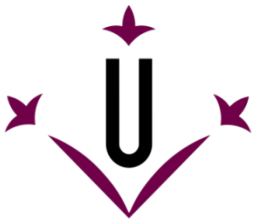

Universitat de Lleida

Document downloaded from:

http://hdl.handle.net/10459.1/69381

The final publication is available at:

https://doi.org/10.1016/j.lwt.2016.07.018

Copyright

cc-by-nc-nd, (c) Elsevier, 2016

Està subjecte a una llicència de
Reconeixement-NoComercial-SenseObraDerivada 3.0 de Creative Commons

Accepted Manuscript

Antioxidant activity of thermal or non-thermally treated strawberry and mango juices by *Saccharomyces cerevisiae* growth based assays

Isabel Odriozola-Serrano, Judit Puigpinós, Gemma Oms Oliu, Enrique Herrero, Olga Martín-Belloso

PII: S0023-6438(16)30421-2

DOI: 10.1016/j.lwt.2016.07.018

Reference: YFSTL 5592

To appear in: *LWT - Food Science and Technology*

Received Date: 22 February 2016

Revised Date: 3 June 2016

Accepted Date: 6 July 2016

Please cite this article as: Odriozola-Serrano, I., Puigpinós, J., Oms Oliu, G., Herrero, E., Martín-Belloso, O., Antioxidant activity of thermal or non-thermally treated strawberry and mango juices by *Saccharomyces cerevisiae* growth based assays, *LWT - Food Science and Technology* (2016), doi: 10.1016/j.lwt.2016.07.018.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Title:

Antioxidant activity of thermal or non-thermally treated strawberry and mango juices by *Saccharomyces cerevisiae* growth based assays

Authors: Isabel Odriozola-Serrano[1]
Judit Puigpinós[2]
Gemma Oms Oliu[1]
Enrique Herrero[2]
Olga Martín-Belloso[1*]

[1]Department of Food Technology,
University of Lleida - Agrotecnio Center
Rovira Roure 191, 25198 Lleida, Spain

[2]Department of Basic Medical Sciences
University of Lleida,- IRBLleida
Rovira Roure 80, 25198 Lleida, Spain

*Author to whom correspondence should be addressed.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

ABSTRACT

The antioxidant activity of strawberry or mango juice [untreated or treated by high intensity pulsed electric fields (HIPEF) or heat] has been studied on a *Saccharomyces cerevisiae* yeast strain exposed to different oxidants (*tert*-butyl hydroperoxide, diamide and diethyl maleate). Cellular biomass production was higher in the juices compared to the synthetic medium, which contained all required amino acids, nitrogen bases and vitamins needed for growth, indicating that growth-limiting nutrient concentrations in the natural juices were higher. In addition, the inhibitory effect of *tert*-butyl hydroperoxide or diamide in yeast growth was lower in the juices compared to synthetic medium, supporting an antioxidant effect of both juices. In contrast, juices did not confer significant protection against diethyl maleate. HIPEF or thermal treatments did not negatively influence the antioxidant activity of the juices. In fact, HIPEF-treated strawberry juices presented enhanced antioxidant effect on yeast cell growth in the presence of *tert*-butyl hydroperoxide compared to fresh juice in terms of adaptation period and the final cellular biomass.

Key words: oxidative stress, yeast strains, fruit juices, high intensity pulsed electric fields, thermal treatment.

ACCEPTED MANUSCRIPT

1. **INTRODUCTION**

Several methods have been developed to determine the antioxidant capacity of juices; the most frequently used are *in vitro* methods based on capturing or scavenging free radicals generated in the reaction or the reduction of metals ions (Huang, Ou & Prior, 2005). Recent studies suggested that, although *in vitro* methods are widely used due to their simplicity, they just provide a slight approximation to the antioxidant capacity, since these methods did not reflect cellular and physiological conditions such as bioavailability or metabolisms (Belinha et al., 2007). Cellular models, even with some limitations, allow a better approximation to the antioxidant protection in more complex systems; whereby they can be used as a good prediction tool of antioxidant capacity (Stinco, et al., 2015). *Saccharomyces cerevisiae* has been extensively used as a cellular model to determine the antioxidant capacity of foods and beverages, because this yeast reacts to oxidative stress and generates a response (Herrero, Ros, Bellí & Cabiscol, 2008). The response of *S. cerevisiae* against oxidative stress is well characterized and involves post-transcriptional mechanisms in addition to the transcriptional ones, eventually resulting in the activation of enzymatic and non-enzymatic response strategies to cope with the oxidant insult (Jamieson, 1998; Gasch, 2007). Thioredoxins (NADPH-dependent) and glutaredoxins (glutathione-dependent) are the enzymatic systems involved in repairing the oxidant alterations of protein thiol groups (Herrero, et al., 2008; Grant, 2001; Lillig, Berndt & Holmgren, 2008; Toledano, Delaunay-Moisan, Outten, & Igbaria, 2013). On the other hand, superoxide dismutases, catalases and thioredoxin- and glutathione-dependent peroxidases are the enzyme systems that detoxify reactive oxygen species with the exception of hydroxyl radical, which is the most toxic oxidant (Wood, Schroder, Harris & Poole 2003; Leitch, Yick & Culotta, 2009). Ascorbic acid, vitamin E or polyphenols are other compounds that may protect

ACCEPTED MANUSCRIPT

yeast cells against oxidants, although the specific mechanisms of protection are not well characterized (Krzepilko et al., 2004; Raspor *et al.*, 2005; Monteiro, Horta, Pimenta, Augusto & Netto, 2005; Escoté *et* al., 2012; Kwolek-Mirek, Zadrag-Tecza & Bartosz, 2012).

