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ORIGINAL PAPER

Reduction of *Zygosaccharomyces rouxii* Population in Concentrated Grape Juices by Thermal Pasteurization and Hydrostatic High Pressure Processing

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Abstract
Zygosaccharomyces rouxii is the most frequent spoilage yeast species detected in concentrated grape juice. In order to reduce *Z. rouxii* populations and consequently extend the microbiological shelf life of this product, different programs of thermal pasteurization and high hydrostatic pressures processing were evaluated. Results showed that pasteurization temperatures higher than 75 °C are necessary to reduce *Z. rouxii* population in concentrated grape juice. Reduction of 7 logarithms can be reached after 90 s at 75 and 80 °C, and 5 s at 85 °C of pasteurization treatment. High hydrostatic pressure treatment above 500 MPa for 2 min are necessary to reduce 7 logarithms of *Z. rouxii* population and to significantly extend the shelf life of concentrate grape juice. Extension of holding times from 3 to 5 min, at the different high hydrostatic pressures evaluated, did not improve the *Z. rouxii* population reduction, nor the shelf life extension of concentrate grape juice. In conclusion, thermal pasteurization and high hydrostatic pressure could be suitable treatments to achieve the reduction of *Z. rouxii* population below the recommendation limit (10^2 CFU/g) and extension of the microbiological shelf life of concentrate grape juice.

Keywords Concentrated grape juice · *Zygosaccharomyces rouxii* · Thermal pasteurization · Hydrostatic high pressure

Introduction

Grape juice and by-products constitute an important portion of the worldwide food industry. Argentina grape production is mainly industrialized, where wine and concentrated grape juices are the two mayor types of commercial products. Mendoza and San Juan provinces are the main manufacturers of concentrated grape juices in Argentina and their whole production is mainly exported to demanding markets as the USA, Japan, and South Africa (INV 2018). Concentrated grape juices have a great importance as additives in several massively consumed products and due to their natural qualities; they are used to elaborate baby food, pharmaceutical products, and soft drinks (Bruzone 1998).

Concentration of grape juice involves a number of steps including strong clarification and acidification of must, followed by cooled filtration and finally juice concentration using falling film evaporator with multiple effects (Rojo et al. 2017). The objective of concentrating the juice is to eliminate water until a concentration of sugars and an osmotic pressure is reached, inhibiting microbial development. Consequently, concentrated juices are microbiologically more stable than other fruit products and can be usually stored at room temperature without any additional treatment (ICMSF 1980). However, these products are not free of microbiological spoilage risk. Osmophilic yeasts represent the primary spoilage cause in high sugar food and drink industries, where the genus *Zygosaccharomyces* is the most frequently described spoilage yeast (Worobo and Splittstoesser 2005; Martorell et al. 2007).

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A previous study carried out in Argentina showed that *Zygosaccharomyces rouxii* was the only species isolated from spoiled concentrated grape juice, and it was also detected at a higher frequency in unspoiled samples (Combina et al. 2008; Rojo et al. 2014). The particular physiological characteristics of *Z. rouxii* make it very difficult to control, mainly because of its resistance to different preservatives (Martorell et al. 2007; Fleet 2011; Stratford et al. 2013). Previous studies carried out in order to assess the individual effects of different chemical preservatives to control the growth of *Z. rouxii* in concentrated grape juice, showed that none of them were capable of completely inhibiting yeast growth (Rojo et al. 2015). So, several efforts have been made by the industry to avoid concentrated grape juice spoilage using a nonchemical approach, where pasteurization turns to be the most commonly process used. Traditional pasteurization refers to heat treatment of food (usually below 100 °C) in order to destroy microorganisms of public health significance (Silva and Gibbs 2010).

In the industries, the thermal program specification used to pasteurize concentrated grape juice is often set by the equipment supplier. The parameters used have been usually set for other food industries without any special consideration on substrate composition and target microorganism (Rojo et al. 2017). Proper design of industrial thermal pasteurization processes requires the knowledge of target microorganism and the food composition. Thus, it results important to establish a pasteurization program that allows inactivating the spoilage yeast, and consequently increasing the concentrated grape juices shelf life (Queirós et al. 2015; Milani et al. 2015).

