BIO Web of Conferences **7**, 02013 (2016) DOI: 10.1051/bioconf/20160702013
39th World Congress of Vine and Wine

The Ultraviolet radiation (UV-C) for the microbiological stabilization of red wine

Fábio Matias[1], Ana F. Pinto[1], Isabel Torgal[1], Marco Alves[2], Joana Grácio[2], and Helena Mira[1]

[1] Departament of Food Technology, Biotechnology and Nutrition, Instituto Politécnico de Santarém, Escola Superior Agrária, 2001-904 Santarém, Portugal
[2] INOV'LINEA – Centro de Transferência de Tecnologia Alimentar – TAGUSVALLEY – Abrantes, Portugal

Abstract. The traditional procedure for the control of the microbiological stability of wine consists of the addition of sulfur dioxide (SO_2), which acts as an antimicrobial agent and also as an antioxidant. The search for alternative methods of microbiological control is important and necessary, since SO_2 is a potential allergen and consumers are increasingly looking for healthier and preservative free products. Ultraviolet radiation was tested as an innovative technology that can help reduce the amount of sulphur dioxide used in winemaking. The object of this study was to optimize the process conditions compared to the results obtained previously, and to evaluate the efficiency of microbiological stabilization and its influence on the physico-chemical characteristics, the phenolic composition and sensory profile. Thus, red wine with very low content of sulphur dioxide was subjected to UV-C radiation in two different doses 424J/l e 778J/l, and the preparation of a control wine was carried out to which 30 mg/l sulfur dioxide was added. The wines (control=UV0, UV1 and UV2) were analyzed over time (from 0 to 4 months). The results show that treatment with a lower dosage is effective in the microbiological control of the product. The wines subjected to treatment with UV-C showed an increase in intensity of colour, and the treatment does not affect the flavour and taste of the wine.

Resumo: O procedimento tradicional de controlo da estabilidade microbiológica no vinho consiste na adição de dióxido de enxofre (SO_2), que atua como agente antimicrobiano e também antioxidante. A procura de métodos alternativos de controlo microbiológico é importante e necessária, dado que o dióxido de enxofre é um potencial alérgeno e os consumidores procuram cada vez mais produtos saudáveis e livres de conservantes. A radiação ultravioleta tem sido estudada como uma tecnologia inovadora que pode auxiliar na redução do teor de dioxido de enxofre em enologia. O objectivo deste trabalho foi optimizar as condições do processo, face aos resultados já obtidos anteriormente, e avaliar a eficiência na estabilização microbiologica, e a sua influência nos parâmetros fisico-quimicos, na composição fenólica, e nas caracteristicas sensoriais. Assim, o vinho tinto com teor muito baixo em SO_2 foi submetido a radiação UV-C com duas doses diferentes, 424J/L e 778J/L, e procedeu-se ainda à preparação de um controlo, a que foi adicionado 30 mg/L de dióxido de enxofre. Os vinhos (UV0, UV1 e UV2) foram analisados ao longo do tempo (de 0 a 4 meses). Os resultados mostram que o tratamento com menor dose é eficaz no controlo microbiológico do produto. A análise sensorial mostrou que o tratamento com radiação UV-C não afectou o aroma e o sabor dos vinhos, inclusivamente estes vinhos foram mais pontuados no descritor intensidade da cor.

1. Introduction

The microorganisms play a magisterial role in wine production, where *Saccharomyces cerevisae* and *Oenococcus oeni* play an important role in alcoholic and malolactic fermentation respectively. However, certain species of yeasts and bacteria can cause spoilage defects, with decrease of the quality of the wine [1–3]. The spoilage defects are usually recognized by haze formation, production of CO_2, increase in acetic acid or volatile acidity, off-flavours as volatile phenols and volatile sulphur, and viscosity of wine [4]. The addition of sulphur dioxide (SO_2) during different steps of the production process is a well-established practice in winemaking, due to its multiple and diverse properties [5]. The SO_2 acts as microbiological control agent in musts and wines and as enzyme inhibitor to prevent must browning and wine oxidation [6,7]. Although this addition is considered to be almost inevitable in the production of wine, it is also known that an excess SO_2 intake can cause health problems in sensitive people, particularly headaches, allergies, gastric irritation, nausea, and difficulties in breathing in asthma patients [8–10]. In this point of view, the International Organization of Vine and Wine (OIV) has been progressively reducing the maximum concentration authorized in wines, which is 150 mg/l for red wines and 200 mg/l for white wines nowadays (Regulation (EC) No 606/2009). In the same way, since November 25th 2005, it is mandatory to put on the label the phrase "containing sulphites" when the concentration of SO_2 exceeds 10 mg/l (Directive 2003/89/EC). In addition to the legislative norms, consumers are becoming much

