Chapter III

Effect of sorghum crude phenolic extracts on *Bacillus cereus*, *Escherichia coli* and *Listeria monocytogenes*

3.1 Abstract

Freeze-dried phenolic extracts obtained from defatted bran fractions of condensed tannin (red) and condensed tannin-free sorghum (white) varieties were evaluated for their antimicrobial activities against *Bacillus cereus (B. cereus)* ATCC 1178, *Escherichia coli (E. coli)* ATCC 25922 and *Listeria monocytogenes (L. monocytogenes)* ATCC 7644 pathogenic bacteria. The extracts were tested at 1 , 2 , 4 and 20 % concentrations (w/v) in methanol using the paper disc diffusion method and absolute methanol was used as a control. The condensed tannin-free sorghum crude phenolic extract at concentrations 1 , 2 and 4 % had no inhibitory effects on the bacteria tested but was effective against Gram-positive bacteria, *B. cereus* ATCC 1178 and *L. monocytogenes* ATCC 7644 at a concentration of 20 %. The condensed tannin sorghum crude phenolic extract was effective against *B. cereus* ATCC 1178 and *L. monocytogenes* ATCC 7644 at concentrations 1, 2, 4 and 20 %. None of the tested sorghum extracts inhibited the Gram-negative bacteria, *E. coli* ATCC 25922. Phenolic extracts from condensed tannin sorghum may be used as antimicrobial agents to prevent the growth of Gram-positive bacteria, *B. cereus* ATCC 1178 and *L. monocytogenes* ATCC 7644.

Keywords: Sorghum; phenolic extracts; antimicrobial activity; *B. cereus*; *E. coli*; *L. monocytogenes*

3.2 Introduction

Microbiological activity is the principal mode of spoilage of foods and is often responsible for the loss of quality and safety of food (Jayaprakasha *et al*., 2003). Synthetic additives have mostly been employed to prevent the spoilage of foods. Examples of synthetic additives include chemical antimicrobials such as formic and propionic acid (Duffy and Power, 2001).

There are however concerns over pathogenic microorganisms in foods due to increases in outbreaks of foodborne diseases (Tauxe as cited by Jayaprakasha *et al.*, 2003) and increases in consumer's resistance to the use of synthetic additives (Duffy and Power, 2001). Recently, interest has been focused on the use of plant extracts rather than synthetic additives to prevent spoilage of foods (Baydar *et al*., 2004) as they sometimes show antioxidant as well as antimicrobial activity (Smid and Goris as cited by Jayaprakasha *et al*., 2003). Extracts of herbs and spices are mostly used for this purpose (Baydar *et al*., 2004). Phenolic extracts from different plant sources such as green tea, cinnamon, curry, mustard, herbs, spices and grapes have been shown to have antioxidant as well as antimicrobial activity (Sakanaka *et al*., 2000; Jayaprakasha *et al*., 2003; Baydar *et al*., 2004).

Tannins (a group of polyphenolic compounds) from different sources have also been shown to be inhibitory to the growth of different bacteria (Scalbert, 1991; Chung *et al*., 1993; Chung *et al*., 1998b). Phenolic compounds such as tannic acid, propyl gallate, (Chung *et al*., 1993; Chung *et al*., 1998b) and methyl gallate (Chung *et al*., 1998b) have been reported to be inhibitory to the growth of various intestinal bacteria including *Bacteroides fragilis* ATCC 25285 and *Clostridium perfringens* ATCC 13124.

Various studies have shown that sorghum grain contains a wide range of phenolic compounds (Beta *et al*., 1999; Awika *et al*., 2003). They are mostly concentrated in the outer layers of the sorghum kernel (Hahn *et al*., 1984). These compounds have been shown to have many favourable effects on human health such as lowering of human low-

density lipoprotein (Frankel *et al*., 1995) as well as exertion of other physiological effects such as reducing blood pressure (Chung *et al*., 1998a). These phenolic compounds, (especially the tannins), also appear to be responsible for the astringency of many plant materials and can have an effect on the colour, appearance and nutritional quality when added to the diet or when found naturally in high levels in certain foodstuffs (Strumeyer and Malin, 1975; Haslam, 1989; Hahn *et al*., 1984; Murty and Kumar, 1995; Bvochora *et al*., 2004).

There is however, no information on the antimicrobial properties and inhibitory effects of phenolic compounds from sorghum. The objective of this study was therefore to evaluate the antimicrobial activity of crude phenolic extracts prepared from bran fractions of condensed tannin and condensed tannin-free sorghum varieties against pathogenic microorganisms namely *B. cereus* ATCC 1178, *E. coli* ATCC 25922 and *L. monocytogenes* ATCC 7644.

3.3 Materials and methods

3.3.1 Sorghum grain samples and reagents

As previously described in Chapter 2 sections 2.3, 2.3.1 and 2.3.2

3.3.2 Bacterial cultures used for evaluation of antimicrobial activity of sorghum crude phenolic extracts (CPE)

The antimicrobial activity of CPE was evaluated against three pathogenic bacteria, i.e. *B. cereus* ATCC 1178, *E. coli* ATCC 25922 and *L. monocytogenes* ATCC 7644. The Agricultural Research Council (ARC) at Irene, Pretoria, South Africa supplied the bacterial cultures.

3.3.3 Preparation of CPE from bran fractions of condensed tannin and condensed tannin-free sorghum varieties for evaluation of antimicrobial activity

Bran (20 g) was defatted with 200 ml hexane for 1 h and the hexane extract was discarded. A bran fraction-to-solvent ratio of 1:4 (w/v) was used in the preparation of CPE. Samples (1 g) of each of the milled and defatted sorghum bran fractions were suspended in 4 ml of extractant (75 % acetone). The bran fractions were extracted for 2 h by vortex mixing at 5 min intervals. After 2 h of extraction, samples were centrifuged for 6 min at 3500 rpm (Selecta Medifridge, UK) and the supernatants were collected.

