12th HPBB CONFERENCE

International Conference on High Pressure Bioscience and Biotechnology HPBB 2024

Lyon, Oct. 1st - Oct. 3rd

Booklet

The International Association of High Pressure Bioscience and Biotechnology (HPBB)

INSTITUT NATIONAL DES SCIENCES APPLIQUÉES LYON

12th HPBB CONFERENCE

Organizing committee

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Anaïs Cario, ICMBS, Bordeaux, France

12th HPBB CONFERENCE

Welcome to HPBB 2024

Dear Colleagues and Friends,

A very warm welcome to the International Conference on High Pressure Bioscience and Biotechnology, HPBB 2024.

It is with great pleasure that we invite you to the 12th HPBB which will be held in Lyon from September 30th to October 3rd.

For this edition, we have opted to highlight the work of three very young High Pressure researchers from the three main fields relevant to HPBB :

- Dr. Stewart Gault from the University of Edimburgh in the Physics/chemistry section
- Dr. Jacob Winnikoff from Harvard University in the Microbiology section
- Dr. Tomas Bolumar from Max Rubner Institut in the Fodd Sciences section

We hope that you will enjoy the presentations ant take part in the discussion about the latest developments in the field.

We also hope that you will enjoy the food and architecture of the capital of gastronomy.

On behalf of the HPBB 2024 organizing committee,

Philippe M. Oger
CNRS Research Director
Chair

Gold Sponsor of HPBB 2024

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12th HPBB Program

September, 30th

18h00 **Pre-meeting-welcome-get-together and lab visits**
(Bâtiment Pasteur, INSA de Lyon, 11 Avenue Jean Capelle)

October, 1st

8h30 **Registration and welcome**

9h15 **Conference opening**
Phil Oger, Local organization

Food Sciences session 1 (Chair Marie de Lamballerie)
Keynote 1 : High-pressure Processing (HPP) of Meat Systems: Molecular Changes, Quality Aspects and Industrial Applications
Tomas BOLUMAR (Max Rubner-Institut, Germany)

10h15-10h45 **Coffee and Posters**

10h45 **Food Sciences Session 2 (Chair Marie de Lamballerie)**

O1 : Impact of hyperbaric storage and moderate pressure pasteurization on the volatile compounds of watermelon juice
Vasco Lima (University of Aveiro, Portugal)

O2 : The effects of nonionic kosmotropes under high pressure: implications for protein-flavonoid non-covalent Interactions
Avi Shpigelman (Israel Institute of Technology, Israel)

Industrial sponsor
Irian Technologies (Moissac, France)

12h00-13h30 **Lunch and Posters**

13h30-15h00 **Food Sciences Session 3 (Chair Avi Shpigelman)**

O3 : Moderate-pressure-pasteurization of salmon in combination with oxygen scavenger: a new strategy for improving color
Alireza Mousakhani Ganjeh (University of Aveiro, Portugal)

O4 : Development of a Secondary Model for Full Geeraerd Under High Hydrostatic Pressure
Alpas Hami (METU-Food Engineering Department, Turkey)

O5 High pressure combined with biopreservation: effects on acceptability of cooked ham
Laurence Pottier (Ecole Nationale Vétérinaire, Agroalimentaire et de l'Alimentation Nantes-Atlantique, France)

Industrial sponsor
Hiperbaric (Burgos, Spain)

15h00-16h15 **Coffee and Posters**

16h15-17h00 **Microbiology session 1 (Chair Anaïs Cario)**

O6 : Hyperbaric inactivation - a novel tool for Alicyclobacillus acidoterrestris spores' destruction in commercial apple juice as affected by a previous HPP and PEF pre-treatment
Carlos Pinto (University of Aveiro, Portugal)

O7 : Size distribution of Escherichia coli cells after high hydrostatic pressure treatment
Kazutaka Yamamoto (National Agriculture and Food Research Organization, Japan)

12th HPBB Program

October, 2nd

8h30 **Welcome**

8h50 **Microbiology Session 2 (Chair Phil Oger)**

Keynote 2 : Homeocurvature and homeoviscosity: unifying principles of membrane adaptation
Jacob WINNIKOFF (Harvard University, USA)

O8 : Real-time imaging of bacterial motility with high-pressure microscopy
Nishiyama Masayoshi (Kindai University, Japan)

10h00-10h30 **Coffee and Posters**

10h30-12h00 **Microbiology Session 3 (Chair Phil Oger)**

O9 : Cellular Transfection using Rapid Decrease in Hydrostatic Pressure
Shudi Huang (University of Toronto, Canada)

O10 : Influence of high hydrostatic pressure with benzoic acid addition on the energy metabolism and inactivation of budding yeast *Saccharomyces cerevisiae*
Toru Shigematsu (Niigata University, Japan)

O11 : Mechanosensing in Yeast: The Role of the Cell Wall Integrity Pathway in *Saccharomyces cerevisiae* under High-Pressure Stress
Fumiyoshi Abe (Aoyama Gakuin University, Japan)

O12 : Combining extreme microfluidics and *in situ* analyses techniques to get close insights into deep-sea vents microbial ecosystems
Emma Bohuon (ICMCB, Bordeaux, France)

12h00-13h30 **Lunch and Posters**

13h30-15h00 **Physics/Chemistry Session 1 (Chair Judith Peters)**

Keynote 3 : The limits to life at high pressures and their astrobiological relevance
Stewart GAULT (Edinburgh University, UK)

O13 : Pressure effect on protein cluster formation induced by multivalent ions
Marcel Wolf (TU Munich, Germany)

O14 : Unravelling the mechanisms of adaptation to high pressure in proteins
Antonino Caliò (LIPhy, Grenoble, France)

15h00-16h15 **Coffee and Posters**

16h15-17h00 **Physics/Chemistry session 2 (Chair Roland Winter)**

O15 : Probing the pressure stability of R-Phycocyanin from red macroalgae Porphyra haitanensis
Simeon Minic (University of Belgrade, Serbia)

O16 : Probing biomolecular conformational landscapes by High-Pressure NMR spectroscopy (HP-NMR)
Ewen Lescop (ICSN, Gif-sur-Yvette, France)

19h30-23h00 **Gala dinner: Brasserie Georges** (30 Cour de Verdun Perrache, 69002 Lyon)

12th HPBB Program

October, 3rd

8h30 **Welcome**

8h50-10h00 **Physics/Chemistry session 3 (Chair Roland Winter)**

O17 : Effects of pressure, temperature, salts, and osmolytes on biomolecular liquid-liquid phase separation
Ryo Katahara (Ritsumeikan University, Japan)

O18 : High pressure effects of nanoplastics on structural integrity of lipid membranes
Judith Peters (LIPhy, Grenoble, France)

O19 : Inhibitor binding in rare excited states of the Ras oncogene protein revealed by high pressure macromolecular crystallography
Nathalie Colloc'h (Caen University, France)

10h00-10h30 **Coffee and Posters**

10h30-11h45 **Physics/Chemistry session 4 (Chair Ryo Kitahara)**

O20 : Next steps towards the understanding of the drivers of evolution to HHP adaptation in Archaea
Phil Oger (INSA Lyon, France)

Keynote 4 : Life in Multi-Extreme Environments - A Physicochemical View
Roland Winter (TU Dortmund, Germany)

11h45-12h00 **Presentation of next HPBB**

12h00 **Conference closing and farewell**

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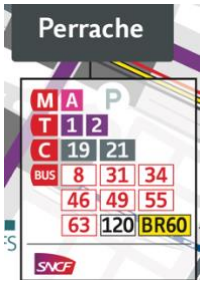
Gala dinner

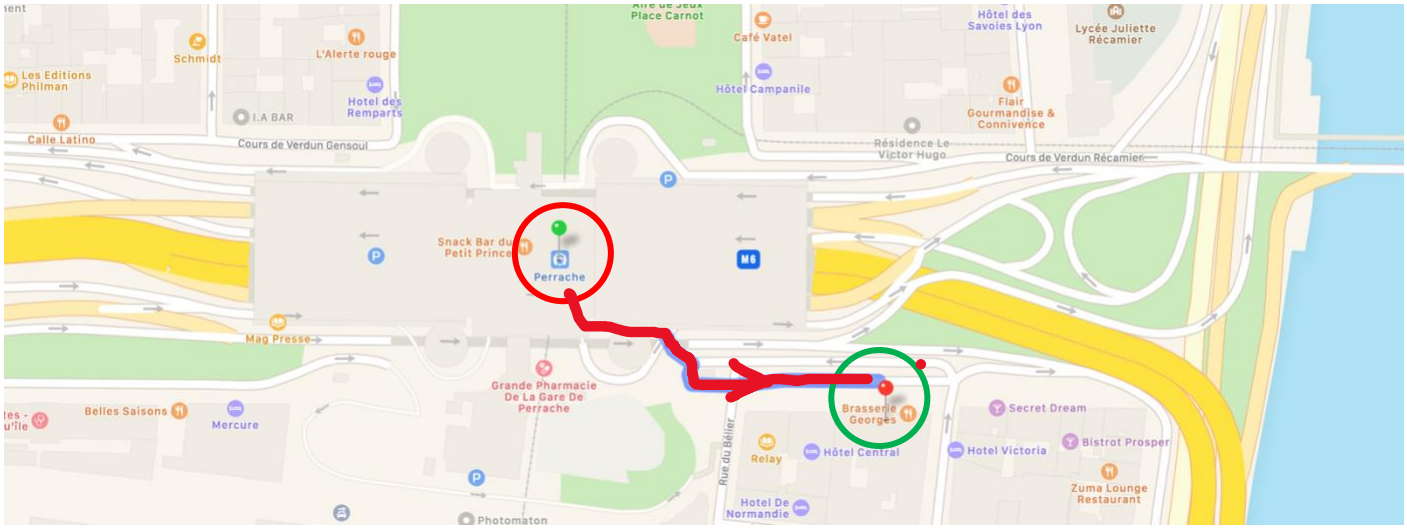
The gala dinner will take place at the
Brasserie Georges
30 Cour de Verdun Perrache, 69002 Lyon
from 19h30 to 23h00

Brasserie Georges has been a Lyonnais institution since 1836 and is a benchmark for Lyonnais cuisine.
Its motto has always been "Good beer, Good food"

The brasserie is located in the Perrache railway station neighbourhood.
It is is very easily accessible by public transports.

Metro line A: Perrache station
Tram T1 and T2 : Perrache station
Bus C19 and C21 : Perrache bus stop





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Posters

Food Sciences

F1 : **Cheng Yuchun** (National Taiwan Ocean University, Taiwan)
Effects of High Hydrostatic Pressure Treatment on Microbial Flora and Chemical Characteristics in Coffee Cherry

F2 : **Tavares Jéssica** (University of Aveiro, Portugal)
Hyperbaric food pasteurisation and preservation as a methodology to increase shelf-life with colour changes in raw poultry meat

F3 : **Matos Gabriela** (University of Aveiro, Portugal)
Hyperbaric storage of egg yolk to assure microbiological safety while minimizing impacts on functional and quality parameters

F4 : **Lopes Rafaela** (University of Aveiro, Portugal)
Moderate Pressure Pasteurization of Raw Red Meat: Color and Texture changes

F5 : **De Lamballerie Marie** (Ecole Nationale Vétérinaire, Agroalimentaire et de l'Alimentation Nantes-Atlantique, France)
High hydrostatic pressure as a denaturing treatment for easy-to-prepare gels from soybean and amaranth proteins

F6 : **Chalain Anatole** (Ecole Nationale Vétérinaire, Agroalimentaire et de l'Alimentation Nantes-Atlantique, France)
Development of a pressure spectrophotometer system for applications in understanding pressure phenomena in food science

F7 : **Chevalier-Lucia Dominique** (Université de Montpellier, France)
High pressure-induced nanoaggregates of potato proteins to generate curcumin-loaded complexes

Microbiology

M1 : **Saraiva Jorge** (University of Aveiro, Portugal)
Portugal Hyperbaric storage at room-like temperatures as a new food preservation methodology to inhibit spores' germination and development – the case-studies of *Bacillus subtilis* and *Clostridium perfringens* spores

M2 : **Erdem Furkan** (METU-Food Engineering Department, Turkey)
Predictive Approach on Inactivation of *E. coli* O157:H7 using high hydrostatic pressure (HHP) processing

fects on acceptability of cooked ham

Physics/Chemistry

P1 : **Martins Ana P** (University of Aveiro, Portugal)
Portugal Hyperbaric storage as a cell preservation methodology – Can blood be stored under pressure?

