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ORIGINAL PAPER

Effect of different fermentation conditions on the kinetic parameters and production of volatile compounds during the elaboration of a prickly pear distilled beverage

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Abstract An experimental design considering thermal treatment of must, yeast strain, prickly pear variety and degree of ripeness was chosen to evaluate the fermentation behavior and generation of volatile compounds, during the elaboration of a distilled beverage from prickly pear. Four Mexican prickly pear varieties were characterized physically and two of them were selected for fermentation studies. The thermal treatment of the must showed the highest statistical influence on fermentation behavior and production of volatile compounds, followed by prickly pear variety, then yeast strain and finally the degree of ripeness was the least statistically significant factor. The growth rate increased when the thermal treatment was applied whereas the ethanol production rate and alcoholic efficiency were unaffected. The results also suggested that thermal treatment was effective for inhibition of microbial contamination. As regards volatile compounds production, acetic acid and methanol decreased while other volatiles increased when the thermal treatment was applied. Despite the influence of thermal treatment, prickly pear variety strongly influences the volatile profile of fermented musts.

Keywords Prickly pear · Fermentation behavior · Volatile compounds · Distilled beverage

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Introduction

In Mexico, there are more than 100 species of the *Opuntia* genus, producing prickly pears [6]. They are well distributed in semi-arid zones around the country, although they are concentrated in the north-central part of Mexico. These plants produce a great variety of prickly pear fruits, which provide sugars in a range of 12 to 15% (w/w), weight 110–160 g [12] and they differ also in chemical composition [10, 15, 17, 20, 31, 32]. Prickly pears contain volatile compounds that are found also in other fruits; most of these compounds being alcohols. Nevertheless, the principal difference in the composition of volatiles, with respect to other fruits, is the presence of different levels of nonen-1-ols, which produce a melon-like flavor [14]. The variation in biochemical composition in prickly pears not only depends on the kind of fruit, but the color, size, seed content, sugars, proteins, fats, pectins and non-volatile organic acids of different prickly fruits, that change during fruit development and ripening [1, 19]. In the specific case of prickly pear fruits from the north-central part of Mexico, the differences in their color and pulp, seeds and peel percentage (w/w) depends upon whether they were grown in the wild or developed in cultivars [26].

There are only a few production options in the north-central part of Mexico, because of the aridity, and prickly pear represents an important source of revenues for the farmers of this region. However, only the best fruits can be sold in fresh form, resulting in a loss of up to 60%. Transformation options are therefore required for prickly pear. Traditionally, in Mexico, prickly pear has been transformed into semi-solid sweets and solid jams called "quesos" [14]. The "cardona" variety is used as the raw material for elaboration of the fermented indigenous beverage named "colonche" [11]. Wine has also been obtained from the pulp of prickly pear fruits after the adjustment of the must, to avoid bacterial contamination [7] and combustible alcohol was obtained from cladodes and fruits [28].

This work is the first study of the evaluation of the effect of the yeast strain, type and ripeness degree of prickly pear and thermal treatment of must on the fermentation behavior and the production of volatiles, in order to obtain the conditions for the elaboration of a fermented and distilled prickly pear beverage, with economic potential.

Materials and methods

Selection of prickly pear varieties

According to information of INIFAP (National Institute for Forestry and Agriculture of Mexico), four agronomically important *Opuntia* spp. varieties from San Luis Potosí (Mexico) were selected for a preliminary characterization. The varieties were: "amarilla montesa" (orange, 10–15 cm large, oval shape), "roja lisa (red, 10–15 cm large, oval shape), "esmeralda" (green–yellow, 13–18 cm, round shape) and "cristalina"(green–yellow, 9–13 cm large, oval shape). For preliminary characterization; the pH, Brix degree, sugar content by the Miller method [24], juice density and the relation of peel, pulp and seed (w/w) were determined.

