

Nisin, Carvacrol and Their Combinations Against the Growth of Heat-Treated *Listeria monocytogenes* Cells

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Summary

Listeria monocytogenes is a Gram-positive microorganism responsible for one of the most serious food-borne diseases in the world, listeriosis. The aim of this study is to evaluate the combined effect of a heat pretreatment with the use of antimicrobials, nisin and carvacrol, on the growth of *L. monocytogenes*, and their potential uses as food preservatives. Carvacrol showed a dose-dependent inhibitory effect, while nisin did not, it decreased the growth rate of *L. monocytogenes* up to 20 %, and it increased lag time for approx. 25 % at any of the concentrations tested (0.13–0.39 μM). When both antimicrobials were combined, a synergistic effect was observed. This effect was further increased when they were combined with a heat pretreatment for 15 min at 55 °C, where no growth was observed for at least 15 days, even at the lowest concentration tested. The effect was proved both in tryptic soy broth and in carrot juice. This study indicates the potential use of carvacrol and nisin applied simultaneously for preservation of minimally processed foods.

Key words: nisin, carvacrol, *Listeria monocytogenes*, combined antimicrobial processes

Introduction

Listeria monocytogenes has become one of the most important foodborne pathogens in the last 20 years (1). Several foodborne disease outbreaks, which have caused severe and often fatal infections in susceptible human hosts, have been linked to this pathogen (2).

Listeria can be inactivated by exposure to a variety of food processing treatments including heating at high temperatures, freezing and exposure to acids or sanitizing compounds, but also it may survive adverse conditions, such as vacuum, ultraviolet rays and conventional pasteurisation (3). Heat inactivation is one of the most frequently used preservation techniques to extend the shelf life of food products. Lately, an increased interest in mild heat treatments is observed because of the increased market demand for minimally processed food. *L. monocytogenes* is one of the most heat-resistant non-sporeforming pathogenic microorganisms present in food (4).

Lactic acid bacteria produce a wide range of antimicrobial substances including bactericidal peptides known as bacteriocins. Nisin, produced by *Lactococcus lactis* ssp. *lactis*, can have bactericidal effects against a broad range of Gram-positive bacteria including some lactic acid bacteria, *L. monocytogenes* and spore-forming bacteria like *Clostridium* and *Bacillus* species (5,6). Nisin inhibits the outgrowth of germinating spores and causes lysis of vegetative cells (7). Its primary target is the cytoplasmic membrane of vegetative cells. Nisin permeabilizes the cytoplasmic membrane by forming pores in the membrane, resulting in a rapid efflux of small molecules (8–10). Pore formation caused by this cationic lantibiotic involves the local perturbation of the bilayer structure and a membrane potential-dependent or pH gradient-dependent reorientation of these molecules from a surface-bound into a membrane-inserted configuration (5,11,12). The efflux of cellular constituents results in a complete collapse of the proton motive force (8,13–15). Nisin is Generally Recognized As Safe (GRAS status), it is the only bacteriocin

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that has been approved by the World Health Organization (WHO) as a food preservative and is currently permitted in over 50 countries (6). Nisin can be used in processed cheese, canned food, and other food products, but the practical application is still limited because of its low stability and activity at high pH and its limited efficacy in certain food matrices (16).

Many herbs and spices have been used for millennia to provide distinctive flavours, but they also exhibit antimicrobial activity (17). Carvacrol is a phenolic compound present in the essential oil fraction of *Origanum* (18), commonly known as oregano. These sorts of phenolic compounds are hydrophobic and their primary site of toxicity is the membrane. They accumulate in the lipid bilayer according to a partition coefficient that is specific for the applied compound, leading to disruption of the membrane structure and function (19,20). As a result, the permeability increases and the activity of the enzymatic systems, including those involved in energy production and structural component synthesis, is affected (21–23). A critical concentration of the compound is needed to cause leakage of cellular constituents (24). In practice, carvacrol is added to different products, e.g. baked goods (15.75 ppm), nonalcoholic beverages (28.54 ppm), or chewing gum (8.42 ppm) (17,25).