more health-conscious and, as a result, prefer healthy products free of chemical preservatives or additives [11–13]. Thus, researchers and the wine industry are looking for innovative methods that can reduce or even eliminate the use of SO_2 as preservative without significantly changing the quality attributes of wine. With this objective in mind several emerging technologies have been explored including pulsed electric fields [14], ultrahigh pressure [6,8], ultrasound [6,15] or UV irradiation [4,8,12,16,17] and natural products, including bacteriocins [6,8,18], lysozyme [6,8,18] chitosan [8,20, 21] and glutation [22,23].

The application of ultraviolet C light (UV-C, 200–280 nm) was successfully used to inactivate microorganisms in water and various types of liquid foods and beverages, such as fruit juices, soft drinks, beer and wine [4,12,16,17,24–31].

The microbial inactivation is due to the rearrangement of the nucleic acid bonds, which block DNA transcription and replication, and eventually cause cell death [4,32]. The UV-C radiation is efficient in reducing yeasts, lactic acid bacteria and acetic acid bacteria in grape juice and wine [4,12,33]. However, UV-C sensitivity also differs among microorganisms, species, strains and growth stage of the culture [4,25]. UV-C efficacy was largely affected by dosage, turbidity and colour of the liquid product, and initial microbial load [4,25].

The aim of this study was to optimize the process conditions, to compare to the results obtained previously [34], and evaluate the efficiency of microbiological stabilization, and its influence on the physical-chemical characteristics, the phenolic composition and sensory profile.

2. Materials and methods

2.1. Red Wine

The red wine of 2015 was produced in the winery of the Escola Superior Agrária de Santarém in the Tejo region, a Portuguese wine region. The wine is a blend of Tinta Roriz, Syrah and Alicante Bouschet. The chemical composition is as follow: 13.5% (v/v) alcohol, pH 3.78; 4.33 $g.l^{-1}$ total acidity, 0.65 $g.l^{-1}$ volatile acidity, 3 $mg.l^{-1}$ free molecular SO_2 and 12 $mg.l^{-1}$ total SO_2 (the wine has low content of sulphur dioxide).

2.2. Application of UV-C to wine

The wine was treated at the Tagusvalley-InovLinea technology center, using a pilot-scale UV-C reactor **UV-Therm** developed and patented by Ypsicon. The reactor is constituted of 4 UV-C germicidal lamp that emit light at the specific wavelength of 254 nm, comprised within the range UV-C (200–280 nm). The equipment allows flows of up to 100 $l.h^{-1}$

The applied dosages in a single pass of the wine through the system was 424 $J.l^{-1}$ (UV1) and 778 $J.l^{-1}$ (UV2). Sulphur dioxide was added to the wine to reach about 30 $mg.l^{-1}$ free molecular SO_2, this wine was not exposed to the UV-C light, which served as the control (UV0). After the treatment, the wines were bottled, and the evolution of the samples was monitored over time (0, 1, 2, 3, 4 months). Each trial was conducted in duplicate ($n = 3 \times 2$).

2.3. Chemical and polyphenol analysis

The concentration of alcohol (%v/v), pH, total acidity (expressed in g/l of tartaric acid), volatile acidity (g/l of acetic acid) were determined by the official methods OIV [35], free and total molecular SO_2 (mg/l) were determined by an adapted Paul method.

The total content of the phenolic compounds was measured by the absorbance at 280 nm, total anthocyanins were evaluated according to the method of of Ribéreau-Gayon and Stonestreet [36]. Total tanins were analysed as described by Ribéreau-Gayon *et al.* [36]. A Perkin Elmer – LAMBDA 25 UV/Vis spectrophotometer was used.

2.4. Microbiology analysis

Washed and sterilized bottles were used to bottle the wine.

For the microbiology analysis a membrane filtration method was used with sterile nitrocellulose filters of 0.45 μm in a Millipore ramp.

