COLD PLASMA AT ATMOSPHERIC PRESSURE FOR ELIMINATING *BRETTANOMYCES* FROM OAK WOOD.

Lucía González-Arenzana[1], Ana Sainz-García[2], Ana González-Marcos[2], Rodolfo Múgica-Vidal[2], Ignacio Muro-Fraguas[2], Rocío Escribano-Viana[1], Isabel López-Alfaro[1], Fernando Alba-Elías[2] and Elisa Sainz-García[2].

[1] Institute of Grapevine and Wine Science (ICVV). Finca La Grajera, Ctra. de Burgos Km.6 (Lo-20, salida 13), 26007 - Logroño, La Rioja Spain.

[2] Department of Mechanical Engineering. University of La Rioja. C/ San José de Calasanz 31, 26004 - Logroño, La Rioja, Spain

lucia.gonzalez@unirioja.es

Research presented at Enoforum Web Conference, prizewinner of Enoforum Contest 2021. Conference videos and articles are available on www.infowine.com

1. INTRODUCTION

The use of oak barrels in winemaking is a bold enological practice because wood plays a key role in wine's organoleptic attributes. Wood barrels are usually employed for wine ageing but nowadays the diversification of products by winemakers has made barrels an essential tool for developing different oenological activities such as white wine alcoholic fermentations or red wine malolactic fermentations. Among a wide diversity of wood types, oak is still the most usually employed for barrels.

During the aging in barrels, wood provides wine with different attributes that depend directly on the wood origin and toasted, on the wine quality and on the conditions that characterized this period. Moreover, not only a change in the organoleptic features happens but important oenological processes such as natural clarification, slow microoxigenation and a general stabilization of wine color also happen.

Currently, the high cost of the oak barrels make winemakers develop a "re-using strategy" (García-Alcaraz et al., 2020). Barrels have usually a live extension of 3 or 4 years and during this period they are an important tool for elaborating and blending different wines, because the initial toasted characteristics tend to get reduced over time. In other cases, the oldest barrels that are in good condition are destined to ageing of spirits.

Nevertheless, porous natural surface of wood becomes the maintenance of barrels a challenge in winemaking. Biomass, less, tartrates, etc., proceeding from natural clarification, might probably block up the pores causing a loss of transference between wood surface and wine. Sometimes, this pores are ecological niches for bacteria and yeasts that are able to survive to harsh wine conditions and even penetrating till a depth of 8 mm (Suárez, Suárez-Lepe, Morata, & Calderón, 2007).

Consequently, the microbial population that is contained in wines could eventually take shelter in wood pores. Among these microorganisms present in wines *Brettanomyces bruxellensis* is the most feared species by winemakers all over the World, in fact, it is considered the "winemakers' nightmare". *Brettanomyces* genus refers to an asexual form what means that species belonging to that genus are able to generate spores under difficult conditions while *Dekkera* is the genus that gathers species that only shows sexual forms (Agnolucci, Tirelli, Cocolin, & Toffanin, 2017). The species present in wines are *B. bruxellensis* and *D. bruxellensis* and both have a similar ecological and oenological behaviour being considered

spoilage yeasts responsible for the “Brett character”. This “Brett character” is mainly characterised by the presence of volatile phenols in wines. In effect, these yeasts synthetize volatile phenols that when reach concentrations higher than the threshold limit are perceived as disgusting for wine consumers and even in low rates they cause a loss in the fruity and varietal odour of wines (Renouf et al., 2007). Additionally, these yeasts can also synthetize biogenic amines and they have the capacity of metabolising the ethanol as the only carbon source causing a direct increment of acetic acid concentration rendering vinegar aromas (Palacios, Borinaga, & Carrillo, 2012).

Talking in general about “Brett”, these yeasts are survivors that can be found in grapes, musts, after the wine alcoholic fermentation, after the malolactic fermentation, during ageing, bottle, etc. so that its presence is not only limited to wood barrels ageing although this stage is the most risky for wine quality because, as said before, they can occupy wood pores for years. They are told to support low pH, relatively high SO_2 doses, high alcoholic contents, low nitrogen and vitamins concentrations, low dissolved oxygen rates, etc. (Renouf, Lonvaud-Funel, & Coulon, 2007).

