Employment of different processes for the production of strawberry vinegars: Effects on antioxidant activity, total phenols and monomeric anthocyanins

C. Ubeda[1], R. Callejón[1], C. Hidalgo[2], M.J. Torija[2], A.M. Troncoso[1], M.L. Morales[1*]

[1]Área de Nutrición y Bromatología. Facultad de Farmacia. Universidad de Sevilla. C/ P. García González nº2, E- 41012. Sevilla. Spain.

[2]Departamento de Bioquímica y Biotecnología. Facultad de Enología. Universitat Rovira i Virgili. C/ Marcel·lí Domingo s/n. E- 43007. Tarragona. Spain.

[*]Corresponding author: mlmorales@us.es; Tel.: 34-954-556760; Fax.: 34-954-233765

Abstract

The use of strawberry surpluses for the production of added value products seems to be a good solution choice to avoid the waste of this fruit. We produced strawberry vinegars through double fermentation (alcoholic and acetous) from three different harvests of *Fragaria x ananassa var. Camarosa.* The objective was to study the evolution of antioxidant activity, total phenols and monomeric anthocyanins during the vinegar production process. These parameters increased when sulphur dioxide and pectolytic enzymes were added to substrates. Inoculation with the *Saccharomyces cerevisiae* strain RP1 produced wines with half the anthocyanins with respect to the spontaneous fermentations. The use of wood barrels, particularly cherry wood barrels, had a positive effect on all the parameters determined. All measured parameters decreased during the double fermentation process. In general, the acetification stage led to a high loss of antioxidant compounds. Moreover, the production of these vinegars at a semi-pilot scale yielded final commodities with the best values for antioxidant activity, total phenols and monomeric anthocyanins comparing with the vinegars obtained in 2008 and 2009 harvest.

Keywords: antioxidant activity; monomeric anthocyanins; strawberry; vinegar; wine.

1. Introduction

Strawberries are a widely researched fruit for their nutritional and health benefits as well as their organoleptic properties. This fruit is rich in vitamins, minerals, fibre and phytochemicals. In addition, strawberries contain potentially bioactive compounds and are a great source of phenolic compounds such as flavonoids and phenolic acids (Aaby, K., Skrede, G., & Wrolstad, R. E, 2005; Määttä-Riihinen, K. R., Kamal-Eldin, A., & Törrönen, A. R, 2004; Seeram, N. P., Lee, R., Scheuller, H. S., & Heber, D, 2006). All of these phenolic compounds have been shown to prevent oxidative processes, particularly those caused by reactive oxygen species (ROS) (Aaby, K., Ekeberg, D., & Skrede, G, 2007; Cerezo, A. B., Cuevas, E., Winterhalter, P., Garcia-Parrilla, M. C., & Troncoso, A. M, 2010a). These compounds make strawberries a highly antioxidant fruit (Aaby et al., 2005; Wolfe, K. L., Kang, X., He, X., Dong, M., Zhang, Q., & Liu, R. H, 2008) with potential health benefits. Among the numerous healthy properties described in the literature are anti-proliferative effects on cancer cells (Meyers, K. J., Watkins, C. B., Pritts, M. P., & Liu, R. H, 2003; Olsson, M. E., Andersson, C. S., Oredsson, S., Berglund, R. H., & Gustavsson, K, 2006) and the antioxidant and anti-inflammatory effects that have been shown to reduce cardiovascular disease risk factors in several prospective cohort studies (Hannum, 2004).

According to the latest data from the FAO (FAOStat, FAO 2011), Spain is the second-largest strawberry producer in the world; a large portion of this production is harvested in Huelva (Andalucía). Every year, part of the crop is discarded for various reasons, including size or deformations of the berries, or overproduction which leads to surpluses. Because vinegar is generally an inexpensive product, its production requires low-cost raw materials, such as sub-standard fruit and seasonal agricultural surpluses (Solieri & Giudici, 2009). In addition, there is a growing demand for fruit vinegars,

which are sold as a health food (Shau-mei & Chang, 2009). The use of strawberries of second quality, which are still suitable for human consumption, to production healthy vinegars with special organoleptic nuances may be a good method to reduce losses due to discarding the fruit.

For this purpose, we have produced strawberry vinegars using second-quality strawberries employing two-stage fermentation and assessed different conditions and treatments. The aim of this work was to evaluate the changes in the antioxidant activity (AA), total phenols index (TPI) and total monomeric anthocyanins (TA) during the production process of strawberry vinegar. In addition, an adequate extraction method to perform these determinations was designed.

2. Materials and methods

2.1. Chemicals

The reagents acetone, methanol, Folin-Ciocalteu reagent, ethanol, di-potassium hydrogen phosphate (anhydrous), sodium di-hydrogen phosphate 1-hydrate, potassium chloride, sodium acetate and sodium carbonate (anhydrous) were purchased from Merck (Darmstadt, Germany). Fluorescein sodium and gallic acid were supplied from Fluka (Madrid, Spain). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azobis (2-methylpropionamidine) dihydrochloride (AAPH) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Samples

For the optimisation of the extraction process, we used strawberries (*Fragaria ananassa var. camarosa*) acquired at the market. The fruit was crushed in our laboratory, distributed into amber glass flasks and frozen at -20º C.

