Effects of heat treatments in combination with high hydrostatic pressures (HHP) on the viability and physiological state of *Clostridium* species

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Resumen

Clostridium es un microorganismo esporulado anaerobio cuyas esporas presentan un amplio rango de valores D_{100} de 16 a 124 minutos dependiendo de la cepa y otros factores. Tratamientos térmicos más severos serían necesarios para inactivar las esporas bacterianas en la industria alimentaria pero estos podrían afectar la calidad de los alimentos. Por ello, la industria alimentaria está buscando tecnologías alternativas. Las altas presiones hidrostáticas en combinación con calor es una interesante alternativa que muestra un efecto sinérgico que mejora la inactivación de los microorganismos. Después del tratamiento, algunos microorganismos dañados pueden permanecer en el producto. Se ha mostrado que los microorganismos dañados de algunas especies pueden adaptarse a diferentes tipos de estrés y desarrollar resistencias cruzadas comprometiendo la calidad y seguridad alimentaria. Por tanto, es necesario optimizar las tecnologías alternativas y asegurar que éstas causan el mínimo daño subletal.

Palabras clave: conservación de alimentos; tecnologías no térmicas; microorganismos esporulados; daño subletal; adaptación al estrés

Abstract

Clostridium is an anaerobic spore-forming microorganism whose spores show a wide range of D100 values from 16 to 124 minutes depending on the strain and other factors. More severe heat treatments would be necessary to inactivate the bacterial spores in the food industry but these could affect the food quality. The food industry is looking for alternative technologies and it is reported that the high hydrostatic pressures in combination with heat show a synergistic effect which improves the inactivation of microorganisms. After the treatment some damaged microorganisms could remain in the product. It is reported that damaged microorganisms of some species adapt themselves to several stressful conditions and develop cross-resistances compromising the food quality. Thus, it is necessary to optimize the alternative methods and ensure they cause the minimum sublethal damage.

Keywords: food preservation; alternative technologies; spore-forming microorganisms; sublethal damage; stress adaptation

1. Introduction

Clostridium is one of the most frequent heat-resistant spore-forming pathogen [1; 2].

Currently, heat treatments are still the most used methods to preserve food. However, due to the high heat resistance of some bacterial spores, more severe heat treatments would be necessary [3; 4]. This might decrease the food quality.

The high hydrostatic pressures have emerged as a powerful alternative to the heat treatments. However, the optimization of this emergent technology is required. For this purpose, suitable scientific data are necessary.

The aim of this bibliographic research is to obtain an overview of the combined use of the preservation technologies for microbial inactivation as well as of the effect of such technologies on the microbial population in order to validate and optimize the high hydrostatic pressures combined with heat for food preservation.

2. Materials and Methods

Published papers in SCI journals related to this subject were used to carry out this bibliographic research. Scientific databases such as Sciencedirect and Scopus were the searching tools to obtain such information. Reports from the food safety authorities were also used.

3. Results

Generally, the conventional heat treatments are well established and traditionally applied to pasteurize and sterilize food. However, in recent years, occurrence of more heat resistant spores often evoked even more severe heat treatments [3; 4].

Clostridium is an anaerobic, Gram-positive, spore-forming microorganism and it is considered as one of the most frequent spore-forming pathogen [1]. In fact, bacterial toxins produced by *Clostridium* were one of the most frequently identified agents in the UE causing 12 deaths [2]. *Clostridium perfringens* is the third most common cause of bacterial disease foodborne in the United States causing around 250,000 cases each year [5]. Some authors have reported that the spores of *Clostridium perfringens* are able to survive 15-22 minutes at 100ºC in meat gravy [6] while others reported that *Clostridium perfringens* survives 1 hour at 100ºC [7] and shows a wide range of D_{100} values from 16 to 124 minutes depending on the strain [8; 9]. It has also been reported that depending on the enterotoxin gene location of *Clostridium perfringens*, this microorganism shows a higher or a lesser heat resistance [8]. Thus, the current heat resistance data of *Clostridium perfringens* spores are very different. However, there are no data in the literature about the heat resistance of *Clostridium perfringens* spores and the behaviour of this microorganism under non-isothermal conditions. On the other hand, *Clostridium difficile* is also a spore-forming microorganism which produces severe enteric diseases in humans worldwide [10]. There is no much data in the literature about the heat resistance of its spores, as this microorganism was not frequently related to food. Yet, its presence in animal food is increasingly documented since 2006 [11; 12; 13; 14; 15] and it has been shown that it survives the minimum cook temperature recommended for cooking meat [16] and at least 15 minutes are required to inactivate it at 100ºC [17].

More severe heat treatments, aimed at inactivating heat-resistant spore-forming microorganisms, could damage the organoleptic and nutritional quality of food. In addition, the current trend of consumers is to demand less processed and more natural products. In order to satisfy this, the food industry is looking for alternative technologies [18]. These alternative technologies would produce a series of log reductions of the initial load that are recognized as safe [19], which means that an important number of damaged microorganisms would remain in the product [20;21]. As the presence of these microorganisms might be dangerous for consumers, the preservation by mild treatments or alternative technologies must be properly validated. Thus, suitable scientific data are necessary to optimize and validate the alternative technologies.

The high hydrostatic pressures have emerged as a powerful alternative to the heat treatments and in combination with heat show a synergistic effect which improves the inactivation of microorganisms. It might be an effective method for spore inactivation [22; 17]. A study showed that *Clostridium sporogenes* spores are resistant to 1500 MPa at room temperature; yet, the addition of a mild heat treatment (60ºC) during the process of high pressures resulted in spore counts 5 log units lower in phosphate buffer, meat and in carrot juice [23].

It is relatively important to know the damage the microorganisms suffer during a technologic treatment and their ability to recover in suboptimal conditions [24;25;26] Some damaged microorganisms could remain in a product after the processing by any preservation technology [20;21] and they might be able to adapt themselves to several stressful conditions [27; 28]. When this happens, the food safety is compromised, being this a real challenge for the food industry [29]. Thus, an important issue in food safety is to check that the alternative methods of preservation inactivate specific microorganisms causing the minimum sublethal damage and avoiding or minimizing the stress adaptation [20;21] In previous projects of our group, damaged cells of *L. monocytogenes, Cronobacter sazakazakii, Salmonella* and *E. coli* O157:H7 has been detected after treatments by alternative technologies or mild heat treatments combined or not with natural antimicrobials [30; 31; 32]. It has also been reported that high heating rates are more lethal for the vegetative cells of *E. coli* than slow heating rates [33], although the influence of the heating rate on bacterial spores is not very clear [31; 34]. There are several methods available to assess the extension and nature of the sublethal damage within a microbial population such as cultivation in selective agars, cell staining using epifluorescence techniques and flow cytometry [35; 36; 37]. It is also important to take into account the capability of the microorganisms for recovery in fluctuating conditions of parameters such as temperature, pH or activity water (real circumstances in food). Recently, it has been reported the influence of these fluctuations in parameters such as storage temperature, pH or activity water on the capability for recovery and microbial proliferation [38; 39].

4. Conclusions

Research on the behaviour of the microbial population which survives both conventional heat treatments and alternative technologies (high hydrostatic pressures combined with heat) is necessary. This data will allow optimizing the preservation technique by high hydrostatic pressures in such way that it will produce the maximum death to the microorganism, minimizing the existence of sublethal damage.

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