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Influence of Calcium Lactate-calcium Gluconate Combination and Other Calcium Salts or Mixtures on the Fate of Salmonellae in Artificially Inoculated Orange Juice

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ABSTRACT

This study was undertaken to investigate the influence of a calcium lactate calcium gluconate combination (CaL-CG) and other calcium salts or mixtures on the fate of salmonellae in artificially-inoculated orange juice. A non-fortified orange juice was supplemented with each calcium salt or mixture at 10 or 30% of the Dietary Reference Intake value for calcium. The fortified juice samples (pH 3.6 or 4.1) were inoculated with a three-strain mixture of salmonellae at 10^5 CFU/ml and stored at 4 or 10°C for 7 weeks. The juice samples were assayed once a week for the populations of salmonellae. The orange juice supplemented with CaL-CG had significantly lower *Salmonella* populations ($P < 0.05$) than did the control juice at both pH levels and storage temperatures. At 4 and 10°C, the mean populations of salmonellae in the low pH juice supplemented with CaL-CG were numerically lower than the *Salmonella* populations in the low pH juice supplemented with calcium lactate (CaL) and numerically higher than the *Salmonella* population in the low pH juice supplemented with calcium lactate-calcium citrate (CaL-CC) and calcium lactate-tricalcium phosphate mixtures. In the high pH juice stored at 4°C, Cal-CG was less inhibitory to *Salmonella* cells than not only CaL but also CaL-CC. The worst performance of CaL-CG was observed in the high pH juice stored at 10°C. While CaL-CG could be used as a calcium supplement in both high and low acidity beverages at refrigeration temperatures, it might be particularly useful as a replacement for CaL in low pH beverages, in which it could improve the quality of the products.

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FIGURE 1. Survival of salmonellae in orange juice samples with a pH value of 3.6 and a calcium concentration equivalent to 30% (A) or 10% (B) DRI value for calcium at 4°C. CaL: calcium lactate, CaL-CC: calcium lactate and calcium citrate (1:1); CaL-TCP: calcium lactate and tricalcium phosphate (1:3); CaL-CG: calcium lactate and gluconate, CON: non fortified control.

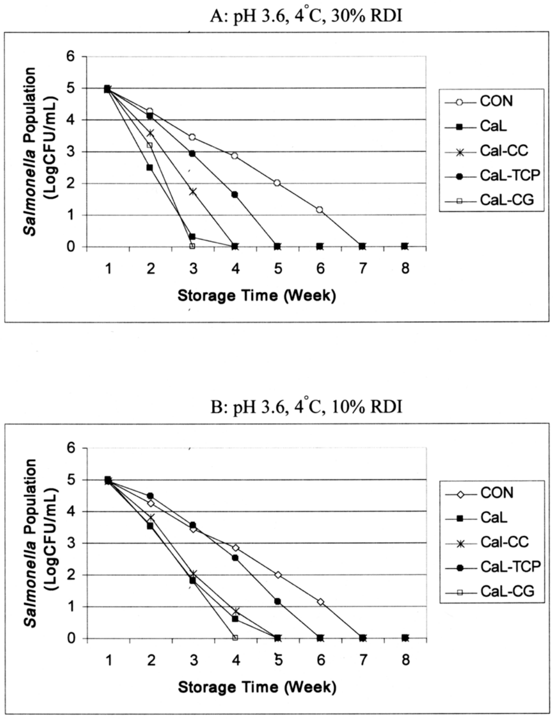
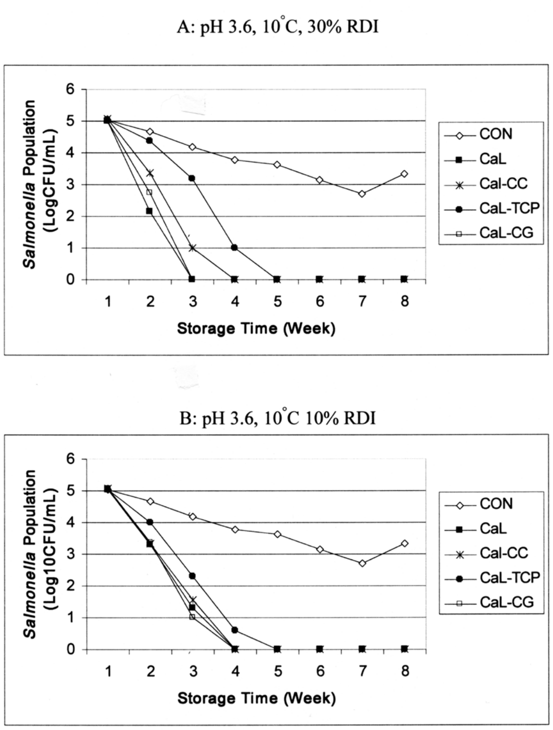


