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Short Communication

Antibacterial effects of the sauce from cassava

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Casareep **is a sauce that is made from the sap of cassava (*Manihot esculenta*) and is reputed to extend the life of cooked meats. This preliminary investigation of *casareep's* preservative properties indicates that it has antibacterial activity against both gram-positive and gram-negative organisms and that it does prevent the growth of bacteria in cooked beef. Our investigations have determined that the sauce is bacteriostatic at lower concentrations and bactericidal at higher concentrations and that *Bacillus subtilis* was more sensitive to *casareep* than *Escherichia coli.***

Key words: Antibacterial, bacteriostatic, bactericidal, *casareep*, cassava, *Manihot esculenta*, food preservative.

INTRODUCTION

Cassava is the tuber of the perennial woody shrub *Manihot esculenta*. Grown as an annual, it is a major source of low cost carbohydrates for populations in many parts of Africa and the rest of the humid tropics. In rural settings of the Americas, the tubers are grated and the sap is extracted through squeezing or pressing. The residual solid is dried over a fire and then fermented or cooked to make a meal. In the hinterlands of Guyana, the sap is collected and evaporated down to a viscous, caramel-like liquid known as *casareep*, which is used for the distinctive flavor it imparts to meats during cooking (Shier, 1848). The *casareep* sauce is believed to have preservative properties as it is reputed to extend the life of cooked meats (Shier, 1848). *Casareep* has been used since times preceding the availability of synthetic food preservatives and refrigeration in the hinterlands of Guyana and its use continues (Dewprashad and Vaz, 2005). With immigration, use of the sauce has expanded into many international urban communities. *Casareep* is readily obtained, for example, in the neighborhood markets of Caribbean immigrant communities in New York City.

The medicinal chemistry of *Manihot esculenta* and/or its tuber has not been extensively studied. In one study, extracts from the leaves of the plant were found to exhibit broad spectrum antibacterial activity but no specific antibacterial agents were isolated nor identified (Zakaria et al., 2006). The antibacterial activity of the tuber has not been investigated. However, there are indications that there is an accumulation of flavan-3-ols (Buschmann et al., 2002) in the tubers. These compounds and their polymers have antifungal activity but antibacterial properties have not been reported (Li et al., 1999). The antibacterial effect of *casareep* has not been previously investigated. This paper presents the results of a preliminary investigation of the antimicrobial properties of *casareep*.

MATERIALS AND METHODS

Effect of *casareep* on the growth common household microbes in beef broth

Casareep (sold under the brand name *Lall's Pomeroon Cassava Casareep*) was obtained from a New York City supermarket. The label states that the product was manufactured by traditional methods in the hinterlands of Guyana. The label does not indicate the presence of preservatives and traditionally, preservatives are not added to casareep (Shier, 1848). The laws in Guyana and USA require that any added preservative be declared. In addition, one of the authors (BD) grew up in Guyana and was involved in the manufacture of casareep and is aware that preservatives are not added to the product. Also, contact was made with current manufacturers (in Guyana) who indicated that preservatives are not added. As such, it can be concluded that the product had no added

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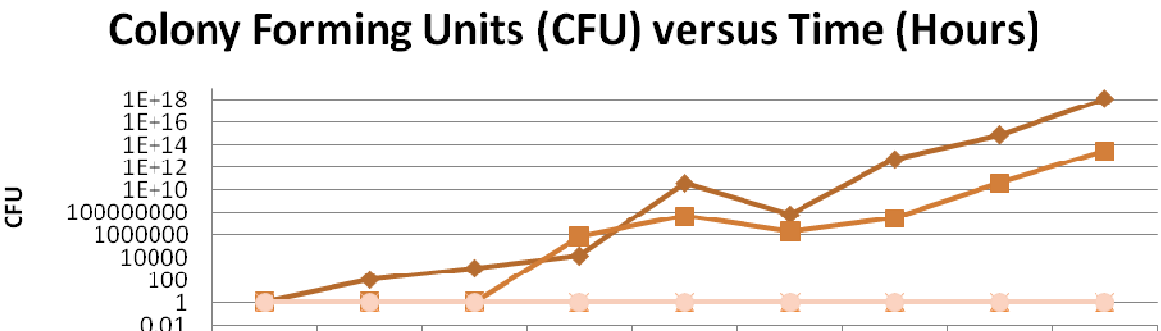

	0	12	24	36	48	60	72	84	96
0% casareep	1	100	1.00E+03	1.30E+04	3.70E+10	6.00E+07	5.00E+12	8.00E+14	1.30E+18
7.50%	1	1	1	6.50E+05	4.30E+07	1.90E+06	2.90E+07	3.80E+10	2.90E+13
10%	1	1	1	1	1	1	1	1	1
15%	1	1	1	1	1	1	1	1	1
20%	1	1	1	1	1	1	1	1	1
30%	1	1	1	1	1	1	1	1	1

Figure 1. Bacterial growth in beef broth in presence of *casareep.*

preservative. Nevertheless, a HPLC analysis of the product was done to determine the presence of benzoate, the only preservative used in vegetable based products in Guyana. The analysis was done using a standard literature procedure and no benzoate was detected (Altiokka et al., 2007).

