

DISCLAIMER

Please read this disclaimer carefully before accessing, participating in, reading or making any other use of the following informational webinar and any documents, information, recommendations, options or opinions obtained from or provided in connection with or ancillary thereto.

This webinar is provided by BevZero Services, Inc., a California corporation (the “Company”). The Company makes no representations or warranties of any kind or type, and expressly disclaims any and all warranties, express or implied, including without limit the accuracy or suitability of information, suitability for a particular purpose, fitness for an intended use, or any particular outcome from any use of the information provided in the webinar and related materials (including without limit hand-outs, presentation documents, exhibits, visual aids, and recordings “related materials”). The information contained in the webinar and the related materials are not intended to, and do not, constitute advice of any kind or the rendering of consulting, or other professional services, or any opinion of the suitability of the services the Company provides for any particular person, company or application; each application and use of the Company’s services and technology is unique, and each potential use or application thereof must be carefully adapted to, adjusted to and crafted for each specific use. A particular outcome in one application of the Company’s technology or services is no guarantee, warranty or representation as to how such technology or services might perform in another application, and any such projections are expressly disclaimed. Registering for or attending a webinar is not and does not create a contract with the Company to provide any services, or commit the Company to providing any consulting services, opinions, or advice, all of which would need to be agreed upon in a separate contract with the Company.

The opinions expressed in the webinar, if any, and related materials are those of the Company only. To the extent permitted by law, by proceeding to participate in the webinar, you agree to waive any and all claims for any loss, liability, damage, cost or expense, including any indirect or consequential damages or lost profit, whether arising in negligence or otherwise, suffered in connection with the access to, participation in, or use of the information from the webinar and the related materials. By participating in the webinar, you acknowledge that the information and materials contained in the webinar are the intellectual property of the Company, and while it is believed to be accurate could contain inaccuracies. Nothing in this webinar shall serve to create any right or license in or to the Company’s intellectual property. The content of the information provided in the Webinar is for general information and use only, and subject to change at any time and without notice. If you have any questions about any of the foregoing, before participating in the webinar, you should consult with an attorney, or other qualified professional.



Microbial Stabilization of Non-Alcoholic Beverages International Discussion

April 26th, 2022



POLARCLAD



Webinar Formalities

- This webinar is being recorded, and will be posted on our website
- Please refrain from using the chat box during the presentation, there will be a 15-minute Q&A at the end of the discussion.
- For technical difficulties, you can use the chat box and a team member will assist you. Chrome web browser is recommended.
- If the chat box is distracting, you can toggle it closed.
- Please post your questions in the designated Q&A chat box.



Outline

Background

Fermented Beverages and Microbial Ecology
Hurdle Technologies

Microbial Stabilization Techniques
Additive Techniques
Physical Techniques

Market Demand and Trends

BevZero South Africa
Gustav Fouche, Managing Director
Dylan Dowell-Ellis, Production Manger

BevZero Spain
Silvia Cedeno Daguerre, General Manager
Gregorio Blanco, Quality and R&D Manager

BevZero United States
Kayla Winters, Director of Product Services and Winemaking
Chris Anderson, Head of Brewing Operations