By combining nisin with plant essential oils, the restrictions in its use as a food preservative might be overcome and the range of applications could be expanded. Since both compounds act on the cytoplasmic membrane, an additive or synergistic effect and lower dosage of both compounds would be necessary to cause an inhibitory effect (16). Synergism is observed when the effect of the combined substances is greater than the sum of the individual effects, while an additive effect is observed when the combined effect is equal to the sum of the individual effects, and antagonism corresponds to a lesser effect of one or both compounds when they are applied together than when they are applied individually (26). The synergistic antimicrobial effect of nisin and carvacrol on the viability of *L. monocytogenes* cells has been observed before (16). The actual mechanism of synergy is not known. It has been proposed that carvacrol might enhance the action of nisin by increasing the life time of pores created by nisin or by increasing the number or size of the formed pores (16). The effect of a heat pretreatment on the sensitivity of *Bacillus cereus* to antimicrobials has also been investigated (27). So far, the viability of heat-treated *L. monocytogenes* cells exposed to the combination of nisin and carvacrol has not been studied. The aim of this study is to evaluate the combined effect of a heat pretreatment with the use of antimicrobials nisin and carvacrol on the growth of *L. monocytogenes* and their potential uses as preservatives of minimally processed foods.

Materials and Methods

Bacterial strains and culture conditions

The strain of *L. monocytogenes* CECT 4031 used in this study was supplied by the Spanish Type Culture Collection. During this investigation it was maintained on slants of tryptic soy agar (TSA) supplemented with

0.6 % (*m/V*) yeast extract (TSAYE) (both from Scharlau Chemie, Barcelona, Spain) at 4 °C.

The culture was grown for 24 h at 37 °C in tryptone soy broth (Scharlau Chemie) supplemented with 0.6 % (*m/V*) yeast extract (TSBYE). The strains were then subcultured in TSBYE for another 24 h at 37 °C, until the stationary phase of growth was reached, at a concentration of approx. 10^9 cells/mL. Working cultures were then divided into two aliquots of which one served as the nonthermal control culture and the other was subjected to a heat treatment. The nonthermal control culture was appropriately diluted to 10^3 cells/mL, while the culture exposed to the thermal treatment was diluted to 10^6 cells/mL. In this way, both cultures had the same initial number when starting growth.

Chemicals

Nisin and carvacrol (both from Sigma-Aldrich Chemie, Steinheim, Germany) stock solutions (0.3 mM and 0.5 M, respectively) were made in 95 % ethanol and stored at 4 °C.

Preparation of carrot juice

Carrot juice was prepared from fresh raw carrots of the Nantesa variety provided by a local factory of minimally processed foods. They were peeled, washed, minced and filtered through a metal sieve. The juice was dispensed in tubes, heated with steam for 15 min, and stored at –40 °C. Natural inhibitors present in carrots were likely to be destroyed by this heat treatment (28). The final pH was 6.14 ± 0.10 . Just before experiments, the tubes were heated again with steam for 15 min and the temperature was adjusted to 37 °C.

Heat treatment

A mild heat pretreatment for 15 min at 55 °C was selected as it resulted in a three-log reduction of cells. This heat treatment was applied to the culture grown in TSBYE for 24 h at 37 °C by placing the test tubes in a preheated water bath (Frimengruppe Press-Daimier, Dresden, Germany). Plate counts in TSAYE of the appropriate dilutions in buffered peptone water (BPW) (Scharlau Chemie) were performed before and after the thermal treatment, in order to check the extent of the achieved inactivation. Plates were incubated for 24 h at 37 °C.

Growth curves of *L. monocytogenes* in TSBYE

The effect of nisin and carvacrol, individually and combined, on the growth of *L. monocytogenes* CECT 4031 suspended in TSBYE at 37 °C was studied. Controls without the addition of natural compounds but with the same concentration of ethanol were prepared. Cell suspensions were exposed to different concentrations of nisin (0.13, 0.26 and 0.39 μ M) or carvacrol (0.11, 0.22, 0.33, 0.44 and 0.66 mM) alone and to combinations of both compounds (0.13 μ M nisin plus 0.11 mM carvacrol, and 0.13 μ M nisin plus 0.22 mM carvacrol). In order to study the effects of these compounds on *L. monocytogenes* lag time duration and growth rate, the natural compounds were added to the growth medium prior to the inocula (10^3 CFU/mL). Absorbance ($A_{620 \text{ nm}}$) was measured with a spectrophotometer (ZUZI 4110RS, Auxilab, Beriáin, Spain) at differ-

ent time intervals during exposure. At least three replicate experiments per condition were performed.

