


## Article

# Glow Discharge Plasma Processing for the Improvement of Pasteurized Orange Juice's Aroma and Off-Flavor

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**Abstract:** Orange juice is one of the most popular juices sold in the world. Although very popular, most orange juice sold in the market is pasteurized, presenting off-flavor compounds and aromas different from the freshly squeezed juice. This study investigated how green chemistry, through the application of glow discharge plasma, can help improve the aroma and mitigate off-flavors of pasteurized orange juice. Orange juice was processed at different plasma flow rates (10 to 30 mL/min) for 10 to 30 min. Glow discharge plasma processing induced several chemical reactions enabling positive changes in the volatile profile of pasteurized orange juice. Chemical pathways were proposed and correlated to the changes in aroma and mitigation of off-flavor compounds. Glow discharge plasma increased the concentration of terpenoid and aldehyde compounds that give the orange juice its fresh-like characteristics and reduced the off-flavor compound concentration by 61%.

**Keywords:** cold plasma; citrus sinensis; terpenoids; aldehydes



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## 1. Introduction

Orange juice is one of the most popular juices sold around the world. It can be sold freshly squeezed, concentrated, or pasteurized. Pasteurized orange juice is produced from freshly squeezed oranges or reconstituted juice. The concentrated and the pasteurized juices go through thermal processes that extend their shelf-life. Most volatiles are lost due to thermal degradation during the concentration and pasteurization of orange juice. Industries bypass part of the juice or add orange essential oil to premium quality orange juice to improve its flavor. Unfortunately, this addition does not fully reconstitute the juice's natural aroma [1].

Novel technologies are being studied and developed to improve food quality. In most studies, non-thermal technologies, such as high hydrostatic pressure (HPP) [2], high-pressure homogenization (HPH) [3], and supercritical CO<sub>2</sub> processing [4], are used in substitution for thermal processes to preserve thermolabile compounds, such as aroma compounds [5]. Although industrial processing of orange juice with novel technologies, such as high-pressure processing and pulsed electric field processing, is current practice, scale-up problems and economic viability when processing commodities such as orange juice are still disadvantages of cold plasma technologies. Thus, orange juice and many other commodities are still processed using thermal technologies, which are fast and cheap compared to several other processes.

Lower-priced orange juice is often reconstituted and pasteurized orange juice that contains a reasonable amount of off-flavor compounds. Orange essential oil or the aroma recovered from the juice concentration process is added to premium-quality orange juice to improve the flavor and masquerade off-flavors. However, undesired off-flavor compounds remain in the juice.

Previous studies from our group [6] showed that cold plasma technology could induce changes in terpenoid compounds, the main class of compounds present in orange juice.

Plasma processing induces hydrogenation, scission, hydrolysis, isomerization, dehydrogenation, ring-opening, and ring-closure reactions that can be controlled by setting the proper operating conditions [6,7]. Theoretically, these reactions can transform the low-odor limonene into more distinguished orange-like odor compounds, such as  $\alpha$ -pinene and 3-carene, produce orange-characteristic aldehydic compounds, such as octanal and decanal, and mitigate off-flavor compounds, such as  $\alpha$ -terpineol, by transforming it into other terpenoid compounds [6,8]. These plasma-induced reactions fall within the scope of green chemistry processing since they are similar to naturally occurring reactions in plants that do not require the addition of any reagent or solvent. Furthermore, cold plasma operates at low temperatures (below 30 °C), reducing any further degradation of the nutritional compounds.

This study aimed to identify and discuss the chemical reactions induced by glow discharge plasma on pasteurized orange juice and evaluate if this plasma technology could be applied to improve the aroma profile of pasteurized orange juice while controlling or mitigating the contents of off-flavors.

## 2. Materials and Methods

### 2.1. Materials

Pasteurized orange juice (Del Valle 100% Laranja, Norsa Refrigerantes S.A., Maracanau, Brazil) was used in this study. The juice was bought from a local supermarket (Fortaleza, Brazil) and was chosen because of its large quantities of aroma compounds and presence of off-flavors [9,10].

### 2.2. Plasma Processing

A PE-50 gas discharge plasma system (Plasma Etch, Carlson City, NV, USA) working at a radio frequency of 50 kHz and electrical potential difference of 80 kV was used for the gas discharge plasma experiments. Plasma was generated in synthetic air. The samples were placed in the processing chamber (190 × 220 × 90 mm). The pressure was maintained at 0.3 bar inside the chamber. The experiments were performed with varying air flow rates (10, 20, and 30 mL/min) and processing times (10, 20, and 30 min). The selection of this range of operating conditions was based on our group's previous studies [11–13]. The samples (15 mL) were placed inside polypropylene tubes and subjected to glow discharge plasma treatment. Control samples consisted of untreated orange juice. All experiments were carried out in triplicates.

### 2.3. GC-MS Analysis

Orange juice (5.0 mL) was added to a 20 mL vial and equilibrated for 20 min at 40 °C with constant stirring (500 rpm). An internal standard, 3-hexanone with a final concentration of 10  $\mu$ M, was added to the samples prior to equilibration and extraction. A manual SPME holder with a DVB/CAR/PDMS (50/30  $\mu$ m) of a 2 cm-length fiber was used to capture the volatile compounds [14]. The SPME fiber was exposed for 20 min to the sample headspace, maintaining a constant temperature of 40 °C. After the extraction, the volatiles were directly desorbed on the GC liner and maintained for 10 min at 250 °C for fiber reconditioning. Samples were analyzed in a gas chromatograph coupled with a mass spectrometer (Thermo ISQ model) following the methodology previously published in the literature [15]. The injector and the interface temperatures were 250 °C working in splitless mode. Chromatographic separations were performed using a (5%-phenyl)-methylpolysiloxane column (30 m × 0.25 mm ID × 0.25  $\mu$ m film). The temperature programming started at 40 °C for 4 min, increased to 80 °C at 2.5 °C·min<sup>-1</sup>, increased to 100 °C at 5.0 °C·min<sup>-1</sup> and held at this temperature for 1 min, and then increased to 250 °C at 10.0 °C·min<sup>-1</sup>, maintaining the final temperature for 1 min. The ion trap detector was operated in the EI mode at 70 eV and 200 °C with a mass scan range of 50 to 400 *m/z*. The carrier gas was helium, which was set at a 1.0 mL·min<sup>-1</sup> flow rate. The mass spectra were compared with the NIST and Wiley mass spectral libraries.

#### 2.4. Odor Profile

The volatile compounds were grouped according to their primary odor description [16]. The odor activity value (OAV) was calculated based on the mass fraction of each substance divided by its odor threshold in water [17]. The OAV of each substance was summed according to their primary odor description. The five main odor description notes that give orange juice its characteristic aroma were plotted: citrus, aldehydic, herbal, terpenic, and floral. The values for the odor threshold in water were obtained in the literature [18–23].

