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# Screening the Antioxidant Activity of Thermal or Non-Thermally Treated Fruit Juices by In Vitro and In Vivo Assays

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**Abstract:** The health benefits of fruit juices have been associated with their high content of antioxidant compounds. Commercial juice has been traditionally heat-processed to destroy microorganisms and enzymes. However, high temperatures induce undesirable changes in the nutritional value of the juice. High-intensity pulsed electric fields (HIPEF) are being studied as an alternative to heat treatments. In addition, in vitro and in vivo methods have been recommended to determine the antioxidant potential of juices in a complementary manner. Thus, the antioxidant activity of untreated, high-intensity pulsed electric fields (HIPEF) or heat-treated fruit juices (tomato, apple, pineapple and orange) was studied using in vitro (TEAC, DPPH, FRAP and Folin-Ciocalteu) and in vivo assays (*Saccharomyces cerevisiae*). Vitamin C and total phenolic compounds in these juices were determined. The highest antioxidant activities (12.01 mmol of Trolox/L) were obtained through the Folin-Ciocalteu assay in orange juices. The lowest values (0.119 mmol of Trolox/L) were found in apple juice analysed by the FRAP assay. Vitamin C content varied from 10 mg/L (orange juice) to 344 mg/L (orange juice). The highest concentration of total phenolic compounds was determined in orange juice (1238 mg/L), whereas the lowest value was found in tomato juices (149 mg/L). The effect of HIPEF and thermal processing on the antioxidant potential of juices depended on the fruits used to prepare the juices and the antioxidant activity assay conducted. Vitamin C concentration was directly related to the antioxidant activity analysed by Folin-Ciocalteu and FRAP methods and the *S. cerevisiae* growth rate. *S. cerevisiae* yeast can be used as a feasible in vivo assay to further determine the antioxidant activity of fruit juices.

**Keywords:** fruit juices; antioxidant activity; health-related compounds; high intensity pulsed electric fields; thermal treatment; oxidative stress; yeast strains



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## 1. Introduction

The process of ageing and several diseases such as cancer, Alzheimer's, and Amyotrophic Lateral Sclerosis have been strongly related to modifications in proteins, lipids and nucleic acids as a consequence of oxidative stress [1,2]. Enzymatic and/or non-enzymatic factors are involved in cellular defence mechanisms against reactive oxygen species (ROS)-induced oxidative stress. Enzymatic defence encompasses enzymes such as superoxide dismutases, catalases, and glutathione peroxidases, which act as detoxifying oxidant compounds [3]. Non-enzymatic antioxidants such as vitamins and phenolic compounds are associated with the elimination and detoxification of pernicious components produced by ROS [4]. In addition, ascorbic acid (vitamin C) may prevent free radical-induced damage to DNA [5], avoiding cell dysfunction and decrease LDL- induced leukocyte adhesion [6]. A

diet rich in phenolic compounds correlated with a reduced risk of coronary heart diseases since phenols stop the continuous progress of lipid peroxidation induced by ROS [7].

Several in vitro methods have been used to determine the antioxidant activity of food and beverages because antioxidants respond differently to different radical and oxidant sources. A single method could not accurately reflect all the radicals and antioxidants in complex matrixes. In general, the most important in vitro tests are based on two major mechanisms: the hydrogen atom transfer (HAT) methods, which measure the classical ability to quench free radicals by hydrogen donation, and the single electron transfer (SET) methods, which detect the ability to transfer one electron to reduce a compound such as radicals and metals. In this way, the Trolox equivalent antioxidant capacity (TEAC) is based on the ability of the antioxidants to scavenge the blue-green radical cation 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS), the determination of the free radical-scavenging effect of antioxidants on 2,2-diphenyl-1-picrylhydrazyl (DPPH), and the ferric reduction-antioxidant power assay (FRAP) are the most widely used. However, in vitro methods do not reflect cellular and physiological conditions. Living cell assays have proven to be useful for routine analyses, producing reliable results in the identification of biological activities. The yeast *Saccharomyces cerevisiae* has been used as a eukaryotic model organism to screen the in vivo antioxidant activity of different health-related compounds (such as vitamin C and phenolic compounds). Some studies have directly used these in vivo tests with yeast strains to determine the antioxidant activity of juices [8–11]. *S. cerevisiae* reacts to oxidative stress by inducing diverse defence systems. This adaptive response is reflected in changes in growth rate depending on external conditions. Since ROS are also endogenously generated by physiological processes such as aerobic respiration or protein folding at the endoplasmic reticulum, the antioxidant systems also operate in basal conditions of growth independent of external oxidant insults. Thus, mutants in some of the enzymatic antioxidant systems are compromised in their growth rate.