For the production of the vinegars, we employed three different batches of strawberries (*Fragaria ananassa var. camarosa*) from the Huelva area (Spain), corresponding to

three harvests: 2008, 2009 and 2010. The production processes were performed in the laboratories of the Dept of Biochemistry and Biotechnology, Faculty of Oenology, Univ Rovira i Virgili (Tarragona). In 2008 and 2009, the substrate employed were purees prepared in the laboratory using a beater. In 2010, we used a commercial puree provided by the Hudisa Company (Huelva). Sulphur dioxide (60 mg/L), sucrose and two types of pectolytic enzymes (Depectil extra-garde FCE® and Depectil clarification® from Martin Vialatte Oenologie, Epernay, France), both at a concentration of 15 mg/L, were added to the puree. After this point, the procedures were slightly different in each harvest.

2008 harvest

One portion of the strawberry puree was pressed to study the effect of two types of starting substrates (semi-solid and liquid) (Table 1). Six glass containers were filled with 6 L of fruit substrate (four purees and two liquids). Half of the containers of each type of substrate were inoculated with the yeast *Saccharomyces cerevisiae* QA23 at a concentration of $2x10^6$ cells/mL, and spontaneous alcoholic fermentation was allowed to occur in the other half. All wines were spontaneously acetified keeping it in the same containers. Two final treatments were tested in vinegars: pasteurization or centrifugation. The average acetic degrees in the 2008 strawberry vinegars were 4.8.

2009 harvest

For the vinegar production in 2009, eight glass vessels were filled with 6 L of strawberry puree each. Half of these vessels were inoculated with the yeast strain *Saccharomyces cerevisiae* RP1, isolated during the 2008 spontaneous alcoholic fermentation, and spontaneous alcoholic fermentation was allowed to occur in the other half. All of the wines obtained from the inoculated alcoholic fermentation were mixed and dispensed in three different types of containers: a glass vessel and oak or cherry wood barrels. Samples were then inoculated with a strain of acetic acid bacteria isolated

from the 2008 acetification. Wines from the spontaneous alcoholic fermentation were processed in the same way and left to acetify spontaneously. The vinegars obtained were pasteurised. Inoculated vinegars from the 2009 harvest reached an acetic degree of 5.5 (glass container), 6.6 (oak barrel) and 6.3 (cherry barrel).

A portion of the puree from the 2009 strawberries was concentrated by heating in a water bath at 80ºC during 10 hours, to test another method of increasing the sugar content; the resulting product was a cooked must (Table 1). The sucrose final concentration was 140 g/L. One litre of this substrate was fermented by a spontaneous process and one litre was inoculated with the RP1 strain of yeast. The inoculated wines (IWs) were acetified with the same acetic acid bacteria isolated in 2008, and the spontaneous wines (SWs) were left to acetify spontaneously.

2010 harvest

In this harvest, the pectolytic enzymes added were Rohapect® (12 mg/hL) and the pH was adjusted to 3.5 with 2 g/L $CaCO_3$. In this case, 45 L of puree were fermented in a stainless steel container on a semi-pilot scale, after inoculation with *S. cerevisiae* RP1. The acetous fermentation was performed in a cherry wood barrel. The vinegar had an acetic degree of 6.3.

All vinegars from 2009 and 2010 harvest were pasteurized as final treatment.

Forty-one samples, taken throughout these production processes, were analysed. The codes and characteristics of the samples are shown in Table 1. In addition, five commercial vinegars were also analysed to carry out comparative studies: Aceto Balsamico, red wine and white wine vinegars, apple vinegar and sherry vinegar.

2.3. Sample-extraction procedure

The consistency of the samples (purees, wines and vinegars) made it necessary to establish an extraction system prior to analysis. The method employed was based on the

126 extraction procedures designed and optimised previously by Ubeda, Hidalgo, Torija,
127 Mas, Troncoso & Morales (2011a). Twenty grams of sample were mixed in a beaker
128 with 40 ml of extract for 10 min while shaking at 800 rpm. The sample was then
129 subjected to ultrasonication followed by a centrifugation at 4000 rpm for 15 min. The
130 supernatant was recovered, and the pellet was re-extracted with 40 ml of solvent
131 following the same procedure. Both extracts were subsequently mixed, and the organic
132 solvent was removed under vacuum. Finally, the extract was filtered, and MilliQ water
133 was added to a final volume of 15 ml. Every extraction was performed in duplicate. We
134 tested different condition to get the maximum values of AA, TPI and TA as well as
135 economy of solvent used and time. Thus, the parameters studied to select the best
136 extraction conditions were: type of solvent (acetone, methanol or ethanol), percentage
137 of solvent (80% or 100%) and ultrasonic extraction time (15, 25, 35 or 50 min).

