

Antimicrobial properties of phenolic compounds from sorghum

By

Tshiwela Norah Khadambi

**Submitted in partial fulfillment of the requirements for the degree
MSc (Agric) Food Science and Technology**

in the

**Department of Food Science
Faculty of Natural & Agricultural Sciences
University of Pretoria
Pretoria**

June 2005

Declaration

I declare that this dissertation that I hereby submit for the degree of MSc (Agric) Food Science and Technology at the University of Pretoria is my own work and has not been previously submitted by me for a degree at any other University or institution of higher education.

Tshiwela Norah Khadambi

Dedication

I dedicate this dissertation to my late father Mr Johannes Khadambi, my mother Mrs Gladys Khadambi, my sister Joyce Khadambi, my brothers Michael and Khathutshelo Khadambi, my sister-in-law Nancy Khadambi, as well as to the rest of Khadambi family.

Acknowledgement

I would like to acknowledge the following people whose efforts, inspiration and support made it possible for me to successfully complete my research project and my Masters studies as a whole.

Head of department, Prof A. Minnaar for accepting me into the department, I guess that was the starting point to a dream come true. My project supervisor, Prof E.M. Buys, my co-supervisor, Dr K.G. Duodu for your confidence in my abilities, your support and guidance throughout the program.

Other staff members and post-graduate students for your assistance, in many different ways I cannot explain.

My sponsors, Foodbev SETA for helping me further my studies by their generous offer for financial assistance.

My academic mentor, Mr S.E. Lidovho, I thank you for your support and guidance throughout my studies, your commitment was phenomenal.

My parents, Mr Johannes Khadambi and Mrs Gladys Khadambi, I will always cherish all the good things that you made me realise. You made me realise that “Knowledge is power” even though you didn’t say that in words but through your loving support. My brothers Michael and Khathutshelo, my sister Joyce, my sister-in-law Nancy, as well as my friend Sylvia Mudau, for having gone out of your way to offer me financial support and always being there for me when I needed you the most.

My heart felt gratitude goes to my Lord who through His mercy has blessed me with a circle of people mentioned above, who each in their own special ways contributed to the completion of my program and for having given me strength and taken good care of me throughout my studies, for that I will always be thankful.

Abstract

Antimicrobial properties of phenolic compounds from sorghum

By

Tshiwela Norah Khadambi

Supervisor: Prof E.M. Buys
Co-supervisor: Dr K.G. Duodu
Department: Food Science
Degree: MSc (Agric) Food Science & Technology

Sorghum grains contain phenolic compounds that have been shown to have many favourable effects. In this study the levels of phenolic compounds in condensed tannin and condensed tannin-free sorghums have been determined and the antimicrobial activity of phenolic extracts from bran fractions of the respective sorghums has been further evaluated against pathogenic bacteria *Bacillus cereus* ATCC 1178, *Escherichia coli* ATCC 25922 and *Listeria monocytogenes* ATCC 7644.

Defatted bran fractions prepared from a condensed tannin sorghum variety (red) and a condensed tannin-free sorghum variety (white) were analysed for their content of total phenols and condensed tannins. Total phenols were determined using the Folin-Ciocalteu method and condensed tannins with the vanillin-HCL method. Total phenols and condensed tannins of the bran fractions were extracted with aqueous acetone (75 % v/v) and acidified methanol (1% HCL v/v in methanol) respectively, using a bran-to-solvent ratio of 1:4 (w/v). Red sorghum bran contained a higher amount of total phenols and condensed tannins (33.18 mg tannic acid equivalent/g and 117.98 mg catechin equivalent/g of the bran fractions, respectively) than white sorghum bran (6.81 mg tannic acid equivalent/g and 8.52 mg catechin equivalent/g of the bran fractions, respectively).

Freeze-dried sorghum crude phenolic extracts (CPE) obtained from defatted bran fractions of condensed tannin and condensed tannin-free sorghum varieties were evaluated for their antimicrobial activities against *Bacillus cereus* ATCC 1178, *Escherichia coli* ATCC 25922 and *Listeria 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 CPE 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 CPE 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.