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Food Sciences

Keynote : Tomas BOLUMAR (Max Rubner-Institut, Germany)
High-pressure Processing (HPP) of Meat Systems: Molecular Changes, Quality Aspects and Industrial Applications

Oral presentations

O1 : **Vasco Lima** (University of Aveiro, Portugal)
Impact of hyperbaric storage and moderate pressure pasteurization on the volatile compounds of watermelon juice

O2 : **Avi Shpigelman** (Israel Institute of Technology, Israel)
The effects of nonionic kosmotropes under high pressure: implications for protein-flavonoid non-covalent Interactions

O3 : **Alireza Mousakhani Ganjeh** (University of Aveiro, Portugal)
Moderate-pressure-pasteurization of salmon in combination with oxygen scavenger: a new strategy for improving color

O4 : **Alpas Hami** (METU-Food Engineering Department, Turkey)
Development of a Secondary Model for Full Geeraerd Under High Hydrostatic Pressure

O5 : **Laurence Pottier** (Ecole Nationale Vétérinaire, Agroalimentaire et de l'Alimentation Nantes-Atlantique, France)
High pressure combined with biopreservation: effects on acceptability of cooked ham

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High pressure-induced nanoaggregates of potato proteins to generate curcumin-loaded complexes

High-pressure Processing (HPP) of Meat Systems: Molecular Changes, Quality Aspects and Industrial Applications

Bolumar Tomas*[1]

[1]Max Rubner-Institut. Department of Safety and Quality of Meat. Kulmbach – Germany

Abstract

HPP is the most successfully implemented non-thermal processing technology in the food industry. This presentation gives a comprehensive overview of HPP applied to meat systems, with meat products representing about a quarter of the HPP foods. The extensive research undertaken during the last three decades has characterised the different effects of HPP on meat systems, along with witnessing its industrial uptake and global commercial expansion. This talk introduces the fundamental principles of HPP for preservation and its effect on meat's molecular components and quality traits. Furthermore, it outlines HPP technology's current main industrial applications in the meat sector. Highlighting other promising applications, such as HPP for enabling salt reduction, *sous-vide*-like meat tenderisation and advances towards its use as an industrial sterilisation method, will be presented to the audience to encourage reflection and debate about the HPP method in future meat processing.

Keywords: high pressure, meat products, shelf life, tenderisation, salt reduction, sterilisation

*Speaker

Impact of hyperbaric storage and moderate pressure pasteurization on the volatile compounds of watermelon juice

Vasco Lima*[1], Rebeca Cruz[2], Susana Casal[2], and Jorge Saraiva†[1]

[1]LAQV-REQUIMTE, Chemistry Department, University of Aveiro – Portugal
[2]LAQV-REQUIMTE, Department of Chemistry, Faculty of Pharmacy, University of Porto – Portugal

Abstract

Hyperbaric storage (HS) is an emerging method for food preservation under mild pressures at room temperature (RT), offering a potential alternative to conventional refrigeration. This technique promises virtually no energy costs and substantially reduced greenhouse gas emissions. More recently, moderate-pressure pasteurization (MPP) has been explored for its pasteurization capabilities, using hydrostatic pressures up to 200–250 MPa for a few hours. Watermelon juice, with its rich nutritional profile - vitamins, minerals, functionally important amino acids, carotenoids, and phenolic compounds - is known for its health benefits. However, it is highly perishable due to its high pH (5.2–6.7) and water activity (0.97–0.99), resulting in rapid microbial growth and enzymatic activity, and a short shelf-life. Its aroma, a key quality attribute, consists mainly of volatile C6 and C9 aldehydes, ketones, and alcohols.

This work aimed to study the impact of these pressure-based methodologies on watermelon juice by analysing samples through headspace solid phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS). Two separate studies were conducted: 1) watermelon juice treated with MPP at RT (18-23 ∘C) for 3 hours at 200 MPa, and 2) watermelon juice stored for 4 weeks either under HS at RT (18-23 ∘C) at 75 MPa or under conventional refrigeration at 4 ∘C and atmospheric pressure (control). The results showed that MPP at RT did not significantly alter the overall volatile content of the WJ. Still, some volatile compounds were significantly affected, and new volatiles were detected. During the 4-week storage, refrigerated juice exhibited a higher concentration of compounds associated with microbial activity (like ethanol), an increase in total volatile content (_˜41%) was influenced by the rise in alcohols (mainly (Z)-3-nonen-1-ol and (E,Z)-3,6-nonadien-1-ol) and terpenoid content and a decrease of aldehydes like hexanal (and the disappearance of several aldehydes detected at lower concentrations in the fresh WJ), with ketones remaining relatively stable; in contrast, HS resulted in a decrease in total volatile content (_˜37%), primarily due to reduced alcohol levels, while ketones and terpenoids increased (especially due to 6-methyl-5-hepten-2-one and geranial, respectively), and aldehyde levels remained largely unchanged.

Although HS did not successfully preserve the characteristic volatiles of WJ through time, MPP at RT was able to maintain the fresh WJ volatile profile with minimum changes. This suggests that MPP is a viable alternative to the traditional thermal treatments, preserving

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the aroma while offering a more environmentally friendly process with minimal energy requirements.

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Thanks are also due to FCT/MCTES for financing the PhD grant of Vasco Lima (SFRH/BD/146080/2019) and the CEEC contract of Rebeca Cruz (2022.00965.CEECIND).

Keywords: watermelon juice, hyperbaric storage, moderate pressure pasteurization, volatile compounds

The effects of nonionic kosmotropes under high pressure: implications for protein-flavonoid non-covalent Interactions

Shahar Plaut , Zoya Okun[1], and Avi Shpigelman*†[1]

[1]Technion - Israel Institute of Technology [Haifa] – Israel

Abstract

Kosmotropic co-solutes, known for their ability to increase the structural order of water, play a significant role in stabilizing proteins against various external stresses, including temperature and high hydrostatic pressure. Many salts are well-known kosmotropic agents that affect protein stability. Some carbohydrates, polyalcohols, and uncharged organic polymers also demonstrate kosmotropic abilities. It has been suggested that nonionic kosmotropes maintain the structural and functional integrity of proteins by being excluded from their hydration layer or surface area. As a result, the native protein structure is kept, and the solubility lowers due to a preferential exclusion of cosolvent from the protein surface. It was reported that the secondary protein structure unfolding by pressure significantly differs from thermal. It is also well known that flavonoids can non-covalently interact with proteins, and such interaction can be affected by external factors such as increased temperature, due to the modification of protein structure in solution. On the other hand, very little information, if any, exists on the impact of pressure on non-covalent protein-flavonoid interactions, despite the fact that such interactions can affect both the protein and flavonoid techno-functionality (digestibility, solubility, antioxidant capacity, stability of the flaovnoid etc.). To our knowledge, scarce attempts, if any, were made to study the impact of pressure, protein-flavonoid physical interactions, and nonionic kosmotropy to better define the possible outcomes of pressure on protein structure and physical interactions.
Our study investigated the impact of nonionic kosmotropes, specifically sucrose, on the interactions between ovalbumin (used as a model protein) and a series of flavonoids with varying functional groups under high-pressure processing conditions. The objective was to elucidate how sugars, as nonionic kosmotropes, modulate protein-flavonoid interactions under high pressure compared to their effects at elevated temperatures. Additionally, we aim to correlate these interactions with the kosmotropic effect of sugar in a binary (protein-sugar) system under pressure.

Circular dichroism (CD) spectroscopy confirmed changes in protein secondary structure upon heating (65∘C-90∘C) and high-pressure application (300-500 MPa). The addition of 1M sucrose significantly preserved the α-helical content in the protein structure after both thermal and high-pressure treatments at different intensities. This preservation of the original structure was also observed as a smaller particle size increase after thermal and pressure treatments due to aggregation, with the kosmotropic effect being more pronounced under high-pressure conditions than during thermal treatment. Surface hydrophobicity increased

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more than 10-fold due to thermal and pressure treatments; however, this increase was significantly mitigated by the presence of sugar, highlighting the kosmotropic effect.

Our findings indicate that flavonoids interact non-covalently with ovalbumin in a structure-dependent manner, with glycoside presence and flavonoid planarity reducing binding. Both high-pressure and thermal treatments enhanced flavonoid-ovalbumin interactions (up to more than 100% increase in relative binding), without causing significant flavonoid degradation. Notably, thermal processing led to a greater increase than high-pressure processing (HPP), in line with the effect on surface hydrophobicity. The presence of sucrose at 1 M didn't affect the binding where no external stress was applied, yet the kosmotropic effect significantly reduced the increase in interaction, and at high pressure (20 min at 500 MPa), the interaction with 1M sucrose was not different than the interaction without the application of pressure. A notable correlation was observed between flavonoid structure-dependent hydrophobicity and the effects of sucrose and HPP on the ternary system, where fluorescent quenching confirmed that hydrophobic forces primarily governed these interactions.

This study also confirmed that ovalbumin's innate antioxidant capacity might be enhanced under high pressure without significant interference from flavonoid interactions.
Our study highlights the significant influence of kosmotropic effects on regulating protein stability and functionality at high pressure. These findings hold both fundamental significance and potential applications in the development of advanced nutraceutical formulations and the broader comprehension of osmolyte utilization in food and pharmaceutical sciences.

Keywords: High pressure, kosmotropy, protein interactions, flavonoids

Moderate-pressure-pasteurization of salmon in combination with oxygen scavenger: a new strategy for improving color

Alireza Mousakhani Ganjeh*[1,2], Carlos A. Pinto[1], Susana Casal[2], and Jorge A. Saraiva†[1]

[1]LAQV-REQUIMTE, Department of Chemistry – University of Aveiro, 3810-193 Aveiro, Portugal
[2]LAQV-REQUIMTE, Department of Chemistry, Faculty of Pharmacy, University of Porto, 4050-313 Porto – Portugal

Abstract

Recently, a novel pressure-based food preservation methodology (found during hyperbaric storage/HS experiments), called low-pressure-long-time (LPLT) or Moderate Pressure Pasteurization (MPP), revealed potential to inactivate microorganisms at the level of pasteurization (extending shelf-life), by hyperbaric inactivation/HI, on protein-rich foods that are heat and pressure sensible, with minor/no changes in fresh-like characteristics.
This work aimed to evaluate the physical properties of salmon pasteurized by MPP in combination with an oxygen scavenger (OS) to minimize the presence of oxygen. For this work, a pressure level of 100 MPa at room temperature (18-23 ^{o}C) was used to posterize packaged salmon (with/without OS) for up to 7 days.

The results showed that using OS led to a significant 24% reduction in total color changes compared to MPP treatment without OS. This effect can be attributed to the removal of oxygen from the samples. According to the suppliers of the OS products, these oxygen scavengers can reduce the level of oxygen within the packaging from the 20.9% normally present in the air to less than 0.01%. By effectively reducing oxygen inside of the packaging, oxidation could be minimized, resulting in improved color retention.
Regarding drip loss, it is evident that the use of OS in conjunction with MPP resulted in a reduction in salmon drip loss compared to using MPP alone. After 1, 3, and 7 days of treatment time in MPP/OS conditions, the reduction percentages were 64, 40, and 39%, respectively, compared to using MPP without OS over the same treatment times. In terms of cooking loss, a similar pattern was observed, with the amount increasing over time for all treatments. It was noted that using MPP/OS, there was a lower cooking loss compared to MPP without OS. The results showed that the maximum total loss (the sum of drip loss and cooking loss) was achieved in the samples treated by MPP and 7 days under pressure (32%). However, using MPP in combination with OS shows that total loss was 14, 22, and 24%, respectively, at the first, third, and seventh days of storage under pressure, which is significantly lower than MPP without OS. High pressure processing (HPP) often leads to increased drip loss and reduced water-holding capacity (WHC). This is primarily attributed to the disruption of proteins' electrostatic and hydrophobic interactions, resulting in changes in protein conformation and denaturation of important myofibrillar and/or sarcoplasmic proteins, ultimately leading to exudation from the muscles. As a result of using OS, it was

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observed a decrease in drip loss, cooking loss, and total loss which may be attributed to the ability of OS to reduce oxygen content with this decreasing protein disruption during but pasteurisation. Also, the findings indicated that WHC decreased with longer storage time in both treatments. Nevertheless, WHC in samples treated with MPP/OS was notably higher compared to those treated with high pressure alone (MPP). The WHC results demonstrate that the use of an oxygen scavenger can effectively reduce protein disruption caused by MPP. This, in turn, can enhance water holding capacity and minimize drip, cooking, and total loss in salmon. Over 1, 3, and 7 days of storage under pressure, the reduction in WHC was 0.84, 0.18, and 1.01% respectively, as compared to using MPP in combination with OS over the same storage periods. Additionally, the results revealed a linear correlation between cooking loss and WHC (WHC=-0.317(cooking loss)+99.856 (R2=0.9354)). The TPA results indicated that the use of an oxygen scavenger can affect the texture properties of salmon. In terms of hardness, the highest level of hardness was observed for MPP/OS after 7 days at 100 MPa. The findings for MPP/OS demonstrated that an increase in treatment time resulted in increased hardness. Conversely, the hardness of MPP without OS exhibited different patterns, with hardness decreasing over the storage period. This divergence in behavior may be attributed to accelerated protein denaturation in the MPP without OS samples. The increase in hardness of pressurized fish muscles and the strengthened myofibrillar structure may be attributed to the unfolding of actin and sarcoplasmic proteins, as well as the formation of new hydrogen-bonded networks. However, when high pressure is applied without OS, it leads to the denaturation of muscle proteins and adversely affects the microstructure of salmon muscle, resulting in decreased hardness. These results also align with the outcomes of WHC and cooking loss tests, indicating that MPP with protein denaturation may lead to increased cooking loss and reduced WHC. Overall, MPP/OS has proven to be a highly promising and effective pasteurization method, offering several benefits with minimal physical property alterations in salmon.