Growth curves were obtained by plotting the $A_{620\text{ nm}}$ against exposure time. Although the measurement of the absorbance of a culture is only an estimation of its growth, it might help to compare the growth rate and lag phase duration under different conditions. For this purpose, growth curves were fitted with the function of Baranyi *et al.* (29) in order to obtain the main growth parameters (*i.e.* specific growth rate and lag time). Only growth curves with at least 10 points were used for modelling, as suggested by the authors.

When no growth was observed after 15 days of incubation, plate counts were performed in order to check the number of microorganisms present in the culture. If the plate counting showed some growth, the culture was further incubated.

Analysis of variance and Duncan's multiple range test were done using the statistical program Statgraphics Plus v. 5.1 (Statpoint Technologies, Inc, Warrenton, VA, USA). Differences between data were considered significant when $p \leq 0.05$. The experiments were replicated three times for each treatment.

Growth curves of *L. monocytogenes* in carrot juice

The effect of nisin and carvacrol, individually and combined, on the growth of *L. monocytogenes* CECT 4031 in carrot juice at 37 °C was studied. Controls without the addition of natural compounds but with the same concentration of ethanol were prepared. Tubes with 4.5 mL of carrot juice were exposed to different concentrations of nisin (0.13 μM) or carvacrol (0.33 and 0.66 mM) alone or combined for 24 h at 37 °C to verify their inhibitory effect. In order to study the effects of these compounds on *L. monocytogenes* growth, the natural compounds were added to the growth medium prior to the inocula (10^3 CFU/mL). Appropriate dilutions in BPW were pour plated in TSAYE and incubated at 37 °C for 24 h.

Results

Effect of nisin and carvacrol on the growth of *L. monocytogenes* cells in TSBYE

When *L. monocytogenes* CECT 4031 in lag phase without a heat pretreatment was exposed in TSBYE to increasing concentrations of nisin alone (0.13, 0.26 and 0.39 μM ; Fig. 1a), or carvacrol alone (0.11, 0.22, 0.33, 0.44 and 0.66 mM; Fig. 1b), both the onset of growth was delayed and the growth rate decreased (Table 1). Nevertheless, the behaviour was different for each antimicrobial agent. When exposed to 0.13 μM of nisin, the lag phase increased from 13.84 to 17.03 h and the growth rate decreased from 0.26 to 0.23 h^{-1} . However, when the concentration of nisin was further increased, up to 0.39 μM , growth parameters were not largely affected and no significant differences were found between lag phases at these concentrations (Table 1).

On the contrary, the behaviour of *L. monocytogenes* was more carvacrol concentration-dependent (Table 1). Concentrations higher than 0.11 mM progressively in-

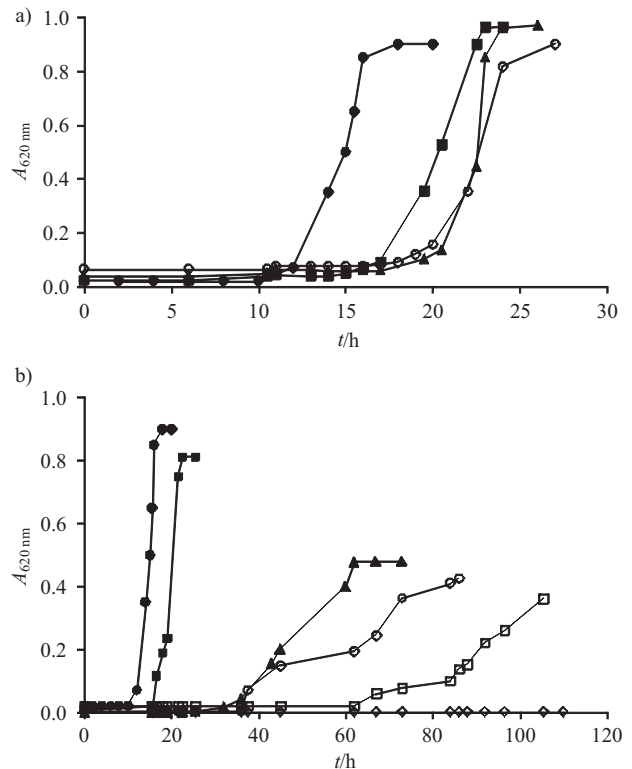


Fig. 1. Effect of different concentrations of: a) nisin and b) carvacrol on the growth of *L. monocytogenes* CECT4031 in TSBYE at 37 °C. a) ● control, ■ 0.13 μM nisin, ▲ 0.26 μM nisin, ○ 0.39 μM nisin; b) ● control, ■ 0.11 mM carvacrol, ▲ 0.22 mM carvacrol, ○ 0.33 mM carvacrol, □ 0.44 mM carvacrol, ◇ 0.66 mM carvacrol