Refrigerated juices are a fast-growing segment within the beverages industry, partly due to the fact that these products are a good source of vitamin C and phenolic compounds such as flavonoids and phenolic acids. Great interest has been focused in strawberry juice because of its extremely high content of folate and vitamin C (37-65 mg/100 ml) (Klopotek, Otto & Böhn, 2005; Odriozola-Serrano, Soliva-Fortuny & Martín-Belloso, 2008; Giampieri, Tulipani, Alvarez-Suarez, Quiles, Mezzetti & Battino, 2012). To a lesser extent, strawberry juices are a source of healthy essential amino acids, fatty acids, iodine, magnesium, copper, iron, fructose and phosphorus. It has also been shown that mango juices are a good source of antioxidants vitamins such as vitamin C and vitamin E as well as carotenoids (β-carotene) and polyphenols which possess various beneficial effects on human health (Duarte, Barros, Delgadillo, Almeida & Gil, 2002; Gonzalez-Aguilar, Celis, Sotelo-Mundo, de la Rosa, Rodrigo-Garcia & Alvarez-Parrilla, 2008). Nutritionally, glucose, fructose and sucrose are the most abundant components of these juices. Amino acids, monounsaturated fatty acids, riboflavin, niacin, vitamin B_6, potassium, iron and phosphorus are essential constituents which can be found in low amounts in mango juice. The extension of the shelf-life of juices is commonly achieved by thermal processing with the inactivation of microorganisms and enzymes. However, heat treatments reduce the sensory and nutritional qualities of these products (Deliza, Rosenthal & Silva, 2003). Therefore, high intensity pulsed electric fields (HIPEF) is being developed as a non-thermal emerging technology for the preservation of foods. HIPEF processing, has been shown to effectively inactivate microorganisms in mango

ACCEPTED MANUSCRIPT

and strawberry juices at levels equivalent to those achieved by heat pasteurization without greatly affecting their nutritional and sensory properties (Mosqueda-Melgar, Raybaudi-Massilia & Martín-Belloso, 2012; Salinas-Roca, Élez-Martínez, Welti-Chanes & Martín-Belloso, 2013; Odriozola-Serrano, Garde-Cerdán, Soliva-Fortuny & Martín-Belloso, 2013a; Odriozola-Serrano, Aguiló-Aguayo, Soliva-Fortuny & Martín-Belloso, 2013b). Based on these considerations, the aim of the present work was to determine the potential antioxidant protective effect of fruit juices preserved by HIPEF and heat using the growth of *S. cerevisiae* cells as index of models of antioxidant activity. The yeast cells were exposed to different oxidants such as *tert*-butyl hydroperoxide, diamide and diethyl maleate to induce oxidative stress by different mechanisms.

2. MATERIALS AND METHODS

2.1.Reagents

SC medium was from Difco™, Detroit, MI, USA. Glucose, *t*-BOOH, diamide and DEM were from Sigma-Aldrich Co., St. Luois, MO, USA.

2.2.Juices preparation

Ten kg of strawberries (*Fragaria ananassa* Duch, cultivar Camarosa) and mangoes *(Mangifera indica* L cultivar Kent) were purchased from a local supermarket (Lleida, Spain), and kept at 4 ºC before being treated. 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 to guarantee the uniform distribution of electric field and avoid possible partial electric discharges. Juices were quickly frozen at -46 ºC and kept in darkness prior to treatment. Table 1

ACCEPTED MANUSCRIPT

122 presents the physicochemical parameters of the juices The pH and soluble solids content
123 of the juice were determined with a CRISON 2001 pH-meter (Crison, Barcelona, Spain)
124 and with a temperature-compensated refractometer Atago RX-1000 (Atago Company
125 Ltd., Tokyo, Japan), respectively. The color of juices was directly measured with a
126 Minolta CR-400 spectrophotocolorimeter (Konica Minolta Sensing, Inc., Osaka, Japan).
127 The CIE L* (lightness), CIE a* (red-green) and CIE b* (yellow-blue) were read using a
128 D_{75} light source and setting the observer angle at 10º and electric conductivity (Testo
129 240 conductivimeter; Testo GmBh & Co, Lenzkirch, Germany).
130

131 **2.3.Pulsed electric fields equipment**

132 High intensity pulsed electric field treatments were carried out using a continuous flow
133 bench scale system (OSU-4F, Ohio State University, Columbus, OH, USA), that
134 provides squared-wave pulses within eight co-field flow chambers arranged in series.
135 Each chamber had a treatment volume of 0.012 cm^3, delimited by two stainless steel
136 electrodes and separated by a gap of 0.29 cm. The flow rate of the process was adjusted
137 to 60 mL/min and controlled by a variable speed pump (model 752210-25, Cole Palmer
138 Instrument Company, Vermon Hills, IL, USA). The treatment temperature was kept
139 below 40 ºC using a cooling coil, which was connected before and after each pair of
140 chambers and submerged in an ice-water shaking bath. The thermocouples were
141 attached to temperature readers and isolated from the atmosphere with an insulation
142 tape. The temperatures of the inlet and outlet of each pair of chambers were recorded
143 every 0.1 s during HIPEF treatment. Juices were subjected to bipolar square-wave
144 pulses of 4 μs and 35 kV/cm field strength at frequencies of 100 Hz during 1700 μs
145 (strawberry juices) and 200 during 1500 μs (mango juices). These conditions were

ACCEPTED MANUSCRIPT

146 selected to obtain safe juices (Mosqueda-Melgar, et al., 2012; Salinas-Roca, et al.,
147 2013).Three replicates of each treatment were carried out.

148 **2.4.Thermal treatment**

149 In order to compare the effect of HIPEF treatment to that of the conventional thermal
150 treatment (TT), strawberry and mango juices were subjected to heat processes at 90 ºC
151 for 60 s, which conditions are usually conducted by fruit juices industry to ensure safety
152 of juices (Nagy, Chen & Shaw,1993). Juices were thermally-treated in a tubular heat
153 exchanger. A gear pump was used to maintain the juice flow rate through a stainless
154 steel heat exchange coil immersed in a hot water shaking bath (Universitat de Lleida,
155 Lleida, Spain). Three replicates of the treatment were carried out.

