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Control of *Hanseniaspora osmophila* and *Starmerella bacillaris* in strawberry juice using blueberry polyphenols

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ABSTRACT

The aims of the present work were to isolate and identify the principal yeasts present in spoiled Argentine strawberry juice, identify polyphenols present in four blueberry cultivars and use these blueberry extracts in the control of yeasts using strawberry juice as food system model. *Hanseniaspora osmophila* and *Starmerella bacillaris* were identified for the first time in Argentine strawberry juice. The blueberry extracts assayed showed antifungal activity against *H. osmophila* and *S. bacillaris* through individual phenolic compounds such as quercetin, kaempferol and chlorogenic, ρ-coumaric and ellagic acid. The cytotoxicity assay demonstrated that the blueberries were not toxic to humans and that they did not modify the sensorial qualities of strawberry juice. No viable *S. bacillaris* and *H. osmophila* cells were detected after 7 days in strawberry juice supplemented with 150 μg/ml *Blue Crisp* or *Millennium* extract, inoculated with the isolated spoilage yeasts and conserved at 4 °C. This is the first evidence of *S. bacillaris* and *H. osmophila* in spoiled Argentine strawberry juice and blueberry extracts could be a good natural and non-toxic alternative to prevent growth of these yeasts. Blueberry extracts could be feasible alternatives to improve the microbiological quality without impact on the organoleptic properties of polyphenol-enriched strawberry juice.

1. Introduction

The growing importance of yeasts regarding food spoilage is partly because of the use of modern technologies in traditional food processing, but also as a result of numerous new food and beverage formulations and the tendency to reduce the use of preservatives to avoid yeast spoilage (Loureiro & Malfeito-Ferreira, 2003).

Tucumán is the leading producer of strawberries and blueberries in northern Argentina and consumption of strawberry juice is high. The contaminating microbiota that can be present in strawberry juice is mainly constituted of yeasts, as they can tolerate and grow at high osmotic pressure, low pH and low temperature. Some yeasts are extraordinarily resistant to chemical preservatives such as sodium benzoate and potassium sorbate (ICMSF, 1998) commonly used in the juice industry, which reduces the shelf-life of commercial natural strawberry juice if they are spoilage yeasts. Nevertheless, currently there are no reports available about identification of yeasts present in spoiled Argentine strawberry juices.

Actually, besides the use of chemical preservatives such as benzoate or sorbate, pasteurization is probably the most common preservation method applied in the juice industry to eliminate pathogens from juices, but this process may cause a loss of vitamins, minerals, fresh color or flavor (Burt, 2004). During the past decade, there has been an increasing interest in the use of biologically active compounds from natural sources, because consumers are looking for safer and healthier food without addition of chemicals and without the use of thermal treatment. In nature there are many different types of antimicrobial compounds; natural products of higher plants provide a variety of antimicrobial agents, probably demonstrating novel mechanisms of action (Barbour et al., 2004; Rodríguez-Vaquero & Manca de Nadra, 2008). Berries are rich in phenolic compounds, and several researchers have reported the content and antimicrobial activity of phenolic compounds in berries (Vallejo, Aredes-Fernández, Farías, & Rodríguez-Vaquero, 2013). Blueberry (*Vaccinium* spp.) contains relatively high amounts of acids and phenolic compounds (Kalt et al., 2008) that display potential health benefits such as protection against cancer and cardiovascular diseases (Almeida, Farah, Silva, Nunam, & Glória, 2006; Santos, Almeida, Lopes, & Souza, 2006; Serafini et al., 2009). However, at

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present there is no evidence of antifungal activity of phenolic blueberry extracts against yeasts isolated from commercial deteriorated natural juice in Argentina.

The aims of this study were to 1) isolate and identify yeasts present in deteriorated Argentine strawberry juice, 2) extract and characterize antifungal activity of four phenolic extracts from blueberries widely grown in Argentina and 3) control isolated yeasts and carry out sensorial assays in strawberry juices supplemented with blueberry extracts.