Keywords: Food preservation, Moderate, pressure, pasteurization, oxygen scavenger, Physical Properties, Fish

Development of a Secondary Model for Full Geeraerd Under High Hydrostatic Pressure

Hami Alpas*†[1,2] and Furkan Erdem[2]

[1]Hami Alpas – Turkey
[2]METU-Food Eng.Dept. – Turkey

Abstract

Aim: This study aims to refine the Full Geeraerd model for microbial inactivation under High Hydrostatic Pressure (HHP) in tomato juice. The goal is to enhance the accuracy of the model for predicting inactivation at these pressure levels and reparametrize the equation to enable its use in a variety of pressures.
Method: Tomato juice was inoculated with E. coli O157:H7 (ATCC 43888) at a concentration of approximately 10ˆ8 cfu/mL. HHP treatments were applied at 150-450 MPa and validated at 200-400 MPa for durations of 5 to 25 minutes. Enumeration of E. coli O157:H7 was performed using tryptic soy agar with 0.6% yeast extract as non-selective media and MacConkey agar as selective media. Incubation periods were 18-24 hours for non-selective media and 36-48 hours for selective media. The Full Geeraerd model, parameterized with kmax and Nres, was evaluated for its effectiveness under these conditions.

Results: Reparametrized Full Geeraerd model was not sufficient for accurately predicting microbial inactivation at pressures below 250 MPa. At 200 MPa, a 0.5 log reduction was observed after 5 minutes, and a 0.7 log reduction after 10 minutes. At 300 MPa, a 2.5 log reduction was achieved after 5 minutes, and a 2.9 log reduction after 10 minutes. At 400 MPa, a 4.2 log reduction was observed after 5 minutes, and a 4.8 log reduction after 10 minutes. Adjustments to the model parameters were necessary to improve prediction accuracy at these lower pressures.

Conclusion: The refined Full Geeraerd model now provides a more accurate representation of microbial inactivation under HHP conditions, particularly at pressures below 250 MPa. Accurate model parameterization is crucial for ensuring the reliability of HHP as a non-thermal food processing method. Future research should focus on optimizing pressure levels and processing times to further enhance microbial safety in food products.
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Keywords: HHP, Secondary model, Foodborne pathogens, E.coli O157:H7

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High pressure combined with biopreservation: effects on acceptability of cooked ham

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Abstract

Current health recommendations aim to reduce sodium nitrite levels in cured meats. In this context, we studied the stabilization of "home-made" nitrite-reduced cooked ham (1) using biopreservation combined with high pressure.
Cooked ham was "home"- manufactured (1). The muscle used was Longissimus dorsi (4 days post-mortem, pH between 5.5 and 6). Brining was carried out by adding 10 % brine to the meat mass (Nitrite: 25 ppm; NaCl: 1.76 %; Lactose: 0.22 % and Sucrose: 0.07 %). Batch mixing was carried out in a paddle mixer (GLASS GmbH & Co. Co, Frankfurt, Germany), for 17 h (work: 7.5 rpm - 15 min, rest: 15 min). The brined meat pieces were then molded and vacuum-packed. Cooking was carried out in stages to reach 65 ∘C (core temperature). The cooked hams were cooled at room temperature for 20 min, stored at 4 ∘C for 3 days and then cut into 1.5 cm cubes. Control samples were vacuum-packed and stored 10 days at 4 ∘C followed by 20 days at 8 ∘C (accelerated aging). Treated samples (biopreserved and pressurized BLc+HP) were first inoculated by spraying a suspension (106 CFU/g of ham) of the protective strain Lactococcus lactis CH-HP15 (2), then placed at 4 ∘C for 1 h, and transferred to PA/PE bags and vacuum-packed (80 mbar) for high-pressure processing (1).

Enumerations were realized on PCA (total aerobic mesophilic counts) and M17 (lactococci counts) plates. After enumeration, 15 to 20 colonies were picked from PCA plates for identification (Isolation on FTA® membrane, 16S rDNA PCR amplification then sequencing).

A sensory evaluation was carried out to assess the odor (triangular test, 30 untrained panelists), taste and global appreciation (hedonic test, 60 untrained panelists) of ham (control and BLc+HP).

The bacterial enumeration after accelerated aging showed a progressive increase in mesophilic aerobic counts that was similar in the control and treated samples. Moreover, most aerobic bacteria count was composed of lactic bacteria. To assess the efficiency of the combined treatment, species identification was carried out on the biopreserved and pressurized samples at D+1, D+15 and D+30. The results of the identification showed that at D+1 and D+15, 100 % of the bacteria isolated corresponded to L. lactis, the bioprotective strain. However, at D+30, we observed the growth of another species of lactic acid bacterium, Carnobacterium

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sp. (33 %).

Regarding olfactory sensory analysis, the judges noted no significant difference in odour between the samples at D+1 but perceived significant differences at D+15. A hedonic analysis showed a clear preference regarding the overall appreciation and the taste of the treated product. The panelists noted the development of milky and/or fruity aromas.

The use of L. lactis CH-HP15 combined with high-pressure treatment would be an interesting process for maintaining the microbiological quality of nitrite-reduced cooked ham. After high-pressure treatment, L. lactis could grow and dominate the endogenous microbiota during storage, inhibiting spoilage bacteria and pathogens, although other bacterial species could be observed at the end of the shelf-life, as previously observed (2). However, modification of odours probably linked to the metabolic activity of L. lactis was also observed as storage progressed. It would be interesting to identify the volatile compounds formed.

Further studies remain to be carried out in order to promote the recovery of the biopreservation bacterium and to verify on different products the possibility of extending the shelf-life of meat products even in the absence of nitrites or other preservatives.

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Keywords: combined treatment, acceptability, ham

Effects of High Hydrostatic Pressure Treatment on Microbial Flora and Chemical Characteristics in Coffee Cherry

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Abstract

To alter or enhance flavors, it is common to add strains for fermentation or use emerging pretreatment methods to change fermentation flavors. The purpose of this study is to use high hydrostatic pressure processing (HPP) to change the coffee structure and alter the action of endogenous microorganisms, hoping to shorten the drying time and change the microbial flora of coffee, resulting in a change in flavor. During different fermentation periods, the moisture content of the Control (CON), 100 MPa, and 200 MPa groups showed a decreasing trend. Scanning electron microscopy analysis indicated structural damage caused by HPP, making the drying rate faster from day 0 to day 4 compared to the CON. HPP reduced the number of mesophilic and lactic acid bacteria, with slight changes to the microbial flora of cherry coffee. 16S rRNA sequencing identified two major categories of microorganisms: the family *Leuconostocaeae* and the *Enterobacteriaceae*. *Leuconostoc* showed the highest relative abundance at both the beginning and end of fermentation in all three groups, making it the most common genus during the coffee fermentation process. ITS sequencing identified only one yeast species *Hanseniaspora* commonly found in coffee cherry. Sensory evaluations of the three groups revealed unique characteristics: the CON displayed caramelized flavors. After the 100 MPa treatment, a tropical fruit profile emerged. Following the 200 MPa treatment, floral notes were observed, although the specific types of floral aromas could not be precisely identified. In the electronic tongue analysis, the 100 MPa treatment had a higher astringency, the 200 MPa treatment had a more pronounced bitterness, and body was also higher than the other two groups. Overall, the populations of mesophilic and lactic acid bacteria decreased due to high pressure. The microbial flora consisted of *Leuconostocaeae*, *Enterobacteriaceae*, and *Hanseniaspora*. In sensory evaluations, the CON showed a caramel flavor, whereas treatment at 100 MPa resulted in a peppery taste, and treatment at 200 MPa produced a floral aroma. Electronic tongue analysis indicated higher astringency at 100 MPa and increased bitterness at 200 MPa. High hydrostatic pressure treatment induced changes in the microbial flora of coffee cherry, while also altering the coffee's flavor characteristics.

Keywords: High hydrostatic pressure processing, Coffee cherry, Fermentation, Next generation sequencing, Sensory evaluations

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Hyperbaric food pasteurisation and preservation as a methodology to increase shelf-life with colour changes in raw poultry meat

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Abstract

Global meat consumption is shifting towards poultry as a result of major changes in human eating habits over the past three decades and, is expected to account for 41% of the global meat market by 2030 (OECD/FAO, 2021).
The quality of poultry meat is made up of many characteristics, with colour (described as pale, pink or yellow) being one of the most important and is often used as an index of quality and a determining factor for consumers when making a purchase (Altmann et al., 2023; Pathare et al., 2013). The poultry microbiome is a complex and dynamic community of spoilage and pathogenic microorganisms that are well established from the day of hatching to the day of slaughter, through all stages of food retailing and up to consumption. Conventional preservation methodologies (such as chilling, freezing, vacuum packaging and modified atmosphere packaging) have been shown to be not fully effective in maintaining the microbiological quality and safety of poultry meat and to prevent colour changes during storage. The aforementioned hurdle technologies only delay microbial development and current pasteurisation methodologies (either thermal or non-thermal pasteurisation by high pressure processing) alter the physicochemical properties of the meat to give a cooked-like appearance or cause significant colour changes in fresh meat, making it less appealing to consumers (Santos et al., 2021).

The aim of this research was to study the effect of hyperbaric preservation (food storage under low pressure between 25 and 100 MPa, for days or months) and moderate pressure pasteurisation (low pressure between 100 and 200 MPa, up to 72 hours), two pressure-based

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methodologies, on the quality parameters of raw poultry breast meat, such as colour and pH variation, as well as the evaluation of the endogenous microbiome, allowing its commercialisation as safe(r) raw meat with extended shelf-life. Colour and pH parameters were evaluated on raw poultry breast meat vacuum packed at two pressure level (100 and 125 MPa) at 20-25 ∘C, for up to 28 days. In addition, the endogenous microbiome was also evaluated under the same experimental conditions, considering the main groups of spoilage microorganisms (total aerobic mesophilic and facultative anaerobic microorganisms, *Pseudomonas* spp., Enterobacteriaceae, coliforms and *Escherichia coli*, lactic acid bacteria, yeasts and moulds).

The results showed that pressures of 100 and 125 MPa have a significant effect on surface colour even after 24 hours under pressure, with a colour difference of 9.779 ± 1.655 and 14.281 ± 1.453, respectively, and to a lesser extent in the refrigeration control samples (3.478 ± 1.180). Nevertheless, the small variation observed is perceived by the human eye and influences the consumer's decision. At 100 MPa the colour difference remained stable up to 14 days under pressure, and after 28 days of storage, a similar colour difference was observed as after 24 hours. At 125 MPa, the colour difference gradually increased with time, remaining similar for the first 7 days under pressure and then increased significantly up to 28 days (19.930 ± 1.595). At refrigerated temperature and atmospheric pressure (4 ∘C—0.1 MPa) the colour differences ranged from 1.896 ± 0.941 to 4.549 ± 1.904 at 2 and 21 days of storage, respectively. Regarding the pH results, at 100 MPa the values remained constant, while at 125 MPa an increasing pattern was observed, and for the refrigerated samples the pH values showed no clear pattern. Considering the endogenous microbial load, the results showed that 125 MPa was more effective than 100 MPa in inactivating bacteria and fungi. The detection level of 1.60 or 2.60 log CFU/g meat (depending on the enumeration method) was reached for all microbial parameters evaluated after 28 days or even earlier, depending on the pressure applied. Under refrigerated conditions, the endogenous microorganisms increased over a period of up to 28 days, reaching the microbiological limits for rejection concerning total aerobic and anaerobic facultative microorganisms, as well as lactic acid bacteria groups.

These results show that, depending on the final objective of the food technologist and the industry, always bearing in mind the consumer demands and expectations, it is possible to commercialise pressurised poultry meat as a raw product after 1 to 28 days under low-mild pressures at 20-25 ∘C without any safety concerns. In fact, even instrumentally assessed colour changes may be acceptable to the consumer, as the acceptable colour range depends on a variety of factors such as consumer preferences or characteristics, including age, ethnic origin, and the environment at the time of purchase.