After preparation, the extracts were concentrated by evaporation at 30 °C under vacuum using a rotary evaporator to remove most of the acetone and freeze-dried to obtain a fine powder. The freeze-dried sorghum CPE in powder form were vacuum packed (Busch Vacuum technique, Germany) in laminated plastic bags and stored in a box made of cardboard at –20 °C in the dark until required for evaluation of antimicrobial activity.

The total phenol and condensed tannin contents of the freeze-dried CPE could not be determined due to inadequate yield especially with the condensed tannin-free sorghum variety. However, it would be expected that the concentration of phenolic compounds in the freeze-dried CPE would be much higher than in the sorghum bran fractions (Sikwese, 2005).

3.3.4 Preparation of bacterial cultures for antimicrobial activity evaluation

Cultures of *B. cereus* ATCC 1178 and *L. monocytogenes* ATCC 7644 were grown in Tryptone Soy Broth medium (Sigma-Aldrich, Johannesburg, South Africa) at 30 °C, for 24 h. *E. coli* ATCC 25922 was grown in the same medium at 35 °C for 24 h. Suspensions (250 µl) (Baydar *et al*., 2004) of the bacteria adjusted to 10^7 cfu/ml final cell concentration as confirmed by the McFarland standard (http://www.campmicro.com/macfarland.htm), were added to bottles containing 25 ml sterile tryptone soy agar (Sigma-Aldrich, Johannesburg, South Africa) (*E. coli* ATCC 25922 and *L.*

monocytogenes ATCC 7644) and nutrient agar (Sigma-Aldrich, Johannesburg, South Africa) (*B. cereus* ATCC 1178) maintained at 45 °C. Respective agars for each bacterial culture were then poured into petri dishes (90 mm diameter) (Sigma-Aldrich, Johannesburg, South Africa) and left to solidify at 4 °C for 1 h (Baydar *et al*., 2004).

3.3.5 Evaluation of sorghum CPE for antimicrobial activity

The paper disc diffusion method (Baydar *et al*., 2004) was used to evaluate the antimicrobial effect of the CPE from bran fractions of condensed tannin and condensed tannin-free sorghum varieties.

Sterilised antibiotic assay paper discs (6 mm diameter) (Sigma-Aldrich, Johannesburg, South Africa) were soaked in 50 µl of 1, 2, 4 and 20 % (w/v) solutions of condensed tannin and condensed tannin-free sorghum CPE prepared in absolute methanol. Methanol was used as a control (Baydar *et al*., 2004).

The soaked discs were placed on the plates inoculated with the bacterial cultures and incubated at 30 °C for 24 h for *B. cereus* ATCC 1178 and *L. monocytogenes* ATCC 7644. *E. coli* ATCC 25922 was incubated at 35 °C for 24 h. After incubation, the diameter of the clear inhibition zone formed around the disc in the medium was measured and expressed in millimetres (mm) as its antimicrobial activity (Baydar *et al*., 2004). All the analyses were carried out in triplicate and all samples were plated in triplicate to give a total of 9 values (Baydar *et al*., 2004).

3.4 Statistical analysis

Analysis of variance (ANOVA) was performed to determine whether the bacteria (*B. cereus* ATCC 1178 and *L. monocytogenes* ATCC 7644), percentage crude phenolic extracts (1, 2, 4 & 20 %) and sorghum varieties (condensed tannin and condensed tannin-free) significantly influenced the size of inhibition zones. Significance level was $p \leq 0.05$.

Since no inhibition was noted for *E. coli* ATCC 25922, the data for *E. coli* ATCC 25922 was not included in the ANOVA to ensure a normal distribution.

3.5 Results and discussion

3.5.1 Antimicrobial effect of condensed tannin and condensed tannin-free sorghum varieties on *B. cereus* ATCC 1178, *E. coli* ATCC 25922 and *L. monocytogenes* ATCC 7644

There were statistically significant differences ($p \leq 0.05$) between the condensed tannin sorghum CPE and condensed tannin-free sorghum CPE regarding the level of inhibition exhibited on the bacteria used in this study namely, *B. cereus* ATCC 1178, *E. coli* ATCC 25922 and *L. monocytogenes* ATCC 7644 (Table 4). The overall average inhibition given by the bacteria was 2.7 and 0.4 mm for condensed tannin sorghum CPE and condensed tannin-free sorghum CPE, respectively (Figure 12). Only condensed tannin sorghum CPE had any inhibitory effect at 1, 2 and 4 % (Figure 13).

Even though the inhibition levels increased for both sorghum varieties with increasing concentration, the increase in inhibition was not equal between the two sorghum varieties, indicated by a significant ($p \leq 0.01$) sorghum variety and concentration interaction (Table 4). The greatest inhibitory effect of condensed tannin sorghum CPE was at a concentration of 20 % with a mean inhibition zone of 5.2 mm compared to 1.4 mm for the condensed tannin-free sorghum CPE (Figure 13). The mean inhibition increased from 1, 2 to 3 mm as the concentration increased from 1 to 4 % for the condensed tannin sorghum CPE while the condensed tannin-free sorghum CPE showed no inhibition at these concentrations (Figure 13). This may be due to the lower levels of phenolic compounds determined in the condensed tannin-free sorghum bran fraction that were insufficient to attain considerable bacterial inhibition. This observation is in agreement with that of Baydar *et al*. (2004).