Lauren Barrett
Manager, Global Beverage
Innovation
USA



Gustav Fouche
Managing Director / Enologist
SOUTH AFRICA



Dylan Dowell-Ellis
Product Manager / Enologist
SOUTH AFRICA



**Silvia Cedeno
Daguerre**
General Manager / Enologist
SPAIN



Gregorio Blanco
Quality and R&D Manager
SPAIN



Kayla Winter
Director, Product Services
USA



Chris Anderson
Head of Brewing Operations,
USA



Fermented Beverages and Microbial Ecology

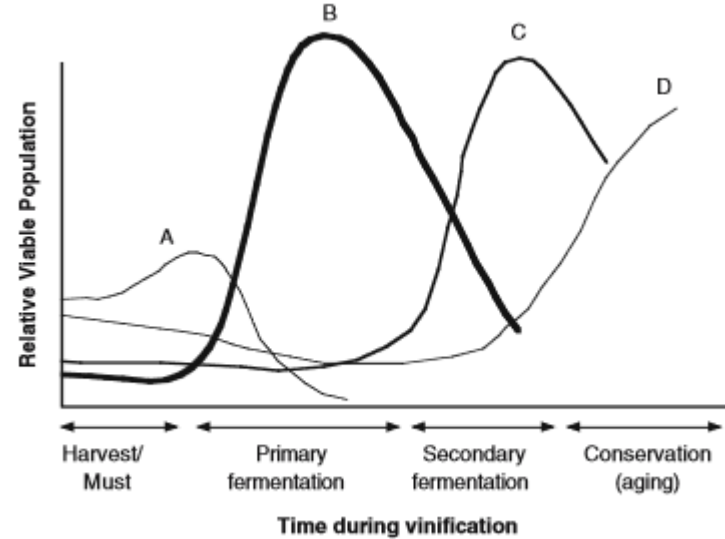


BACKGROUND

Fermented Beverages and Microbial Ecology

- Fermentation helps to reduce microbial diversity where only adapted, niche, relatively safe microorganisms can survive compared to other non-fermented beverages (i.e., juices).
- Fermented beverages contain antimicrobial metabolites (i.e., ethanol, organic acids, microbial peptides and SO₂) which have an inhibitory effect against spoilage and pathogenic microbes.
- Log Reduction (i.e., 10⁻¹:10, 10⁻⁶:1,000,000)

Microbial Evolution During Vinification



General growth of (A) *non-Saccharomyces*, (B) *Saccharomyces*, (C) *Oenococcus oeni* and (D) spoilage yeast and bacteria during vinification. Extracted from (Fugelsang and Edwards 2006).



Hurdle Technologies



- Hurdle technology is a set of methods used for inactivation of microorganisms in food preservation.
- Hurdles are food preservation factors, which are combined to achieve certain food quality and stability, considering:
 - Temperature
 - pH
 - Redox potential
 - Water activity
 - Preservatives
 - Competitive microorganisms

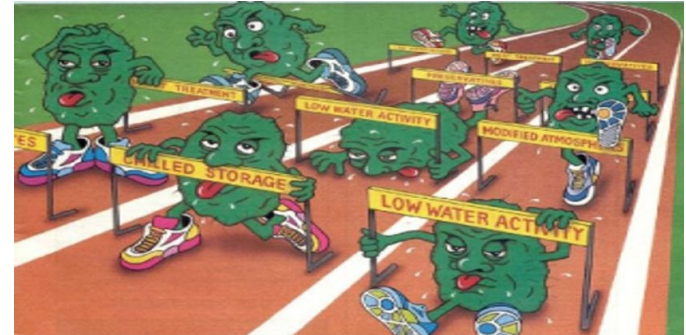


Image from Disha Sinha, Hurdle Technology, July 24, 2020, PMG Engineering.com

(Putnik et al., 2020)



Microbial Stabilization Techniques and Considerations

Multitargeted Preservation

Hurdle Technologies

Physical Techniques

Thermal and Non-thermal

Additive Techniques

Chemical and Clean Label Preservatives



Chemical Additive Preservation

Additive Preservatives	Regulatory Reference	Advantages	Disadvantages
Sulfur Dioxide (SO₂)	E220, 21 CFR §182.3637, 27 CFR § 24.246	<ul style="list-style-type: none">• Widely available• Effective at low pH• Antimicrobial• Antioxidant• Inexpensive	<ul style="list-style-type: none">• Allergen labeling required• Microbial resistance• High levels of SO₂ can contribute to can liner degradation and impact sensory• Less effective at higher pH (>3.50)
Velcorin (Dimethyl decarbonate, DMDC, C₄H₆O₅) *Processing Aid	E242, 21 CFR §172.133, 27 CFR § 24.246	<ul style="list-style-type: none">• Antimicrobial• Effective at low pH• Classified by the FDA as a direct secondary food additive, no label requirement	<ul style="list-style-type: none">• Specialized equipment and personal• Expensive• Yeast cell population should be below 500 cells/mL• Not as effective against lactic and acetic acid bacteria• Recommended to combine with other chemical preservatives (i.e., SO₂)• Highly toxic within active time range (up to 48 hours after addition)