2. Materials and methods

2.1. Isolation and identification of yeasts from deteriorated strawberry juice

Yeasts were isolated from spoiled commercial strawberry juice (Tucumán, Argentina) with a pH of 2.9, and with off-odor, a brown color and presence of lumps and precipitate at the bottom. Samples of deteriorated juices were plated onto YMPG agar medium (Oxoid Ltd., London, England), pH 5.5, and 1.0% chloramphenicol (YMPG-C) was added in order to suppress bacterial growth. YMPG-C plates were aerobically incubated at 28 °C for 48 h. Presumptive identification of isolates was performed based on morpho-physiological criteria (Yarrow, Kurtzman, & Fell, 1998). Genotypic identification of yeasts was carried out using chromosomal DNA isolated according to the protocol described by Hoffman and Winston (1987). Molecular identification of the selected isolated yeasts was carried out by amplification and sequence analysis of the fragment containing the genes encoding ribosomal RNA (rRNA): 18S, 5.8S and 26S. Universal primers ITS1 and ITS4 were used to amplify the internal transcribed spacer 1 (ITS1), 5.8S rRNA, and internal transcribed spacer 2 (ITS2) sequences, while primers NL-1 and NL-4 were used to amplify the 26S rRNA D1/D2 domain. PCR amplifications of a fragment containing ITS1, 5.8S rRNA, and ITS2 sequences were performed according to Lott, Kuykendall, and Reiss (1993) while that of 26S rRNA D1/D2 domain were carried out according to Kurtzman and Robnett (1998). DNA sequencing of both strands was performed using the dideoxy chain termination method with an ABI Prism 3730 DNA analyzer, at the DNA sequencing facility at Macrogen Inc., Korea, using ABI Prism BigDye® terminator cycle sequencing Ready Reaction Mix (PE Biosystems) according to the manufacturer's protocol. Sequence comparisons were performed using the BLAST tool available within the GenBank database. ClustalW software (Thompson, Higgins, & Gibson, 1994) was used for local alignment of multiple sequences. Phylogenetic and molecular evolutionary analyses were carried out with MEGA 5.2 (Tamura et al., 2012) by using neighbor-joining analysis (Saitou & Nei, 1987). For construction of phylogenetic trees, only sequences belonging to type strains of closely related species, whose names have been validly published in public databases, were considered. Sequences obtained from isolates of selected yeasts were submitted to the public Genbank database.

2.2. Extraction and characterization of the low molecular weight phenolic fraction (LMPF) in different blueberry cultivars

The 4 cultivars selected, *Misty*, *Blue Crisp*, *O'Neal* and *Millennium*, are widely cultivated in Tucumán. The extraction method used was described by Ghiselli, Nardini, Baldi, and Scaccini (1998). Total phenolic compounds in blueberry cultivars were measured using the Folin Ciocalteu method (Singleton & Rossi, 1965) adapted by Cicco, Lanorte, Paraggio, Viggiano, and Lattanzio (2009) for microtechnics. Phenolic compounds in the 4 blueberry extracts were identified and quantified by HPLC analysis coupled to a diode array detector according to the technique by Fanzone et al. (2011). Toxicological assessment was carried out with fresh human blood according to the technique by Shubha et al. (2016).

2.3. Antifungal activity of blueberry LMPF and individual phenolic compounds against isolated yeasts

The agar diffusion test was used to assay antifungal activity of blueberry LMPF and individual phenolic compounds, and the assay was carried out according to Rodríguez-Vaquero, Alberto, and Manca de Nadra (2007). Cycloheximide (500 μg/ml) was used as a positive control and sterile water or ethanol was used as negative control. After 48 h of incubation the inhibition zones were measured to an accuracy of 0.5 mm and the antifungal effect was calculated as the mean of three replication assays. For each blueberry LMPF the IC_{50} and IC_{90} (inhibitory concentration of 50 and 90% of yeast viability, respectively) were determined following the CLSI guidelines (Clinical and laboratory Standards Institute, 2006); tubes with Mueller-Hinton broth supplemented with serial dilutions of blueberry LMPF were inoculated individually with isolated yeasts. Cultures were incubated at 28 °C during 24–48 h, and the number of viable cells was determined using successive dilutions.