Since thermal pasteurization has been widely accepted as an effective preservation method for killing pathogens in food products, with minimal loss of desired food quality, new technologies that can satisfy the goals of pasteurization have rapidly grown in recent years. In this context, nonthermal technologies are emerging, with special emphasis in hydrostatic high pressure processing (Patrignani and Lanciotti 2016). Hydrostatic high pressure (HHP) considered a promising and attractive technology consists in a process where pressure is transferred uniformly and instantly to food without heating the system (Mok et al. 2006). The HHP uses water as a pressure-transmitting medium to transmit isostatic pressure to foods, usually in the 100–600 MPa range, independently of size, shape, or food composition (Balasubramaniam et al. 2008; Oey et al. 2008). The HHP produces the volume reduction of the biological unit causing structural alterations in biomolecules, lipid membranes, protein structure, or cell division (Royer 1995; Bartlett 2002). The main advantage provided by HHP technology relies on its ability to inactivate vegetative microorganisms extending shelf life of food products without substantial modifications of nutritional, functional, and sensorial properties of food (Mota et al. 2013). Currently, HHP is being widely used in a whole range of products such as fruit juices (Raso et al. 1998; Donsí et al. 2010; Wang et al. 2016; Chang et al. 2017;) and table olives (Argyri et al. 2014). Nonthermal preservation techniques could provide an alternative to traditional pasteurization for concentrated grape juice industry not explored yet.

The aim of this work was to evaluate thermal and cold pasteurization using HHP to reduce *Z. rouxii* population and to extend the microbiological stability in concentrated grape juice.

Materials and Methods

Yeast Strains

Four *Z. rouxii* strains (MR4, MT6, MC8. and MC9) isolated from spoilt Argentinean concentrated grape juices and previously identified by molecular sequencing of the D1/D2 domain of 26S ribosomal gene were used in this study (Combina et al. 2008; Rojo et al. 2014). Strains belonged to the vine and wine microorganisms collection from INTA (Mendoza, Argentina). Thermal pasteurization assay was carried out using a *Z. rouxii* MC8 strain selected due to its greater thermo-resistance, observed in a preliminary study (Rojo et al. 2012), whereas a cocktail of *Z. rouxii* strains were used to evaluate the effect of HHP.

Inoculum Preparation and Sample Inoculation

Z. rouxii strains were grown on YPD broth (40 g/L glucose (Biopack Co.); 5 g/L bacteriological peptone (Britania Co.); and 5 g/L yeast extract (Britania Co.) during 24 h at 28 °C. Later, strains were adapted to osmotic condition by growing in a high sugar culture media MYGF (195 g/L glucose (Biopack Co.); 195 g/L fructose (Biopack Co.); 20 g/L malt extract (Britania Co.); and 5 g/L yeast extract (Britania Co.) adjusted to pH 4.5 by citric acid addition during 72 h at 28 °C. Finally, the 2% *v*/*v* of strain cultures were inoculated in concentrated grape juice at 68 °Brix and pH 3.2, and were incubated at 28 °C overnight to reaching an active and adapted population of 10^7 CFU/g.

Thermal Pasteurization

The inoculated concentrated grape juice was submitted to pasteurization treatments using different temperatures (70, 75, 80, and 85 °C) combined with different holding times (5, 10, 20, 30, 45, 60, 75, and 85 s). The assay was performed in triplicate in thin-walled stainless steel tubes containing 2.5 mL of concentrated juice. The tubes were placed in a thermostatic bath to achieve the desired temperature. Temperatures were recorded by thermal couples connected to a digital thermometer introduced into two control tubes with the same volume of concentrated grape juice. The samples were maintained in the

water bath for different holding times and afterwards rapidly cooled in ice. Aliquots of 0.1 and 1 g of samples were spread onto WL Nutrient Agar medium (Oxoid Co.), before dilution in 30% (*w*/*v*) glucose solution if necessary, and incubated during 72 h at 28 °C. Curves of the surviving yeasts (Log CFU/g) were constructed as a function of time and were fitted using the double Weibull simplified model (Coroller et al. 2006). The model was as follows:

$$\mathrm{Log}\, N_t = \mathrm{Log}\left\{N_0/(1+10^{\alpha})*\left[10^{\alpha-(t/\delta 1)\hat{}p}+10^{-(t/\delta 2)\hat{}p}\right]\right\} \quad (1)$$

where N_t is the number of survivors, N_0 is the inoculum size, t is the time, p is a shape parameter, and δ is the treatment time for the first decimal reduction. The subscripts 1 and 2 indicate the two different subpopulations that differ in their levels of resistance to stress. Subpopulation 1 is more sensitive to stress than subpopulation 2 ($\delta_1 < \delta_2$). Parameter α includes the relation of the fractions of each subpopulation and is a derivate of the parameter f originally proposed for the Weibull model $\alpha = \log(N_{01}/N_{02})$. The α value is the graphic difference between log (N_0) and the logarithm of the population size where the inflection is observed. In theory, the α value can be equal to all real numbers and represent the thermo-sensitive subpopulation. Parameter p represents the shape of the curve.

In order to evaluate microbiological shelf life, a second experiment was done. Plastic bottles containing 100 mL of *Z. rouxii* inoculated concentrated grape juice were treated with the preselected pasteurization programs and stored at 23 ± 0.5 °C during 210 days. Bottles without pasteurization processing were used as controls. Each treatment was carried out in three independent replicates. The samples were monitored weekly to record the visual signs of product spoilage (gas production and turbidity).

Cold Pasteurization by High Hydrostatic Pressure (HHP) Processing

Four high hydrostatic pressures (300, 400, 500, and 600 MPa) were applied during three holding times (2, 3, and 5 min). The assay was performed in plastic bottles containing 100 mL of inoculated concentrated grape juice. Bottles without HHP processing were used as controls. Each treatment was carried out in four independent replicates. A high hydrostatic pressure equipment with a 2-L capacity (Stansted Fluid Power Ltd., High Pressure Iso-Lab System Model: FPG9400:922, UK) and a maximum working pressure of 900 MPa. A mix of distilled water and propylene glycol (70/30) was used as the compression fluid. Rate of pressure increasing was 5 MPa/s. High-pressure treatments were carried out at an initial temperature of 21–24 °C, which was only modified by adiabatic heating. Aliquots of 0.1 and 1 g of samples were spread onto WL Nutrient Agar medium (Oxoid Co.), before dilution in 30% (*w*/*v*) glucose solution if necessary, and incubated during 72 h at 28 °C. Bottles containing HHP-treated and control samples were kept at 28 ± 0.5 °C during 49 days for microbiological shelf life evaluation. Weekly, viable cell count and visual observation were made to detect signs of spoilage. When spoilage was recorded, no further measures were done. The storage temperature was chosen to favor the recovery and development of the yeast after the treatment.

Statistical Analysis

All analyses were carried out with InfoStat software (Di Rienzo et al. 2015). Results were analyzed using two-way ANOVA and comparisons were performed by a LSD Fisher test. Differences were considered significant at ≤ 0.05 ($p \leq 0.05$).

Results

In order to determine *Z. rouxii* resistance to thermal pasteurization, *Z. rouxii* strain MC8 inoculated in concentrated grape juice was subjected to the different thermal pasteurization programs described in the materials and methods section. The survival curves were constructed with the experimental data independently obtained of each replica. The curves were fitted applying the double Weibull simplified model (Coroller et al. 2006). This model was used since thermal inactivation produced biphasic shapes of survival curves with two subpopulations with different thermo-resistance (Fig. 1). One advantage of using this simplified model is that all parameters can be graphically interpreted. For instance, δ value (first decimal reduction time) could be considered equivalent to the D value (time required to inactivate 90% of the studied microorganisms) and reflects the thermo-resistance of the most sensitive (δ_1) and resistant (δ_2) subpopulations. Moreover, the α value corresponding to the decrease to the ratio for the most resistant cells occurred as the temperature increased. A thermo-sensitive subpopulation quickly died at all the evaluated temperatures, as was evidenced by the δ_1 values (Table 1). This heat-sensitive subpopulation was different depending on the temperature used, as evidenced by the α parameter. Thus, for the lowest temperature evaluated (70 °C), the α value was 2.5 log CFU/g, leaving a residual population (resistant to this temperature) of 4.5 log CFU/g that remain viable for a long time at this temperature, showing a high value of δ_2 (Table 1). Therefore, this temperature was not lethal for *Z. rouxii* MC8 in concentrated grape juice. In treatments with temperatures greater than 75 °C, the thermal-resistant subpopulation was lower as temperature increased, reaching a null fraction of thermo-resistant cells at 85 °C, where α value was equal to

