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Exploiting the synergism among physical and chemical processes for improving food safety

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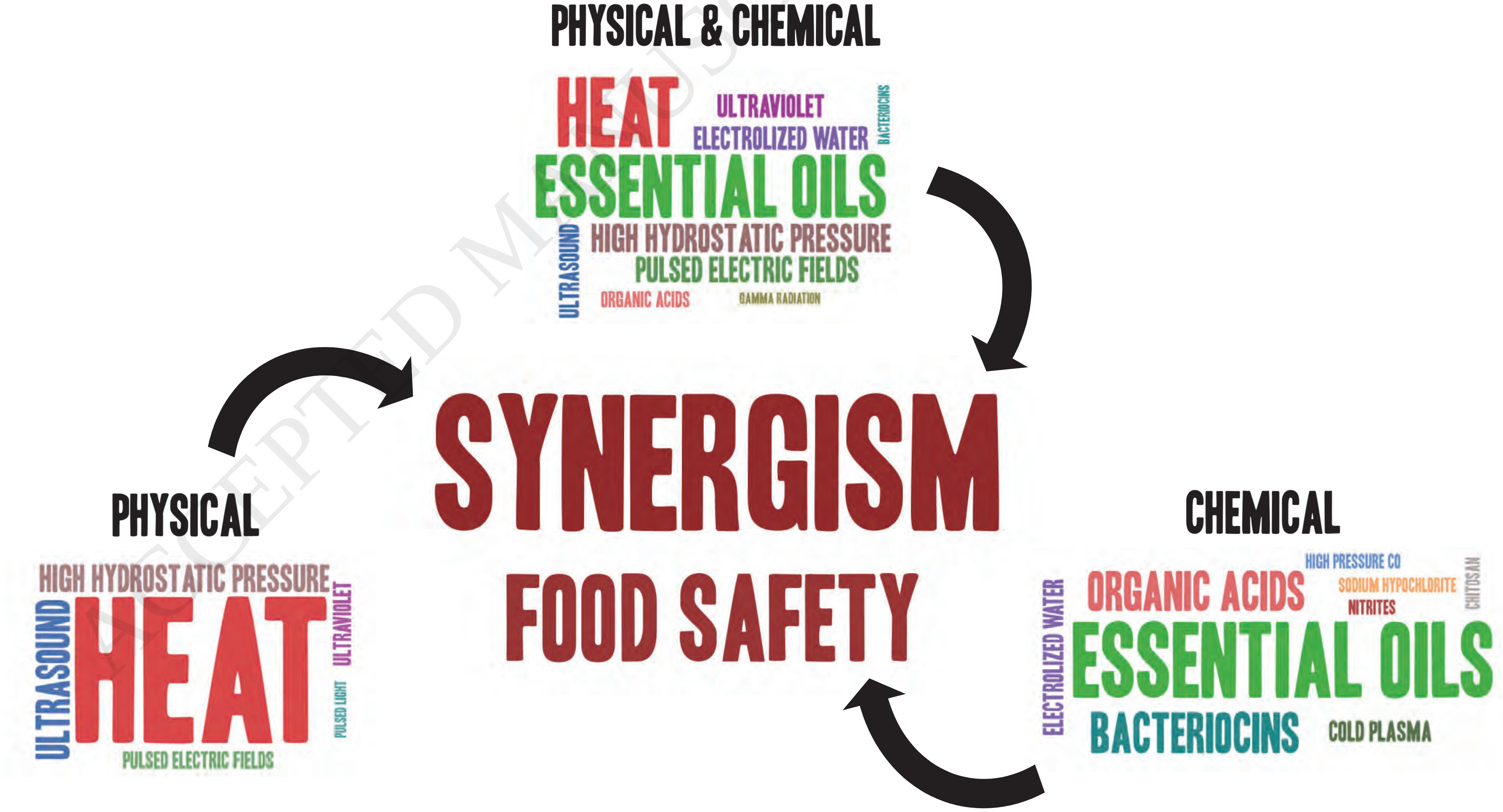