Exactly 50 mL of each sample (non diluted and diluted 1/10) was filtered through the membrane and then aseptically transferred onto Plate Count Agar (Biokar) for the enumeration of the total aerobic mesophilic microorganisms and Rose Bengal Calf Medium (Liofilchem) for the enumeration of Yeast and Moulds, and placed in a 30°C incubator for three days and in a 25°C incubator for five to seven days respectively.

All microbiological analysis was executed in duplicate from two different bottles. In the first month an additional sample without any treatment was analysed.

2.5. Sensory analysis

The sensory analysis was performed by a panel of six expert panellists, members of the 'Comissão Vitivinícola Regional of Tejo (CVRTejo)', trained wine tasters that have previous experience. The attributes of the wine, corresponding to the visual appearance, aroma and taste senses, as well as the harmony (overall judgment) were evaluated by the tasters.

2.6. Statistical analysis

The data was analysed by Analysis of Variance using SPSS 21.0 for Windows. The significance of the results was evaluated using Tukey test. Differences were considered significant for $p < 0.05$.

3. Results and discussion

The results of the microbiology analysis are presented in Table 1. Relatively to the aerobic mesophilic microorganisms (30°C) enumeration, the initial microbial value decreased right after treatment with either the addition of SO_2 or after the addition of UV-C radiation. This decrease was constant with time for the wines treated with UV-C and was more intense with the higher dose of UV-C where no microbial growth was detected from the 3rd month on.

With regard to the enumeration of yeast and molds, a decrease in the microbial number was detected after the treatments were applied and with time, where no microbial growth was detected from the 3rd month on in the wine treated with either the addition of SO_2 or after the addition

Table 1. Results from the microbiology analysis: enumeration of the aerobic mesophilic microorganisms (30°C) and yeast and molds.

	Aerobic mesophilic microorganisms 30°C (CFU/50 mL)			Yeast and Molds (CFU/50 mL)		
	UV0	**UV1**	**UV2**	**UV0**	**UV1**	**UV2**
Before treatment		3.95×10^2			7.90×10^2	
After	$2.10^{d} \times 10^2 \pm$	$1.75^{cd} \times 10^2 \pm$	$1.65^{cd} \times 10^2$	$3.5^{a} \pm 3.53$	$6.15^{b} \times 10 \pm$	$5.65^{b} \times 10 \pm$
treatment	1.13×10^2	4.90×10	$\pm 5.10 \times 10$		0.49×10	1.34×10
1st month	$3.00^{bc} \times 10 \pm$	$5.50^{bc} \times 10 \pm$	$2.40^{bc} \times 10 \pm$	$1.00^{a} \pm 0.00$	$6.50^{a} \pm 3.54$	$4.50^{a} \pm 2.12$
	2.80×10	1.83×10	2.12×10			
2nd month	$3.30^{bc} \times 10 \pm$	$2.00^{bc} \times 10 \pm$	$1.10^{ab} \times 10 \pm$	$2.00^{a} \pm 0.00$	$2.00^{a} \pm 1.41$	$2.00^{a} \pm 1.41$
	0.78×10	0.49×10	0.14×10			
3rd month	$4.00^{a} \pm 1.41$	$1.50^{a} \pm 0.71$	nd	nd	nd	nd
4th month	$2.00^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	nd	nd	nd	nd

Enumeration values (CFU/ 50ml) ± standard deviation; nd – less than 1 CFU/50 mL. Means followed by different letters in a column or a row are significant at $p \leq 0.05$ (Turkey test).

Table 2. Mean values (± standard deviations) of the chemical analyses, after UV-C treatment.

	Alcohol content (% Vol.)	**Density ($g.l^{-3}$)**	**pH**	**Total acidity (g Tar. $ac.l^{-1}$)**	**Volatile acidity (g Acet. $ac.l^{-1}$)**	**Free SO_2 ($mg\ .l^{-1}$)**	**Total SO_2 ($mg\ .l^{-1}$)**
UV0	$13.5^{a} \pm 0.00$	$992^{a} \pm 0.00$	$3.78^{a} \pm 0.00$	$4.4^{a} \pm 0.04$	$0.65^{a} \pm 0.004$	$30^{b} \pm 0.23$	$59^{b} \pm 0.00$
UV1	$13.5^{a} \pm 0.00$	$992^{a} \pm 0.00$	$3.81^{b} \pm 0.00$	$4.3^{a} \pm 0.09$	$0.63^{a} \pm 0.083$	$3^{a} \pm 0.45$	$12^{a} \pm 0.00$
UV2	$13.5^{a} \pm 0.00$	$992^{a} \pm 0.00$	$3.81^{a} \pm 0.00$	$4.3^{a} \pm 0.05$	$0.65^{a} \pm 0.002$	$3^{a} \pm 0.23$	$12^{a} \pm 0.23$

Means followed by different letters in a column are significant at $p \leq 0.05$ (Turkey test).