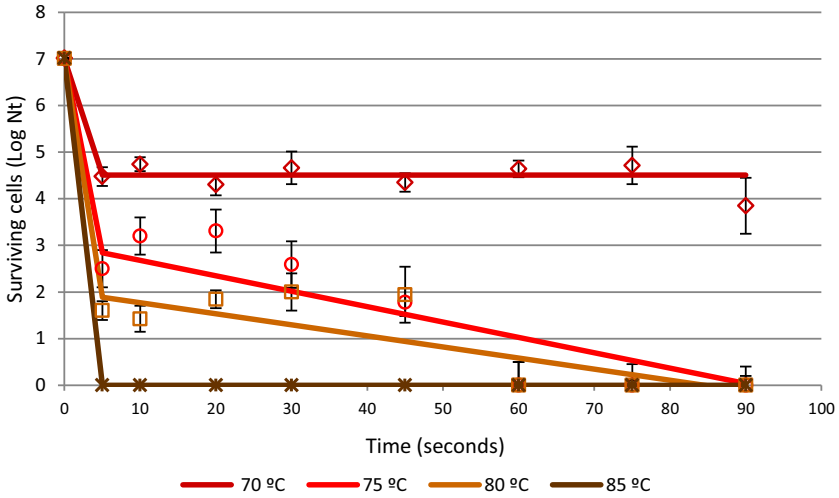

Fig. 1 Surviving curves for *Zygosaccharomyces rouxii* submitted to different thermal pasteurization programs. Averages and standard deviation of experimental data are indicated by symbols and error bars. Double Weibull simplified model fitted curves are showed in solid lines

the value of the initial total population (Log N_0) and the value of δ_2 could not be estimated. The time necessary for the second decimal reduction (δ_2) value was 6 and 5 s at 75 and 80 °C, respectively (Table 1).

These results clearly show that the application of temperatures lower than 75 °C is not lethal for the assessed populations of *Z. rouxii* in concentrated grape juices. Temperatures above 75 °C can be used for thermal pasteurization of this product, obtaining a reduction of 7 logarithms after 90 s at 75 and 80 °C, and after 5 s at temperatures above 85 °C (Fig. 1). Moreover, commercial sterility is demanded by many markets, which means the need of a thermal treatment able to accomplish a 12 log cycle reduction *Z. rouxii* population. To achieve this reduction, pasteurization of concentrated grape juices applying any of the three programs, 75 °C/160 s, 80 °C/160 s or 85 °C/10 s, is strongly recommended since no spoilage was recorded during 210 days at storage at 23 ± 0.5 °C (data not shown).

The application of high hydrostatic pressure as a cold pasteurization method was next evaluated. Viable cell counts of *Z. rouxii* in concentrated grape juice at 68 °Brix treated with four different hydrostatic pressures during three different holding times is shown in Table 2. The lower pressure evaluated (300 MPa) did not produce any significant reduction of viable populations in none of the holding times evaluated. On the other hand, pressures of 400 MPa, 500 MPa, and 600 MPa produced a significant reduction in the viable population after 2 min of treatment, maintaining the same reduction regardless the holding time evaluated. It is important to note that none of the applied treatments allowed the reduction of the populations below the detection limit of the technique (1 CFU/g), leaving an average of 2.44 ± 0.12 Log CFU/g viable cells.