FIGURE 2. Survival of salmonellae in orange juice samples with a pH value of 3.6 and a calcium concentration equivalent to 30% (A) or 10% (B) DRI value for calcium at 10°C. CaL: calcium lactate, CaL-CC: calcium lactate and calcium citrate (1:1); CaL-TCP: calcium lactate and tricalcium phosphate (1:3); CaL-CG: calcium lactate and gluconate, CON: non fortified control.



INTRODUCTION

Orange juice has been recognized as a vehicle of transmitting foodborne diseases *(3, 4, 5, 6, 10).* In 1989, 67 individuals became ill in a New York hotel after consumption of orange juice *(1).* The outbreak was linked to infected kitchen workers. In 1995, unpasteurized orange juice consumed at a Florida theme park was epidemiologically linked to 62 confirmed cases of *Salmonella* Hartford infection *(8).* A probable source of this contamination was amphibians that carried the pathogen into the juice processing facility. In the summer of 1999, an outbreak of salmonellosis in the western United States and Canada sickened 298 people and claimed the life of one individual *(7).* Unpasteurized orange juice was identified as the vehicle of transmission. Laboratory research has shown that the survival of *Salmonella* could be influenced by orange juice pH and storage temperatures. Cells of salmonellae survived in detectable numbers for up to 27 days at pH 3.5, 46 days at pH 3.8, 60 days at pH 4.1 and 73 days at pH 4.4 *(11).* Oyarzábal et al. *(9)* reported that salmonellae were able to survive for 12 weeks in orange juice concentrates stored at -23°C.

Calcium fortification of orange juice has become increasingly popular in recent years. The calcium salts and mixtures used for orange juice fortification have included tricalcium phosphate (TCP), calcium lactate-tricalcium phosphate combination (CaL-TCP), calcium citrate malate complex and calcium citrate (CC). Some of these supplements, such as CaL-TCP, not only deliver calcium but also preserve orange juice by inhibiting the growth of microorganisms *(13).*

The goal of this study was to compare the inhibitory effect of a calcium lactate-calcium gluconate combination (CaL-CG) with that of other calcium salts and mixtures, including CaL (calcium lactate) and calcium citrate mixture (CaL-CC) and CaL-TCP, toward the cells of salmonellae in artificially inoculated orange juice.

FIGURE 3. Survival of salmonellae in orange juice samples with a pH value of 4.1 and a calcium concentration equivalent to 30% (A) or 10% (B) DRI value for calcium at 4°C. CaL: calcium lactate, CaL-CC: calcium lactate and calcium citrate (1:1); CaL-TCP: calcium lactate and tricalcium phosphate (1:3); CaL-CG: calcium lactate and gluconate, CON: non fortified control.