In a study done by Baydar *et al*. (2004) on total phenolic contents and antimicrobial activities of grape (*Vitis vinifera* L) extracts the authors associated the extent of the inhibitory effects of the grape seeds and bagasse (berry without seed and juice) extracts on the bacteria tested with their total phenolic compound concentration. The grape seed extract which was found to have highest levels of total phenolics (627.98 and 667.87 mg gallic acid equivalent/g when extracted with acetone: water: acetic acid (90:9.5:0.5) and ethyl acetate: methanol: water (60:30:10), respectively showed an inhibitory effect at 20 % against all bacteria tested, including *B. cereus*, *E. coli* and *L. monocytogenes.* Bagasse extract with lower levels of phenolics (45.44 and 29.55 mg gallic acid equivalent with ethyl acetate: methanol: water (60:30:10) and acetone: water: acetic acid (90:9.5:0.5) respectively) did not inhibit any of the bacteria at the same extract concentration.

In this study (results shown in Chapter 2 section 2.4.2) the condensed tannin sorghum bran fraction was found to contain higher levels of total phenols, 33.18 mg tannic acid equivalent/g of sample and condensed tannins, 117.98 catechin equivalent/g of sample when compared to the condensed tannin-free sorghum bran fraction which contained total polyphenols of 6.81 tannic acid equivalent/g of sample and condensed tannins of 8.52 catechin equivalent/g of sample. The above is the phenolic content of the bran samples, it is however expected that the total phenol and condensed tannin content of the freeze-dried extract would thus follow a similar trend (Sikwese, 2005). In that case, the greater the amount of phenolic compounds in the extract would imply the greater the inhibitory effect of that particular extract on the test microorganism involved, provided that the test microorganism is sensitive to the extract as it was found in a study by Baydar *et al*. (2004).

Table 4. Statistical analysis of the effect of CPE from bran fractions of condensed tannin and condensed tannin-free sorghum at concentrations of 1, 2, 4 and 20 % on the inhibition (mm) of *B. cereus* ATCC 1178, *E. coli* ATCC 25922 and *L. monocytogenes* ATCC 7644 bacteria

Factors	Degrees. of Freedom	P
Bacteria (*B. cereus* ATCC 1178 & *L. monocytogenes* ATCC 7644)	1	<0.01
Sorghum (condensed tannin & condensed tannin-free)	1	<0.01
Concentration (1, 2, 4 & 20 %)	3	<0.01
Bacteria*Sorghum	1	<0.01
Bacteria*Concentration	3	<0.01
Sorghum*Concentration	3	<0.01
Bacteria*Sorghum*Concentration.	3	0.01

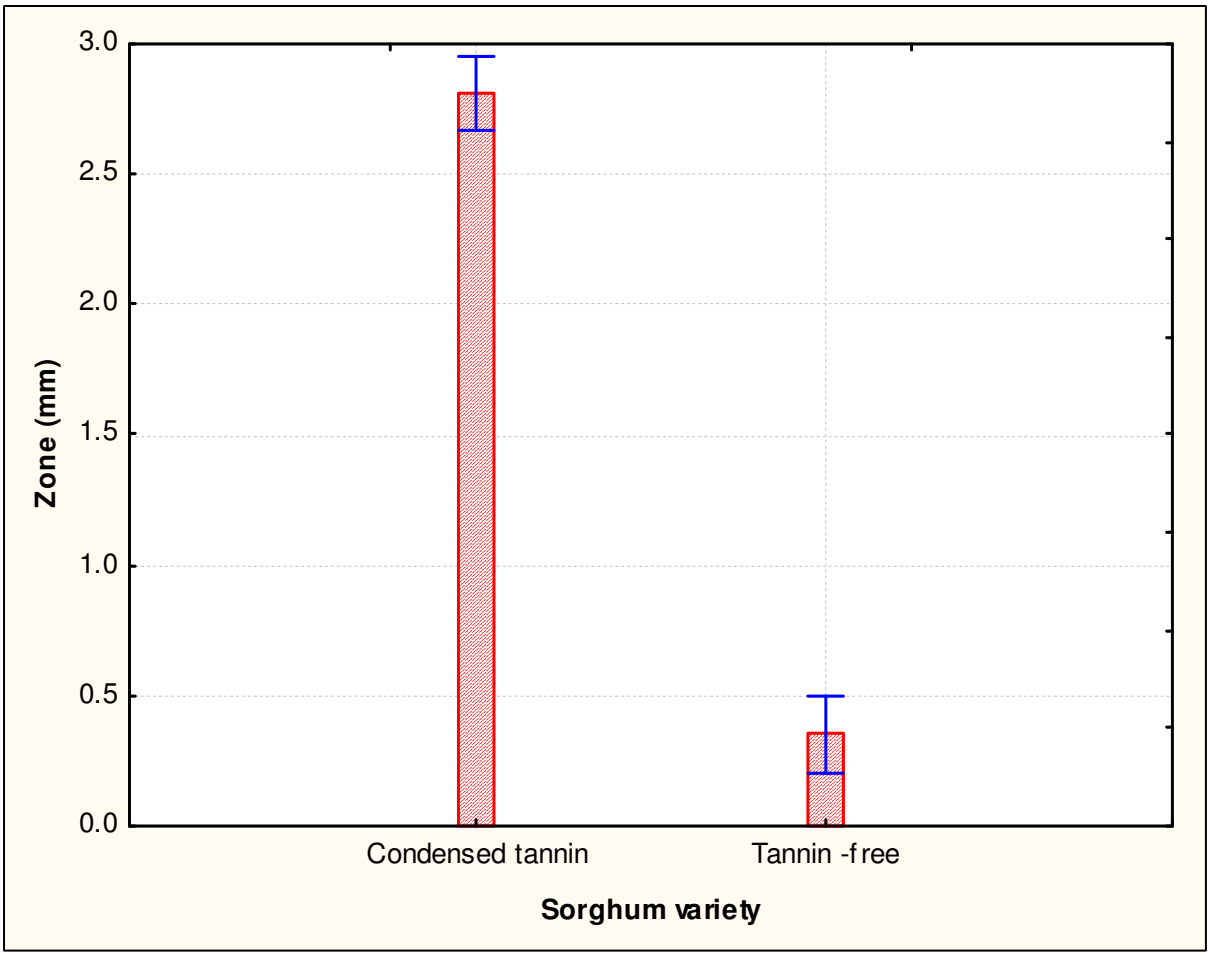

Figure 12. Effect of sorghum crude phenolic extracts from bran fractions of condensed tannin and condensed tannin-free sorghum varieties on growth inhibition.