creased the lag phase and decreased growth rate, to 0.66 mM, which not only inhibited growth, but even inactivated microorganisms, as indicated by plate counts performed after a 15-day incubation. Increasing concentrations of carvacrol also had an effect on the maximum absorbance, which was progressively lower.

An exponential correlation was observed for both the lag time and the growth rate in relation to the concentration of carvacrol in the growth medium (Fig. 2), with correlation coefficients (R^2) higher than 0.95 in both cases. This means that the growth of this strain of *L. monocytogenes* could be easily predicted as a function of carvacrol concentration by simply substituting this concentration in Eqs. 1 and 2:

$$\log \lambda = 1.556 \times c + 1.135 \quad /1/$$

$$\log \mu = -4.285 \times c - 0.620 \quad /2/$$

where λ is the lag phase duration (h), c is the carvacrol concentration (mM) and μ the growth rate (h^{-1}).

The effect of nisin and carvacrol added simultaneously on the growth of *L. monocytogenes* CECT 4031 in lag phase in TSBYE broth at 37 °C was also studied. When *L. monocytogenes* was exposed to 0.13 μM nisin and 0.11 mM carvacrol, the onset of growth was significantly delayed (from 13.84 to 57.54 h) and the growth rate significantly reduced (from 0.26 to 0.09 h^{-1}), leading to an important synergistic effect, at least in the lag time duration: 0.13 mM nisin alone only increased lag time

Table 1. Growth parameters (lag time and growth rate) of *L. monocytogenes* CECT 4031 in TSBYE at 37 °C, with or without heat pretreatment, exposed to different concentrations of nisin and carvacrol added separately or simultaneously

		λ/h		μ/h^{-1}	
		without HT	with HT	without HT	with HT
<i>c</i> (nisin)/ μ M	0	(13.84±0.02) ^{Aa}	(14.84±0.18) ^{Ab}	(0.26±0.0005) ^{Ga}	(0.25±0.01) ^{Da}
	0.13	(17.03±1.46) ^{Ba}	(23.31±0.5) ^{Bb}	(0.23±0.003) ^{Eb}	(0.07±0.001) ^{Aa}
	0.26	(18.42±1.90) ^B	>15 days	(0.24±0.003) ^F	N.G.
	0.39	(18.60±1.83) ^B	>15 days	(0.21±0.027) ^D	N.G.
<i>c</i> (carvacrol)/mM	0	(13.84±0.02) ^{Aa}	(14.84±0.18) ^{Ab}	(0.26±0.0005) ^{Ga}	(0.25±0.01) ^{Da}
	0.11	(18.96±0.93) ^{Bb}	(13.95±0.5) ^{Aa}	(0.11±0.0007) ^{Ca}	(0.26±0.01) ^{Db}
	0.22	(33.18±4.52) ^{Ca}	(32.57±0.001) ^{Ca}	(0.015±0.003) ^{Ba}	(0.16±0.01) ^{Cb}
	0.33	(41.37±9.43) ^C	n.d.	(0.009±0.001) ^A	N.D.
	0.44	(67.19±7.92) ^{Eb}	(39.89±3.53) ^{Da}	(0.004±0.002) ^{Aa}	(0.12±0.011) ^{Bb}
	0.66	>15 days	>15 days	N.G.	n.d.
<i>c</i> (nisin)/ μ M+ <i>c</i> (carvacrol)/mM	0	(13.84±0.02) ^{Aa}	(14.84±0.18) ^{Ab}	(0.26±0.0005) ^{Ga}	(0.25±0.01) ^{Da}
	0.13+0.11	(57.54±5.33) ^D	>15 days	(0.09±0.04) ^C	N.G.
	0.13+0.22	>15 days	>15 days	N.G.	N.G.

n.d.=not determined, N.G.=no growth after 15 days, HT=heat treatment at 55 °C for 15 min. The results represent mean values±standard deviation of three replicates, the same capital letter indicates that there are no significant differences in columns, the same lower case letter indicates that there are no significant differences in rows between lag times or growth rate of the heat-treated and non-treated cells

