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Food borne bacteria isolated from spices and fate of *Cronobacter sakazakii ATCC 29544* in black pepper exposed to drying and various temperature conditions

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Food-borne disease outbreaks caused by spices have been on the increase in the recent years, although they are rarely reported in the developing countries. The purpose of this work therefore, is to show that spices sold in selected retail outlets in Durban South Africa may contain unacceptable level of pathogenic microorganisms including *Cronobacter sakazakii* which may survive desiccation and high temperature. Selected spices were purchased from four retail outlets in Durban and examined for the presence of aerobic bacteria (AB), aerobic sporeformers (ASF), anaerobic sporeformers (AnSF), *Staphylococcus aureus* and *C. sakazakii* using ISO methods. Black pepper was inoculated with 10^8 cfu/ml of *C. sakazakii* ATCC 29544 and subjected to pasteurization temperature, desiccation temperature and they were later stored in refrigeration and room temperatures for 72 h. The mean values of AB, ASF, AnSF and *S. aureus* in the sampled spices were 2.98, 3.05, 1.82 and 1.67 log cfu/g, respectively; however analysis of variance showed that *S. aureus* was significantly low in cinnamon as compared to other spices. *C. sakazakii* was recovered in 50% of the samples tested. The result of the impact of processing temperature on black pepper inoculated with *C. sakazakii* ATCC 29544 showed that pasteurization at 72°C for 15 s was unable to eradicate all the pathogens. Desiccation (58°C for 50 min) combined with low temperature storage however was able to eliminate the pathogen. The sampled spices constitute a public health risk and *C. sakazakii* ATCC 29544 was able to survive high temperature such as pasteurization. Also, spices contaminated with CS may still grow after desiccation if stored at room temperature.

Key words: Bacteria, pathogens, *Cronobacter sakazaki*, temperature, desiccation.

INTRODUCTION

The history of spices dates back to 3000 to 200BC when the Arabs traded them among the early civilizations (ACH Food, 2012). Spices are normally used to add flavour and other organoleptic or sensory properties to food such as aroma among others. They are used all over the world to prepare many varieties of foods (Mousumi, 2003). According to Thomas et al. (2012), spices have also been used for medicinal, religious ritual and cosmetics purposes. Many spices have antimicrobial properties and because of this benefit, it is commonly used to process meat, which is majorly susceptible to spoilage (Thomas et al., 2012).

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Different workers have written on some common spices which were also used in this study, that is, cinnamon, black pepper, chili pepper and masala (Farrell, 1985; Perry et al., 2007; Prasad et al., 2000). These spices and others not studied in this work may however be sources of contamination of food borne pathogens because of the manner they are processed and stored in developing countries. Problem of contamination should not be seen as a localized one because the world is a global village. The spices produced in Africa may end up being used to make delicacies in Europe or US. According to CDC (2012), food-borne disease outbreaks caused by imports were on the increase between 2009 and 2010 in which fish and spices were reported to be the most common sources.

According to a FAO report (2008a), spices and herbs including agricultural products may be contaminated from different sources and this may occur during storage, distribution or during processing stage of the spices. For example, the old method, that is, spreading on the ground for the spices to access the sun automatically exposes the spices to risk of being contaminated. However, a better procedure such as drying the spices in an enclosed system may help reduce the problem of contamination. The levels of microbial contamination that is contained in spices and herbs will therefore depend on the pathogen reduction treatment that was performed on them (FAO, 2008a).

A recent study conducted on the spices used for local meat (kilichi) in Northern Nigeria showed that the spices were heavily contaminated with pathogenic bacteria: *Escherichia coli*, *Salmonella* sp., and *Clostridium* sp. isolated from some of the samples (Shamsuddeen, 2009). Sagoo et al. (2009) also reported on the potential public health risk of spices and herbs after isolating high counts of *Bacillus cereus*, *Clostridium perfrigens* and *E. coli*.

