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Comparing the antimicrobial effectiveness of NaCl and KCl with a view to salt/sodium replacement

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ABSTRACT

A study using a small range of pathogenic bacterial species (*Aeromonas hydrophila*, *Enterobacter sakazakii*, *Shigella flexneri*, *Yersinia enterocolitica* and 3 strains of *Staphylococcus aureus*) has shown that potassium chloride has an equivalent antimicrobial effect on these organisms when calculated on a molar basis. Combined NaCl and KCl experiments were carried out and data was analysed using a modification to the Lambert and Lambert [Lambert, R.J.W., and Lambert, R., 2003. A model for the efficacy of combined inhibitors. Journal of Applied Microbiology 95, 734–743.] model for combined inhibitors and showed that in combination KCl is a direct 1:1 molar replacement for the antimicrobial effect of common salt. If this is a general finding then, where salt is used to help preserve a product, partial or complete replacement by KCl is possible.

1. Introduction

Salt (NaCl) is generally added to foodstuffs to 1. improve taste and 2. as a preserving agent. Indeed, historically, salt 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 integrity. Furthermore, consumers want products with reduced sodium levels (e.g. 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.

The most obvious replacement for salt (NaCl) in food products is potassium chloride (KCl). Strong et al. (1970) reported that 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 demonstrably greater effect than NaCl. Beuchat (1974), however, reported that at equivalent a_w NaCl and KCl had equivalent effects against *Vibrio parahaemolyticus*; it was reported 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). More recently, Boziaris et al. (2007) have reported that equal-molar concentrations of NaCl or KCl exerted similar inhibitory effects against *Listeria monocytogenes* in terms of lag phase duration, growth or death rate and that NaCl can be replaced by KCl without risking the microbiological safety, with respect to *L. monocytogenes*, of the product. They also stated that in order to confirm this observation as general, a greater number of organisms need to be studied.

In the work reported herein, we simply wanted to answer the following question: can KCl be a direct or partial replacement for NaCl? Since the area of investigation is potentially vast, we concentrated our initial efforts on a few species of pathogenic bacteria with which we already had extensive modelling expertise on and which complimented other published work.

2. Materials and methods

2.1. Culture preparation

Aeromonas hydrophila (ATCC 7092), *Enterobacter sakazakii* (1387-2NL), *Staphylococcus aureus* (ATCC 6538, ATCC 25923 (labeled as ST121 in this report), ST55 (isolated from pasta)), *Yersinia enterocolitica* (ATCC 9610) or *Shigella flexneri* (ATCC 12022) was grown overnight in a flask containing 80 ml tryptone soya broth (TSB; Oxoid CM 129) shaking at 30 °C. The cells were harvested, centrifuged to a pellet (512 g, 10 min, 15 °C), washed and re-suspended in peptone water (0.1%). A standard inoculum was produced by diluting the culture to an OD of 0.5 at 600 nm. This standardized culture was then further diluted in TSB to produce the starting inoculum (approx 1×10^5 CFU ml^{-1} in the microtitre plate).

2.2. Experimental method

Experiments were carried out either using half-fold dilutions using the method of Lambert and Pearson (2000) or by using linear dilutions

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Table 1
Modelled parameters values for NaCl and KCl inhibition for pathogens used in this study

Organism	Humectant	P_1 (mg l^{-1}) (SErr)	P_1 (mol l^{-1}) (SErr)	P_2 (SErr)	MIC (mg l^{-1})	MIC (mol l^{-1}) (95% CI)
E. sakazakii	NaCl	48,180 (160)	0.824 (0.003)	1.609 (0.010)	89,810	1.537 (1.516–1.561)
	KCl	62,970 (390)	0.845 (0.005)	1.587 (0.0182)	118,200	1.586 (1.545–1.630)
A. hydrophila (ATCC 7966)	NaCl	32,290 (330)	0.552 (0.006)	2.080 (0.050)	52,230	0.893 (0.857–0.932)
	KCl	41,870 (410)	0.562 (0.006)	1.910 (0.0395)	70,670	0.948 (0.911–0.987)
Y. enterocolitica (ATCC 9610)	NaCl	32,700 (262)	0.560 (0.0045)	2.034 (0.061)	53,460	0.915 (0.873–0.961)
	KCl	37,930 (172)	0.509 (0.0023)	1.859 (0.030)	64,950	0.871 (0.848–0.895)
S. flexneri (ATCC 12022)	NaCl	37,040 (170)	0.634 (0.003)	2.189 (0.036)	58,490	1.000 (0.977–1.025)
	KCl	43,410 (291)	0.582 (0.004)	1.673 (0.031)	78,900	1.0589 (1.022–1.096)
S. aureus 121	NaCl	89,600 (500)	1.533 (0.009)	2.190 (0.0476)	141,500	2.421 (2.349 –2.498)
	KCl	114,900 (554)	1.542 (0.007)	2.114 (0.0366)	184,400	2.474 (2.411–2.539)
S. aureus 6538	NaCl	88,100 (710)	1.508 (0.012)	2.176 (0.0603)	139,500	2.388 (2.292–2.488)
	KCl	115,700 (590)	1.552 (0.008)	1.984 (0.0363)	191,500	2.569 (2.496–2.645)
S. aureus 55	NaCl	87,650 (470)	1.500 (0.008)	1.875 (0.0341)	149,400	2.557 (2.482–2.634)
	KCl	114,400 (800)	1.534 (0.010)	1.817 (0.0408)	198,400	2.661 (2.558–2.770)

from stock solutions of sodium chloride or potassium chloride for the effect of individual inhibitors or the method of Lambert and Lambert (2003) for combined inhibitors.