Chemical Additive Preservation

Additive Preservatives	Regulatory Reference	Advantages	Disadvantages
Sodium Benzoate (C ₇ H ₅ NaO ₂)	E212, 21 CFR §582.3733	<ul style="list-style-type: none">• Widely available• Antimicrobial• Effective at low pH• Effective on yeast and molds• Inexpensive	<ul style="list-style-type: none">• Microbial resistance (Zygosaccharomyces, Brettanomyces)• Bacteria inhibition requires higher dosages• Decreased solubility at lower pH.• Benzoate and ascorbic acid (vitamin C) should not be used together, as they may react to form benzene, which is a known carcinogen• Labeling required
Potassium Sorbate (C ₆ H ₇ KO ₂)	E202, 21 CFR §182.3640, 27 CFR § 24.246	<ul style="list-style-type: none">• Widely available• Antimicrobial• Effective on yeast and molds• Inexpensive	<ul style="list-style-type: none">• Poor solubility in water• Not effective against acetic and lactic acid bacteria.• Risk of “geranium” taint from bacterial metabolism• Microbial resistance (Lactic acid bacteria, Zygosaccharomyces, Brettanomyces)• Oxidation of sorbate can produce browning and aldehydes; free SO₂ concentrations should be between 30-40 ppm to protect from oxidation.• It’s recommended to combine with other chemical preservatives (Benzoates & SO₂)• Labeling required



Additive Preservation and Clean Label Ingredients

1. **Natural ingredients:** No artificial flavors, artificial colors, artificial, preservatives, or synthetic additives.
2. **Simplicity:** Less chemicals and recognizable ingredients that do not sound chemical or artificial.
3. **Transparency:** Information on how ingredients are sourced and how products are manufactured.
4. **Minimal processing:** Processing using techniques that consumers don't understand to be artificial.



CONSIDER:

- Compatibility, Efficiency and Sensory Impact
- FDA Food Additive Status List
- FDA Generally Recognized as Safe (Gras)
- FDA Natural vs. USDA Organic Program
- Supplier SDS, COA, Third Party Data.



Microbial Stabilization Techniques and Considerations

Multitargeted Preservation

Hurdle Technologies

Physical Techniques

Thermal and Non-thermal

Additive Techniques

Chemical and Clean Label Preservatives



Thermal Technologies

- **Low temperature long time (LTLT)**
 - Spray or in batch tunnel pasteurization at 60-100°C/140-212 °F for 15-30 minutes with pasteurization in package.
 - **Benefits:** Ensures in package stability, is widely available and commonly used.
 - **Challenges:** Negative sensory impact (cooked flavors), color and nutrient degradation, time consuming, water usage, energy usage, space.



Ed Michalski Tunnel and Batch Pasteurization vs.
Artificial Preservatives to Food and Beverages April
1st 2021, prowm.com

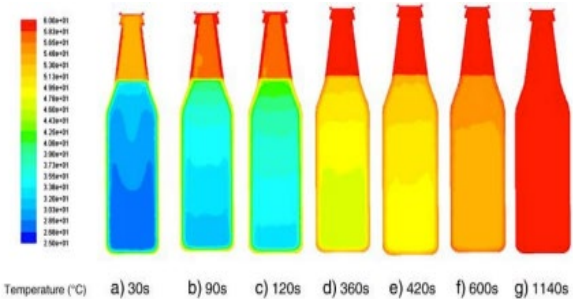


Image from Bhuvaneswari E, Anandharamakrishnan C. Heat transfer analysis of pasteurization of bottled beer in a tunnel pasteurizer using computational fluid dynamics. Innovative Food Science & Emerging Technologies. 2014 Jun 1;23:156–63.