PHYSICAL & CHEMICAL
HEAT
ULTRAVIOLET
ELECTROLIZED WATER
BACTERIOCINS
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HIGH HYDROSTATIC PRESSURE
PULSED ELECTRIC FIELDS
ORGANIC ACIDS
GAMMA RADIATION
SYNERGISM
FOOD SAFETY
PHYSICAL
HIGH HYDROSTATIC PRESSURE
ULTRASOUND
HEAT
ULTRAVIOLET
PULSED LIGHT
PULSED ELECTRIC FIELDS
CHEMICAL
HIGH PRESSURE CO
ORGANIC ACIDS
SODIUM HYPOCHLORITE
NITRITES
CHITOSAN
ELECTROLIZED WATER
ESSENTIAL OILS
BACTERIOCINS
COLD PLASMA

Graphical abstarct

Highlights

- Synergisms among food preservation processes improve food safety
- Mild heat increase the lethal effects of non-thermal technologies
- EOs improve lethality of both thermal and non-thermal treatments
- Promising synergisms are obtained by the interaction of chemical constituents

Abstract

This article provides an overview of recent published information on the subject of synergistic lethal effects that emerge from the combination of physical and/or chemical processes applied to enhance food safety.

Despite important recent advances in non-thermal technologies, the greatest synergistic lethal effects emerge from combining them with traditional, relatively mild heat treatments. The combined application of antimicrobials has shown that their main constituents interact effectively, and great synergistic effects have been described with the capacity of either inhibiting or inactivating pathogens. Moreover, natural antimicrobials are more effective when pathogens are previously damaged sublethally by the application of physical technologies. Such combinations allow for a considerable reduction of treatment intensity and costs, along with a noticeable improvement in food quality and safety.

Keywords

Synergism, combined treatments, hurdle technology, food preservation, food safety.

Introduction

In the food safety sector, a successful synergism arises when two or more processes (hurdles) are applied simultaneously and/or successively, and the bacteriostatic or bactericidal effect obtained against pathogens is greater than that obtained by the hurdles applied separately (Figure 1). Valuable synergisms have been described and analysed in the last two decades [1,2], ever since Leistner and Gorris [3] described the "hurdle theory". The present paper provides an overview of recent literature on the occurrence of synergisms when physical and/or chemical hurdles are combined in order to be applied as a tool for designing more effective treatments to improve food safety.

Synergisms resulting from the combination of physical technologies

Thermal inactivation has played a major role in food preservation; nevertheless, the adverse effects of high-intensity heat treatments on food quality have led researchers to develop alternative non-thermal technologies. Among them, High Hydrostatic Pressure (HHP), Pulsed Electric Fields (PEF), ultraviolet light (UV), ultrasound (US), and Pulsed Light (PL) have attracted great interest in view of their capability to inactivate microorganisms without altering the organoleptic and nutritional properties of foods. HHP, like heat treatments, affects several kinds of cell structure, causing disruption of cell envelopes and protein denaturation with the purpose of inducing cell death. The other four non-thermal technologies are mainly focused on one target: PEF and US cause damages in cell envelopes, while UV and PL induce cellular lesions in genomic DNA. However, the high resistance displayed by certain pathogenic microorganisms under specific environmental conditions is restricting those technologies' use. Thus, the search for new, complex processes that combine different technologies in order to guarantee food safety and improve quality and shelf-life continues to represent a major challenge.

Despite advances in non-thermal technologies, most of the proposed synergisms continue to combine them with the application of mild heat (Table 1). The combination of HHP with heat is one of the most effective known synergisms. According to Ates, *et al.* [4], >6-log$_s$ of *Listeria monocytogenes* were inactivated when HHP (625 MPa) and heat (40 ºC) were applied to model soup. A great synergism was also observed against bacterial spores: while HHP (600 MPa) caused the inactivation of only 0.5-logs of *Bacillus cereus* spores in beef slurry, it succeeded in inactivating 4.9-logs in combination with heat (70 ºC) [5].

PEF achieves a higher lethality when applied together with mild heat. Katiyo, *et al.* [6] demonstrated that PEF (35 kV/cm) at 50 ºC inactivated >5-logs of *Escherichia coli* and *Salmonella* Enteritidis in apple juice.

Mild heat also enhanced UV lethality. UV-C (10.6 kJ/m^2) at 45-50ºC caused 6-logs reduction of *E. coli* in juice carrot-orange blend after 15 min, compared to single UV-C treatment (2.5-logs) [7]. Gayan, *et al.* [8] observed a 20-70% increase in the lethality of foodborne pathogens (*E. coli, Salmonella* Typhimurium, *L. monocytogenes* and *Staphylococcus aureus*) in several liquid foods, such as vegetable and chicken broth or orange and apple juice, thanks to the combination of UV with heat. This was brought about thanks to DNA damage caused by reactive oxygen species.