Analytical procedures

Yeast cell growth was determined by counting the cell number, using a Neubauer chamber. Sugar consumed was determined according to the Miller method [24]. For ethanol and volatiles production, 5 ml of sample was added to 5 ml of distilled water, the mixture was distilled and the first 5 ml were recovered. Ethanol was quantified colorimetrically using chromic acid, according to Boehringer [4]. The quantification of volatile compounds was done by gas chromatography: analyses were carried out in a Hewlett-Packard 5890 gas chromatograph with a FID detector (USA), 1 μl of distilled was injected into a HP-Innowax PEG 60 m × 0.32 mm i.d., 0.25 μm column (Agilent technologies), the temperature program was 50°C for 6 min, then 10°C min^{-1} to 160°C and 20°C min^{-1} to 220°C was maintained for 8 min. Injector and detector temperature were 250°C. The carrier gas used was helium.

Experimental design

A multi-factorial experimental design 2^k with four factors and one replicate was employed as follows: fruit variety (amarilla montesa and esmeralda), degree of ripeness (completely ripe and almost ripe), *Saccharomyces cerevisiae* yeast strains (low (MG) and high (GU4) aroma producers, isolated from tequila must, CIATEJ collection) and thermal treatment of must (with or without treatment). Must treatment consisted of boiling the prickly pear juice for 5 min in order to hydrolyze some compounds and to reduce the initial microbial population.

Fermentation

Fermentations were performed at room temperature (30–33°C) in an orbital shaker (New Brunswick Scientific, NJ, USA), in 500 ml flasks containing 200 ml of prickly pear juice, according to the different combinations of the experimental design with one replicate. The inoculum was 2×10^6 cells ml^{-1} from an overnight culture. Samples were taken during the fermentation to determine cell growth, sugar consumption, ethanol and volatile compounds production. Data presented are the averages of two replicates.

Statistical analysis

The program Statgraphics (version 4.0, Manugistics Inc, Rockville, MD, USA) was used for statistical analysis of the data. After data analysis, Pareto charts were obtained and are presented in two tables, one for kinetic parameters, and one for volatile compounds, indicating the statistical significance of the factors studied.

Results and discussion

Selection of prickly pear varieties

Table 1 presents the physical characterization of the prickly pear fruits studied. The "roja lisa" and "amarilla montesa" varieties, especially the unripe fruits (Table 1), contain the highest amount of peel. The results of physical characterization are in agreement with other reports [2, 12]. Seed content was higher in "cristalina" and "roja lisa" varieties, while the lowest seed content was observed in the "esmeralda" prickly pear. The greatest amount of juice, which constitutes the must to be fermented, was found in the "esmeralda" variety. The pH of the juices was between 3.2 and 4.8, with the exception of "esmeralda" unripe fruit with a value of 5.8. The Brix degree (percentage of dissolved solids) as an indicator of the sugar content, was highest in the “roja lisa” and “amarilla montesa varieties. The “esmeralda” variety was selected for fermentation studies because of its higher content of juice, the “amarilla montesa” variety was selected because of its high Brix degree and because of the interest of prickly pear producers. The mean content of sugars was 119 g l^{-1} and 118 g l^{-1} for “amarilla montesa” and “esmeralda” varieties, respectively (Table 1).

Fermentation behavior

In the fermentation tests with these two prickly pear varieties, the four factors studied had a statistically significant influence on the kinetic parameters, except on the ethanol production rate and on alcoholic efficiency (Table 2). As prickly pear juice contains sugars, vitamins, minerals and also some amino acids [10, 15, 17, 20, 32], the composition of juice, either with or without thermal treatment, provides enough requirements for fermentation, and this could explain the low influence of thermal treatment in the ethanol production rate and its efficiency.

Contrary to ethanol production parameter, the yeast growth rate was highly affected by the thermal treatment (Table 2; Fig. 1); the average values were in the range of 5–7 and 1–3 cells × 10^6 ml^{-1} h^{-1} with and without thermal treatment, respectively. The thermal

Table 1 Physical characterization of four prickly pear varieties from San Luis Potosí (Mexico) with different ripeness degree