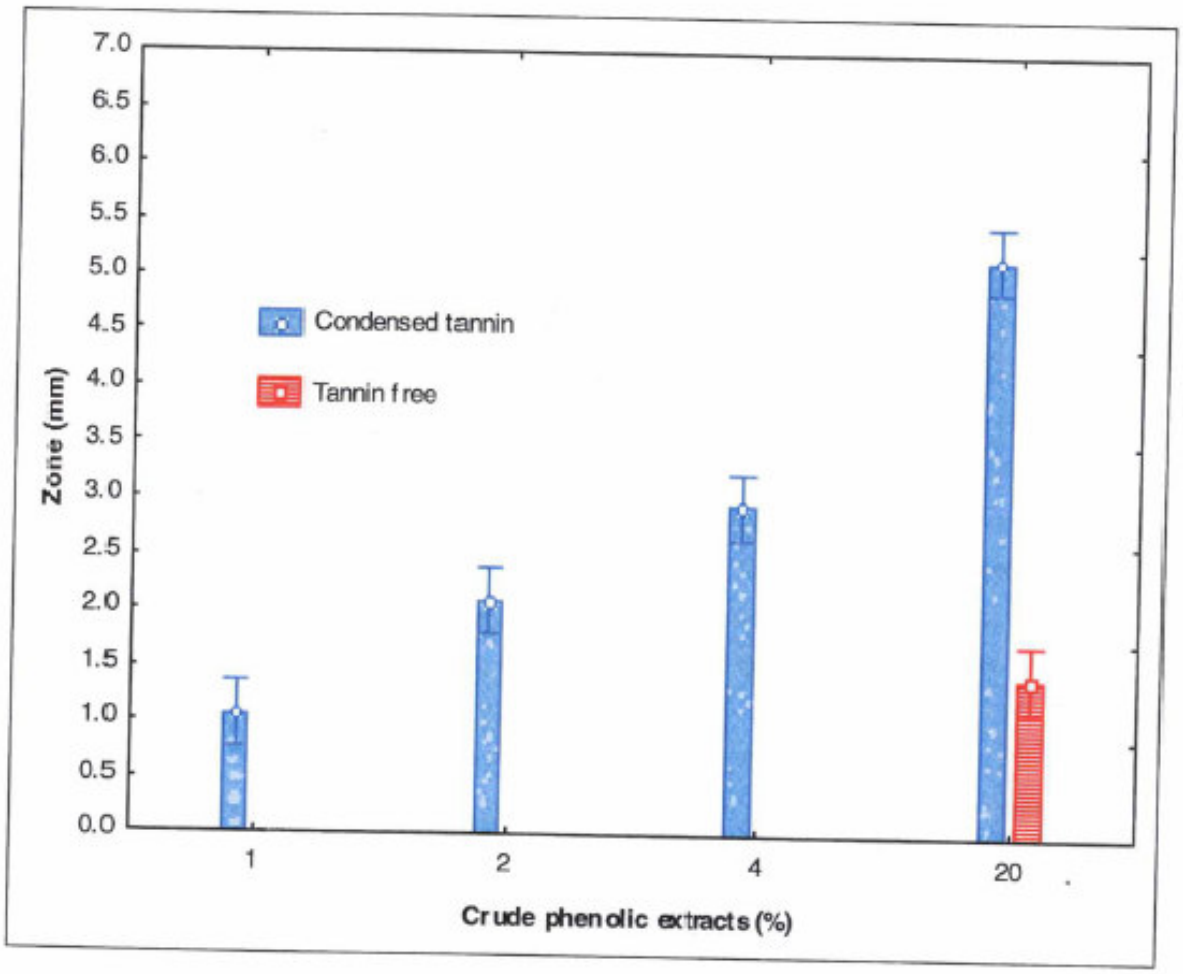

Figure 13. Effect of sorghum crude phenolic extract from bran fractions of condensed tannin vs. condensed tannin-free sorghum varieties on bacterial inhibition at 1, 2, 4 and 20 % concentrations.

3.5.2 Resistance of Gram-positive bacteria, *B. cereus* ATCC 1178 and *L. monocytogenes* ATCC 7644 to inhibition by condensed tannin and condensed tannin-free sorghum CPE in comparison with Gram-negative bacteria, *E coli* ATCC 25922

There were significant differences ($p \leq 0.05$) among the inhibition of the different bacteria by both CPE tested (Table 4). Even though both Gram-positive bacteria, *B. cereus* ATCC 1178 and *L. monocytogens* ATCC 7644 were susceptible to inhibition by both extracts, it was evident from the results that *B. cereus* ATCC 1178 was more vulnerable to both extracts. Mean inhibition zones of 3.9 mm and 0.49 mm for the condensed tannin and condensed tannin-free sorghum CPE respectively as compared to *L. monocytogenes* ATCC 7644 which was inhibited at mean inhibition zones of 1.7 mm and 0.2 mm for the condensed tannin and condensed tannin free sorghum CPE respectively were observed (Figure 14a). Both bacteria were more susceptible to inhibition by the condensed tannin sorghum CPE than the condensed tannin-free sorghum CPE (Figure 14a). However, *E. coli* ATCC 25922 appeared to be resistant to inhibition by both the condensed tannin and condensed tannin-free sorghum CPE (Table 5) and was not inhibited by any of the extracts tested at all the concentrations and was therefore not included in the figures.

