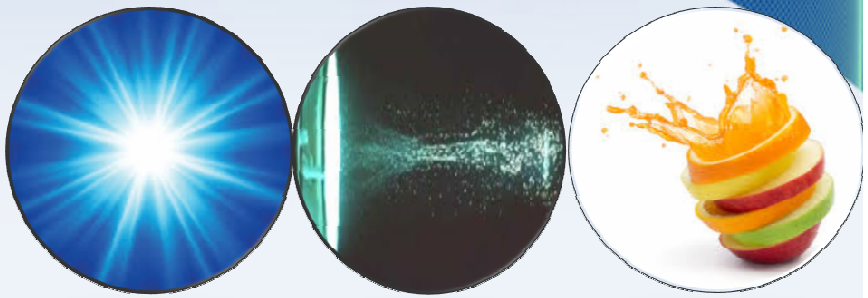


Combined use of Pulsed Light and High Intensity Ultrasound technologies to preserve apple juice. Study of microbial inactivation and induced damage

Sandra Guerrero, PhD, CFS

6th Global Summit and Expo on Food & Beverages

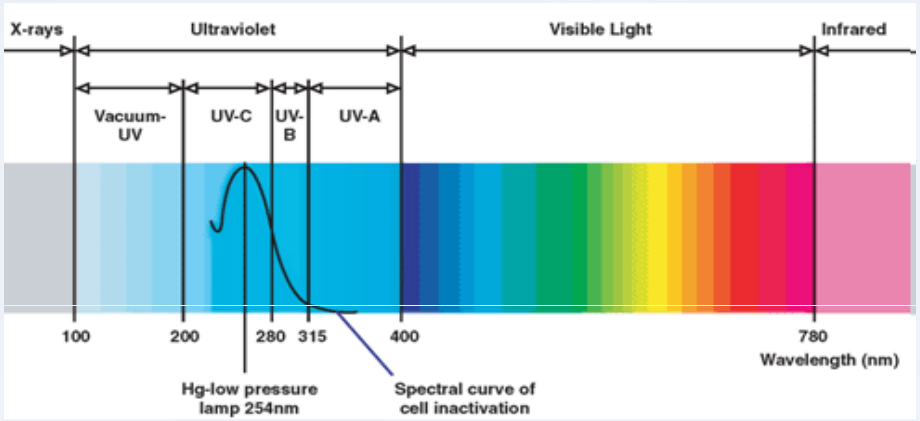
Emerging preservation factors

- High Pressure Processing(HPP)
- Pulsed Electric Fields (PEF)
- **Ultrasound (US)**
- Continuous UV-C light
- **Pulsed Light Technology (PL)**

These technologies may inactivate microorganisms at sublethal temperatures, reducing detrimental effects on food quality

PL- Fundamental

- Intense and short duration pulses of broad spectrum light from UV to near IR (λ 200 – 1100 nm) produced using (Xenon) discharge lamps



One pulse:

Duration: 1 μs to 0,1 s

1 – 20 flashes per second

Energy density ≈ up to 50 J/cm² per pulse

Inactivation mechanisms

I - Photochemical: DNA modification, protein desnaturalization and other cell alterations avoiding reproduction } **UV-C**

II- Photothermal: cell disruption by localized heating produced by light absorption at doses >0,5 J/cm²

III- Photophysical: cell structures´damage and lost due to high energy peaks

} **PL**

PL limitations in fruit juices

- Superficial effect (penetration depth)
- Heating effect depending on the fluence
- Less effectiveness in juices with suspended solids (shadowing effect) , higher absortivities and turbidities

HURDLE APPROACH

Objective

Saccharomyces cerevisiae KE162 & Alyciclobacillus acidoterrestris ATCC 49025 spores response to pulsed light treatment combined with high intensity ultrasound as starting basis for processing design to obtain improved or more safe fruit juices

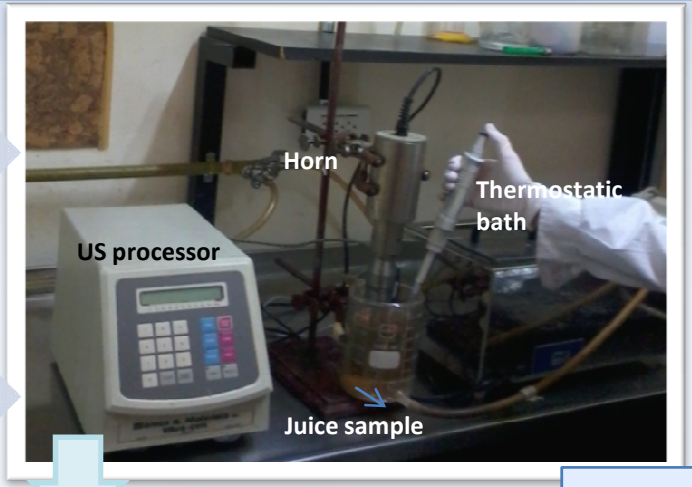
Treatments

Commercial Apple juice-CAJ without additives

- pH 3.5
- °Brix 10.5
- $A_{254\ nm}$ 0.049
- Turbidity (660 nm): 0.063
- Particle size(nm):1.67

Natural apple juice-NAJ Fresh squeezed centrifuged (5000 rpm, 10 min)

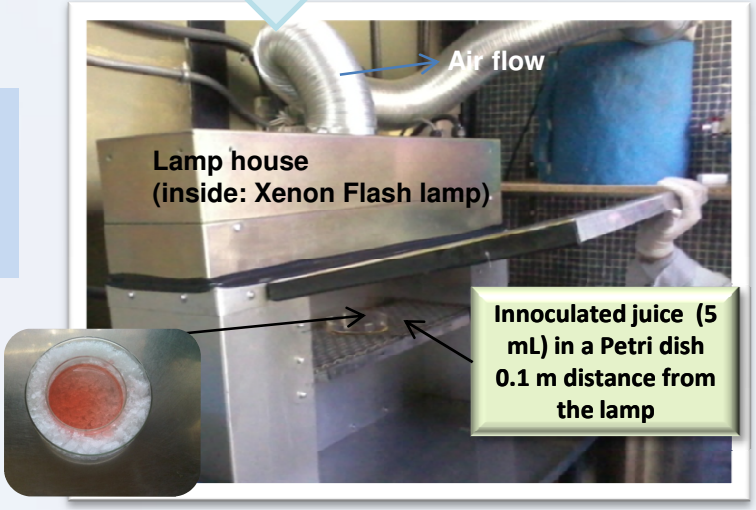
- pH 3.5
- °Brix 12.6
- $A_{254\ nm}$ 0.110
- Turbidity (660 nm): 0.07
- Particle size(nm):712



US TREATMENT

Vibracell 600, Sonic & Materials, NewTown, CT, USA)
20 kHz , 600 watts,
WA: 95.2 µm (80 %)
10-30 minutes; 20 or 44 °C