Prickly pear variety	Composition (%, w/w)			Brix degree (sugar content)	pH
	Peel	Seeds	Juice		
Roja lisa					
Ripe	46.56	16.64	36.78	14.4 (ND)	4.5
Unripe	51.07	19.46	29.46	13 (ND)	3.5
Amarilla montesa					
Ripe	45.69	15.62	38.67	13 (118 g l^{-1})	4.8
Unripe	52.42	19.54	28.03	14 (119 g l^{-1})	3.2
Cristalina					
Ripe	45.14	22.37	32.48	13.1 (ND)	3.6
Unripe	38.48	19.39	42.12	13.1 (ND)	4.3
Esmeralda					
Ripe	43.32	13.81	42.86	13.2 (125 g l^{-1})	4.1
Unripe	41.53	11.90	46.56	12 (111 g l^{-1})	5.8

ND not determined

Table 2 Statistical analysis summary for kinetic parameters

Factors and interactions	Kinetic parameters									
	$r_{p\,max}$ (g l^{-1} h^{-1}) $R^2 = 33.1$		$r_{x\,max}$ (cells × 10^6 l^{-1} h^{-1}) $R^2 = 72.9$		$r_{s\,max}$ (g l^{-1} h^{-1}) $R^2 = 71.3$		Initial sugar consumed (%) $R^2 = 78.8$		Alcoholic efficiency (%) $R^2 = 63.4$	
	RC	PV	RC	PV	RC	PV	RC	PV	RC	PV
Constant	1.27		4.24		2.85		93.4		81.3	
A = Ripeness degree	0.05	0.287	−0.07	0.81	0.46	6 × 10^{-4}*	0.77	0.19	−2.99	0.09
B = Prickly pear variety	0.037	0.474	0.11	0.71	0.19	0.09	2.90	1 × 10^{-4}*	0.16	0.92
C = Yeast strain	0.065	0.219	−0.41	0.16	0.38	3x10^{-3}*	2.06	2 × 10^{-3}*	1.31	0.45
D = Thermal treatment	−0.046	0.380	2.00	2 × 10^{-4}*	0.06	0.60	−2.49	3 × 10^{-4}*	−1.29	0.45
AB	−0.07	0.174	−0.12	0.67	0.06	0.57	0.87	0.14	−1.43	0.41
AC	0.012	0.807	0.01	0.96	0.30	0.01*	0.47	0.42	−1.17	0.49
AD	0.027	0.598	−0.21	0.46	0.02	0.86	0.25	0.65	2.16	0.22
BC	0.012	0.813	−0.16	0.58	0.20	0.08	−0.80	0.17	−0.80	0.64
BD	0.010	0.842	−0.10	0.72	0.17	0.15	0.94	0.11	0.66	0.70
CD	−0.08	0.105	−0.12	0.67	0.14	0.22	1.09	0.07	−2.60	0.14

$r_{p\,max}$ maximum ethanol production rate, $r_{x\,max}$ maximum growth rate, $r_{s\,max}$ maximum sugar consumption rate, R^2 R-squared of lineal fitted model, *RC* Regression coefficients of fitted model, *PV* *P*-value or significance level (α = 0.05)