Ineffective barrel disinfestation is a problem that affects a big amount of wineries, in fact, wines with “Brett characters” can be found in most of the winemaking areas of the World. Currently, winemakers are using chemical additives such as chitosan or sulphur dioxide to limit *B. bruxellensis* presence in wines but the most extended method is the hot water or vapor cleansing combined with the sulphur dioxide pills burning. When the combustion of the pills takes places, the sulphur dioxide that is produced has biocidal effects. Curiously, every cleansing actuation do not seem effective, because “Brett” problems persist. Furthermore, Directive 98/8/CE2 of the European Commission prohibits the use of sulphur dioxide for the sanitization of barrels and a moratorium of this directive which ends in 2022 (Palacios et al., 2012). Winemakers have not an effective method to replace sulphur burning to manage *B. bruxellensis* contaminations in oak wood barrels.

This situation has led the oenology industry to search for alternative and cutting-edge technologies that guarantee the effective, economical, viable and sustainable sanitization of oak barrels and that could be considered. Some of these emerging technologies have been, for instance, ozone, hydrogen peroxide, ultrasounds, high hydrostatic pressure, microwaves, thermal treatments, etc. (Costantini et al., 2016; González-Arenzana et al., 2013; Guzzon et al., 2017; Marko et al., 2005; Porter, Lewis, Barnes, & Williams, 2011; Renouf et al., 2007; Schmid, Grbin, Yap, & Jiranek, 2011; Yap, 2009). Needless to mention that these technologies should be careful with the wood features for facilitating a steady cession of wood physicochemical characteristics to wine organoleptic profile (Stadler, Schmarr, & Fischer, 2020).

Considering these alternative technologies, the Atmospheric Pressure Cold Plasma (APCP) technology has been considered an adequate alternative for sorting out the difficulties regarding barrel sanitization. APCP is a non-equilibrated, ionized gas generated at ambient temperature and at atmospheric pressure. During the process different energetic species such as positive and negative ions, free electrons and radicals, molecules and UV photons neutral and excited atoms have been described (Niemira, 2012; Scholtz, Pazlarova, Souskova, Khun, & Julak, 2015). Until now, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are thought to be the main molecules responsible for biocide effect (Iuchi et al., 2018). This technology offers some advantages for being safe and not expensive technology, only consuming compressed air and electricity to generate the plasma and because the plasma generation happens at atmospheric pressure and at room temperature. Eventually, no toxic or waste chemicals are produced during APCP treatment.

The direct APCP treatment over surfaces is a useful technology for food disinfection. Many researchers define APCP like an adequate technique to improve the microbiological quality of

a wide vegetable and animal foods, without changing their physicochemical properties. Several studies have demonstrated their biocide effect over food borne bacteria, moulds and yeast (Misra & Jo, 2017). This study was focused on *Brettanomyces* sensibility to APCP treatments on the surface of artificially contaminated oak wood (Sainz-García et al., 2021) as an effective and sustainable alternative to burning sulphur pills for barrel sanitization.

2. MATERIALS AND METHODS

2.1. MICROBIAL STRAINS, CULTURE AND SAMPLE PREPARATION

This study was conducted with *Brettanomyces bruxellensis* CECT 11045 from the Spanish Type Culture Collection (CECT). This yeast was grown in Glucose Yeast Peptone (GYP) broth at 28º C for 48 h. After incubation, when the cultures reached the stationary phase (10^8-10^9 cells/ml), cells were collected by centrifugation at 10000 x g at 4ºC for 30 minutes. The pellet obtained was suspended in 50 ml of Ringer Solution (RS) and inoculated in 450 ml of sterile synthetic wine (yeast extract 4 g/L, glycerol 2 g/L, DL-Malic 6 g/L, ethanol 100 mL/L).

Fragments of 50x30x10 mm of American oak staves with a medium toasted were used in triplicate, so that each sample was composed of three oak stave fragments. These three fragments of each sample were placed into a glass Tupper of 1L of volume and then they were sterilized at 121 ºC and 1 bar for 20 minutes. After this, the samples were contaminated by immersion in the 500 mL synthetic wine inoculated with *B. bruxellensis* in stationary phase. The oak fragments were for 48 h in orbital shaking (100 rpm) at 27º C to facilitate the penetration of the yeast into the wood pores.