No inhibition was noted at concentrations of 1, 2, and 4 % for the condensed tannin-free sorghum CPE for both *B. cereus* ATCC 1178 and *L. monocytogenes* ATCC 7644. Only 1.9 mm and 1.1 mm mean inhibition zones were noted for *B. cereus* ATCC 1178 and *L. monocytogenes* ATCC 7644 at 20 % concentration respectively (Figure 14b). The vulnerability of *B. cereus* ATCC 1178 to inhibition by the condensed tannin extract increased with increase in concentration of the extract (Figure 14b and Figure 15). The mean inhibition zones for the condensed tannin sorghum CPE on *B. cereus* ATCC 1178 bacteria significantly ($p \leq 0.05$) increased from 1.5 mm at 1 %, 3.1 mm at 2 %, 4.4 mm at 4 % and 6.6 mm at 20 % of the CPE compared to that of *L. monocytogenes* ATCC 7644 where mean inhibition zones of 0.5 mm at 1 %, 1.0 mm at 2 %, 1.4 mm at 4 % and 3.6 mm at 20 % were observed (Table 5 and Figure 14b). However, no inhibition was

observed for Gram-negative bacteria, *E. coli* ATCC 25922 by both the condensed tannin and condensed tannin-free sorghum extracts (Table 5).

Table 5. Inhibition (mm) of *B. cereus* ATCC 1178, *E. coli* ATCC 25922 and *L. monocytogenes* ATCC 7644 by CPE from bran fractions of condensed tannin and condensed tannin-free sorghum varieties (n=9)

Bacteria	Sorghum CPE	Concentration (%)	Inhibition (mm) (±SD)
B. cereus ATCC 1178	Condensed tannin	1	1.5 (0.2)
		2	3.1 (0.2)
		4	4.4 (0.2)
		20	6.6 (0.2)
	Condensed tannin-free	1	0
		2	0
		4	0
		20	1.7 (0.2)
L. monocytogenes ATCC 7644	Condensed tannin	1	0.5 (0.2)
		2	1.0 (0.2)
		4	1.4 (0.2)
		20	3.6 (0.2)
	Condensed tannin-free	1	0
		2	0
		4	0
		20	1.0 (0.2)
E. coli ATCC 25922	Condensed tannin	1, 2, 4 & 20	No inhibition
	Condensed tannin-free	1, 2, 4 & 20	No inhibition

SD Standard deviation

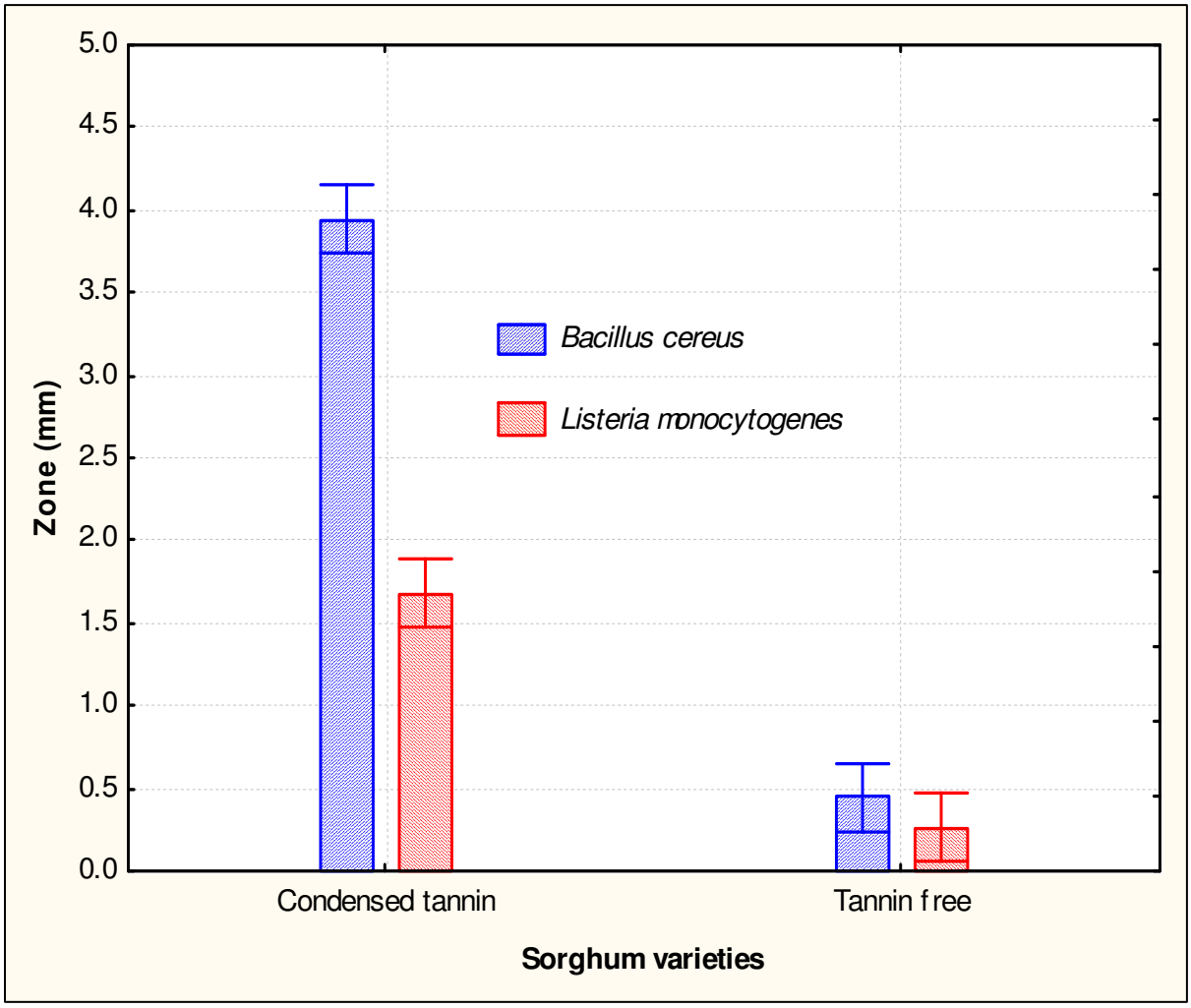

Figure 14a. Effect of condensed tannin and condensed tannin-free sorghum crude phenolic extract on inhibition of *B. cereus* and *L. monocytogenes.*