2.4. Yeast viability in strawberry juice supplemented with blueberry LMPF and sensorial quality

Commercial pasteurized strawberry juice (pH 3.12, viscosity 1.357, 16 °Brix, bright red color) in 1-L tetra bricks was purchased at a supermarket in Tucumán, Argentina. One hundred ml of juice were poured in 200 ml flasks and supplemented with 50 μg, 100 μg or 150 μg of sterile blueberry LMPF per ml of juice under aseptic conditions immediately after opening. Then, yeast cells were washed and inoculated individually to obtain a concentration of approximately 4 log cfu/ml and samples were incubated at 4 °C protected from light, to simulate initial contamination. Inoculated juice without additives was used as yeast growth control. Samples were taken after 0, 7 and 14 days of incubation at 4 °C. Yeast viability was determined using successive dilutions on YMPG-C agar medium.

A qualitative descriptive assay was used to assess sensory attributes of strawberry juice supplemented with 50, 100 and 150 μg/ml blueberries LMPF at day 0 and 7. A panel of ten members with sensory evaluation experience conducted the evaluation of strawberry juice. Four attributes were evaluated: odor, flavor, color and turbidity on a scale from 1 to 4. Odor and flavor scored from 1 (deteriorated juice) to 4 (fresh juice), color scored from 1 (roseate juice) to 4 (red juice) and turbidity scored from 1 (clean juice) to 4 (high turbidity). The limit of acceptance for each attribute was 2.5; lower values for any of the attributes were considered to have passed the shelf-life.

2.5. Statistical analysis

All experiments were repeated three times with duplicate samples and viable plate counts from three replications. Data were analyzed by ANOVA using Minitab (Minitab Inc., PA, USA). Multiple means comparison was carried out by Duncan's multiple range tests ($p < 0.05$).

3. Results

Table 1 shows the morpho-phenotypic characterization of 100 yeasts isolated from deteriorated juice based on morpho-physiological criteria. The results demonstrate that 49 of the 100 isolates seem to be the same yeast (Group I) and 51 isolates share the same/similar characteristics. Isolates of group I and II were presumptively identified as *Hanseniaspora* and *Starmerella* genus, respectively. Partial sequence analysis of rRNA of the isolates showed that Group I bore a remarkably close relationship, sharing 100% similarity with strains of the genus *Hanseniaspora* and Group II shared 95% identity with the genus *Starmerella* (Fig. 1a and b). Partial rRNA sequences of *H. osmophila* and *S. bacillaris* were submitted to the public Genbank database under access numbers KJ880968 and KJ880972, respectively (Vallejo, Delgado,

Table 1
Phenotypic characterization of isolated yeasts from deteriorated strawberry juice.

	Group I	Group II
Number of isolated yeasts	49 isolates	51 isolates
Macro-morphology	White and glossy colonies with raised center and flat periphery.	Butyrous colonies with well delimited periphery.
Micro-morphology	Lemon-shaped; occur single or in pairs.	Cells are ellipsoidal; occur single or in pairs.
Classification	non-*Saccharomyces*	non-*Saccharomyces*
Sporulation	Yes	Yes
Ascospores/asci	1–2	1–2
Pseudomycelium	Yes	No
Growth at different temperatures	20 °C (v) 28 °C (+) 37 °C (−)	20 °C (v) 28 °C (+) 37 °C (v)
ACF	No	No
D-Glucose fermentation	+	+
Sucrose fermentation	-	+
Raffinose fermentation	-	+
Trehalose fermentation	-	+
Maltose assimilation	+	–
Cellobiose assimilation	+	–
D-Mannitol assimilation	-	+
Raffinose assimilation	-	+

ACF: Amylaceous compound formation.
v: variable.