Nowadays, fruit juices have become very popular because consumers associate them with healthy products, leading to an increase in their consumption during the last years. Although citrus, apple, and pineapple juices have dominated the fruit juice production ranking for many years, other juices, such as tomato, have gained market share within the past decade. Thermal treatments remain at least one of the most applied forms of stabilisation for preserving and extending the shelf-life of juices because of their excellent performance against microorganisms. However, many of the health-related compounds from juices can be affected by the elevated temperature reached during processing. The high demand for healthy products has forced the food industry to develop new technologies that produce less destruction to the nutritional quality of food products. In this context, non-thermal technologies such as high-intensity pulsed electric fields (HIPEF) have received attention because they preserve juices while avoiding the negative effects of heat pasteurisation. In this way, several authors have established the antioxidant potential of HIPEF-treated juices using in vitro methods. However, to the best of our knowledge, no information is currently available concerning the effect of HIPEF treatment on the antioxidant activity of juices determined by in vivo tests. Because antioxidants respond differently to different radical and oxidant sources, in vitro and in vivo methods should be carried out in a complementary manner to determine the antioxidant potential of HIPEF juices.

Therefore, the present work aimed to evaluate and compare the antioxidant activity of different fresh, thermally, and non-thermally treated fruit (tomato, apple, pineapple, and orange) juices through in vitro and in vivo methods.

## 2. Materials and Methods

### 2.1. Juices Preparation

Ten kg of tomato (*Lycopersium esculentum* Mill.), apple (*Malus domestica* Borkh), pineapple (*Ananas comosus* Merr) and orange (*Citrus sinensis* L.) fruits were purchased from a local supermarket (Lleida, Spain), and kept at 4 °C before being processed. The fruits were washed, drained and chopped. Then, the squeezed juices were centrifuged at 24,000× *g* for

15 min, and the supernatants were filtered using a steel sieve with a mesh of 2 mm. Juices were quickly frozen at −46 °C and kept in darkness prior to treatments. Soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Fukaya, Japan), pH (crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), colour (Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) and electric conductivity (Testo 240 conductivimeter; Testo GmBh & Co., Lenzkirch, Germany) of juices were determined (Table 1).

**Table 1.** Analytical characteristics of juices.

| Parameters [a] | Tomato Juice | Apple Juice | Pineapple Juice | Orange Juice |
|---|---|---|---|---|
| Soluble solids (°Brix) | 4.65 ± 0.13 | 10.82 ± 0.09 | 13.51 ± 0.05 | 13.56 ± 0.07 |
| pH | 4.23 ± 0.01 | 4.28 ± 0.03 | 3.56 ± 0.02 | 3.56 ± 0.01 |
| Colour | | | | |
| L* | 23.21 ± 0.04 | 25.32 ± 0.01 | 60.35 ± 0.07 | 40.25 ± 0.12 |
| a* | 6.83 ± 0.17 | 0.41 ± 0.09 | −3.20 ± 0.11 | 6.83 ± 0.13 |
| b* | 5.21 ± 0.09 | 3.85 ± 0.04 | 20.31 ± 0.07 | 27.52 ± 0.15 |
| Electrical conductivity (S/m) | 0.63 ± 0.21 | 0.21 ± 0.02 | 0.36 ± 0.11 | 0.38 ± 0.05 |

[a] Results are the mean ± SD of three measurements.

### *2.2. Pulsed Electric Fields Equipment*

Pulse treatments were carried out using a continuous flow bench-scale system (OSU-4F, Ohio State University, Columbus, OH, USA) that provides square-wave pulses within eight co-field flow chambers arranged in series. Each chamber had a treatment volume of 0.012 $cm^3$, and the two stainless steel electrodes and separated by a gap of 0.29 cm. The process was adjusted to 60 mL/min and controlled by a variable speed pump (model 752210-25, Cole Palmer Instrument Company, Vernon Hills, IL, USA). Electric field strength, treatment time, pulse frequency and pulse width were selected according to previous studies to achieve safe juices [12,13]. Juices were subjected to HIPEF treatments consisting of 4 µs bipolar square-wave pulses at 35 kV/cm field strength for 1500 µs and 100 Hz pulse frequency (tomato juice), for 1575 µs and 180 Hz pulse frequency (apple juice), for 1700 µs and 235 Hz pulse frequency (orange juice) as well as for 2000 µs and 200 Hz pulse frequency (pineapple juice).

### *2.3. Thermal Treatment*

Tomato, apple, pineapple and orange juices were heat-treated at 90 °C for 60 s in a tubular heat exchanger to have safe thermally-treated juices [14].

### *2.4. Packaging and Storage Conditions*

Fresh, HIPEF-treated or thermally-treated juices were poured directly from the treatment system, leaving the minimum amount of headspace volume. Juices were kept under refrigeration (4 °C) in darkness prior to the analysis.

### *2.5. Sample Extraction*

To obtain the hydrophilic extracts for phenolic compound and antioxidant activities determination, 25 mL of juice were mixed with 25 mL of a 70:30 methanol: water ($v/v$) solution, then centrifuged at 6000× $g$ for 15 min at 4 °C (Centrifuge Medigifer; Select, Barcelona, Spain).