138 **2.4. Assays and Methods**

139 **2.4.1. ORAC-FL assay**

140 The Oxygen Radical Absorbance Capacity assay (ORAC-FL) was performed in a Black
141 96-well microplate, following the procedure described in Ubeda et al. (2011a). This
142 assay was conducted in a Multi-detection plate reader (Synergy HT, Vermont, USA)
143 located at the Centre for Research, Technology and Innovation at the University of
144 Seville (CITIUS). All reaction assays were performed in triplicate. Results were
145 expressed as μmol Trolox equivalents (TE)/kg of sample.

146 **2.4.2. DPPH radical scavenging assay**

147 To determine the radical scavenging capacity, the DPPH assay described by Brand-
148 Williams, Cuvelier, & Berset (1995) was used. For this test, we used an UV/Vis
149 spectrophotometer U-2800 Digilab coupled to a Peltier themostatic system (Hitachi,

Tokyo, Japan). Results were expressed as µmol Trolox equivalents (TE)/kg of sample. The assays were performed in triplicate.

2.4.3. Total Phenols Index

This parameter was determined in triplicate, using the Folin-Ciocalteu method following the procedure described in Waterhouse (2001). Results were expressed as mg gallic acid/L.

2.4.4. Total monomeric anthocyanins

The determination of total monomeric anthocyanin content (TA) was measured following the pH-differential method described in Giusti & Wrolstad (2001). TA was expressed as pelargonidin-3-glucoside (Plg-3-glu), which is the major anthocyanin in strawberry fruit with a $\lambda_{vis\text{-}max}$ at 510 nm (Swain, 1965). Two buffers were prepared: potassium chloride buffer pH=1 (0.025 M), and sodium acetate buffer pH=4.5 (0.4 M). We measured the absorbance at 510 and 700 nm against a cuvette filled with distilled water as a blank.

We then calculated the absorbance of the diluted sample (A) as follows:

$$A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}$$

The monomeric anthocyanin concentration in the original sample was calculated using the following formula:

$$TA[\text{Plg-3-glu (mg/L)}] = (A \times MW \times DF \times 1000) / (\varepsilon \times 1)$$

Where

A = Sample absorbance

MW= Molecular weight of Plg-3-glu (487.5)

DF= Dilution factor

ε= Absorption coefficient of Plg-3-glu (17330)

174 The results were expressed as mg Plg-3-glu/kg of sample.

175 **2.5. Statistical analysis**

176 All statistical analysis was performed using the Statistica version 7.0 software package
177 (Statsoft, Tulsa, USA).

178 **3. Results and discussion**

179 **3.1. Selection of the best extraction conditions**

180 Several factors, such as solvent composition, time of extraction, temperature, pH, solid-
181 to-liquid ratio and particle size, may significantly influence solid-liquid extractions
182 (Azizah, A. H., Ruslawati, N. M. N., & Tee, T. S, 1999; Pinelo, M., Del Fabbro, P.,
183 Manzocco, L., Nunez, M J., & Nicoli, M. C, 2005). In our case, the parameters that
184 were evaluated to determine the best extraction conditions were the type of solvent, the
185 solvent-water ratio and ultrasonication time. The criteria used to select the extraction
186 parameters were the maximum values of antioxidant activity, total phenols,
187 anthocyanins and time and solvent savings.

188 The type of solvent is one of the most influential variables in the extraction process. We
189 tested acetone, ethanol and methanol. The extraction with methanol gave the worst
190 results in all the assays. As shown in Figure 1, acetone yielded the highest values for
191 DPPH (8327 µmol Trolox equivalents (TE)/kg) and TPI (2090 gallic ac. mg/kg), with
192 significant differences in this last parameter. However, we obtained the best results for
193 the ORAC assay (24329 µmol TE/kg) and for the TA determination (26.78 mg Plg-3-
194 glu/kg) using ethanol, but no significant differences were found between these values
195 and those with acetone (26.30 mg Plg-3-glu/kg). Henríquez, C., Carrasco-Pozo, C.,
196 Gomez, M., Brunser, O., & Speisky, H, (2008) reported that the antioxidant activity of
197 strawberry extracts obtained with acetone/water was higher than that with ethanol/water
198 and aqueous extracts. Taking into account this and other studies (Garcia-Viguera, C.,

Zafrilla, P., & Tomás-Barberán, F. A, 1998; Pinelo et al., 2005) and our results, we selected acetone for the strawberry extractions.

The solvent-water ratios assayed were 100 and 80:20 (acetone:water) (Figure 2). The best results for all the parameters measured were obtained using a ratio of 80:20.

Finally, the extraction potential of ultrasound technique depends on the application time, so, we assayed 15, 25, 35 and 50 mins. The ultrasonication time chosen was 25 mins, since at this time ORAC, TPI and TA reached the highest values (Figure 3).

3.2. Changes in AA, TPI and TA during the production of strawberry vinegars

3.2.1. Substrate pre-treatments

Three different strawberry purees were employed in this study. These purees presented similar values for all parameters, except the high values of TA in the substrate of the 2009 harvest. After the pre-treatments (pectolytic enzymes and SO_2 addition), we observed significant increases in almost all of the measured parameters, comparing P1 and P2 samples of each harvest (Tables 2-4). Considering the increases percentage, we observed a good correlation between the DPPH with TA (r^2=0.998) and with TPI (r^2=0.971) percentages. This could mean that these phenolic compounds are responsible for a percentage of the increases of AA.

