

ORIGINAL ARTICLE

Production and characterization of a novel distilled alcoholic beverage produced from blueberry (*Vaccinium corymbosum* L.)

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Abstract – Introduction. The cultivation of underutilized berries and the process production of high-value-added products, such as fruit-based spirits, could have a beneficial effect on the economy of disadvantaged rural mountain areas of Spain. However, production of a distilled alcoholic beverage from the blueberry has not been reported before. **Materials and methods.** The pulp of blueberries var. Bluecrop was fermented with *Saccharomyces cerevisiae* IFI83, distilled by using a steam drag distillation system and the volatile compounds were determined by gas chromatography. **Results and discussion.** In the distillate obtained, the mean concentrations of ethanol (45.3 mL 100 mL⁻¹ distillate), volatile substances (317.1 g hL⁻¹ absolute alcohol) and methanol (261.0 g hL⁻¹ absolute alcohol) were in accordance with the specifications that the European Council (Regulation 110/2008) fixed for these compounds. In addition, the ratios [3-methyl-1-butanol/2-methyl-1-propanol] and [2-methyl-1-propanol/1-propanol] were 2.60 and 1.34, respectively, indicating that the distilled alcoholic beverage has a good organoleptic quality. **Conclusion.** The results suggest that blueberry can be successfully used for the production of a novel spirit with a good sensory quality that is safe for the consumers.

Keywords: Spain / blueberry / *Vaccinium corymbosum* / ethanol distilling / solid-state fermentation / volatile compounds

Résumé – Production et caractérisation d'une nouvelle boisson alcoolisée distillée produite à partir de grande myrtille (*Vaccinium corymbosum* L.). **Introduction.** La culture d'espèces de baies sous-utilisées et leur transformation en produits à haute valeur ajoutée, tels que les spiritueux à base de fruits, pourraient avoir un effet bénéfique sur l'économie des régions rurales et défavorisées des montagnes d'Espagne. La production d'une boisson alcoolisée distillée à partir de grandes myrtilles ou bleuets n'a pourtant jamais été signalée auparavant. **Matériel et méthodes.** La pâte de bleuets var. Bluecrop fermentée avec *Saccharomyces cerevisiae* IFI83, a été distillée en utilisant un système de distillation par train de vapeur d'eau ; les composés volatils ont été déterminés par chromatographie en phase gazeuse. **Résultats et discussion.** Dans le distillat obtenu, les concentrations moyennes d'éthanol (45,3 mL 100 mL⁻¹ distillat), de substances volatiles (317,1 g hL⁻¹ alcool absolu, aa) et de méthanol (261,0 g hL⁻¹ aa) étaient conformes aux spécifications que le Conseil européen (règlement 110/2008) fixe pour ces composés. De plus, les rapports [3-méthyl-1-butanol/2-méthyl-1-propanol] et [2-méthyl-1-propanol/1-propanol] ont été de 2,60 et 1,34, respectivement, indiquant que la boisson alcoolisée distillée a une bonne qualité organoleptique. **Conclusion.** Les résultats suggèrent que la grande myrtille peut être utilisée avec succès pour la production d'un nouveau spiritueux de bonne qualité organoleptique et sanitaire pour les consommateurs.

Mots clés : Espagne / grande myrtille / *Vaccinium corymbosum* / distillation éthanolique / fermentation à l'état solide / composés volatils

1 Introduction

The processing of berries, mainly those underutilized fruits, for production of high-value-added products, such as fruit-based spirits, could have a beneficial effect on the economy of disadvantaged rural mountain areas of Spain,

where climatic conditions are less favorable and where steep slopes hamper machine use. This approach is based on the fact that different berries have been successfully used as fermentation substrates to produce different commercialized alcoholic beverages [1, 2].

However, the reproducibility of the fermentation process is very difficult because production of these alcoholic beverages has been carried out by using different fermentation methods

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(spontaneous or inoculated submerged liquid fermentations), under uncontrolled conditions and in some cases, the kinetics of fermentation process has not been described [1, 2].