*Statistically significant

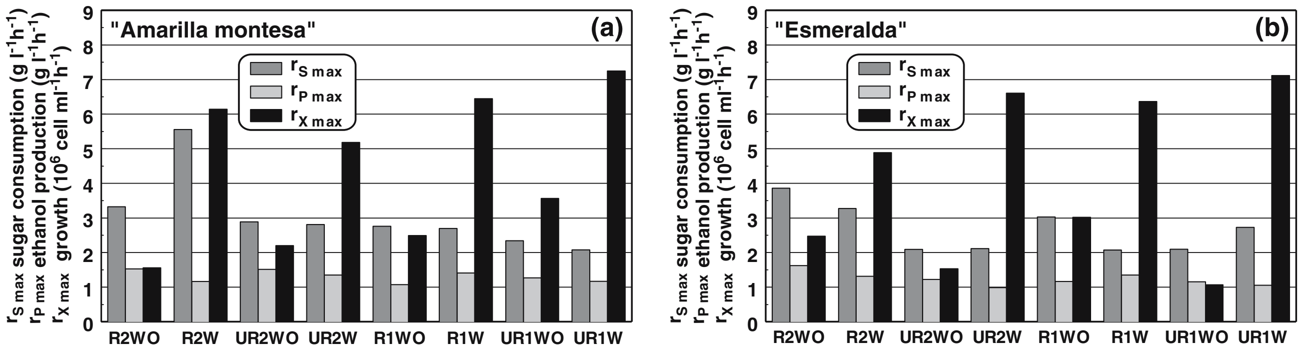

Fig. 1 Maximum ethanol production ($r_{p\,max}$), growth ($r_{x\,max}$) and sugar consumption ($r_{s\,max}$) rates in "amarilla montesa" (**a**) and "esmeralda" (**b**) prickly pear must fermentations. Conditions code: *R/UR* ripe/unripe, *1/2* MG/GU4 strain, *W/WO* with/without thermal treatment

treatment increased the levels of some compounds such as amino acids and sugars by hydrolysis, and assimilation of them resulted in improved growth. On the other hand, as a consequence of its composition, prickly pear juice is very susceptible to microbial contamination. Thermal treatment caused a reduction in initial microbial contamination, which permitted a better growth of the inoculated yeasts.

The sugar consumption rate was principally dependent on the degree of ripeness and less on the yeast strain (Table 2 ; Fig. 1). The average values were 3.3 g l^{-1} h^{-1} and 2.4 g l^{-1} h^{-1} for ripe and unripe prickly pear musts, respectively. The initial sugar consumption rates depended primarily on the fruit variety and next on thermal treatment. The content of initial sugar varied with ripeness degree, especially in the "esmeralda" prickly pear variety with an initial sugar content of 125 and 111 g l^{-1} for ripe and unripe "esmeralda" prickly pear, respectively (Table 1). The initial sugar consumption rate was higher for the "amarilla montesa" prickly pear. The thermal treatment decreased the initial sugar consumption rate in ripe "esmeralda" prickly pear (Table 2; Fig. 2). In this case, the less significant factor was the yeast strain (Table 2). The average values of sugar consumed were 96.3 and 90.5% for "amarilla montesa" and "esmeralda" prickly pear varieties, respectively. Sugar content in prickly pear varies with the degree of ripeness [1, 19] and fruit variety [12]. Thus, if more initial sugar is available, this could affect the rate of sugar assimilation. Additionally, the principal sugars in prickly pear are glucose, fructose and sucrose [31]. As the capability of glucose and fructose assimilation varies between the yeasts strains [3], this could be another factor that has an influence on the rate of sugar assimilation. Therefore, the complex correlation of sugar content and composition of the "amarilla montesa" and "esmeralda" prickly pear varieties and the fermentation capacity of the yeasts strains used in the conditions tested, could be the cause of the influence on initial sugar consumed and sugar consumption rate.

Volatile compounds production

The volatile compounds are important for beverage flavor, as they contribute to different desirable sen-

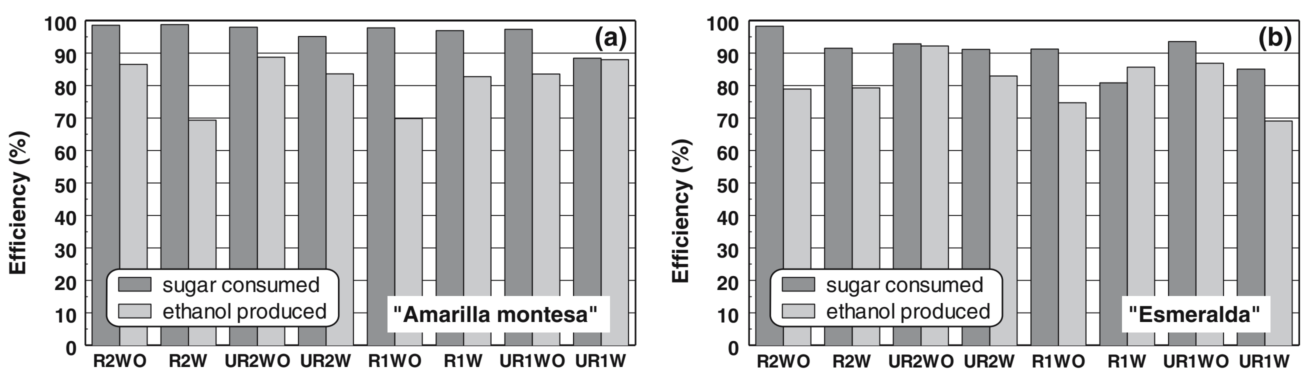

Fig. 2 Initial sugar consumed and alcoholic efficiency for "amarilla montesa" (**a**) and "esmeralda" (**b**) prickly pear must fermentations. Conditions code as in Fig. 1