Previous studies have shown that pectolytic enzyme treatment is very useful for the release of phenols and anthocyanins from different kinds of berries (Meyer, 2002; Klopotek, Y., Otto, K., & Boehm, V, 2005). These enzymes were effective for the release of other phenolic compounds such as ellagic acid, which has been described as the main phenolic compound in berries from the *Fragaria* (strawberry) genus, representing 51% of the compounds analysed (Häkkinen, S. H., Heinonen, I. M., Kärenlampi, S. O., Mykkänen, H. M., Ruuskanen, J., & Törrönen, A. R, 1999). On the other hand, SO_2 protects against oxidation (Delteil, D., Feuillat, M., Guilloux-Benatier,

M., Sapis, J. C, 2000) and may be extracting anthocyanins and phenolic compounds. This effect was observed in blueberries (Lee & Wrolstad, 2004).

The 2008 liquid substrate had significantly lower values for all parameters when compared to the puree substrate.

The cooked must from 2009 harvest had more AA than the original substrate. Because of this result, and taking into account that the starting substrate was concentrated 2.13 times, it seems that the AA was affected by the heating as expected. In addition, anthocyanins were strongly affected by this treatment, decreasing 84%. This same effect was observed by Verbeyst, L., Oey, I., Van der Plancken, I., Hendrickx, M., & Van Loey, A, (2010), who showed that anthocyanins are more rapidly degraded at higher temperatures on strawberry puree.

3.2.2. Alcoholic fermentation

Alcoholic fermentation was associated with a decrease in all parameters studied. The decline was statistically significant in most cases when the substrate employed was a puree, except in the case of cooked must, in which AA increased obtaining a very high antioxidant product. The decrease in anthocyanins was larger than in the rest of parameters (63-85%). This result is similar to the values obtained in other studies (decrease of 69-79%) (Klopotek et al., 2005). In general, the final values of AA and TPI in wines were similar in the three harvests.

In 2008, we found significantly differences between types of alcoholic fermentation, i.e. inoculation (IW) and spontaneous (SW) for DPPH, TPI and TA values. Total phenolic content was higher in SWs, and anthocyanin contents were higher in IWs, regardless the type of substrate used (semi-solid or liquid). In the wine from the liquid substrate, we observed that the AA and the TPI were lower than semi-solid substrate. However, the levels of anthocyanins in both types of wines were similar.

In the 2009 wines, strawberry SWs had higher significantly values of TA than inoculated wines, even in wines made from cooked must, showing a trend contrary to that observed in the wine production of 2008. It is important to note that the yeast strain (RP1) employed for the production of 2009 IWs was isolated from the 2008 spontaneous alcoholic fermentation. For this reason, we believe that the diminution of TA may be related in some way to the yeast strain involved in fermentation. There are several possible explanations: the adsorption of anthocyanins to the cell walls of the used yeast strain (Morata, A., Gomez-Cordoves, M. C., Colomo, B., & Suarez, J. A, 2005) and condensation reactions with acetaldehyde (Bosso & Guaita, 2008). Perhaps the *Saccharomyces* strains involved in the 2008 spontaneous fermentations had a greater tendency to adsorb these molecules than the strain used in the inoculated processes.

The condensation reactions involve a loss of the aldehyde and the diminution of anthocyanins. We have previously reported (Ubeda, C., Callejón, R. M., Hidalgo, C., Torija, M. J., Mas, A., Troncoso, A. M., Morales, M. L, 2011b) wines obtained by spontaneous alcoholic fermentations in 2008 and inoculated in 2009 contained less acetaldehyde and TA (mentioned above) than their corresponding opposite type of fermentation. In any case, the yeast strain had a greater influence in TA values than the strawberry harvest.

Finally, in the alcoholic fermentation at semi-pilot scale in a stainless steel tank (2010), the loss of AA, TPI and TA was smaller than the losses in the 2008 and 2009 harvests. Probably, the difference found may be due to the lower volume to size of contact surface with oxygen ratio in the stainless steel tank.

3.2.3. Acetous fermentation

In most cases, the acetification process was associated with a decrease in the parameters studied, being TA the most affected. Some of the loss of anthocyanins can be attributed

to polymerisation or condensation reactions with other phenols, as noted in vinous substrates (Andlauer, W., Stumpf, C., & Fürst, P, 2000; Cerezo, A. B., Cuevas, E., Winterhalter, M., Garcia-Parrilla, M. C., & Troncoso, A. M, 2010b). Again, as occurred in alcoholic fermentation, we observed the lowest decreases in all of these parameters in the 2010 samples.

