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Effect of storage conditions on the volatile composition of wines obtained from must stabilized by PEF during ageing without SO_2

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ABSTRACT

The aim of this work was to study the effect of the storage conditions on the evolution of volatile composition of white wines aged in bottles without the addition of SO_2. Therefore *Parellada* must was stabilized by pulsed electric fields (PEF) and fermented without the employment of SO_2 and later on the wine was aged in bottles, without addition of this preservative, at low and controlled temperature and at room temperature. The obtained results showed that the concentration of some important compounds for wine aroma such as isoamyl acetate, and ethyl esters of fatty acids was higher in the wines stored at low and controlled temperature than in those aged at room temperature. However, the temperature favoured the formation of total alcohols during the aging of wines in the bottles. Consequently, from the point of view of the aromatic quality, the conservation of white wines obtained from fermentation of must processed by PEF and aged in bottles without the addition of SO_2 was improved under controlled storage conditions than at room temperature.

Industrial relevance: SO_2 is used as a preservative agent in wine due to its multi-action in the wine conservation. Although neither carcinogenicity nor mutagenic effects have been found in SO_2, this compound has an influence on human health. For that reason, several competent international organizations (WHO, FAO, OIV) have set down maximum limits for wines as well as promote a reduction of its concentration in wines. Therefore, potential industrial applications of this work include the possibility to produce and store wines without SO_2. This has been achieved by stabilizing the musts with PEF. The wines produced under these conditions can be conserved without this additive when used under controlled conditions of storage.

1. Introduction

The compounds formed during alcoholic fermentation have a decisive influence on the volatile composition of wine. The major volatile products of yeast metabolism, ethanol, glycerol and carbon dioxide make a relatively small yet fundamental contribution to wine flavour. The main groups of compounds that form the *fermentation bouquet* are esters, alcohols and acids and, to a lesser extent, aldehydes (Lambrechts & Pretorius, 2000). After the alcoholic fermentation the white wine is bottled for its commercialisation and later consumption. There is a tendency among an important sector of the market to demand white wines that are organoleptically characterized for their noticeable fruity and fresh aroma, pale colour and elegant acid taste. Therefore, the evolution of the product in the bottle before its consumption is very important. The wines in the sale places remain at room temperature and once acquired by the consumer they stay, generally, at room temperature too. For this reason, it is important to study how a bottle of wine evolves at room temperature and compare it with the evolution at low and controlled temperature.

Pulsed electric field (PEF) technology has been used to preserve fruit juice and to delay the spoilage by microorganisms (Elez-Martínez, Escolà-Hernández, Soliva-Fortuny, & Martín-Belloso, 2004, 2005; Sen Gupta, Masterson, & Magee, 2005). Recently, this technology has been implemented for the production of commercial fruit juices in the USA whereas some important food processors of the EU are trying this technology at a pilot plant level. A previous study showed that, when grape must is treated by pulsed electric fields (PEF), the sulphur dioxide concentration could be reduced to safer levels or even eliminated without an important effect on the volatile compound content of the final product (Garde-Cerdán, Marsellés-Fontanet, Arias-Gil, Ancín-Azpilicueta, & Martín-Belloso, 2008). It has also been reported that PEF treatments also decrease the activity of enzymes such as peroxidases and polyphenoloxidases in grape juice (Marsellés-Fontanet, & Martín-Belloso, 2007), in apple and pear

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extracts (Giner, Gimeno, Barbosa-Cánovas, & Martín, 2001), peach purée (Giner, Ortega, Mesegué, Gimeno, Barbosa-Cánovas, & Martín, 2002) and orange juice (Elez-Martínez, Aguiló-Aguayo, & Martín-Belloso, 2006). The proposed mechanism of enzymatic inactivation might be related with the change of specific structures of the enzymes (Zhong et al., 2007).

Sulphur dioxide is widely used in food industry since it is a good antioxidant agent and an excellent antimicrobial compound. It is known that it has an influence on human health although neither carcinogenicity nor mutagenic effects have been found (Romano & Suzzi, 1993). Nevertheless it would be important to reduce the dose of SO_2 in wine to avoid an accumulative effect due to the fact that it is a very common additive in many food products being also present in the atmosphere as a consequence of the industrial activity. For that reason, several competent international organizations (WHO, FAO, OIV) have set down maximum limits for wines as well as promote a reduction of its concentration in foodstuffs, specifically in wines.

Until now, we have not found any study with a relationship between wine aged without SO_2 in bottles with different storage conditions and its volatile composition. Consequently, the aim of this work was to study and compare the evolution of the volatile composition of white wines aged in bottles at room temperature and at low and controlled temperature, without the addition of sulphur dioxide. To do it, must of the variety *Parellada* was stabilized by pulsed electric fields (PEF) and inoculated with a *Saccharomyces cerevisiae* yeast strain and it was fermented without SO_2. Afterwards the obtained wine was aged at room temperature (weighted average temperature, 23 °C) and at low and controlled temperature of 5 °C during 6 months without the employment of SO_2.