Thermal Technologies

- **Aseptic Processing (AP)**
 - Product is sterilized prior separately from packaging where products is packaged in sterilize containers under extremely clean environments. Products do not need
- **High temperature short time (HTST)**
 - Flash Pasteurization (~75°C/167°F for 15-30 seconds with rapid cooling).
 - Continuous system, “Hot filling” usually combined with aseptic packaging. .
- **Ultra-high temperature (UHT)**
 - (135-150°C/275-302°F for 3-5 seconds with rapid cooling).
 - Continuous system, “Hot filling” usually combined with aseptic packaging.



Image from Pablo Coronel, Strategies for Aseptic Food Processing, foodqualityandsafety.com, 2021



Benefits: Readily available, increased shelf life, less sensory alterations than tunnel and shorter processing.

Challenges: Expensive, risk for overheating, sensory alterations (light struck and scorched faults), color and nutrient degradation, water and energy consumption, risk of contamination at packaging.

Non-Thermal Technologies

Non-Thermal technologies use lethal agents other than heat to reduce or eliminate microorganisms that might be harmful or cause spoilage.

- Demonstrates “pasteurization” effects
- Variable efficacy on enzymes
- Combination with heat for commercial sterilization
- Milder treatments result in refrigerated/cold chain foods with less cooked flavor
- Clean labeling

1. Sterile Filtration (0.2 μ m)
2. High Hydrostatic Pressure
3. Pulsed electric fields
4. Ultrasound
5. Ultraviolet



Ultra High Pressure (UHPH) and High Dynamic Homogenization (HDH)

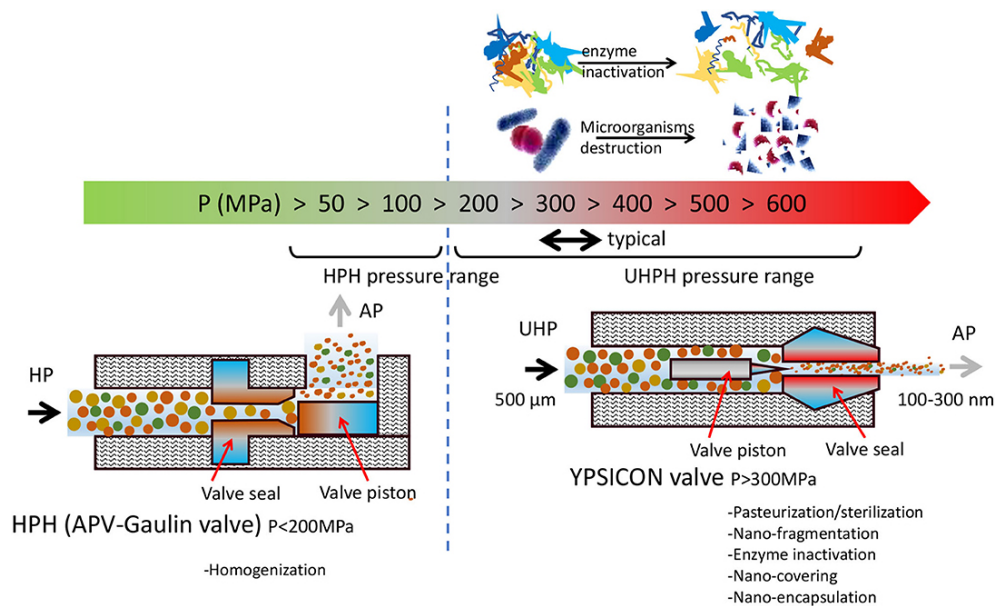


Image from Morata, A., & Guamis, B. (2020). Use of UPHH to obtain juices with better nutritional quality and healthier wines with low levels of SO₂. *Frontiers in nutrition*, 241.

- **Benefits:** UPHH is continuous, gentle, with high antimicrobial effect (6-log reduction), has a short processing time, with no significant impact on sensory and bioactive compounds even with multiple treatments.
- **Challenges:** HDH is in batch and has limited effect on enzyme deactivation. Both may require multiple passes and higher in valve temperature to inactivate bacteria and spores. Not as readily available commercially.