The solid-state fermentation of blueberry could allow the production, in a reproducible way, of a spirit with its own distinctive aromatic profile composed of the aromas present in the fruit and those which arise during fermentation, distillation and storage processes [1, 3]. This fact could contribute not only to the valorization of the underutilized blueberry fruits by their incorporation in a production process of an alcoholic beverage, but also to the stimulation of the cultivation and harvesting of this berry. However, to our knowledge, studies dealing with the inoculated solid-state fermentation of blueberry to produce distilled alcoholic beverages are lacking.

Thus, the main goal of this work was to evaluate the potential of blueberry to be used as a substrate for producing, in a reproducible way, a novel distilled alcoholic beverage of good quality, by solid-state fermentation of the fruit with *Saccharomyces cerevisiae* IFI83 and the subsequent distillation of the fermented pulp.

2 Materials and methods

2.1 Yeast strain and fermentation substrate

Saccharomyces cerevisiae IFI83, a high ethanol-producing strain, was obtained from the yeast collection of the Institute for Industrial Fermentations (IFI), Spanish National Research Council (CSIC), Madrid, Spain. The pulp from blueberry fruit (*Vaccinium corymbosum* L., var. Bluecrop) used as a fermentation substrate contained the following mean composition (g 100 g⁻¹ fresh pulp): glucose, 9.49 ± 0.55; total protein, 0.98 ± 0.08; moisture content, 80 ± 0.88; ashes, 0.29 ± 0.02; pH, 3.27 ± 0.02.

Fermentations were conducted in 150-mL Erlenmeyer previously sterilized flasks, containing exactly 50 g crushed fruit, which were supplemented with 0.5 mL of a sterile mixture of salts composed of NH₄Cl and KH₂PO₄ to get nitrogen and phosphorus supplements of 200 and 136 µg g⁻¹ fresh pulp, respectively [1]. After inoculation with 0.4 mL of a cell suspension of *Saccharomyces cerevisiae* IFI83 adjusted previously to give an initial concentration of 5 × 10⁵ cells g⁻¹ pulp, the flasks were mixed thoroughly, covered with cotton plugs and incubated under static conditions at 18 °C. Samples as whole flasks in triplicate were withdrawn at regular intervals for analytical determinations. In each case, the fermented pulp was mixed with 100 mL distilled water in an Ultraturrax and then centrifuged (15,000 × g for 5 min). The concentrations (on a wet basis) of glucose and fermentation products (ethanol and glycerol) in the supernatants were measured by HPLC and expressed as g 100 g⁻¹ fruit pulp. All of the analyses were done in triplicate and the results were expressed as means with their respective standard deviations [1].

2.2 Distillation and aromatic compound determination

Fermented pulp (in triplicate) was distilled using a steam drag distillation system equipped with a distilling flask fixed to

a rectifying column, which allowed the fractional distillation and concentration of volatile compounds on the basis of their volatility.

The first volume of distillate (0.2 L) obtained in the first phase of the distillation process at temperatures between 70–85 °C, was removed as “head”. The intermediate fraction called the “heart” (2.2 L) obtained in the temperature range of 85–95 °C was used for volatile compounds determination. The last volume of distillate (1.0 L) obtained at 95–99 °C was removed as the “tail” [1].

Volatile compounds present in the distilled “heart” fractions of blueberry (BLBD) were determined in triplicate by gas chromatography on an Agilent 6890 (Agilent Technologies, Waldbronn, Germany) equipped with a split/splitless injector, electronic flow control (EFC) and a flame ionization detector (FID) as described previously [4]. The ethanol concentration in the distillates was expressed as mL 100 mL⁻¹ distillate, and the concentration of the other volatile compounds was expressed as g hL⁻¹ absolute alcohol (aa).

Chromatographic analysis of each heart fraction was performed in triplicate (runs). After averaging the distilled samples (three) and run (three) replicates, the experimental results were presented as means ± standard deviations.