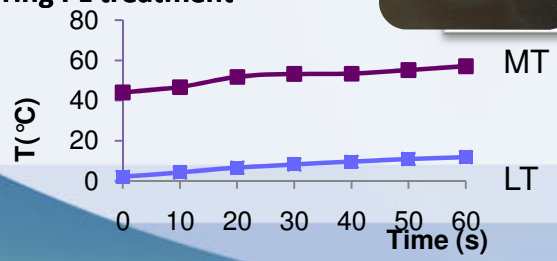
Inoculation with *S.cerevisiae* KE162 cells or *A. acidoterrestris* ATCC4025 spores (stationary phase, final cell density :10^7 -10^8 CFU/mL)



PL TREATMENT

RS-3000B Steripulse-XL system (Xenon Corporation, Wilmington, MA, USA), 3pulses/s (pulse width = 360 µs)
71.6 J/cm² (60s);
$T_{initial}$ - T_{final}: ~ 2-12 °C; 44–56 °C

Juice temperature profile during PL treatment



US+PL TREATMENT

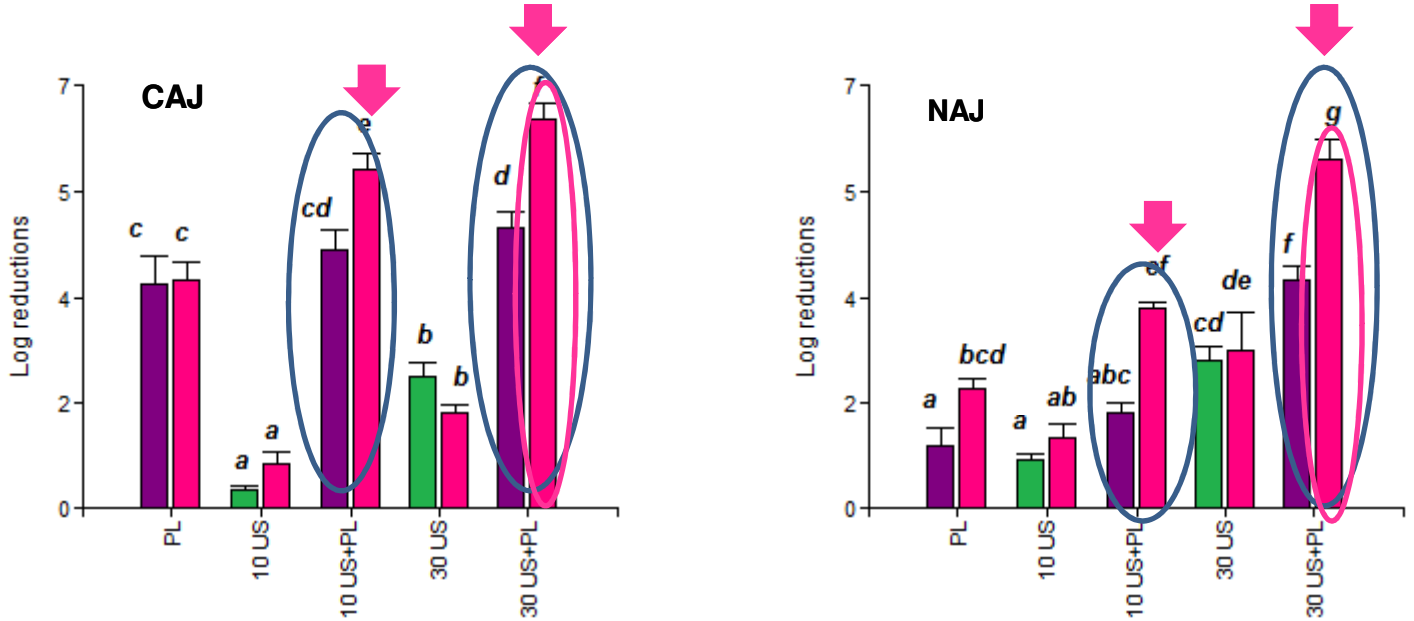
LT treatments
US :10min (10US) or 30 min(30US), T= 15°C +
PL: 60 s (60PL), T_{PLi}= 2°C, T_{PLf}= 12 °C
MT treatments
US :10min (10US) or 30 min(30US), T= 44 °C+
PL: 60 s (60PL), T_{PLi}= 44°C, T_{PLf}= 56 °C

Food safety engineering - Some indicators:

- Bulk methods: Inactivation studies as function of dose
- Single cell analysis
  - Flow cytometry (FCM)
  - Transmission Electron Microscopy (TEM)

S. cerevisiae inactivation achieved by single and combined treatments

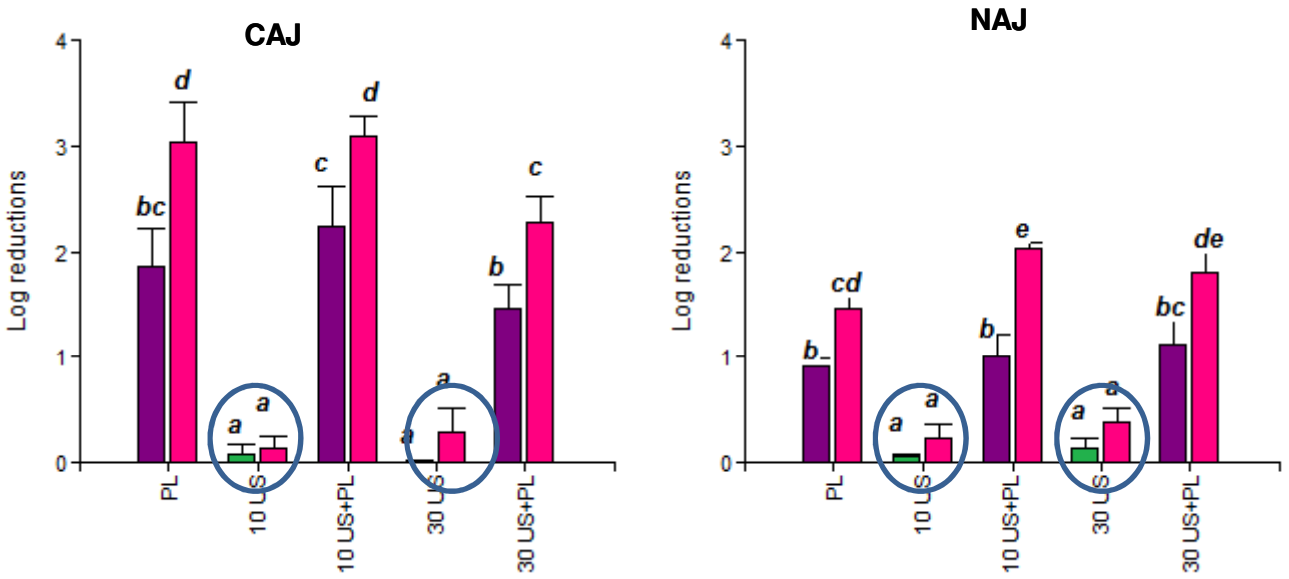
US: 15 °C (■); 44 °C (■); **PL**: 2-12 °C (LT, ■) ; 44 -56 °C (MT, ■)



- Higher final temperatures significantly increased the effect of the combined US+ PL treatments for both juices
- US+PL treatments increased ***S. cerevisiae*** inactivation obtained by single treatments showing in general **additive** or **less than additive effect**.
- The combined treatment **30 US/PL/ MT** was the most effective one achieving **5.8 – 6.4 log reductions** in apple juice

