.**

## Keywords

**Ozonated Water, Vitamin C, Polyphenol, Carotenoid**

## 1. Introduction

Mango (*Mangifera indica* L.), Anacardiaceae family, is one of the most popular tropical fruits, followed by banana, pineapple, papaya and avocado. Studies about its antioxidant properties were made. This plant has parts, as the stem bark, leaves and fruit pulp, which are known for various biomedical applications, including the free radicals elimination [1] [2], anti-inflammatory [3] and anticancer [4].

Reactive oxygen species (ROS) generated during the normal metabolic processes can easily start the membrane lipid peroxidation [5]. To decrease the ROS action, plants produce substances that scavenging free radicals, de-

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nominated antioxidants, which are compounds that inhibit or lag the oxidation of other molecules, inhibiting the outset or the spread of the chain oxidation reactions. Natural antioxidants are a wide range of compounds including phenolic compounds, nitrogen compounds and carotenoids [6]. Among the compounds with antioxidant action, phenolic compounds possess a series of pharmacological properties that make them active in biological systems. Several studies have been conducted evaluating their antioxidant effects, aiming chronic degenerative diseases prevention such as cardiovascular diseases, atherosclerosis, and rheumatoid arthritis, among others [7].

Furthermore, the phenolic compounds which exist mostly as esters or glycosylated forms in plants provide health benefits by several mechanisms including: free radical cleaning, protection and regeneration of others dietary antioxidants (e.g. vitamin E) and pro-oxidants metals ions chelating. However, the types and levels of phenolic compounds vary dramatically among vegetables [8].

Vegetables are usually sanitized for consumption and this step is critical and paramount importance for the microbiological quality. At this stage, it is important to check the sanitizer which should also be effective in addition to insurance toxicologically. Generally, chlorine-based compounds it is used, which may present some limitations due to the formation of some by-products such as organochlorine residues, trihalomethanes (THM) and haloacetic acids, which are mutagenic, toxic and carcinogenic. Likewise, they can remain in food, water, wash water and surfaces of food contact [9].

According to studies, some chlorinated compounds are inactivated with organic matter presence limiting their use [10]-[12]. It is believed that the hypochlorite activity losses its effectiveness after reacting with nitrogenous compounds in food, resulting in halogenated organic compounds [13] [14].

An alternative to chlorine use is the ozone. It is considered an alternative sanitizer due to its characteristics of instability and no by-products formation which may cause human health and environment issues [15] [16]. Compared to chlorine and other similar sanitizer agents, ozone needs lower concentrations and shorter food contact to an effective disinfectant action [17].

Food and Drug Administration of the United States of America (USA), recently approved the ozone as a sanitizer, applied in aqueous or gaseous forms and combined with good manufacturing practices. The US Department of Agriculture also approved this sanitizer in 1997 [16].

The aim of this study was to determine whether ozonated water use could induce increases in antioxidant compounds, contributing to obtaining mangoes "Palmer" with higher nutritional quality.

## 2. Material and Methods

Mango cultivar "Palmer" were provided by Ogata Citrus, localized in Taquaritinga, São Paulo, Brazil (latitude 21˚37'11"S, longitude 49˚04'25"W and altitude 429 meters). The fruits were harvested in the early morning hours, selected, standardized in size and maturity degree and subjected to sanitization treatments by immersion in water, chlorinated water (10 minutes sodium hypochlorite 100 $mg \cdot L^{-1}$) or ozonated water for 10 and 20 minutes, obtained from an ozonator (Degradatox/OZ Engenharia, Indústria de Equipamentos Geradores de Ozônio-LTDA, Porto Alegre, Rio Grande do Sul, Brazil). After the sanitization process, the mangoes were stored at 15˚C ± 1˚C and 85% ± 5% RH for seven days, followed by 4 days of storage at room temperature (simulating the trading period), totaling 11 days after harvested. The fruits were separated into pulp and peel and the analyzes were performed on fresh material. Parts of the fruit (pulp and peel) were powdered in liquid nitrogen and stored at −80˚C, and parts oven dried at 60˚C ± 2˚C and ground in a Willey mill. Each sanitization treatment contained 2 fruits per repetition, with four repetions, amounting 32 fruits.