sory characteristics. Amyl alcohols and their esters contribute to banana aroma [22], 2-phenylethanol contributes to rose aroma [34], and α-terpineol and other terpenoids are responsible for fruity and floral aroma [16]. Ethyl acetate and acetaldehyde produce a fruity and a fermented aroma, respectively [23], while acetic acid is not a desirable aroma in alcoholic beverages because it is considered a product of a poorly controlled fermentation [30]. Methanol is desirable in concentrations ranging from 8 to 269 mg l^{-1}, but not more than 500 mg l^{-1}, because it could affect the beverage quality [23] and consumers' health [25]. In general, for the production of major (acetic acid, amyl alcohols and methanol) and minor (acetaldehyde, α-terpineol, n-propanol, ethyl acetate and 2-phenylethanol) volatile compounds, the thermal treatment and fruit variety were statistically the most important, while ripeness degree did not have a significant effect (Table 3). For minor volatile compounds, the production of acetaldehyde was higher in "amarilla montesa" than in "esmeralda" prickly pear with the strain GU4.Thus, prickly pear variety and yeast strain influence the production of this compound (Table 3; Fig. 3a, b). Acetaldehyde is directly correlated with yeast metabolism in alcoholic fermentation, because this compound is produced by decarboxylation of pyruvate, which is the direct product of glycolysis. This reaction is performed by pyruvate-decarboxylase [27], which requires thiamine [27]. Therefore, if there were differences in thiamine concentration in "amarilla montesa" and "esmeralda" prickly pear varieties, it could be possible that the levels of this vitamin affect the rate of acetaldehyde production and different levels of acetaldehyde.

For *n*-propanol production, the factors, prickly pear variety and thermal treatment have the principal influence (Table 3), and yeast strain was less significant (Table 3), which is in accordance with the data in Fig. 3a, b. In the case of ethyl acetate, only the thermal treatment had a statistically significant influence (Table 3). Ethyl acetate is produced principally by the action of alcohol acetyl-transferase in *Saccharomyces cerevisiae* [22]; however, ethyl acetate is naturally present in prickly pear fruit [14]. In our case, it seems that levels of ethyl acetate in the product were more dependent upon the initial content of this compound in the prickly pear used.

The production of 2-phenyl-ethanol is affected principally by thermal treatment and less by fruit variety (Table 3; Fig. 3a, b). For the major volatile compounds, the thermal treatment had the principal influence on the production of amyl alcohols. When the thermal