A. Acidoterrestris inactivation achieved by single and combined treatments

US: 15 °C (■); 44 °C (■); **PL**: 2-12 °C (■) ; 44 -56 °C (■)

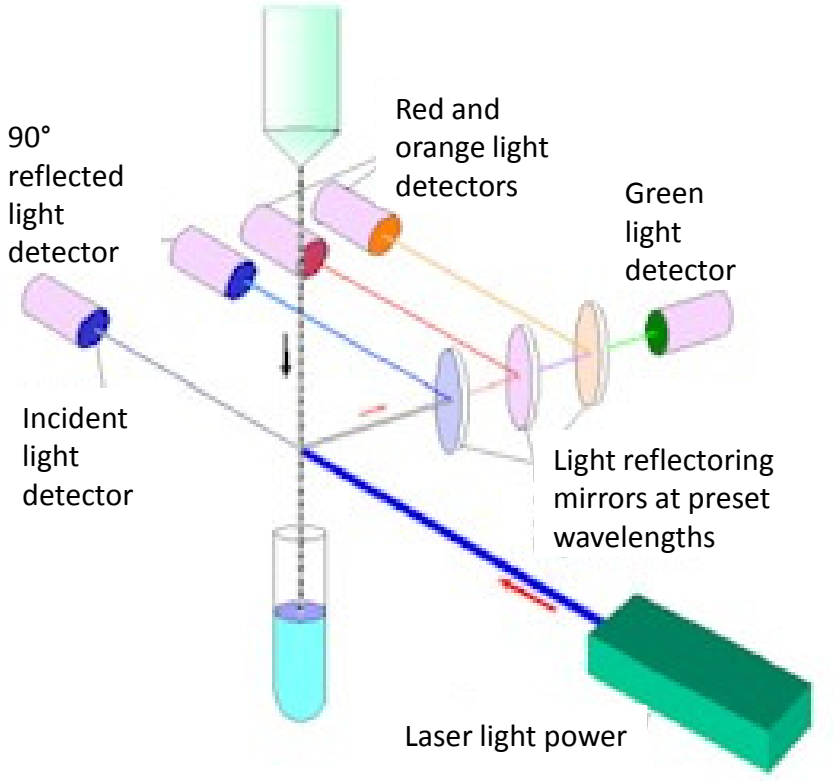


- US was innefective inactivating ***A. acidoterrestris*** **spores**
- Combined US+PL treatments did not improve single PL treatments
- PL treatments with MT final temperature significantly improved inactivation

Food safety engineering - Some indicators:

- Bulk methods: Inactivation studies as function of dose
- Single cell analysis
  - Flow cytometry (FCM)
  - Transmission Electron Microscopy (TEM)

Flow cytometry: fundamentals & procedure



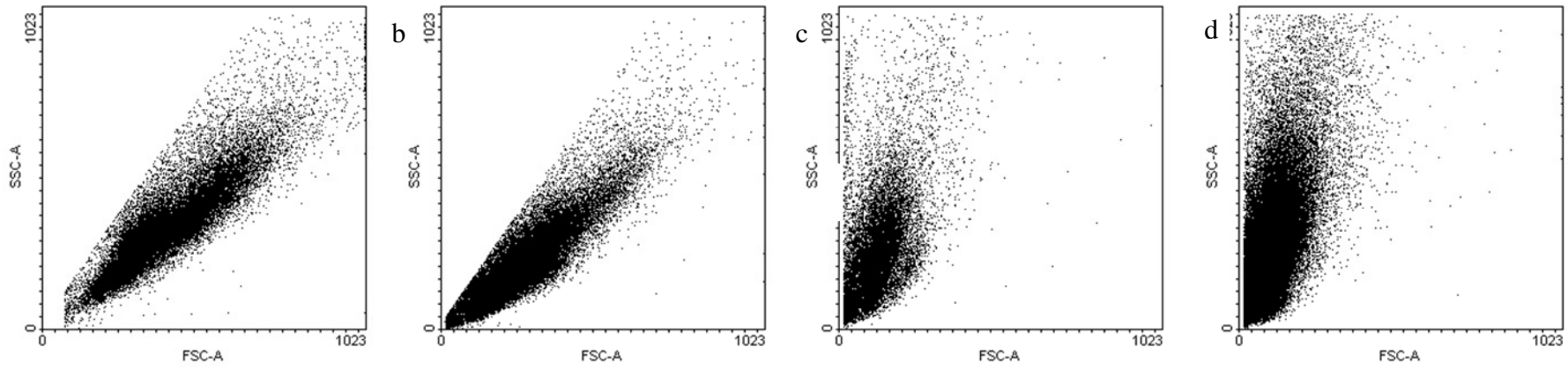
Through **light deviation** to different angles and, the **fluorescence signals**

Cell parameter information at single cell level

(size, surface granularity, physiological status)

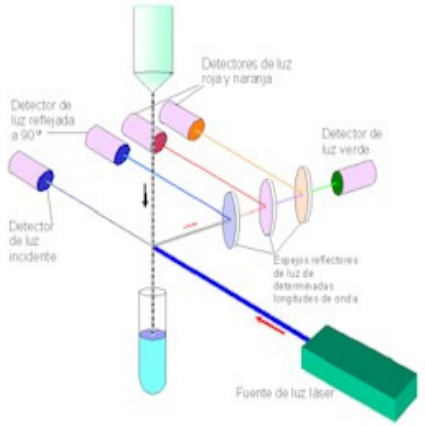
FLOW CYTOMETRY:

Dot plots representing forward scatter light (FSC) versus side scatter light (SSC) of *S. cerevisiae* cells in NAJ submitted to different treatments/MT

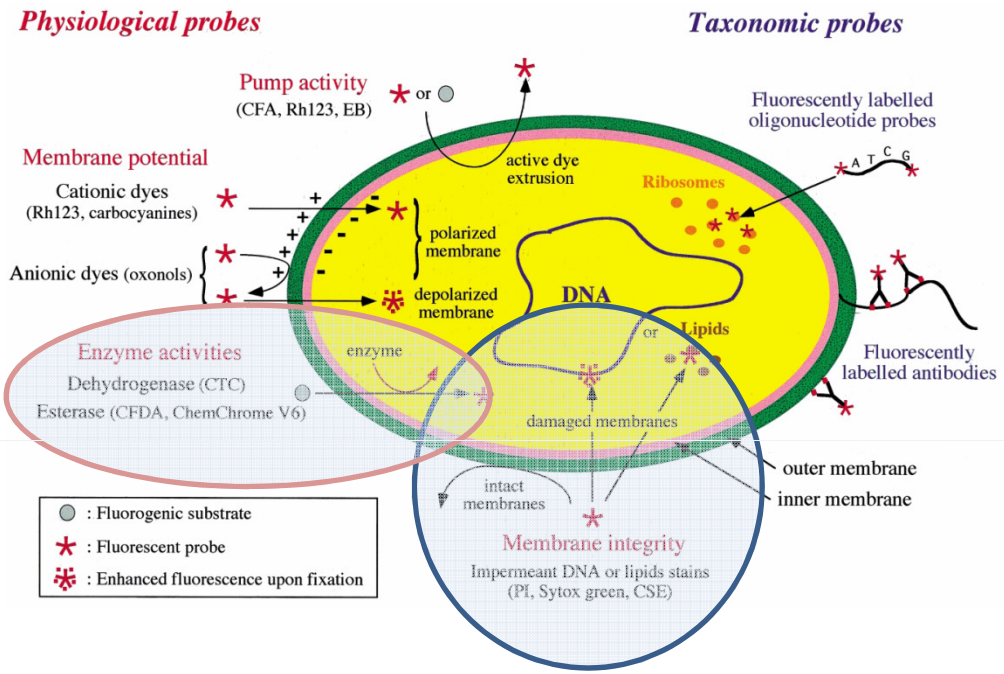


Untreated cells **PL** **10 US** **10 US+PL**

- For the combined treatment cell size dramatically decreased possibly due to damage in the membrane integrity and cytoplasmic disorder