2.2. TREATMENT WITH ATMOSPHERIC PRESSURE COLD PLASMA

The equipment used for APCP was PlasmaSpot® (MPG, Luxenburg) with a dielectric barrier discharge. It has of two coaxial electrodes, with an Al_2O_3 dielectric tube between them. The inner electrode is grounded and the external one is connected to a high voltage source operating at 68 kHz. The plasma was generated with three different gases: Argon (99.999%), compressed air and Nitrogen (99.999%) and at a flow of 40 slm, 80 slm and 40 slm, respectively.

The plasma power was 90 W for Argon and 500 W for air and Nitrogen. Batches of three oak wood fragments were exposed to APCP device at a 12-passes treatment (Figure 1). During these treatments, the plasma device was moving across the surface of the oak wood samples with a pattern scan. A linear speed of 100 mm/s, a pitch of 4 mm and a gap of 10 mm were the movement parameters used. Table 1 shows the plasma conditions for each batch of treatments.

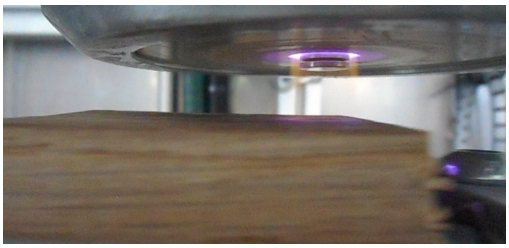

Figure 1. Plasma treatment process of one oak wood fragment (Sainz-García et al., 2021).

Table 1. Conditions of the plasma treatments (Sainz-García et al., 2021).

Plasma Gas	Gas Flow (slm)	Plasma Power (W)
Argon	40	90
Air	60	500
Nitrogen	60	500

2.3. MICROBIAL INACTIVATION

Every oak fragments (treated and untreated) were individually brushed with an automatic wood planer till a deep of 1 cm. The chips were gathered in sterile plastic bags and then weighted. After this, 300 mL of sterile Trypticasein Soy Broth (TSB) recovering medium were added. Sealed bags were horizontally shaking at 100 rpm for 24 h at 25ºC. After incubation in TSB, the liquid was recovered and centrifuged (10000 rpm; 30 min; 4º C). Pellets were suspended in RS, serially diluted, and spread on GYP culture media and incubated at 28º C. After 48 h of incubation period, colonies forming units (CFU) were counted in plates with growth between 30-300 colonies and expressed in each sample as the average CFU per gram of wood with the corresponding standard deviation, represented by error bars, and in logarithmic scale.

2.4. THERMAL CHARACTERIZATION

Wood sample surface temperatures were monitored using a K-type Teflon-coated thermo couple connected to a data logger Testo 167T4. Probes were attached to the sample surface and temperatures were recorded every 1 s while samples treatment.

2.5. MORPHOLOGICAL CHARACTERIZATION

A HITACHI S-2400 Scanning Electron Microscope (SEM) analyzed the surface morphology of the treated and untreated wood samples. Sample surfaces were coated with a thin layer of palladium and gold using a plasma sputtering apparatus before the SEM examination to make them conductive.

2.6. STATISTICAL ANALYSIS

Results of microbial counts in logarithmic scale (log CFU/ wood gr) were also reported from triplicates with average values and with their standard deviation, represented in error bars.

3. RESULTS AND DISCUSSION

This study analysed the inactivation rate of the APCP technology on *B. bruxellensis* used for contaminating oak barrel pores. The APCP was generated with three gases (Argon, air and Nitrogen) and each plasma gas was applied to stave fragments as samples in order to investigate wine barrel disinfection. Thermal and morphological characterization of the process were also determined. The results were extracted, and individually analysed, from a previous study (Sainz-García et al., 2021).

Figure 2 shows microbial viable and cultivable average population of *B. bruxellensis* (log CFU/ wood gram) achieved after brushing the treated and the untreated fragments.