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Keywords: raw poultry meat, food safety, hyperbaric storage, moderate pressure pasteurisation, colour, food quality

Hyperbaric storage of egg yolk to assure microbiological safety while minimizing impacts on functional and quality parameters

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Abstract

Hen egg yolk is an important ingredient in a wide range of food products, due to its excellent functional properties and pleasant organoleptic characteristics. Salmonellosis is recognized to be one of the principal infections transmitted by egg products, and combining with being a highly perishable ingredient it is necessary to be thermally pasteurized. Nevertheless, this process usually causes considerable detrimental effects on functional properties and refrigeration is still needed to extend shelf-life. (1) Moreover, refrigeration is highly reported to have significant economic and environmental impacts. (2)

Hyperbaric storage is a novel pressure-based food preservation methodology that uses low pressures (25-100 MPa), being a possible alternative to refrigeration, since it can inhibit microbial population of many food products, at room temperature, maintaining quality properties. In addition, since almost no energy is needed to store food products under pressure, it has the potential to be a more environmentally sustainable food preservation methodology. (3) Therefore, the aim of this work was to evaluate hyperbaric storage as a nonthermal preservation methodology on egg yolk, to assure its microbial safety and minimizing the impacts on functional properties, thus, avoiding thermal pasteurization and evaluate at what extent this methodology can replace RF.

Experiments at different pressure levels (50/75/100 MPa) at room temperature (18-23ºC) were carried out up to 60 days in egg yolk. Microbiological evaluation was assessed through counts of inoculated pathogenic microorganisms (≈7.3 log CFU/mL), namely *Salmonella enterica* and *Staphylococcus aureus*, for the established pressure/time conditions. Then, egg yolk was evaluated for functional and quality parameters, being chosen for this purpose the condition with lower pressure to minimize the impact on the matrix, but still ensuring microbiological safety. Thus, its pH, color, soluble protein content, free sulfhydryl content, and viscosity were evaluated during storage at 50MPa, and compared to refrigeration.

Microbiological results showed that during hyperbaric storage the inoculated population in egg yolk, *S. enteritidis* and *S. aureus,* were reduced, in most cases, to counts below 1 log CFU/mL. At 50 MPa, it was achieved a 3.43 log reduction of *S. enteritidis* and 5.20 log

*Speaker

reduction of *S. aureus*, after 60 days of storage. At 75 and 100 MPa, after 60 days, both microorganisms were reduced more than 5 log units.

Regarding the functional properties assessed in egg yolk, apparent viscosity showed a slight increase of 1.14-fold after 60 days at 50MPa. Despite this, when compared to refrigeration, pH, color, soluble protein, and free sulfhydryl content maintained unchanged in most cases. This indicates that this innovative nonthermal pressure methodology has great potential to assure microbial safety, while maintaining the egg yolk quality properties, comparable to those maintained through refrigeration.

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Keywords: egg yolk, hyperbaric storage, food safety, proteins

Moderate Pressure Pasteurization of Raw Red Meat: Color and Texture changes

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Abstract

Moderate Pressure Pasteurization (MPP) has emerged as a promising pressure-based technique for pasteurization of highly perishable and heat/pressure-sensitive foods, such as raw red meat (1). This method offers several advantages for the product, including a higher microbial inactivation/safety, leading to extended shelf life; superior energy efficiency, as energy is only required for pressurization and depressurization; and no need for temperature control. MPP is also superior to the other pressure-based pasteurization method, HPP, High Pressure Processing, which has already been shown to have a significant and undesirable effect on high-protein foods such as meat (2). The main issue of HPP is the alteration of product's colour and texture due to the protein unfolding and denaturation at higher pressures (3).
Therefore, the aim of this work was to evaluate the effect of MPP on the colour and texture-related proteins (sarcoplasmic and myofibrillary, respectively) of raw meat, important parameters for the consumer.

Red raw meat (pork and bovine) was pasteurized using MPP (125 and 200 MPa) at room temperature (RT) and refrigeration temperature (RF) for up to 48 hours. The color and texture of both raw and cooked red meat were evaluated. Additionally, analyses were conducted to determine the amount of sarcoplasmic and myofibrillar proteins, as well as the changes in these proteins induced by MPP.

At first glance, the results showed that MPP can be used to pasteurize meat with negligible impact on its properties unlike HPP; in fact, at 125 MPa, the differences were imperceptible to the consumer, especially in color and texture and even at 200 MPa the effect on the appearance of the meat was minimal. Also, these colour differences in red meat vanished when the product was cooked.

Analysis of the protein quantity indicated a decrease in concentration, with a higher impact for pork meat and in the sarcoplasmic proteins, but this impact remained constant for hours long. The study also revealed that protein and lipid oxidation occur, but leans to stabilization, preventing further nutrient loss.

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The use of MPP showed an impact that was independent of pressurization time. This could be an important breakthrough, because it allows to extend the time of pressurization to reach a higher impact in microbial safety without affecting the nutritional value of meat.

Overall, MPP proved to be a highly promising and effective pasteurization method for sensitive proteinaceous products, offering substantial benefits with minimal changes in red meat.

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Keywords: Red Meat, Moderate Pressure Pasteurization, Proteins, Oxidation

High hydrostatic pressure as a denaturing treatment for easy-to-prepare gels from soybean and amaranth proteins

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Abstract

One of the most important components for proper human nutrition is good quality proteins since an adequate intake of essential amino acid is required, especially in populations that have a tendency to lose muscle mass, such as the elderly or patients with certain diseases. At the same time, one of the issues of these populations can be dysphagia, which is treated with safe swallowing foods, such as thickened liquids or soft solids, to avoid aspiration. The alimentary matrixes such as gels are a good option for this kind of foods which can be obtained in a variety of ways. An interesting manner is a cold-set gelation, which requires a three steps processes: first, a protein dispersion at low concentration is submitted to denaturation, in a second step, the dispersion is dried and finally the gel is obtained just by the addition of water.
High hydrostatic pressure (HHP) is a technology with different applications in the food industry, among which is its potential to generate changes in the structures of biopolymers such as proteins. HHP at 600 MPa (or higher) is capable of completely denature proteins by affecting Van der Waals forces, hydrophobic interactions and hydrogen bonds. These changes can lead to protein aggregation, which in turn can be useful for the formation of a gel matrix.

Some plant proteins present good gelling capacity when subjected to certain treatments. Soybean proteins have been largely studied because they have a good amino acid composition, and exhibit an interesting gelling ability. On the other side, amaranth is an Andean pseudo cereal with high protein content (12-18 %wt), well balanced amino acid profile and some peptides released upon digestion exert beneficial effects on human health even dough its incorporation in current diets is emerging. Since the main globulins in in amaranth protein isolate (API) and soybean protein isolate (SPI) share the structure (legumin- and vicilin-like), they may form mixed aggregates when subjected to denaturing treatments, and develop versatile technological properties.

The aim of this work was to evaluate the cold-set gelation ability of different mixtures of SPI and API denatured by HHP in the presence of calcium, by applying an experimental central composite design with two factors, API:SPI (10:70-30:70) and Ca:protein ratios (0.075-0.250

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mmolCa:g protein). The gels were evaluated according to their rheological characteristics, water holding capacity (WHC), and color in order to find an optimal condition for the production of a powder that easily generates gels. Protein dispersions were prepared at 4 %wt total protein, with the corresponding CaCl2 concentrations, neutralized (with NaOH), packaged in polyethylene bags for HHP processing (600 MPa – 5 min – 20 ∘C), and then they were freeze dried. Finally, the powders were dispersed in water at 13 %wt total protein for gel formation assays. Results were analyzed by multiple response surface.

The WHC adjusted to a linear model and SPI:API was the only significant factor, increases in API proportion led to a decrease, probably due to excessive aggregation of amaranth protein and consequent decrease in water-protein interactions. The values found were within a range from 42.2 ± 0.4 % to 99.4 ± 0.4 %, the minimum corresponded to a API:SPI ratio of 30:70, meanwhile the maximum corresponded to 10:90 even so, these results implied a high WHC given that the determinations were carried out at very intense conditions (15000 g - 15 min – 4 ∘C). The same behavior was found for apparent viscosity (γ=50 s-1), which decreased, as did thixotropy, with increasing API:SPI ratio. Luminosity fitted to a quadratic model, with increases as API:SPI and Ca:P ratios increased, which was due to the increase in the fraction of insoluble protein. The excessive degree of aggregation of API proteins would generate a discontinuous network and consequently a weak structure, which was evidenced by the decrease in thixotropy. For storage modulus it was not possible to find a significantly fitting surface response, however a negative tendency was observed as the API proportion increased.

Under the studied conditions, despite sharing some structural features, API and SPI proteins do not seem to interact during gelation, but API seems to behave as a filler without contributing to the gel structure. On the other side, for a protein gel to form, not only the content of proteins and their conformation are important, but also a proper balance of attractive and repulsive interactions is necessary, which can be modulated by the addition of ions. At low salt concentrations gelation is difficult to occur due to high electrostatic repulsion between proteins, and an excessive salt concentration could lead excessive aggregation and precipitation. As the Ca:protein ratio increased, the appearance of the gels became opaque and ivory, due to insolubilization.

For the optimization, WHC, storage modulus and viscosity were maximized in order to obtain a structured gel and luminosity was minimized to avoid excessive aggregation. The obtained condition was 10:90 API:SPI ratio with a Ca:protein ratio of 0.25 mmol Ca/g protein. The respective gels presented proper apparent viscosity (0.9 ± 0.2 Pa.s), classified as 1J according to the Japanese Society of Dysphagia Rehabilitation; this level of apparent viscosity is associated with foods such as jelly, pudding or mousse. Also, whit this formulation the gel had a great WHC (82 ± 2 %).

As a control, an attempt was made to form gels with the individual isolates, SPI formed translucent gels with high WHC (99.7 ± 0.2%) and thixotropy (6410 ± 155 Pa/s). However, API samples formed no gels, but precipitated after HHP treatment, with low WHC (25.8 ± 0.4%) and thixotropy (145 ± 5 Pa/s).
As conclusion, it was possible to obtain a gel with high protein content (a 150 g portion would cover 38% of daily protein intake, FAO) using two different plant proteins by applying HHP as denaturing treatment.

Keywords: soy and amaranth proteins, instant gel, denaturation, calcium

Development of a pressure spectrophotometer system for applications in understanding pressure phenomena in food science

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Abstract

High-pressure processing (HPP) is a widely used technique in various industries, particularly in the food sector, where it is applied to enhance food safety and quality by inactivating microorganisms and modifying biochemical and structural properties. Understanding the effects of high pressure on different matrices is crucial for optimizing these processes and ensuring product integrity. This study focuses on the development and utilization of a specialized research installation designed to analyse absorbance spectra of samples subjected to high pressure of up to 600 MPa. The primary objective of this research was to develop a high-pressure cell coupled with an optical fiber spectrophotometer capable of measuring absorbance spectra under controlled temperature and pressure conditions. By applying hydrostatic pressure up to 600 MPa, this set-up aims to investigate the structural, biochemical, and microbiological effects of high-pressure treatment on various samples thanks to absorbance spectra results. The findings from these analyses are intended to contribute to a deeper understanding of the impact of high-pressure processing, particularly in the context of food science.
The research installation consists of a custom-designed high-pressure cell from Unipress Equipment Division (Warsaw, Poland), which is thermoregulated and connected to a manual pump for the application of hydrostatic pressure up to 600 MPa. The cell is integrated with an optical fiber spectrophotometry system through sapphire windows that allow the transmission of light. The light source, comprising both deuterium and halogen lamps from IDIL Fibres Optiques (Lannion, France), generates a beam that traverses the sample contained within the cell. The transmitted light is then analysed using a FLAME-S UV-Visible spectrophotometer from OceanInsight (Orlando, Florida, USA), which records the absorbance spectra of the sample across a range of wavelengths between 180 nm and 870 nm. The signal reading and analyses are performed using the OceanView software by OceanInsight.

Various samples such as proteins were subjected to incremental pressure up to 600 MPa, with absorbance spectra recorded at each pressure level. The data obtained were then analysed to identify shifts in absorbance peaks, changes in spectral patterns, and potential correlations with pressure-induced structural or biochemical modifications.

An experiment was conducted to assess the influence of hydrostatic pressure on the pH

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of samples within the high-pressure cell. According to Samaranayake and Sastry (2013), an increase in pressure to 800 MPa results in a pH drop of approximately -0.69 ± 0.07 pH units in pure water. Given that pH is a crucial factor in the manipulation and stability of biomolecules, it was essential to estimate the pH changes occurring within our high-pressure setup.

To achieve this, we employed a colorimetric method based on the work of Lai et al. (2016). This technique involves analysing the absorbance spectra of a meta-cresol purple (mCP) solution, a pH indicator sensitive to changes within the range of 5 to 10, which is a typical pH range for most food matrices. By measuring the absorbance at two specific wavelengths and the molar extinction coefficients of the two deprotonated forms of mCP molecule, we were able to calculate the pH drop between atmospheric pressure and 600 MPa.