Flow cytometry: fundamentals & procedure



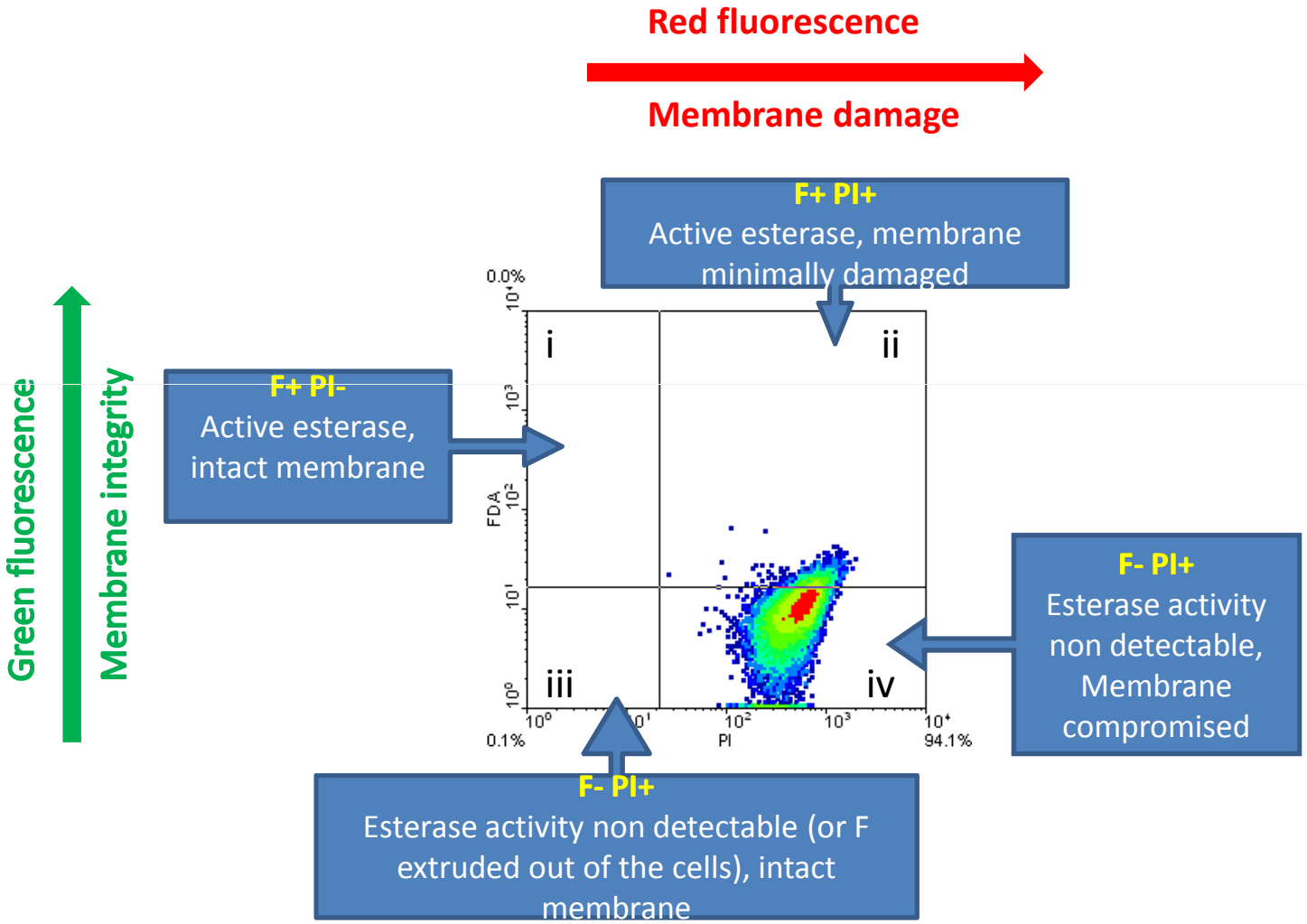
(Joux *et al.*, 2000)

FCM Procedure

(BD FACSAria II; New Jersey, USA; Flow speed: 200 cells/s; 20.000 events/sample).

- *S. cerevisiae* cells were double stained with fluorescein diacetate (FDA) and propidium iodide (PI).
- Fluorescent and scattering signals of individual cells were collected as logarithmic signals.
- Green fluorescence of cells stained with F was collected at channel FL1 (525 ± 15 nm), while red fluorescence of cells stained with PI was collected at channel FL2 (620 ± 15 nm)
- WinMDI 2.8 was used for the analyses of data. Measurements were performed by triplicate and % cells ± standard deviation was calculated.

Viability assessed by FCM



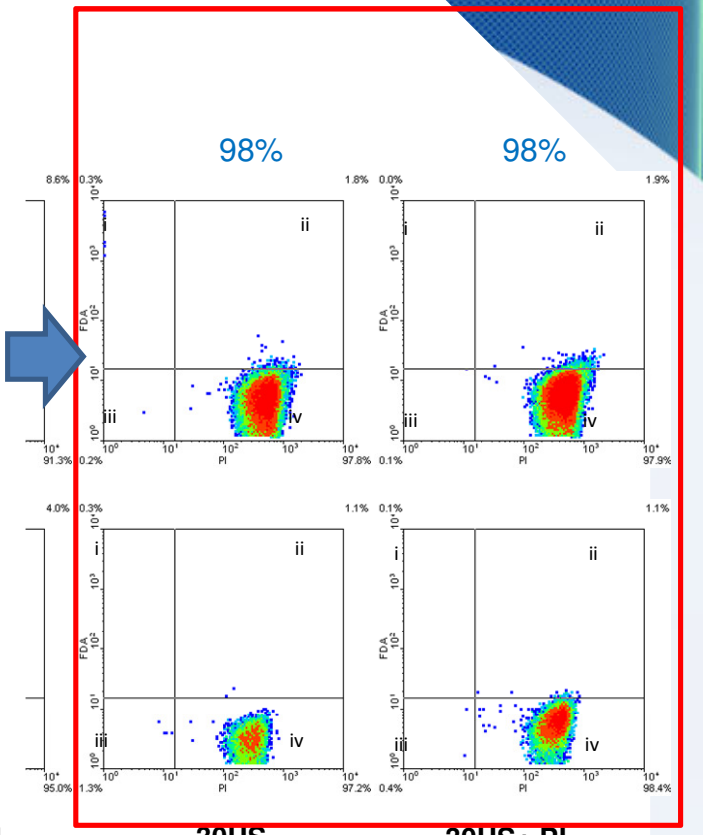
Fluorescence density plots of *S. cerevisiae* in response to staining with FDA and PI after single and combined MT treatments(US : 44 °C PL: 44-56 °C)

CAJ

- No differences in the percentages of $F^{-}PI^{+}$ subpopulation were observed for despite the observed differences in the inactivation degree.
- Cell sorting of fractions in gate 4 showed that cells could not be able to recover in **30US+PL** but 1 x 10^2 CFU/mL were recovered in **30US** system at T_{US} 44 °C.