### *2.6. Determination of Health-Related Compounds*

#### 2.6.1. Vitamin C

Vitamin C determination was carried out by HPLC [15]. After the extraction of juices with 7.2 g of DL-1,4-dithiotreitol/L, the mixture was centrifuged at 22,100× $g$ for 15 min at 4 °C. The supernatant was vacuum-filtered through Whatman No. 1 paper and passed

through a Millipore 0.45 µm membrane. An HPLC system using a reverse-phase C18 Spherisorb® ODS2 (5 µm) stainless steel column (4.6 mm × 250 cm) was used to quantify vitamin C. Sulphuric acid adjusted to pH = 2.6 was used as the mobile phase. Detection was performed with a 486 Absorbance Detector (Waters, Milford, MA, USA) set at 245 nm. The quantification of vitamin C was carried out by comparing the measured signal of the sample with the calibration line of ascorbic acid (Scharlau Chemie, Barcelona, Spain). Results were expressed as mg of vitamin C per litre of juices.

#### 2.6.2. Determination of Phenolic Compounds

The determination of total phenolic compounds was conducted by the Fast Blue Assay [16]. Ten mL of the hydrophilic extract or standards were diluted 1:20 with water and mixed with 1 mL of Fast Blue BB diazonium salt (1 g/L) and 1 mL of NaOH (0.5 g/L). Absorbance was measured at 420 nm. The concentration of total phenolic compounds was determined by comparing the absorbance of the samples with standards of gallic acid (Scharlau Chemie, Barcelona, Spain). Results were expressed as mg of gallic acid per litre of juices.

### *2.7. Determination of Antioxidant Activity Using In Vitro Methods*

The in vitro antioxidant capacity was studied by evaluating the free radical scavenging effect on the DPPH, the TEAC, FRAP and the Folin-Ciocalteu assays [17–20]. The final antioxidant activity determined throughout all methods was calculated using a regression equation between the Trolox concentration (0–20 µM) and the absorbance changes. The final results were expressed as micromole Trolox equivalents per litre of juice.

### *2.8. Determination of Antioxidant Activity Using an In Vivo Method*

#### 2.8.1. Yeast Strains, Media and Growth Conditions

The commercial baker's yeast strain Plus Vital (from Lesaffre Yeast Corp., Maisons-Alfort, France) was grown at 30 °C in tomato, apple, pineapple, and orange juice media without adding supplements. Alternatively, yeast cells were grown in a synthetic complete (SC) medium containing all the nutrients needed for supported an exponential growth rate similar to undefined complex growth media [21].

#### 2.8.2. Growth Measurements of Treated Cultures

The growth of the yeast strain was automatically recorded for 30 h at 600 nm in a PowerWave XS (Biotek) apparatus at a controlled temperature (30 °C). The cell numbers initially inoculated in SC or fruit juice medium was $2 \times 10^6$. The oxidant *tert*-butyl hydroperoxide ($t-$BOOH) was added at different concentrations (0–2 mM) to induce oxidative stress in cells. $t-$BOOH is a stable peroxide with a higher diffusion rate across biological membranes than hydrogen peroxide and which oxidises different types of macromolecules inside the cell. For each growth curve, the following growth parameters were determined in the above conditions: maximal growth rate (µ, calculated as $\log_{10}OD_{600} \cdot h^{-1}$) during the exponential growth phase of the curve; maximal biomass growth (B), biomass measured as $OD_{600}$ after 30 h of growth; and lag time (λ), the period between cell inoculation and growth resumption [22]. For each growth condition, two parallel cultures were made, and three independent experiments were made for each growth medium and concentration.

### *2.9. Statistical Analysis*

Treatments were conducted in duplicate, and three replicate analyses were carried out for each sample to obtain the mean value. Statistical analysis was performed using the Statgraphics Plus v.5.1 Windows package (Manugistics, Inc., Rockville, MD, USA). Data were analysed by multifactor analysis of variance, and a Duncan multiple-range test was employed to find differences among means. Significant differences were considered at the $p < 0.05$ level. Principal component analysis (PCA) was carried out to obtain relationships among variables. The loadings plot was used to summarise the main relationship between

different variables and also highlight relationships between variables themselves. Variables that appear close together in this plot correlated positively. The score plot represents the projection of each sample into PC, defining different groups.

## 3. Results and Discussion

### *3.1. Health-Related Compounds*

Initial vitamin C content ranged from 10 (apple juice) to 344 mg of ascorbic acid/L (orange juice) (Table 2). Sánchez-Moreno et al. [23] reported that citrus juices, especially orange, are rich sources of vitamin C (54–62 mg/100 g) and suggested that drinking orange juice (500 mL/day) reduces oxidative stress. Pineapple juice is one of the juices that contain a higher concentration of vitamin C (158–580 mg/L) [24,25]. In the present work, the results obtained for vitamin C in pineapple juice (230 mg/L) are in the range of those published in the literature. Both HIPEF and heat processing did not modify the vitamin C concentration of apple juices. Consistently, vitamin C contents were not affected by HIPEF in apple juices after treatments of 35 kV/cm for 94 ms [26]. On the other hand, the vitamin C content of HIPEF and heat-treated orange, pineapple and tomato juices decreased drastically after the treatments compared to the untreated juices (Table 2). In the same line, some authors observed significant losses of vitamin C after HIPEF and thermal treatments in different fruit juices due to the sensitivity of the vitamin to different processing factors such as temperature, oxygen, and light [27,28]. However, HIPEF-treated tomato juice presented higher contents of vitamin C than those heat-treated juices. The retention of vitamin C in tomato juice treated by heat was about 79%, whereas in HIPEF-treated tomato juice, retention of 86% was obtained (Table 2). Min et al. [29] reported higher amounts of vitamin C in tomato juice treated by HIPEF than in samples processed by the conventional thermal treatment.