Table of contents	Page
List of Figures	i
List of Tables	iii
Chapter 1: Introduction, statement of the problem and literature review	1
1. 1 Introduction and statement of the problem	2
1.2 Literature review	4
1.2.1 Sorghum (<i>Sorghum bicolor</i> (L.) Moench)	4
1.2.2 Sorghum production and utilisation	4
1.2.3 Phenolic compounds in sorghum	5
<i>1.2.3.1 Phenolic acids</i>	5
<i>1. 2.3.2 Flavonoids</i>	7
<i>1.2.3.3 Tannins</i>	10
1.2.4 Sorghum morphology with reference to phenolic compounds	12
<i>1.2.4.1 Pericarp</i>	13
<i>1.2.4.2 Endosperm</i>	14
<i>1.2.4.3 Germ</i>	14
1.2.5 Modes of classification of sorghums based on phenolic content	15
<i>1.2.5.1 Classification based on extractable tannin content</i>	15
<i>1.2.5.2 Classification based on presence or absence of</i> <i>condensed tannins</i>	15
<i>1.2.5.2.1 Condensed tannin sorghum</i>	15
<i>1.2.5.2.2 Condensed tannin-free sorghum</i>	16
1.2.6 Significance of phenolic compounds in sorghum	17
1.2.7 Natural antimicrobials in foods	18
1.2.8 Determination of antimicrobial activity	18
1.2.9 Antimicrobial activities and inhibitory effects of various phenolic compounds from different plant sources	19

1.2.10 Mechanisms of antimicrobial activity of phenolic compounds	21
1.2.10.1 <i>Inhibition of bacterial enzymes and substrate deprivation</i>	21
1.2.10.2 <i>Chelation of essential minerals</i>	21
1.2.11 Foodborne pathogens	24
1.2.11.1 <i>Bacillus cereus and its characteristics</i>	25
1.2.11.1.1 <i>Growth requirements of B. cereus with reference to temperature, pH and water activity</i>	25
1.2.11.1.2 <i>Pathogenicity and foodborne illnesses associated with B. cereus</i>	26
1.2.11.1.2.1 <i>Diarrhoeal syndrome</i>	26
1.2.11.1.2.2 <i>Emetic syndrome</i>	27
1.2.11.1.3 <i>Prevalence in foods and epidemiological issues</i>	27
1.2.11.2 <i>Escherichia coli and its characteristics</i>	28
1.2.11.2.1 <i>Growth requirements of E. coli with reference to temperature, pH and water activity</i>	28
1.2.11.2.2 <i>Pathogenicity and foodborne illnesses associated with E. coli</i>	29
1.2.11.2.3 <i>Prevalence in foods and epidemiological issues</i>	29
1.2.11.3 <i>Listeria monocytogenes and its characteristics</i>	30
1.2.11.3.1 <i>Growth requirements of L. monocytogenes with reference to temperature, pH and water activity</i>	30
1.2.11.3.2 <i>Pathogenicity and foodborne illnesses associated with L. monocytogenes</i>	31
1.2.11.3.3 <i>Prevalence in foods and epidemiological issues</i>	31

1.2.13 Hypothesis and objectives	33
1.2.13.1 Hypothesis	33
1.2.13.2 Objectives	33
Chapter II: Extraction of phenolic compounds and quantification of the total phenol and condensed tannin content of bran fraction of condensed tannin and condensed tannin-free sorghum varieties	34
2.1 Abstract	35
2.2 Introduction	36
2.3 Materials and methods	38
2.3.1 Sorghum grain samples	38
2.3.2 Reagents	38
2.3.3 Testing for the presence of pigmented testa in sorghum grains	38
2.3.4 Preparation of sorghum bran fractions	39
2.3.5 Determination of total phenol content of sorghum bran fractions	40
2.3.6 Determination of condensed tannin content of sorghum bran fractions	40
2.3.7 Statistical analysis	41
2.4 Results and discussion	41
2.4.1 Chlorox bleach test for the sorghum grains	41
2.4.2 Total phenol and condensed tannin content of sorghum bran fractions	43
2.5 Conclusions	45
Chapter III: Effect of sorghum crude phenolic extracts on <i>Bacillus cereus</i> , <i>Escherichia coli</i> and <i>Listeria monocytogenes</i>	46
3.1 Abstract	47
3.2 Introduction	48