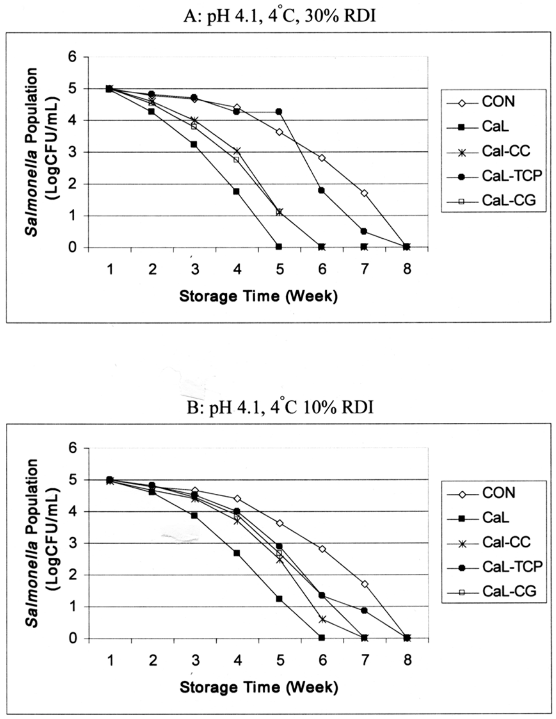
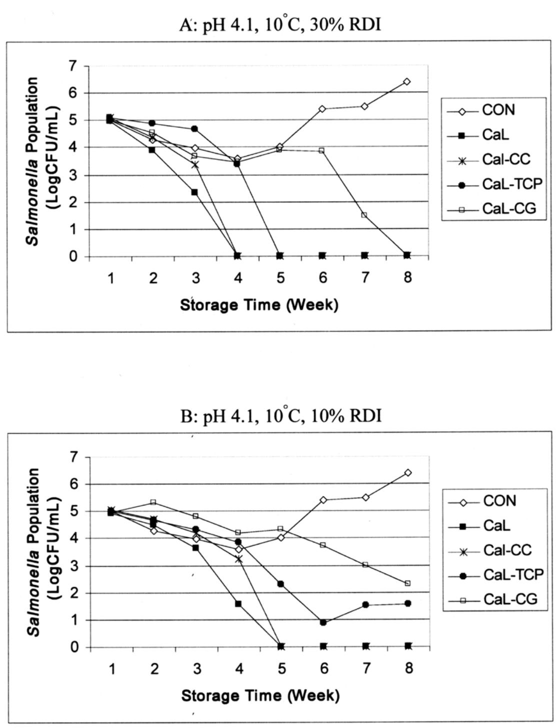


FIGURE 4. Survival of salmonellae in orange juice samples with a pH value of 4.1 and a calcium concentration equivalent to 30% (A) or 10% (B) DRI value for calcium at 10°C. CaL: calcium lactate, CaL-CC: calcium lactate and calcium citrate (1:1); CaL-TCP: calcium lactate and tricalcium phosphate (1:3); CaL-CG: calcium lactate and gluconate, CON: non fortified control.



MATERIALS AND METHODS

Orange juice and *Salmonella* strains

Pasteurized, pulp-free, non-fortified orange juice was purchased from a local supermarket in Griffin, GA. Microbiological media used in this study—tryptic soy agar (TSA), tryptic soy broth (TSB) and bismuth sulfite agar (BSA)—were purchased from Becton, Dickinson & Company (Sparks, MD) and prepared according to manufacturer's specifications. The *Salmonella* mixture was comprised of *S.* Baildon, *S.* Gaminara and *S.* Hartford. All the *Salmonella* strains were from our laboratory collections.

Inoculum preparation

The *Salmonella* cultures were grown individually on TSA plates at 37°C for 24 h. A colony of cells of each culture was transferred into 10 ml of TSB and incubated at 37°C for 16 h. Following incubation, the three *Salmonella* cultures in an equal volume were pooled to constitute a three-strain cocktail. The *Salmonella* mixture was centrifuged at 4,000 × g for 20 min at 4°C. The supernatant was then discarded and the cell pellet re-suspended in pasteurized, pulp-free, non-fortified orange juice to obtain a cell concentration of *ca.* 10^7 colony-forming units (CFU)/ml.