Table 3. Mean values (± standard deviations) of the chemical analyses over time.

	pH			**volatile acidity (g Acet · ac · l^{-1})**			**free SO_2 ($mg \cdot l^{-1}$)**			**total SO_2 ($mg \cdot l^{-1}$)**		
	UV0	**UV1**	**UV2**	**UV0**	**UV1**	**UV2**	**UV0**	**UV1**	**UV2**	**UV0**	**UV1**	**UV2**
1st month	$3.71^{a} \pm 0.000$	$3.74^{ab} \pm 0.007$	$3.73^{ab} \pm 0.007$	$0.71^{a} \pm 0.006$	$0.69^{a} \pm 0.027$	$0.70^{a} \pm 0.004$	$24^{b} \pm 0.68$	$2^{a} \pm 0.42$	$2^{a} \pm 0.23$	$53^{b} \pm 2.26$	$10^{a} \pm 0.45$	$11^{a} \pm 0.68$
2nd month	$3.71^{a} \pm 0.014$	$3.74^{ab} \pm 0.007$	$3.73^{ab} \pm 0.007$	$0.67^{a} \pm 0.017$	$0.69^{a} \pm 0.002$	$0.69^{a} \pm 0.013$	$24^{b} \pm 0.45$	$2^{a} \pm 0.23$	$2^{a} \pm 0.23$	$49^{b} \pm 1.58$	$12^{a} \pm 0.00$	$11^{a} \pm 0.68$
3rd month	$3.72^{a} \pm 0.000$	$3.76^{b} \pm 0.007$	$3.76^{b} \pm 0.007$	$0.69^{a} \pm 0.019$	$0.70^{a} \pm 0.015$	$0.69^{a} \pm 0.006$	$24^{b} \pm 0.67$	$2^{a} \pm 0.00$	$2^{a} \pm 0.00$	$48^{b} \pm 0.68$	$10^{a} \pm 0.00$	$11^{a} \pm 0.22$
4th month	$3.71^{a} \pm 0.007$	$3.75^{b} \pm 0.000$	$3.7^{ab} \pm 0.014$	$0.71^{a} \pm 0.003$	$0.71^{a} \pm 0.016$	$0.69^{a} \pm 0.013$	$23^{b} \pm 0.73$	$2^{a} \pm 0.04$	$2^{a} \pm 0.04$	$47^{b} \pm 0.13$	$10^{a} \pm 0.04$	$10^{a} \pm 0.09$

Analysis of variance was used to compare data (dose × time). Means followed by different letters in a column or row are significant at $p \leq 0.05$ (Turkey test).

of the two doses of UV-C radiation. These results agree with other studies performed in wines and musts [4, 16, 17].

Table 2 shows the results of physico-chemical analysis after UV-C radiation treatment and addition of SO_2, indicating a light increase of pH and the total acidity decreased. These parameters, obtained over time, are present in Table 3 and practically no variations are revealed. This finding is in agreement with other authors [16] that reported UC-V treatment does not change the physical and chemical parameters such as alcohol content, density, pH, total acidity or volatile acidity.

The results for polyphenols are shown in Table 4. During the experiment in sensory evaluation, the panelists found that this technology originated some impact on colour, towards its stabilization. There is a decrease in total anthocyanins. However, this does not adversely affect the colour. This is possibly due to radiation causing copolymerization or self-association of individual anthocyanins causing a stabilization of colour; this aspect was always referred to by the tasters in the sensory evaluation, over the months.

The results of the sensory analysis (Figure 1) show that the wine, after the treatment with UV-C radiation, appears impaired when compared with the wine control (with SO_2 added). This observation indicates that treatment may momentarily contribute to a reduction in the quality of the wine, as with other technologies such as filtration. But, over time, the wines subjected to ultraviolet radiation

Table 4. Results of the analysis to wine polyphenols.