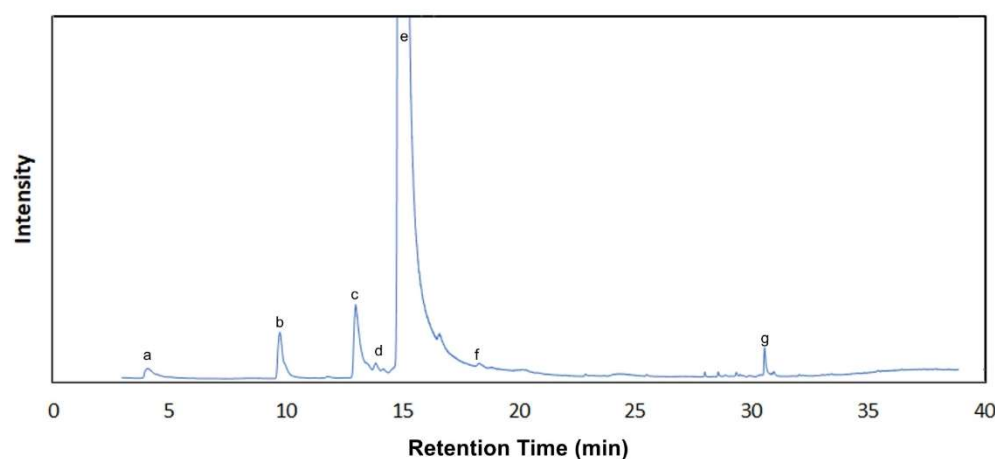
#### 2.5. Statistical Analysis

Statistical analysis was conducted using Statistica v.13 (Tibco Software). Fisher's LSD test at a 95% confidence level was used for mean comparison.

### 3. Results and Discussion

#### 3.1. Identification of the Volatile Profile

Figure 1 shows a chromatogram and the identification of orange juice's main volatile substances.



**Figure 1.** Representative chromatogram of pasteurized orange juice: (a) ethyl butanoate, (b)  $\alpha$ -pinene, (c) 3-carene, (d) terpinolene, (e) limonene, (f)  $\gamma$ -terpinene, and (g) eremophila-1(10),11-diene.

Table 1 presents the identified volatile compounds and their respective retention times (RTs), Kovats indices, odor thresholds in water, and primary odor descriptions. A total of 24 substances were identified in the chromatograms, accounting for  $98 \pm 1\%$  of the total peak area. Seven other substances ( $2 \pm 1\%$  of the total peak area) were detected in trace amounts; thus, it was not possible to identify them with accuracy.

Nine compounds (ethyl butanoate,  $\alpha$ -pinene,  $\beta$ -pinene, 3-carene, terpinolene, p-cymenene, limonene,  $\gamma$ -terpinene, and eremophila-1(10),11-diene) accounted for  $94 \pm 1\%$  of the total peak area, being the main compounds found in pasteurized orange juice. The main group of substances found in the pasteurized orange juice and in the plasma-treated juice were terpenoids, mainly limonene. The juice also presented small amounts of alcohols (including terpenyl alcohols), aldehydes, esters, organic acids, sesquiterpenes, and terpenyl hydroperoxides. The compounds found in this work were similar to the volatile profiles published by other authors [9,24,25].

**Table 1.** Volatile compounds identified in orange juice and their respective retention times (RTs), Kovats indices, odor thresholds in water, and primary odor descriptions. Odor thresholds and descriptions were taken from the literature [18–23].

Compound	Retention Time (min)	Kovats Index	<i>m/z</i>	Odor Threshold in Water (mg/L)	Odor Description
<i>Alcohols</i>					
1-Octanol	17.97	1084	56.0; 41.0; 70.0	0.875	Waxy
$\alpha$ -Linalool	19.10	1107	71.0; 93.0; 55.0	0.006	Floral
<i>p</i> -Mentha-2,8-dienol	20.40	1133	73.0; 91.0; 117.0	–	–
4-Terpineol	23.11	1188	71.0; 111.0; 43.0	6.400	Spicy
$\alpha$ -Terpineol	23.83	1204	59.0; 93.0; 136.0	0.330	Woody
<i>Aldehydes</i>					
Octanal	13.79	999	43.0; 41.0; 56.0	0.0069	Aldehydic
Nonanal	19.52	1115	41.0; 43.0; 57.0	0.001	Aldehydic
Decanal	24.30	1221	41.0; 43.0; 55.0	0.030	Aldehydic
Perillaldehyde	26.91	1311	68.0; 79.0; 107.0	0.030	Herbal
Dodecanal	29.84	1425	41.0; 43.0; 57.0	0.002	Aldehydic
<i>Terpenoids</i>					
$\alpha$ -Pinene	9.56	914	93.0; 92.0; 77.0	0.062	Herbal
Camphene	10.36	930	93.0; 121.0; 41.0	1.980	Terpenic
Sabinene	11.86	960	93.0; 91.0; 77.0	0.037	Woody
$\beta$ -Pinene	12.69	977	93.0; 41.0; 69.0	2.540	Herbal
3-Carene	13.53	994	93.0; 91.0; 77.0	0.044	Citrus
Terpinolene	14.11	1006	93.0; 121.0; 136.0	0.200	Herbal
Limonene	14.97	1023	93.0; 68.0; 136.0	1.040	Citrus
$\gamma$ -Terpinene	16.48	1054	93.0; 91.0; 136.0	0.260	Terpenic
<i>p</i> -Cymene	18.54	1095	119.0; 134.0; 91.0	0.120	Terpenic
<i>Other Compounds</i>					
3-Amino-2,3-dihydrobenzoic acid	2.67	774	166.0; 150.0; 147.0	–	–
Ethyl butanoate	4.56	812	71.0; 43.0; 88.0	0.100	Fruity
<i>p</i> -Mentha-6,8-diene-2-hydroperoxide	28.97	1383	109.0; 93.0; 107.0	–	–
Germacrene D	30.04	1438	119.0; 161.0; 91.0	–	–
Eremophila-1(10),11-diene	31.09	1506	107.0; 93.0; 161.0	–	–

### 3.2. Modifications in the Volatile Profile

Table 2 shows the volatile profile of pasteurized orange juice subjected to glow discharge plasma. All conditions significantly affected the volatile profile of the juice ( $p < 0.05$ ) compared to the control (untreated pasteurized orange juice). Orange juice volatiles are marked by the high amount of limonene, which accounts for over 90% *w/w* of its volatiles. In terms of mass fraction, other important volatiles are  $\alpha$ -pinene, 3-carene, *p*-cymene,  $\beta$ -pinene, and  $\gamma$ -terpinene.

**Table 2.** Mass fraction of the volatile compounds identified in orange juice in the control sample and plasma-treated samples. Same letters (a–h) indicate statistically similar values at a 95% confidence level.