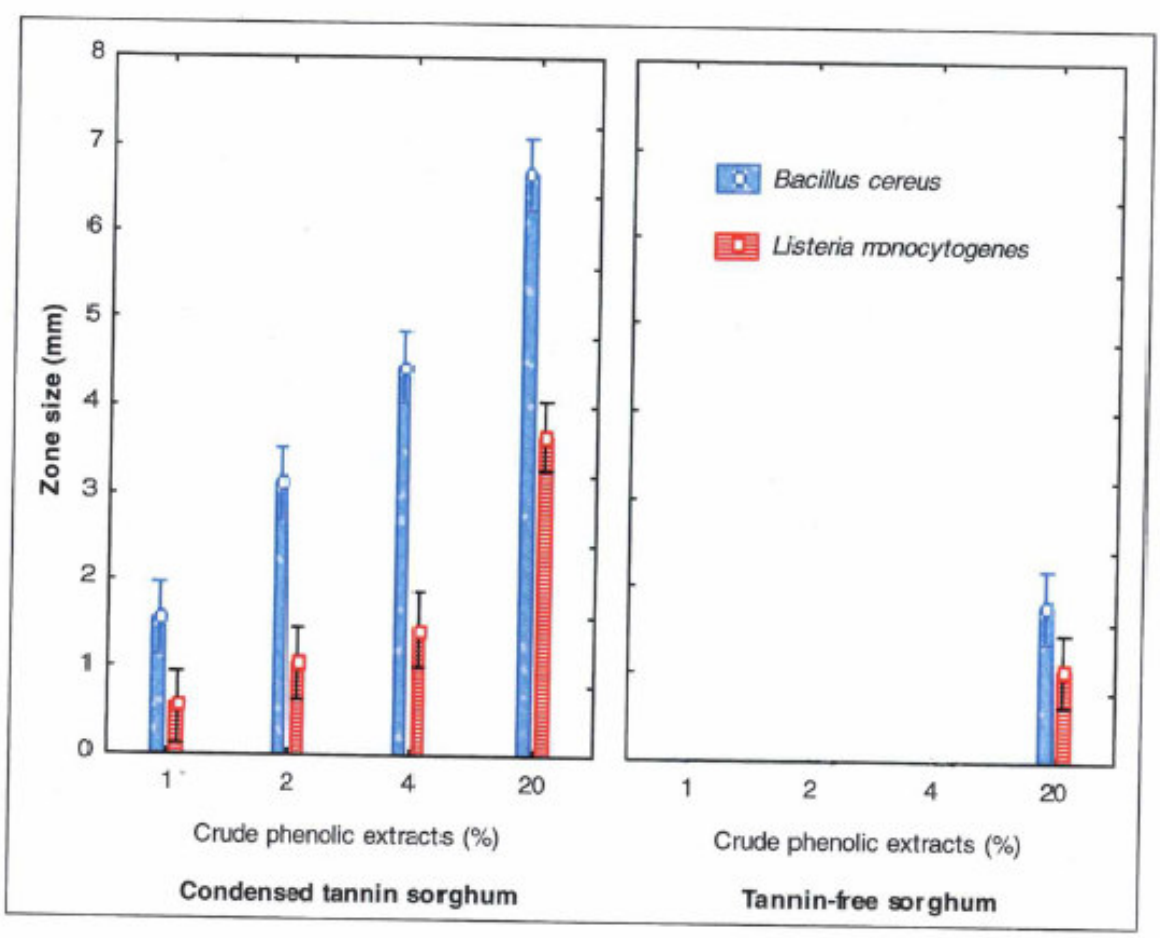

Figure 14b. Effect of increase in concentration (1, 2, 4 and 20 %) of sorghum crude phenolic extract from condensed tannin and condensed tannin-free sorghum on growth of *B. cereus* and *L. monocytogenes.*