We estimated the pH decrease in buffered solutions with initial pH values ranging from 5 to 9. The results indicate pH drops between -0.44 and -0.59 pH units across the pressure range of 0.1 MPa to 600 MPa. These findings suggest a consistent decrease in pH as pressure increases, with the magnitude of the pH change depending on the initial pH of the solution and the buffering power of the medium.

Following the initial pH experiments, we conducted a series of preliminary tests using the newly developed high-pressure installation to assess the absorbance spectra of various protein samples in real-time. The objective was to establish a baseline understanding of the spectral data obtainable for future studies. We subjected samples of ovalbumin, myofibrillar proteins, and haemoglobin to pressure up to 600 MPa. These proteins were selected because of their relevance in food science, and their varying structural complexity, which are expected to exhibit distinct spectral changes under high-pressure conditions.
The primary advantage of this new installation lies in its ability to collect real-time absorbance spectra of samples under high pressure, rather than analysing them post-treatment. This capability allows for the direct observation and study of protein unfolding kinetics, the reversibility of high-pressure treatment on various matrices, and the monitoring of conformational changes in biomolecules. Looking ahead, the next step will be to adapt this system to integrate a fluorescence spectrophotometer, which would significantly expand the range of samples that can be analysed. This enhancement would allow for the study of additional molecular interactions and provide a more comprehensive understanding of the effects of high-pressure processing on complex biological systems.

Keywords: spectrophotometer, optical fiber, in, situ measurement

High pressure-induced nanoaggregates of potato proteins to generate curcumin-loaded complexes

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Abstract

There is great interest nowadays in adding hydrophobic bioactive compounds such as curcumin to the diet. However, curcumin is characterized by a very low water solubility and a chemical instability. Therefore, the development of protein complexes able to bind and protect curcumin is a major current challenge.
The aim of this work was to generate high pressure-induced nanoaggregates from a patatin-rich potato protein isolate and evaluate the efficiency of these nanoaggregates to bind and protect curcumin.

First of all, the aggregation kinetics of 4% (w/w) patatin-rich dispersions (pH 6 and 7) processed by high hydrostatic pressure (400 and 600 MPa) at 20∘C were investigated. Nano-sized aggregates obtained for a pressurization time of 8 h were then selected and characterized in terms of denaturation yield, surface hydrophobicity and zeta potential. To optimize the efficiency of curcumin complexation to potato protein nanoaggregates, the curcumin addition before or after high pressure treatment was compared. Finally, the antioxidant activity of the curcumin-protein nanocomplexes was also studied just after high pressure treatment and after cold storage for 10 days.
This study allowed to select the processing parameters to applied in order to optimize the complexation efficiency of curcumin to high pressure-induced nanoaggregates and also preserve the antioxidant activity of the processed dispersions.

Keywords: high pressure, potato proteins, aggregation, curcumin, complexation

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12th HPBB CONFERENCE

Microbiology

Keynote : Jacob WINNIKOFF (Harvard University, USA)
Homeocurvature and homeoviscosity: unifying principles of membrane adaptation

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Hyperbaric inactivation - a novel tool for Alicyclobacillus acidoterrestris spores' destruction in commercial apple juice as affected by a previous HPP and PEF pre-treatment

O7 : **Kazutaka Yamamoto** (National Agriculture and Food Research Organization, Japan)
Size distribution of Escherichia coli cells after high hydrostatic pressure treatment

O8 : **Nishiyama Masayoshi** (Kindai University, Japan)
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O9 : **Shudi Huang** (University of Toronto, Canada)
Cellular Transfection using Rapid Decrease in Hydrostatic Pressure

O10 : **Toru Shigematsu** (Niigata University, Japan)
Influence of high hydrostatic pressure with benzoic acid addition on the energy metabolism and inactivation of budding yeast *Saccharomyces cerevisiae*

O11 : **Fumiyoshi Abe** (Aoyama Gakuin University, Japan)
Mechanosensing in Yeast: The Role of the Cell Wall Integrity Pathway in *Saccharomyces cerevisiae* under High-Pressure Stress

O12 : **Emma Bohuon** (ICMCB, Bordeaux, France)
Combining extreme microfluidics and *in situ* analyses techniques to get close insights into deep-sea vents microbial ecosystems

Posters

M1 : **Saraiva Jorge** (University of Aveiro, Portugal)
Portugal Hyperbaric storage at room-like temperatures as a new food preservation methodology to inhibit spores' germination and development – the case-studies of *Bacillus subtilis* and *Clostridium perfringens* spores

M2 : **Erdem Furkan** (METU-Food Engineering Department, Turkey)
Predictive Approach on Inactivation of *E. coli* O157:H7 using high hydrostatic pressure (HHP) processing

K2 Microbiology

Homeocurvature and homeoviscosity: unifying principles of membrane adaptation

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Abstract

Phospholipid membranes are one of the most acutely pressure- and temperature-sensitive structures in the cell. Phospholipid composition has evolved to compensate for pressure- and temperature-perturbation so that the physical properties of membranes in all organisms are similar under their native environmental conditions. Some organisms can also actively adjust their lipid composition as part of acclimatory response. Fifty years ago, the need to maintain membrane fluidity and lateral diffusion within certain margins, i.e. homeoviscosity, was identified as a selective force driving membrane lipid composition. **Recently, we discovered an additional, distinct driver of lipid adaptation occurring primarily in response to hydrostatic pressure. This second mode of adaptation is called "homeocurvature"** because it controls the molecular shape, and thus monolayer curvature, of phospholipids at their native conditions. Correct phospholipid shape establishes stress forces within the membrane that are essential for fission, fusion, and protein function. Homeocurvature emerged from a comparative study of ctenophores, or comb jellies, ranging from the surface to 4 km deep and from the tropics to the Arctic. **Evidence for this new principle, including its demonstration in engineered bacteria, is briefly presented, followed by a discussion of the interplay between pressure, temperature, homeocurvature and homeoviscosity.** The specific adaptive modes are most clearly identified when they are at cross-purposes and require a tradeoff. **The potential prevalence of homeocurvature is considered, with data from the literature and from ongoing research supporting its independent occurrence in bacteria, archaea, fungi, and metazoa.**

Keywords: Membranes, convergent adaptation, phospholipids, homeocurvature, homeoviscosity, deep sea

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Hyperbaric inactivation – a novel tool for Alicyclobacillus acidoterrestris spores' destruction in commercial apple juice as affected by a previous HPP and PEF pre-treatment

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Abstract

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Abstract:

Alicyclobacillus acidoterrestris is a gram-positive, thermophilic microorganism that produces highly resistant spores that represent and atypical case of an endospore able to germinate and outgrowth in acidic food products, as it is generally accepted that most endospores are unable to germinate/outgrowth at pH levels below 4.6, with the acidity hurdle blocking the nutrient receptors of the spores (1). Indeed, this particular endospore represents a threat to the industry, as it is rather prevalent in fruit juices and concentrates and, in its vegetative form, it is able to produce guaiacol, which is responsible for the off-flavours and odours in fruit juices and concentrates contaminated with this microorganism.

Lately, high pressure processing (HPP) has been widely used for nonthermal pasteurization of foods, although, as for any pasteurization procedure (either thermal or nonthermal) it is unable to destroy bacterial spores, although, the combination of high temperatures (above 70 ∘C) with HPP has shown to be efficient for endospore inactivation. Nevertheless, the combination of these temperatures with HPP may also causes considerable changes to the food products (although in a lower extension compared with intense thermal processes aiming the inactivation of spores, such as sterilization) (2).

In a previous study, hyperbaric storage (HS) at uncontrolled room temperatures (RT) has shown to be quite efficient to inhibit endospore development (3), and in some cases resulting in *A. acidoterrestris* inactivation in commercial apple juice, with the results pointing out that these spores could be reduced below detection limits within 48 h, which led to the hypothesis that an increase of the set pressure level could result in higher inactivation rates, in a range of pressures usually higher than those used in hyperbaric storage (up to 100 MPa) (4), but lower than those used in HPP (above 450 MPa), leading to a new concept aiming endospore inactivation, called hyperbaric inactivation (HI). This principle states the use of hydrostatic pressures between 150-250 MPa to inactivate microorganisms that are usually

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resistant to HPP, such as bacterial spores.

In the present study, hyperbaric inactivation was performed, using *A. acidoterrestris* endospores inoculated in commercial apple juice (pH 3.70) as case-study. The HI conditions were set to 150, 200 and 250 MPa up to 24 h, at uncontrolled room temperature (20-23 ∘C). At the same time, HPP (600 MPa, 3 min, 17-18 ºC) and pulsed electric fields (30 kV/cm, 80 μs, 1400 Hz) were performed to infer how a previous endospore activation step would impact their behaviour under HI conditions.

The inactivation kinetics results followed non-linear inactivation models for all the evaluated pressures. A pre-activation by HPP allowed to reduce *A. acidoterrestris* endospore counts below quantification limits after 24h, with at least 4.54 log units' reduction, while a pre-activation by PEF delayed endospore inactivation while under HI conditions, being the inactivation rates slower for higher HI pressures. Non-pre-activated *A. acidoterrestris* endospores (that were not pre-activated by HPP or PEF) showed a slower inaction rate compared to HPP (especially at 150 MPa) but faster than those observed for PEF. This result may be related to a direct opening of the dipicolinic acid channels of the endospores caused by HPP, leading to a quicker endospore inactivation upon exposure to HI conditions, contrary to PEF, which only is able to cause slight vibrations on the calcium-dipicolinic acid complex in the core, being unable to trigger its release to the external media and thus, unable to trigger the first stage of the germination process.

These results seem to indicate that HI can be used to inactivate bacterial spores in food products, with wider potential, to improve the safety such products, and particularly faster if the spores are pre-activated by HPP.

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Keywords: Hyperbaric storage, Hyperbaric inactivation, Alicyclobacillus acidoterrestris spores, HPP, PEF

Size distribution of Escherichia coli cells after high hydrostatic pressure treatment

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Abstract

Food processing by high hydrostatic pressure (HHP) (1) may induce lethal inactivation (death) of bacterial injury as well as sublethal inactivation (injury) (2). The inactivation can be discussed from several aspects such as membrane damage, protein denaturation, ribosome dissociation, reactive oxygen species, and colony formation (2). It is reported that cell aggregation/clumping may enhance HHP resistance of bacteria (3). In this study, the effect of HHP on the size distribution of *Escherichia coli* ATCC25922 was studied using a zeta potential analyzer and a laser light scattering analyzer.
Escherichia coli cells, which are in general rod-shaped with an approximate dimension of 1 μm x 3 μm, were suspended in phosphate-buffered solutions (pH 7.4; hereafter, PB) containing NaCl at 0.9 % (as physiological saline) and 10.0 % (as brine), respectively. The cell suspensions (ca. 10 log CFU/ml) were treated with HHP at 600 MPa and 25 ∘C for 10 min and stored under refrigeration for further analysis. The suspensions were applied to a zeta-potential analyzer (Zetasizer Nano ZS, Malvern, UK) for evaluation of an average cell size as well as to a laser diffraction particle size analyzer (LS 13 320, Beckman Coulter, USA) for measuring cell size distribution.

Average cell size was smaller in HHP-treated cells in 0.9 % NaCl PB while larger in HHP-treated cells in 10.0 % NaCl PB than their untreated cells. It seemed that the average size of the cells would not give sufficient information to understand the cell size effect. Therefore, size distribution analysis was carried out. In the size distribution analysis, unimodal curve was obtained for untreated suspension in 0.9 % NaCl PB, bimodal curves for untreated suspension in 10.0 % NaCl PB and HHP-treated suspension in 0.9 % NaCl PB. Trimodal curve was obtained for HHP-treated suspension in 10.0 % NaCl PB. Main peaks were observed for all the cell suspensions at around 0.8 μm. A shoulder peak was observed for untreated cells in 10.0 % NaCl at around 1.2 - 2.0 μm. Furthermore, the shoulder peak was enlarged by HHP treatment of the cell suspensions in 0.9 % and 10.0 % NaCl. In addition, the third peak was observed at 4 - 6 μm for HHP-treated suspension in 10.0 % NaCl PB. It was indicated that HHP treatment at 600 MPa induced cell aggregation/clumping and that 10.0 % NaCl PB promoted the aggregation/clumping. It was reported that higher initial cell counts resulted in lower inactivation of *E. coli* and bacterial spore with possible contributions of cell clumps (3). Aggregation/clumping of cells in 10.0 % NaCl and/or HHP-treatd suspensions might contribute to HHP resistance which is often observed in bacterial cell suspensions of high initial counts.