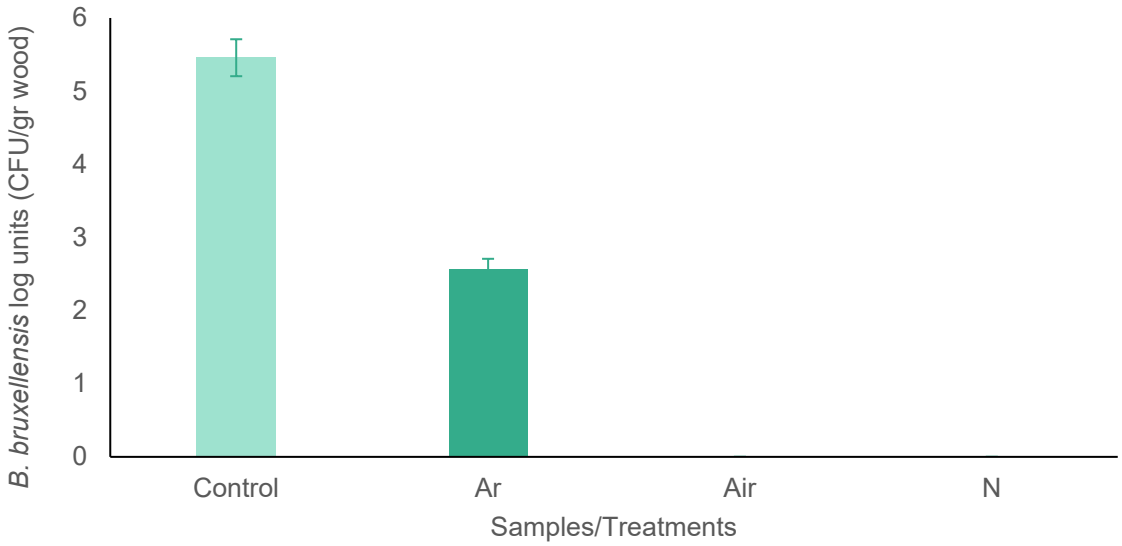

Figure 2. Average Brettanomyces bruxellensis population in log CFU/g in untreated oak fragments (Control) and after APCP treatment with Argon (Ar), Air and Nitrogen (N).

Regarding this figure 2, the first successful result was the achievement of an effective artificial contamination of staves that reached 5.46 log units of *B. bruxellensis* CFU per gram of wood. Moreover, after using Argon for APCP generation, remaining viable population was 2.57 log CFU/gr of wood, so that it meant a significant inactivation of 2.89 log units. The most important of this result is that the yeast population remained lower than 3 log units that is considered no risky for wine organoleptic deviations (Renouf et al., 2007). Eventually, when APCP was generated with Air or with Nitrogen we did not find *B. bruxellensis* population meaning an absolute inactivation of *Brettanomyces* viable and cultivable population. Differences were not found with the inactivation achieved by air APCP and nitrogen APCP, what was in agreement with the described by several authors (Mošovská et al., 2019; Xiao et al., 2016).

In Table 2 average and maximum temperature reached during the different treatments are displayed. The treatments of APCP generated with Air and Nitrogen were more powerful than the APCP generated with Argon (Table 1). It has been described that the use of a more energetic plasma could lead to higher disinfection efficiency (Feng et al., 2009). Evidently, these two powerful treatments were also responsible of highest average temperatures over the wood surface although always lower than 55 ºC, critic temperature that could cause direct microbial inactivation (González-Arenzana et al., 2015). Most of these alternative APCP treatments are able to inactivate microorganisms due to a synergy between the energy applied and the temperature that this energy triggers (Ehlbeck et al., 2011; Iuchi et al., 2018; Patil, Bourke, & Cullen, 2016) although, in this specific case, the temperature increase did not mean a problem because wood is a quite resistant surface.

Although average temperatures of the three APCP treatments were apparently not limiting the microorganism surveillance, electronic images of wood surface after treatments were studied to determine if APCP treatments have caused some type of damage.

Table 2. Average temperatures of the sample surface during plasma treatments (Sainz-García et al., 2021).