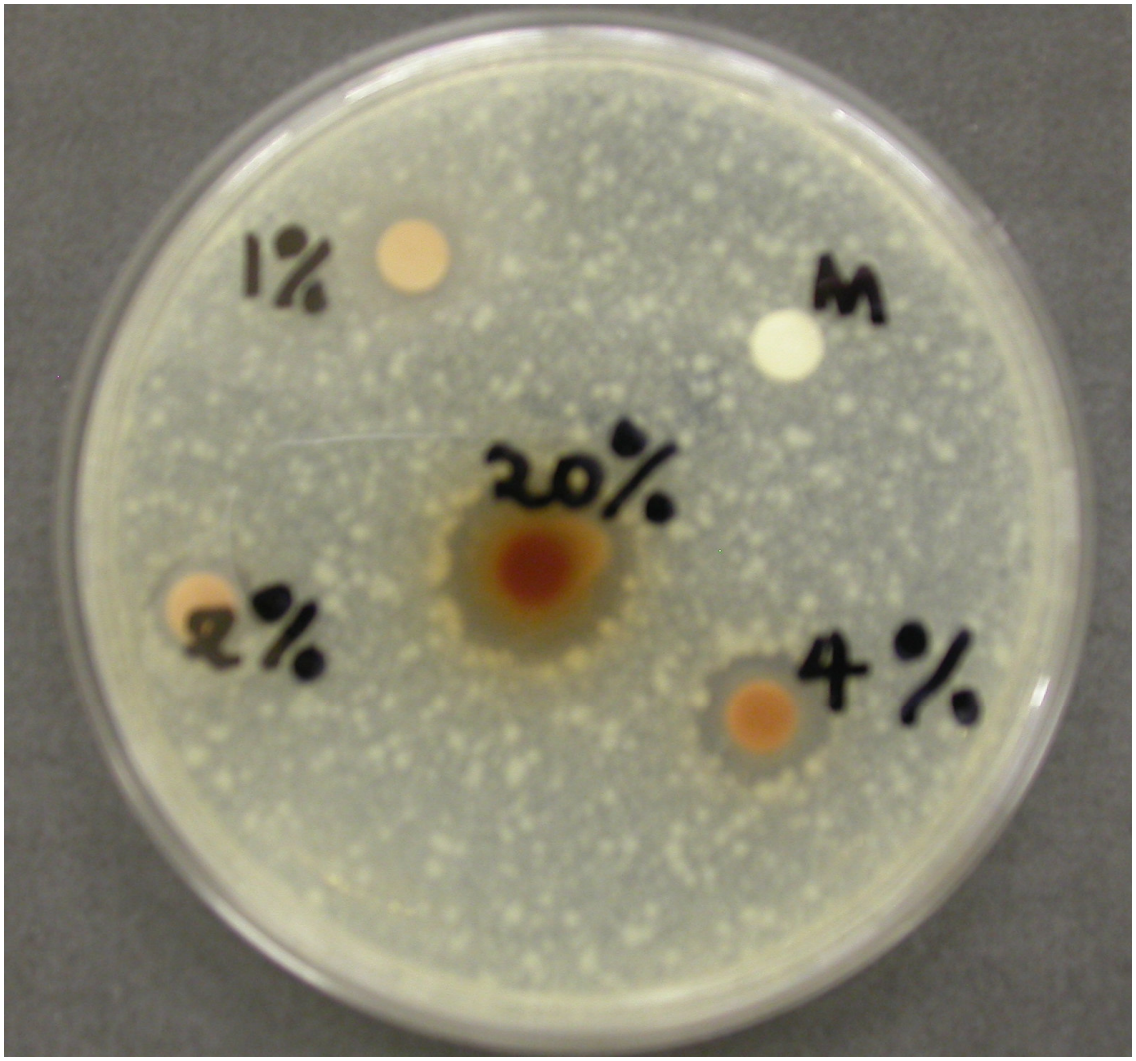

Figure 15. Inhibitory effect of condensed tannin sorghum CPE on *B. cereus* ATCC 1178.

In contrast to this study, Jayaprakasha *et al.* (2003) have shown that grape seed extracts prepared by extraction with acetone: water: acetic acid (90:9.5:0.5) and with methanol: water: acetic acid (90:9.5:0.5) exhibited an antibacterial effect against both Gram-negative and Gram-positive bacteria. Both extracts were found to be more effective against Gram-positive bacteria when compared to Gram-negative bacteria (Jayaprakasha *et al*., 2003). Gram-positive bacteria were completely inhibited at 850-1000 ppm, while Gram-negative bacteria were inhibited at 1250-1500 ppm concentration of grape seed extracts (Jayaprakasha *et al*., 2003). Microorganisms tested in a study of Jayaprakasha *et*

al., (2003) included *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Baydar *et al*. (2004) also reported the inhibition of Gram-positive bacteria, *B. cereus* and *L. monocytogenes* by grape seed extracts at a concentration of 20 % and Gram-negative bacteria, *E. coli* by grape seed extract at a concentration of 20 %.

However in the present study, both sorghum CPE were found to have no inhibitory effects on the Gram-negative bacteria tested, *E. coli* ATCC 25922. Basile, Sorbo, Giordano, Lavitola and Cobianchi, (1998) have also shown increased sensitivity of Gram-positive bacteria as compared to Gram-negative bacteria towards *Pleurochaete squarrosa* (Byrophyta) extract. Again, in contrast to the results obtained in the present study, Puupponen-Pimiä *et al*., (2001) reported the antimicrobial properties of phenolic compounds from berries on Gram-negative bacteria including *E. coli* strains but not Gram-positive bacteria. Cloudberry extracts, raspberry and strawberry extracts were found to exhibit strong inhibitory effects against *Salmonella* when the agar diffusion method was used to screen for antimicrobial activity of the extracts (Puupponen-Pimiä *et al*., 2001). These variations were associated with differences in cell structures between Gram-positive and Gram-negative bacteria and the authors concluded that different bacteria show different sensitivities towards phenolic compounds (Puupponen-Pimiä *et al*., 2001). This may possibly explain the apparent contradictions among the different studies that have been carried out on the antimicrobial activities of different plant extracts on different bacterial species.

The variations in sensitivity between Gram-negative and Gram-positive bacteria to inhibition by plant essential oils and other different extracts have also been supported by other researchers including Shelef; Farbood, McNeil and Ostovar as cited by Smith-Palmer, Stewart and Fyfe, (1998) and Duffy and Power, (2001). It is not known precisely why Gram-negative bacteria should be less vulnerable, but it may possibly be related to their outer membrane (OM) and periplasmic space both of which are not present in Gram-positive bacteria (Duffy and Power, 2001). The OM provides the bacterial surface with strong hydrophilicity and acts as a strong permeability barrier to foreign molecules

(Nikaido and Vaara as cited by Smith-Palmer *et al*., 1998). In addition, the periplasmic space contains enzymes which are capable of breaking down foreign molecules introduced from outside (Duffy and Power, 2001).