C. sakazakii may also be associated with spices as they are known to grow on dry food, for example cereals and powder infant formula (PIF). They are able to grow adequately on fresh-cut apple, cantaloupe, watermelon, cabbage, carrot, cucumber, lettuce and tomato at 25°C and at 12°C for other types of produce (Beuchat et al., 2009). Furthermore, *Cronobacter sakazakii* survives better in dried formula and cereal at low water activity (0.25 to 0.30) making it a difficult pathogen to remove during processing (Beuchat et al., 2009). The pathogen has emerged as a cause of gastroenteritis and a rare cause of neonatal meningtitis, septicemia and enterocolotis (Kim et al., 2011; Dumen, 2010).

C. sakazakii also presents a major challenge before the stakeholders in the produce industry because it is able to form biofilms along with other food borne pathogens such as *Listeria monocytogenes* and *Salmonella* spp. Pathogens that form biofilms are difficult to be eradicated from surfaces and equipment by sanitizers (Beuchat et al., 2009; Sofos, 2009; Fu et al., 2011). Furthermore, *C. sakazakii* is also a persistent pathogen in a manufacturing environment; however the underlying survival mechanisms remain to be fully understood (FAO, 2008b, Fu et al., 2011)

It must however be noted that there is limited information or no reported work on the bacterial pathogens associated with spices sold and consumed in South Africa. Neither did we come across information on isolation of *C. sakazakii* in dried spices. It will also be informative to study the behaviour of *C. sakazakii* in spices when subjected to some hurdle technologies such as high temperature and desiccation.

Therefore, the aim of this study was to determine the level of microbial hazards in spices sold in selected markets in Durban, South Africa and to study the impact of processing and storage temperature on survival of *C. sakazakii* ATCC 29544 in a selected spice, that is, black pepper.

MATERIALS AND METHODS

Collection of the samples

Spices (cinnamon, black pepper, chili pepper and masala) were purchased from four different open retail markets in Durban, in each retail store, the spices were collected using a scoop that the store uses when serving their customers; spices were collected into a sterile packaging nylon.

Bacterial analyses of samples

Spices were examined for the presence of *C. sakazakii, Staphylococcus aureus*, aerobic sporeformers, anaerobic sporeformers, and aerobic colony counts (aerobic bacteria) were done.

Aerobic colony counts

Dilution series from 25 g of spices macerated with 225 ml of water prepared using buffered peptone water (BPW) (Merck Ltd; Wadeville, Gauteng, South Africa) and 0.1 ml each of the dilutions were pour-plated with Nutrient Agar (Merck Ltd; Wadeville, Gauteng, South Africa) and incubated at 30°C for 72 h (ISO, 1991).

Aerobic and anaerobic sporeformers

A 25 g of spices was macerated with 225 ml of water and 20 ml of the sample was heated in a sterile test tube in a water bath (75°C) for 20 min (Austin, 1998). Serial dilutions were pour-plated. A set of plates were incubated aerobically at 37°C for 48 h, while the other set of plates were incubated an-aerobically in an anaerobic jar with anaerocult (Merck Ltd; Wadeville, Gauteng, South Africa) at 37°C for 48 h.

Staphylococcus aureus

S. aureus was determined according to ISO (1999). About 0.1 ml

Table 1. Results of aerobic colony counts, aerobic sporeformers, anaerobic sporeformers and *S. aureus* in spices from four retail outlets.

Retail outlet	Spices	ACC (log cfu/g)	ASF (log cfu/g)	AnSF (log cfu/g)	*S. aureus* (log cfu/g)
A	Black pepper	2.33	3.31	2.81	2.78
	Cinammon	1.53	2.97	1.22	1.41
	Chilli pepper	3.02	1.98	1.02	1.48
	Masala	4.29	4.03	1.35	2.73
B	Black pepper	4.24	4.30	2.97	2.96
	Cinammon	2.78	2.72	1.72	0.96
	Chilli pepper	2.89	2.68	0.35	1.23
	Masala	3.46	3.10	1.46	4.04
C	Black pepper	4.23	4.16	3.00	1.64
	Cinammon	2.21	1.81	1.79	0.00
	Chilli pepper	3.03	2.00	1.35	1.99
	Masala	2.98	3.85	1.75	2.21
D	Black pepper	4.53	3.33	3.32	2.04
	Cinammon	0.00	1.65	0.98	0.00
	Chilli pepper	2.31	3.34	2.05	1.53
	Masala	3.00	3.32	3.04	1.74

each of the dilutions was released on Baird Parker (Merck) Agar plates containing egg-yolk tellurite solution (Oxoid). Plates were incubated at 37°C for 24 h. Catalase test was performed on positive colonies and confirmed with Staphylase test (Merck Ltd; Wadeville, Gauteng, South Africa).