In 2008, vinegars were subjected to two different final treatments. In assessing the antioxidant activity (Table 2), we observed that the ORAC and DPPH values were slightly higher in pasteurised vinegars than in centrifuged vinegars. The centrifugation procedure removes suspension particles being able to produce losses of antioxidant compounds. Moreover, this result could also be explained by the formation of Maillard reaction products such as melanoidins that are produced by the heat of pasteurisation. Several authors who have studied vinegar melanoidins have concluded that contribute to the total antioxidant capacity of it (Xu, Q., Tao, W., & Ao, Z, 2007).

In the 2009 (Table 3), spontaneous and inoculated acetifications were performed. However, the spontaneous fermentation stopped, so we only obtained inoculated vinegars. Regarding the effect of the type of container used in the acetification, the vinegar produced in glass vessel displayed the lowest values for all the parameters studied. These results were expected due to concentration phenomena and compounds extraction in wood barrels. The vinegar from cherry barrel had the highest AA, at levels significantly different from the oak vinegar. From the oak barrel, we obtained vinegar with the highest amount of total phenols and anthocyanins, but significant differences were not found with the vinegar from cherry barrel. These results were similar to those of Cerezo, A. B., Tesfaye, W., Torija, M. J., Mateo, E., Garcia-Parrilla, M. C., & Troncoso, A. M, (2008), who reported a generally decreasing trend of TPI and TA in vinegars acetified in cherry and oak barrels, being slightly lower in oak. The lower final

299 levels of TA in vinegar from cherry barrel may be explained by the different porosity of
300 wood (higher in cherry wood than in oak). Oxygen permeation through the wood
301 favours the formation of stable anthocyanin-derived compounds (Cano-López, M.,
302 Pardo-Minguez, F., López-Roca, J. M., & Gómez-Plaza, E, 2006), decreasing
303 monomeric anthocyanins. According to these results, it seems that cherry wood barrel is
304 the best to produce high antioxidant strawberry vinegars rich in phenols.
305 Vinegars from cooked must had the highest AA and TPI of all of the vinegars produced.
306 Otherwise, the 2010 vinegars produced on a semi-pilot scale had the highest AA and
307 TA values of all the vinegars obtained from strawberry purees without heating. As
308 mentioned above, the important losses of TA that occurred in the 2008 and 2009
309 vinegars did not occur in 2010, where losses were only around 50% from wine to
310 vinegar. These results indicate that the production of vinegars on a semi-pilot scale
311 allowed getting vinegars with better antioxidant properties.
312 Finally, we compared our vinegars with common vinegars from the market. The results
313 are given in Figure 4. Vinegars produced in this research project were surpassed only by
314 the Aceto Balsamico. Cooked must vinegar had AA and TPI values close to this one.

315 **4. Conclusions**

316 The addition of SO_2 and pectolytic enzymes to the substrate increased AA, TPI and TA.
317 Although the cooked must vinegar presented the highest AA and TPI values, this
318 substrate must be discarded for the strawberry vinegars production at an industrial scale
319 because of their obtaining process is very slow and complex. Concerning the
320 acetification stage, the use of wood barrels was an improvement in all of the parameters
321 determined; specifically, cherry barrels were the best to produce high antioxidant
322 strawberry vinegars rich in phenols. The most appropriate final treatment was the
323 pasteurisation with reference to AA. All measured parameters decreased during the

double fermentation process. In general, acetic fermentation was associated with higher decreases in AA and polyphenols than alcoholic fermentation, except in the semi-pilot scale case. Moreover, anthocyanins were severely influenced by this process. So, for substrate selection the parameter more important to take into account is the TA content. We also noted that the production of these vinegars on a semi-pilot scale resulted in final products with the best antioxidant properties and phenolic content. The antioxidant properties of these vinegars point to them as products with potential health benefits that could make them competitive commodities in the market.

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449 **Figure captions**

450 Figure 1. ■ ORAC, □ DPPH (left axis) and □ TPI (right axis) values for the different
451 extraction solvents tested in strawberries acquired at the market. The bars in the same
452 assay with different letters show significant differences (p<0.05) (ORAC assay: a, b, c;
453 IPT: A, B, C; DPPH test: α, β, γ).

454 Figure 2. Effect of solvent percentages. a) ■ ORAC and □ DPPH values. b) ■ TPI and
455 □ TA values of strawberries acquired at the market. The bars in the same assay with
456 different letters show significant differences (p<0.05) (ORAC and TPI assays: a, b;
457 DPPH and TA tests: A, B).

458 Figure 3. Effect of different ultrasonication times a) —▲— ORAC and —■— DPPH
459 values. b) —✳— TPI and —●— TA values of strawberries acquired at the market. The
460 markers in the same assay with different letters show significant differences (p<0.05)
461 (ORAC and TPI assays: a, b, c; DPPH and TA tests: A, B, C).

462 Figure 4. Comparison of ■ ORAC, □ DPPH (left axis) and ■ TPI (right axis) values of
463 strawberry vinegars with commercial varieties. Sample codes: F9MCV (mean value of
464 all vinegars from cooked must), F9V (mean value of all vinegars from 2009 harvest)
465 and F8V (mean value of all vinegars from 2008 harvest).