In order to evaluate the microbiological shelf life of concentrated grape juice after HHP processing, treated samples were stored at 28 °C for 49 days or until visible spoilage. Holding times (2, 3, and 5 min) showed similar results regarding the viable cell counts and the time needed to show alteration during storage in all the hydrostatic pressures applied. Consequently, in the Fig. 2, only the viable cell counts of the samples treated during 2 and 5 min with the different hydrostatic pressures are shown. After 7 days of storage, the samples treated with the lowest pressure (300 MPa) showed visible spoilage of the product, similar to control samples without treatment. The samples treated with 400 MPa showed a small, but statistically significant, decrease in the viable cell counts at 7 days; however, signs of spoilage appeared after 14 days of storage. Finally, samples treated with 500 and 600 MPa showed a significant reduction in viable cell counts after 7 days of storage. This reduction trend continued during storage, showing counts smaller than 1 viable cell in 1 g of sample (0.1 Log CFU/g) after 14 days of storage. Cell counts continued below the detection limit of the technique until the end of the assay as well as no visible spoilage was observed after 49 days (Fig. 2).

In summary, three different results have been observed regarding the pressure used to reduce *Z. rouxii* population in concentrated grape juice. In the first one, no effect of the treatment (300 MPa) on the viability of the cells was observed, showing a similar behavior as untreated juices. In the second one, a significant reduction of the cultivable population was

Table 1 Thermo-resistance parameters for *Z. rouxii* MC8 strain in concentrated grape juice treated with different pasteurization temperatures according to double Weibull simplified model

Temperature (°C)	α (Log CFU/g)	δ_1 (seconds)	δ_2 (seconds)	R^2
70	2.5 ± 0.3	$5 \pm 2 \times 10^{-4}$	$1 \pm 3 \times 10^{5}$	0.91
75	4.0 ± 0.5	$5 \pm 3 \times 10^{-5}$	6.0 ± 0.9	0.93
80	5.0 ± 0.3	$1 \pm 3 \times 10^{-8}$	5.0 ± 0.7	0.94
85	7.0 ± 0.1	$1 \pm 1 \times 10^{-9}$	NE	1.00

NE not estimated by the model

Table 2 Number of viable cells (Log CFU/g ± SD) of *Z. rouxii* cocktail strains in concentrated grape juice treated with different hydrostatic pressures during increasing holding times

Holding time (min)	Pressure (MPa)			
	300	400	500	600
0	7.03 ± 0.07a	7.03 ± 0.07a	7.03 ± 0.07a	7.03 ± 0.07a
2	6.82 ± 0.23a	2.52 ± 0.30b	2.62 ± 0.13b	2.41 ± 0.21b
3	6.74 ± 0.08a	2.56 ± 0.17b	2.45 ± 0.51b	2.20 ± 0.28b
5	6.76 ± 0.16a	2.38 ± 0.28b	2.37 ± 0.20b	2.43 ± 0.13b

Different letter means statistical differences into the same column (LSD Fisher test, $\alpha = 0.05$)

observed immediately after the treatment (400 MPa), although the cells were able to recover and spoil the product in a short storage time. Finally, in the third one, in spite of a small population of viable cells could be counted immediately after treatment, no positive counts were observed in the samples after 7 days of storage. Consequently, these treatments (500 and 600 MPa) produce a significant extension of microbiological shelf life of the product.

Discussion

Thermal pasteurization of concentrated grape juice at the end of the production process is a widely used practice in the industry. However, no optimization of pasteurization parameters has been done specifically for this substrate.

It has been shown that sugar exerts a protective action on some microorganisms. The protective action of sugar is related to the decrease in water activity which causes an increase in thermo-resistance (Ball and Olson 1957; Bean 1983). However, this study confirms that the main spoilage yeast in concentrate grape juice, *Z. rouxii*, showed sensitivity to thermal treatments in a substrate with high concentrations of sugars, even in low pasteurization temperatures as 75 °C. This study provides with useful recommendations for the industry to extend the microbiological shelf life of concentrated grape juice regarding the employment of different pasteurization programs. Nonetheless, it is also important to remember that microbiological recontaminations usually occur in the postpasteurization stages, mainly due to defects in the sanitary procedures during packaging and storage of the pasteurized juices (Rojo et al. 2017).

Moreover, a known limitation of thermal pasteurization relies on quality changes in the product, such as the destruction of vitamins, aroma and color, and the development of off-tasting flavor (Chang et al. 2017). In this regard, reduction in the nutritional value by pasteurization is not an important issue in concentrated grape juice industry because it has a limited nutritional value. Glucose and fructose constitute approximately 80–90% of the soluble solids of the musts, proteins represent only 0.2–0.3 g/L and mineral elements are present in very low concentrations (20–0.3 mg/100 mL) (Carreño et al. 2001). However, due to the high concentration of sugars, the concentrated grape juice is susceptible to caramelization if the pasteurization temperatures are not strictly controlled.

