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Short Paper

Comparing the effect of NaCl and KCl on the growth of *Listeria monocytogenes* with a view to NaCl replacement

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Summary

In this study, the effects of various concentrations of NaCl and KCl and partial replacement of NaCl with KCl on the growth characteristics of *L. monocytogenes* were evaluated. It was found that *L. monocytogenes* can grow in the presence of 1-9% NaCl and 1-11% KCl. The higher the concentration of salt used, the longer the lag phase induced. In addition, it was observed that *L. monocytogenes* tolerate KCl better than NaCl when using the same percents in broth. In an attempt to partially replace NaCl with KCl, it was found that the level of substitution of NaCl with KCl can be at least 25% without risking the microbiological safety, with respect to *L. monocytogenes* of the product, but not as high as 50%.

Key words: *Listeria monocytogenes*, KCl, NaCl, Replacement

Introduction

Listeria monocytogenes is a foodborne pathogen that can cause serious invasive disease in humans. This microorganism is widespread in the environment and is able to survive and grow under environmental conditions that are lethal or inhibitory to many other non-sporeforming foodborne pahogens. This pathogen is remarkably salt tolerant and can survive under high salt concentrations (McClure *et al*., 1989).

NaCl is one of the most commonly employed agents for food conservation, allowing considerable increase in storage time by reducing water activity (Jamshidi *et al*., 2008). Historically, NaCl was among the very few effective preserving methods known. With the advent of refrigeration, better processing, packaging, transport and storage, there is less need for high salt levels to maintain product quality and safety. Furthermore, in recent years there has been a tendency for reducing sodium in foods due to its relationship with hypertension, but where salt has been added as a preservation hurdle, removal or reduction of the salt will reduce shelf-life and could affect safety in more microbiologically fragile products. Potassium chloride (KCl) is the most obvious replacement for salt (NaCl) in food products (Bidlas and Lambert, 2008). A previous report on the survival of microorganisms at low a_w values revealed that the response to a_w was solute dependent and for the growth of *Clostridium perfringens*, solute identity had a bearing on the amount of growth for a given a_w, with KCl having a demonstratably greater effect than NaCl (Strong *et al*., 1970). In addition, NaCl was found to be more inhibitory than glycerol for *Salmonella* cells at the same a_w (Marshall *et al*., 1971). Beuchat (1974), however, reported that at equivalent a_w, NaCl and KCl had equivalent effects against *Vibrio parahaemolyticus*. Furthermore, it was observed that in fermented meat products, the replacement of NaCl with KCl did not affect the degree of inhibition and or inactivation, but did alter the taste of the foodstuffs (Gimeno *et al*., 1999, 2001).

Therefore, the aims of this study were

the comparative evaluation of: 1) the effect of various concentrations of NaCl and KCl on the growth characteristics of *L. monocytogenes* in broth; and, 2) the effect of partial substitution of NaCl with KCl on the growth of *L. monocytogenes* at different temperatures.

Materials and Methods

The stock culture of *Listeria monocytogenes* (a food isolate) was stored at -20°C in Tryptic Soy Broth (Merck, Germany) supplemented with 25% (vol/vol) sterile glycerol (Merck, Germany). To prepare the inoculum, 0.1 ml of stock culture was added to 10 ml of TSB and incubated without shaking for 18 to 24 h at 35°C. NaCl and KCl were added to TSB prior to autoclaving and pH was adjusted to 6.0 with HCl after autoclaving. For growth studies, two experiments were carried out. In the first experiment, the behaviour of *L. monocytogenes* in TSB (pH = 6.0) was determined at 30°C in the presence of 1, 3, 5, 7, 9, and 11% NaCl or KCl. To achieve this purpose, according to the correlation between optical density (O.D.) and viable cell count, portions (10 ml each) of sterile TSB containing 1, 3, 5, 7, 9 and 11% NaCl or KCl were inoculated with 100 µl of diluted *L. monocytogenes* culture to produce an initial level of ca 10^5 CFU/ml. It was confirmed by plating 100 µl on TSA. For the inoculated TSB medium, 300 µl were dispensed in six wells and the same volume of non-inoculated medium was dispensed in four wells of micro-titre plates in order to determine the O.D. of the growth medium and to detect possible contamination. A synergy HT microplate reader (BioTek Instruments) was used to follow the growth of *L. monocytogenes* in the micro-titre plates. Optical density was read every one hour for the first 24 h. and then every 2 h until 70 h. at a wavelength of 600 nm (Cheroutre-Vialette *et al*., 1998).