	Total phenols index			Total anthocyanins (mg.l^{-1})			Total tanins (g.l^{-1})		
	UV0	**UV1**	**UV2**	**UV0**	**UV1**	**UV2**	**UV0**	**UV1**	**UV2**
After Treatment	$57.4^{a} \pm 0.42$	$56.3^{a} \pm 0.28$	$56.7^{a} \pm 0.07$	$348.3^{de} \pm 13.84$	$323.2^{bcd} \pm 5.19$	$310.7^{bc} \pm 1.30$	$3.13^{ab} \pm 0.076$	$2.97^{a} \pm 0.030$	$3.10^{ab} \pm 0.076$
1st month	$56.1^{a} \pm 0.85$	$54.4^{a} \pm 0.64$	$54.5^{a} \pm 1.34$	$369.1^{e} \pm 3.46$	$329.6^{bcd} \pm 2.16$	$329.0^{bcd} \pm 6.49$	$3.18^{b} \pm 0.004$	$3.18^{b} \pm 0.031$	$3.12^{ab} \pm 0.061$
2nd month	$57.9^{a} \pm 1.83$	$56.0^{a} \pm 1.06$	$57.2^{a} \pm 0.92$	$338.2^{bcd} \pm 15.15$	$316.5^{bc} \pm 12.11$	$306.4^{bc} \pm 1.30$	$3.26^{bc} \pm 0.075$	$3.23^{bc} \pm 0.017$	$3.20^{bc} \pm 0.006$
3rd month	$58.6^{a} \pm 1.84$	$57.1^{a} \pm 0.42$	$58.6^{a} \pm 0.92$	$325.9^{bcd} \pm 11.69$	$306.4^{bc} \pm 8.22$	$300.0^{b} \pm 1.73$	$3.19^{b} \pm 0.009$	$3.23^{bc} \pm 0.007$	$3.22^{bc} \pm 0.003$
4th month	$55.3^{a} \pm 2.40$	$56.7^{a} \pm 1.20$	$54.1^{a} \pm 0.42$	$295.7^{b} \pm 10.38$	$245.5^{a} \pm 1.73$	$246.6^{a} \pm 13.84$	$3.41^{c} \pm 0.104$	$3.28^{bc} \pm 0.059$	$3.30^{bc} \pm 0.070$

Analysis of variance was used to compare data (dose x time). Means followed by different letters in a column or row are significant at $p \leq 0.05$ (Turkey test).