An increased lethality was also achieved by the simultaneous application of US and mild temperatures, called thermosonication (TS). Interesting synergisms after TS (20-24 kHz/400-750 W/50-58 ºC) were demonstrated in juices, reducing 5-logs of *E. coli*, as recommended by the FDA [9,10]. Li, *et al.* [11] were able to ascertain that sublethal injuries caused by heat in the membrane of *S. aureus* were the underlying cause.

In addition, synergistic effects were observed among non-thermal technologies, such as HHP and UV applied successively. Pretreatment with UV light (0.82-8.45 J/cm^2) prior to HHP (300-600 MPa) led to relevant synergisms against pathogens (*L. monocytogenes*, *S. aureus*, *E. coli* O157:H7 and *S.* Typhimurium) in apple juice, achieving >6-logs at the maximum treatment intensity [12].

However, a consecutive treatment of US (20 kHz/600 W) and PL (0.0175 J/mL) against *E. coli* and *S.* Enteritidis in apple juice only resulted in an additive effect [13].

The successive combination of more than two physical technologies also displayed interesting results. A synergism was observed when applying HHP, US, and heat, in that order: a 5-min treatment (350 MPa/560 W/40ºC) achieved 5.85-logs of *E. coli* inactivation [14]. A greater rate of inactivation is thereby achieved thanks to the microbial sublethal damages caused by the first treatments.

Synergisms resulting from the combination of antimicrobial compounds

Many synergisms resulting from the combination of several chemical compounds are currently being described (Table 1). Widespread consumer rejection of synthetic compounds have led to an increase in this type of investigation. Certain studies thus describe the combination of traditional food preservatives, such as nitrites [15] or sodium hypochlorite [16], with natural compounds and organic acids in order to decrease the doses applied.

Research in natural antimicrobials is being particularly encouraged, since they are attracting worldwide interest as an alternative to synthetic preservatives. Among them, plant essential oils (EOs) and their individual constituents (ICs) are being extensively studied; their negative effects on organoleptic properties nevertheless restrict their use in foods to a certain extent. Recent studies have observed that combinations among EOs/ICs lead to synergisms that permit the reduction of doses applied. According to Barbosa, *et al.* [17], antimicrobial properties of oregano and rosemary EOs were enhanced by their simultaneous application: apart from a bacteriostatic effect, a greater bactericidal effect against pathogens such as *L. monocytogenes*, *E. coli* and *S.* Enteritidis was ascertained in broth and leafy vegetables. Other authors reported similar synergistic effects between *Cymbopogon citratus* and *Allium cepa* EOs at low doses in spinach and lettuce in order to avoid consumer rejection of foodstuffs [18]. With the purpose of reducing EO doses, certain authors have calculated the optimal ratio among different EOs in order to achieve the greatest antimicrobial effect with the lowest amount of EOs [19].

Not all combinations of natural antimicrobials resulted in a synergistic bactericidal or bacteriostatic effect: the combination of mustard and cinnamon EO [20] only led to additive effects. Thus, the effect achieved by combining EOs depends on the specific compounds, their mechanisms of inactivation, and the food matrix. Furthermore, synergistic effects were described when EOs were added to edible coating and films (active packaging) with modified atmosphere packaging for purposes of food preservation [21-24].

Enhanced antimicrobial effects were also detected when combining EOs with other preservatives such as nisin in milk [25,26], organic acids in leafy vegetables [16,27], chitosan in laboratory broth [28] or cold nitrogen plasma applied to eggshell or lettuce [29,30]. Conversely, only a negligible bactericidal effect was observed when combining EOs with electrolyzed water (EW) against *E. coli* in nutrient broth medium [31]. Cold plasma also developed a strong synergism when applied in combination with organic acids and SDS in red chicory against *E. coli* and *L. monocytogenes* [32].

Other natural antimicrobials synthesized by microorganisms, such as nisin, displayed interesting bacteriostatic and bactericidal effects in combination with citric acid against *Cronobacter sakazaki* and *E. coli* [33]. Bacteriostatic synergistic effects resulting from the combination of nisin, lactobionic acid, and thymol against *L. monocytogenes* were also observed [26]. The magnitude of the synergism depended on the target bacteria, mainly because of divergent characteristics of envelopes in Gram-positive and Gram-negative bacteria. For instance, combined treatments of nisin and high-pressure carbon dioxide caused a higher inactivation of *S. aureus* than of *E. coli* [34].