C. sakazaki

Ten milliliters of the macerated solution was added to 90 ml of buffered peptone water and incubated for 24 h after which 1 ml was introduced into Lauryl Sulphate Tryptose Broth Modified (mLST) and incubated for 24 h. After incubation, a loopful was streaked onto the surface of the *C. sakazaki* isolation chromogenic agar plate and incubated at 44°C for 24 h according to the method described by Guillaume-Gentil et al. (2005).

Reference strain

C. sakazakii ATCC 29544 was purchased from Anatech Analytical Technology Westville Durban South Africa. The strain was cultured in Lauryl Sulphate Tryptose Broth (for 24 h at 37°C and then stored at 4°C. The working stock culture was sub-cultured into Lauryl Sulphate Tryptose Broth twice a month.

Inoculation and growth after desiccation/pasteurization treatments

The black pepper was purchased at a super market and it was tested for the presence of *C. sakazakii* to ensure that the pathogen was absent. The black pepper was weighed in each of the 16 test tubes; each test tube contained 1 g of the black pepper.

10^8 cfu/g of *C. sakazakii* ATCC 29544 solution was prepared by suspending colonies of *C. sakazakii* ATCC 29544 in a 9 ml of prepared saline solution, it was then compared with the mc farland standard which was used as a reference to adjust the turbidity of bacteria. 1 ml of the 10^8 cfu/g of *C. sakazakii* ATCC 29544 was inoculated in each of the 16 test tubes weighed with 1 g of the black pepper. The spice was divided and exposed to different treatments which were desiccation and pasteurization. After the treatment, the treated spices were divided into 2 groups, that is, one group was stored in refrigeration temperature and the other group was stored in the room temperature. The spices were stored for different time intervals of less than an hour, 24, 48 and 72 h. The spice was then microbiologically analyzed for the presence of *C. sakazakii* ATCC 29544.

Statistical analysis

Analysis of variance (ANOVA), $p \leq 0.05$, (Tulsa, Oklohama, USA, 2003) was used to determine whether there were significant differences between the levels AB, ASF, AnSF and *S. aureus* in the spices from the four retail outlets. ANOVA was also used to determine whether there were significant differences between growths of *C. sakazakii* ATCC 29544 in different treatments and temperature storage. The experiments were repeated twice.

RESULTS AND DISCUSSION

It was observed from Table 1 that black pepper and masala generally had higher level of ACC, ASF, AnSF and *S. aureus* in all the four retail outlets, while they were lower in cinnamon and chili pepper. In fact in the two

Table 2. Statistical analysis of mean aerobic colony counts, aerobic sporeformers, anaerobic sporeformers and *S. aureus* in spices from four retail outlets.

Spices	ACC (log cfu/g)	ASF (log cfu/g	AnSF (log cfu/g)	*S.aureus (log cfu/g)*
Cinnamon	1.9950[b]	2.6750[a]	1.14275[b]	0.5925[b]
Chilli pepper	2.7850[ab]	2.7525[a]	1.1925[b]	1.5575[a]
Masala	3.2900[ab]	3.0625[a]	1.900[b]	2.1750[a]
Black Pepper	3.8325[a]	3.7075[a]	3.0250[a]	2.3550[a]

Means with the same letters are not significantly different.

Table 3. Prevalence of *C. sakazakii* in spices purchased from four retail outlets in Durban.

Type of spice	No. of sample	No. of positive sample	Positive (%)
Cinnamon	6	2	33
Black pepper	6	3	50
Masala	6	4	67
Chilli pepper	6	3	50
Total no of sample	24	12	50

Table 4. Effect of temperature treatments on black pepper inoculated with *C. sakazaki*.