NAJ

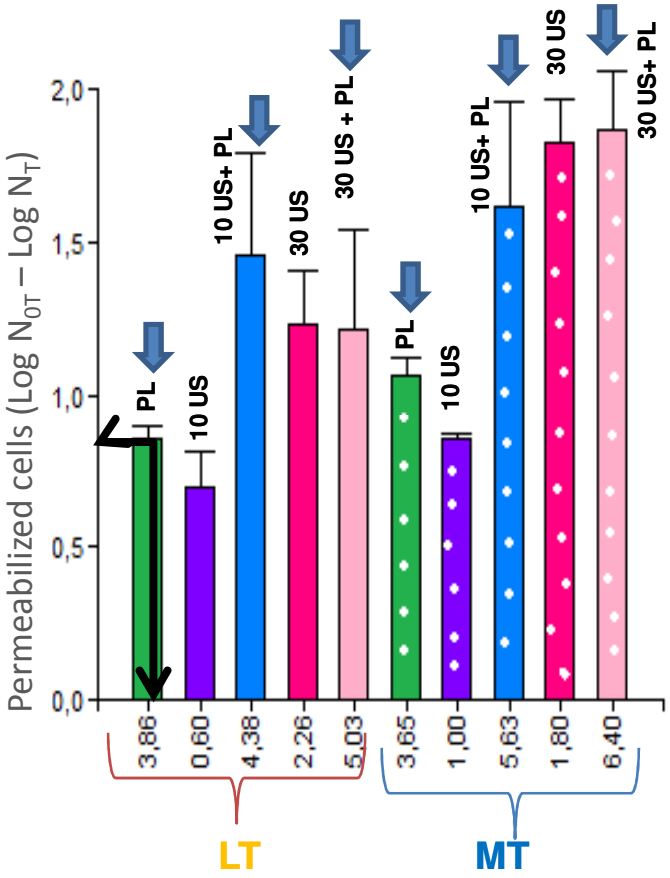
This suggests that membrane permeabilization occurred, but other mechanisms contribute to yeast inactivation.



0	PL	10 US	10US+ PL	30US	30US+ PL
1 %	68%				98%

- A great proportion of **double-stained cells ($F^{+}PI^{+}$)** were detected when **single PL** was applied (~26 % cells).
- cells with esterase activity and partially damaged too
- The presence of this subpopulation is not detected by traditional method and could affect the product shelf life

Permeabilized *S. cerevisiae* cells in CAJ determined by PI uptake as a function of non viable cells determined by CFU method



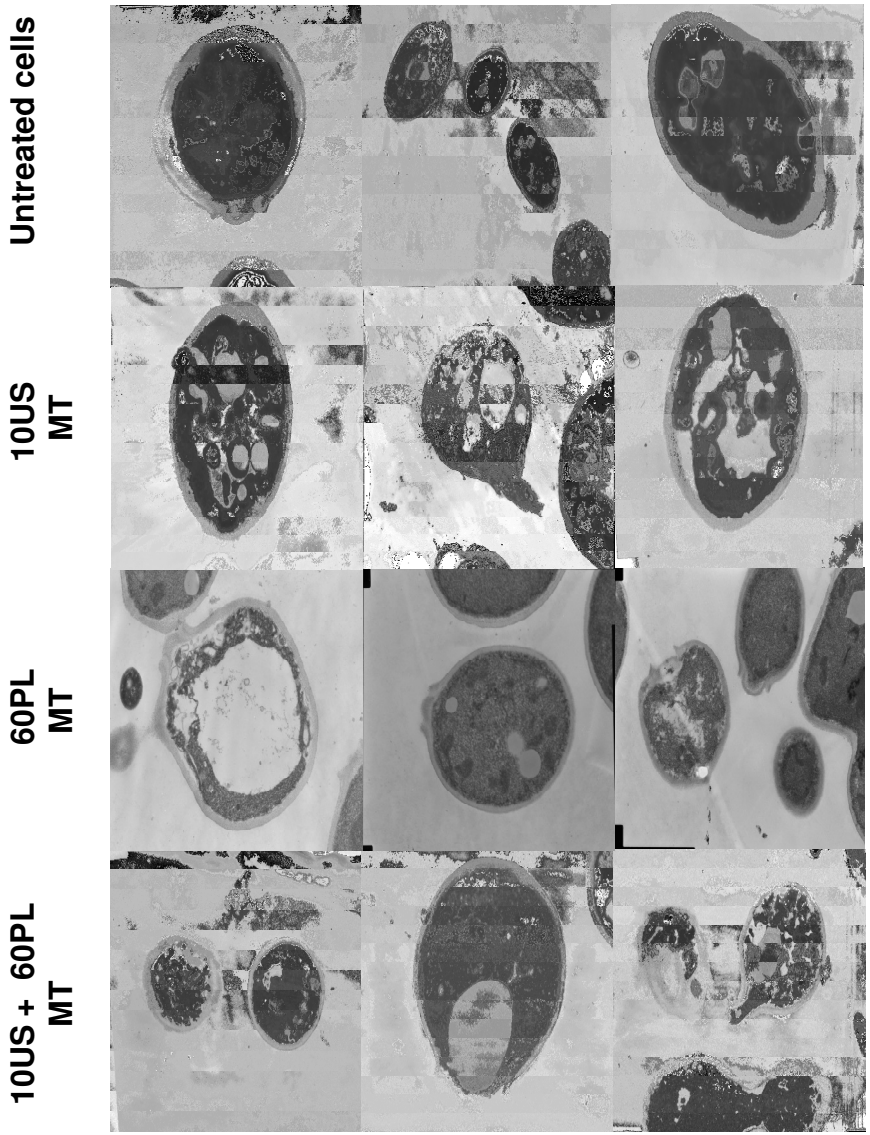
- The number of non viable cells for all PL treatments was **higher** than the number of permeabilized cells

Membrane integrity is critical but, there are other factors responsible for cell viability

Food safety engineering - Some indicators:

- Bulk methods: Inactivation studies as function of dose
- Single cell analysis
  - Flow cytometry (FCM)
  - Transmission Electron Microscopy (TEM)

TEM examination of *S. cerevisiae* KE162 *in apple juice (CAJ) with different treatments*



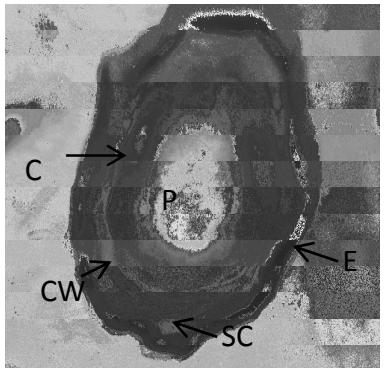
- Intact yeast cells showed well defined organelles and membranes