Plasma Gas	Average temperature (ºC)
Argon	33.7
Air	54.3
Nitrogen	53.0

In the following Figure 3, four images are shown. These pictures were taken with an electronic microscope. Apparently, the untreated samples were quite similar to Nitrogen and Argon APCP treatments but significant differences of surface structure were neither defined after air APCP treatment. Then, APCP seems not to cause damage on wood surface independently on the energy and on the temperature reached. Moreover, it is suggested that the bulk capacity of the oak wood would not be affected (Acda, Devera, Cabangon, & Ramos, 2012). These observations were similar to the described in other studies (Acda et al., 2012; Novák et al., 2015) and are really interesting for the wine barrel re-using.

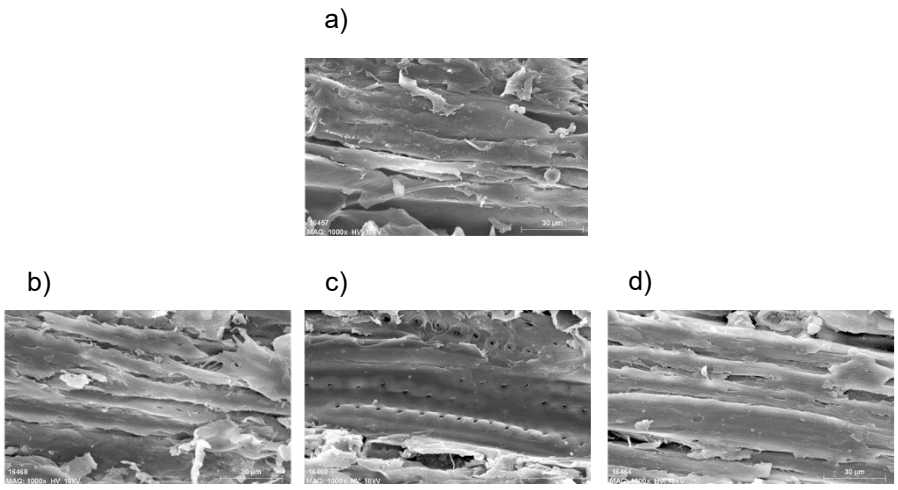

Figure 3. SEM images of the: a) untreated sample and samples treated with: b) Air plasma, c) Nitrogen plasma and d) Argon plasma (Sainz-García et al., 2021).

There are several recent studies focused on the APCP ability to inactivate microorganisms present on food or beverages contact surfaces. The types and quantity of ROS, RNS, UV radiation, charged particles, electric field and heat and their synergic effect have been suggested as the most probably cause for the microbial inactivation caused by APCP (Xu et al., 2021). In fact, it is known the dependence of the characteristics and concentrations of ROS and RNS generated during APCP treatments on the type of the plasma gas used (Schmidt-Bleker, Winter, Bösel, Reuter, & Weltmann, 2015). The obtained results were similar to the results of other studies in which lower concentrations of reactive species were identified in Argon APCP compared to other gases such as air (Seo et al., 2010; Yoon et al., 2016). Taking into consideration all the described results, it seems that less energetic APCP that generates lower reactive species concentration caused lower disinfection rates.

Regarding the yeast *B. bruxellensis* inactivation with APCP treatments, there is not enough research what makes difficult the understanding of the way that APCP treatment inactivates this yeast. In a recent study, Xu et al. (2021) have demonstrating that the –OH generated with APCP caused important damages in the cellular membrane what directly affects their permeability and their osmotic equilibrium. Most of the technologies that have been studied for replacing sulphur burning, such as microwaves, pulsed electric fields, high hydrostatic pressures, etc. have been described for displaying similar effects although following different routes (González-Arenzana et al., 2016; González-Arenzana et al., 2013; González-Arenzana et al., 2015).

4. CONCLUSIONS

In this study, the application of the APCP on the surface of oak wood artificially contaminated with *B. bruxellensis* was tested. Three different gases were used to generate the APCP (Argon, air and Nitrogen). The effect of reactive species during the APCP treatments generated with air and Nitrogen could play the main role on *B. bruxellensis* inactivation and apparently the thermal effect was not the main cause of the cell inactivation. Moreover, SEM images did not show important morphological modifications on wood surface after APCP treatment.