2. Materials and methods

2.1. Samples and vinification

The grape variety used was *Vitis vinifera* var. *Parellada*. The grape was de-stemmed, crushed, pressed and finally filtered. The must was processed by pulsed electric fields (PEF), as described by Garde-Cerdán, Arias-Gil, Marsellés-Fontanet, Ancín-Azpilicueta, and Martín-Belloso (2007). A laboratory scale PEF unit (Ohio State University, Columbus, OH, USA) was used to treat the must. The pulse generator module consists of a high voltage generator (OSU-4F), which can supply differences of electric potential between the electrodes of the treatment chambers of up to 12 kV, and a pulse generator unit model 9412A (Quantum Composers, Inc., Bozeman, MT, USA), which can render square wave pulses of up to 10 µs and 2000 Hz. PEF treatment was performed with bipolar electric field pulses of 4 µs width and with an electric field strength of 35 kV/cm. The pulse repetition rate was 1000 Hz and the total PEF treatment time was 1 ms, which was calculated as the product of the pulse width and the number of pulses delivered to must.

The fermentations were made in duplicate with two aliquots of PEF processed grape juice (3 l). Each one was inoculated with the active dry *S. cerevisiae* subsp. *cerevisiae* (Na33 strain). This yeast was selected by the Estación de Viticultura y Enología de Navarra (Olite, Spain) and commercialised by Lallemand España. The Na33 strain was inoculated in the musts in a proportion of 0.2 g/l. For this, 0.65 g of dry yeast was rehydrated in a sterile flask in 7.5 ml of distilled water with 0.07 g of sucrose (number of viable cells/ml $\geq 2\times10^9$); it was kept in this medium for 30 min at 35 °C. The musts were inoculated while being mixed to obtain a homogeneous distribution.

The fermentations took place in spheric glass fermentors of 3.5 l with two outlets, one for sample extractions capped with a plastic septum and the other with a CO_2 trap to eliminate carbon dioxide. The fermentors were placed over magnetic stirrers (Ikamag RCT basic, Milian SA, Geneve, Switzerland) to ensure a homogeneous fermentation. The fermentations were carried out in a hot–cold incubator (Selecta, Barcelona, Spain) at a controlled temperature of 18 °C. The fermentations were measured daily for sugar concentration through a refraction index at 20 °C, using a refractometer ABBE model 325 (Misco, Cleveland, USA) and through an enzymatic measure (reactives from Chema Italia, Rome, Italy) using a multi-parametric analyser Enochem (Tecnología Difusión Ibérica, Barcelona, Spain). All recipients and materials, which were in contact with the samples, were previously sterilized. The wine was stabilized and clarified by decantation during 48 h at −4 °C. The obtained wine was stored for 6 months in 500 ml bottles, which were sealed with corks, in a dark place at room temperature (highest temperature, 27 °C; lowest temperature, 15 °C; weighted average temperature, 23 °C) and at controlled temperature of 5 °C in a hot–cold incubator (Selecta).

Sampling was done at the end of the fermentation when the reducing sugar content was below 2.5 g/l, after 3 and at 6 months of ageing. Two samples were taken from different fermentors and bottles.

2.2. Standard wine making process

Several bottles of wine were elaborated following a standard process. It was performed using the same grape variety and fermenting conditions although as the grape juice was not PEF treated the fermentation process begun spontaneously by the native yeast species. As in the standard wine making method it was added 50 mg/l of sulphur dioxide to grape juice to avoid the spoilage before fermentation. Bottle ageing was carried out in the same place that the PEF processed the samples. The sampling procedure was also the same.

2.3. Enological parameters

The reducing sugar and volatile acidity analyses were made in a multi-parametric analyser Enochem by enzymatic methods. The pH was determined by using a pH-meter Metrohm 702 (Metrohm, Herisau, Germany). The total acidity was determined by the method described by the Office International de la Vigne et du Vin (1990). The alcoholic level of the final wine was determined by using a Salleron-Dujardin ebulliometer (Paris, France). The analytical measure and the fermentation process were performed twice. Therefore, the values shown in Table 1 are the average of 4 analyses.

2.4. Analysis of volatile compounds by gas chromatography

The analysis was carried out using two different methods because of the great difference of volatilities of the studied compounds and the wide interval of concentrations (Fraile, Garrido, & Ancín, 2000). The method outlined by Fraile et al. (2000) was used to analyse the compounds of high volatility and high concentration such as ethyl acetate, *n*-propanol, isobutanol, and isoamyl alcohols. In this method the sample of 0.5 µl is injected (split ratio 1:50) into a gas chromatograph device Shimadzu GC-14B (Shimadzu, Kyoto, Japan) and the separated analytes quantified with a flame ionisation detector

Table 1
Enological parameters of *Parellada* wine obtained from must stabilized by PEF and from standard fermentation

	PEF wine	Standard wine	
Alcohol level	8.4±0.3	8.5±0.2	% (v/v)
pH	3.23±0.01	3.56±0.03	–
Total acidity[a]	4.32±0.08	3.32±0.04	g/l
Volatile acidity[b]	–	0.50±0.01	g/l
Reducing sugar	1.52±0.02	1.66±0.01	g/l

All parameters are given with their standard deviation (n=4).
[a] Expressed as tartaric acid.
[b] Expressed as acetic acid.