**Table 2.** Effect of high intensity pulsed electric field (HIPEF) and thermal treatment (TT) on vitamin C content of juices.

| Parameters | Treatments | Tomato Juice * | Apple Juice * | Pineapple Juice * | Orange Juice * |
|---|---|---|---|---|---|
| Vitamin C (mg of ascorbic acid/L of juice) | Fresh | $128 \pm 5^{aC}$ | $10 \pm 1^{aD}$ | $231 \pm 13^{aB}$ | $344 \pm 2^{aA}$ |
| | HIPEF | $110 \pm 1^{bC}$ | $12 \pm 3^{aD}$ | $145 \pm 4^{cB}$ | $276 \pm 3^{cA}$ |
| | TT | $102 \pm 2^{cC}$ | $11 \pm 1^{aD}$ | $160 \pm 5^{bB}$ | $291 \pm 4^{bA}$ |
| Total phenolic compounds (mg of gallic acid/L of juice) | Fresh | $167 \pm 2^{aD}$ | $547 \pm 5^{aB}$ | $276 \pm 7^{aC}$ | $1238 \pm 49^{aA}$ |
| | HIPEF | $154 \pm 1^{bD}$ | $551 \pm 12^{aB}$ | $280 \pm 11^{aC}$ | $1185 \pm 58^{aA}$ |
| | TT | $149 \pm 1^{cD}$ | $546 \pm 14^{aB}$ | $247 \pm 15^{bC}$ | $1190 \pm 68^{aA}$ |

* Data shown are mean ± standard deviation ($n = 6$). Different lower case letters in the same column for each parameter indicate significant differences among treatments ($p < 0.05$) for each juice. Different capital letters in the same row indicate significant differences among juices for the same treatment.

The concentration of total phenolic compounds in juices varied from 149 (tomato juice) to 1238 mg of gallic acid/L of juice (orange juice) (Table 2). Apple juices presented a total phenolic content of 547 mg of gallic acid/L. These values are within the range of those observed in other studies for juices. Total phenolic compounds in apple juices (400–600 mg of gallic acid/kg of juice) change related to the apple cultivar and various cultivation practices conducted [30]. Regarding the literature, orange juices presented a concentration of phenolic compounds between 3897 to 7550 mg of gallic acid/L with three hydroxybenzoic acids and seven hydroxycinnamic acids as the main phenolic acids in samples [10,31].

The effect of HIPEF and thermal processing on the total phenolic content of juices depended on the fruits used to prepare the juices. No changes in the total polyphenolic content of apple and orange juices were observed irrespective of the technology applied.

Agcam et al. [31] reported that the content of phenolic acids in orange juice did not significantly change after the PEF-treatment. Regarding pineapple juices, HIPEF technology did not cause significant losses of phenolic compounds compared to the thermal alternative

(11% less). However, total phenolic contents in HIPEF (154 mg of gallic acid/L) and thermally processed (149 mg of gallic acid/L) tomato juices were slightly but significantly lower than in the untreated juice (167 mg of gallic acid/L) (Table 2). These results agree with those published for tomato juices treated under HIPEF conditions similar to those used in the present work [32].