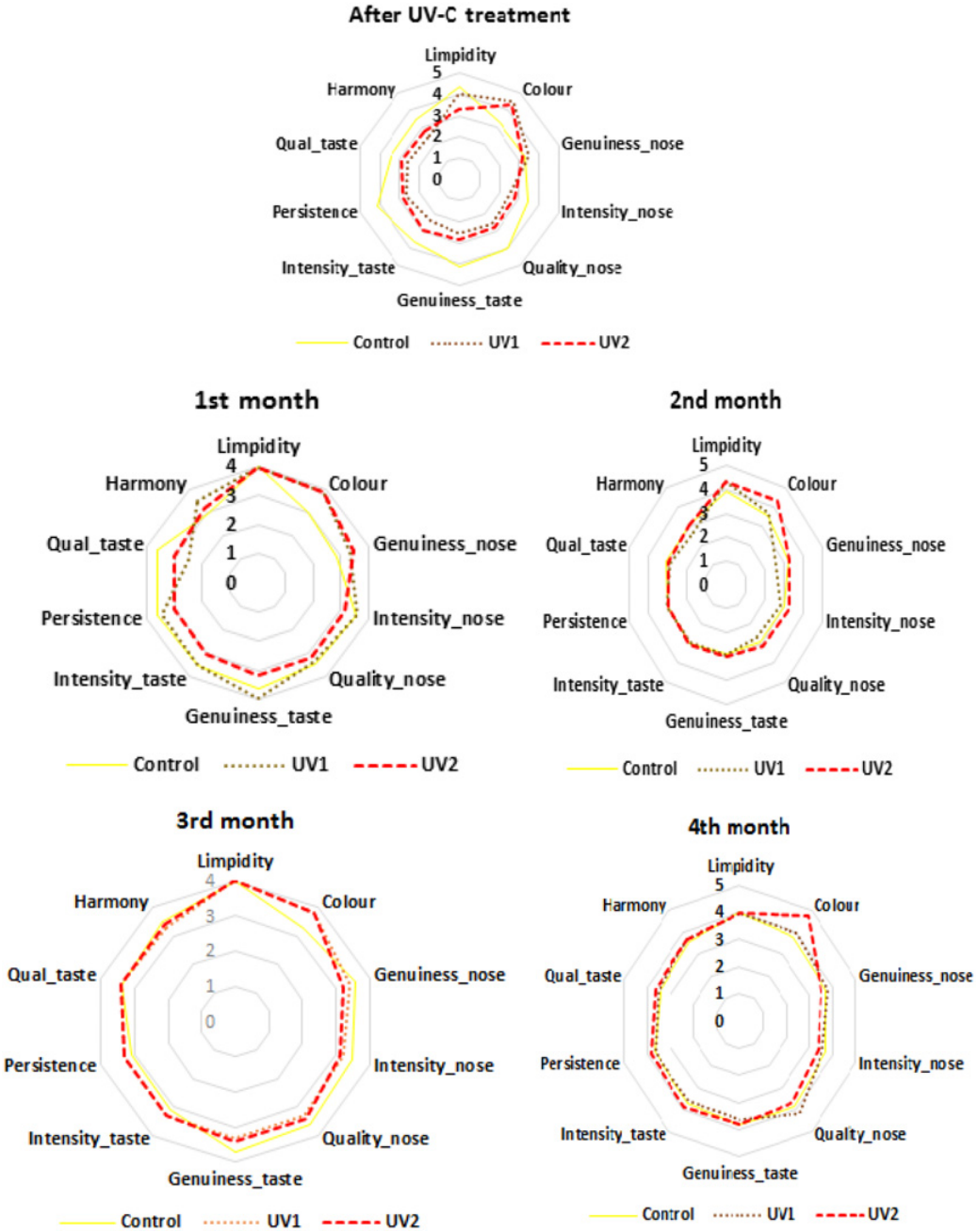

Figure 1. Comparison of the results of the sensory analysis over time.

had similar sensory characteristics to the wine control, indicating that treatment does not affect the flavour and taste of the wine. These results proved promising compared with our previous results where higher doses were used [34]. The colour is a characteristic of the wine which will benefit from this treatment, suggesting that the treatment promotes UV colour stabilization.

4. Conclusions

At the end of 4 months, the results show that the UV-C technology is effective in the microbiological control for wine, for the doses used. This technology did not affect the physico-chemical parameters or the content of polyphenols. The aroma and flavour of the wine was not affected, and colour stabilization was promoted.

The use of the recommended doses was shown to be effective in the microbiological control of the wine.

Future work will entail following up of this wine for a year. The application of this treatment to white wine is also intended.

We are grateful to the tasters for their availability and commitment, namely Carmen Santos (Wtejo), Carlos Eduardo (Enoport), João Sardinha (Adega Cooperativa de Alcanhões), Maria Vicente (Casa 1927), Martta Simões (Quinta da Alorna) and Pedro Gil (Adega Cooperativa do Cartaxo).