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Keywords: high hydrostatic pressure, Escherichia coli, cell size, cell size distribution, salt

Real-time imaging of bacterial motility with high-pressure microscopy

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Abstract

Similar to the meaning of the proverb "One picture is worth a thousand words", it is important to observe targets in situ. This is a basic reason why optical microscopy is a simple and powerful method. In fact, various types of microscopes are used in a wide range of research areas. High-pressure microscopy is one of the powerful techniques to visualize the effects of hydrostatic pressures on research targets. It could be used for monitoring the pressure-induced changes in the structure and function of molecular machines in vitro and in vivo. We have developed a high-pressure microscope that is optimized both for the best image formation and for the stability to hydrostatic pressure up to 150 MPa (1). By using the developed apparatus, Using this device, we have investigated the pressure responses of molecules(2), cells(3), and individuals(4). Bacterial motility is also one of the most pressure-sensitive cellular processes. In previous studies, we studied the mechanism how applied pressures effect on the swimming motion and rotation of flagellar motors in E. coli cells (1, 5). Here, we report that application of 5 MPa of pressure change the rotational direction of flagellar motors in *Escherichia coli* cells. Bacterial flagellar motor is a molecular machine that converts an ion flux to the rotation of a helical flagellar filament. The rotational direction switch between counterclockwise (CCW) and clockwise (CW) direction in response to environmental conditions. Rotation of single flagellar motors on tethered cells was measured using high-pressure microscopy measurements (5). At 0.1 MPa (ambient pressure), the cells rotated in both directions. When the pressure was increased from 0.1 to 5 MPa, all cells rotated exclusively in a CCW direction. And then, several cells started to rotate in both directions. The CW bias value, a possibility that motors rotate in the CW direction, gradually increased, and then reached to around the initial value at 0.1 MPa. After the release of pressure, most of the cells immediately started to rotate in CW direction. The CW bias value gradually decreased with a time, and reached to almost the initial value before application of pressure. Similar results were observed in swimming E. coli cells. Our results could be explained by a simple model, applied pressure forces to increase a concentration of the phosphorylated CheY, but also inhibit binding to flagellar motors (6). Application of pressure produces two opposing effects on flagellar rotations.
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Keywords: High, pressure microscopy, Bacterial motility, Flagellar motor, E. coli

Cellular Transfection using Rapid Decrease in Hydrostatic Pressure

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Abstract

The modification of cellular properties through the introduction or removal of nucleic acids is one of the most fundamental methods employed in molecular biology. Several methods have arisen to promote this process; these include the condensation of nucleic acids with calcium, polyethylenimine or modified lipids, electroporation, viral production, biolistics, and microinjection. An ideal transfection method would: (1) be low cost, (2) exhibit high levels of biological safety, (3) offer improved efficacy over existing methods, (4) be free of costly consumables, (5) work efficiently at any scale from microlitres to litres, (6) work efficiently on cells that are difficult to transfect by other methods, and (7) be capable of utilizing the widest array of existing genetic resources to facilitate its use in research, biotechnology, and clinical settings. We describe here Pressure-jump-poration (PJP), which addresses all of these issues. We show that the instantaneous depressurization to atmospheric pressure from _˜50 MPa results in the transfection of even difficult to modify primary cell types such as embryonic stem cells. The results demonstrate that PJP can be used to introduce an array of genetic modifiers in a safe, sterile manner. Finally, PJP-induced transfection in primary versus transformed cells reveals a surprising dichotomy between these classes which may provide further insight into the process of cellular transformation.
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Keywords: transfection, transformation, pressure jump, pressure

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Influence of high hydrostatic pressure with benzoic acid addition on the energy metabolism and inactivation of budding yeast Saccharomyces cerevisiae

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Abstract

We previously found that high hydrostatic pressure (HHP) inactivation of *Saccharomyces cerevisiae* synergistically increased with addition of four food additives, benzoic acid, sorbic acid, adipic acid and caproic acid (1). To investigate the mechanism of this synergistic effect of the food additives on HHP inactivation, we analyzed the effect of benzoic acid addition on HHP inactivation and on energy metabolism. In this study, *S. cerevisiae* wildtype haploid strain KA31a and its piezosensitive mutant a924E1, which had a deletion of *COX1* gene region in the mitochondrial DNA (2), were used and compared.
The growth characteristics of strains KA31a and a924E1 cultivated in YPD medium with benzoic acid addition at 0 to 12 mM were analyzed. For both strains, growth curves with benzoic acid addition at 3 mM and 6 mM were essentially comparable with those without benzoic acid addition (0 mM), indicating no apparent inhibitory effect of benzoic acid, although their growths were slightly inhibited depending on the concentration of benzoic acid, with lower specific growth rates during logarithmic growth phases than those without benzoic acid addition. The final cell concentrations also showed lower than those without benzoic acid additions. When both strains were cultivated with benzoic acid addition at 8 to 12 mM, their growths were significantly inhibited. The initial pH of cultivation broth with benzoic acid addition at 0, 6 and 8 mM were approximately 6.0, 5.0 and 4.8, respectively. The specific growth rates (μ) during logarithmic growth phase of strains KA31a and a924E1 cultivated in YPD medium, in which pH was adjusted to 4.8 and 5.0 by HCl addition, were approximately 0.5 and 0.4 h-1, respectively, which were comparable with those cultivated in YPD medium with pH of 6.0. The decreased specific growth rates by benzoic acid addition would be caused not by acidic conditions but by benzoic acid. High concentrations of benzoic acid addition would thus cause inhibitory effect on the growth of strains KA31a and a924E1. Since the acid dissociation constant pKa of benzoic acid is 4.2. The benzoate (anion form) would be larger amount than benzoic acid (protonated form) under at pH 5.0 and 4.8 with molar ratios of 6.3 and 4.0, respectively. Because cell surface exhibits a negative charge, only benzoic acid (protonated form) could permeate into the cells. Benzoic acid, permeated into cells, would inhibit certain intracellular metabolic reactions, and thus caused the growth inhibition.

The inactivation of strains KA31a and a924E1 subjected to HHP treatment at 225 MPa for 120 s without benzoic acid were only 0.2-log and 0.4-log by colony counting. In contrast,

*Speaker

the HHP inactivation of these strains KA31a and a924E1with benzoic acid addition at 6 mM increased to be 1.8-log and 7.8-log, respectively. The intracellular benzoic acid concentration of strain a924E1 after the HHP treatment was approximately 1.4-fold higher than that without HHP treatment, suggesting that HHP treatment caused permeation of benzoic acid into inside of the cells. After HHP treatments, the cells were subjected to scanning electron microscopy (SEM) observation. For both strains, cells with wrinkled surface were observed after HHP treatment with benzoic acid addition at 6 mM, whereas cells with wrinkled surface were not observed after HHP treatment without benzoic acid addition, as well as cells suspended in benzoic acid without HHP treatment. This result indicated that HHP treatment with the benzoic acid addition would cause a injury on the cell surface in both strains.

The metabolites related to glycolysis and TCA cycle were analyzed for strains KA31a and a924E1 cultivated in YPD medium with or without benzoic acid addition at 6 mM for 24 h, For both strains, apparent inhibition of the reaction from glucose-6-phosphate to fructose-1,6-bisphosphate when they were cultivated with benzoic acid. The metabolome analysis of TCA cycle in strain KA31a cultivated with benzoic acid addition showed that lower concentrations of citric acid and malic acid and that a higher concentration of succinic acid, compared with those without benzoic acid addition. These results suggested that benzoic acid caused a restriction in the glycolysis pathway, resulting in the low concentrations of metabolites of TCA cycle. Moreover, benzoic acid was suggested to inhibit reaction from succinic acid to malic acid. In the mutant train a924E1 cultivated in YPD medium without benzoic acid addition, the concentrations of metabolites related to TCA cycle were different from those in strain KA31a possibly due to the deletion in *COX1* gene. However, effect of benzoic acid addition on the metabolites related to TCA cycle in strain a924E1 was shown to be comparable with that of strain KA31a. Lower concentrations of citric acid and malic acid and a higher concentration of succinic acid were observed in strain a924E1 cultivated with benzoic acid addition than those without benzoic acid addition. These results suggested that in strain a924E1, as well as strain KA31a, benzoic acid addition in YPD medium would cause restrictions in the reaction step from glucose-6-phosphate to fructose-1,6-bisphosphate in glycolysis and the reaction step from succinic acid to malic acid in TCA cycle.

These results suggested that HHP treatment with benzoic acid caused an increased permeation of benzoic acid into inside of the cells, which would inhibit the energy metabolisms, resulting in synergistically increased HHP inactivation. This synergistic microbial inactivation with HHP and food additives would provide new food processing method, which enables efficient pasteurization with lower levels of hydrostatic pressure, compared with conventional HHP processing.

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Keywords: high hydrostatic pressure, Saccharomyces cerevisiae, inactivation, synergistic effect, food additives, benzoic acid

Mechanosensing in Yeast: The Role of the Cell Wall Integrity Pathway in Saccharomyces cerevisiae under High-Pressure Stress

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Abstract

Microorganisms are frequently exposed to extreme environmental stresses, such as heat, cold, salinity, and nutrient deprivation. These stressors are initially detected by sensor proteins, which activate specific intracellular signaling pathways to mediate adaptive responses. In addition to these chemical and thermal stresses, organisms also face physical stresses like tension, shear forces, and hydrostatic pressure. This study investigates the mechanisms by which *Saccharomyces cerevisiae* responds to high hydrostatic pressure, focusing on the roles of osmoregulation and the cell wall integrity (CWI) pathway, with particular emphasis on the transmembrane mechanosensor Wsc1 and the aquaglyceroporin Fps1.
High pressure at 25 MPa promotes water influx into yeast cells, leading to an increase in cell volume and a loss of the plasma membrane invaginated structures, known as eisosomes. This triggers the activation of the CWI pathway via Wsc1, resulting in the phosphorylation of Slt2, a mitogen-activated protein kinase downstream in the pathway. Concurrently, Fps1 undergoes phosphorylation, driven by downstream components of the CWI pathway, leading to increased glycerol efflux. This glycerol efflux contributes to a reduction in intracellular osmolarity, helping cells to prevent further water influx under high pressure.

Understanding the mechanisms of high-pressure adaptation through the CWI pathway in yeast may offer insights into cellular mechanosensation across different organisms, including mammalian cells.

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Keywords: Yeast, Water influx, CWI pathway, Mechanosensor Wsc1, Aquaglyceroporin Fps1

*Speaker

Combining extreme microfluidics and in situ analyses techniques to get close insights into deep-sea vents microbial ecosystems

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Abstract

Deep in the Earth oceans, deep-sea vents (DSVs) offer particular ecosystems thriving with a wide range of steep physical and chemical gradients. These ecosystems host a broad variety of microorganisms with unique capabilities, pushing the boundaries of life as well as extended applications in biotechnology (*e.g.* using extremophilic microorganisms). Conventional cultivation techniques enable to reproduce closely the deep-sea vents conditions in the lab (*e.g.* high temperature, high pressure, *etc.*) but are often time-consuming techniques (given the large number of experiments needed to perform basic growth studies for instance). Furthermore, combined analyses techniques offer limited *in situ* characterization, introducing some biases in our understanding of DSVs microbial communities.
Extreme microfluidics which mixes the advantages of microfluidics (*i.e.* size reduction, fast screening, *in situ* analyses, high reproducibility, *etc.*) with fluid systems used under high-pressure (*i.e.* up to 800 bar) and high temperature conditions, are modern tools particularly well adapted to investigate archaea living in extreme conditions such as deep-sea vents (Cario *et al.*, 2022). Indeed, they overcome the previously cited limitations and propose fast screening approaches as well as *in situ* monitoring in real and extreme conditions, both in batch and continuous modes.

By combining both extreme microfluidics and *in situ* analyses (*i.e.* microscopic and spectroscopic), my main PhD research objectives are i) to perform non-invasive fast-screening phenotyping (*i.e.* growth monitoring and molecular fingerprinting) of DSVs microorganisms; and ii) determine both their adaptation strategies and their boundaries (while coping with high pressure, heavy metals concentrations and thermo-chemical gradients conditions).

In this presentation, I will introduce first the microfluidics set-up used in this study as well as the different strategies developed to investigate DSVs microorganisms at lab scale. I will then present the growth monitoring of model DSVs strains using the microfluidics set up and the integration of modern data analyses. Eventually, I will give some insights on how

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I use spectroscopy techniques (combined to microfluidics) to access molecular vibrational modes of specific cellular biochemical molecules (such as proteins, carbohydrates, nucleic acids, lipids, etc.). The possibility to combine extreme microfluidics and *in situ* characterization techniques would help to determine the physiological states, phenotypic traits, and metabolic activities of the cells according to the different conditions tested.

Overall, this study uncovers microbial growth strategies using deep-sea vents model strains, and provides insights in archaeal biosignatures using *in situ* high pressure and high temperature microscopy coupled to spectroscopy. The ultimate goal is to use an artificial microbial consortium (and eventually a real one) in order to identify several microbial phenotypes in a sample for subsequently conducting to a better understanding of DSVs microorganisms' lifestyle.

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Cario, A., Larzillière, M., Nguyen, O., Alain, K., & Marre, S. (2022). High-pressure microfluidics for ultra-fast microbial phenotyping. *Frontiers in Microbiology*, *13*, 866681.