Sawer, Berry and Ford as cited by Alzoreky and Nakahara, (2003) associated the resistance of Gram-negative bacteria towards antibacterial substances to lipopolysaccharides in their OM providing the bacterium with a hydrophilic surface (Naikado as cited by Helander, Alakomi, Latva-Kala, Mattila-Sandholm, Pol, Smid, Gorris and Von Wright, 1998). Due to the presence and features of these unique lipopolisaccharide macromolecules in the outer leaflet of the OM, many Gram-negative bacteria are inherently resistant to hydrophobic antibiotics (Nohynek *et al*., (s.a)). Nevertheless, Al-Adham, Dinning, Eastwood, Austin and Collier as cited by Park, Moon, Song, Kim, Chung and Yoon, (2001) also indicated that the microbial cell membrane acts as a permeability barrier between the cytoplasm and the cell's external environment, and regulates flux of solutes into and out of the cytoplasm.

According to Basile *et al*. (1998) conventional antibiotics are often more active against Gram-positive bacteria than Gram-negative bacteria. Small hydrophilic solutes were shown to be able to pass the OM through abundant porin proteins providing hydrophilic transmembrane channels, whereas the OM serves as a penetration barrier towards macromolecules and to hydrophobic compounds, and it is for this reason that Gram-negative bacteria are relatively resistant to hydrophobic antibiotics (Naikado and Vaara; and Naikado as cited by Helander *et al*., 1998). This may possibly explain why Gram-positive bacteria, *B. cereus* ATCC 1178 and *L. monocytogenes* ATCC 7644 were susceptible to inhibition by the sorghum CPE as compared to the Gram-negative bacteria, *E. coli* ATCC 25922 that did not show any susceptibility to inhibition by both the condensed tannin and condensed tannin-free sorghum CPE tested.

On the other hand, Russell and Chopra as cited by Park *et al*. (2001) described phenols as predominantly membrane-active agents that damage cell membrane and cause release of intracellular constituents causing intracellular coagulation of cytoplasmic constituents

which in turn result into cell death or inhibition of cell growth. It was also hypothesised in this work that CPE from sorghum bran fractions of condensed tannin and condensed tannin-free sorghum may exhibit antimicrobial properties because they bind with proteins. Phenolic compounds in the sorghum CPE would presumably damage the cell membrane and bind and precipitate intracellular proteins, which would lead to death or inhibition of cell growth.

Microorganisms have been shown to produce siderophores (low molecular weight chelating agents that have the affinity for iron (III), bind and solubilise iron and supply it to the cell) (Cloete, 1999). In a number of studies, iron deprivation has been suggested to be the mechanism through which the tannins (high molecular weight polyphenolic compounds) exhibit their antimicrobial activity on different microorganisms (Scalbert, 1991; Mila *et al*., 1996; Chung *et al*., 1998b). The tannins have also been reported to act like the siderophores to chelate iron from the medium and therefore make the iron unavailable (Chung *et al*., 1998b) and hence prevent the microorganisms from growing. In a study done by Chung *et al*. (1998b) tannic acid (a hydrolysable tannin) was found to be inhibitory to the growth of intestinal bacteria, *Bacteroides fragilis* ATCC 25285, *Clostridium crostridiiforme* ATCC 25537, *Clostridium perfringens* ATCC 13124, *Clostridium paraputrificum* ATCC 25870, *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 13047 and *Salmonella* Typhimurium TA98 by acting like siderosphore to chelate iron from the medium and make iron unavailable for utilisation by the microorganisms. The inhibitory effect of tannic acid by iron deprivation on intestinal bacteria, *E. coli* ATCC 25922 was evident as the growth of the culture was restored by the supplementation of additional iron (Chung *et al*., 1998b). Hence in this case tannic acid inhibited the growth of *E. coli* ATCC 25922 through iron deprivation. On the contrary, in this study no inhibition by the condensed tannin or condensed tannin-free sorghum CPE against *E. coli* ATCC 25922 was observed. Compounds such as methyl gallate and propyl gallate, which are forms of gallic acid, were also found to be inhibitory to the growth of intestinal bacteria mentioned above, even though the author suggested that their effect on bacteria probably occurs by a different mechanism other than iron-chelating (Mila *et al*., 1996).

Mila *et al*. (1996) confirmed that iron-chelating properties of polyphenols limit the growth of microorganisms unless the microorganisms developed particular efficient biochemical systems to displace iron (III) from the polyphenol/iron complex that is formed during the binding of iron by polyphenols. No work was done in this study to investigate the mechanisms by which phenolic compounds from sorghum CPE's exhibit their antimicrobial activity on bacteria. The above mentioned mechanism may possibly explain the inhibitory effect of CPE from bran fractions of condensed tannin and condensed tannin-free sorghum on the growth of *B. cereus* ATCC 1178 and *L. monocytogenes* ATCC 7644 Gram-positive pathogenic bacteria. However, this mechanism may not necessarily be the only mechanism through which tannins and related compounds exhibit their inhibitory effect to the growth of various microorganisms.

3.6 Conclusions

Crude phenolic extracts from bran fractions of condensed tannin and condensed tannin-free sorghum varieties exhibit antimicrobial activity against the growth of the pathogenic Gram-positive bacteria, *B. cereus* ATCC 1178 and *L. monocytogenes* ATCC 7644 but are ineffective against the Gram-negative bacteria *E. coli* ATCC 25922. The condensed tannin sorghum CPE (which contains higher levels of total phenols) shows a greater degree of inhibition than the condensed tannin-free sorghum CPE. It appears that the extent of bacterial inhibition by the sorghum CPE may be related to the levels of phenols present in the extracts. Gram-positive bacteria tested are more sensitive to condensed tannin CPE while Gram-negative bacteria tested is resistant to the CPE. Both condensed tannin and condensed tannin-free sorghum CPE exhibit greater inhibition at higher concentrations. Although higher concentrations of CPE may be required for the condensed tannin-free sorghum CPE to achieve satisfactory inhibition against bacteria, lower concentrations of the condensed tannin sorghum CPE may be sufficient to achieve significant inhibition of bacteria.