In conclusion, APCP could be a promising alternative to burning of sulphur pills in the oak barrels sanitization, sorting out the problems related to their re-using. Further research should be carried out to know the effectiveness of APCP against other microorganisms, even those that are in viable but not culturable forms. After all, it would be interesting testing this technique in naturally contaminated staves, in other wood types, from different origins and with different toasted levels. Eventually, research should be aimed to face the scaling up of this technique applied for completing its adaptation to the facilities of wineries.

ACKNOWLEDGEMENTS

The author E. Sainz-García, as postdoctoral researcher of the University of La Rioja, thanks the postdoctoral training program that is funded by the Plan Propio of the University of La Rioja. The author I. Muro-Fraguas, thanks the program of pre-doctoral contracts for the training of research staff funded by the University of La Rioja.

FUNDING

This work was supported by the Ministerio de Economía, Industria y Competitividad of Spain (MINECO) (project PID2019-105367R “Programa Estatal de I + D + i Orientada a los Retos de la Sociedad”) and 50% co-funded by the European Regional Development Fund (FEDER) “A way to make Europe”; and the European Regional Development Fund, granted to the Autonomous Community of La Rioja (project PR- 07-18), within the 2014–2020 Operational Program. FEDER of La Rioja.

REFERENCES

Acda, M. N., Devera, E. E., Cabangon, R. J., & Ramos, H. J. (2012). Effects of plasma modification on adhesion properties of wood. *International Journal of Adhesion and Adhesives*, *32*(1), 70–75. https://doi.org/10.1016/j.ijadhadh.2011.10.003

Agnolucci, M., Tirelli, A., Cocolin, L., & Toffanin, A. (2017). *Brettanomyces bruxellensis* yeasts: impact on wine and winemaking. *World Journal of Microbiology and Biotechnology*, *33*(10). https://doi.org/10.1007/s11274-017-2345-z

Costantini, A., Vaudano, E., Cravero, M. C., Petrozziello, M., Piano, F., Bernasconi, A., & Garcia-

Moruno, E. (2016). Dry ice blasting, a new tool for barrel regeneration treatment. *European Food Research and Technology*, *242*(10), 1673–1683. https://doi.org/10.1007/s00217-016-2667-3

Ehlbeck, J., Schnabel, U., Polak, M., Winter, J., Von Woedtke, T., Brandenburg, R., Weltmann, K. D. (2011). Low temperature atmospheric pressure plasma sources for microbial decontamination. *Journal of Physics D: Applied Physics*, *44*(1). https://doi.org/10.1088/0022-3727/44/1/013002

Feng, H., Sun, P., Chai, Y., Tong, G., Zhang, J., Zhu, W., & Fang, J. (2009). The interaction of a direct-current cold atmospheric-pressure air plasma with bacteria. *IEEE Transactions on Plasma Science*, *37*(1), 121–127. https://doi.org/10.1109/TPS.2008.2008438

García-Alcaraz, J. L., Flor Montalvo, F. J., Martínez Cámara, E., Pérez de la Parte, M. M., Jiménez-Macías, E., & Blanco-Fernández, J. (2020). Economic-environmental impact analysis of alternative systems for red wine ageing in re-used barrels. *Journal of Cleaner Production*, *244*. https://doi.org/10.1016/j.jclepro.2019.118783

González-Arenzana, L., Sevenich, R., Rauh, C., López, R., Knorr, D., & López-Alfaro, I. (2016). Inactivation of *Brettanomyces bruxellensis* by High Hydrostatic Pressure technology. *Food Control*, *59*, 188–195. https://doi.org/10.1016/j.foodcont.2015.04.038

González-Arenzana, L, Santamaría, P., López, R., Garijo, P., Gutiérrez, A. R., Garde-Cerdán, T., & López-Alfaro, I. (2013). Microwave technology as a new tool to improve microbiological control of oak barrels: A preliminary study. *Food Control*, *30*(2), 536–539. https://doi.org/10.1016/j.foodcont.2012.08.008