Compound	Control Sample (Untreated)	Plasma Treatment 10 mL/min 10 min	Plasma Treatment 10 mL/min 20 min	Plasma Treatment 10 mL/min 30 min	Plasma Treatment 20 mL/min 10 min	Plasma Treatment 20 mL/min 20 min	Plasma Treatment 20 mL/min 30 min	Plasma Treatment 30 mL/min 10 min	Plasma Treatment 30 mL/min 20 min	Plasma Treatment 30 mL/min 30 min
3-Amino-2,3-DBA	0.03 ± 0.01 <sup>bc</sup>	0.03 ± 0.01 <sup>bc</sup>	0.00 ± 0.00 <sup>d</sup>	0.06 ± 0.01 <sup>a</sup>	0.03 ± 0.01 <sup>bc</sup>	0.02 ± 0.01 <sup>cd</sup>	0.04 ± 0.01 <sup>ab</sup>	0.04 ± 0.01 <sup>ab</sup>	0.03 ± 0.01 <sup>bc</sup>	0.01 ± 0.01 <sup>cd</sup>
Ethyl butanoate	0.21 ± 0.02 <sup>c</sup>	0.24 ± 0.03 <sup>b</sup>	0.21 ± 0.02 <sup>c</sup>	0.24 ± 0.03 <sup>ab</sup>	0.25 ± 0.03 <sup>ab</sup>	0.25 ± 0.03 <sup>ab</sup>	0.30 ± 0.03 <sup>a</sup>	0.24 ± 0.02 <sup>b</sup>	0.23 ± 0.02 <sup>bc</sup>	0.23 ± 0.02 <sup>bc</sup>
α-Pinene	0.94 ± 0.04 <sup>d</sup>	0.79 ± 0.03 <sup>f</sup>	1.55 ± 0.05 <sup>a</sup>	1.32 ± 0.05 <sup>b</sup>	0.82 ± 0.03 <sup>ef</sup>	1.27 ± 0.05 <sup>b</sup>	0.65 ± 0.03 <sup>g</sup>	1.06 ± 0.05 <sup>c</sup>	0.86 ± 0.03 <sup>e</sup>	1.53 ± 0.05 <sup>a</sup>
Camphene	0.07 ± 0.01 <sup>cd</sup>	0.06 ± 0.01 <sup>d</sup>	0.14 ± 0.02 <sup>a</sup>	0.13 ± 0.02 <sup>ab</sup>	0.07 ± 0.01 <sup>cd</sup>	0.10 ± 0.02 <sup>bc</sup>	0.06 ± 0.01 <sup>d</sup>	0.10 ± 0.02 <sup>bc</sup>	0.05 ± 0.01 <sup>d</sup>	0.13 ± 0.02 <sup>ab</sup>
Sabinene	0.02 ± 0.01 <sup>bc</sup>	0.01 ± 0.01 <sup>c</sup>	0.03 ± 0.01 <sup>ab</sup>	0.02 ± 0.01 <sup>bc</sup>	0.02 ± 0.01 <sup>bc</sup>	0.01 ± 0.01 <sup>c</sup>	0.05 ± 0.01 <sup>a</sup>	0.02 ± 0.01 <sup>bc</sup>	0.01 ± 0.01 <sup>c</sup>	0.02 ± 0.01 <sup>bc</sup>
β-Pinene	2.21 ± 0.05 <sup>d</sup>	1.75 ± 0.05 <sup>e</sup>	3.09 ± 0.05 <sup>a</sup>	2.49 ± 0.05 <sup>c</sup>	1.77 ± 0.05 <sup>e</sup>	2.15 ± 0.05 <sup>d</sup>	1.19 ± 0.05 <sup>g</sup>	2.31 ± 0.05 <sup>b</sup>	1.37 ± 0.05 <sup>f</sup>	2.61 ± 0.05 <sup>b</sup>
3-Carene	0.46 ± 0.03 <sup>c</sup>	0.32 ± 0.03 <sup>d</sup>	0.61 ± 0.03 <sup>a</sup>	0.54 ± 0.03 <sup>b</sup>	0.31 ± 0.03 <sup>d</sup>	0.45 ± 0.03 <sup>c</sup>	0.26 ± 0.03 <sup>d</sup>	0.46 ± 0.03 <sup>c</sup>	0.29 ± 0.02 <sup>d</sup>	0.54 ± 0.03 <sup>b</sup>
Octanal	0.14 ± 0.02 <sup>cd</sup>	0.11 ± 0.02 <sup>d</sup>	0.18 ± 0.02 <sup>ab</sup>	0.18 ± 0.02 <sup>ab</sup>	0.10 ± 0.02 <sup>d</sup>	0.16 ± 0.02 <sup>bc</sup>	0.20 ± 0.02 <sup>a</sup>	0.11 ± 0.02 <sup>d</sup>	0.17 ± 0.02 <sup>ab</sup>	0.19 ± 0.02 <sup>a</sup>
Terpinolene	0.11 ± 0.02 <sup>a</sup>	0.08 ± 0.02 <sup>ab</sup>	0.11 ± 0.02 <sup>a</sup>	0.10 ± 0.02 <sup>a</sup>	0.06 ± 0.02 <sup>bc</sup>	0.08 ± 0.02 <sup>ab</sup>	0.06 ± 0.02 <sup>bc</sup>	0.09 ± 0.02 <sup>ab</sup>	0.05 ± 0.02 <sup>c</sup>	0.10 ± 0.02 <sup>a</sup>
Limonene	92.08 ± 0.09 <sup>b</sup>	93.57 ± 0.10 <sup>a</sup>	89.21 ± 0.08 <sup>e</sup>	90.46 ± 0.10 <sup>d</sup>	93.38 ± 0.10 <sup>a</sup>	91.62 ± 0.08 <sup>c</sup>	93.62 ± 0.10 <sup>a</sup>	91.54 ± 0.08 <sup>c</sup>	93.35 ± 0.10 <sup>a</sup>	90.10 ± 0.10 <sup>d</sup>
γ-Terpinene	0.34 ± 0.03 <sup>ab</sup>	0.25 ± 0.03 <sup>cd</sup>	0.40 ± 0.03 <sup>a</sup>	0.34 ± 0.03 <sup>ab</sup>	0.28 ± 0.03 <sup>ac</sup>	0.31 ± 0.03 <sup>b</sup>	0.21 ± 0.03 <sup>d</sup>	0.33 ± 0.03 <sup>b</sup>	0.21 ± 0.03 <sup>d</sup>	0.38 ± 0.03 <sup>ab</sup>