2.3 Statistical analysis

Hierarchical Cluster Analysis (HCA) was used to compare the volatile composition of the blueberry distillate with those of twenty alcoholic beverages including: i) four Bacanora distillates obtained from four regions of the Sonora state in north-western México: the Central and Southern Region (BacCS), the Sonora River (BacSR), the High Sierra (BacHS) and the Sierra Baja (BacSB) [5]; ii) four commercial Galician orujo spirits: Albariño, Mencia, Godello and Treixadura [6]; iii) two Greek distillates: Mouro [7] and Koumaro [2]; iv) two Portuguese spirits: bagaceiras [8] and a whey distillate [3]; v) a Serbian home-made spirit beverage (Drenja) from cornelian cherry fruits [9] and, vi) seven distilled alcoholic beverages obtained from Thai rice collected from Roi-et (ThaiRoi), Yasothorn (ThaiYas), Nakhonsawan (ThaiNak), Lopburi (ThaiLop), Supanburi (ThaiSup), Chiangmai (ThaiChiang) and Lumpang (ThaiLump) provinces in Thailand [10]. The Euclidean distance was used as the dissimilarity criterion and the average linkage (in the variant of unweighted pair-group average) was used as the amalgamation (linkage) method. Both the statistical analyses and the dendrogram plot were performed by using the Hierarchical Cluster Analysis module of the software package IBM® SPSS® Statistics 20.0 for Windows (Release 20.0.1; SPSS Inc., Chicago, IL, 2011).

The data (mean concentration of each volatile compound) were standardized (z-score) before clustering, to ensure an equal contribution of each compound to the discrimination process, by using the following formula:

$$z_i = \frac{y_i - \bar{y}}{sd_y}$$

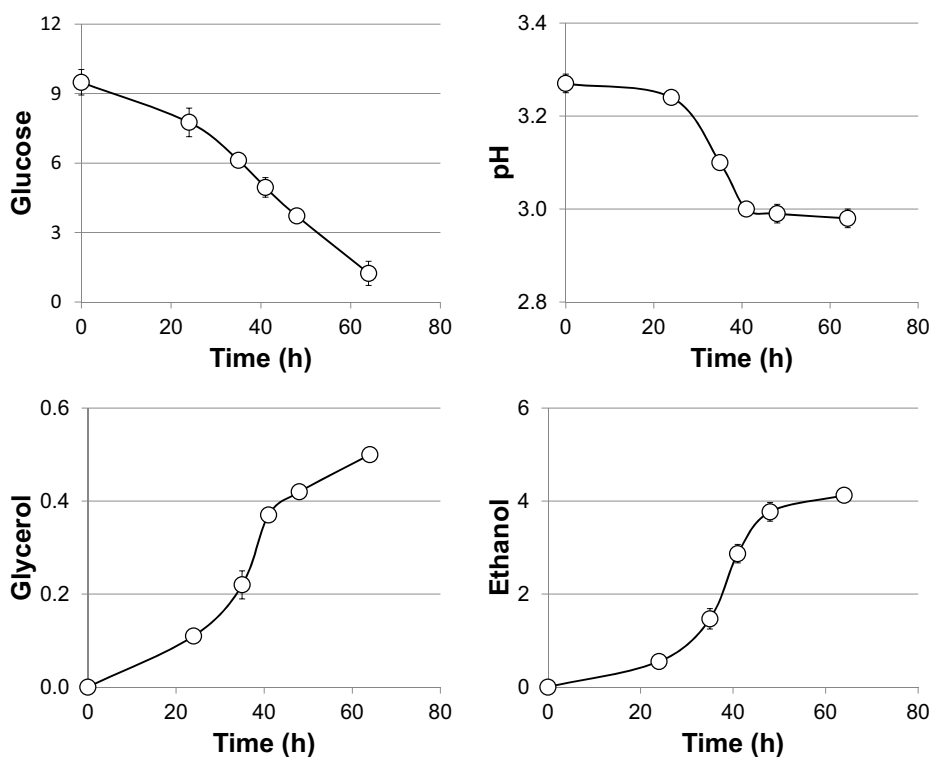


Figure 1. Kinetic of the solid-state fermentation of the blueberry pulp inoculated with *S. cerevisiae* IFI83. Time courses of the culture pH and the concentrations (in g 100 g⁻¹ fruit pulp) of ethanol, glycerol and glucose. Data reported are means \pm standard deviations (error bars) of three repeated experiments and three replicate measurements.

where z_i is the z score, y_i is the original value of each variable, \bar{y} is the mean of all values of y , and sd_y is the standard deviation of that mean.