González-Arenzana, Lucía, Portu, J., López, R., López, N., Santamaría, P., Garde-Cerdán, T., & López-Alfaro, I. (2015). Inactivation of wine-associated microbiota by continuous pulsed electric field treatments. *Innovative Food Science & Emerging Technologies*, *29*, 187–192. https://doi.org/10.1016/j.ifset.2015.03.009

Guzzon, R., Bernard, M., Barnaba, C., Bertoldi, D., Pixner, K., & Larcher, R. (2017). The impact of different barrel sanitation approaches on the spoilage microflora and phenols composition of wine. *Journal of Food Science and Technology*, *54*(3), 810–821. https://doi.org/10.1007/s13197-017-2527-6

Iuchi, K., Morisada, Y., Yoshino, Y., Himuro, T., Saito, Y., Murakami, T., & Hisatomi, H. (2018). Cold atmospheric-pressure nitrogen plasma induces the production of reactive nitrogen species and cell death by increasing intracellular calcium in HEK293T cells. *Archives of Biochemistry and Biophysics*, *654*, 136–145. https://doi.org/10.1016/j.abb.2018.07.015

Marko, S. D., Dormedy, E. S., Fugelsang, K. C., Dormedy, D. F., Gump, B., & Wample, R. L. (2005). Analysis of oak volatiles by gas chromatography-mass spectrometry after ozone sanitization. *American Journal of Enology and Viticulture*, *56*(1), 46–51.

Misra, N. N., & Jo, C. (2017). Applications of cold plasma technology for microbiological safety in meat industry. *Trends in Food Science & Technology*, *64*, 74–86. https://doi.org/http://dx.doi.org/10.1016/j.tifs.2017.04.005

Mošovská, S., Medvecká, V., Gregová, M., Tomeková, J., Valík, Ľ., Mikulajová, A., & Zahoranová, A. (2019). Plasma inactivation of Aspergillus flavus on hazelnut surface in a diffuse barrier discharge using different working gases. *Food Control*, *104*(March), 256–261. https://doi.org/10.1016/j.foodcont.2019.05.003

Niemira, B. A. (2012). Cold Plasma Decontamination of Foods. *Annual Review of Food Science and Technology*, *3*(1), 125–142. https://doi.org/10.1146/annurev-food-022811-101132

Novák, I., Popelka, A., Špitalský, Z., Mičušík, M., Omastová, M., Valentin, M., Šlouf, M. (2015). Investigation of beech wood modified by radio-frequency discharge plasma. *Vacuum*, *119*, 88–94. https://doi.org/10.1016/j.vacuum.2015.04.038

Palacios, A., Borinaga, I., & Carrillo, D. (2012). Estudio comparativo de sistemas de desinfección de barricas de vino como alternativas al empleo del sulfuroso. *Enólogos*, *77*, 46–54.

Patil, S., Bourke, P., & Cullen, P. J. (2016). *Principles of Nonthermal Plasma Decontamination*. *Cold Plasma in Food and Agriculture: Fundamentals and Applications*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-801365-6.00006-8

Porter, G. W., Lewis, A., Barnes, M., & Williams, R. (2011). Evaluation of high power ultrasound porous cleaning efficacy in American oak wine barrels using X-ray tomography. *Innovative Food Science & Emerging Technologies*, *12*(4), 509–514. https://doi.org/10.1016/j.ifset.2011.06.007

Renouf, V., Lonvaud-Funel, A., & Coulon, J. (2007). The origin of *Brettanomyces bruxellensis* in wines: A review. *Journal International Des Sciences De La Vigne Et Du Vin*, *41*(3), 161–173.