- Presence of vacuoles ; broken cell wall with partial release of cellular content
- Disorganized inner structure, but cell wall non broken

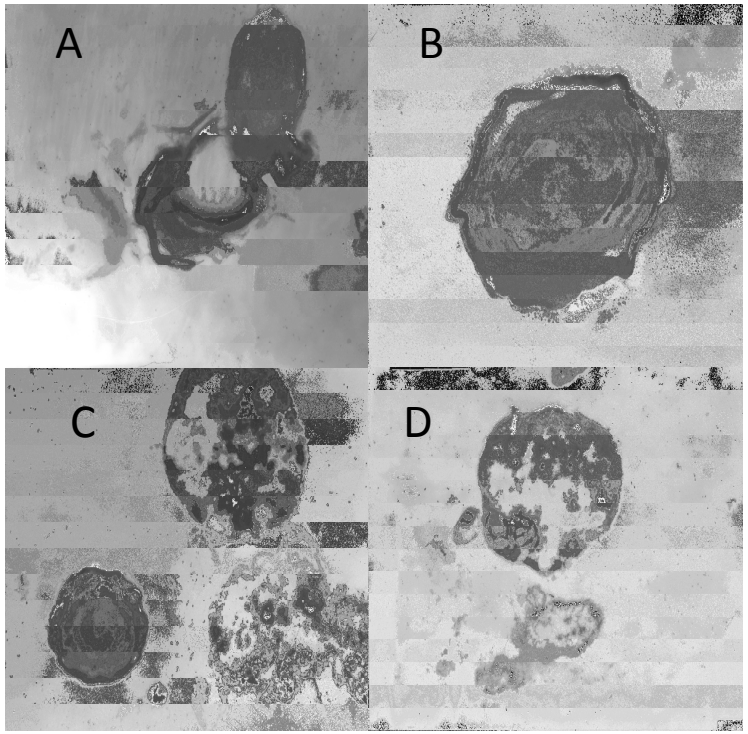
- Cells more rounded with unnatural shape, with less electronic density.
- Inner content looked coagulated, extremely vacuolated and coarse.

- Cells deeply damaged with organelle disruption and generalized rupture of membranes
- Presence of subcellular fragments out of the cell
- Swollen damaged cell wall, vacuoles and shrinkage of cytoplasmic material from cell wall
- Rupture of cell wall and release of inner content

TEM images of *A. acidoterrestris* ATCC 49025 in CAJ treated by PL (60 s, 44-56 ºC ,~ 3 log red)



- Intact endospore showed well defined protoplast (P), cortex (C), Core wall (CW), multilayered spore coat (SC) and Exosporium (E)



- Presence of subcellular fragments out of the spore (A)
- Separation of exosporium from the spore coat (B)
- Inner content looked coagulated (C,D)
- Spores with unnatural shape and deeply damaged (A,C,D)
- Spores that totally or partially lost their content ("ghost spores") (C,D)

Conclusions

- **Temperature build up in the juice due to PL treatment** may be used as an **additional microbial stress factor**

- Loss of membrane integrity and metabolic activity were observed by **FCM analysis**
- FCM allowed the detection of **double stained cells** which were not detected by PCM.
- In PL treatments**, membrane integrity** was not the only factor in determining cell viability.

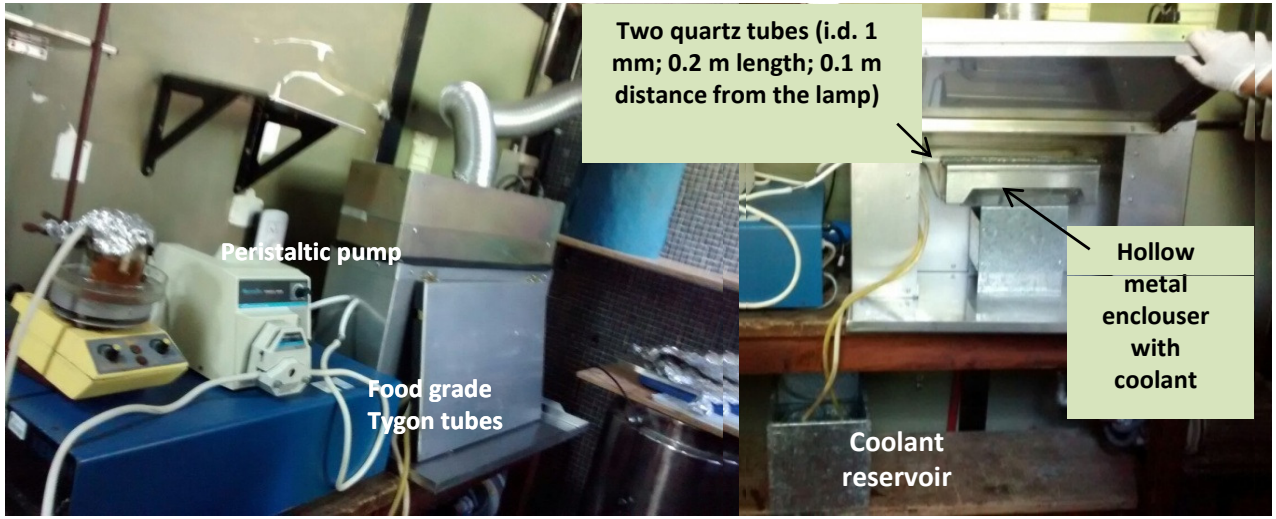
- **TEM observations revealed different changes in cell structure.**
- PL treated cells exhibited mainly significant disorder inside the cells, while US affected cell wall and membrane too.
- Alterations in the inner content and in the cell wall deepened when the combined US + PL treatment was applied.

In food safety engineering , inactivation, FCM and TEM studies were altogether useful indicators as starting basis for processing design to obtain improved or more safe fruit juices

THANK YOU !

Universidad de Buenos Aires
UBA
Buenos Aires - Argentina

Sandra N. Guerrero
Natural and Exact Science School
Buenos Aires University
sguerrero@di.fcen.uba.ar


Peristaltic pump
Food grade
Tygon tubes
Two quartz tubes (i.d. 1 mm; 0.2 m length; 0.1 m distance from the lamp)
Hollow metal enclouser with coolant
Coolant reservoir

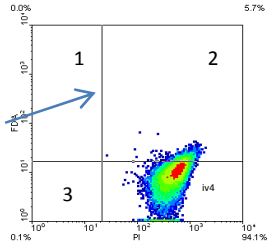
Two stress factors that have different action modes to overwhelm the target cells´ repair systems

