# MICROBIAL INACTIVATION BY ULTRASOUND

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## ABSTRACT

The Food Technology group of the University of Zaragoza has investigated the effect of ultrasound and its combination with other factors on the microbial inactivation since the early 90's. Our group designed an equipment that allows to apply ultrasound under pressure treatments at sublethal (Mano-Sonication; MS), and at lethal temperatures (Mano-Thermo-Sonication; MTS) to liquid products. The influence of physical and environmental factors on the resistance of vegetative and spore cells to MS and MTS has been investigated. The MS and MTS processes could be an alternative to current heat treatments of heat-sensitive foods or in products where microorganisms were particularly resistant.

## INTRODUCTION

Heat, compared to other food preservation methods, has the important advantage of ensuring food safety and long preservation due to its destructive effect on enzymes and microorganisms. However, the non-specific effect of heat can cause reductions in nutritive and sensorial quality of foods and impairs their functional properties. To avoid unwanted effects of heat many attempts have been made to design alternative procedures for food preservation and sanitation [1]. High Hydrostatic Pressures, Pulsed Electric Fields, High Intensity Magnetic Fields and Ultrasound are the main alternative processes.

Ultrasound is defined as sonic waves with frequencies over the threshold of human hearing (16-20 kHz). Ultrasound generators generate these high frequency waves based on the properties of some piezoelectric materials. These vibrate with a specific amplitude at the ultrasonic frequency when voltages are applied as high frequency pulses [2]. Recently, high intensity (<100 kHz; >10 $Wcm^{-2}$) and low intensity (>100 kHz, <1 $Wcm^{-2}$) ultrasound have been considered for food industry applications. Low intensity ultrasound are used as analytical techniques to obtain information about different physical-chemical properties (composition, particle size, etc.) or to control several processes (flow of fluids, overfilling of containers, etc.) [3]. High intensity ultrasound have been suggested for different applications including microbial and enzymatic inactivation from foods. When these high intensity ultrasound propagates in liquid media, the cavitation phenomena is generated. Cavitation consists on the formation, growth and suddenly implosion of bubbles. Due to the implosion, the molecules around the bubble hit each other violently creating spots of very high temperature (5500 °C) and peaks of pressure ($10^4$-$10^5$ kPa) [4]. It is estimated that these temperatures and pressures in the spots have a life lower of 1 µs, and the liquid heating and cooling speed is in the range of $10^9$ °C/s [5]. The high intensity ultrasound effects are dependant on the number and intensity of bubbles implosioning per unit of time, the characteristics of the treatment and the characteristics of the treatment media.

## MICROBIAL INACTIVATION BY ULTRASOUND

The microbial inactivation effect of ultrasound is known since the early 30's [6]. Since then, several investigations have been carried out to study the inactivation effect of ultrasound [7, 8], and ultrasound combined with other agents [9, 10]. At the early 70's, it was observed that heat sensitivity of spores increased with a previous ultrasonic treatment [11]. Subsequently, it was demonstrated that a simultaneous heat and ultrasound treatment (thermoultrasonication) had a higher lethal effect than a heat treatment at the same temperature [12]. At the early 90's, the Food Technology group of the University of Zaragoza designed an equipment called Mano-Thermo-Sonicator. With this equipment, the microbial inactivation effect of ultrasound and its combination with pressure (Mano-Sonication; MS) or with pressure and heating (Mano-Thermo-Sonication; MTS) was investigated.

Microbial inactivation by ultrasound depends on several factors that can be classified under treatment conditions, microbial characteristics and environmental factors.

### Treatment Conditions

Time and amplitude of the ultrasonic treatment, and hydrostatic pressure and temperature of the medium are the main factors that influence on the microbial inactivation by ultrasound.

#### Treatment time

Ultrasonic microbial inactivation increases with the treatment time. An exponential relationship between the survival fraction and the treatment time was observed. Based on this, the microbial resistance to ultrasonic waves can be estimated from the decimal reduction time (D value) that is defined as minutes of treatment at a given temperature needed for the number of survivors to drop one log cycle.