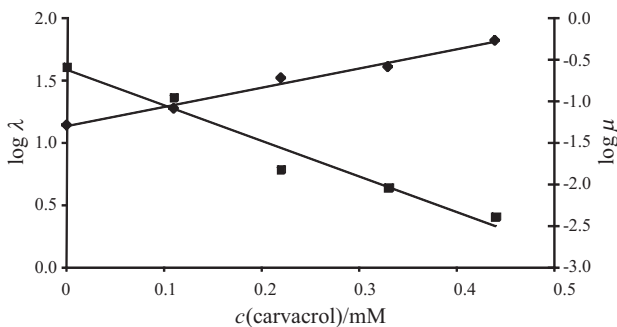


Fig. 2. Growth parameters of *L. monocytogenes* CECT 4031 in TSBYE at 37 °C as a function of carvacrol concentration. ◆ lag time (λ), ■ growth rate (μ)

for approx. 4 h, and 0.11 mM carvacrol alone for approx. 5 h, but the combination of both antimicrobials increased lag time for more than 40 h, which is much more than the addition of both increases. Regarding growth rate, the effect was only additive at these concentrations. When increasing concentrations of carvacrol were combined with this same concentration of nisin (0.13 μ M), the growth of *L. monocytogenes* was completely inhibited for at least 15 days. Plate counts performed after this 15-day incubation indicated that not only the growth was inhibited, but the microorganisms were even inactivated, as microbial counts were under the detection limit (<1 CFU/mL). Again, synergistic effects were observed.

Effect of a heat pretreatment on the growth of L. monocytogenes cells in TSBYE

L. monocytogenes cells grown in TSBYE at 37 °C were exposed to a thermal treatment for 15 min at 55 °C. This thermal treatment decreased the counts by three log

cycles. When these heat-treated *L. monocytogenes* cells were grown in TSBYE at 37 °C, the growth rate did not significantly differ from that obtained with the cells not previously exposed to a heat treatment and lag phase was only increased for 1 h (Table 1 and Fig. 3).

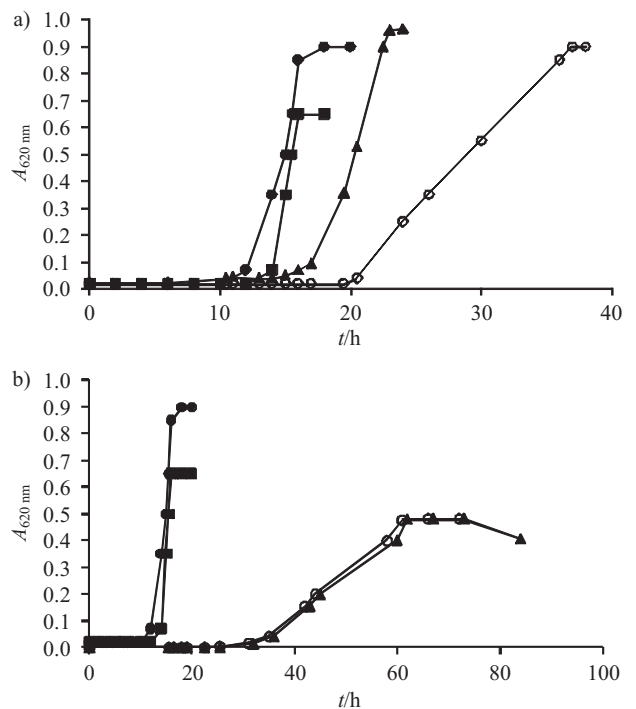


Fig. 3. Effect of combined concentrations of: a) nisin and b) carvacrol on the growth in TSBYE at 37 °C of heat-treated (HT: 15 min at 55 °C) or non-treated *L. monocytogenes* CECT 4031 cells. a) ● control, ■ HT, ▲ 0.13 μ M nisin, ○ 0.13 μ M nisin+HT; b) ● control, ■ HT, ▲ 0.22 mM carvacrol, ○ 0.22 mM carvacrol+HT

Effect of nisin and carvacrol on the growth of heat-treated *L. monocytogenes* cells in TSBYE

The growth of heat-treated *L. monocytogenes* cells was dramatically affected by exposure to nisin. At the lowest nisin concentration tested (0.13 μM), growth rate was significantly reduced (Fig. 3a). At higher concentrations, the growth was completely inhibited for at least 15 days (Table 1). Again, plate counts performed after the incubation showed microbial counts under the detection limit.