High Pressure Processing (HPP)

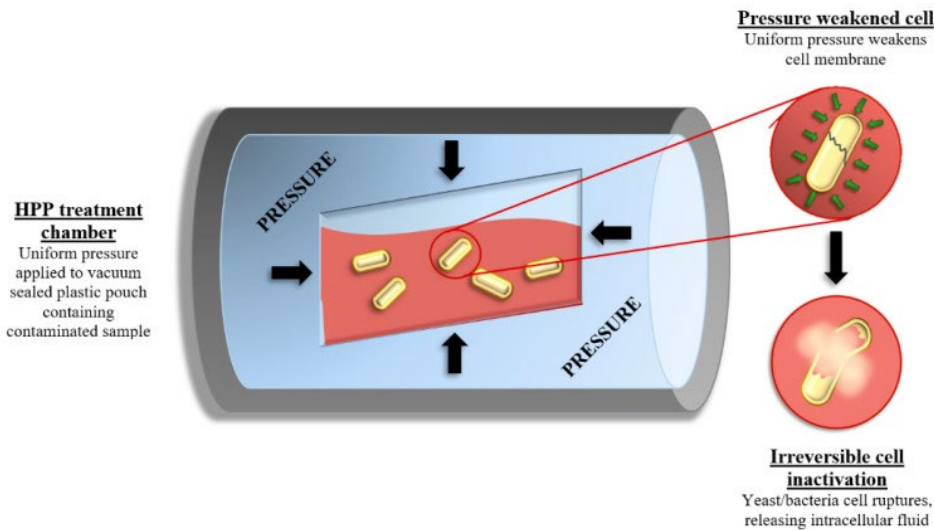


Image from Silva, F. V., & van Wyk, S. (2021). Emerging non-thermal technologies as alternative to SO₂ for the production of wine. *Foods*, 10(9), 2175.

- **Benefits:** In Batch and In Packaging options, strong microbial inactivation (7 log reduction), can operate below room temperature, instantaneous transfer of pressure regardless of liquid characteristics.
- **Challenges:** In packaging (discontinuous) is more widely available but present packaging. There is a high capital investment but are become more available. Limited availability with in-batch options.

- **Pulsed Electric Fields (PEF)**

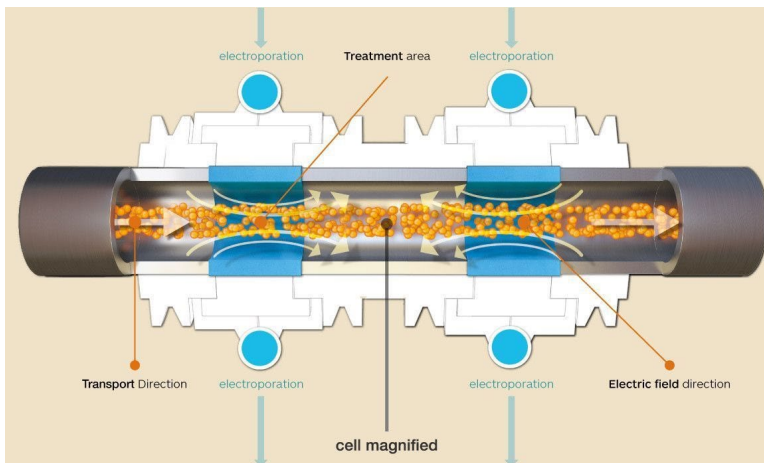


Image from Pulsed Electric Field preservation i3-food project.eu 2020.



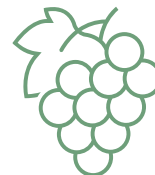
- **Benefits:** Continuous, fast, effective antimicrobial (log reduction 5) low energy consumption, minimal alterations to wine chemistry. Minimal effect on wine and beer chemistry.
- **Challenges:** Liquids should be de-gassed, not as effective with bacteria, high voltage safety risks, can develop light-struck character in beer.



Non- Thermal Technologies

- **Ultrasound**

- **Benefits:** In batch and continuous process, versatile (homogenization, extraction, emulsion, de-gassing).
- **Challenges:** Time consuming, cavitation can modify to color, sensory, antioxidants and polysaccharides. Less effective against certain yeast and bacteria.