References

[1] M. du Toit, I.S. Pretorius. S. Af. J. Enol. Vitic., **21**, 74–96 (2000)
[2] M. Malfeito-Ferreira. Ann Microbiol **61**, 95–102 (2010)
[3] E.J. Bartowsky, P.A. Henschke, Int. J. Food Microbiol. **125**, 60–70 (2008)
[4] I.N. Fredericks, M. du Toit, M. Krügel, Food Microbiology, **28**, 510–517 (2011)
[5] P. Ribereau-Gayon, D. Dubourdie, B. Doneche, A. Lonvaud, *Handbook of enology* (Vol. I). Chichester, West Sussex, England: John Wiley & Sons, Ltd. (2006)
[6] E.J. Bartowsky, Letters in Applied Microbiology, **48**, 149–156 (2009)
[7] C.M. Oliveira, A.C.S Ferreira, V. De Freitas, A.M.S. Silva, Food Research International, **44**, 1115–1126 (2011)
[8] M.C. Santos, C. Nunes, J.A. Saraiva, M.A. Coimbra, European Food Research and Technology, **234**, 1–12 (2012)
[9] H. Vally, N.L.A. Misso, V. Madan, Clin Exp Allergy **39**, 1643–1651 (2009)
[10] H. Vally, P.J. Thompson PJ, Addict Biol **8**, 3–11 (2003)
[11] T. Bjørndal, J. Fernandez-Polanco, A. Lappo, A. Lem, *Consumer trends and prefences in demand for food.* BERGEN: Centre for Applied Research at NHH. (2014)
[12] L. Rizzotti, N. Levav, F. Fracchetti, G.E. Felis, S. Torriani, Food Control, **47**, 407–412 (2015)
[13] M. Costanigro, C. Appleby, S.D. Menke. Food Quality and Preferences, **31**, 81–89 (2014)
[14] T. Garde-Cerdan, A.R. Marselles-Fontanet, M. Arias-Gil, C. Ancin-Azpilicueta, O. Martin-Belloso. Eur Food Res Technol **227**, 401–408 (2008)
[15] P. Piyasena, E. Mohareb, R.C. McKellar. Int J Food Microbiol **87**, 207–216 (2003)
[16] V. Falguera, M. Forns, A. Inarz. LWT-Food Science and Technology **51**, 59–64 (2013)
[17] M. Lorenzini, F. Fracchetti, V. Bolla, E. Stefanelli, F. Rossi, S. Torriani. *International Organization of vine and Wine – 33rd World Congress of Vine and Wine, 8th General Assembly of the OIV* (2010)
[18] H.A. Nel, R. Bauer, G.M. Wolfaardt, L.M.T. Dicks. Am J Enol Vitic 53, 191–196 (2002)
[19] F. Sonni, M.J. Cejudo-Bastante, F. Chinnici, N. Natali, C. Riponi. J. Science Food Agriculture, **89**, 688–695 (2009)
[20] D. Ferreira, D. Moreira, E.M. Costa, S. Silva, M.M. Pintado, J.A. Couto. J. Chitin and Chitosan Science, **1**, 240–245
[21] F. Chinnici; N. Natali, C. Riponi, J. Agric. Food Chem. **62**, 9868–9875 (2014)
[22] L. Medina, O. Pascual, F. Fort, Canals, J.M.F. Zamora, *In Enologia 2.015. Innovation en vitivinícola*,430–343 (2015)
[23] P. Comuzzo, F. Battistutta, M. Vendrame, M.S. Páez, G. Luisi, R. Zironi. Food Chemistry, **168**, 107–114 (2015)
[24] T.K. Gachovska, S. Kumar, H. Thippareddi, J. Subbiah, F. Williams, Journal of Food Science, **73,** M412–M417 (2008)
[25] T. Koutchma, Food and Bioprocess Technology, **2**, 138–155 (2009)
[26] C.M.A.P. Franz, I. Specht, G. Cho, V. Graef, M.R. Stahl, Food Control, **20**, 1103–1107 (2009)
[27] E. Gayán, M.J. Serrano, S. Monfort, I. Álvarez, S. Condón, Food Bioprocess Technology, **6**, 3006–3016 (2012)
[28] E. Gayán, M.J. Serrano, S. Monfort, I. Álvarez, S. Condón, Journal of Food Engineering, **113**, 598–605 (2012)
[29] M. Guevara, M.S. Tapia, V.M. Gómez-Lopez, Food and Bioprocess Technology, **5**, 803–807 (2012)
[30] M. Gouma, E. Gayán, J. Raso; S. Condón, I. Álvarez, Innovative Food Science & Emerging Technologies, **32**, 146–155 (2015)
[31] G. Lu, C. Li, P. Liu, H. Cui, Y. Yao, Q. Zhang, Food Control, **21**, 1312–1317 (2010)
[32] J.A. Guerrero-Beltrán, G.V. Barbosa-Canovas, **10**, 137–148 (2004)
[33] J.A. Guerrero-Beltrán, J. Welti-Chanes, G.V. Barbosa-Cánovas, J. Food Process Eng. **32**, 916–932 (2009)
[34] Alves, M., Grácio, J., Simões, M, Mira, H. (to be published)
[35] Organization International de la Vigne e du Vin. Methods of analysis of wines and must. `http://www.oiv.int/oiv/info/enmethodinternationalwsenvin.` (2014)
[36] P. Ribéreau-Gayon, E. Stonestreet, *Bull. Soc. Chim.,* **9**, 2649–2652 (1965)