156

157 **2.5.Packaging and storage conditions**

158 Fluid handling systems were sanitized prior to processing. Polypropylene sterilized
159 bottles of 100 mL were filled with fresh, HIPEF-treated or thermally-treated juices
160 which were poured directly from the treatment system, leaving the minimum amount of
161 headspace volume. Each bottle contained 90 ml of juice. Once the containers were
162 tightly closed, the juices were kept under refrigeration (4 ºC) in darkness prior the
163 analysis.

164

165 **2.6.Yeast strains, media and growth conditions**

166 The commercial baker's yeast strain Plus Vital (from Lesaffre Yeast Corp., Maisons-
167 Alfort, France) was employed. Cells were grown with shaking at 30ºC in synthetic
168 complete (SC) medium with 2% glucose (Sherman, 2002). Batch cultures in these
169 conditions had an exponential phase doubling time of 90 min. This medium contained
170 all required amino acids, as well as nitrogen bases and vitamins needed for growth, and

ACCEPTED MANUSCRIPT

supported an exponential growth rate similar to undefined complex growth media (Sherman, 2002). Alternatively, yeast cells were grown in strawberry or mango juice at 30ºC to stimulate yeast growth.

2.7.Growth measurements of treated cultures

Growth of the yeast strain subjected to parallel treatments was automatically recorded (optical density at 600 nm, OD_{600}) at one-hour intervals during 30 hours, using individual 0.5 ml cultures in wells of shaken microtiter plates sealed with oxygen-permeable plastic sheets, in a PowerWave XS apparatus (BioTek, Winooski, VT, USA) at controlled temperature (30ºC). Identical cell numbers ($2x10^6$) from exponential batch cultures in SC medium were inoculated initially in each culture medium (SC or the respective juice). For each growth condition (control untreated cells or cells treated with a particular concentration of the oxidant) two parallel cultures were made, and three independent experiments were made for each growth medium, oxidants and concentration. The following oxidants were added inducing oxidative stress in cells: *tert*-butyl hydroperoxide (*t*-BOOH), diamide and diethyl maleate (DEM). For each growth curve the following parameters were recorded (Blomberg, 2011): 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 hours of growth; and lag time (λ), time period between cell inoculation and growth resumption.

2.8.Statistical analysis

The Mann-Whitney non-parametric test was employed for pair wise comparisons between mean values of different biological conditions, using the JMP package. Significant differences were considered at the $p < 0.05$ level.

ACCEPTED MANUSCRIPT

3.RESULTS AND DISCUSSION

As can be seen in table 1, pH of both juices were lower than 4.5, thus the microorganisms that are capable of growing in those juice are the lactic bacteria, yeasts, and molds depending upon the environments (Parish, 1991). Soluble solids content of mango juices (11.8 ± 0.1) was significantly higher than the strawberry (7.5 ± 0.3) juices, suggesting higher amount of soluble sugars in the former juice. Strawberry juices exhibited the highest a* (4.3) value and the lowest b* (2.7) comparing both juices, which can be related to the high amount of red pigments. Electrical conductivity is a property of food that measures the food's ability to conduct an electric current and it is a function of product characteristics composition (sugar and salt content) and pH, among others. Electric conductivity of strawberry juice (0.36 ± 0.04) was higher than those obtained by mango juices (0.28 ± 0.02), indicating that current will be better conducted in strawberry juices.

On the other hand, the potential protective effect of strawberry juice on growth of yeast cells was initially tested with the addition of an alkyl *t*-BOOH as oxidant. Peroxides are metabolically generated during aerobic respiratory metabolism of eukaryotic cells. As other peroxides, *t*-BOOH causes extensive oxidative damage on cellular lipids, proteins and nucleic acids (Halliwell & Gutteridge, 2007). As a control, the growth of yeast cells without and with *t*-BOOH in synthetic SC medium rich in amino acids, as well as nitrogen bases and vitamins was measured. In this SC medium, *t*-BOOH concentrations of 1.5 mM or higher totally inhibited yeast cell growth; while, yeast cells only resumed growth at 1 mM concentration after a significantly larger lag time (λ) compared to yeast cells without *t*-BOOH (Figure 1A). In contrast, in fresh strawberry juice medium, growth of yeast cells was totally inhibited by the *t*-BOOH only at 2 mM concentration, while at 1 mM no inhibitory effect on growth was observed in comparison to yeast cells

ACCEPTED MANUSCRIPT

221 treated with *t*-BOOH. It is also remarkable that total biomass production was
222 considerably higher (about two-fold) in fresh and HIPEF-treated strawberry juice than
223 in the SC medium without oxidant. This means that some nutrients and antioxidants are
224 limited in the SC medium for the yeast cells employed in the study, while being
225 available at higher levels in the juice. When HIPEF- or thermally-treated strawberry
226 juice was the growth medium, growth of yeast cells upon the *t*-BOOH was further
227 improved compared to fresh juice, and cells were able to grow even at 2 mM *t*-BOOH
228 (Figure 1A). This enhanced protective effect was more evident in the case of HIPEF-
229 treated juices, especially when the λ parameter is considered for comparisons. Amino
230 acids are important food compounds, not only contributing to the nutritive value of
231 foods, but also providing health benefits such as antimutagenicity (Myung, Park,
232 Jung, Park & Soon, 2005) as well as reduction in blood sugar (Gibbs, Zougman, Masse
233 & Mulligan, 2004) and coronary hearth diseases (Anderson, Johnstone & Cook-Newell,
234 1995). Many studies have indicated that amine compounds such as amino acids,
235 biogenic amine and polyamines have antioxidant activity in food. Their mechanisms of
236 protection can be explained by chelation of pro-oxidative metal trace and by
237 regeneration of oxidized primary antioxidants (Marcuse, 1960). Total free amino acids
238 concentration increased significantly after HIPEF treatment of strawberry juice
239 compared to the fresh juice (Odriozola-Serrano, et al., 2013a). In addition, strawberry
240 juice is a rich source of health-related compounds with antioxidant activity such as
241 vitamin C and anthocyanins. Vitamin C is commonly recognized as one of the major,
242 naturally occurring antioxidants in fruits with protective effects against various
243 oxidative stress-related diseases (Omaye & Zhang, 1998). Heat and HIPEF-treated (35
244 kV/cm for 1700 μs with 4-μs bipolar pulses at 100 Hz) strawberry juices contained
245 similar amounts of anthocyanins, ellagic and *p*-coumaric acids than fresh strawberry

ACCEPTED MANUSCRIPT

juices (Odriozola-Serrano, et al., 2013b). Therefore, the protective effect of strawberry juices could mainly be attributed to the amino acids content rather than to the vitamin C concentration. However, more research is needed to fully assess the antioxidant protection of health-related compounds and other secondary metabolites present in our juices.