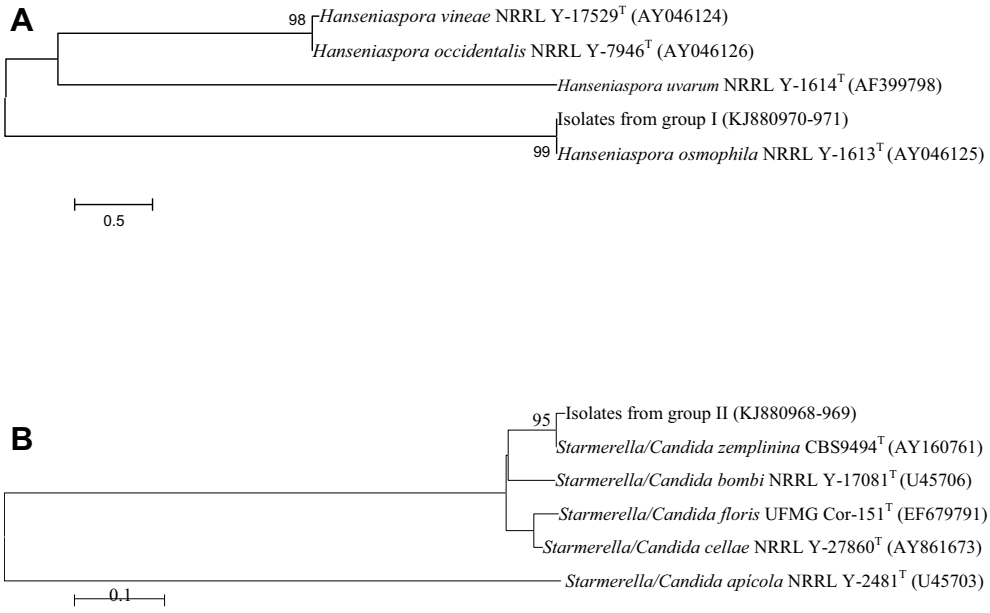

Fig. 1. Phylogenetic trees of the yeast *H. osmophila* (a) and *S. bacillaris* (b) based on rRNA partial sequencing. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 2.03653190 is shown (a) and 1.36407958 (b). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

Strasser, Rollán, & Rodríguez-Vaquero, 2014a,b). Phylogenetic trees of isolates from Group I and Group II revealed that isolates from Group I showed a close phylogenetic relationship with reference strains of *H. osmophila* validated at GenBank, whereas isolates from Group II were closely related to reference strains of *S. bacillaris*.

The phenolic content of the four blueberry LMPFs is shown in Table 2. LMPF of the *Blue Crisp* cultivar had the highest concentration of total phenolic compounds, followed by the *Millennium* cultivar. Significant differences ($p < 0.05$) dependent of cultivars were observed in concentration of phenolic compounds. LMPF obtained from *Misty* and *O´Neal* cultivars showed a 23 and 22% lower concentration of total phenolic compounds than that found in *Blue Crisp*. The majority of compounds in the 4 blueberry LMPF was chlorogenic acid, followed by quercetin. Gallic acid was only identified at a low concentration in LMPF of the *Millennium* cultivar, and catechin was only identified in LMPF of the *Blue Crisp* cultivar. ρ-coumaric, caffeic and ferulic acid were present in all blueberry cultivars. Cytotoxicity of blueberries LMPFs are given in Fig. 2. Hemolysis after addition of blueberry LMPFs was similar to that of the negative control ($p > 0.05$), demonstrating that blueberry LMPF is not toxic and could be safely applied to food.

Antifungal activity results demonstrate that all blueberry LMPFs and several individual phenolic compounds present in blueberry LMPF inhibited growth of *H. osmophila* and *S. bacillaris* (Table 3). Quercetin, kaempferol and ρ-coumaric, ellagic and chlorogenic acid showed highest antifungal activity, and they are probably responsible for the antifungal activity of blueberry LMPF. Table 4 shows the IC_{50} and IC_{90} for both yeast species. IC_{50} was around 50–56 μg/ml, whereas IC_{90} was around 90–100 μg/ml for both yeasts.

In control juice, *S. bacillaris* and *H. osmophila* grew 1.25 and 0.79 logarithmic cycles, respectively, after 7 days of incubation at 4 °C

Table 2
Total phenolic compounds and phenolic LMPF profile of four blueberry cultivars.