### 3.2. Antioxidant Activity

#### 3.2.1. In Vitro Methods

Several methods have been used to evaluate the antioxidant profile of food products. The experimental conditions and the specificity of the free radical used have been reported to greatly affect the antioxidant activity results. As seen in Table 3, in this study, the highest antioxidant activity values were obtained through the Folin-Ciocalteu assays (3.23–12.01 mmol of Trolox/L), followed by those determined by the TEAC method (1.74–7.07 mmol of Trolox/L). The lowest values were found in samples analysed by the FRAP assay leading to minimum values in the range of 0.119 to 0.910 mmol of Trolox/L. FRAP actually measures only the reducing capability based upon the ferric ion, which is not relevant to antioxidant activity mechanistically and physiologically (Prior et al., 2005). In addition, Ou et al. [33] reported that the FRAP assay could not be used to determine the total antioxidant power of many fruits and vegetable extracts because of the coloured compounds present in these matrixes, which can give some interferences. Regarding fruit juices, the antioxidant activity ranged from 0.119 (apple juices through the FRAP assay) to 12.01 mmol of Trolox/L (orange juice through the Folin-Ciocalteu assay) (Table 3). Tomato and apple juices presented similar antioxidant activity values throughout the FRAP and Folin-Ciocalteu assays, while the antioxidant activity in terms of DPPH radical was higher in tomato (1.38–1.44 mmol of Trolox/L) than in apple (0.41–0.68 mmol of Trolox/L) juices. Orange juices show an antioxidant activity between one and a half and seven times higher than tomato and apple juices, depending on the antioxidant activity method used. The highest values of antioxidant activity throughout the DPPH assay were found in pineapple juices (3.33–4.04 mmol of Trolox/L), whereas the antioxidant activity of this juice analysed by the other methods was always similar or significantly lower than those determined in orange juices. Therefore, methods did not provide comparable antioxidant values for the same fruit juice. Multiple reaction characteristics and mechanisms are usually involved in the determination; thus, no single assay will accurately reflect the overall antioxidant activity in a complex matrix such as food products. In addition, the different results from the four assays could be explained by the fact that those methods are based on different chemistry principles. Mechanistically, antioxidant activity methods are based on either a single electron transfer (SET) reaction or a hydrogen atom transfer (HAT) reaction between an oxidant and a free radical. Regarding SET-based methods such as FRAP, a single electron is transferred from the antioxidant molecule to the oxidant. For the HAT-based methods, a radical initiator is used to generate a peroxyl radical, which will abstract a hydrogen atom from the antioxidant. Although TEAC, DPPH and Folin-Ciocalteu assays are usually classified as SET methods, in these reactions, reagents may be neutralised either by direct reduction via electron transfer or by radical quenching via H atom transfer [34].

Regarding treated juices, non-significant depletion of the antioxidant activity according to the FRAP, Folin-Ciocalteu and TEAC assays was observed in apple juices processed by HIPEF and thermal treatment, whereas a reduction of the antioxidant activity (40%) through the DPPH assay was obtained in those juices. However, the antioxidant activity of tomato juices did not significantly change because of the HIPEF and thermal treatment application, irrespective of the antioxidant activity method used (Table 3). HIPEF treatments do not lead to significant changes in the overall antioxidant activity of tomato juices [32]. Elez-Martínez and Martín-Belloso [28] reported high retention of the antioxidant capacity determined by the DPPH method in a PEF-treated cold vegetable soup in which tomato was the main component. In pineapple and orange juices, the antioxidant activity assessed by the FRAP and TEAC methods was not affected by HIPEF treatment. In contrast, the antioxidant

activity determined by DPPH and Folin-Ciocalteu assays decreased after this non-thermal process. Antioxidant activity in orange and carrot juices was not influenced by HIPEF treatment using the TEAC method for measuring total antioxidant activity [35]. On the contrary, the antioxidant activity in pineapple juices evaluated by DPPH, TEAC and FRAP was enhanced with thermal treatments by about 9.1%, 15.3%, and 21.5%, respectively (Table 3). Maillard reaction products, which can be formed due to intense heat treatment or prolonged storage, generally exhibit strong antioxidant properties [36].

**Table 3.** Effect of high intensity pulsed electric field (HIPEF) and thermal treatment (TT) on in vitro antioxidant activity of juices.

| Methods | Treatments | Antioxidant Activity of Juices (mmol of TROLOX/L of Juice) * | | | |
|---|---|---|---|---|---|
| | | Tomato | Apple | Pineapple | Orange |
| DPPH | Fresh | $1.44 \pm 0.11^{aC}$ | $0.68 \pm 0.09^{aD}$ | $3.70 \pm 0.06^{bA}$ | $2.15 \pm 0.10^{aB}$ |
| | HIPEF | $1.38 \pm 0.06^{aC}$ | $0.41 \pm 0.12^{bD}$ | $3.33 \pm 0.05^{cA}$ | $1.87 \pm 0.11^{bB}$ |
| | TT | $1.44 \pm 0.04^{aC}$ | $0.45 \pm 0.03^{bD}$ | $4.04 \pm 0.15^{aA}$ | $2.26 \pm 0.16^{aB}$ |
| TEAC | Fresh | $1.74 \pm 0.09^{aD}$ | $4.73 \pm 0.03^{aB}$ | $3.39 \pm 0.02^{bC}$ | $6.58 \pm 0.02^{bA}$ |
| | HIPEF | $1.83 \pm 0.03^{aD}$ | $4.69 \pm 0.08^{aB}$ | $3.46 \pm 0.20^{bC}$ | $6.63 \pm 0.14^{bA}$ |
| | TT | $1.84 \pm 0.04^{aD}$ | $4.78 \pm 0.16^{aB}$ | $3.91 \pm 0.18^{aC}$ | $7.07 \pm 0.56^{aA}$ |
| FRAP | Fresh | $0.138 \pm 0.009^{aC}$ | $0.135 \pm 0.044^{aC}$ | $0.609 \pm 0.014^{bB}$ | $0.904 \pm 0.045^{aA}$ |
| | HIPEF | $0.135 \pm 0.001^{aC}$ | $0.119 \pm 0.040^{aC}$ | $0.602 \pm 0.012^{bB}$ | $0.910 \pm 0.047^{aA}$ |
| | TT | $0.135 \pm 0.002^{aC}$ | $0.138 \pm 0.035^{aC}$ | $0.740 \pm 0.038^{aA}$ | $0.656 \pm 0.002^{bB}$ |
| Folin-Ciocalteu | Fresh | $3.38 \pm 0.15^{aB}$ | $3.30 \pm 0.27^{aB}$ | $10.79 \pm 0.52^{aA}$ | $12.01 \pm 1.11^{aA}$ |
| | HIPEF | $3.26 \pm 0.11^{aB}$ | $3.23 \pm 0.21^{aB}$ | $9.80 \pm 0.35^{bA}$ | $10.46 \pm 0.94^{bA}$ |
| | TT | $3.28 \pm 0.01^{aC}$ | $3.76 \pm 1.20^{aC}$ | $8.66 \pm 0.09^{cB}$ | $10.48 \pm 1.64^{bA}$ |