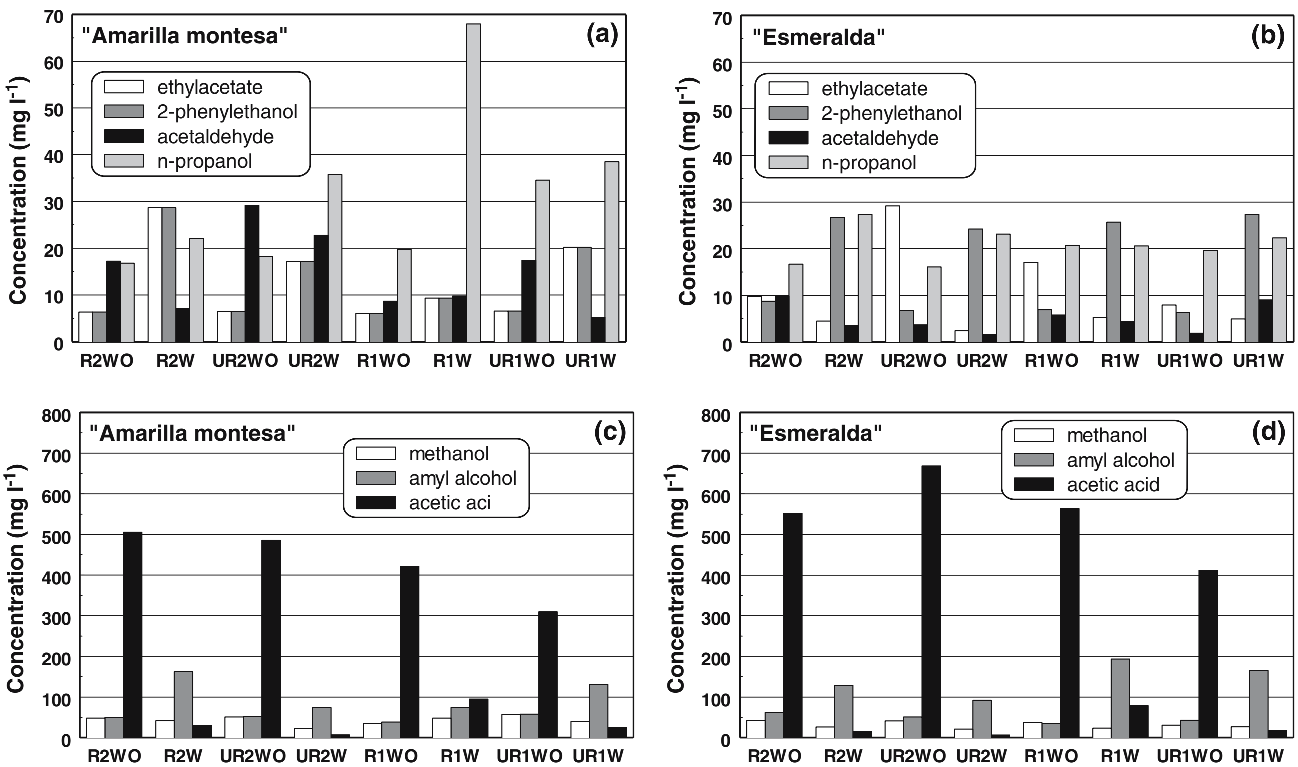

Fig. 3 Minor (**a**, **b**) and major (**c**, **d**) volatile compounds in "amarilla montesa" (**a**, **c**) and "esmeralda" (**b**, **d**) prickly pear fermentations. Conditions code as in Fig. 1

Table 3 Statistical analysis summary for volatile compounds

Factors and interactions	Volatile compounds															
	Acd $R^2 = 50.4$		α-Te $R^2 = 94.2$		Et ace $R^2 = 68.1$		2-Phe $R^2 = 86.3$		Pro $R^2 = 72.2$		Acet $R^2 = 91.06$		Amy $R^2 = 83.9$		Meth $R^2 = 54.4$	
	RC	PV	RC	PV	RC	PV	RC	PV	RC	PV	RC	PV	RC	PV	RC	PV
Constant	9.83		0.56		8.85		14.6		26.2		262		87.9		36.8	
A = Ripeness degree	−0.05	0.97	−0.004	0.92	−1.72	0.08	−1.30	0.11	1.81	0.29	−1.26	0.94	−5.19	0.29	−2.44	0.23
B = Prickly pear variety	4.85	0.01*	0.378	9×10^{-4}*	−1.29	0.18	−1.98	0.02*	5.44	2×10^{-3}*	−27.1	0.11	−8.18	0.10	5.98	0.01*
C = Yeast strain	3.08	0.04*	0.067	0.12	0.68	0.47	1.30	0.11	−5.19	5×10^{-3}*	30.9	0.07	13.1	0.05	0.25	0.90
D = Thermal treatment	−1.88	0.26	−0.53	3×10^{-4}*	−3.90	4×10^{-4}*	7.82	1×10^{-4}*	5.95	4×10^{-3}*	−227	1×10^{-4}*	39.5	2×10^{-4}*	−5.71	7×10^{-3}*
AB	0.002	0.99	−0.06	0.14	1.54	0.11	−1.58	0.06	1.56	0.36	23.4	0.17	−15.6	0.00*	−1.90	0.34
AC	0.717	0.67	0.03	0.46	−1.01	0.29	−0.11	0.88	−0.406	0.81	−6.11	0.71	−1.26	0.79	−0.48	0.81
AD	2.24	0.19	0.017	0.68	1.83	0.06	−1.38	0.09	3.89	0.03*	15.8	0.35	−4.85	0.32	1.40	0.48
BC	1.32	0.43	−0.02	0.63	−1.47	0.12	0.75	0.35	−3.31	0.06	−8.96	0.59	−8.47	0.09	−2.04	0.31
BD	−1.54	0.36	−0.34	0.00*	1.94	0.05	−1.58	0.06	3.41	0.05	31.9	0.07	−9.13	0.07	0.97	0.62
CD	−0.18	0.91	−0.08	0.06	−0.87	0.35	0.98	0.22	−1.83	0.28	−32.1	0.07	8.05	0.10	−2.64	0.19