Keywords: microfluidics, deep sea vents, phenotyping, extremophiles

Hyperbaric storage at room-like temperatures as a new food preservation methodology to inhibit spores' germination and development – the case-studies of Bacillus subtilis and Clostridium perfringens spores

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Abstract

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Abstract:

Bacterial spores are a major problem for the food industry, and, consequently, for consumers themselves, as these are survival structures originated from bacteria as a response to a stress source. These spores are able to survive pasteurization processes (either thermal and nonthermal processes), and some of them are from pathogenic bacteria, which are responsible for food poisoning outbreaks, thus raising food safety issues (1). According to the pH of the food product, if it is $\leq$4.6, the pH will naturally inhibit the development of spores, while if the pH is $>$ 4.6 the foods have to be refrigerated to temporarily inhibit the germination and outgrowth of spores, with refrigeration being energetically expensive and environmentally harmful (2). Recently, a new food preservation methodology was proposed, called hyperbaric storage, stating storage pressure control, instead of temperature control as it is the case of refrigeration/freezing processes. This method uses hydrostatic pressures up to 150-200 MPa at naturally variable, uncontrolled room temperatures to hurdle microbial development (3).

In this work, the performance of hyperbaric storage (75, 150 and 200 MPa at uncontrolled room temperature – 18-23 ^{o}C) to hurdle the development of spores of *Bacillus subtilis* and *Clostridium perfringens* spores was accessed in a model system (brain-heart infusion broth, BHI-broth). Additionally, the effect of pH on the spores' behaviour under hyperbaric storage was also accessed, being the spores inoculated in BHI-broth at pH 4.50, 6.00 and 7.50. At the same time, control samples stored at atmospheric pressure and room temperature, and under refrigeration (5 ^{o}C) were also studied)

At pH 4.50, neither spore germination nor outgrowth was observed, regardless of the storage conditions. At atmospheric pressure at room temperature (0.1 MPa, 18-23 ^{o}C), at pH 6.00 and 7.50, quickly resulted in spore germination and outgrowth after a few days for both spores. For refrigerated samples, *B. subtilis* spores were able to germinate and develop after

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10-15 days, while for *C. perfringens* spores the germination and development of the spores was inhibited for 30 days.

When it comes to hyperbaric storage, the results showed different behaviours for the *B. subtilis* and *C. perfringens* spores while under hyperbaric storage conditions. For *B. subtilis* spores, a gradual inactivation was observed, which was considerably higher than that observed for *C. perfringens* spores, with an overall spore inactivation of 5 log units for the former (in some cases below detection limits) and about 2.5 log units for the latter, regardless of the pH level. The inactivation followed non-linear models, such as the Weibull and the Biphasic models. Additionally, phase-contrast microscopy images revealed that the spores do not reach the vegetative state (i.e., do not form a vegetative bacteria) before being inactivated, which is particularly important for the cases where toxin production (such as cereulide or enterotoxins) takes place.

In conclusion, hyperbaric storage without temperature control allowed not only to inhibit both spores' germination and development, but also resulted in their gradual inactivation along storage, which is remarkable considering that spores require very high temperatures (above 100 ^{o}C) to be inactivated, and it was possible to achieve high levels of inactivation at room temperature (without applying any heat). As such, this methodology can simultaneously be seen both as a storage methodology and as a nonthermal method to inactivate bacterial spores at room temperature.

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Keywords: Hyperbaric storage, Bacillus subtilis, Clostridium perfringens, spores' inactivation

Predictive Approach on Inactivation of E. coli O157:H7 using high hydrostatic pressure (HHP) processing

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Abstract

Aim: Tomatoes and their derivatives are highly valued for their nutritional content, rich in phenols and carotenoids. High hydrostatic pressure (HHP) processing, a non-thermal method of pasteurization, has been suggested as a substitute for traditional pasteurization techniques. This study aimed to assess the impact of increased pressure on the survival of *E. coli* O157:H7 (ATCC 43888) and use this data to perform a predictive analysis through modeling the inactivation kinetics.
Method: Roma tomato juice was utilized as the inoculation medium, with an inoculation level of approximately 10ˆ8 cfu/mL for *E. coli* O157:H7 (ATCC 43888). HHP treatments were administered at 150, 250, 350, and 450 MPa over durations ranging from 5 to 25 minutes, in 5-minute intervals, at 25∘C. Post-treatment, the samples were plated on MacConkey (selective) and tryptic soy yeast extract(non-selective) agars. Selective media were incubated for 36-48 hours, while non-selective media were incubated for 18-24 hours. Enumeration from selective media indicated sublethal injury. A variety of mathematical models, including Weibull, Geeraerd, Modified Gompertz, and linear models, were employed to evaluate the inactivation kinetics.

Results: The impact of pressure on microbial viability was statistically significant ($p < 0.001$). At 150 MPa, microbial reduction ranged up to 1.65 logs in non-selective media and up to 2.2 logs in selective media after 25 minutes. At the highest pressure of 450 MPa, a reduction of approximately 7 logs was observed in non-selective media after 25 minutes, with counts in selective media falling below detectable levels. Among the models tested, the Geeraerd model excelled, demonstrating superior conformance to the experimental data with an R2 value of 0.995 and an RMSE of 0.09. This model, verified by supplementary experiments, provided an excellent predictive analysis of E. coli O157:H7 inactivation kinetics under various HHP conditions.
Conclusion: The reduction in microbial load achieved with the 450 MPa treatment for 25 minutes was satisfactory, marking a significant advance in HHP processing efficacy. The Geeraerd model stood out in predictive accuracy, underlining its utility in simulating the behavior of *E. coli* O157:H7 under extreme pressure conditions, further substantiated by additional experimental validations.

Keywords: E. coli, HHP, pasteurization, modelling

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Posters

P1 : **Martins Ana P** (University of Aveiro, Portugal)
Portugal Hyperbaric storage as a cell preservation methodology – Can blood be stored under pressure?

The limits to life at high pressures and their astrobiological relevance

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Abstract

Life as we know it exists in a diverse range of strange and brutally extreme environments, where the very limits of biochemistry are pushed right to the edge. When seeking life elsewhere in the solar system we use these known limits to life to guide our search.
However, the solar system presents environments that terrestrial life has never experienced and hence never become adapted to. Therefore, we have a poor understanding of the habitability of some of the more exotic environments we find in the solar system, with the Martian deep subsurface providing a prime example. Potential liquid water environments on Mars are theorised to be deep underground, experience high pressures and deep subzero temperatures, with high concentrations of eutectic salts such as perchlorates in order to maintain the liquid environment. As no known life could tolerate such conditions, I have instead explored what the limits of biophysics can tell us about the habitability of such environments.
In this talk I will present work showcasing the delicate interplay between temperature, pressure, and perchlorate concentrations in how they ultimately combine to affect biochemistry. I will then focus on what is known about the low temperature limits for life and how pressure and salts may increase the potential for life in deep subzero environments.

Keywords: biophysics, microbiology, calorimetry, protein stability, enzyme activity, vitrification

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Pressure effect on protein cluster formation induced by multivalent ions

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Abstract

We have shown that a patchy particle model can describe the phase behavior of a system of acidic globular proteins such as BSA in the presence of multivalent salts such as YCl3. The phase diagram of the studied system as a function of salt concentration and temperature is quite complex, showing reentrant condensation, metastable liquid-liquid phase separation, cluster formation and crystallization. In particular, a lower critical solution temperature is observed which suggests that hydration plays an essential role in the ion-mediated protein interactions.

This is also visible by changing the solvent from H2O to D2O. It leads to an increasing attraction potential between the proteins and the formation of clusters is observed. By neutron spectroscopy a slowing down of the protein short-time self-diffusion as a function of available yttrium ions per protein is observed. The effect is enhanced by increasing the temperature of the sample.

Using temperature as a control parameter has some disadvantages because temperature influences both the thermal energy and the density of the system. Furthermore, only a small temperature range is available for studying proteins since high temperatures lead to denaturation. As opposed to temperature, pressure influences only the density and can be considered to have milder effects.

Here we will present results from pressure dependent neutron spectroscopy experiments. In contrast to the previous studies at and above room temperature we found, that the slowing down of the short-time self-diffusion is less pronounced. This effect will be discussed with the help of pressure dependant SAXS measurements.

Keywords: BSA, QENS, SAXS

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Unravelling the mechanisms of adaptation to high pressure in proteins

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Abstract

The adaptation of proteins to high pressure is still an open debate, but understanding it could shed light on the origins of life (1), lead to a better understanding of protein dynamics, and deliver new tools to engineer pressure-resistant enzymes for biotechnological purposes. While the thermodynamic and dynamical properties of model proteins under pressure have been extensively studied (2), the evolutionary aspects of their adaptation are still unclear.

Disentangling the contributions of pressure adaptation from those of another adaptation, such as high or low temperature, is a difficult task and, in fact, genomic studies were unable to determine a clear pattern among the order of Thermococcales.

Recent experiments by our group focused on whole cells of two closely related species (*Thermococcus barophilus,* Tba, and *Thermococcus kodakarensis,* Tko) that grow at the same optimal temperature (85◦C) but differ only for the optimum pressure (400 bar for Tba, 1 bar for Tko), and they highlighted the differences in the dynamics of the two organisms' proteomes (3,4). To take this investigation to the molecular level, we studied the *Phosphomannose Isomerase* and the *Ribosomal protein S24e* from the two organisms with Elastic and Quasi-elastic Incoherent Neutron Scattering, 2-D NMR Spectroscopy and X-ray crystallography. Our results evidence that the substitutions of amino acids enhancing pressure stability are those in the hydrophobic core, which eliminate cavities, and those on the surface, which modulate the interaction of the proteins with the surrounding water layer and give them the right flexibility to perform their function under high pressure (fig. 1).

Keywords: Protein dynamics, Neutron Scattering, Adaptation to High Pressure

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Probing the pressure stability of R-Phycocyanin from red macroalgae Porphyra haitanensis

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Abstract

Red macroalgae *Porphyra*, known as Nori, is commonly used as food worldwide due to its high abundance of nutrients and alternative proteins, like coloured phycobiliproteins (PBPs). R-phycocyanin (R-PC), a highly abundant PBPs in *Poprphyra*, has a characteristic purple colour and numerous bioactive properties. R-PC is an attractive protein that is useful in the food industry. However, its highly limited thermal stability implies alternative approaches for treating food-containing R-PC to preserve both the outstanding colour and bioactive properties of R-PC.

We aimed to probe the *in situ* stability of R-PC at high-pressure (HP) conditions (up to 4,000 bar) by combining absorption, fluorescence, and small-angle X-ray (SAXS) techniques. Unfolding of R-PC is a multiphase process comprising, first, the conformational changes of R-PC oligomeric form (trimers) at low pressure, followed by the dissociation of trimers to monomers (above 160 MPa), until finally, the unfolding of subunits (above 300 MPa). A decrease in pressure induces only partial refolding of R-PC to monomer-like forms, with colour preservation of 50% compared to the initial absorbance before pressure treatment. In contrast, the thermal treatment of R-PC has an irreversible and detrimental effect on R-PC colour, demonstrating the advantage of HP for the colour and bioactive properties preservation of R-PC over thermal treatment.

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For the first time, R-PC unfolding is depicted under HP conditions. The proposed mechanism can be of substantial significance for developing new approaches using high pressure for R-PC stabilization and enhancing the potential for applications of this bioactive protein in the food industry.

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Keywords: R, Phycocyanin, high pressure, SAXS, absorption, fluorescence, unfolding, Porphyra

Probing biomolecular conformational landscapes by High-Pressure NMR spectroscopy (HP-NMR)

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Abstract

NMR spectroscopy under High-Pressure has become a useful biophysical tool to explore the structural and dynamic properties of biomolecules such as proteins in solution. High-Pressure NMR is routinely used in our laboratory to explore the conformational landscape of biomolecules. In this communication, we will show that pressure can highlight the binding competent conformational state of the anti-apoptotic TCTP protein to the cancer-related Mcl-1 and we will illustrate how high-pressure NMR allows us exploring the intimate dynamic coupling between lipid and membrane proteins such as OmpX porin or the BLT2 G-Protein Coupled Receptor in nanometric bilayer membranes. Our lab will continue to extent the power of HP-NMR.