3 Results and discussion

3.1 Solid-state fermentation of blueberry

The time courses of glucose, ethanol, glycerol and pH variation in the fermentation of blueberry (BLB) pulp with *S. cerevisiae* IFI83 are presented in *figure 1*. During the first 24 h of fermentation, the glucose concentration dropped from 9.49 to 7.76 g 100 g⁻¹ fruit pulp, whereas the ethanol and glycerol levels increased from 0 to 0.55 and 0.11 g 100 g⁻¹ fruit pulp, respectively. In this period, the culture pH only decreased from 3.27 to 3.24.

The rates of glucose consumption evolved in parallel with those of ethanol and glycerol synthesis (*figure 2*). Thus, from the 24 h to 41 h of incubation, the rates of glucose consumption and products (ethanol and glycerol) synthesis increased until reaching concentrations of 4.95, 2.87 and 0.37 g 100 g⁻¹ fruit pulp, respectively (*figure 1*). Finally, the three rates decreased from the 41 h, until the end of the culture (*figure 2*). However, the decreases in the rates of glycerol and ethanol production were more pronounced than the decrease in glucose consumption rate, probably because the carbon source was also consumed to support the growth and energy maintenance requirements of *S. cerevisiae* IFI83 [11].

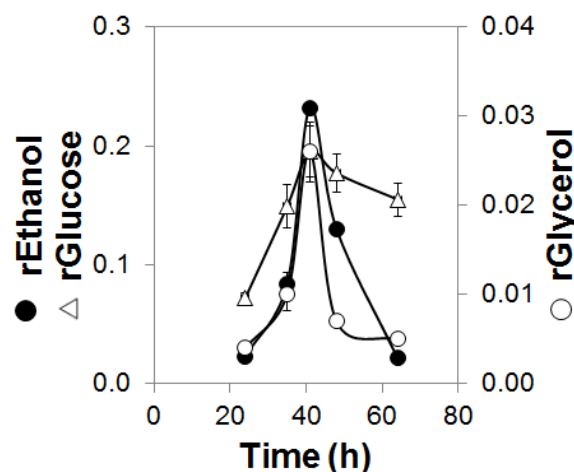


Figure 2. Absolute rates (r , in g 100 g⁻¹ h⁻¹) of glucose consumption and product (ethanol and glycerol) synthesis obtained in the solid-state fermentation of the blueberry pulp inoculated with *S. cerevisiae* IFI83. Data reported are means \pm standard deviations (error bars) of three repeated experiments and three replicate measurements.

The final concentrations of glucose, ethanol and glycerol reached after 64 h of incubation were 1.24, 4.13 and 0.50 g 100 g⁻¹ fruit pulp, respectively (*figure 1*). The ethanol yield from glucose consumed (0.50 ± 0.01 g g⁻¹) obtained in this culture corresponds to 97.8% of the theoretical ethanol yield from glucose (0.51 g g⁻¹).

Table I. Concentrations (in g hL⁻¹ absolute alcohol, except for ethanol) of volatile compounds present in the heart fractions of the three blueberry distillates (BLBD) obtained after fermented fruit pulp distillation. Values are means ± standard deviations (3 replications, *n* = 3).

no.	Analyzed compounds	Mean ± SD	Concentrations fixed by the EEC ^y
1	Ethanol (mL 100 mL ⁻¹ distillate)	45.26 ± 0.10	37.5 to 86.0
2	Methanol	261.00 ± 1.76	< 1,000
3	1-Propanol	42.87 ± 0.32	
4	1-Butanol	0.29 ± 0.02	
5	2-Methyl-1-propanol	57.66 ± 0.55	
6	2-Methyl-1-butanol	31.90 ± 0.31	
7	3-Methyl-1-butanol	150.23 ± 2.91	
	Total alcohols (3-7)	282.96 ± 2.38	
8	1-Hexanol	0.90 ± 0.01	
9	Benzyl alcohol	0.44 ± 0.02	
10	Ethyl acetate	18.10 ± 0.31	
11	Ethyl lactate	0.05 ± 0.00	
12	Acetaldehyde	14.45 ± 0.23	
13	Acetals	0.15 ± 0.03	
	Total volatile substances (3-13)	317.05 ± 2.45	> 200

^y EEC: Council Regulation No. 110/2008 of 15 January 2008 for fruit distillates.