1-Octanol	0.21 ± 0.02 <sup>cd</sup>	0.14 ± 0.01 <sup>e</sup>	0.35 ± 0.02 <sup>a</sup>	0.28 ± 0.02 <sup>b</sup>	0.17 ± 0.01 <sup>d</sup>	0.22 ± 0.02 <sup>c</sup>	0.14 ± 0.01 <sup>e</sup>	0.24 ± 0.02 <sup>bc</sup>	0.13 ± 0.02 <sup>e</sup>	0.31 ± 0.02 <sup>a</sup>
p-Cymenene	2.44 ± 0.08 <sup>d</sup>	1.72 ± 0.05 <sup>f</sup>	3.24 ± 0.07 <sup>a</sup>	2.66 ± 0.07 <sup>c</sup>	1.74 ± 0.05 <sup>f</sup>	2.18 ± 0.06 <sup>e</sup>	1.09 ± 0.05 <sup>h</sup>	2.68 ± 0.05 <sup>c</sup>	1.25 ± 0.05 <sup>g</sup>	2.83 ± 0.05 <sup>b</sup>
α-Linalool	0.20 ± 0.03 <sup>bc</sup>	0.26 ± 0.03 <sup>b</sup>	0.17 ± 0.02 <sup>a</sup>	0.21 ± 0.03 <sup>bc</sup>	0.22 ± 0.03 <sup>bc</sup>	0.22 ± 0.03 <sup>bc</sup>	0.37 ± 0.03 <sup>a</sup>	0.21 ± 0.03 <sup>bc</sup>	0.34 ± 0.03 <sup>a</sup>	0.20 ± 0.03 <sup>bc</sup>
Nonanal	0.02 ± 0.01 <sup>bc</sup>	0.01 ± 0.01 <sup>c</sup>	0.03 ± 0.01 <sup>bc</sup>	0.05 ± 0.01 <sup>a</sup>	0.01 ± 0.01 <sup>c</sup>	0.03 ± 0.01 <sup>b</sup>	0.04 ± 0.01 <sup>ab</sup>	0.01 ± 0.01 <sup>c</sup>	0.03 ± 0.01 <sup>bc</sup>	0.04 ± 0.01 <sup>ab</sup>
p-m-2,8-dienol	0.01 ± 0.01 <sup>b</sup>	0.06 ± 0.01 <sup>b</sup>	0.01 ± 0.01 <sup>b</sup>	0.08 ± 0.01 <sup>b</sup>	0.14 ± 0.01 <sup>b</sup>	0.09 ± 0.01 <sup>b</sup>	0.28 ± 0.01 <sup>b</sup>	0.01 ± 0.01 <sup>b</sup>	0.32 ± 0.01 <sup>b</sup>	0.01 ± 0.01 <sup>b</sup>
4-Terpineol	0.06 ± 0.02 <sup>ab</sup>	0.06 ± 0.01 <sup>ab</sup>	0.04 ± 0.01 <sup>b</sup>	0.05 ± 0.01 <sup>b</sup>	0.04 ± 0.01 <sup>b</sup>	0.05 ± 0.01 <sup>b</sup>	0.08 ± 0.01 <sup>a</sup>	0.05 ± 0.01 <sup>b</sup>	0.08 ± 0.01 <sup>a</sup>	0.05 ± 0.01 <sup>b</sup>
α-Terpineol	0.15 ± 0.02 <sup>bc</sup>	0.13 ± 0.03 <sup>bc</sup>	0.08 ± 0.02 <sup>d</sup>	0.16 ± 0.02 <sup>bc</sup>	0.13 ± 0.02 <sup>c</sup>	0.13 ± 0.02 <sup>c</sup>	0.22 ± 0.04 <sup>a</sup>	0.13 ± 0.02 <sup>c</sup>	0.20 ± 0.03 <sup>ab</sup>	0.11 ± 0.02 <sup>cd</sup>
Decanal	0.08 ± 0.01 <sup>c</sup>	0.08 ± 0.01 <sup>c</sup>	0.18 ± 0.02 <sup>b</sup>	0.21 ± 0.03 <sup>b</sup>	0.08 ± 0.01 <sup>c</sup>	0.19 ± 0.02 <sup>b</sup>	0.30 ± 0.02 <sup>a</sup>	0.09 ± 0.01 <sup>c</sup>	0.23 ± 0.02 <sup>b</sup>	0.23 ± 0.03 <sup>b</sup>
Perillaldehyde	0.00 ± 0.00 <sup>f</sup>	0.06 ± 0.01 <sup>d</sup>	0.04 ± 0.00 <sup>e</sup>	0.07 ± 0.01 <sup>d</sup>	0.14 ± 0.01 <sup>c</sup>	0.08 ± 0.01 <sup>d</sup>	0.23 ± 0.02 <sup>b</sup>	0.00 ± 0.00 <sup>f</sup>	0.29 ± 0.02 <sup>a</sup>	0.01 ± 0.00 <sup>f</sup>
p-m-6,8-d-2-h	0.01 ± 0.00 <sup>d</sup>	0.02 ± 0.00 <sup>c</sup>	0.04 ± 0.01 <sup>b</sup>	0.05 ± 0.01 <sup>ab</sup>	0.02 ± 0.00 <sup>c</sup>	0.05 ± 0.01 <sup>ab</sup>	0.06 ± 0.01 <sup>a</sup>	0.02 ± 0.00 <sup>c</sup>	0.05 ± 0.01 <sup>ab</sup>	0.06 ± 0.01 <sup>a</sup>
Dodecanal	0.01 ± 0.00 <sup>b</sup>	0.02 ± 0.01 <sup>ab</sup>	0.02 ± 0.00 <sup>ab</sup>	0.02 ± 0.00 <sup>ab</sup>	0.01 ± 0.00 <sup>b</sup>	0.02 ± 0.01 <sup>ab</sup>	0.03 ± 0.01 <sup>a</sup>	0.01 ± 0.00 <sup>b</sup>	0.03 ± 0.01 <sup>a</sup>	0.02 ± 0.00 <sup>ab</sup>
Germacrene D	0.01 ± 0.00 <sup>b</sup>	0.01 ± 0.00 <sup>b</sup>	0.02 ± 0.01 <sup>b</sup>	0.02 ± 0.00 <sup>ab</sup>	0.01 ± 0.00 <sup>b</sup>	0.02 ± 0.01 <sup>ab</sup>	0.03 ± 0.01 <sup>a</sup>	0.01 ± 0.00 <sup>b</sup>	0.02 ± 0.00 <sup>ab</sup>	0.02 ± 0.01 <sup>ab</sup>
Er-1(10),11-diene	0.19 ± 0.02 <sup>d</sup>	0.23 ± 0.02 <sup>cd</sup>	0.24 ± 0.02 <sup>cd</sup>	0.27 ± 0.02 <sup>bc</sup>	0.22 ± 0.02 <sup>d</sup>	0.30 ± 0.03 <sup>b</sup>	0.49 ± 0.03 <sup>a</sup>	0.22 ± 0.02 <sup>d</sup>	0.42 ± 0.04 <sup>a</sup>	0.30 ± 0.03 <sup>b</sup>