Keywords: NMR, protein, lipid, structure, dynamics, molecular interactions, high, pressure

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Effects of pressure, temperature, salts, and osmolytes on biomolecular liquid-liquid phase separation

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Abstract

Fused in sarcoma (FUS) is an intrinsically disordered RNA-binding protein known to form liquid droplets via liquid-liquid phase separation (LLPS), and it has been extensively studied in biophysical and biochemical research. Irreversible aggregation of FUS in the cytosol leads to familial amyotrophic lateral sclerosis (ALS). Consequently, understanding the aggregation mechanism and developing inhibitors to prevent this process are of high importance. We identified two distinct LLPS states for FUS: normal LLPS (LP-LLPS), which is stable below 2 kbar, and aberrant LLPS (HP-LLPS), which is stable above 2 kbar and promotes irreversible aggregation (1-4). Additionally, FUS-LLPS is destabilized by increasing salt concentrations but stabilizes again at higher salt levels; reentrant LLPS occurs at concentrations above 2 M of NaCl in a buffer solution, referred to as high-salt LLPS (5).
We investigated the impact of trimethylamine N-oxide (TMAO) and urea, known osmolytes, on three FUS-LLPS states: LLPS states at atmospheric pressure with low- and high-salt concentrations, and a re-entrant LLPS state above 2 kbar (6). Temperature and pressure-scan turbidity measurements demonstrated that TMAO stabilizes LLPS while urea destabilizes it. These findings are attributed to the excluded volume effect of TMAO (preferential hydration) and urea's preferential interaction with proteins. Additionally, TMAO was found to counteract the effects of equimolar urea on LLPS, a previously unreported phenomenon. The concept of the m-value for osmolyte-induced protein folding and unfolding is applicable to the effects of osmolytes on LLPS. In summary, biomolecular LLPS can be modulated by preferential hydration and small osmolytes' interaction with proteins, promoting LLPS formation even in extreme conditions such as high salt, high urea, and high pressure.

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Keywords: pressure, trimethylamine N, oxide, liquid–liquid phase separation, fused in sarcoma, preferential hydration, m, value

High pressure effects of nanoplastics on structural integrity of lipid membranes

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Abstract

Plastic pollution increasingly threatens the environment (1), including extreme environmental layouts under e.g. high-pressures, salinities or temperature, where life strives. Microplastics, nanoplastics and molecular products of plastic degradation have been found in both sandcoasts as well as in the deepest parts of the oceans (2). Styrene oligomers (SO), the low-molecular weight products of polystyrene (PS) degradation, are potentially dangerous for the health of mammals and ecosystems, as they are widespread in ecosystems and accumulate and perturb the functioning of cellular membranes (3).
We use different neutron-based methods at the Institute Laue Langevin (ILL), by taking advantage of the isotope-selective interactions of neutrons with different elements (e.g. proteum vs. deuterium hydrogen isotopes) allowing to finely and controllably tune the scattering length density (SLD) in biologically-relevant samples. We obtain valuable insights into how membrane structure and thermodynamics are affected by SO as a model of pollutant.

By means of SANS, we have proven that low molecular weight (Mw _˜ 500 g/mol) SO incorporated within h-DPPC large unilamellar liposomes can affect membrane thermodynamics (3).

Moreover, using 100% D2O and a custom-made pressure cell (4), we performed specular and off-specular neutron reflectometry (NR) at (5) at different (temperatures T, and pressures P) values for supported h-DPPC bilayers at the solid (Silicon)-liquid interface using the same size also (Mw _˜ 500 g/mol) of (d-SO) oligomer, with 30% mol presence in the h-DPPC bilayer. When compared at the same pressure (P = 1 bar) in the h-DPPC gel phase (T = 21 ∘C < T = 41 ∘C), the presence of d-SO perturbs the whole lipid bilayer manifested by an increase in (i) both tail thickness and (ii) the tail SLD.

Finally, neutron diffraction in h-DPPC multilamellar stacks under high-pressure shows spatial perturbations in the lamellar stack upon presence of SO.

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We anticipate that our study will broaden our understanding of how plastic oligomer incorporation, under the influence of pressure (e.g. deep sea) might affect cell viability and oceanic pollution.

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Keywords: Nanoplastics, lipid membranes, neutron scattering

Inhibitor binding in rare excited states of the Ras oncogene protein revealed by high pressure macromolecular crystallography

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Abstract

Pressure is an ideal tool to explore the conformational landscape of proteins, since it allows to increase the population of high-energy states functionally relevant, but rare at ambient pressure. High Pressure Macromolecular Crystallography (HPMX) is thus an ideal tool to study with a high precision excited states of proteins. Roger Fourme was a pioneer in this domain, developing a diamond anvil cell (DAC) especially suited for biological macromolecules (1-3).
Moreover, HPMX is a powerful method to induce transitions allowing drug binding in proteins that are in low-populated conformations at ambient conditions, enabling the design of specific inhibitors. These properties will be illustrated with the example of Ras.

Ras in an oncogenic protein involved in a large number of cancers, however the development of efficient inhibitors of Ras is still challenging, since Ras proteins possess multiple conformational states.

Using HPMX, we have been able to induce an in-crystallo phase transition, allowing a precise description of the different segments of Ras which adopt transient intermediates states corresponding to conformers which interact with different regulators and effectors (4). Moreover, high pressure has driven Ras toward an excited state where an inhibitor targeted for this rare but functionally important state can bind, allowing a precise description of its binding site (5).

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Keywords: High Pressure macromolecular crystallography (HPMX), drug design, in crystallo transition

Life in Multi-Extreme Environments - A Physicochemical View

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Abstract

Elucidating the details of the formation, stability, interactions, and reactivity of biomolecular systems under extreme environmental conditions, including high and low temperature, high salt concentrations in brines, high osmotic as well as high hydrostatic pressure, is of fundamental biological, astrobiological and biotechnological importance. Bacteria and archaea are able to survive in the deep ocean or subsurface of Earth, where pressures up to more than 100 MPa (1000 bar) are reached. The deep subsurface of Mars may host high concentrations of ions in brines, such as aggressive perchlorates, but we know little about how these conditions and the resulting osmotic and hydrostatic stress conditions would affect the habitability of such environments for cellular life. By combining various biophysical techniques, including calorimetry, confocal fluorescence microscopy, fluorescence, smFRET, UV/Vis, CD, FTIR, and NMR spectroscopy as well as small-angle X-ray and neutron scattering, the combined effects of temperature, osmotic and hydrostatic pressure and cosolutes on the structure, solvational properties, dynamics and intermolecular interactions of proteins, nucleic acids, and lipid membranes have been studied and selected results are presented here. In recent years, it became clear that assembly processes based on liquid-liquid phase separation (LLPS) of protein and protein-nucleic acid mixtures, acting as membrane-less organelles, play also an important role in cellular self-assembly processes. We discuss also the combined effects of temperature, pressure, crowding, and cosolvents on LLPS phenomena of biomolecular systems and discuss the effects of LLPS on the pressure dependence of ligand binding and enzymatic reactions. Knowledge of these effects is essential to our understanding of life exposed to such harsh conditions and of the physical limits of life in general. In addition, we discuss strategies that not only help us understand the adaptive mechanisms of organisms that thrive in such harsh geological settings, but could also have important ramifications in biotechnological and pharmaceutical applications.

Keywords: high hydrostatic pressure, high salt, high osmotic pressure, proteins, membranes, nucleic acids, liquid, liquid phase separation

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Hyperbaric storage as a cell preservation methodology – Can blood be stored under pressure?

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Abstract

In some low/middle-income countries, blood preservation for transfusion remains a challenge (1). Therefore, it is essential to develop new techniques that can improve blood preservation or storage in more difficult circumstances. Hyperbaric storage (HS) is a new preservation methodology that allows the storage of products under pressure (up to 100 MPa) for variable time periods and has been studied almost exclusively in food products (2). When used at uncontrolled room temperature, this methodology is a quasi energetically costless procedure since, after pressure generation, no additional energy is required to keep it along storage, allowing energetic savings (3). So, hyperbaric storage was studied in this work as a potential new blood preservation methodology. Swine blood, with CPDA-1, was stored for 35 days under pressure (25 – 75 MPa) at room and refrigeration temperatures and compared to a conventional whole blood preservation method (refrigeration). To study whole blood during storage, quantification of hemolysis, pH, and a multivariate and metabolic composition analysis (NMR) were performed. Subsequently, a preliminary study with human blood was also performed to confirm its potential storage using hyperbaric storage under the same conditions than swine blood and to evaluate some important parameters such as lactate and ATP quantification and RBCs viability.

The results demonstrate that pressures above 50 MPa, regardless of temperature, promote cell lysis. However, at lower pressures (25 – 45 MPa) at room temperature (≈ 17 – 25 ∘C), it was possible to keep hemolysis values below 0.8 % (allowed limit for blood transfusion) up to 12 days with minimal energy consumption. At the same pressures but combined with refrigeration temperatures (5 ∘C) better hemolysis results were obtained with values below the limit during 35 days of storage. However, when compared to the conventional method (refrigeration), the values were approximately the same considering the maximum allowed value of 0.8 %. The metabolic composition analysis revealed a lower lactate and a higher glucose concentration in blood stored under pressure, which may indicate a decrease in red blood cell metabolic rates when pressure and refrigeration temperatures are combined. The preliminary study with human blood revealed a similar behaviour than the studies with swine blood (at room temperature) and, when pressure and low temperature were combined,

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better quality parameters were also obtained.

This study highlights the potential of using hyperbaric storage as a whole blood preservation technique, and additionally, when used at room temperature is quasi energetically costless. To have a deeper insight, additional research and analysis are required, such as flow cytometry for microvesicles detection and microscopy for morphology characterization, some of which are under way.

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Keywords: Blood preservation, Hyperbaric storage, Hemolysis, Metabolic profile

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12th HPBB CONFERENCE

Visit Lyon

Notre-Dame de Fourvière – Fête des Lumières 2021
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Aerial view of the quays of the Rhône river

12th HPBB CONFERENCE

Visit Lyon

Top left :	Notre-Dame de Fourvière (on top of the Fouvière hill).
Top right :	Place des Terreaux (in front of the City Hall)
Bottom left :	Lafayette bridge (crossing the Rhône river to join the Lafayette park to the Lyon Opera house)
Bottom right :	Lyon's Skyline

A brief history of HPBB conference

1992 **La Grande Motte** (France)
Joint Seminar on High Pressure and Biotechnology and High Pressure and Food Science
Organized by **Prof. Claude Balny** (INSERM, Bordeaux, France)
September 13, to September 17

1995 **Kyoto** (Japan)
International Conference on High Pressure Bioscience and Biotechnology
Organized by the late **Prof. Rikimaru Hayashi** (Kyoto University, Japan) and **Prof. Claude Balny** (INSERM, Bordeaux, France) November 5, to November 9, 1995

1998 **Heidelberg** (Germany)
International Conference on High Pressure Bioscience and Biotechnology
Organized by **Prof. Horst Ludwig** August 30 - September 3, 1998

2000 **1st** **Kyoto** (Japan). First International Conference on High Pressure Bioscience and Biotechnology
Organized by the late **Prof. Rikimaru Hayashi** (Kyoto University, Japan) and **Prof. Claude Balny** (INSERM, Bordeaux, France)
November 26, 2000, to November 30, 2000)

2002 **2nd** **Dortmund** (Germany)
Organized by **Prof. Roland Winter** (Technische Universität Dortmund)
September 16, 2002, to September 19, 2002

2004 **3rd** **Rio de Janeiro** (Brazil)
Organized by **Prof. Jerson Silva** (University of Rio de Janeiro)
September 27, 2004, to September 30, 2004

2006 **4th** **Tsukuba** (Japan, 2006)
Organized by **Prof. Kazuyuki Akazaka** (Kinki University)
September 25, 2006, to September 29, 2006

2008 **5th** **La Jolla (USA)**
Organized by **Prof. Douglas H. Bartlett** (Scripps Institute of Oceanography, UCSD)
September 15, 2008, to September 19, 2008

2010 **6th** **Freising** (Germany)
Organized by **Prof. Rudi Vogel** (Weihenstephan Science Center, TU München)
August 28, to September 1, 2010

2012 **7th** **Otsu** (Japan)
Organized by **Prof. Kazuyuki Akasaka** (Kinki University)
October 29, to November 2, 2012

2014 **8th** **Nantes** (France)
Organized by **Prof. Marie de Lamballerie** (ONIRIS, Université de Nantes)
July 15, 2014, to July 18, 2014

2016 **9th** **Toronto** (Canada)
Organized by **Prof. Tigran V. Chalikian** (Pharmacy University of Toronto, Canada)
July 25, to July 29, 2016

2018 **10th** **Numazu** (Shizuoka, Japan)
Organized by **Prof. Fumiyoshi Abe** (Aoyama Gakuin University, Shibuya City, Japan)
September 18, to September 22, 2018

2020 **(pre-11th)** cancelled in person in 2020
Organized online in 2021 by **Prof. Vibeke Orlien** (University of Copenhagen)
July 6, to July 8, 2021

2022 **11th** **Copenhagen** (Denmark)
Organized by **Prof. Vibeke Orlien** (University of Copenhagen)
July 5, to July 8, 2022

2024 **12th** **Lyon** (France)
Organized by **Dr. Philippe M. Oger (CNRS/INSA Lyon)**
September 30, to October 3, 2024

Microbiology of Extreme Environments

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