* Data shown are mean ± standard deviation ($n = 6$). Different lower case letters in the same column for each parameter indicate significant differences among treatments ($p < 0.05$) for each juice. Different capital letters in the same row indicate significant differences among juices for the same treatment.

#### 3.2.2. In Vivo Methods

*S. cerevisiae* cells have been used in in vivo tests to study the antioxidant activities of phenolic compounds and vitamin C present in fruit juices and/or wine. However, in most of these studies, individual compounds are employed on yeast strains in a standard growth medium. Some studies have directly used wine [8] or fruit juices [9,10] in these in vivo tests with yeast strains. Thus, we tried to relate the antioxidant properties of the fruit juices analysed above with several growth parameters of an industrial strain of *S. cerevisiae* when growing in these juices in the absence or presence of an externally added oxidant (the alkyl peroxide *t*−BOOH). The study by Stinco et al. [10] demonstrated the protective antioxidant effects of orange juice. In the present work, in the absence of the oxidant *t*−BOOH, the four fruit juices studied allowed better growth conditions when compared with the synthetic SC medium, which was especially evident in the case of pineapple and orange juices for the final biomass parameter (Figure 1). In contrast, the exponential growth rate in apple juice was only slightly higher than in the SC medium. Both pineapple and orange juices, besides allowing adequate nutritional conditions, conferred protection against *t*−BOOH up to the maximum oxidant concentration tested. In orange and pineapple juice medium, no inhibitory effect on the growth of yeast cells was observed relative to untreated cells at 2 mM concentration. Heat and HIPEF treatments did not affect the antioxidant conditions of the pineapple juice medium (Figure 1). In the case of orange juice, its antioxidant properties were partially disturbed when 1.5 or 2 mM concentrations of *t*−BOOH were tested, particularly in heat-treated juices. Upon treatment with *t*−BOOH, neither tomato nor apple juices conferred significant antioxidant protection, taking SC as the control for comparisons. In these two juices, a 1.5 mM or higher concentration of the oxidant inhibited growth. Nevertheless, tomato juice treated by HIPEF improved the antioxidant properties of the medium, allowing yeast cell growth after a long adaptation period (Figure 1).