#### Hydrostatic pressure

Yeast [13], bacterial vegetative cells and spores [14, 15] inactivation by ultrasound increases with the hydrostatic pressure of the treatment medium. The inactivation effect of ultrasound increased exponentially with pressure in the range of 0 to 200-300 kPa. Over 300 kPa, new increments of the pressure hardly improved the inactivation effect of MS. Figure 1 shows the ultrasonic resistance of *Escherichia coli* at different pressures of treatment.

The influence of the hydrostatic pressure on the lethal effect of ultrasonic waves is related to the effect of pressure on the cavitation phenomena. When the pressure is raised, the bubble implosion intensity increases and the number of bubbles implosioning per unit of time is reduced [16]. Therefore, although at higher pressures the intensity of the implosion is stronger, the lower number of bubbles collapsing would explain the decrease of the efficiency of the ultrasound lethal effect over a determine pressure value.

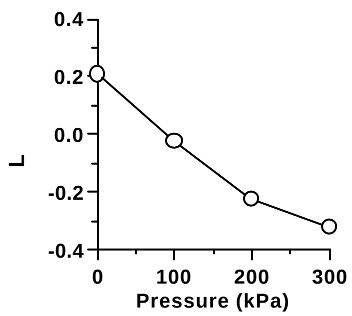


**Fig. 1.** *E. coli* resistance to ultrasonic treatments (20 kHz, 117 µm) at different hydrostatic pressures.

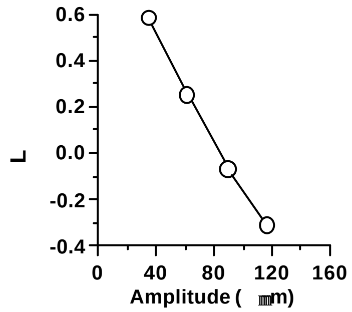


**Fig. 2.** *E. coli* resistance to ultrasonic treatments (20 kHz, 200 kPa) at different ultrasonic amplitudes.

Ultrasonic amplitude

It has been observed that the inactivation of both bacterial vegetative cells and spores exponentially increases with the amplitude of the ultrasonic waves [14, 15]. Figure 2 shows the influence of the amplitude on the resistance of *E. coli* to an ultrasonic treatment. The higher inactivation rate at greater amplitudes could be due to an increase in the number of bubbles undergoing cavitation per unit of time [17] or to an increase in the volume of liquid in which cavitation is liable to occur [4].

Temperature

In order to reduce the intensity of heat treatments, the microbial inactivation effect of combining treatments of ultrasound under pressure and heat (Mano-Thermo-Sonication) has been investigated. An additive effect of this combination has been observed on the inactivation of most of vegetative cells investigated [14, 21, 29] and a synergistic effect for *Streptococcus faecium* [14] and for *Bacillus subtilis* spores [15].

Microbial Characteristics

Microorganism

It is generally admitted that cells of a bigger size are more sensitive to ultrasound [9] and rod-shaped bacteria more than coccal forms [8]. The Gram-positive are more resistant than Gram-negative, aerobic more than anaerobic species [9] and bacterial spores more than vegetative cells [18].

In spite of the different resistance of vegetative cells to ultrasound, the influence of the hydrostatic pressure and the ultrasonic amplitude on the inactivation effect of these treatments is independent of the microorganism. Thus, two mathematical equations, one for the influence of the pressure and another one for the influence of the amplitude have been developed. These equations allow to predict the inactivation effect of a determine ultrasonic process [14].

### Growth phase

The influence of the growth phase on the microbial resistance to ultrasonic waves is not clear. While some authors have found out a non-influence of this factor on the inactivation effect of ultrasound [7], others have observed that cells of *E. coli* at the exponential growth phase were more sensitive to ultrasonic treatments than that at the stationary phase [19].

### Growth temperature

Regardless of the influence of this factor on the microbial inactivation by heat or high hydrostatic pressure, the resistance of different microorganisms, such as *Listeria monocytogenes* and *Yersinia enterocolitica* to ultrasonic treatments, was independent of the growth temperature [20].

### Sublethal heat treatments

Microbial thermo-resistance increases to a great extent when microorganisms are heat-shocked. This higher resistance could be due to cells synthesized specific proteins called "heat shock proteins" that protect bacteria against heat.