3.3 Materials and methods	49
3.3.1 <i>Sorghum</i> grain samples and reagents	49
3.3.2 <i>Bacterial cultures used for evaluation of antimicrobial activity of sorghum crude phenolic extracts (CPE)</i>	49
3.3.3 <i>Preparation of CPE from bran fractions of condensed tannin and condensed tannin-free sorghum varieties for evaluation of antimicrobial activity</i>	50
3.3.4 <i>Preparation of bacterial cultures for antimicrobial activity evaluation</i>	50
3.3.5 <i>Evaluation of sorghum CPE for antimicrobial activity</i>	51
3.4 Statistical analysis	51
3.5 Results and discussion	52
3.5.1 <i>Antimicrobial effect of phenolic compounds from condensed tannin and condensed tannin-free sorghum varieties on B. cereus ATCC 1178, E. coli ATCC 25922 and L. monocytogenes ATCC 7644</i>	52
3.5.2 <i>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</i>	57
3.6 Conclusions	65
Chapter IV: General discussion	66
4.1 Methodology evaluation	67
4.2 Relative levels of total phenols and condensed tannins in sorghum CPE	71
4.3 Antimicrobial activities of sorghum CPE and the effects of phenolic compound concentration and bacterial species on inhibition	73
Chapter V: Conclusions and recommendations	76
Chapter VI: References	79

List of figures	Page
Figure 1. Some phenolic acid monomers identified in sorghum	6
Figure 2. Basic flavonoid ring structure	8
Figure 3. The 3-deoxyanthocyanidins in sorghum	9
Figure 4. Chemical structures of hydrolysable tannins, galloyl and hexahydroxydiphenoyl	11
Figure 5. Structure of proanthocyanidin (tannin) polymer	11
Figure 6. Diagram of sorghum caryopsis showing the pericarp [epicarp, mesocarp, testa] where phenolic compounds are found, endosperm (E) (aleurone layer, corneous, and floury) and the germ	13
Figure 7. Fluorescence photomicrograph of sorghum bran cross-section, showing structural differences between a non-tannin sorghum without a testa (left) and a tannin sorghum with a pigmented testa (right). Al, aleurone layer; CW, cell wall; E, endosperm; En, endocarp; Ep, epicarp; M, mesocarp; T, pigmented testa	16
Figure 8. Relationship between the modes of classification of sorghums based on phenolic content	17
Figure 9. Polyphenol/iron (III) lattice formed upon complexation of iron (III) (dark spot) by polyphenol (star) containing five <i>o</i> -dihydroxyphenyl functional groups (triangles)	23

Figure 10.	Insoluble tannin-ferric ion complexes	24
Figure 11.	Appearance of sorghum grain samples before and after the chlorox bleach test	42
Figure 12.	Effect of sorghum crude phenolic extracts from bran fractions of condensed tannin and condensed tannin-free sorghum varieties on bacterial growth	55
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	56
Figure 14a.	Effect of condensed tannin and condensed tannin-free sorghum crude phenolic extract on inhibition of <i>B. cereus</i> and <i>L. monocytogenes</i>	59
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 <i>B. cereus</i> and <i>L. monocytogenes</i>	60
Figure15.	Inhibitory effect of condensed tannin sorghum CPE on <i>B. cereus</i> ATCC 1178	61
Figure16.	Possible reaction between orthodihydroxyphenyl group of phenolic compound and Fe ³⁺ ions	75

List of tables	Page
Table 1. Contents of major phenolic acids in sorghum	7
Table 2. Anthocyanin content of sorghum brans	10
Table 3. Total phenol content (expressed as mg tannic acid equivalent/g) and condensed tannin content (expressed as mg catechin equivalent/g) of sorghum bran fractions on a dry matter basis	45
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 <i>B. cereus</i> ATCC 1178, <i>E. coli</i> ATCC 25922 and <i>L. monocytogenes</i> ATCC 7644 bacteria	54
Table 5. Inhibition (mm) of <i>B. cereus</i> ATCC 1178, <i>E. coli</i> ATCC 25922 and <i>L. monocytogenes</i> ATCC 7644 by CPE from bran fractions of condensed tannin and condensed tannin-free sorghum varieties (n=9)	58