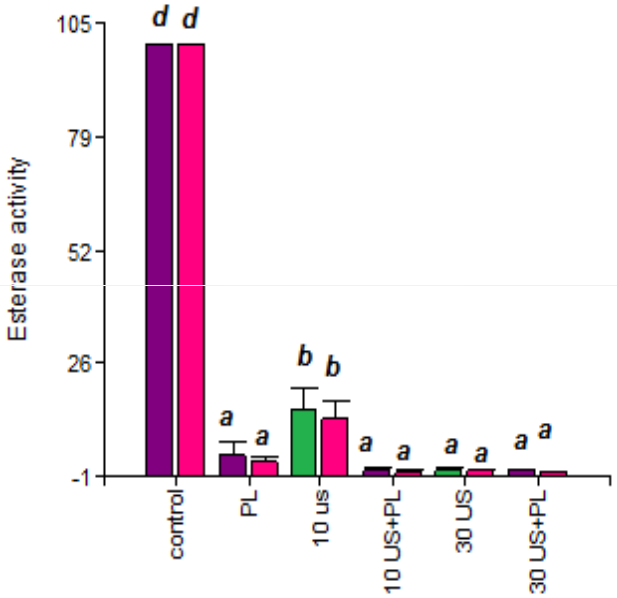
Possible arrangements

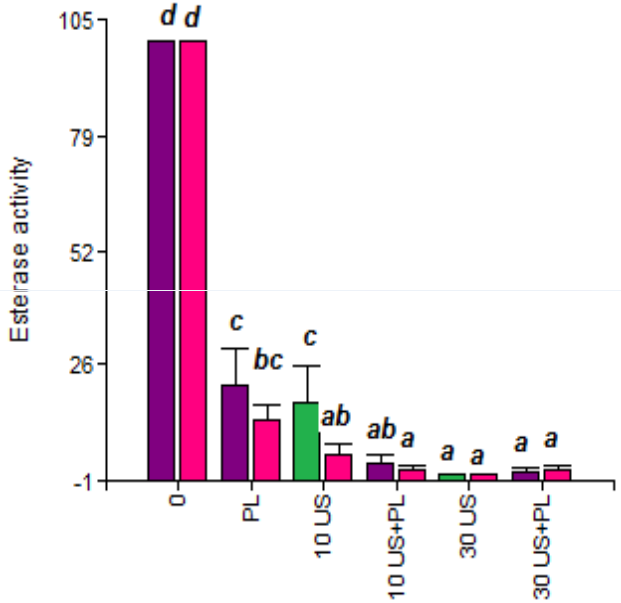
- Two or more stress factors **simultaneously** applied to inactivate pathogens and deteriorative microorganisms
- One or more stress factors to **inactivate/damage** microorganisms and then, on **sequential mode**, one or more factors **to avoid growth/survival** of sub lethally damaged or resistant cells
- Two or more stress factors **sequentially** applied

Esterase enzyme activity

$$\%EA = \left(\frac{\#1_T}{\#1_{Ctrl}}\right) \times 100$$



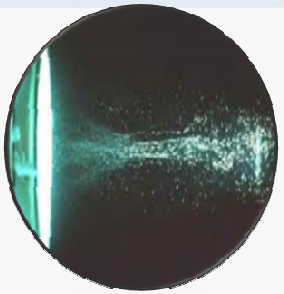


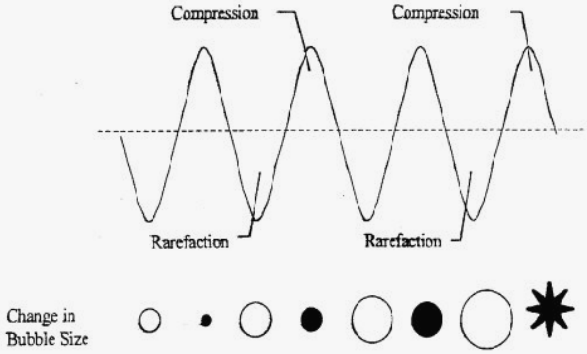


US: 20 °C (■); 44 °C (■) **PL**: 2-12 °C (■) ; 44 -56 °C (■)

High intensity ultrasound (US)

- Energy generated by sound waves of 20 kHz or more
- Microbial inactivation by “cavitation ” phenomenon.



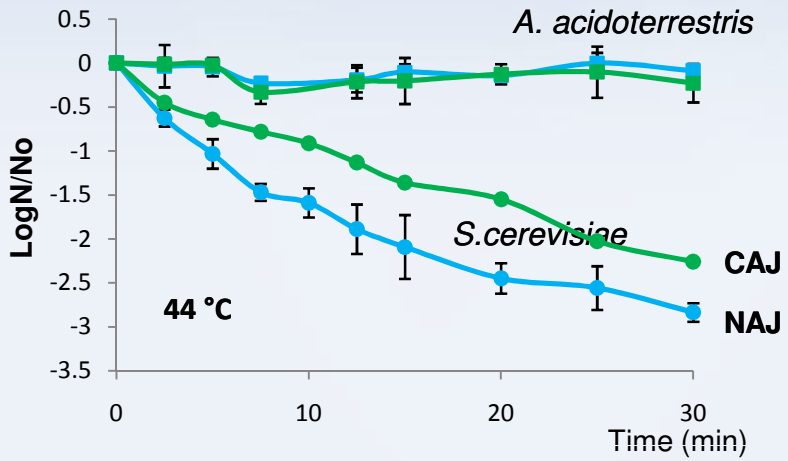


Gas or vapour microbubles formed during a tension cycle, violently collapse (compression cycle) generating high pressure shock waves and high local heating

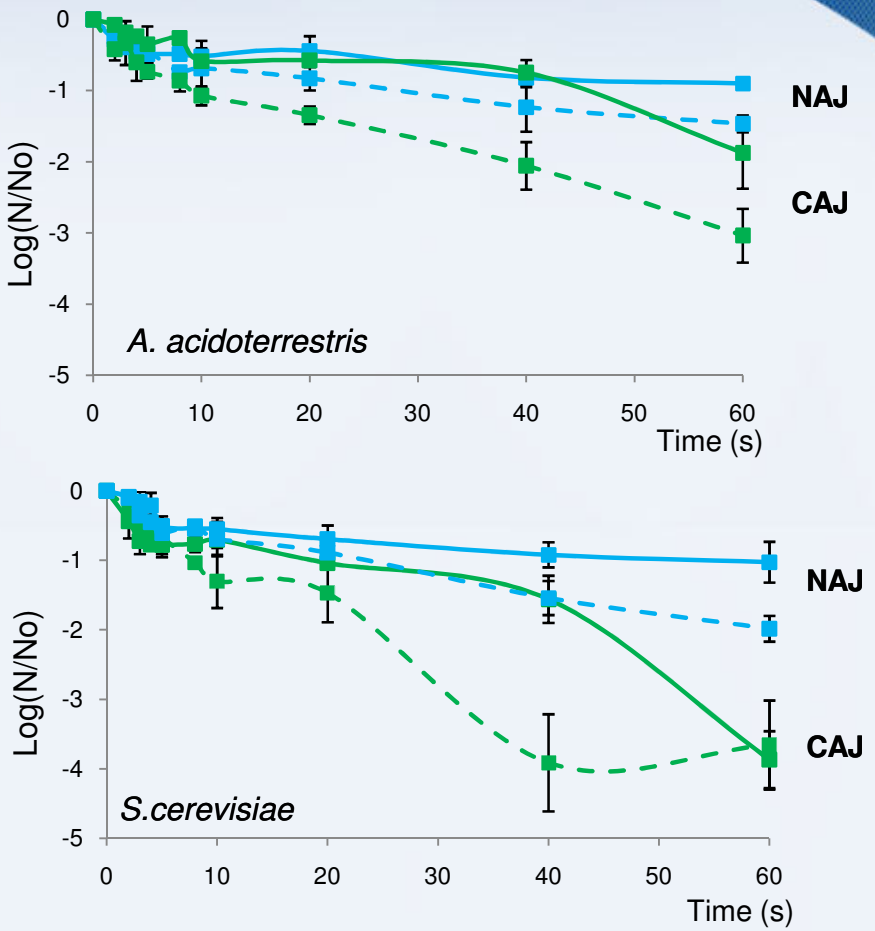
- Influencing factors: wave amplitude, temperature, volume, sample properties and composition
- Actual/potential applications: No commercial food products. Limited to product modification and process efficiency improvements (enhancement of mass and heat transfer, degassing of liquids, cleaning of surfaces)