Two additional oxidants causing oxidative stress, diamide (Kosower, Kosower & Wertheim, 1969) and DEM (Plummer, Smith & Sies, Bend, 1981) were also added to yeast cells. These compounds oxidize thiol groups of proteins and also of glutathione, which is the main redox regulator in the cells. Consequently, they have a narrower oxidant effect than *t*-BOOH, although their alteration of the equilibrium between reduced and oxidized glutathione may secondarily inhibit the antioxidant activity of enzymes dependent on reduced glutathione such as glutathione peroxidases and glutathione transferases (Herrero et al., 2008). Strawberry juice allows growth of yeast cells at diamide concentrations of 2 and 3 mM, whereas, cells do not grow in SC medium, at the same amount of oxidant (Figure 2A). This confirms the protective effect of the juice upon oxidative stress related to the oxidize thiol groups of proteins and glutathione of the yeast cells. In this case, no significant differences among fresh, HIPEF or heat treated strawberry juices medium were observed when comparing the three growth parameters (Figure 2A). DEM had a much milder inhibitory effect on yeast cell growth than *t*-BOOH or diamide. Considering the SC medium, the adaptation period was delayed in yeast cells with a concentration as high as 8 mM of DEM compared to those without oxidant, while non-significant differences in growth rate and final biomass were observed (Figure 2B). Similar enhancement of growth rate and biomass parameters was observed in cells yeast grown in strawberry juice with addition of 8 mM of DEM as oxidant. The only significant protective effect of the strawberry

ACCEPTED MANUSCRIPT

juice compare to SC medium was on shortening the differences in the lag time between cultures with or without oxidant (Figure 2B). All these results confirm that the observed antioxidant protective role of strawberry juice against *t*-BOOH can be extended to the diamide oxidant, although in this last case such protection is not enhanced neither by HIPEF or thermal treatments. These differences could be related to the different antioxidant systems directly acting for peroxide detoxification (mainly catalases and peroxidases) and for repairing diamide-mediated thiol oxidation (mainly thioredoxins and glutathione-dependent glutaredoxins). The low oxidant activity of DEM in the experimental conditions could be related to the progressive inactivation of the oxidant molecules during incubation of the cultures. This would explain why the only inhibitory effects observed are on the lag time at the initial stages of the cultures.

The effect of the three oxidants on yeast cells was also studied in mango juice. In this case, exponential growth rate of untreated cultures was higher in synthetic SC medium than in mango juice, although the final accumulated biomass was higher in the latter (Figure 3). As in the case of strawberry juice, this would be related with the higher nutrient and antioxidant content in juices, delaying nutrient exhaustion and consequent growth arrest. Comparison of the absolute values of the growth rate and biomass parameters in fresh strawberry (Figure 1 and 2) and mango (Figure 3) juices evidence that strawberry juice provides richer conditions for the yeast cells to growth than mango. High oxidant concentrations (1.5 mM for *t*-BOOH, and 2.5 mM for diamide) totally inhibiting yeast cell growth in SC medium, whereas a protective effect on growth was observed in juices when any of the three growth parameters was considered (Figure 3A and 3B). The mango juice-based protection was significant for the *t*-BOOH and diamide, but it was much more intense for the latter oxidant. In contrast, no protection by mango juice against DEM action was observed similarly to the strawberry juice

ACCEPTED MANUSCRIPT

(Figure 3C). The fact that mango protection is more effective against a specific thiol oxidant as diamide than in the case of oxidants with wider effects as *t*-BOOH points to the existence of disulfide-reducing activities in the mango juice. The surprising observation that no protection exist against DEM neither by strawberry nor by mango juice may be a consequence of the low toxicity of this oxidant against the reporter yeast cells employed in this study, perhaps due to deficient entry of the oxidant into the cells. Thus, the only statistically significant differences between yeast cells growth with or without oxidant in SC medium occurred in the lag time, even at a concentration of DEM as high as 8 mM (compared with diamide at 2-3 mM), and a similar situation was observed in yeast cells grown in strawberry juice. In mango juice, DEM toxicity was even moderately exacerbated compared with SC medium in particular when the biomass parameter was considered (Figure 3C). In these conditions, the modest inhibitory effects by DEM could be caused by mechanisms different from thiol oxidation that would not be protected by the juice.

4.CONCLUSION

Employing a *S. cerevisiae* yeast cells has allowed us to discriminate between the ability of two different fruit juices to sustain growth of yeast cells and to provide antioxidant protection during oxidative stress conditions. Strawberry juice presents substantially richer nutritional conditions than mango juice for yeast growth, and thus yeast cells display higher growth rate and reach a higher final culture biomass in the former juice. In any case, both juices provide protection to the yeast cells against oxidative stress acting at different biochemical levels as the organic *t*-BOOH or the thiol oxidant diamide. The antioxidant properties of the two juices are not lost in HIPEF or thermal-treated juices, and even HIPEF treatment of strawberry juice causes enhanced growth of

ACCEPTED MANUSCRIPT

the yeast cells. This supports that HIPEF treatment of juices induces some chemical conversion resulting in more effective antioxidant molecules. The yeast cells reported model provides an easy-to-use biological system to further characterize the specific molecules of the juices responsible for the antioxidant protective effects.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

5. REFERENCES

-Anderson, J.W., Johnstone, B.M., & Cook-Newell, M.E. (1995). Meta-analysis of the effects of soy protein intake on serum lipids. *The New England Journal of Medicine, 333 (5)*, 276-282.