Figure 2 shows a heat map showing the changes in the mass fraction of the volatile components with the untreated juice as a basis for comparison. Darker colors indicate changes above 50% in the component's mass fraction, and lighter colors indicate changes below 50%. The changes observed in the limonene content were lower than 3%, which could be understood as a minor change. However, limonene is converted into compounds with a much lower odor threshold; thus, it makes a much higher contribution to odor perception. Therefore, even though the changes induced by the plasma treatment were small, these changes had a significant impact on odor perception in terms of the volatile compound profile. The changes in odor perception are presented and discussed in Section 3.3 (Modifications in the Aroma Profile).



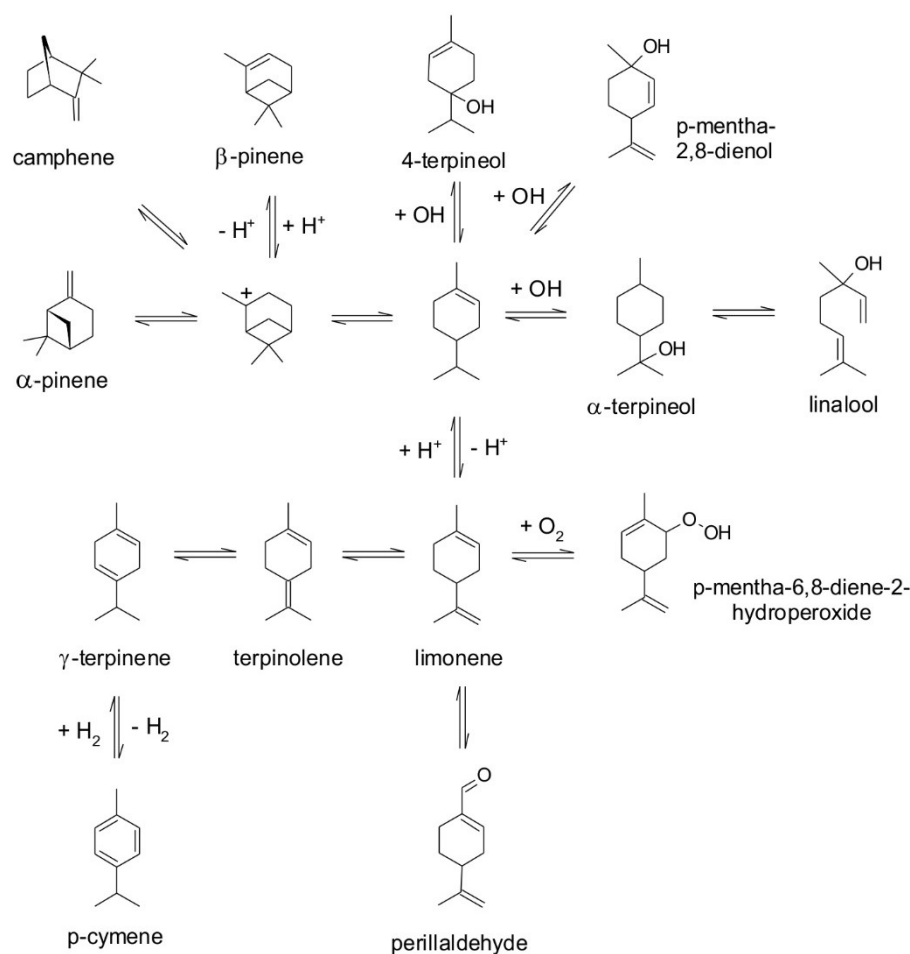
**Figure 2.** Heat map showing the changes in the mass fraction of volatile components with the untreated juice as a basis for comparison. Blue and red colors indicate an increase and decrease in concentration, respectively. Light colors indicate changes between 0 and 50% in the component's mass fraction. Darker colors indicate changes above 50% in the component's mass fraction.

A heat map analysis shows a significant variation in the intensity of the changes induced by the glow discharge plasma. Overall, the treatments carried out for 30 min at 20 mL/min and 20 min at 10 mL/min and 30 mL/min presented bigger changes.

The mass fraction of limonene, terpinolene,  $\gamma$ -terpinene, and 3-carene consistently decreased after plasma application. The contents of  $\beta$ -pinene and p-cymene also presented a greater tendency to decrease. However, the concentration of  $\alpha$ -pinene, camphene, p-mentha-2,8-dienol, decanal, perillaldehyde, p-mentha-6,8-diene-2-hydroperoxide, dodecanal, germacrene D, and eremophila-1(10),11-diene showed a tendency to increase after plasma treatment.

The analysis of the glow plasma systems and their effects on products can be complicated because one can analyze the plasma flow rate, residence time, and fluence, where fluence is defined as the product of the flow rate and residence time ( $F \times t_R$ ), by measuring the accumulated amount of plasma species that came in contact with the sample. Another point is the flow rate. Higher flow rates do not necessarily mean that more of the same plasma species will act in the system. As the same electrical potential difference is applied to a higher flow rate of gas, different plasma species will be produced with different outcomes for the product. Furthermore, higher flow rates mean a lower plasma residence time inside the treatment chamber since a vacuum pump controls the pressure and plasma content in the system. Herein, the discussion is based on the fluence and residence time because they showed a higher correlation with the data.

The main class of compounds in orange juice are terpenoids. Figure 3 shows the chemical reaction pathways observed in terpenoid compounds during glow discharge plasma processing.



**Figure 3.** Putative chemical pathways observed in pasteurized orange juice during glow discharge plasma treatment.

Reactive oxygen species, such as oxygen singlet, superoxide radical, hydroxyl radical, ozone, and hydrogen peroxide, are formed during plasma application and contribute to the production of peroxides and alcohols [26,27]. Direct oxidation of limonene and the terpenyl radical was observed due to the formation of p-mentha-6,8-diene-2-hydroperoxide, perillaldehyde, and p-mentha-2,8-dienol, which increased significantly after plasma treatment. The selectivity of these reactions followed the order: perillaldehyde > p-mentha-2,8-dienol > p-mentha-6,8-diene-2-hydroperoxide. The highest yield of these three oxygenated products occurred at a flow rate of 20 mL/min, which was already shown to be the flow rate that leads to high aroma modification [6]. The highest yield of these oxygenated products occurred at a fluence of 600 for 20 and 30 mL/min flow rates.

The increase in oxygenated products was also marked by the decrease in  $\gamma$ -terpinene, p-cymene, and terpinolene, which are in equilibrium with limonene (Figure 3). The mass balance of components indicates that this equilibrium shifts from the limonene isomers toward oxygenated products.

The content of compounds derived from the pinenyl radical ( $\alpha$ -pinene,  $\beta$ -pinene, camphene, sabinene, and 3-carene) did not present a clear trend. Camphene,  $\alpha$ -pinene, and sabinene were the compounds most affected by the plasma treatment, exhibiting the greatest percentual changes. Overall, the increase in pinenyl-derived compounds occurred at fluences which produced less oxygenated products. Thus, the pinenyl-derived compounds are favored under operating conditions that do not induce extended oxidation and hydrolysis of limonene and the terpenyl radical.

In a previous work of our group on plasma processing of camu-camu juice [6], the selectivity followed the order:  $\beta$ -pinene >  $\alpha$ -pinene > camphene. There was a slightly

higher formation of  $\beta$ -pinene instead of  $\alpha$ -pinene, but with camphene still showing the lowest selectivity among these pinenyl radical-based compounds. This selectivity was the same with orange juice when considering the mass balance of components (not the percentual change).