These studies not only describe the potential of treatments combining different chemical compounds for purposes of food preservation, but also assist researchers in identifying the main constituents that determine the efficacy of each antimicrobial substance, and the mechanism of interaction that justifies each synergism. In this regard, efficient food preservation strategies can be designed by coordinating molecular biology techniques with computer analysis in order to identify target structures, involved metabolic pathways, and microbial response to treatments [35,36]. In contrast with physical technologies, however, the lethal activity of antimicrobial compounds is highly influenced by the amount of bacterial concentration present in the foodstuffs [37]: when interpreting results, this must be taken into account.

Synergisms resulting from the combination of physical technologies and antimicrobial compounds

The search for new preservation processes that guarantee food safety and improve food quality has led research into combining physical technologies with food preservatives, particularly with natural antimicrobial compounds (Table 1). The synergism observed is brought about by alterations of microbial cell envelopes via physical technologies, which facilitate the access of antimicrobials to the target [38].

The notable bactericidal effect of EOs/ICs in combination with mild heat has been widely demonstrated [39] against planktonic and sessile cells that usually present a high resistance to common disinfectants [40]. Cell membrane disruption and loss of cell membrane potential are considered to be the main causes of bacterial inactivation by heat and ICs [41].

The combination of EOs/ICs with non-thermal technologies also results in synergistic lethal effects. Wang, *et al.* [42] observed a synergism between PEF and carvacrol against *S. aureus*, even when carvacrol was added to the recovery agar medium. This effect was associated with cell permeabilization by PEF, allowing the access of carvacrol to genomic DNA. An improved antimicrobial activity of EOs/ICs combined with PEF has been described for mandarin and cauliflower infusion (5%) against *S.* Typhimurium at 20 kV/cm, or citral (0.2 µL/mL) against *E. coli* (30 kV/cm) [43]. Nevertheless, under the same experimental conditions, EOs applied with heat were more effective than EOs with PEF [39].

HHP has been combined with EOs, leading to divergent results. Sanz-Puig, *et al.* [44] reported a synergistic effect against *S.* Typhimurium when HHP (200 MPa) was combined with cauliflower or mandarin infusion. However, *L. monocytogenes* inactivation by HHP (200-300 MPa) was not improved when combined with thyme extract [45]. Thus, a synergistic, additive, or antagonistic effect of HHP lethal activity can be observed in combined processes depending on the antimicrobial compounds applied, their mode of action, and dosage [46]. Moreover, de Carvalho, *et al.* [47] demonstrated that the addition of *Mentha piperita* EO in the form of nanoemulsions improves the lethality of mild heat, PEF, or HHP treatments against *E. coli* O157:H7 in tropical fruit juices.

Successful synergism between physical and chemical processes is sometimes due to the bactericidal effect of the physical technology and the subsequent bacteriostatic effect of the antimicrobial during food shelf-life. For example, whilst US (130 W/20 kHz) reduced the initial microbial contamination in skim milk, citrus extract delayed growth of survivors [48]. Moreover, the application of gamma radiation at low doses (0.5-1 kGy), followed by the application of small amounts of oregano or lemongrass EO with citrus extract and lactic acid, was effective in controlling food pathogens in cauliflower such as *L. monocytogenes* and *E. coli* [49]. Those authors also tested the application of UV-C (5-10 kJ/m^2) in combination with the same compounds, and obtained a less effective but nonetheless promising alternative treatment against *E. coli*.

Apart from EOs/ICs, other natural preservatives such as organic acids and bacteriocins have been tested in combination with physical food preservation technologies. Thus, a synergy between UV-A and gallic or lactic acid inactivated 4.7-logs of *E. coli*, whereas the individual treatments, applied separately, reduced <1-log of initial population [50]. According to Wang, *et al.* [51], once gallic acid reaches the cytoplasm, it induces the formation of reactive oxygen species (ROS) together with UV-A, thereby resulting in lethal oxidative damage. With regard to bacteriocins, the combination of nisin with PEF (30kV/cm) caused up to 3.7-log$_{10}$ or 1.8-logs reduction of exponential- and stationary-phase *E. coli* cells, respectively [52].