-Belinha, L., Amorim, M.A., Rodrigues, O., De Freitas, V., Moradas-Ferrerira, P., Mateus, N. et al. (2007). Quercetin increases oxidative stress resistance and longevity in Saccharomyces cerevisiae. *Journal of Agricultural and Food Chemistry*, 55, 2446-2451. -Blomberg, A. (2011). Measuring growth rate in high-throughput growth phenotyping. *Current Opinion in Biotechnology*, *22*, 94-102.

-Deliza, R., Rosenthal, A., & Silva, A.L.S. (2003). Consumer attitude towards information on non-conventional technology. *Trends in Food Science and Technology*, *14*, 43-49.

-Duarte, I.F., Barros, A., Delgadillo, I., Almeida, C., & Gil, A.M. (2002). Application of FTIR spectroscopy for the quantification of sugars in mango juice as a function of ripening. *Journal of Agricultural and Food Chemistry, 50*, 3104-3111.

-Escoté, X., Miranda, M., Menoyo, S., Rodríguez-Porrata, B., Carmona-Gutiérrez, D., Jungwirth, H., Madeo, F., Cordero, R.R., Mas, A., Tinahones, F., Clotet, J., & Vendrell. J. (2012). Resveratrol induces antioxidant defence via transcription factor Yap1p. *Yeast*, *29*, 251-263.

-Gasch, A.P. (2007). Comparative genomics of the environmental stress response in ascomycete fungi. *Yeast*, *24*, 961-976.

-Giampieri, F., Tulipani, S., Alvarez-Suarez, J.M., Quiles, J.L., Mezzetti, B., & Battino, M. (2012). The strawberry: composition, nutritional quality, and impact on human health. *Nutrition*, *28*, 9-19.

ACCEPTED MANUSCRIPT

-Gonzalez-Aguilar, G. A., Celis, J., Sotelo-Mundo, R. R., de la Rosa, L. A., Rodrigo-Garcia, J., & Alvarez-Parrilla, E. (2008). Physiological and biochemical changes of different fresh-cut mango cultivars stored at 5° C. *International Journal of Food Science and Technology*, *43*, 91-101.

-Grant, C.M. (2001).Role of the glutathione/glutaredoxin and thioredoxin systems in yeast growth and response to stress conditions. *Molecular Microbiology*, *39*, 533-541.

-Halliwell, B., & Gutteridge, J.M.C. (2007). *Free Radicals in Biology and Medicine* Edition 4th. Oxford, England: Oxford University Press.

-Herrero, E., Ros, J., Bellí, G., & Cabiscol, E. (2008). Redox control and oxidative stress in yeast cells. *Biochemica et Biophysica Acta*, *1780*, 1217-1235.

-Huang, D., Ou, B., Prior, R. The chemistry behind antioxidant capacity assays. *Journal Agricultural and Food Chemistry*, 53, 1841-1856.

-Jamieson, D.J. (1998). Oxidative stress responses of yeast *Saccharomyces cerevisiae*. *Yeast*, *14*, 1511-1522.

-Klopotek, Y., Otto, K., & Böhn, V. (2005). Processing strawberries to different products alters contents of vitamin C, total phenolics, total anthocyanins, and antioxidant capacity. *Journal of Agricultural and Food Chemistry*, *53*, 5640-5646.

-Kosower, N.S., Kosower, E.M., & Wertheim, B. (1969). Diamide, a new reagent for the intracellular oxidation of glutathione to the disulfide. *Biochemical and Biophysical Research Communications*, *37*, 593-596.

ACCEPTED MANUSCRIPT

-Krzepilko, A., Swiecilo, A., Wawryn, J., Zadrag, R., Koziol, S., Bartosz, G., & Bilinski, T. (2004). Ascorbate restores lifespan of superoxide-dismutase deficient yeast. *Free Radical Research, 38*, 1019-1024.

-Kwolek-Mirek, M., Zadrag-Tecza, R., & Bartosz, G. (2012). Ascorbate and thiol antioxidants abolish sensitivity of *Saccharomyces cerevisiae* to disulfuram. *Cell Biology and Toxicology, 28*, 1-9.

-Leitch, J.M., Yick, P.J., & Culotta, V.C. (2009). The right to choose: multiple pathways for activating copper, zinc superoxide dismutase. *The Journal of Biological Chemistry, 284*, 24679-24683.

-Lillig, C.H., Berndt, C., & Holmgren, A. (2008). Glutaredoxin systems. Biochimica et Biophysica Acta, *1780*, 1303-1317.

-Marcuse, R. (1960). Antioxidative effect of amino-acids. *Nature*. 186, 886-887.

-Monteiro, G., Horta, B.B., Pimenta, D.C., Augusto, O., & Netto, L.E. (2007). Reduction of 1-Cys peroxiredoxins by ascorbate changes the thiol-specific antioxidant paradigm, revealing another function of vitamin. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 4886-4891.

-Mosqueda-Melgar, J., Raybaudi-Massilia, R.M., & Martín-Belloso, O. (2012). Microbiological shelf-life and sensory evaluation of fruit juices treated by high-intensity pulsed electric fields and antimicrobials. *Food and Bioproducts Processing, 90*, 205-214.

-Myung, Y.L., Park, S.-Y., Jung, K.O., Park, K.-Y., & Soon, D.K. (2005). Quality and functional characteristics of chungkukjang prepared with various Bacillus sp. isolated from traditional chungkukjang. *Journal of Food Science, 70(4)*, M191-M196.

ACCEPTED MANUSCRIPT

-Nagy, S., Chen, C.S., & Shaw, P.E. (1993). *Fruit juice processing technology*. Florida: Auburndale, Agscience.