Acd acetaldehyde, *α-Te* α-terpineol, *Et ace* ethyl acetate, *2-Phe* 2-phenylethanol, *Pro* propanol, *Acet* acetic acid, *Amy* amyl alcohols, *Meth* methanol, R^2 *R*-squared of fitted model, *RC* regression coefficients of lineal fitted model, *PV* P-value or significance level ($\alpha = 0.05$)

*Statistically significant

treatment was applied, an increase was observed (Fig. 3c, d). As degradation or biosynthesis of amino acids generate precursors of higher alcohols [8], it is possible that differences in the composition of amino acids between “amarilla montesa” and “esmeralda” result in differences in n-propanol production. It has been found that there are differences in composition between some Italian prickly pear varieties [1]. On the other hand, the thermal treatment of must, could increase the levels of phenylalanine, leucine and isoleucine because of peptide hydrolysis, and as these amino acids are precursors of amyl alcohols [9] and 2-phenylethanol [35], this could increase the production of these higher alcohols. This increase in higher alcohols could be favorable for the prickly pear distilled beverage, 2-phenylethanol in particular, which is an important sensory component in other fruit-fermented [36] and distilled [21, 29] beverages.

For acetic acid production, the thermal treatment was the most significant factor. While for methanol production, both the thermal treatment and fruit variety had a statistically significant effect (Table 3). When the thermal treatment was applied, a decrease in the production of acetic acid and methanol was observed, especially in acetic acid production (Fig. 3c, d). Methanol in distilled beverages is produced by the breakdown of methoxyl groups from pectins, or by methanol producing bacteria [33]. It is possible that the increased methanol production in musts without thermal treatment was due to microbial contamination with some methanol producing bacteria at the beginning of the fermentation. In the case of acetic acid, the production was higher without thermal treatment for the two prickly pear varieties and it could also be attributed to initial microbial contamination, which was reduced by the thermal treatment of must before the fermentation. As the production of α-terpineol was very low (less than 5 mg l^{-1}), it was not shown in Fig. 3, but its production was influenced principally by the thermal treatment and less by the fruit variety (Table 3). When the thermal treatment was applied, the production of α-terpineol decreased with both prickly pear varieties; the production was higher in the “amarilla montesa” (2 mg l^{-1}) than in the “esmeralda” prickly pear variety (1 mg l^{-1}) (data not shown). *Hanseniaspora* and *Kloeckera* yeasts present at the beginning of most fruit fermentations [30] possess the enzyme β-glucosidase [13], which hydrolyzes terpenic compounds from glycosidic bonds [18]. Therefore, it is possible that the yeasts present at the beginning of fermentation, were inhibited or possibly killed by the thermal treatment, and that was the reason for the low production of α-terpineol when thermal treatment was applied. Nevertheless, it has been found that these compounds are released by heating as a consequence of the breakdown of glycosidic bonds [5], but in our case this mechanism was not possible because the production of α-terpineol decreased with thermal treatment.

Conclusions

Moderate thermal treatment of the must appears to be a simple and efficient way to control microbial contamination and to improve yeast growth and volatile profile, giving adequate sensory properties in order to obtain a new distilled beverage. Prickly pear variety greatly influences the final volatiles profile, indicating that volatile precursors differ between varieties. Thus, it could be interesting to test several prickly pear varieties to obtain distilled beverages with different sensory profiles.

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