3.2 Volatile compounds present in the blueberry distillate

The concentrations of the main volatile compounds present in the “heart” fractions of the three samples of blueberry distillates (BLBD) were compared to the limits of acceptability fixed by the European Council [12] for the contents of ethanol, methanol and volatile substances in fruit distillates (*table I*). The BLBD contain mean levels of ethanol, methanol and volatile substances that are in accordance with the legal specifications fixed by the above-mentioned European Council Regulation for fruit distillates. Although the higher alcohol contents for fruit distillates have not been fixed by the latter regulation, it is considered that a total concentration ≥ 350 g hL⁻¹ absolute alcohol in distilled beverages is indicative of a poor quality, because the beverages take a disagreeable alcoholic flavor [13]. Since the mean concentration of higher alcohols (282.96 ± 2.38 g hL⁻¹ absolute alcohol) in the BLBD samples (*table I*) was lower than 350 g hL⁻¹ absolute alcohol, it can be concluded that this distillate has a good quality.

In addition, this fruit distillate meets other quality criteria. For example, the concentrations of 1-propanol (42.87 ± 0.32 g hL⁻¹ absolute alcohol, equivalent to 194.02 ± 1.02 mg L⁻¹) and 1-butanol (0.29 ± 0.02 g hL⁻¹ absolute alcohol, equivalent to 1.33 ± 0.08 mg L⁻¹) in BLBD distillate (*table I*) were lower than the respective perception thresholds of 800 and 450 mg L⁻¹ for both compounds [6, 14]. This suggests that the fermentations of blueberry pulp and its storage before distillation were carried out in adequate conditions [3].

The mean concentrations of 2-methyl-1-propanol (equivalent to 260.93 ± 1.88 mg L⁻¹) and amyl alcohols (2-methyl-1-butanol (144.39 ± 1.71 mg L⁻¹) and 3-methyl-1-butanol (679.93 ± 14.70 mg L⁻¹) were respectively higher than the perception thresholds of 200 and 65 mg L⁻¹ reported for these compounds [14]. This indicates that the BLBD could have a pleasant flavor and a good body [2]. On the other hand, the ratios between the concentrations

of [3-methyl-1-butanol/2-methyl-1-propanol] (=2.60) and [2-methyl-1-propanol/1-propanol] (=1.34) in BLBD were higher than the unity. Therefore, it could be assumed that this distillate has a good sensory quality [3].

Since the level of 1-hexanol (4.09 ± 0.05 mg L⁻¹) was slightly higher than its perception threshold of 4 mg L⁻¹ [14], but lower than the maximum concentration (20 mg L⁻¹) at which this compound produces a positive influence on the aroma of a distillate [15], it could be considered that this alcohol could have a low positive influence on the herbaceous aroma of the BLBD.

Since the benzyl alcohol contributes to the flowery and sweet-like odors of alcoholic beverages [6], the presence of this aromatic compound in BLBD (0.44 ± 0.02 g hL⁻¹ absolute alcohol) represents a positive characteristic for this beverage.

At concentrations lower than 150 mg L⁻¹, ethyl acetate contributes to a pleasant aroma with fruity properties in the distillates. Contrarily, when its concentration exceeds 150 mg L⁻¹, ethyl acetate imparts an acidic character (vinegar smell) and adds spoilage notes to the alcoholic beverages [3, 15]. Since the mean ethyl acetate level in the BLBD samples (18.10 ± 0.31 g hL⁻¹ absolute alcohol, equivalent to 81.94 ± 1.57 mg L⁻¹) (*table I*) was lower than 150 mg L⁻¹, it could be considered that this ester was present at a suitable concentration in the distillate to confer a pleasant flavor.

Since ethyl lactate was detected at a low concentration (0.05 g hL⁻¹ absolute alcohol) in the BLBD, it could be assumed that this compound contributes to the stabilization of the distillate flavor and softens the harsh flavor characteristics [15].