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472 Figure 1.
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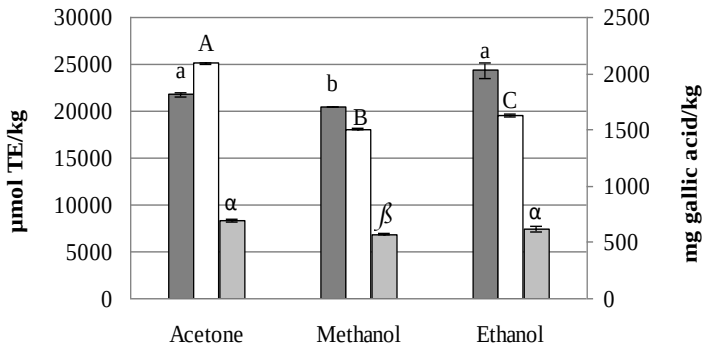

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487 Figure 2.
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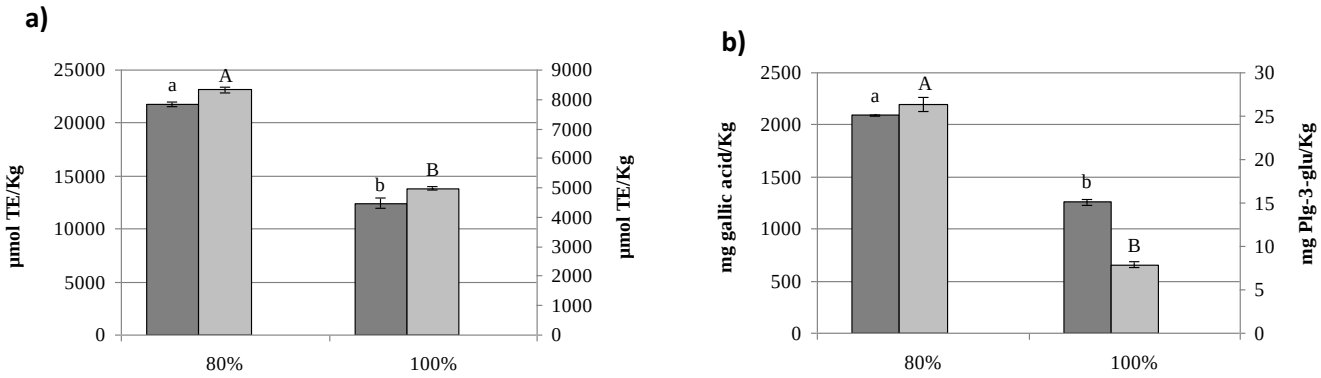

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490 Figure 3.
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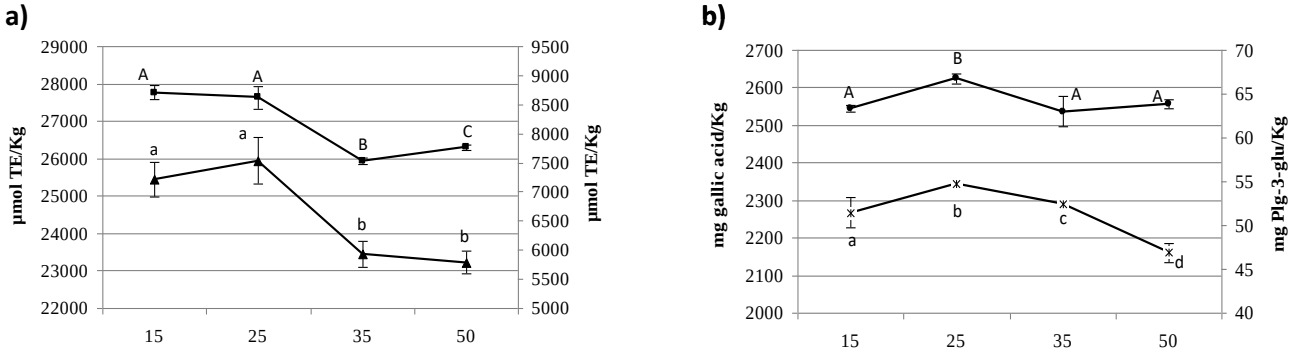

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Figure 4.

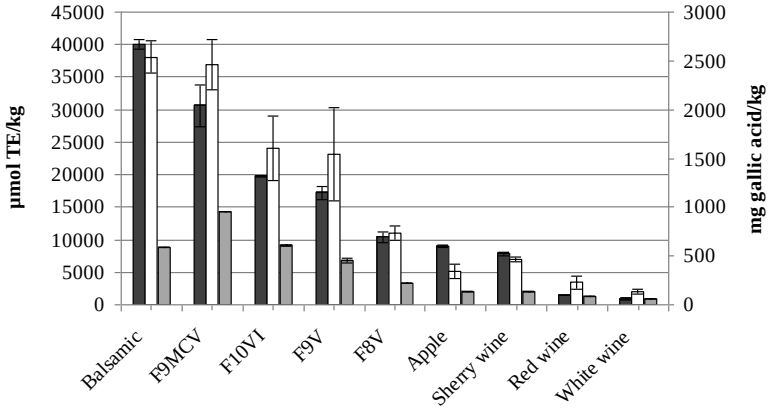
µmol TE/kg
mg gallic acid/kg
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
3000
2500
2000
1500
1000
500
0
Balsamic
F9MCV
F10V1
F9V
F8V
Apple
Sherry wine
Red wine
White wine

Table 1. Samples description.