The increasing requirement for natural products by consumers has encouraged research into nonthermal preservation techniques. HHP processing is a novel food processing technology capable of inactivating microorganisms and some

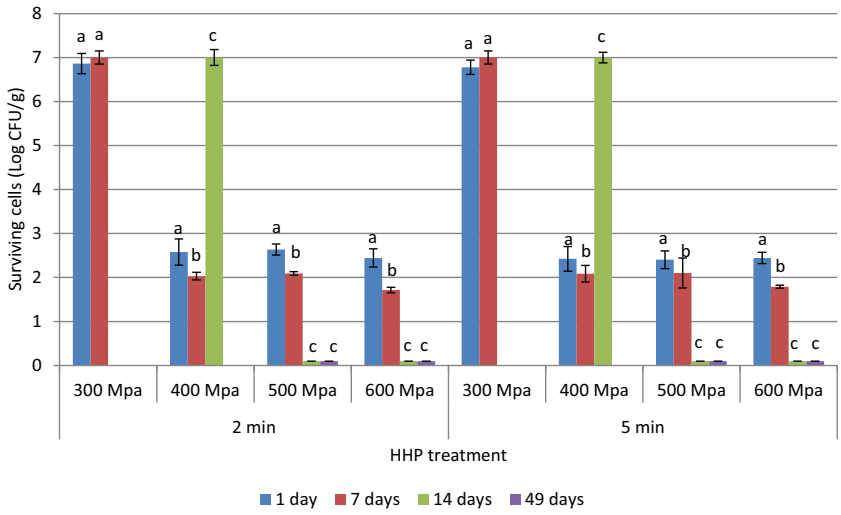

Fig. 2 Number of surviving cells in concentrated grape juices stored at 28 °C after processing at different high hydrostatic pressures during 2 and 5 min. Different letter means statistical differences between storage days into the same HHP treatment (LSD Fisher test, $\alpha = 0.05$)

enzymes in food at room temperature, extending shelf life and reducing the damage caused by high temperatures to heat-sensitive components (Wang et al. 2016). The US Food and Drug Administration have listed HHP as a nonthermal pasteurization method suitable for replacing thermal pasteurization technology. During the last two decades, the effect of HHP on various juice products has been explored; indeed, mandarin, grapefruit, apple, orange, and carrot juices treated by HHP are currently available in the market (Donsí et al. 2010; Wang et al. 2016).

Three different results were observed in this study regarding the application of different programs (value of pressure/time) of HHP to reduce *Z. rouxii* population in concentrated grape juice, which are discussed separately. Firstly, no effect of the treatment (300 MPa) on the viability of the *Z. rouxii* cells was observed. Our results are in line with previous findings by Chang et al. (2017), who found that the microbial content (aerobic plate count, coliform and yeast/mold) in grape juice treated with 300 MPa during 3 min was not significantly different compared with that observed in the control. In contrast, Raso et al. (1998) studied HHP inactivation of *Zygosaccharomyces bailii* vegetative cells suspended in apple, orange, pineapple, cranberry, and grape juices, and found that after 5 min of pressurization at 300 MPa, the population of vegetative cells decreased almost five log cycles in all of fruit juices analyzed. Furthermore, the efficiency of a certain program of HHP depends on the spoilage microorganism as well as the substrate being processed, and it results evident the importance of determining the best program for each particular case.