	LMPF			
	Misty	*Millennium*	*Blue Crisp*	*O´Neal*
Total phenolic content*	4033.00 ± 40.33[a]	4446.00 ± 44.50[b]	5233.00 ± 52.10[c]	4057.00 ± 40.60[a]
Gallic acid	*Nd*	4.90 ± 0.24	*Nd*	*Nd*
Methyl gallate	*Nd*	8.20 ± 0.41[a]	0.60 ± 0.03[b]	1.50 ± 0.07[c]
Caftaric acid	4.10 ± 0.20[a]	3.10 ± 0.15[b]	4.10 ± 0.20[a]	*Nd*
Cutaric acid	6.60 ± 0.33[a]	8.70 ± 0.43[b]	5.50 ± 0.27[c]	5.50 ± 0.27[c]
Procyanidin	105.90[4] ± 5.29[a]	227.90[4] ± 11.39[b]	843.00[4] ± 42.15[c]	168.80[4] ± 8.44[d]
Catechin	*Nd*	*Nd*	13.50 ± 0.67	*Nd*
Chlorogenic acid	305.20 ± 15.26[a]	325.10 ± 16.25[a]	423.40 ± 21.17[b]	98.20 ± 4.91[c]
Trans-caffeic acid	6.00 ± 0.30[a]	22.90 ± 1.14[b]	7.10 ± 0.35[a]	9.40 ± 0.47[c]
Trans-ρ-coumaric acid	2.50 ± 0.12[a]	2.30 ± 0.11[a]	15.20 ± 0.76[b]	1.90 ± 0.10[a]
Ferulic acid	9.10 ± 0.45[a]	3.80 ± 0.19[b]	5.30 ± 0.26[c]	45.00 ± 2.25[c]
Ellagic acid	12.20 ± 0.61[a]	21.90 ± 1.09[b]	14.20 ± 0.71[a]	79.00 ± 3.95[c]
Quercetin-3-glucoside	83.00 ± 4.15[a]	61.00 ± 3.05[a]	171.00 ± 8.55[b]	66.00 ± 3.30[a]
Laricitrin 3-galactoside	3.20 ± 0.16[a]	4.10 ± 0.20[a]	6.90 ± 0.34[b]	3.30 ± 0.16[a]
Isorhamnetin-3-galactoside	3.40 ± 0.17[a]	3.70 ± 0.18[a]	5.00 ± 0.25[b]	2.20 ± 0.11[c]
Kaempferol-3-glucoside	*Nd*	32.00 ± 1.60[a]	5.70 ± 0.28[b]	0.50 ± 0.02[c]
Laricitrin-3-glucoside	*Nd*	1.10 ± 0.05[a]	1.20 ± 0.06[a]	1.40 ± 0.07[a]
Isorhamnetin-3-glucoside	3.20 ± 0.16[a]	3.70 ± 0.18[a]	3.70 ± 0.18[a]	2.90 ± 0.14[a]

*Total phenolic content in blueberries was determined with the Folin Ciocalteu method (μg GAE/g fresh fruit).
Individual phenolic compounds in blueberries were determined with HPLC analysis (μg phenolic compounds/g fresh fruit).
[n] numbers in superscript at concentration represent the number of compounds found within that group.
Nd: Not detected.
Different letters in the same row show significant differences ($p < 0.05$).

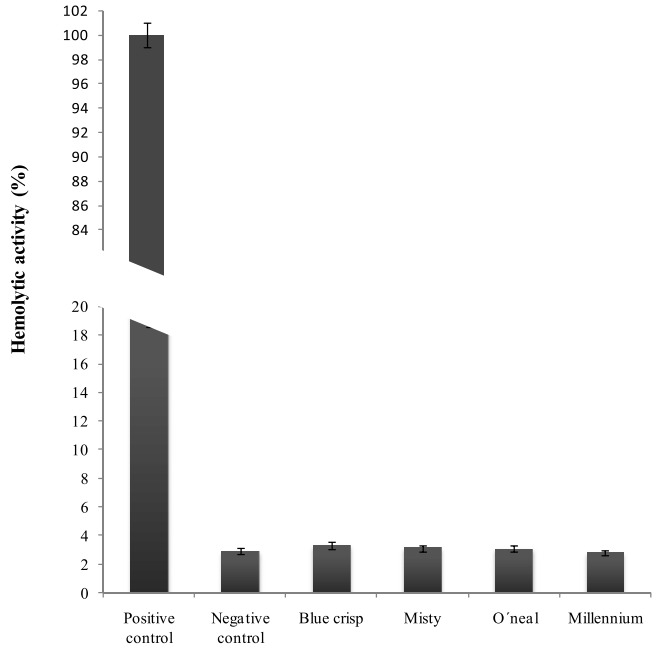

Fig. 2. Hemolytic activity of blueberry LMPFs using human red blood cells.

Table 3
Antifungal activity of LMPF extracts of four blueberry cultivars and individual phenolic compounds.