Pagán *et al*. [21] observed that *L. monocytogenes* heat resistance at 62ºC increased 6 times after a heat shock of 180 min. at 45ºC. However, this shock did not modify its resistance to a treatment of ultrasound under pressure (117 µm, 200 kPa, 40ºC). Besides, the lethal effect of a MTS treatment on cells previously treated with a sublethal heat treatment was synergistic in a determine range of temperatures.

## Environmental Factors

### pH

Commonly, microbial heat resistance decreases in acidic media. However, different authors have observed that the ultrasonic resistance of microorganisms was independent of the acidity conditions of the medium [21, 22].

### $a_w$

It is well known that reduced water activities influences in a great extent the microbial thermal resistance [23, 24]. A decrease of the $a_w$ also increases the microbial resistance to ultrasound, however this increment is lower. While the addition of 50 and 57% of sucrose to the medium increased 30 and 25 times the thermal-resistance of *Salmonella enteritidis* and *L. monocytogenes*, respectively, their MS resistance was only duplicated [20, 25]. A synergistic effect was also observed on the MTS inactivation of these microorganisms in media of reduced water activity.

Composition of the treatment medium

In general, the microbial resistance to ultrasonic treatments increases in food [8]. This protective effect depends on the composition of the product. *L. monocytogenes* showed the same resistance to a MS treatment in skim milk than in McIlvaine buffer of pH 7 [21]. However, the resistance of *Salmonella senftenberg* in liquid whole egg was two times higher than in pH 7 McIlvaine buffer [26].

## MECHANISM OF ACTION OF ULTRASOUND

The lethal effect of high power ultrasound is due to the cavitation phenomena. When bubbles implode in an ultrasonic field, high temperatures and pressures are generated at the collision point. Therefore heat, pressure shock waves, or both could be the responsible for the lethal effect of ultrasound. On the other hand, the very high temperatures and pressures of collapsing bubbles lead to generate free radicals such as $H^{\cdot}$ and $OH^{\cdot}$ [17]. These very reactive radicals could be responsible for the inactivation of bacterial cells by oxidative damage similar to that caused by hydroperoxides [27].

When a bubble implodes, heat is generated in the liquid surrounding the cavity. The volume of liquid heated is very small and the heat dissipates quickly, though the temperature of this region is extraordinarily high for a few microseconds [4, 5]. However, most published data indicate that heat does not contribute to the lethal effect of ultrasound neither at room nor under static pressure. On the other hand, the percentage of damaged cells of a bacterial population increases throughout the heat treatment time, but damaged cells have not been observed under ultrasonic treatments [21, 25].

It has been demonstrated that the lethal effect of ultrasonic waves under pressure on bacterial spores [28] and vegetative cells [19, 20, 29] was the same when cysteamine (a well-known free radical scavenger) was added to the treatment medium. Therefore, any important contribution of the free radicals to the inactivating effect of ultrasound on microorganisms can be discarded.

Therefore, it seems that the pressure shock waves, originated by the implosion of bubbles undergoing cavitation, are the ultimate reason for the inactivating effect of ultrasound. In fact, broken cell walls have been observed with electronic microscopy after an ultrasonic treatment [19]. And there was found a correlation between the number of broken cells, observed with phase-contrast microscopy, and the number of inactivated microorganisms determined by plate counts [29].

## APPLICATION OF ULTRASOUND ON FOOD PRESERVATION

Although the inactivation effect of ultrasound has been known for decades, ultrasound has not been applied for food preservation due to its limited lethal effect. The increase on the lethal effect of ultrasound when is applied under pressure (MS) or combined with pressure and heat (MTS) opens new possibilities to its use instead of traditional heat treatments. On the other hand, several investigations have demonstrated the inactivation effect of ultrasonic waves on enzymes of interest for the food industry [30, 31].

Therefore, MS and MTS processes could be an alternative for preservation of thermal-sensitive liquid foods, especially when raw material is contaminated with very heat-resistant bacterial species, or when food components protect microorganisms to heat. However, up to now, the impossibility to apply to industrial scale the used intensities at laboratory level limits its application.

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