One of the chemicals that is attracting great interest in combined treatments is EW. Synergies between EW and HHP were observed in the inactivation of *B. cereus* spores: although separate treatment with EW (44 mg/L of available chlorine) or HHP (500 MPa) inactivated only <1-logs, a combined treatment reached a 4-logs reduction [53].

Likewise, Luo and Oh [54] reported a considerable lethal effect by combining EW (28-30 mg/L) with heat (60 ºC) and US (40 kHz/400 W), thereby inactivating ca. 4.5-logs of *S.* Typhimurium and *L. monocytogenes* in 5 min. In that case, the damages on the cell membrane caused by heat and US might facilitate the access of EW, thereby enhancing its lethality. However, the lethal effect of EW (4 mg/L of free available chlorine) on *E. coli* and *L. monocytogenes* was not influenced by heat: bactericidal activity was similar at 20 and 50 ºC [55]. Likewise, the simultaneous application of EW with US or UV did not display any relevant synergism against *L. monocytogenes* [56].

The above-described synergistic lethal effects between physical and/or chemical treatments are of great value and might help decrease doses of antimicrobials or reduce treatment intensity, thereby preventing undesirable effects on food quality while ensuring food safety.

Conclusions

This review has summarized some of the most relevant recent publications in the field of combined treatments that associate physical and/or chemical processes for purposes of food preservation. Hundreds of papers related to this topic have been published over the last two years, reflecting the food industry's interest in providing consumers with safe food of the highest quality. Such strategies are considered successful when they can take advantage of synergistic effects against food pathogens. Among the evaluated hurdles, heat and EOs showed the greatest synergistic effects when applied simultaneously. Moreover, it is noteworthy that, although most studies focus on non-thermal technologies for food preservation, some of those technologies' most interesting lethal effects are currently being obtained by combining them with traditional mild heat treatments. The high potential of the latter resides in their ability to cause sublethal damages in bacterial survivors that enhance the action of other preservation technologies. Thus, the described synergisms allowed an overall reduction of treatment intensities or doses, which helped maintain organoleptic and nutritional food properties. Nevertheless, combinations of non-thermal technologies with preservatives, such as nisin or EW, have also displayed notable synergisms that deserve further evaluation as promising alternatives to traditional treatments in the food industry.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the period under review, are highlighted as:

- • of special interest
- •• of outstanding interest

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In this review, physical technologies and chemical preservatives currently applied or of potential use for purposes of food preservation are extensively discussed in an easily comprehensible, well-structured overview that comprises a great number of significant scientific publications.

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This paper shows that the combined treatment of ultraviolet light and heat delivers truly interesting results in the inactivation of pathogenic bacteria in liquid foods. To quantify the synergism observed between UV and heat, the authors carried out a modeling of the experimental results with the purpose of optimizing the combined treatment to achieve the greatest synergistic effects.

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This study demonstrates the synergistic effect between ultrasound and heat in inactivation treatments against *S. aureus*. The authors performed microbiological assays in selective medium, staining with fluorochromes, and cytometry along with microscopy analysis: they found that the amount of sublethal damage in the cell membrane is one of the main factors in the synergism between ultrasounds and heat.

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This study demonstrated the notable synergistic effects of combined treatments of high-pressure carbon dioxide and nisin, applied simultaneously against *S. aureus*. Conversely, only a slight effect against *E. coli* was observed. According to the authors, the outer membrane of Gram-negative bacteria counteracts the synergism between those two technologies, which had proved successful against *S. aureus* (which is Gram-positive).

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The results reported in this paper demonstrate the potential of pulsed electric fields to enhance the bactericidal effect of carvacrol against *S. aureus*. The authors show that carvacrol exerts its effect via two mechanisms: it increases the membrane permeability also brought about by pulsed electric fields, and binds directly to genomic DNA.