Sainz-García, A., González-Marcos, A., Múgica-Vidal, R., Muro-Fraguas, I., Escribano-Viana, R., González-Arenzana, L., Sainz-García, E. (2021). Application of atmospheric pressure cold plasma to sanitize oak wine barrels. *LWT*, *139*, 110509. https://doi.org/https://doi.org/10.1016/j.lwt.2020.110509

Schmid, F., Grbin, P., Yap, A., & Jiranek, V. (2011). Relative efficacy of high-pressure hot water and high-power ultrasonics for wine oak barrel sanitization. *American Journal of Enology and Viticulture*, *62*(4), 519–526. https://doi.org/10.5344/ajev.2011.11014

Schmidt-Bleker, A., Winter, J., Bösel, A., Reuter, S., & Weltmann, K. D. (2015). On the plasma chemistry of a cold atmospheric argon plasma jet with shielding gas device. *Plasma Sources Science and Technology*, *25*(1). https://doi.org/10.1088/0963-0252/25/1/015005

Scholtz, V., Pazlarova, J., Souskova, H., Khun, J., & Julak, J. (2015). Nonthermal plasma - A tool for decontamination and disinfection. *Biotechnology Advances*, *33*(6), 1108–1119. https://doi.org/10.1016/j.biotechadv.2015.01.002

Seo, Y. S., Mohamed, A. A. H., Woo, K. C., Lee, H. W., Lee, J. K., & Kim, K. T. (2010). Comparative studies of atmospheric pressure plasma characteristics between He and Ar working gases for sterilization. *IEEE Transactions on Plasma Science*, *38*(10 PART 2), 2954–2962. https://doi.org/10.1109/TPS.2010.2058870

Stadler, E., Schmarr, H. G., & Fischer, U. (2020). Influence of physical and chemical barrel sanitization treatments on the volatile composition of toasted oak wood. *European Food Research and Technology*, *246*(3), 497–511. https://doi.org/10.1007/s00217-019-03417-7

Suárez, R., Suárez-Lepe, J. A., Morata, A., & Calderón, F. (2007). The production of ethylphenols in wine by yeasts of the genera *Brettanomyces* and *Dekkera*: A review. *Food Chemistry*, *102*(1), 10–21. https://doi.org/10.1016/j.foodchem.2006.03.030

Xiao, D., Cheng, C., Lan, Y., Ni, G. H., Shen, J., Meng, Y. D., & Chu, P. K. (2016). Effects of Atmospheric-Pressure Nonthermal Nitrogen and Air Plasma on Bacteria Inactivation. *IEEE Transactions on Plasma Science*, *44*(11), 2699–2707. https://doi.org/10.1109/TPS.2016.2598564

Xu, H., Zhu, Y., Du, M., Wang, Y., Ju, S., Ma, R., & Jiao, Z. (2021). Subcellular mechanism of microbial inactivation during water disinfection by cold atmospheric-pressure plasma. *Water Research*, *188*. https://doi.org/10.1016/j.watres.2020.116513

Yap, A. (2009). Cleaning and disinfecting barrels with high-power ultrasonics: a new industry benchmark. *Grapegrower and Winemaker*, (551), 89–93.

Yoon, S. Y., Kim, K. H., Seol, Y. J., Kim, S. J., Bae, B., Huh, S. R., & Kim, G. H. (2016). Effects of metastable species in helium and argon atmospheric pressure plasma jets (APPJs) on inactivation of periodontopathogenic bacteria. *Journal of the Korean Physical Society*, *68*(10), 1176–1191. https://doi.org/10.3938/jkps.68.1176

Abstract

In the oenological industry, the maintenance and sanitation of oak barrels has become a fundamental task.

The wood has a porous structure that facilitates the penetration not only of the wine, but of the microorganisms it contains, such as the alterative yeast Brettanomyces bruxellensis.

Although the most widely used method of sanitizing barrels is the burning of sulfur tablets, there is a European directive that will limit this practice, even when an effective alternative has not yet been found.

This research is part of a project that studies the application of cold plasma at atmospheric pressure (APCP) to sanitize oak wood staves. This alternative technology to sulfur is respectful with the environment.

In this study, various fragments of staves artificially contaminated with Brettanomyces bruxellensis were exposed to the APCP device with different plasma gas and distinct plasma strengths. The results showed inactivations of 2.89 logarithmic units (of colony-forming units per milliliter) using argon for plasma generation. Absolute inactivations (5.46 log units) were reached when air or nitrogen was used for plasma generation. Nor any morphological modifications were seen on the surface of the wood after the APCP treatments.

Despite the promise of these results, this line of research should be continued to solve the difficulties that may arise when treating naturally contaminated wood fragments in the wineries, as well as when facing their industrial scale.