Orange juice inoculation and storage

Non-fortified orange juice was transferred into sterile flasks and supplemented with CaL, CaL-CC (1:1), CaL-TCP (1:3) or CaL-CG at a concentration equivalent to 10 or 30% of the Dietary Reference Intake (DRI) value for calcium. The CaL-CG combination (12.6% calcium by weight) was provided by PURAC America, Inc., while the other calcium mixtures were prepared in our laboratory, based on a calcium weight / total weight percentage of 13.5 for CaL, 21 for CC and 37 for TCP. The initial pH of each fortified juice sample was adjusted to 3.6 or 4.1 with 10 N HCl or 10 N NaOH, respectively. The juice samples were distributed into sterile glass bottles and subsequently inoculated with the three-strain mixture of salmonellae already described, at a concentration of ca. 10^5 CFU/ml. Juice samples were mixed

TABLE 1. Mean populations of salmonellae in orange juice samples with different pH values, stored at different temperatures, and supplemented with different calcium salt or mixtures at 10 or 30% DRI value for calcium

	Overall Mean Population of Salmonellae (log CFU/ml)*
DRI	
10%	2.28[a]
30%	2.09[b]
Juice pH	
4.1	2.74[a]
3.1	1.62[b]
Storage temperature	
10°C	2.29[a]
4°C	2.07[b]
Calcium supplements	
Control	3.51[a]
CaL-TCP	2.20[b]
CaL-CG	2.07[b]
CaL-CC	1.70[c]
CaL	1.42[d]

CaL-TCP: calcium lactate-tricalcium phosphate (1:3); CaL-CC: calcium lactate-calcium citrate (1:1); CaL-CG: calcium lactate-calcium gluconate (1:1); CaL: calcium lactate.

* Means within a test parameter (concentration of calcium supplements, juice pH, storage temperature or type of calcium supplements) not followed by the same letter are statistically different.

thoroughly after the inoculation and stored under aerobic condition at either 4 or 10°C for 7 weeks. The inoculated orange juice samples and the un-inoculated controls were sampled once a week.

Microbiological sampling

On each sampling day, the orange juice samples were withdrawn from storage and mixed thoroughly. A volume of 1 ml was taken before the juice sample was returned to storage. Samples were serially diluted with 0.1% buffered peptone water. Appropriate dilutions were inoculated in duplicate on BSA plates. The inoculated plates were incubated for 24 h at 37°C before colonies were enumerated.

Statistical analysis

Two replications were conducted, and each sample was assayed in duplicate. Data collected from the experiments were analyzed by using the general linear model procedure of the Statistical Analysis Software. Significant differences in the cell populations of salmonellae were determined based on a 95% confidence level.

RESULTS AND DISCUSSION

The calcium salt and mixtures evaluated in the study significantly ($P < 0.05$) reduced the mean populations of salmonellae artificially inoculated in orange juice (Table 1). The mean cell populations of the pathogens in orange juice samples supplemented with CaL-TCP, CaL-CG, CaL-CC or CaL were 1.31, 1.44, 1.81 or 2.09 log CFU/ml lower than the mean population of salmonellae in the control sample (Table 1). Statistical analysis revealed that the mean *Salmonella* populations were significantly lower ($P < 0.05$) in juice samples with a pH value of 3.6, stored at 4°C or with a calcium concentration equivalent to 30% of the DRI value for calcium, compared with samples with a pH value of 4.1, stored at 10°C or with a calcium concentration equivalent to 10% of the DRI value for calcium (Table 1).