-Odriozola-Serrano, I., Soliva-Fortuny, R., & Martin-Belloso, O. (2008). Phenolic acids, flavonoids, vitamin C and antioxidant capacity of strawberry juices processed by high-intensity pulsed electric fields or heat treatments. *European Food Research and Technology*, *228*, 239-248.

-Odriozola-Serrano, I., Garde-Cerdán, T., Soliva-Fortuny, R., & Martín-Belloso, O. (2013a). Differences in free amino acid profile of non-thermally treated tomato and strawberry jueces. *Journal of Food Composition and Analysis*, *32*, 51-58.

-Odriozola-Serrano, I., Aguiló-Aguayo, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2013b). Pulsed electric fields processing effects on quality and health-related constituents of plant-based foods. *Trends in Food Science and Technology*, *29*, 98-107.

-Omaye, S.T., & Zhang, P. (1998). Phytochemical interactions: β-carotene, tocopherol and ascorbic acid. In W.R. Bidlack, S.T. Omaye, M.S., Meskin, & D. Jahner (Eds), *Phytochemicals, a new paradigm* (pp. 53-75). Lancaster: Technomic.

-Parish, M.E. (1991). Microbiological concerns in citrus juice processing. *Food Technology*, 45(4), 128-136.

-Plummer, J.L., Smith, B.R., Sies, H., & Bend, J.R. (1981). Chemical depletion of glutathione *in vivo*. *Methods in Enzymology*, *77*, 50-59

-Raspor, P., Plesnicar, S., Gazdag, Z., Pesti, M., Miklavcic, M., Lah, B., Logar-Marinsek, R., & Poljsak, B. (2005). Prevention of intracellular oxidation in yeast: the role of vitamin E analogue, Trolox (6-hydroxy-2,5,7,8-tetramethylkroman-2-carboxyl acid). *Cell Biology International*, *29*, 57-63.

ACCEPTED MANUSCRIPT

-Salinas-Roca, B., Élez-Martínez, P., Welti-Chanes, J., & Martín-Belloso, O. (2013). Inactivation of Listeria innocua and enzymatic changes in mango juice treated by high intensity pulsed electric field (HIPEF). 2013 EFFoST Annual Meeting. Bio-based Technologies in the context of European Food Innovation Systems. Bolognia, Italy.

-Sherman, F. (2002). Getting started with yeast. *Methods in Enzymology, 350*, 3-41.

-Stinco, C.M., Baroni, M.V., Di Paola Naranjo, R.D., Wunderlin, D.A., Heredia, F.J., Meléndez-Martínez, A., & Vicario, I.M. (2015). Hydrophilic antioxidant compounds in orange juice from different fruit cultivars: composition and antioxidant activity evaluated by chemical and cellular based (*Saccharomyces cerevisiae*) assays. *Journal of Food Composition and Analysis 37*, 1-10.

-Toledano, M.B., Delaunay-Moisan, A., Outten, C.E., & Igbaria, A. (2013). Functions and cellular compartmentation of the thioredoxin and glutathione pathways in yeast. *Antioxidants and Redox Signaling 18*, 1699-1711.

-Wood, Z.A., Schroder, E., Harris, J.R., & Poole, L.B. (2003). Structure, mechanism and regulation of peroxiredoxins. Trends in Biochemical Sciences, *28*, 32-40.

ACCEPTED MANUSCRIPT

464 **FIGURE CAPTION**

465 **Figure 1.** Effect of *t*-BOOH on *S. cerevisiae* cells growth in strawberry juices. Cells
466 were grown in synthetic SC medium and in fresh, HIPEF and thermally-treated
467 strawberry juices for 30 hours at 30ºC in the presence of *t*-BOOH. (A) OD_{600} was
468 automatically recorded at 1 hour intervals. Curves correspond to a representative
469 experiment. (B) Data shown are mean values of μ, final biomass and λ of yeast cells ±
470 standard deviation (n=3). Values were calculated from the corresponding curves. #: no
471 growth. *: $p<0.05$, **:$p<0.001$ (Mann-Whitney test, when compared to treatment at the
472 same *t*-BOOH concentration in SC medium).

473

474 **Figure 2.** Effect of diamide (A) or DEM (B) on *S. cerevisiae* cells growth. Cells were
475 grown in synthetic SC medium and in fresh, HIPEF and thermally-treated strawberry
476 juices for 30 hours at 30ºC. Data shown are mean values of μ, final biomass and λ of
477 yeast cells ± standard deviation (n=3). Values were calculated from the corresponding
478 curves. #: no growth. *: $p<0.05$, **:$p<0.001$ (Mann-Whitney test, when compared to
479 treatment at the same *t*-BOOH concentration in SC medium).

480

481 **Figure 3**. Effect of diamide (A) or DEM (B) on *S. cerevisiae* cells growth. Cells were
482 grown in synthetic SC medium and in fresh, HIPEF and thermally-treated mango juices
483 for 30 hours at 30ºC. Data shown are mean values of μ, final biomass and λ of yeast
484 cells ± standard deviation (n=3). Values were calculated from the corresponding curves.
485 #: no growth. *: $p<0.05$, **:$p<0.001$ (Mann-Whitney test, when compared to treatment
486 at the same *t*-BOOH concentration in SC medium).