It were analyzed in mango pulps soluble solide (SS), titrable acidity (TA), pH, Vitamin C, protein, fat and total soluble carbohydrate. In the mango peel and pulps were analysed carotenoids, total polyphenolic compounds and flavonoids.

### 2.1. Soluble Solids Content (SS)

The soluble solids content were obtained by digital refractometer (ATAGO) and expressed in Brix [18].

### 2.2. Titrable Acidity (TA)

The titrable acidity was determined according to [19] and the results were expressed as malic acid equivalent in 100 $g^{-1}$.

### 2.3. pH

For the analysis of pH (model HI 4221, Hanna Instruments Brazil), samples (10 g) were weighed and homogenized with 10 mL of distilled water. The pH value was determined by potentiometry, using pH buffer solution (4.01 and 7.01). Instrument calibration was carried out daily, according to [19].

### 2.4. Vitamin C

A titrimetry method was used for vitamin C determination by using 2,6-dichlorophenol-indophenol as indicator [19]. The results were expressed as mg vit. C 100 $g^{-1}$.

### 2.5. Total Protein Content

The protein content were performed following the Kjeldahl method, the results were expressed as percentage of dry matter and calculated multiplying nitrogen percentage by a factor (6.25) [19].

### 2.6. Total Soluble Carbohydrate

The total soluble sugars were performed on the dry matter by hot extraction and the values were compared with standard glucose curve and expressed in percentage [20].

### 2.7. Fat Content

Fat were analyzed on dry matter [21] and expressed as percentage.

### 2.8. Total Polyphenols

The analysis of total polyphenols was performed in accordance to the Folin-Ciocalteu spectrophotometric method [22]. The sample material, dried and powdered, was weighed and placed into centrifuge tubes containing 50% acetone in water. The samples were incubated in an ultrasonic bath for 20 minutes and centrifuged at 6000 × g (Hettich Zentrifugen, Mikro220R) for 10 minutes. The supernatants were re-extracted and combined. Folin-Ciocalteu reagent was added; after 3 min at 25˚C, a saturated solution of $Na_2CO_3$ was added, and the reaction mixture was incubated for 1 h. The absorbance was measured at 760 nm. Polyphenolic content was expressed as gallic acid equivalent 100 $g^{-1}$.

### 2.9. Total Flavonoids

The analysis of flavonoids contents was conducted according to the spectrophotometric method adapted [23] [24], with adjustments. Briefly, fresh material samples were powdered in liquid nitrogen, weighed and mixed with 10% (w/v) acidified methanol. Subsequently, the samples were placed in an ultrasonic bath for 30 minutes, and a 5% aluminum chloride solution was added. The samples were centrifuged for 20 minutes at 10,000 × *g*. Finally, the samples were filtered, and the absorbance was measured at 425 nm. The results were expressed as mangiferin meq 100 $g^{-1}$.

### 2.10. Total Carotenoids

Carotenoids extraction was performed on fresh weight according to [25] method. It was based on molar absorptivity coefficient of the pigments: chlorophylls, carotenoids and anthocyanins in buffered acetone. Absorbance values were converted into mg of total carotenoids 100 $g^{-1}$ FW using the formulas:

**Anthocyanin** ($\mu mol \cdot mL^{-1}$) = $(0.08173A_{537}) - (0.00697A_{647}) - (0.002228A_{663})$

**Chlorophyll a** ($\mu mol \cdot mL^{-1}$) = $0.01373(A_{663}) - 0.000897(A_{537}) - 0.003046(A_{647})$.

**Chlorophyll b** ($\mu mol \cdot mL^{-1}$) = $0.02405(A_{647}) - 0.004305(A_{537}) - 0.005507(A_{663})$.

**Carotenoids** ($\mu mol \cdot mL^{-1}$) = $\{A_{470} - [17.1.(Cla + Clb)] - 9.479.Anthocyanin\}/119.26$.

### 2.11. Statistical Analysis

The experimental design was randomized, for the pulp analysis mean comparison test was applied (Tukey test at 5% probability). Factorial 2 × 4 (pulp and peel x sanitizations) were applied for the pulp and peel analysis. The software used was Statistica, 7.0 version [26].