LMPF	*Starmerella bacillaris*	*Hanseniaspora osmophila*
Misty	w	+
Millennium	+	+
Blue Crisp	+	+ +
O´Neal	w	+
Gallic acid	+	+
ρ-coumaric acid	+ +	+ +
Ferulic acid	+	+
Caffeic acid	+	+
Chlorogenic acid	+ +	+ +
Ellagic acid	+ +	+ +
Catechin	+	+
Quercetin	+ +	+ +
Kaempferol	+ +	+ +
Control (+)	+ + +	+ + +
Control (−)	-	-

Antifungal activity: Inhibition zone < 1 mm, nil (−); Inhibition zone 1–5 mm, weak (w); Inhibition zone 6–11 mm, moderate (+); Inhibition zone 12–19 mm, high (+ +); inhibition zone > 19 mm, strong (+ + +).
Standard deviation ± 0.5 mm.

(Table 5). In juice supplemented with 50 μg/ml blueberry LMPF, growth of both yeasts was not modified ($p > 0.05$) with respect to control juice, but addition of 100 μg/ml blueberry LMPF reduced growth of *S. bacillaris* compared with control juice ($p < 0.05$). Under similar conditions, cellular death of *H. osmophila* was observed in the presence of blueberry LMPF, after 7 days of incubation. No viable *S. bacillaris* or *H. osmophila* cells were detected in juice after addition of 150 μg/ml LMPF from *Blue Crisp* and *Millennium* cultivars. Addition of 100 and 150 μg/ml of blueberry LMPF showed no evidence of yeast viability in juice after 14 days of incubation.

Sensory analysis demonstrated that addition of 50, 100 or 150 μg/ml blueberry LMPF to strawberry juice did not modify significantly odor, flavor, color or turbidity after 0, 7 or 15 days. Consequently, addition of these blueberry LMPFs could be acceptable in natural juice.

4. Discussion

H. osmophila and *S. bacillaris* were for the first time identified in Argentine strawberry juice. *H. osmophila* is mainly found in different parts of plants, fruits, flowers, soil, honeydew systems of Southern beech and in fruit-based fermented foods such as vinegar and wine (Serjeant, Tang, Anfang, Beggs, & Goddard, 2008). Granchi, Ganucci, Messini, and Vincenzini (2002) showed that *H. osmophila* has detrimental properties similar to those of *Saccharomyces ludwigii*. The phenolic profile of Argentine blueberry LMPF coincided with that found by other authors, who reported chlorogenic acid as the major phenolic acid in blueberries cultivated in Spain and Greece, among other countries

Table 4
IC_{50} and IC_{90} of LMPF extracts of four blueberry cultivars for *H. osmophila* and *S. bacillaris*.

LMPF	*Starmerella bacillaris*		*Hanseniaspora osmophila*	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}
Misty	51.55 ± 1.67[a]	100.00 ± 3.00[a]	52.64 ± 1.58[a]	94.75 ± 2.84[a]
Millennium	55.29 ± 1.54[b]	92.32 ± 2.77[b]	50.50 ± 1.51[b]	90.91 ± 2.73[b]
Blue Crisp	55.12* ± 1.50[b]	95.22 ± 2.71[c]	50.00 ± 1.50[b]	90.00 ± 2.70[b]
O´Neal	65.55 ± 1.67[c]	100.00 ± 3.00[a]	53.36 ± 1.60[a]	96.05 ± 2.88[c]

*μg/ml of phenolic extract.
Different letters in the same column show significant differences ($p < 0.05$).

Table 5
Survival of *S. bacillaris* and *H. osmophila* in strawberry juice supplemented with LMPF from 4 blueberry varieties, after 7 days of incubation at 4 °C.