Treatment	30 min	24 h	48 h	72 h
Desiccation- refrigeration (log cfu/g)	0.00[d]	0.00[e]	0.00[e]	0.00[d]
Desiccation- room temperature (log cfu/g)	0.00[d]	2.01[d]	3.29[d]	3.38[c]
Pasteurization- refrigeration (log cfu/g)	2.17[c]	3.37[c]	4.37[c]	5.40[b]
Pasteurization- room temperature (log cfu/g)	4.20[a]	4.27[b]	5.38[b]	6.44[a]
Control- refrigeration (log cfu/g)	3.37[b]	4.27[b]	4.30[c]	5.18[b]
Control- room temperature (log cfu/g)	4.36[a]	5.45[a]	6.45[a]	7.29[a]

Means with same letters are not significantly different.

retail outlets, no *S. aureus* was isolated from cinnamon.

The result of analysis of variance in Table 2 showed that there was significant difference in the spices mean values of the ACC, AnSF and *S. aureus* from the four retail outlets however there was no significant difference in spices mean values of ASF. Analysis of variance showed that *S. aureus* was significantly low in cinnamon as compared to other spices.

The mean values of ACC, ASF, AnSF and *S. aureus* in the sampled vegetables were 2.98, 3.05, 1.82 and 1.67 log cfu/g, respectively (Table 2).

From Table 3, it is observed that that cinnamon had the lowest percentage (33%) of positive growth of *C. sakazakii*, whereas masala had the highest percentage (67%) of positive growth of *C. sakazakii*. In general, 50% of all the spices sampled were positive for *C. sakazakii.* Cinnamon may have a greater level of antimicrobial properties as compared to the other spices.

Tables 1 to 3 shows that the growth of micro organisms differs in all the spices purchased from different retail outlets, signifying that the hygiene practices were not at the same level in all the outlets. Different retail outlets were supplied spices by different suppliers which had different hygiene practices. The problem of the growth of microorganisms may also have occurred during the pre-harvest or post-harvest.

Table 4, Figures 1 and 2 show that desiccation at 58°C for 50 min and then storage in refrigeration temperature was able to eradicate all *C. sakazakii* ATCC 29544 as there was no growth when it was analyzed after all the different time intervals which were after 30 min, 24, 48 and 72 h. However, it was not the case with the black pepper that was exposed to desiccation and then stored in room temperature. There was no growth when it was analyzed after 30 min of storage; however, after 24 h of storage and later time intervals, growth was detected. This signifies that desiccation treatment may not be effective if the black pepper is stored at room temperature

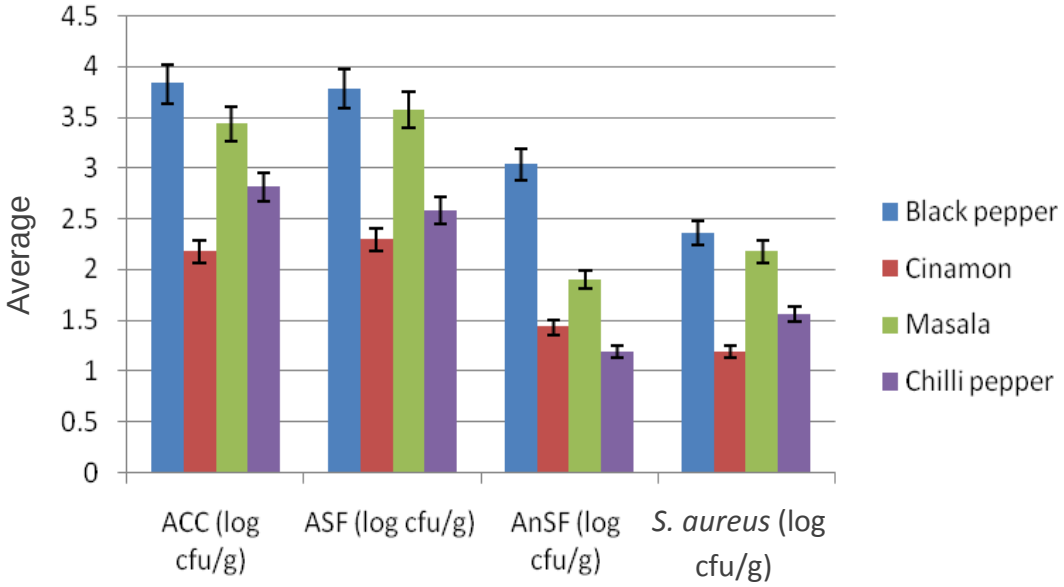

Figure 1. Chart of the average ACC, ASF, AnSF and *S. aureus* results from the four retail outlets.