Solutions of *casareep* (60 ml of 7.5, 10, 15, 20 and 30% v/v) were prepared by mixing freshly prepared tryptic soy agar (TSA) nutrient with *casareep.* Beef cubes (20.0 g) were added to each of the five solutions in a 250 mL beaker. The beakers were covered and boiled for 20 min. A standard was prepared by boiling 20.0 g of beef in 60 ml of water in a covered beaker for 20 min also. The beakers were allowed to cool. The broth was decanted off and exposed to room temperature and the laboratory atmosphere for 4 days. Samples (10 mL) were taken out every 12 h and plated on agar. After twenty-four hours incubation at 37°C, the number of colony forming units (CFUs) were counted from eight different sections of the agar plate and averaged. This experiment was repeated two additional times and the results were averaged shown in Figure 1.

How does *casareep* affect the growth of *Escherichia coli* and *Bacillus subtilis*?

The ability of *casareep* to retard the growth of *E. coli* and *S. aureus*, representative gram negative and gram positive household bacteria, was investigated. *E. Coli* JM 101 (Wild type) was obtained from Wards Natural Science and used. Concentrations of 7.5, 15, 30 and 60% v/v of casareep in TSA nutrient broth (to a volume of 10 ml) were mixed with 0.1 mL of *E. coli* stock solution. A standard of 0.1 ml of *E. coli* stock solution in 10 mL of TSA was also prepared. These cultures were incubated at 37°C, and the numbers of colony forming units (CFUs) were counted for eight different sections of the agar plate every 24 h for three days. The experiment was repeated two additional times. The experiments were repeated using *B. Subtilis* var. niger obtained from Wards Natural Science. The results are averaged and shown in Figure 1.

RESULTS AND DISCUSSION

Effect of *casareep* on the growth of common household microbes in beef broth

The results of increasing concentrations of *casareep* added to beef broth, which were then exposed to ambient air for up to four days, are shown in Figure 1. Only in the standard (0%) and in 7.5% (v/v) *casareep* was significant growth of bacteria observed. Higher concentrations of *casareep* (10, 15, 20 and 30%) appear to prevent the growth of common household microbes. It can be concluded that *casareep* is able to kill microbes that grow in household meat cuisines.

How does *casareep* influence the growth of *E. coli* and *B. subtilis*?

The results, shown in Table 1, indicate that in the *E. coli* cultures incubated with 7.5 and 15% *casareep,* bacterial numbers rise more slowly than in cultures without *casareep*. In 30 and 60% *casareep,* the CFU counts decrease precipitously to zero. These patterns suggest that low concentrations (7.5 and 15% *casareep*) are bacteriostatic and that higher concentrations (30 and 60% *casareep*) are bactericidal.

For *B. subtilis,* the results indicate that only in 0% *casareep* solution did *the* CFUs increase with time. In all other *casareep* solutions, there was no bacterial growth and all the bacteria died. It can be interpreted that *casareep* has bactericidal effects on *B. subtilis* at lower concentration

Table 1. The effect of *casareep* on *E. coli and B. subtilis*.

		CFUs/ml for incubation times of			
Organism	**Casareep (% v/v)**	**0 h**	**24 h**	**48 h**	**72 h**
E. coli	0%	$1.0x10^5$	$1.4x10^{10}$	$3.8x10^{13}$	$9.6x10^{16}$
	7.5%	$3.0x10^5$	$8.9x10^5$	$8.6x10^{10}$	$2.8x10^{12}$
	15%	$2.9x10^5$	$3.3x10^5$	$6.7x10^7$	$6.7x10^9$
	30%	$1.8x10^5$	0	0	0
	60%	$1.0x10^5$	0	0	0
B. subtilis	0%	$1.0 x10^6$	$5.0 x10^9$	$3.2 x10^{12}$	$4.0 x10^{16}$
	7.5%	$4.0 x10^5$	$4.0 x10^5$	0	0
	15%	$6.0 x10^6$	$6.0 x10^6$	0	0
	30%	$8.0 x10^5$	$8.0 x10^5$	0	0
	60%	$1.2 x10^6$	0	0	0

concentrations than it has on *E. coli.*

Conclusion

The results of this preliminary study indicate that *casareep* does prevent the growth of bacteria in cooked beef and has antibacterial properties against *E. coli* and *B. subtilis.* It was also found that the antibacterial effect is bacteriostatic at lower concentrations and bactericidal at higher concentration and that *B. subtilis* is more sensitive to *casareep* than *E. coli.* It seems likely that the antibacterial effect of *casareep* is due to specific chemical(s) present in the sauce that either is/are in cassava or formed during the heating of the cassava sap to form *casareep*. It would be of interest to isolate and identify such chemical(s). It is likely that such an effort could provide leads to new antibacterial compounds. In addition the implication of the results of this study is that cooking meats along with cassava is likely to extend the life of the dish.

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