		Starmerella bacillaris		*Hanseniaspora osmophila*	
		Log cfu/ml	A*	Log cfu/ml	A*
Control juice μg/ml		5.30 ± 0.26[a]	–	4.84 ± 0.24[a]	–
Misty	50	5.00 ± 0.25[a]	0.30 ± 0.01	4.50 ± 0.22[a]	0.34 ± 0.01
	100	4.60 ± 0.23[b]	0.70 ± 0.03	3.58 ± 0.18[b]	1.27 ± 0.06
	150	3.50 ± 0.17[c]	1.80 ± 0.09	*Nd*	*Nd*
Millennium	50	4.95 ± 0.25[a]	0.35 ± 0.02	4.35 ± 0.22[a]	0.49 ± 0.02
	100	4.50 ± 0.22[b]	0.80 ± 0.04	3.39 ± 0.17[b]	1.45 ± 0.07
	150	*Nd*	*Nd*	*Nd*	*Nd*
Blue Crisp	50	4.88 ± 0.24[ab]	0.42 ± 0.02	4.24 ± 0.21[a]	0.60 ± 0.03
	100	4.30 ± 0.21[b]	1.00 ± 0.05	3.19 ± 0.16[b]	1.65 ± 0.08
	150	*Nd*	*Nd*	*Nd*	*Nd*
O´Neal	50	5.10 ± 0.25[a]	0.20 ± 0.01	4.63 ± 0.23[a]	0.21 ± 0.01
	100	4.80 ± 0.24[ab]	0.50 ± 0.02	3.69 ± 0.18[b]	1.15 ± 0.05
	150	4.10 ± 0.20[b]	1.20 ± 0.06	2.50 ± 0.11[c]	2.34 ± 0.12

Initial yeast count: 4.05 log cfu/ml.
A*: reduction in log unit with respect to control juice (without treatment).
Nd: not detected.
Different letters in the same column show significant differences ($p < 0.05$).

(Cardeñosa, Girones-Vilaplana, Muriel, Moreno, & Moreno-Rojas, 2016; Correa-Betanzo et al., 2014; Ferlemi, Makri, Mermigki, Lamari, & Georgakopoulos, 2016; Kjersti, Grimmer, & Holtung, 2013). Dudonné et al. (2015) identified ρ-coumaric, caffeic and ferulic acid in all blueberry cultivars assayed and the authors determined higher concentrations than gallic and protocatechuic acid. In addition, *Ribes nigrum*, a blueberry cultivar grown in Europe, contained mainly ρ-coumaric, ferulic and caffeic acid, with caffeoyl glucoside and flavonols such as myricetin, quercetin, isorhamnetin and kaempferol as the most important compounds (Gavrilova, Kajd-Zanoska, Gjamovski, & Stefova, 2011; Zheng et al., 2012). None of the 4 blueberry LMPFs assayed in the present study were cytotoxic and our results coincide with those reported by Miceli et al. (2014) and they were similar to observations in a hydroalcoholic extract of *Ocimum sanctum* (Shubha et al., 2017).

All 4 blueberry LMPFs studied showed antifungal activity in strawberry juice and highest activity was observed after addition of *Blue Crisp* and *Millennium* LMPF. Differences in antifungal activity could be related to differences in the phenolic compound profile of the extracts. *Blue Crisp* LMPF demonstrated higher concentrations of chlorogenic acid, ellagic acid and kaempferol than the other cultivars. Several authors have reported antifungal activity of chlorogenic and caffeic acid derivatives against *C. neoformans* and *Aspergillus fumigatus* (Jan-Nan & Chao-Mei, 2015). Similar results have been reported by Tomadoni, Cassani, Ponce, Moreira, and Agüero (2016), who demonstrated a decrease in deteriorating microbiota of strawberry juice treated with ultrasound and supplemented with pomegranate extracts combined with vanillin. An important fact is that addition of 50, 100 or 150 μg/ml of the 4 blueberry LMPFs did not modify sensorial characteristics of the strawberry juice, which makes their addition to food acceptable, and similar results have been reported by Cassani, Tomadoni, Viacava, Ponce, and Moreira (2016). The present study has demonstrated the effectiveness of a combined treatment of blueberry LMPF and low temperature to control growth of spoilage yeasts.

5. Conclusion

Argentine blueberry cultivars have a rich phenolic compound profile. The blueberry phenolic extracts showed antifungal activity against *H. osmophila* and *S. bacillaris* in the strawberry juice through individual phenolic compounds such as quercetin, kaempferol and chlorogenic, ρ-coumaric and ellagic acid.

Our study has for the first time demonstrated presence of *Starmerella bacillaris* and *Hanseniaspora osmophila* in spoiled strawberry juice from the Northwest of Argentina and a deeper knowledge of spoilage yeasts is the first step to find a solution. Furthermore, the present study proposes the use of a combined treatment of low temperature and addition of blueberry LMPF as a natural and non-toxic alternative for the control of spoilage yeasts in strawberry juice.

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