In the second experiment, according to the results of the first experiment, three different combinations of NaCl/KCl were selected and the effect of these combinations on the growth of *L. monocytogenes* at 5°C and 30°C were evaluated. For this aim, ca 10^6 CFU/ml *L. monocytogenes* was inoculated into TSB containing 11.0% NaCl (control), 8.25% NaCl-2.75% KCl (treatment 1), 5.5% NaCl-5.5% KCl (treatment 2) and 2.75% NaCl-8.25% KCl (treatment 3). The cultures were incubated at 5°C and 30°C for 35 and 5 days, respectively. Viable counts were then made by surface plating 0.1 ml of the decimal diluted sample each on duplicate plates of TSA. Colonies were counted after 24-36 h of incubation at 35°C (McClure *et al*., 1989). The mean values and the standard deviations were calculated from the data obtained with three separate experiments. Results were analysed using the repeated measure ANOVA and Scheffe tests (SPSS 16). The significance levels are expressed at a 95% confidence level ($P \leq 0.05$) throughout.

Results

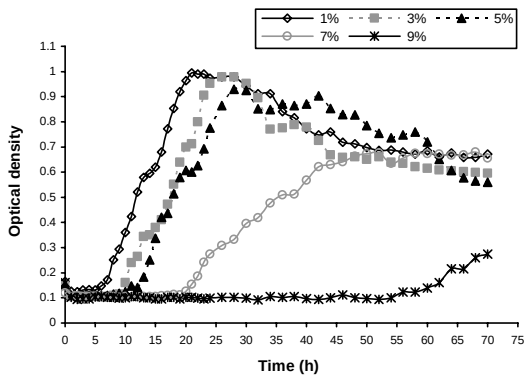
As shown in Figs. 1a and 1b, *L. monocytogenes* can grow in the presence of 1, 3, 5, 7 and 9% NaCl. The higher the concentration of NaCl used, the longer the lag phase induced. For example, the growth occurred in the presence of 7 and 9% NaCl after a lag phase of approximately 20 and 52 h, respectively. According to our results, the growth curve of *L. monocytogenes* was more affected by the presence of NaCl than by the presence of the same concentrations of KCl. Apart from the presence of 1, 3 and 5% NaCl, where no significant differences were observed as compared to treatments having the same percents of KCl, the addition of higher concentrations of salts resulted in a more inhibitory environment in NaCl supplemented broths. For example, *L. monocytogenes* was not able to grow in TSB containing 11% NaCl until 120 h, but in the case of 11% KCl, the growth occurred after about 50 h lag phase.

In the second attempt, the effect of three different combinations of NaCl/KCl was compared with the control (11% NaCl) which was induced in the first experiment, the inhibitory effect on the growth of *L. monocytogenes*. As shown in Figs. 2a and 2b, NaCl at a concentration of 11% (control) exerted an inhibitory effect on the test organism and the viable number of *L.*

monocytogenes decreased markedly, both at 5°C and 30°C. At 5°C, the population was decreased from an initial population of 6.17 Log_{10} CFU/ml to 4.56 Log_{10} CFU/ml during 35 days of incubation and at 30°C the population decreased for 2.16 Log_{10} CFU/ml after 5 days of incubation. A similar pattern of inhibition was observed in the presence of 8.25% NaCl-2.75% KCl (treatment 1). It seems that this level of substitution of NaCl with KCl does not have a significant effect upon the inhibitory action against *L. monocytogenes* ($P>0.05$). However, when the degree of replacement of NaCl was higher, the strain was less affected and there were statistical differences between the control and treatments 2 and 3 conditions ($P<0.05$). For example, at 30°C *L. monocytogenes* was partially inhibited in treatment 2, while it was increased from an initial population of 6.13 Log_{10} CFU/ml to 8.07 Log_{10} CFU/ml in treatment 3. At 5°C, a very slight increase in viable population of *L. monocytogenes* was observed in treatment 2. However, the amount of increase in viable population was much higher in treatment 3, where the population was increased from an initial population of 6.23 Log_{10} CFU/ml to 8.15 Log_{10} CFU/ml at the end of cultivation (day 35).