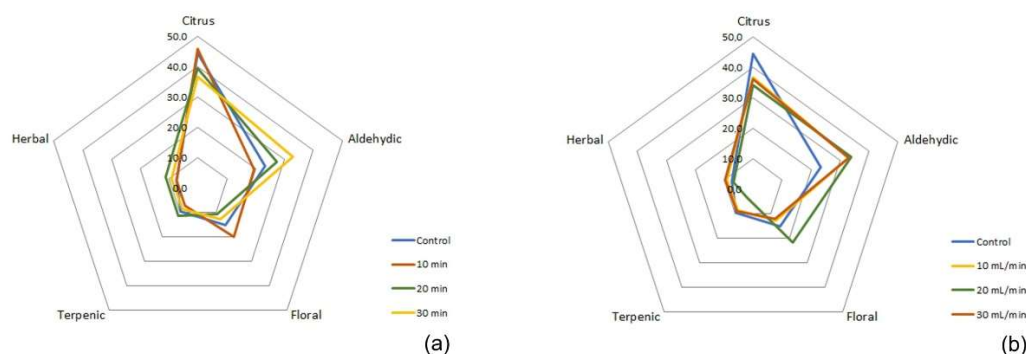
The contents of sesquiterpenoids in orange juice were low, accounting for only 2.5% of the mass of volatiles. Germacrene D and eremophila-1(10),11-diene were the only sesquiterpenoids found in the volatiles of pasteurized orange juice. There was a slight increase in the concentration of these two compounds when processing at 10 mL/min, whereas a higher increase was observed at higher plasma flow rates (20 and 30 mL/min). A significant increase in eremophila-1(10),11-diene (220%), and germacrene D (234%) concentrations were observed at 20 mL/min after 30 min of treatment. The formation of these compounds comes from the farnesyl cation, which can be formed from non-volatile compounds, and thus, is not detected by gas chromatography [28–32]. Further studies using liquid chromatography coupled to a mass spectrometer would be required to fully understand the formation of these sesquiterpenoids in orange juice. Regarding the goal of this study, germacrene D and eremophila-1(10),11-diene do not contribute to orange juice aroma (undetermined odor threshold) and can be considered inert compounds.

The juice presented small concentrations of dodecanal, decanal, nonanal, octanal, and 1-octanol, accounting for 5.9% (*w/w*) of the pasteurized orange juice volatiles. The contents of the aldehydes increased during plasma application, with higher increases at fluences above 400. These substances are typical green leaf volatiles (GLVs) that are naturally formed in fruits, vegetables, and other plants, being formed from fatty acids through the lipoxygenase (LOX) enzymatic route [33]. The lipoxygenase (LOX) enzymatic route produces aldehydes by fatty acid oxygenation, which can further reduce the aldehydes producing alcohols [33]. Orange juice pasteurization reduces LOX activity, but does not fully inhibit it [34]. The formation of aldehydes during plasma treatment may have been caused by the LOX enzymatic route or by the direct oxidation of fatty acids by reactive oxygen species of palmitoleic, oleic, and linoleic acids, which are present in small concentrations in orange juice [35]. The increase in the concentration of these aldehydes followed the order: decanal > dodecanal > nonanal > octanal. This order partially agrees with the reported concentrations of the fatty acids in orange juice (oleic > linoleic > palmitoleic > eicosenoic) that could form such aldehydes. Oleic and linoleic acids can form either decanal or nonanal, depending on the double bond that is cleaved and oxidized. Herein, a higher selectivity toward decanal was observed. Palmitoleic and eicosenoic acids tend to generate octanal and dodecanal, respectively. The results obtained herein indicate that glow discharge plasma tends to preferably cleave fatty acids with more double bonds, which would explain the more significant changes in decanal and dodecanal. The oxidation of octanal to 1-octanol was not significant, and nor was the oxidation of the other aldehydes into their respective alcohols. Decanol, nonanol, and dodecanol were not identified in the volatile compounds of orange juice, probably due to their absence or low concentration in the mixture.

### 3.3. Modifications in the Aroma Profile

Table 1 presents the odor descriptions and thresholds of the volatile compounds of pasteurized orange juice. Figure 4 shows the changes in the orange juice's five primary odor descriptors. The chemical reactions induced by the plasma treatment on the orange juice volatiles directly affected the aroma profile of the juice, as well as some significant changes in the strength of the citrus, aldehydic, and floral descriptors.





**Figure 4.** Aroma profile radar for the pasteurized orange juice and plasma-treated orange juice: (a) treated at 10 mL/min; (b) treated for 30 min.

Considering the pasteurized orange juice's odor activity value (OAV), its aroma is characterized by 44.3% citrus, 23.3% aldehydic, 15.2% floral, 9.7% terpenic, and 7.5% herbal descriptors. The changes induced by the plasma treatment tended to considerably reduce the OAV of the citrus descriptors and increased the aldehydic and floral descriptors' OAV.

The lower contribution of the citrus aroma in the plasma-treated orange juice reflected the reduction in limonene and 3-carene concentrations. Even when treated at low fluences (100 to 200), conditions in which an increase in limonene content was observed, the OAV of the citrus descriptors decreased due to the significant reduction in 3-carene content (>31%), which has a much lower odor threshold (0.044 mg/L) than limonene (1.04 mg/L).

The increase in aldehydic aromas was due to the significant increase in octanal, nonanal, decanal, and dodecanal concentrations. Plasma-treated orange juice presented an aldehydic contribution toward its aroma, ranging between 19.5 to 33.9% of the juice OAV. The aldehydic aroma decreased at low fluences (100 to 200) or short treatment times (10 min) due to the formation of  $\alpha$ -linalool that pushed the aroma toward a more floral scent. At higher fluences or longer treatment times, the aldehydic aroma considerably increased due to the formation of aldehydes and the isomerization of  $\alpha$ -linalool into oxygenated terpenes with higher odor thresholds. Even with the increase in aldehydic and floral descriptors, the glow plasma treatment maintained the citrus descriptors as primary odors in the pasteurized orange juice.

Although there was a partial shift from the citrus descriptors toward the aldehydic descriptors, both octanal and nonanal are considered two important contributors to orange juice flavor because they have an orange-like aroma. Thus, in practice, this aroma descriptor shift does not contribute negatively to the aroma of orange juice.

Significant changes were observed regarding the contribution of the floral descriptor, caused by the changes in the concentration of linalool. This descriptor contributed to 15.2% of the pasteurized orange juice OAV, whereas its contribution in the plasma-treated juice ranged from 10.8 to 21.8%, depending on the operational condition applied. Linalool has a lavender-like smell, which is considered calming and soothing. This fragrance agent is used in most hygiene items, including shampoos, soaps, lotions, and detergents; thus, its smell is well-linked with cleaning products. Its expression in foods may cause a strange perception. In orange juice, linalool is a desirable compound and positively contributes to the orange juice aroma. However, linalool is the precursor of  $\alpha$ -terpineol, which is one of the three most detrimental compounds in orange juice quality, along with 4-vinyl guaiacol and 2,5-dimethyl-4-hydroxy-3-(2H)furanone (DMHF). The formation of high concentrations of linalool should be avoided to ensure a low concentration of  $\alpha$ -terpineol during storage.

The glow discharge plasma treatment increased the contents of aldehydes in the pasteurized orange juice, increasing significantly the aldehydic notes of the juice and contributing to the production of a pasteurized orange juice with a better aroma quality. Thus, glow discharge plasma processing could be employed to improve the aroma of pasteurized orange juice.