## 3. Results and Discussion

### 3.1. Pulp

The quality parameters, SS and pH, were not affected by the sanitizers applied (**Table 1**). The pH and SS values were nearby those reported by some authors [27] [28]. Sanitizer's lack of effect was also found on protein and fat contents (**Table 1**). Protein content of this study was lower than that detected by [29], while the fat content found is close to the described by same authors. However, the fat levels in this work were upper than reported by [30], with four varieties of mango. In this study, mangoes with 11 days after harvested and with ozone or chlorine sanitization have protein and fat levels suitable for consumption.

Regarding total soluble carbohydrate content were higher in mangoes sanitized with ozonated water. This probably means the sanitizing agent could have promoted a stress, increasing on the respiration rate, inducing the polysaccharides reserve solubilization, thereby increasing soluble carbohydrates levels, encouraging the flavor [31]. Findings were consistent with the reported increase in soluble sugar (fructose and glucose content) reported in strawberry [32] and tomato [33], in response to low-level atmospheric ozone.

Mangoes sanitized with ozonated water for 20 minutes increased their vitamin C content. Ozone is an antioxidant and vitamin C has an action against oxidative stress well described [34]. In this study, the highest vitamin C content were noticed on those fruits that had the longest exposure to ozone (**Table 1**), and it may be a response from the vegetable cells to any injury caused by this higher exposure to the oxidant (ozone). Changes in mangoes vitamin C content were described in literature (between 5 - 194 mg·100g$^{-1}$) [28] [29] [35].

The increase in vitamin C content after the ozone use can be referred to the ozone inhibitory effect on enzymes such as ascorbate peroxidase and ascorbate oxidase (responsible for the ascorbic acid degradation), on the other hand, high ozone at damaging concentrations may reduce ascorbic acid by promoting ascorbate oxidase activity [36]. Nutritionally, increased levels of vitamin C promoted by ozonated water can be interesting for the consumer because they can get a fruit with the highest vitamin C content, further the benefits of a fruit with the absence of any agrochemical, as a trihalometane, produced by reactions with chlorine [12].

### 3.2. Pulp and Peel

The total carotenoid in pulp and peel (**Table 2**) were significantly influenced by the ozonated water use. An increase in the carotenoids content, mainly on pulp, can be observed. In tomatoes (*Lycopersicum esculentum*) exposed for 24 hours with ozone enriched air were also found increasing carotenoids content [37]. Studies with kiwifruit revealed that the total carotenoid content was increased after exposure to gaseous ozone (0.3 ppm) [38]. However, there are evidences decreased carotenoids levels can occurr in fresh-cut carrots after ozone exposure [39].

Total polyphenols content in "Palmer" mango pulp were not modified by the ozone use, but by chlorine which induced an increase. Chlorine is a potent sanitizer with strong oxidizing properties, generally effective, comparatively inexpensive, and the most agent used by the food industry for sanitizing products and equipment. NaClO in water increases pH and produces HClO, which is the active disinfectant, being more efficient at a pH range of 6.5 - 7.5 [40]. The chlorine effect may have collaborated to promote an oxidative process, causing polyphenols increase, as a way to scavenging the ROS formation, which could damage the cells.