Discussion

It was reported previously that potassium chloride has an equivalent antimicrobial effect on some microorganisms when calculated on a molar basis (Boziaris *et al*., 2007; Bidlas and Lambert, 2008). As the amount of added salt is generally calculated on a percent basis in the food industry, in this study we used different percents of the salts. According to the results of the present study, although *L. monocytogenes* can grow in the presence of



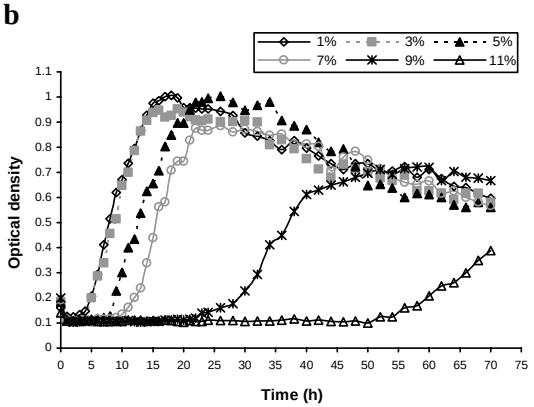


Fig. 1: Growth curves of *L. monocytogenes* in TSB containing various concentrations of NaCl (a) and KCl (b) at 30°C

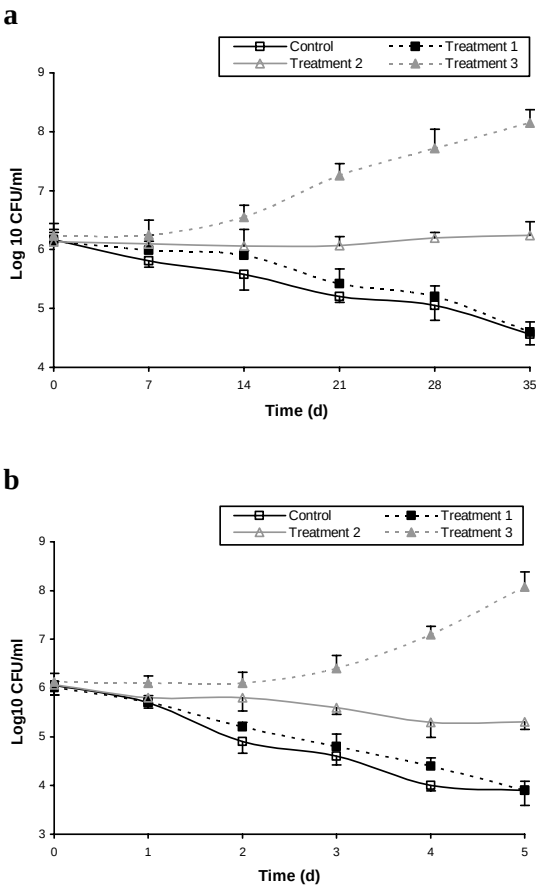


Fig. 2: Behaviour of *L. monocytogenes* in TSB containing 11.0% NaCl (control), 8.25% NaCl-2.75% KCl (treatment 1), 5.5% NaCl-5.5% KCl (treatment 2) and 2.75% NaCl-8.25% KCl (treatment 3) at 5°C (a) and 30°C (b)

up to 9% NaCl and up to 11% KCl, the lag times increased as the concentration of the salts increased. According to McClure *et al*. (1989), *L. monocytogenes* is able to grow in nutrient broth supplemented with up to 10% (w/v) NaCl at pH = 5.0 to pH = 8.0 at 25°C. Furthermore, our results indicate that, *L. monocytogenes* tolerate KCl better than NaCl when using the same percents in broth. On the other hand, KCl has no equivalent antimicrobial effect on *L. monocytogenes* when calculated on a percent basis. It seems that due to a larger a_w effect of NaCl, *L. monocytogenes* was more affected by the osmotic conditions made by NaCl when using the same percents as KCl.

Reduction of NaCl content as an osmotic hurdle is well explained, particularly for fermented meat products. According to the previous reports, replacement of NaCl by KCl in fermented sausages did not affect microbial association and pathogens inhibition and/or inactivation, but only the organoleptic characteristics, especially when the degree of replacement was higher than 40% (Gimeno *et al*., 2001; Gelabert *et al*., 2003). According to Figs. 2a and 2b, the level of substitution of NaCl with KCl can be at least 25% without risking the microbiological safety, with respect to *L. monocytogenes* of the product, but not as high as 50%. While 25% replacement of NaCl with KCl did not alter the inhibitory effect significantly, compared with the control, 50% replacement showed a slight increase in viable population of *L. monocytogenes* at 5°C and a partial inhibition at 30°C. This lower effect is presumably due to the increase in the a_w of the medium as KCl has a lower effect on water activity. We observed that the level of inhibition in the presence of high salt concentrations was greater at 30°C than at 5°C. Although this difference was observed in all treatments and the control, it was more obvious in treatment 2 where the number of viable population was slightly increased at 5°C but decreased for about 0.76 Log_{10} CFU/ml at 30°C. This result is consistent with research described by Mattick *et al*. (2000), They reported that incubation at 37°C resulted in more rapid loss of viability of *Salmonella* than incubation at 21°C at lethal a_w values.

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