The survival trends of salmonellae in orange juice samples with different pH values and calcium concentrations, at different storage temperatures, are shown in Fig. 1–4. The high pH juice supplemented with CaL-CG had an average *Salmonella* population of 2.38 log CFU/ml at 4°C and 3.65 log CFU/ml at 10°C (Table 2), which were significantly lower than the mean *Salmonella* population in the control juice. The mean populations of the pathogens in the low pH juice supplemented with CaL-CG and stored at 10°C were significantly lower than the mean *Salmonella* population in the control juice, whereas the pathogen counts in the same juice stored at 4°C were only numerically different from those in the control juice. CaL was numerically more effective than CaL-CG, CaL-CC and CaL-TCP in the low pH juice (Table 2). In the high pH juice stored at 4°C, the CaL-CG combination inhibited *Salmonella* not only less than CaL but also less than CaL-CC (Table 2). The worst performance of the CaL-CG was observed in the high pH juice stored at 10°C. Although it significantly ($P < 0.05$) reduced the population of salmonellae, the calcium combination was the least effective among all the calcium supplements evaluated in the study.

The mean population of salmonellae in the low pH control juice stored at 4°C (2.14 log CFU/ml) was lower than the population in the juice samples stored at 10°C (3.76 log CFU/ml). However, the populations of salmonellae in calcium-fortified juice samples were greater at 4°C than the populations at 10°C (Table 2). The reason for this is currently unknown, but it could be because bacterial

TABLE 2. Mean populations of salmonellae in low (3.6) or high (4.1) pH orange juice samples stored at 4 or 10°C

	Average Population of Salmonellae (log CFU/ml)			
	pH 3.6, 4°C	pH 3.6, 10°C	pH 4.1, 4°C	pH 4.1, 10°C
Control	2.14[a]	3.76[a]	3.37[a]	4.77[a]
CaL-TCP	1.81[ab]	1.54[b]	2.96[a]	2.51[c]
CaL-CC	1.38[ab]	1.18[b]	2.36[b]	1.88[c]
CaL-CG	1.25[ab]	1.01[b]	2.38[b]	3.65[b]
CaL	1.14[b]	1.01[b]	1.93[c]	1.61[c]
Average	1.54	1.70	2.60	2.88

CaL-TCP: calcium lactate-tricalcium phosphate (1:3); CaL-CC: calcium lactate-calcium citrate (1:1); CaL-CG: calcium lactate-calcium gluconate (1:1); CaL: calcium lactate.

[*]Means in the same column not followed by the same letter are statistically different.

cell membranes tend to be more permissible to the calcium supplements at 10°C than at 4°C or because the solubility of calcium supplements at 4°C was slightly lower than the solubility at 10°C, with the result that more inhibition of salmonellae occurred in calcium-fortified juice samples stored at 10°C. In the high pH juice, however, a similar phenomenon was observed only in the samples supplemented with CaL or CaL-CC (Table 2).

CaL-CG, a relatively new calcium supplement, has the highest solubility among all the calcium salts commonly used for beverage fortification *(12)*. CaL, when applied alone or at high amounts, tends to impart a bitter taste to the beverage due to free calcium ion concentrations. CaL-CG, in contrast, provides a neutral taste to beverage products even at high concentrations. This is primarily due to the ability of CaL-CG to shield the reactive free calcium ions. Gluconic acid has a pKa value of 3.86, similar to that of lactic acid. As a highly polar molecule, it is unable to penetrate the bacterial cell membrane *(2)*. Gluconate itself is therefore not an effective antimicrobial agent. The precise mechanism behind the anti-*Salmonella* activity of CaL-CG is not precisely known. However, it could be related to the presence of undisassociated forms of organic acids in the orange juice samples.

While CaL-CG could be used as a calcium supplement in both high (pH 3.6) and low acidity (pH 4.1) beverages stored at refrigeration temperatures, it might be particularly useful as a substitute for CaL in low pH (ca. pH 3.6) beverages, in which it would be more soluble, inhibit microbial growth, and reduce sourness as well as avoiding the detrimental effect of lactate on the taste of orange juice. Further studies are needed to investigate the interaction among concentrations of calcium supplements, juice pH and storage temperatures.

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