- **Ultraviolet Radiation**

- **Benefits:** Minimal loss of nutrients, sensory and low energy consumption compared to other thermal techniques.
- **Challenges:** UV penetrability (absorbance, solids, color, density) enzyme deactivation is limited, Less effective in red wines, can develop light-struck character in beer.



Non-Alcoholic Beverage Preservation Market Trends and Demands



BevZero South Africa

Gustav Fouche, Managing Director

Dylan Dowell-Ellis, Production Manager



Gustav



BevZero Spain

Silvia Cedeno Daguerre, General Manager

Gregorio Blanco, Quality and R&D Manager






BevZero United States

Kayla Winter, Director of Product Services

Chris Anderson, Head of Brewing Operations



A woman with long brown hair and glasses, eyes closed, in a blurred indoor setting.

Lauren Barrett

Summary

Multitarget Preservation

Hurdle Technologies

UHPP and PEF

US and PEF

Thermal and PEF

Thermal and HPP

Physical Techniques

Non-Thermal

HPP

PEF

UHPP

UV

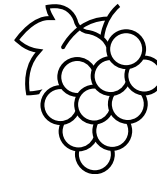
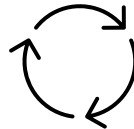
Additive Techniques

Chemical and Clean Label

SO₂

Velcorin (DMDC)

Antioxidant/Antimicrobial
tannins



Resources and Citations

1. Bhuvaneswari E, Anandharamakrishnan C. Heat transfer analysis of pasteurization of bottled beer in a tunnel pasteurizer using computational fluid dynamics. *Innovative Food Science & Emerging Technologies*. 2014 Jun 1;23:156–63.
2. Chiozzi, V., Agriopoulou, S., & Varzakas, T. (2022). Advances, Applications, and Comparison of Thermal (Pasteurization, Sterilization, and Aseptic Packaging) against Non-Thermal (Ultrasounds, UV Radiation, Ozonation, High Hydrostatic Pressure) Technologies in Food Processing. *Applied Sciences*, 12(4), 2202.nd 50 MPa, respectively.
3. Krebs, G., Müller, M., Becker, T., & Gastl, M. (2019). Characterization of the macromolecular and sensory profile of non-alcoholic beers produced with various methods. *Food Research International*, 116, 508-517.
4. Lavefve, L., Marasini, D., & Carbonero, F. (2019). Microbial ecology of fermented vegetables and non-alcoholic drinks and current knowledge on their impact on human health. *Advances in food and nutrition research*, 87, 147-185.
5. Milani, E. A., & Silva, F. V. M. (2022). Pasteurization of beer by non-thermal technologies. *Frontiers in Food Science and Technology*, 2.
6. Morata, A., & Guamis, B. (2020). Use of UHPH to obtain juices with better nutritional quality and healthier wines with low levels of SO₂. *Frontiers in nutrition*, 241.
7. Silva, F. V., & van Wyk, S. (2021). Emerging non-thermal technologies as alternative to SO₂ for the production of wine. *Foods*, 10(9), 2175. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8469166/>
8. Zulkurnain, M., Sulaiman, A., & Balasubramaniam, V. M. (2021). High-Pressure-Based Food-Processing Technologies for Food Safety and Quality. In *Food Processing* (pp. 1-26). CRC Press. Citations and Resource
9. Putnik, P., Pavlič, B., Šojić, B., Zavadlav, S., Žuntar, I., Kao, L., ... & Kovačević, D. B. (2020). Innovative hurdle technologies for the preservation of functional fruit juices. *Foods*, 9(6), 699.
10. Zoecklein, B., Fugelsang, K. C., Gump, B. H., & Nury, F. S. (2013). *Wine analysis and production*. Springer Science & Business Media.
11. Zulkurnain, M., Sulaiman, A., & Balasubramaniam, V. M. (2021). High-Pressure-Based Food-Processing Technologies for Food Safety and Quality. In *Food Processing* (pp. 1-26). CRC Press. Citations and Resource



Thank you for your time!

Q & A



BEVZERO
BEVERAGE INNOVATION



POLARCLAD