Inactivation kinetics by single US and PL treatments

US

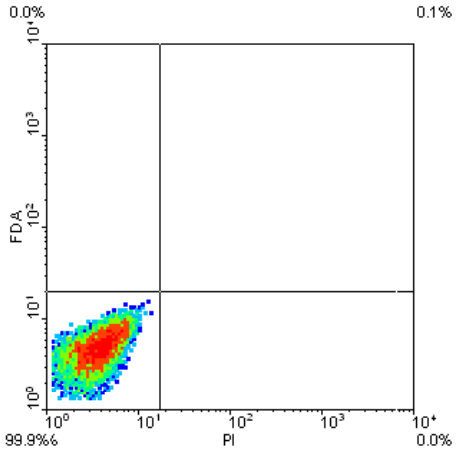


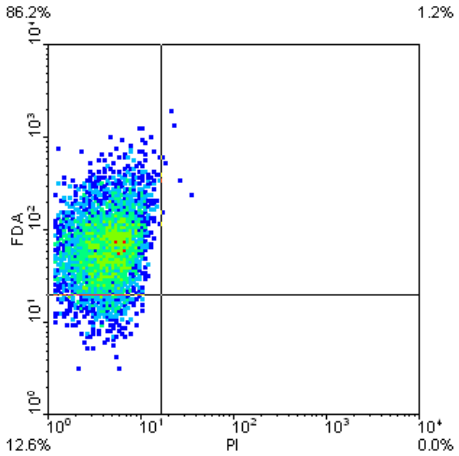
PL

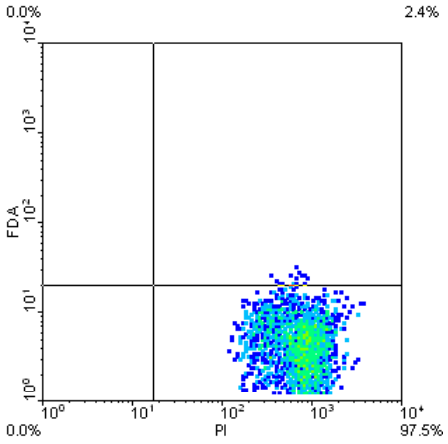


Inactivation curves by single PL treatments at LT(—) and MT(---) in commercial apple juice (—) and natural apple juice (—)

controls

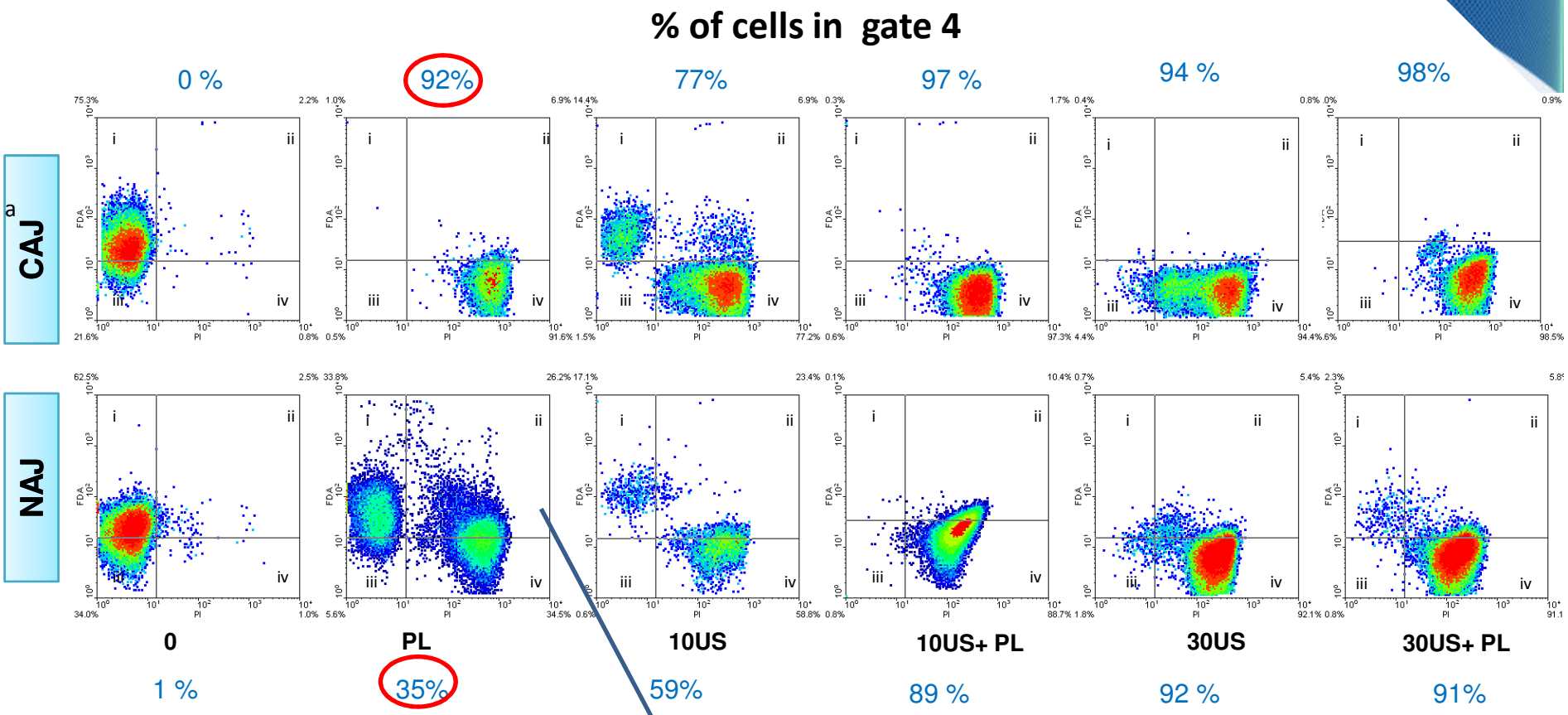






Autofluorescence FDA PI

Fluorescence density plots of *S. cerevisiae* in response to staining with FDA and PI after single and combined LT treatments (US : 15 °C PL: 2-12 °C)



- A great proportion of **double-stained cells (F^+PI^+)** were detected when **60PL** was applied (~26 % cells).
- Cytoplasmic membrane integrity was being affected while cells were capable or retaining esterase activity.
- The presence of this subpopulation could seriously affect shelf life in PL treated foods.