Harvest	Treatment	Puree Sample	Treatment	Sample substrate	Alcoholic fermentation (time)	Wine Sample	Acetification (time)	Treatment or Recipient	Vinegar sample
2008	Crushed	F8P1	SO_2 Pectolytic enzymes Sucrose (50 g/L)	F8P2	Inoculated (4 days)	F8WI1- F8WI4	Spontaneous (2 months)	Centrifugation	F8VIC1-F8SVIC2
								Pasteurization	F8SVIP1-F8SVIP2
					Spontaneous (5 days)	F8WE1- F8WE4		Centrifugation	F8SVEC1-F8SVEC2
								Pasteurization	F8SVEP1-F8SVEP2
	-	F8P2	Pressing	F8L	Inoculated (4 days)	F8LWI	-	-	-
					Spontaneous (5 days)	F8LWE			
2009	Crushed	F9P1	SO_2 Pectolytic enzymes Sucrose (75 g/L)	F9P2	Inoculated (5 days)	F9WI1- F9WI4	Inoculated (2 months)	glass vessel	F9SVIG
								oak barrel	F9SVIO
								cherry barrel	F9SVIX
					Spontaneous (8 days)	F9WE1- F9WE4	Spontaneous (2 months)	glass vessel	-
								oak barrel	-
								cherry barrel	-
			Heating Concentrated	F9MC	Inoculated (7 days)	F9MCWI1-F9MCWI2	Inoculated (5 months)	glass vessel	F9MCVI1-F9MCVI2
					Spontaneous (7 days)	F9MCWE1-F9MCWE2	Spontaneous (2.5 months)	glass vessel	F9MCVE1-F9MCVE2
2010	Crushed	F10P1	SO_2 Pectolytic enzymes Sucrose (65 g/L) $CaCO_3$	F10P2	Inoculated (4 days)	F10WI	Inoculated (1.5 months)	cherry barrel	F10VI

1 Table 2. Changes in 2008 samples on ORAC, DPPH, TPI and TA during strawberry vinegar production (average±standard deviation).

Samples		**ORAC** (μmol TE/kg)	**DPPH** (μmol TE/kg)	**TPI** (mg gallic acid /kg)	**TA** (mg plg-3-glu/kg)
Substrates	**F8P1**	21792 ± 221	8327 ± 99	2090 ± 10	26.3 ± 0.8
	F8P2	26714 ± 910[a]	10116 ± 88[a]	2298 ± 0[a]	69 ± 0[a]
	F8L	20642 ± 111[b]	5907 ± 516[b]	1615 ± 33[b]	43 ± 0[b]
Wines	**F8LWE**	12757 ± 267[b,c]	2837 ± 59[b,c]	868 ± 29[b,c]	12.2 ± 0.2[b]
	F8LWI	13497 ± 227[b,c]	2898 ± 129[b,c]	858 ± 13[b,c]	17.9 ± 0.2[b,d]
	F8SWE1	25314 ± 650	8200 ± 58[b]	1907 ± 26	13.1 ± 0.7[b]
	F8SWE2	24696 ± 70	7879 ± 70[b]	1773 ± 32	12.9 ± 0.6[b]
	F8SWE3	25458 ± 403	7689 ± 82[b]	1757 ± 45	12.4 ± 0.7[b]
	F8SWI1	27987 ± 1227[b]	7241 ± 35[b,d]	1670 ± 9[b,d]	16 ± 0[b,d]
	F8SWI2	25451 ± 429[b]	8004 ± 35[b,d]	1584 ± 19[b,d]	18.0 ± 0.3[b,d]
	F8SWI3	23745 ± 15[b]	6515 ± 67[b,d]	1548 ± 6[b,d]	17.3 ± 0.6[d]
Vinegars	**F8SVE1C**	9202 ± 390[b]	3256 ± 205[b]	769 ± 13[b]	0.4 ± 0.0[b]
	F8SVE1P	9849 ± 413[b]	3368 ± 352[b]	774 ± 23[b]	0.5 ± 0.1[b]
	F8SVE2C	9215 ± 338[b]	3210 ± 129[b]	781 ± 0[b]	1.1 ± 0.2[b]
	F8SVE2P	10869 ± 190[b]	3252 ± 234[b]	683 ± 10[b]	0.6 ± 0.0[b]
	F8SVI1C	10139 ± 341[b,e]	3227 ± 117[b]	751 ± 16[b]	1.3 ± 0.0[b]
	F8SVI1P	11611 ± 89[b,e]	3388 ± 64[b]	744 ± 6[b]	0.9 ± 0.1[b]
	F8SVI2C	11054 ± 40[b,e]	3260 ± 246[b]	694 ± 16[b]	0.8 ± 0.1[b]
	F8SVI2P	11082 ± 86[b,e]	3380 ± 76[b]	712 ± 9[b]	1 ± 0[b]