Regarding the growth of heat-treated *L. monocytogenes* cells exposed to carvacrol, the behaviour was completely different. The growth was even faster than that of non-treated cells, at concentrations of carvacrol up to 0.44 mM (Table 1 and Fig. 3b). At 0.66 mM the growth was inhibited for at least 15 days, similarly to what happened to the cells not exposed to a heat treatment.

The effect of nisin and carvacrol added simultaneously on the growth of heat-treated *L. monocytogenes* CECT 4031 cells in TSBYE at 37 °C during lag phase was also studied. When the heat-treated *L. monocytogenes* CECT 4031 cells were exposed to different combinations of nisin and carvacrol in TSBYE, the growth was inhibited for at least 15 days even at the lowest concentration tested (0.13 μM nisin plus 0.11 mM carvacrol).

Effect of nisin and carvacrol on the growth of *L. monocytogenes* cells in carrot juice

The effect of the combinations of different concentrations of nisin and carvacrol on the viable counts of *L. monocytogenes* CECT 4031 during lag phase cultured at 37 °C in carrot juice (pH=6.14) exposed to a heat pretreatment or not was studied. This part of the research was conducted using plate counts instead of absorbance measurements, because the latter cannot be performed in turbid food media. Hence, plate counts were performed after inoculation and incubation for 24 h at 37 °C. *L. monocytogenes* CECT 4031 showed different behaviour at the different concentrations of the antimicrobials and their combination with a heat pretreatment (Fig. 4). The results showed that *L. monocytogenes* reached the stationary phase of growth at a concentration of $6 \cdot 10^8$ CFU/mL in carrot juice, in 24 h or less at 37 °C with neither heat pretreatment nor antimicrobials added. No significant differences were observed between the control and the

heat-pretreated cells, although these showed a higher variability in the replicates.

When *L. monocytogenes* was grown in the presence of 0.13 μM nisin, significant differences were observed regarding control, even though these differences were small (half a logarithmic cycle). For microorganisms incubated with carvacrol at concentrations of 0.33 and 0.66 mM, a significant and progressive inhibition of growth was observed. When nisin and carvacrol were combined, a further inhibition of growth was observed. A bacteriostatic effect was even shown when the higher concentration of carvacrol (0.66 mM) was combined with 0.13 μM nisin, since no growth was detected after 24 h of incubation (Fig. 4). The application of a heat pretreatment to cells incubated with either nisin or carvacrol resulted in a significant decrease in plate count with respect to non-treated cells incubated with the same amounts of antimicrobials (Fig. 4). The combinations of heat plus 0.66 mM carvacrol resulted in even lower counts than before the incubation.

Discussion

L. monocytogenes showed different behaviour when exposed to increasing concentrations of nisin than when exposed to increasing concentrations of carvacrol. While both antimicrobials inhibited the growth, the effect of carvacrol was progressive as the concentration of this compound increased, while the effect of nisin was less dependent on its concentration. Actually, a good exponential correlation was found between both lag time and growth rate and carvacrol concentration ($R^2 > 0.95$), which enabled the building of a secondary growth model. This deterministic model predicts the growth of *L. monocytogenes* as a function of carvacrol concentration. Other authors have also found a dose-dependent inhibitory effect of carvacrol and other phenolic compounds on *L. monocytogenes* and other Gram-positive bacteria (17,30–32). This different behaviour could depend on the mode of action on the bacterial envelopes for each antimicrobial compound: nisin forms pores in the bacterial membrane (33), while phenolic compounds cause disruption of the membrane function (17). The different modes of action for nisin and carvacrol could explain, at least in part, the synergistic effect observed when both antimicrobials were combined: while a concentration of 0.11 mM carvacrol alone and 0.13 μM nisin alone had little effect on the growth of *L. monocytogenes* in TSBYE broth, the combination of both compounds at these concentrations increased the synergistic effect by more than four times of both antimicrobials in lag time, and when higher concentrations of carvacrol were combined with nisin, the growth was completely inhibited for at least 15 days, showing an even bactericidal effect. Pol *et al.* (34) also found synergistic effects in the combination of nisin and carvacrol in germinating spores of *Bacillus cereus*.