To understand the second set of our results, reduction of the *Z. rouxii* cultivable population immediately after the treatment (400 MPa), although product spoilage in a short storage time, it is important to highlight that the HHP inactivates microorganisms by interrupting the cellular functions responsible for reproduction and survival (Mota et al. 2013; Wang et al. 2016). However, several living organisms are able to withstand such hostile conditions (Abee and Wouters 1999). These microbial cells that survive pressurization also became sublethally injured (Alpas et al. 2000). The study of injury induced by HHP in microorganisms and subsequent recovery has been reported by several research groups, and could explain the second category described in our study (Bozoglu et al. 2004; Bull et al. 2005; Muñoz-Cuevas et al. 2011). In our work, after HHP treatment at 400 MPa, yeasts showed sublethally injured cells that could recover their capacity to grow and spoil the juice under no-limiting conditions during the storage. This behavior has been previously observed by Argyri et al., (2014) who carried out HHP treatment in brine and olives. The authors showed that the HHP treatment of 400 MPa for 15 min resulted in the reduction of the microbial populations in both brines and olives below the detection limit of the enumeration method, although a recovery of yeasts was observed during the subsequent storage for 7 days at 20 °C. In line with this, Pega et al. (2018) evaluated the effect of 400 MPa for 1 min on lactic acid bacteria in fermented dairy beverage using culture-dependent and independent techniques. Results showed that some of the lactic acid bacteria population, which could not be recovered by traditional culturing after HHP, gave positive results by qPCR and RT-qPCR, suggesting that the effect of this pressure may have not been completely lethal (Pega et al. 2018).

Regarding the third results, a significant reduction of the *Z. rouxii* populations in the concentrated grape juices in HHP treatments of 500 and 600 MPa was observed, increasing this reduction throughout the storage period. In this case, treatment could produce a sublethal damage severe enough to impair cell recovery even after several days of storage. In agreement with our study, Argyri et al. (2014) observed no development of acid lactic bacteria and yeasts after HHP treatments of 500 MPa for 30 min. Also, Chang et al. (2017) found that HHP at 600 MPa, caused a significant reduction in microbial content (aerobic plate count, coliform, and yeast/mold counts) in white grape juice and resulted in significantly lower counts compared with that of the control after 20 days of storage at 4 °C (Chang et al. 2017). In this sense, our results suggests that application of pressures over 500 MPa could provide the same reduction in microbial populations as the thermal pasteurization processing often used in the food industry.

It is widely assumed that the magnitude of cell damage produced by HHP treatments depends not only on the organism tolerance, but also on the intensity and duration of pressure and other environmental parameters. However, in our study, an extended holding time did not augment the inactivation of *Z. rouxii* by HHP regardless the applied pressure, reaching the maximum reduction in the minor holding time (2 min). Similarly, other studies have also shown that at moderate pressure ranges, holding time did not increase considerably cell death (Patterson et al. 1995; Alpas et al. 2000; Bozoglu et al. 2004). Overall pressurizing for a longer time, at a lower pressure range (to minimize adverse effects on food texture and color), do not seem to provide a great advantage for microbial inactivation (Alpas et al. 2000; Muñoz et al. 2007).

Even when additional research is needed in order to establish HHP processing as a useful tool for the concentrated grape juice industry, HHP processing has some advantages over thermal pasteurization. HHP is applied uniformly and instantaneously through food material, and can be transmitted in all directions within the shortest period, conveying the pressure to the central point of the food, whereas traditional thermal processing requires a longer period to transmit heat. Regarding energy consumption, a small amount of energy is required to compress a solid or liquid to 500 MPa, compared to heating to 100 °C (Pereira and Vicente 2010). HHP is solely

based on physics, and conducts pressure instantly, can be operated safely, and consumes relatively lower energy; thus, it contributes to the protection of the ecological environment. The main disadvantage is the equipment cost and installation. However, because of the current availability of high-pressure equipment, the cost of products processed using this technology has decreased considerably in the recent years, making more products accessible to consumers (Pereira and Vicente 2010; Wang et al. 2016).

Conclusions

In conclusion, the thermal pasteurization and HHP treatments could be applied to reach populations below the maximum limit accepted for fungi and yeasts count (10^2 CFU/g), and to extend the microbiological shelf life of concentrated grape juice with promising results. Pasteurization temperatures above 75 °C can be used to reduce *Z. rouxii* population in this product. Reduction of 7 logarithms can be reached after 90 s at 75 and 80 °C, and 5 s at 85 °C. High hydrostatic pressure treatment above 500 MPa for 2 min is strongly recommended to reduce *Z. rouxii* population below 7 logarithms and significantly extend the microbiological shelf life of this product.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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