At concentrations higher than 125 mg L⁻¹ in alcoholic beverages, acetaldehyde possesses a pungent irritating odor and can be hazardous to health, since this compound is considered as a source of carcinogenicity [16]. Therefore, the presence of acetaldehyde in the distillates should be diminished to avoid a safety risk to consumers, and because at low levels (< 125 mg L⁻¹) acetaldehyde confers to the alcoholic beverages pleasant characteristics, such as the aroma of

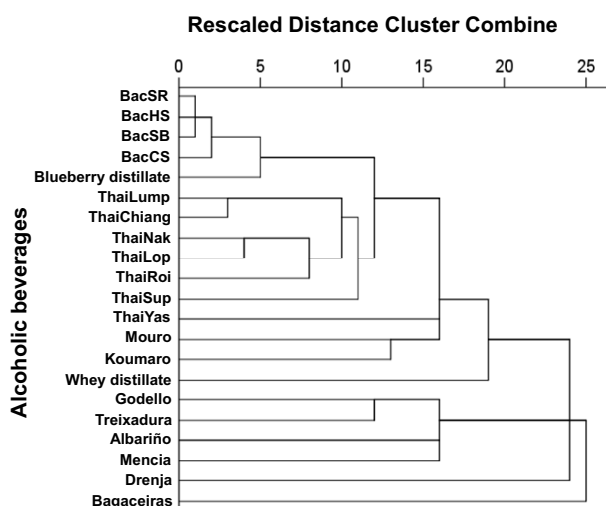


Figure 3. Dendrogram showing the cluster analysis for 21 alcoholic beverages based on their mean concentrations of volatile compounds.

walnuts, sherry and ripe apples [16]. Thus, the mean level of acetaldehyde in BLBD samples ($65.37 \pm 0.87 \text{ mg L}^{-1}$) (table I) was in the range reported to promote these pleasant characteristics.

Acetals were present in the BLBD at a low mean concentration ($0.15 \pm 0.03 \text{ g hL}^{-1}$ absolute alcohol) (table I), but its presence in this distillate is relevant due to its positive contribution to the spirit aroma at low levels [6].

3.3 Comparison of the volatile composition of the BLBD with those of other marketed and non-marketed alcoholic beverages

HCA was carried out to compare the mean volatile composition of BLBD with those of twenty alcoholic beverages obtained from different raw materials (fruits of the forest, grape marc, rice, cornelian cherry, agave and whey) and with the use of different storage, fermentation and distillation conditions (figure 3). It can be noted that the alcoholic beverages were separated into four groups. Although the distilled alcoholic beverages made with the same raw material were mainly grouped together, other factors (e.g., the origin and composition of the substrate, the type of fermentation and strain, as well as the distillation procedure) also seemed to influence the clustering process. In this way, the BLBD was joined to the first group previously formed by the four Bacarona spirits (BacSR, BacHS, BacSB and BacCS), which were obtained from spontaneous liquid fermentation of the wild agave (*Agave angustifolia* Haw) [5].

The second group was formed by six distilled spirits (ThaiLump, ThaiChiang, ThaiNak, ThaiLop, ThaiRoi and ThaiSup) obtained from Thai rice [10]. However, the ThaiYas sample, which was also obtained from Thai rice, but from Yasothorn province, was only joined to the grouping formed by the above-mentioned first two groups (figure 3).

The Greek distillates Mouro [7] and Koumaro [2], which form the third group, were obtained by using similar procedures of fermentation and distillation, but the raw materials

(*Morus nigra* L. and *Arbutus unedo* L., respectively) used in the fermentations were different [2, 7]. The four orujo spirits (Godello, Treixadura, Albariño, and Mencia), which form the fourth group, are produced from white (Albariño, Godello and Treixadura) and red (Mencia) grape varieties by using the same basic process, but the system and time of storage as well as the fermentation and distillation procedures were different [6]. Finally, it can be noted that the whey distillate, Drenja and Bagaceiras were the most different beverages (figure 3).

4 Conclusion

A novel distillate of good sensory quality that is safe for consumers was successfully produced by solid-state fermentation of blueberry with *S. cerevisiae* IFI83. This approach could contribute to the valorization of this fruit by its incorporation in a production process, thus preventing its loss in the forests of Spain and stimulating the cultivation and harvesting of this berry. The posterior commercialization of this distillate could represent an alternative to increase the farmer income in Galicia.

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