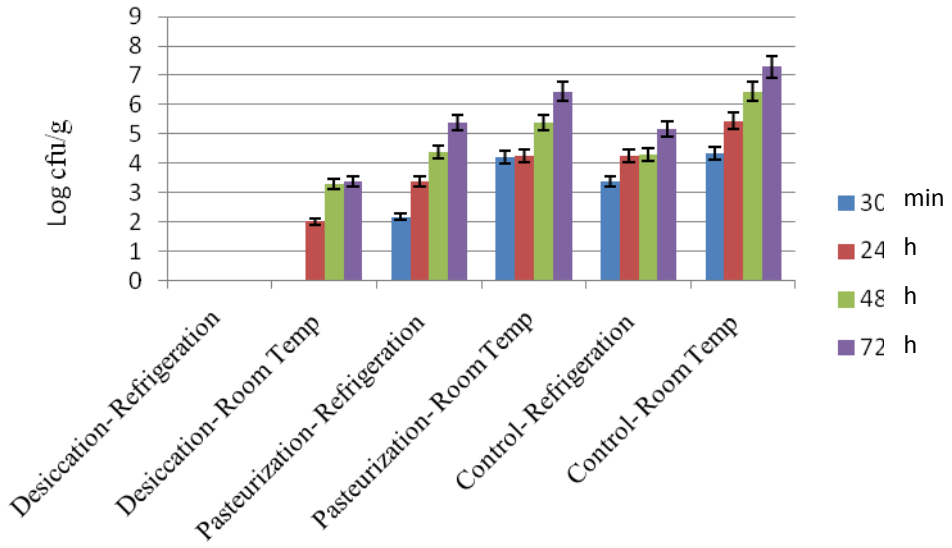

Figure 2. Chart of growth of *C. sakazakii* after being exposed to different treatments and storage at room and refrigeration temperatures.

after being treated.

Surprisingly, flash pasteurization proved to be ineffective. Growth of the pathogen was detected after exposure to pasteurization and storage at room and refrigeration temperatures and at different time intervals, that is, 30 min, 24, 48 and 72 h. *C. sakazakii* ATCC 29544 was able to survive the flash pasteurization temperature possibly because it is one of the most thermo tolerant among the enterobacteriaceae (Nazarowec-White and Farber, 1997).

In all the treatments that were unable to kill the pathogen, it was observed that the pathogen multiplied as the storage time increased, both in room temperature and refrigeration temperature and this was because *C. sakazaki* ATCC 29544 was able to grow at low temperature (Beuchat et al., 2009). However, the frequency of growth of the pathogen in refrigeration temperature is expected to differ from that of the pathogen in room temperature, this was observed in black pepper that was stored in room temperature which

had a heavy growth as compared to the black pepper that was stored in refrigeration temperature. This is because refrigeration works through the principle of slowing down the growth of micro organisms (Brackett, 2007).

This study has shown that the sampled spices constitute a public health risk. This should be taken seriously because South Africa has ample percentage of her population with compromised immune system (Suarez, 2009). Such people including infants and old people have been reported to be easily susceptible to food borne illnesses (CDC, 2006). The study has also brought focus on masala and black pepper as the results have shown that they both had heavy growths as compared to cinnamon and chilli pepper. The reason for this may be because the antimicrobial activity differs in different types of spices (Nanasombat and Lohasupthawee, 2005). Black pepper and masala may have a lower antimicrobial activity as compared to cinnamon and chilli pepper.

This work has also shed light on the behaviour of *C. Sakazakii* ATCC 29544 when exposed to drying and pasteurization temperature. It was observed that *C. sakazakii* can survive high temperature such as pasteurization temperature possibly because some pathogens could adapt to stress or other hurdle technologies. Although, cinnamon was detected as the type of spice with low microbial growth, it remains a problem because pathogens may grow and reproduce at a later stage leading to serious consequences.

CONCLUSION AND RECOMMENDATION

The culture of prevention of contamination of spices with microorganisms, good hygiene practices and food safety is however recommended for the producers, manufacturer and retailers of spices. There is also an urgent need for education of consumers on spices safe handling practices from purchase through consumption.

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