### 3.4. Modifications in the Off-Flavor Concentration

The three main compounds that cause off-flavors in orange juice are  $\alpha$ -terpineol, 4-vinyl guaiacol, and 2,5-dimethyl-4-hydroxy-3-(2H)furanone (DMHF). Only  $\alpha$ -terpineol was present in the pasteurized orange juice used in this study. The formation of  $\alpha$ -terpineol occurs naturally in some oranges, but its concentration increases during orange processing due to limonene oxidation and the isomerization of linalool.

The glow discharge plasma treatment significantly decreased the concentration of  $\alpha$ -terpineol in pasteurized orange juice. The contents of  $\alpha$ -terpineol were reduced at fluences between 200 and 400. A decrease of 61% in the  $\alpha$ -terpineol concentration was observed in the juice treated at 10 mL/min for 20 min. This significant change is desired for the treatment of pasteurized orange juice to reconstitute a more fresh-like flavor.

### 3.5. Further Considerations Regarding the Technology

The development of novel processes usually starts on a lab scale, where the treatment scale is low and the processing times can either be short or long depending on the effects that are being evaluated. If the lab-scale process has potential or proves to be successful, scale-up studies should be carried out to provide pilot- and industrial-scale processes. The studies carried out herein were typical lab-scale experiments. This study has provided proof of the concept that glow discharge plasma can alter the chemical composition of the orange juice aroma and can also reduce the contents of off-flavor compounds.

Cold plasma is still a technology under development. Physicists and electrical engineers are currently studying how to scale up the processing capacity of the power sources, whereas food and chemical engineers and researchers from several areas are working on how to intensify the mass transfer and chemical reactions between plasma and samples of interest. As these studies advance, cold plasma will probably become technically and economically viable for industrial application.

## 4. Conclusions

Glow discharge plasma processing was able to induce several chemical reactions within the terpenoids found in pasteurized orange juice, as well as in the formation of several aldehydes. The chemical changes directly affected the volatiles and, thus, the aroma profile of the juice. The fluence, plasma flow rate, and processing time played essential roles in the selectivity of these reactions, and the proper setting of the operation conditions is crucial to improve the aroma of the pasteurized orange juice. The glow discharge plasma application decreases the concentration of limonene, terpinolene,  $\gamma$ -terpinene, and 3-carene. The concentration of  $\alpha$ -pinene, camphene, p-mentha-2,8-dienol, perillaldehyde, and p-mentha-6,8-diene-2-hydroperoxide increased after the plasma treatment. Thus, the plasma application tends to isomerize terpenyl radical-derived compounds into pinenyl radical-derived compounds and oxygenated compounds.

The chemical changes induced by the glow discharge plasma were able to increase the orange-like aldehydic descriptors of the juice, enhancing its aroma while still maintaining the citrus descriptors as the primary odor of the juice.

The glow discharge plasma was able to significantly mitigate the off-flavor caused by  $\alpha$ -terpineol, which contributed to the improvement in flavor. A decrease of 61% in the  $\alpha$ -terpineol concentration was observed in the juice treated at 10 mL/min for 20 min. The results presented in this study open up the possibilities of using green chemistry concepts to improve the aroma and taste of pasteurized orange juice without causing the addition of artificial aroma compounds.

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## References

1. TetraPak. *The Orange Book*; Tetra Pak International S.A.: Pully, Switzerland, 2017.
2. Umair, M.; Jabbar, S.; Lin, Y.; Nasiru, M.M.; Zhang, J.; Abid, M.; Murtaza, M.A.; Zhao, L. Comparative Study: Thermal and Non-thermal Treatment on Enzyme Deactivation and Selected Quality Attributes of Fresh Carrot Juice. *Int. J. Food Sci. Technol.* **2022**, *57*, 827–841. [[CrossRef](#)]
3. Kruszewski, B.; Zawada, K.; Karpiński, P. Impact of High-Pressure Homogenization Parameters on Physicochemical Characteristics, Bioactive Compounds Content, and Antioxidant Capacity of Blackcurrant Juice. *Molecules* **2021**, *26*, 1802. [[CrossRef](#)]
4. Silva, E.K.; Bargas, M.A.; Arruda, H.S.; Vardanega, R.; Pastore, G.M.; Meireles, M.A.A. Supercritical CO<sub>2</sub> Processing of a Functional Beverage Containing Apple Juice and Aqueous Extract of *Pfaffia Glomerata* Roots: Fructooligosaccharides Chemical Stability after Non-Thermal and Thermal Treatments. *Molecules* **2020**, *25*, 3911. [[CrossRef](#)] [[PubMed](#)]
5. Putnik, P.; Kresoja, Z.; Bosiljkov, T.; Jambrak, A.R.; Barba, F.J.; Lorenzo, J.M.; Roohinejad, S.; Granato, D.; Zuntar, I.; Kovacevic, D.B. Comparison the Effects of Thermal and Non-Thermal Technologies on Pomegranate Juice Quality: A Review. *Food Chem.* **2018**, *279*, 150–161. [[CrossRef](#)]
6. Campelo, P.H.; Alves Filho, E.G.; Silva, L.M.A.; de Brito, E.S.; Rodrigues, S.; Fernandes, F.A.N. Modulation of Aroma and Flavor Using Glow Discharge Plasma Technology. *Innov. Food Sci. Emerg. Technol.* **2020**, *62*, 102363. [[CrossRef](#)]
7. Farias, T.R.B.; Alves Filho, E.G.; Silva, L.M.A.; De Brito, E.S.; Rodrigues, S.; Fernandes, F.A.N. NMR Evaluation of Apple Cubes and Apple Juice Composition Subjected to Two Cold Plasma Technologies. *LWT Food Sci. Technol.* **2021**, *150*, 112062. [[CrossRef](#)]
8. Campelo, P.H.; Alves Filho, E.G.; Silva, L.M.A.; de Brito, E.S.; Rodrigues, S.; Fernandes, F.A.N. Modulation of Aroma and Flavor Using Dielectric Barrier Discharge Plasma Technology in a Juice Rich in Terpenes and Sesquiterpenes. *LWT* **2020**, *130*, 109644. [[CrossRef](#)]
9. Moshonas, M.G.; Shaw, P.E. Flavor and Chemical Comparison of Pasteurized and Fresh Valencia Orange Juices. *J. Food Qual.* **1997**, *20*, 31–40. [[CrossRef](#)]
10. Marcotte, M.; Stewart, B.; Fustier, P. Abused Thermal Treatment Impact on Degradation Products of Chilled Pasteurized Orange Juice. *J. Agric. Food Chem.* **1998**, *46*, 1991–1996. [[CrossRef](#)]
11. Alves Filho, E.G.; Silva, L.M.A.; de Brito, E.S.; Wurlitzer, N.J.; Fernandes, F.A.N.; Rabelo, M.C.; Fonteles, T.V.; Rodrigues, S. Evaluation of Thermal and Non-Thermal Processing Effect on Non-Prebiotic and Prebiotic Acerola Juices Using 1H QNMR and GC–MS Coupled to Chemometrics. *Food Chem.* **2018**, *265*, 23–31. [[CrossRef](#)]
12. Alves Filho, E.G.; Rodrigues, T.H.S.; Fernandes, F.A.N.; de Brito, E.S.; Cullen, P.J.; Frias, J.M.; Bourke, P.; Cavalcante, R.S.; Almeida, F.D.L.; Rodrigues, S. An Untargeted Chemometric Evaluation of Plasma and Ozone Processing Effect on Volatile Compounds in Orange Juice. *Innov. Food Sci. Emerg. Technol.* **2019**, *53*, 63–69. [[CrossRef](#)]
13. Rodríguez, Ó.; Gomes, W.F.; Rodrigues, S.; Fernandes, F.A.N. Effect of Indirect Cold Plasma Treatment on Cashew Apple Juice (*Anacardium Occidentale* L.). *LWT Food Sci. Technol.* **2017**, *84*, 457–463. [[CrossRef](#)]
14. Steffen, A.; Pawliszyn, J. Analysis of Flavor Volatiles Using Headspace Solid-Phase Microextraction. *J. Agric. Food Chem.* **1996**, *44*, 2187–2193. [[CrossRef](#)]
15. Gómez-Ariza, J.L.; García-Barrera, T.; Lorenzo, F. Determination of Flavour and Off-Flavour Compounds in Orange Juice by on-Line Coupling of a Pervaporation Unit to Gas Chromatography–Mass Spectrometry. *J. Chromatogr. A* **2004**, *1047*, 313–317. [[CrossRef](#)]
16. Company, T.G.S. The Good Scents Company Database. Available online: <http://www.thegoodscentscompany.com/> (accessed on 5 March 2022).
17. Grosch, W. Evaluation of the Key Odorants of Foods by Dilution Experiments, Aroma Models and Omission. *Chem. Senses* **2001**, *26*, 533–545. [[CrossRef](#)]
18. Usami, A.; Ono, T.; Marumoto, S.; Miyazawa, M. Comparison of Volatile Compounds with Characteristic Odor in Flowers and Leaves of Nojigiku (*Chrysanthemum Japonense*). *J. Oleo Sci.* **2013**, *62*, 631–636. [[CrossRef](#)]
19. Padrayuttawat, A.; Yoshizawa, T.; Tamura, H.; Tokunaga, T. Optical Isomers and Odor Thresholds of Volatile Constituents in Citrus Sudachi. *Food Sci. Technol. Int. Tokyo* **1997**, *3*, 402–408. [[CrossRef](#)]
20. Xiao, Z.; Chen, J.; Niu, Y.; Chen, F. Characterization of the Key Odorants of Fennel Essential Oils of Different Regions Using GC–MS and GC–O Combined with Partial Least Squares Regression. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2017**, *1063*, 226–234. [[CrossRef](#)]
21. Fazzalari, F.A. *Compilation of Odor and Taste Threshold Values Data*; American Society for Testing and Materials: Philadelphia, PA, USA, 1978.
22. Buttery, R.G.; Ling, L.C.; Light, D.M. Tomato Leaf Volatile Aroma Components. *J. Agric. Food Chem.* **1987**, *35*, 1039–1042. [[CrossRef](#)]
23. Qian, M.C.; Wang, Y. Seasonal Variation of Volatile Composition and Odor Activity Value of “Marion” (*Rubus* Spp. *Hyb*) and “Thornless Evergreen” (*R. Laciniatus* L.) Blackberries. *J. Food Sci.* **2005**, *70*, 13–20. [[CrossRef](#)]