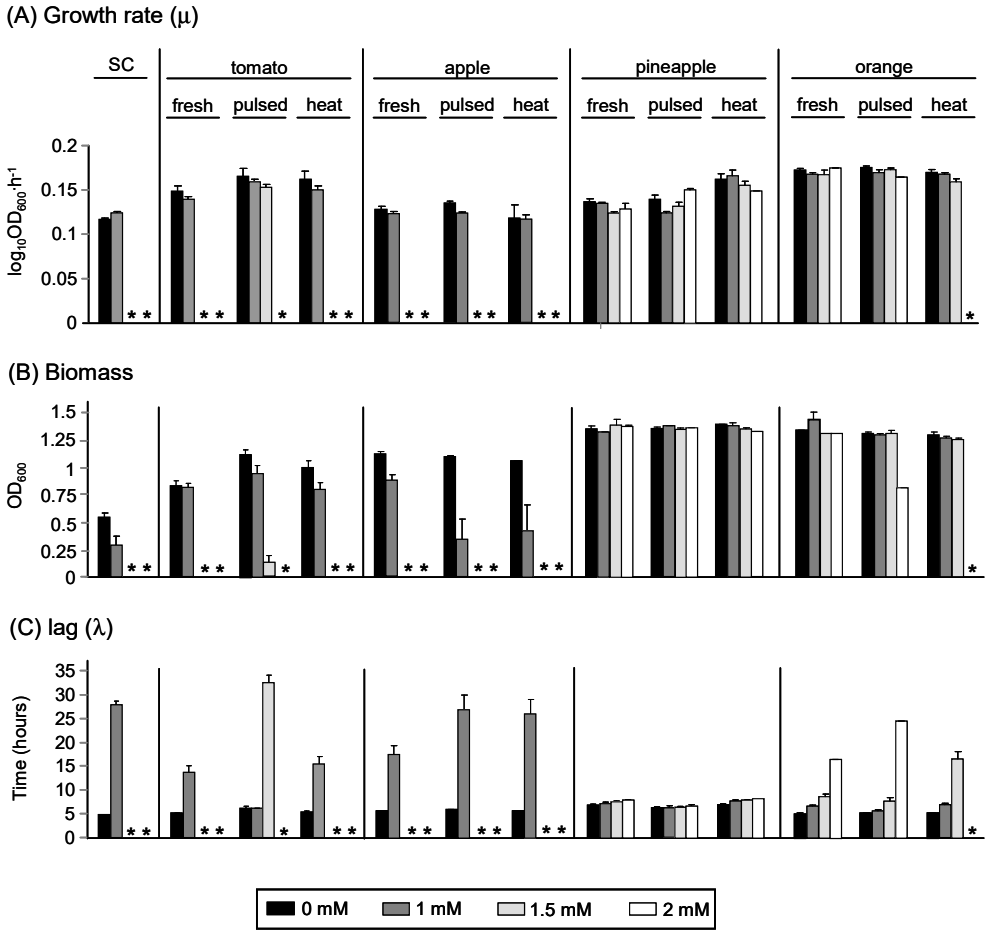


**Figure 1.** Effect of *t*−BOOH on the growth of Plus Vital baker's yeast cells in juices. Cells were grown in synthetic SC medium as well as in tomato, apple, pineapple and orange juices untreated (fresh) or previously treated with a pulsed electric field or heat for 30 h at 30 °C in the presence of the different concentrations of *t*−BOOH (0, 1, 1.5, 2 mM). $OD_{600}$ was automatically recorded at 1 h intervals. Curves correspond to a representative experiment in which cultures in the same medium with increasing oxidant concentrations were grown in parallel. Mean values of μ (**A**), final biomass (**B**) and λ (**C**) from at least three independent experiments of cultures (mean ± standard deviation) were calculated from the corresponding curves. * no growth.

In addition, latency (lag) phase extension indicates the time of adaptation yeast cells require to begin to divide and grow exponentially in a specific media. Yeast cells growing in pineapple juice display a much shorter lag phase than the other juices. Lag phase extension remains almost identical upon adding high doses of *t*−BOOH. This means that pineapple juice allows a better and faster adaptation, counteracting the effects of peroxides. Similarly, but to a lesser extent, orange juice also counteracts *t*-BOOH oxidative effects. Of note, HIPEF and heat treatment do not affect pineapple juice lag phase up to 1.5 mM *t*−BOOH. However, HIPEF treatment extends the lag phase at higher doses (2 mM), whereas yeast cells do not grow after heat treatment.

### *3.3. Relationships between Health-Related Compounds and Antioxidant Activities*

The antioxidant capacity of fruits and vegetables depends on a wide number of compounds. As was previously mentioned, several phytochemicals, especially ascorbic acid, phenolic compounds, thiols, carotenoids, tocopherols, and amino acids, might contribute to the total antioxidant activity of fruit and vegetable juices [37]. In addition, it has been reported that antioxidants may respond differently to different radical or antioxidant sources [34]. A principal component analysis (PCA) was used to determine the relation-

ships between phytochemicals and antioxidant activity. Two principal components (PC1 and PC2) were calculated, accounting for 94.7% of the variability in the original data. As can be seen in Figure 2, values of DPPH were not associated with either ascorbic acid content or phenolic compounds in fruit juices. It has been reported that the DPPH assay is not specific to any particular antioxidant component, thus applying to the overall antioxidant capacity of the sample [38]. In addition, DPPH interpretation is sometimes complicated as carotenoids have spectra that overlap DPPH values at 515 nm. Therefore, the weak relation of DPPH values with the main antioxidant in tomato, orange and pineapple juices might be due to the interference of carotenoids in those juices. In this way, an outstanding correlation between the DPPH radical scavenging capacity and carotenoids concentration (lycopene and β-carotene) in tomato juices has been reported [32]. The TEAC assay has been used to screen the relative radical-scavenging abilities of flavonoids and phenolics through their properties as electron- or H-donating agents [39]. As can be seen in Figure 2, there is a close relationship between TEAC values and the phenolic compounds content of juices. Therefore, the antioxidant capacity of juices determined through the TEAC assay could be mainly attributed to phenolic compounds rather than Vitamin C concentration. The contribution of phenolic compounds to antioxidant activity on vegetables has been reported to be much greater than that of vitamin C through the TEAC assay [40]. Vitamin C content of juices appears to correlate adequately with Folin-Ciocalteu and FRAP assays, demonstrating that both methods are a good indicator of the antioxidant vitamin content of juices (Figure 2). A positive and significant correlation was found between vitamin C and FRAP ($r = 0.70$) values in 18 tropical products from Brasil [41]. Ascorbic acid is a non-phenolic antioxidant capable of reducing the Folin-Ciocalteu regent (polyphosphotungstate-molybdate) to form a blue colour in alkaline pH [20]. In this way, Lester et al. [16] reported a good correlation between the total ascorbic acid content and the Folin-Ciocalteu values of strawberries, demonstrating that other reducing agents such as antioxidant vitamins can be detected through this assay. On the other hand, the growth rate is another parameter that correlates better with vitamin C levels (Figure 2), supporting that the antioxidant effect of these vitamins operates mainly during exponential growth of the cells when, in addition to externally added peroxide, cells are subjected to ROS produced by the metabolism of the actively growing cells. Vitamin C is a central substrate for ROS detoxification [42]. Although *S. cerevisiae* does not endogenously produce vitamin C, its supplementation protects yeast mutants deficient in some of the antioxidant defence systems against external pro-oxidants [43], emphasising the antioxidant role of this small molecule in the yeast system. This protective role of vitamin C is extended to wild-type yeast strains treated with diverse oxidants [44]. At the molecular level, ascorbate acts as a reductant for the activity of *S. cerevisiae* 1-Cys peroxiredoxin [45], a member of the peroxiredoxin family of proteins able to detoxify peroxides. All these facts support the correlation observed in the present study between vitamin C levels in the tested juices and the effects on yeast growth rates, pointing to the antioxidant role of vitamin C in these juices. The score plot of PC1 versus PC2 from the full-data PCA model describes differences between fruit juices. It can be observed that samples with small amounts of vitamin C and phenolic compounds, as well as low antioxidant activity, are situated in the left part of the score plot, whereas pineapple and orange juices rich in health-related compounds appear on the right-hand side. In addition, orange juices score higher on vitamin C, phenolic compounds and antioxidant activity through the TEAC assay since orange juices are located close to those parameters. The plot did not allow to discriminate among differently processed fruit juices. Therefore, the type of juice has more influence on the content of health-related compounds than the treatment applied.