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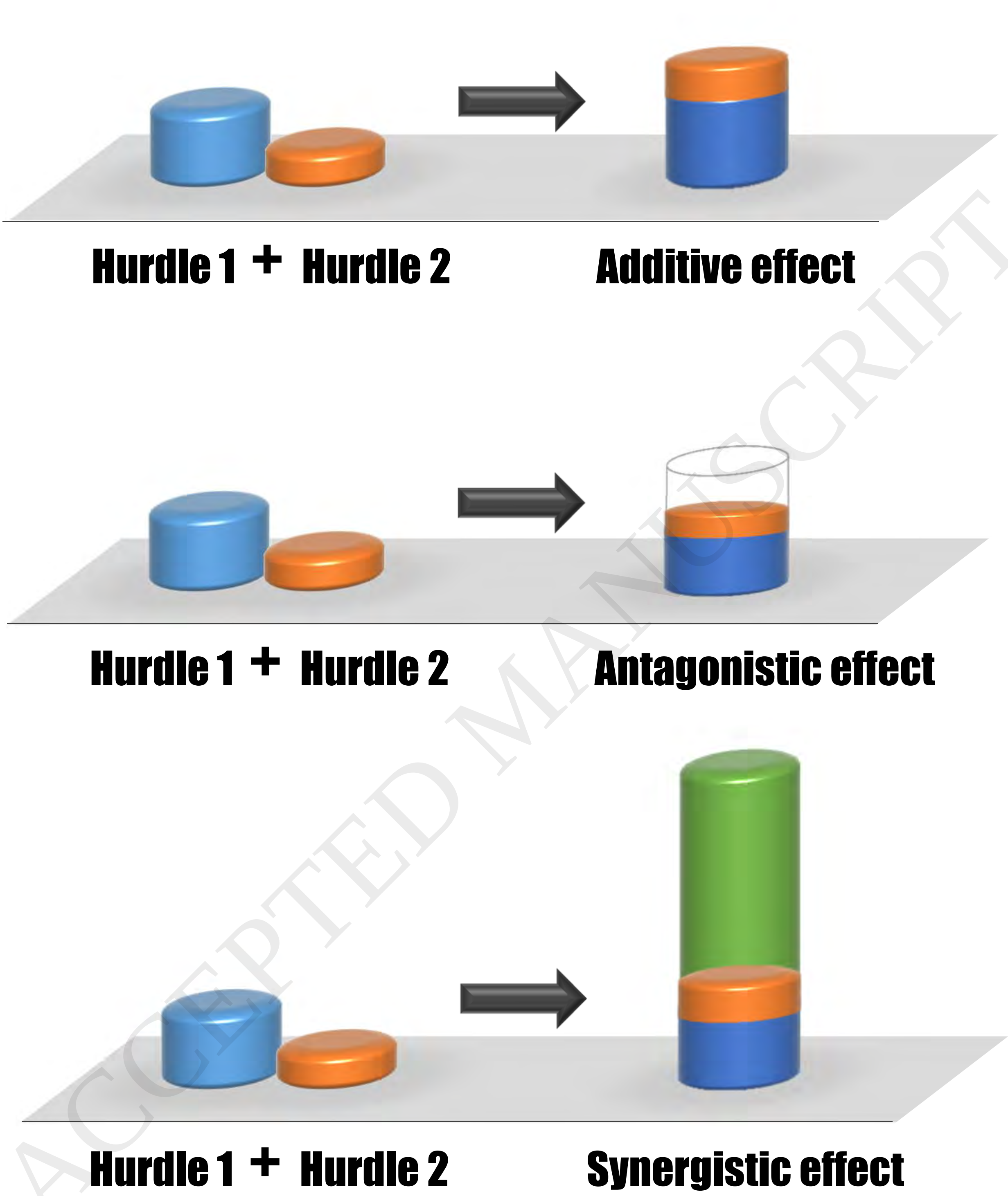
Figure legends

Figure 1. Lethal effect obtained by the combination of two different food preservation processes (hurdle). Additive effect: An effect in which two hurdles used in combination produce a total effect the same as the sum of their individual effects; Antagonistic effect: An effect arising between two hurdles that produces an effect smaller than the sum of their individual effects. Synergistic effect: An effect arising between two hurdles that produces an effect greater than the sum of their individual effects (green area).



Hurdle 1 + Hurdle 2
Additive effect
Hurdle 1 + Hurdle 2
Antagonistic effect
Hurdle 1 + Hurdle 2
Synergistic effect

Table legends

Table 1. Compilation of scientific publications of combined treatments classified based on the synergistic effects among physical technologies and/or chemical preservatives. The letters in the axes indicate the preservation method studied and the numbers showed in the table correspond to the scientific publications displayed as references in the present review.

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