In this study, the flavonoids content in the pulp was much lower (14 and 24 mg mangiferin 100 g$^{-1}$) to that detected in three different mango cultivars (160 and 560 mg mangiferin 100 g$^{-1}$) [41]. Otherwise the ozonated water use induced the increase of total flavonoid content in the pulp (**Table 2**). Polyphenols can end hazardous free radicals propagation, which causes cell lipid peroxidation by transferring an electron equivalent to radicals. Spite of the role these substances play in health maintenance, they are neglected in most food composition surveys [42]. Since ozone can induce oxidative stress in fresh fruits, which promotes many physiological responses, including the synthesis of antioxidants, polyamines, ethylene, phenolic compounds (such as flavonoids) and other secondary metabolites [43], its use as a sanitizer can be propitious for increasing the flavonoids content.

Interesting results have been obtained in mangoes peel, which presented, regardless the sanitization treatment used, higher content of these compounds that have been demonstrated potential as antioxidants. Therefore, better use of the mango peel for ingestion in food could be an excellent source of phenolic compounds.

**Table 1.** Titrable acidity (TA), pH, soluble solids (SS), total soluble carbohydrate, protein and fat content in ripe and sanitized pulp of mango "Palmer".

| | Treatments | | | | |
|---|---|---|---|---|---|
| | **Water** | **Chlorine** | **Ozone 10** | **Ozone 20** | **CV (%)** |
| **TA** (meq malic acid 100 g$^{-1}$) | 0.88 bc | 0.82 c | 1.03 a | 0.99 ab | 6.15 |
| **pH** | 4.0 a | 4.0 a | 4.3 a | 4.4 a | 4.89 |
| **SS** (Brix) | 14.93 a | 13.67 a | 14.73 a | 15.13 a | 4.11 |
| **Carbohydrate** (g·100g$^{-1}$) | 20.56 c | 23.57 b | 23.60 b | 25.67 a | 3.06 |
| **Protein** (g·100g$^{-1}$) | 1.73 a | 1.72 a | 1.71 a | 1.72 a | 0.82 |
| **Fat** (g·100g$^{-1}$) | 0.46 a | 0.47 a | 0.48 a | 0.51 a | 6.54 |
| **Vitamin C** (mg Vit. C 100 g$^{-1}$) | 50.60 b | 53.17 b | 51.93 b | 64.55 a | 3.42 |

Means followed by the same letters (row) were not statistically significant according to the Tukey's test ($p \leq 0.05$).

**Table 2.** Carotenoids, polyphenols, flavonoids contents in ripe and sanitized pulp and peel of mango "Palmer".

| | | Treatments | | | | |
|---|---|---|---|---|---|---|
| | | **Water** | **Chlorine** | **Ozone 10** | **Ozone 20** | **CV (%)** |
| **Carotenoids** (mg·100g$^{-1}$) | **pulp** | 394.90 Ac | 351.83 Ac | 751.32 Aa | 564.84 Ab | 6.15 |
| | **peel** | 257.64 Bb | 283.89 Bab | 337.48 Ba | 295.18 Bab | |
| **Polyphenols** (g eq. Gallic acid 100 g$^{-1}$) | **pulp** | 0.86 Bb | 1.07 Ba | 0.89 Bb | 0.93 Bab | 3.70 |
| | **peel** | 2.94 Aa | 1.84 Ac | 2.43 Ab | 2.31 Ab | |
| **Flavonoids** (meq. mangiferin 100 g$^{-1}$) | **pulp** | 14.16 Bb | 15.08 Bb | 25.82 Ba | 24.50 Ba | 1.87 |
| | **peel** | 484.75 Ac | 652.14 Aa | 495.76 Ab | 594.53 Ab | |

Means followed by the same capital letters (column) and the same low case letters (row) were not statistically significant according to the Tukey's test ($p \leq 0.05$).

## 4. Conclusion

The ozonated water use in mangoes "Palmer" can be profitable to increase substances with an antioxidant potential, as well as the possible consumption of the peel can be interesting for increasing the polyphenol ingestion.

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