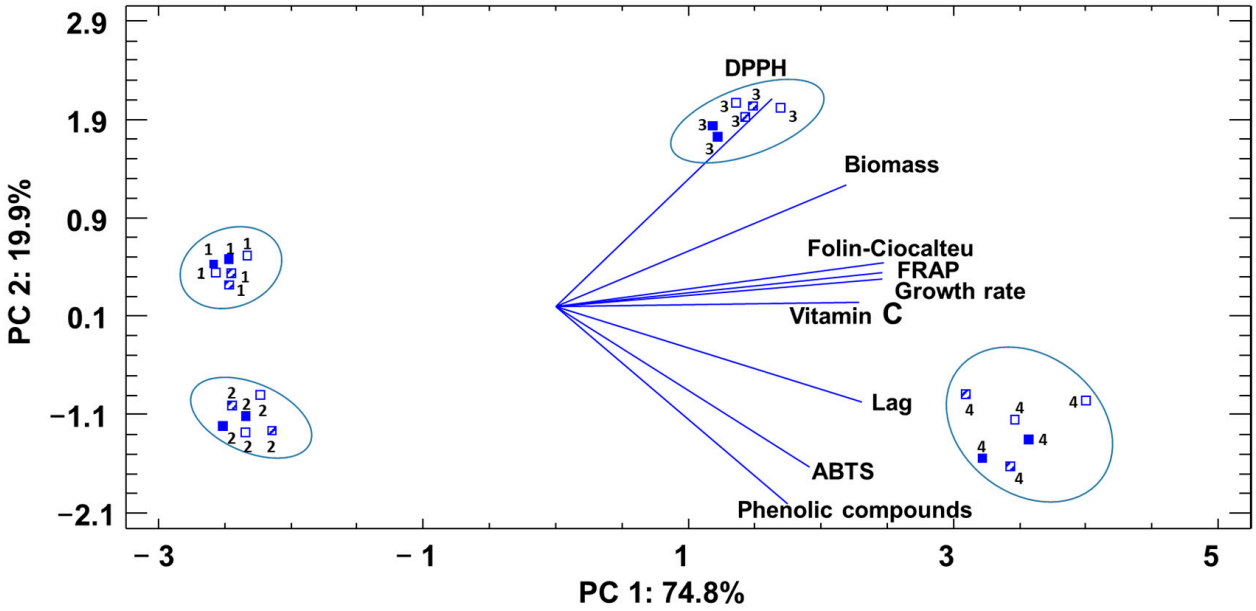


**Figure 2.** Plot of scores and loadings for two principal components (PC1, PC2) of untreated and treated fruit juices: (1) tomato, (2) apple, (3) pineapple and (4) orange juice. Treatments: fresh (□), HIPEF-treated (▨) and thermally-treated juices (■).

## 4. Conclusions

The antioxidant activity assays did not provide comparable antioxidant values for the same fruit juice since multiple reaction characteristics and mechanisms are usually involved in the determination. Among the four juices, pineapple offers the best growth conditions for the yeast cells either in normal conditions or upon oxidative stress. This antioxidant growth protection is maintained upon the pulsed electric field or heat treatments. The antioxidant activity of juices determined through the TEAC assay could be mainly attributed to phenolic compounds rather than Vitamin C concentration. The growth rate of *S. cerevisiae* and antioxidant activity determined by Folin-Ciocalteu and FRAP assays correlates well with vitamin C levels in juices. Therefore, this yeast reporter model provides an easy-to-use biological system to further determine the antioxidant activity of fruit juices. In addition, they can be used as a first step to study the specific molecules responsible for the antioxidant protective effects, but metabolomics studies should also be addressed.

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