487

ACCEPTED MANUSCRIPT

ACKNOWLEDGEMENTS

This study has been carried out with financial support from Universitat de Lleida through a joint Agrotecnio/IRBLleida grant. This work was also supported by the Ministerio de Economía y Competividad (Spain) through the Project BFU2010-17656 and by the Generalitat de Catalunya (2009SGR/196 and 2014SGR/1000). Judit Puigpinós thanks University of Lleida for the predoctoral grant.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 1.- Analytical characteristics of mango and strawberry juices

Parameters[1]		**Mango juice**	**Strawberry juice**
pH		3.42 ± 0.03	3.35 ± 0.04
Soluble solids (ºBrix)		11.8 ± 0.1	7.5 ± 0.3
Color	**L***	38.7 ± 0.2	17.5 ± 0.2
	a*	1.4 ± 0.1	4.3 ± 0.3
	b*	17.4 ± 0.2	2.7 ± 0.2
Electrical conductivity (S/m)		0.28 ± 0.02	0.36 ± 0.04

[1]Results are the mean ± standard deviation of three measurements

ACCEPTED MANUSCRIPT

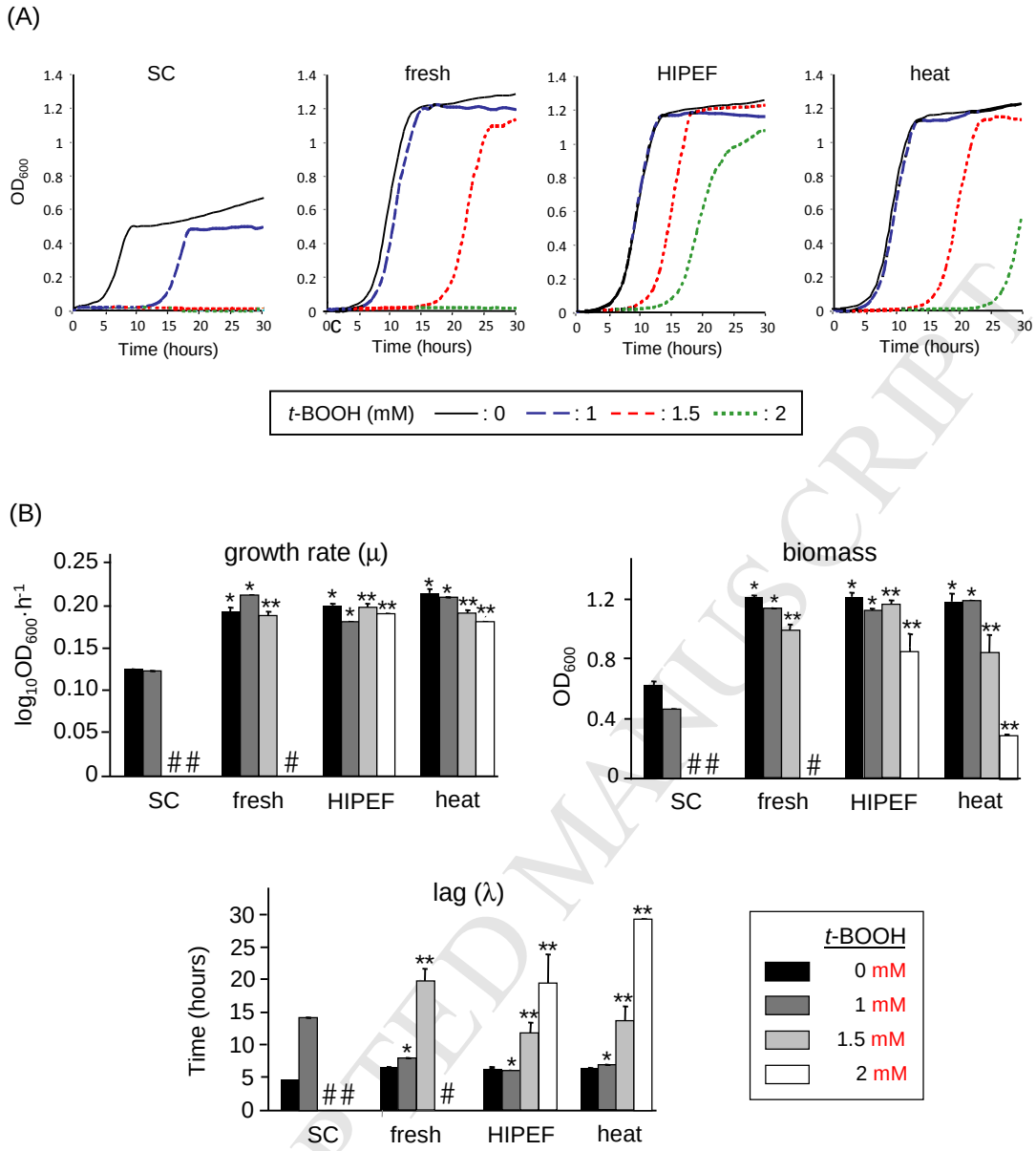

Figure 1

ACCEPTED MANUSCRIPT

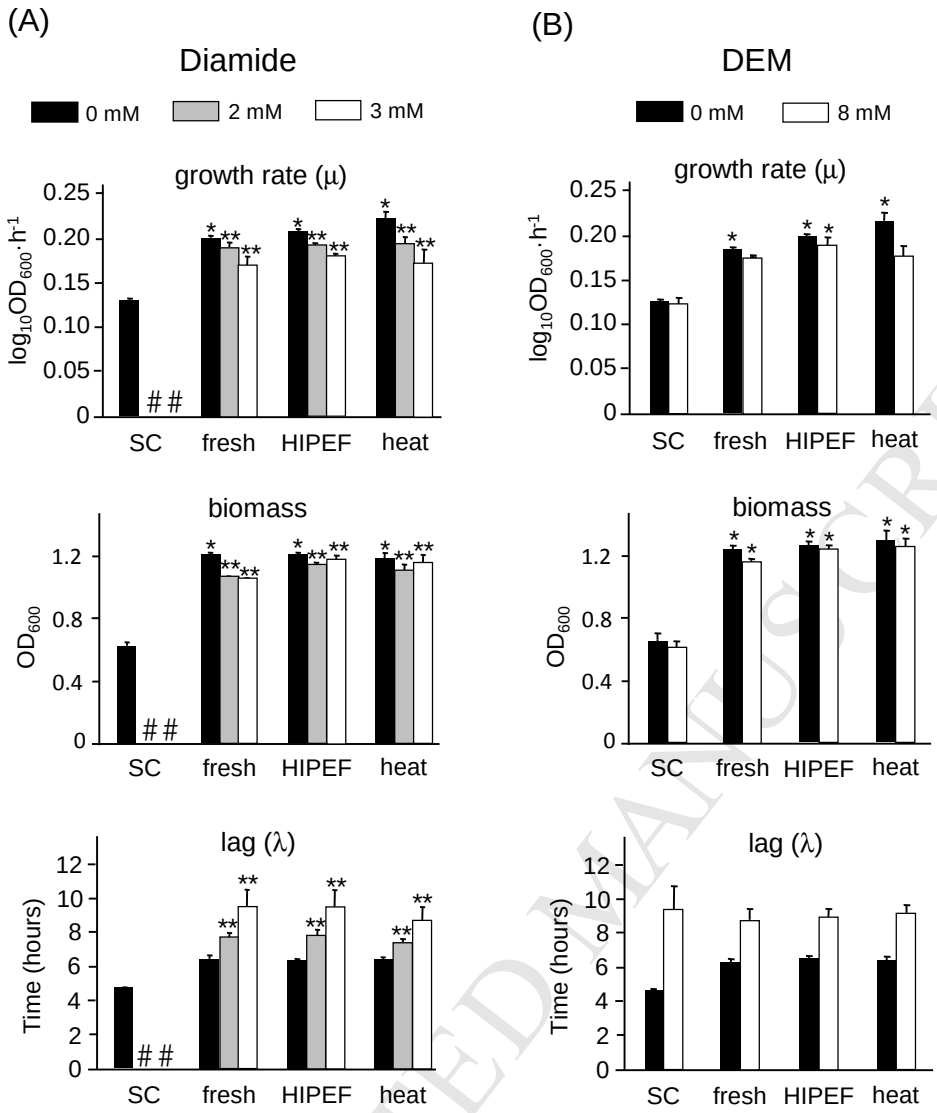

Figure 2

ACCEPTED MANUSCRIPT

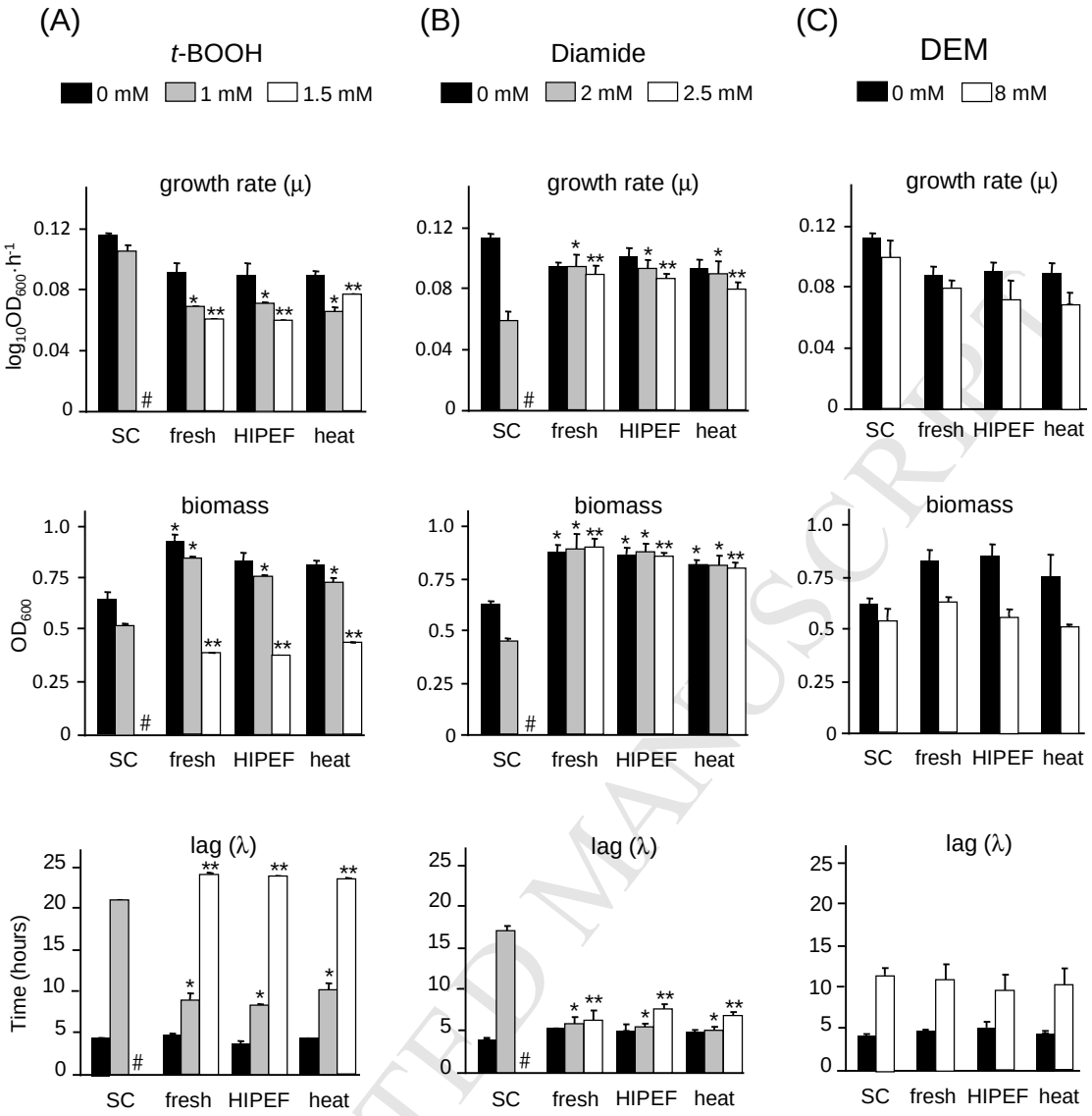

Figure 3

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

HIGHLIGHTS

-Juices provide protective effects against the toxicity of peroxide and thiol oxidant diamide.

-Growth of yeast strain is enhanced in strawberry and mango juices compared to synthetic medium

-Strawberry juice provide better protection on yeast cells against oxidative stress than mango juice

-HIPEF-treated juices presented higher antioxidant effect on yeast cell growth than those thermally treated.

ACCEPTED MANUSCRIPT