Sample codes are located in Table 1.
[a] Significant differences ($p<0.05$) with respect to the initial fruit puree (ANOVA).
[b] Significant differences ($p<0.05$) with respect to the sample from which was produced (ANOVA).
[c] Significant differences ($p<0.05$) with respect to semisolid wines obtained with similar alcoholic process (spontaneous or inoculated) (ANOVA).
[d] Significant differences ($p<0.05$) with respect to spontaneous process (ANOVA).
[e] Significant differences ($p<0.05$) with respect to the vinegars obtained from spontaneous wines (ANOVA).

18 Table 3. Changes in 2009 samples on ORAC, DPPH, TPI and TA during strawberry vinegar production (average±standard deviation).

Samples		ORAC (μmol TE/kg)	DPPH (μmol TE/kg)	TPI (mg gallic acid /kg)	TA (mg plg-3-glu/kg)
Substrates	**F9P1**	23176 ± 868	9964 ± 193	2028 ± 82	173.0 ± 3.7
	F9P2	28998 ± 1893[a]	10117 ± 88	2085 ± 67	183.8 ± 3.1[a]
	F9MC	37472 ± 1419[b]	17897 ± 176[b]	3741 ± 21[b]	27 ± 1[b]
Wines	**F9WE1**	24945 ± 276[b]	6898 ± 132[b]	1853 ± 67	52 ± 1[b]
	F9WE2	25998 ± 795	6992 ± 299[b]	1683 ± 0[b]	55.3 ± 0.4[b]
	F9WI1	25723 ± 564	7079 ± 53[b]	1705 ± 123[b]	26.3 ± 0.6[b,c]
	F9WI2	27771 ± 1086	7135 ± 114	2017 ± 29	30.9 ± 1.1[b,c]
	F9MCWE1	49755 ± 2015[b,c]	19413 ± 141[b,c]	3380 ± 87[b,c]	24.1 ± 1.5[c]
	F9MCWE2	46290 ± 279[b,c]	18493 ± 105[b,c]	3001 ± 63 [b,c]	23.3 ± 2.1[c]
	F9MCWI1	45446 ± 2536[d]	17747 ± 105[d]	3026 ± 29[d]	7.4 ± 0.6 [c,d]
	F9MCWI2	43095 ± 2576[d]	20726 ± 271[d]	3416 ± 53[d]	6 ± 0 [f,d]
Vinegars	**F9VIG**	15163 ± 341[b]	6235 ± 72[b]	1099±55[b]	3.07 ± 0.17[b]
	F9VIO	17446 ± 107[b]	6902 ± 31	1844±56	6.5 ± 0.9[b]
	F9VIX	19077 ± 161[b]	7163 ± 31	1693±45	4.80 ± 0.17[b]
	F9MCVE1	33779 ± 974	14907 ± 103	2377±45	2.9 ± 0.5
	F9MCVE2	31643 ± 1832	14428 ± 41	2480±56	4.0 ± 0.4
	F9MCVI1	30685 ± 1377[e]	14119 ± 305[e]	2536±45[e]	1.70 ± 0.02[e]
	F9MCVI2	26278 ± 1409[e]	14283 ± 123[e]	2377±22[e]	1.79 ± 0.15[e]

Sample codes are located in Table 1.
[a] Significant differences ($p<0.05$) with respect to the initial fruit puree (ANOVA).
[b] Significant differences ($p<0.05$) with respect to the sample from which was produced (ANOVA).
[c] Significant differences ($p<0.05$) with respect to spontaneous wines from F9P2 (ANOVA).
[d] Significant differences ($p<0.05$) with respect to inoculated wines from F9P2 (ANOVA).
[e] Significant differences ($p<0.05$) with respect to inoculated vinegars from F9WI wines (ANOVA).

Table 4. Changes in 2010 samples on ORAC, DPPH, TPI and TA during strawberry vinegar production (average±standard deviation).

Samples		**ORAC** (μmol TE/kg)	**DPPH** (μmol TE/kg)	**TPI** (mg gallic acid /kg)	**TA** (mg plg-3-glu/kg)
Substrates	**F10P1**	20409 ± 431	10218 ± 171	1800 ± 122	46.4 ± 1.6
	F10P2	23783 ± 649[a]	10592 ± 237	1886 ± 79	54.8 ± 1.4[a]
Wine	**F10WI**	22910 ± 315	9652 ± 378[b]	1691 ± 36[b]	20.2 ± 0.5[b]
Vinegar	**F10VI**	19784 ± 117[b]	9113 ± 331	1605 ± 95	10.6 ± 0.9[b]

Sample codes are located in Table 1.
[a] Significant differences ($p<0.05$) with respect to the initial fruit puree (ANOVA).
[b] Significant differences ($p<0.05$) with respect to the sample from which was produced (ANOVA).