24. Perez-Cacho, P.R.; Rouseff, R. Processing and Storage Effects on Orange Juice Aroma: A Review. *J. Agric. Food Chem.* **2008**, *56*, 9785–9796. [[CrossRef](#)]
25. Elss, S.; Kleinhenz, S.; Schreier, P. Odor and Taste Thresholds of Potential Carry-over/off-Flavor Compounds in Orange and Apple Juice. *LWT Food Sci. Technol.* **2007**, *40*, 1826–1831. [[CrossRef](#)]
26. Ito, T.; Uchida, G.; Nakajima, A.; Takenaka, K.; Setsuhara, Y. Control of Reactive Oxygen and Nitrogen Species Production in Liquid by Nonthermal Plasma Jet with Controlled Surrounding Gas. *Jpn. J. Appl. Phys.* **2017**, *56*, 01AC06. [[CrossRef](#)]
27. Takahashi, K.; Satoh, K.; Itoh, H.; Kawaguchi, H.; Timoshkin, I.; Given, M.; MacGregor, S. Production Characteristics of Reactive Oxygen/Nitrogen Species in Water Using Atmospheric Pressure Discharge Plasmas. *Jpn. J. Appl. Phys.* **2016**, *55*, 07FL01. [[CrossRef](#)]
28. Chen, F.; Al-Ahmad, H.; Joyce, B.; Zhao, N.; Kollner, T.G.; Degenhardt, J.; Stewart, C.N., Jr. Within-Plant Distribution and Emission of Sesquiterpenes from *Copaifera officinalis*. *Plant Physiol. Biochem.* **2009**, *47*, 1017–1023. [[CrossRef](#)]
29. Agger, S.; Lopez-Gallego, F.; Schmidt-Dannert, C. Diversity of Sesquiterpene Synthases in the Basidiomycete *Coprinus cinereus*. *Mol. Microbiol.* **2009**, *72*, 1181–1195. [[CrossRef](#)]
30. Konen, P.P.; Wust, M. Analysis of Sesquiterpene Hydrocarbons in Grape Berry Exocarp (*Vitis Vinifera* L.) Using in Vivo-Labeling and Comprehensive Two-Dimensional Gas Chromatography-Mass Spectrometry (GCxGC-MS). *Beilstein J. Org. Chem.* **2019**, *15*, 1945–1961. [[CrossRef](#)]
31. Kumeta, Y.; Ito, M. Characterization of A-Humulene Synthases Responsible for the Production of Sesquiterpenes Induced by Methyl Jasmonate in *Aquilaria* Cell Culture. *J. Nat. Med.* **2016**, *70*, 452–459. [[CrossRef](#)]
32. Dauben, W.G.; Hubbell, J.P.; Vietmeyer, N.D. Acid-Catalyzed Rearrangements of Humulene. *J. Org. Chem.* **1975**, *40*, 479–485. [[CrossRef](#)]
33. Hu, C.J.; Li, D.; Ma, Y.X.; Zhang, W.; Lin, C.; Zheng, X.Q.; Liang, Y.R.; Lu, J.L. Formation Mechanism of the Oolong Tea Characteristic Aroma during Bruising and Withering Treatment. *Food Chem.* **2018**, *269*, 202–211. [[CrossRef](#)]
34. Sampedro, F.; Fan, X. Inactivation Kinetics and Photoreactivation of Vegetable Oxidative Enzymes after Combined UV-C and Thermal Processing. *Innov. Food Sci. Emerg. Technol.* **2014**, *23*, 107–113. [[CrossRef](#)]
35. Nordby, H.E.; Nagy, S. Fatty Acid Profiles of Orange and Tangor Juice Sac Lipids. *Phytochemistry* **1971**, *10*, 615–619. [[CrossRef](#)]