A heat pretreatment did not affect the growth of the survivors, which grew in a similar way as the non-heated cells. However, the heat pretreatment had an obvious effect on the survivors when they were exposed to antimicrobials, *i.e.* in that case their behaviour was different. The application of a heat pretreatment had a synergistic

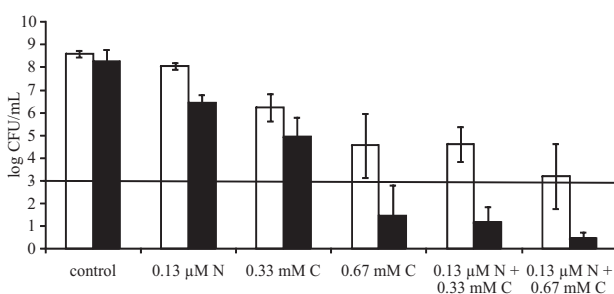


Fig. 4. Effect of different concentrations of nisin and carvacrol alone or combined on the growth of *L. monocytogenes* CECT4031 during 24 h at 37 °C in carrot juice (pH=6.14). White columns, non-heated *L. monocytogenes* cells; black columns, heat-treated *L. monocytogenes* cells (15 min at 55 °C). N=nisin, C=carvacrol. Horizontal line represents the initial concentration of the inocula

effect with nisin, but it was antagonistic with carvacrol. Again, this fact supports the hypothesis of different targets for nisin and carvacrol. While heat damage affects the targets for nisin, hampering the growth of cells exposed to this compound, it seems to cross-protect against carvacrol. Other authors have also found that sequential combinations of mild heat and nisin resulted in significant synergistic decreases in viable counts of *Lactobacillus plantarum* (33) and *B. cereus* (35). However, Periago *et al.* (36) found that *B. cereus* cells developed cross-resistance to nisin after a mild heat treatment. Heat shock proteins may be involved in this antagonistic effect and in the cross-protective effect (37).

However, when combining both antimicrobials, at the lowest concentrations tested for both of them (which only showed slight inhibitory effect against *L. monocytogenes* growth when applied separately), with a heat pretreatment (which did not show any inhibitory effect), the growth was inhibited for at least 15 days. Again, a strong synergistic effect was shown. The synergism between carvacrol and nisin has already been shown in non-heated cells of *B. cereus* (16,27,36) and *L. monocytogenes* (16) and in heat-pretreated cells of *B. cereus* (36). Heat can damage the RNA, ribosomes, cytoplasmic membranes and enzymes. Despite the increased resistance to carvacrol observed after the exposure to heat, the combination of heat, carvacrol and nisin caused irreversible injury to the membrane, as well as to those structures damaged by heat, which was caused by the observed synergistic effect. Synergies, like these described in this research, can be used in the food industry to prevent food spoilage and ensure food safety, while maintaining a high standard of food quality.

When nisin, carvacrol and a heat pretreatment were applied in a food matrix such as carrot juice, the behaviour of *L. monocytogenes* resembled somehow that observed in TSBYE, although with some important differences. Heat-treated cells grew as fast as the control cells (not exposed to a heat pretreatment), and nisin slightly reduced the counts after 24 h of incubation at 37 °C in carrot juice. When nisin was applied after the heat treatment, a stronger inhibitory effect was observed. Carvacrol had a progressive inhibitory effect. However, in this case, the combination of carvacrol with a heat pretreatment had a stronger inhibitory effect, which was even bactericidal when 0.66 mM of carvacrol were combined with the heat pretreatment.

When carvacrol and nisin were combined in the carrot juice, the growth of *L. monocytogenes* was also inhibited and a synergistic effect was observed. The growth was completely inhibited for at least 24 h with a combination of 0.13 µM nisin plus 0.66 mM carvacrol. Similar effects were also found by Periago *et al.* (30) when combining carvacrol and cymene against the growth of *L. monocytogenes* in carrot juice.

Conclusions

Nisin and carvacrol showed inhibitory effects on *L. monocytogenes* growth. When both antimicrobials were combined, synergistic effects were observed. These synergistic effects were even increased when a heat pretreat-

ment was also applied. These effects were observed in a laboratory medium but also in a food matrix such as carrot juice. These synergisms could be applied in the food industry to prevent the growth of *L. monocytogenes* in processed food exposed to mild heat treatments, intended to provide better quality foods.

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