2.3. Data analyses and model fitting

The data obtained from the Bioscreen are tables of optical density (OD) and time. The time to detection was defined as the time to produce an OD=0.2 at 600 nm. The assumption being made was that at an OD=0.2, each well had identical numbers of microorganisms. Furthermore, microscopic checks were performed to see if cell elongation occurred at the highest salt levels used: no such elongation was observed. Data were transformed to the reciprocal in order to stabilise data variance. Wells which showed no visible growth during the period of the experiment were removed from the analysis (censored data).

Previous publications (e.g. Lambert and Bidlas 2007) had used a general model for the fitting of time to detection data (TTD). A modified form of this model, which allows for a definitive MIC for individual inhibitors—the linear exponential model (E–L), was used to analyse the data obtained, Eq. (1).

$$\begin{aligned}&\text{If } [x]=0,\ \ \mathrm{RTD}=P_0\\&\text{Else if } [x]<[P_1],\\&\text{then } \ \mathrm{RTD}=P_0\exp\left(-\left(\frac{[x]}{P_1}\right)^{P_2}\right)\\&\text{Else, } \ \mathrm{RTD}=\frac{P_0}{e}\left(1-P_2\left(\log_e[x]-\log_e P_1\right)\right)\end{aligned} \tag{1}$$

Where $[x]$ is the concentration of the inhibitor, P_i are experimental parameters and e is the exponential, RTD is the reciprocal of the time to detection (min^{-1}).

The minimum inhibitory concentration was calculated from the parameter values obtained for each inhibitor using

$$\mathrm{MIC}=P_1\exp\left(\frac{1}{P_2}\right) \tag{2}$$

For combined inhibitors (combinations of NaCl and KCl) the model of Lambert and Lambert (2003) was modified in a similar way to Eq. (1) allowing a definitive growth–no growth boundary to be constructed for combinations, Eq. (3)

$$\begin{cases}\textit{if} & \sum_{i=1}^{n}[x_i]=0, \mathrm{RTD}=P_0\\ \textit{else if} & \ln[\mathrm{EffC}]<0\\ \textit{then} & \mathrm{RTD}=P_0\exp\left(-[\mathrm{EffC}]^{P_c}\right)\\ \textit{else} & \mathrm{RTD}=\frac{P_0}{e}\left(1-P_c\ln[\mathrm{EffC}]\right)\end{cases} \tag{3}$$

Where $[\mathrm{EffC}]=\sum_{i=1}^{n}\left(\frac{[x_i]}{P_{2i-1}}\right)^{P_{2i}}$ and P_c is the multinomial exponent for the combined system (Lambert and Lambert 2003).

Data were fitted to the equation using non-linear regression using the minimised sum of squares as the search criterion. Analyses were done using the JMP Statistical Software (SAS Institute Cary, NC).

3. Results

3.1. Growth inhibition by sodium chloride and potassium chloride

Three species—the Gram negatives *E. sakazakii* and *A. hydrophila* and the Gram positive *S. aureus* are used below to highlight the results obtained. Three strains of the latter organism were used, due to the importance of humectant activity to control the growth of this organism. The results from these and the other two organisms examined in this study are summarised in Table 1.

3.2. E. sakazakii

Optical density/incubation time data were collected using half-folded dilutions of NaCl or KCl. When analysed using the modified RTD model there was an excellent fit; Fig. 1 shows the results for the effect of KCl on the RTD. At KCl concentrations less than 1×10^4 mg l^{-1} (1%), there is little effect on the rate to detection, i.e. shows uninhibited growth. Above 1%, inhibition increases and above 1.2×10^5 mg l^{-1} (12%) KCl no growth was observed.

Table 1 gives the experimental parameters found in percent and in mol l^{-1}. In terms of mol l^{-1} the MIC of NaCl and KCl are within the 95% confidence interval of the mean (calculated from the parameters P_1 and P_2) given for each humectant. Fig. 2 compares the observed and

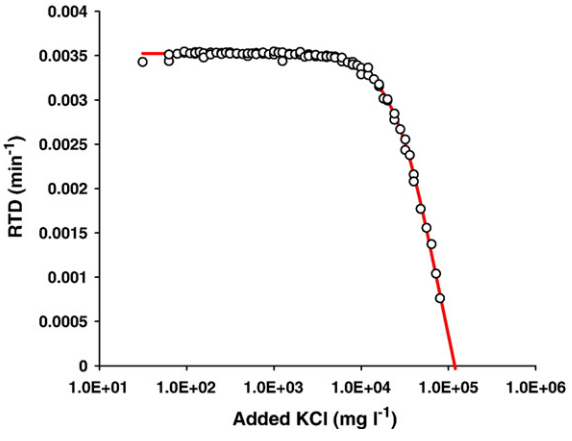

Fig. 1. Effect of KCl on the rate to detection of *Enterobacter sakazakii* in TSB at 30 °C.