

**Tannin binding of kafirin and  
its effects on kafirin films**

**by**

**Mohammad Naushad Emmambux**

**Submitted in partial fulfilment of the requirements for the  
degree**

**PhD Food Science**

**in the**

**Department of Food Science**

**Faculty of Natural and Agricultural Sciences**

**University of Pretoria**

**Pretoria**

**Republic of South Africa**

**March 2004**

## DECLARATION

I hereby declare that the thesis submitted at the University of Pretoria for the award of PhD degree is my work and has not been submitted by me for a degree at any other University or institution of higher education.



Mohammad Naushad Emmambux

January 2004



**TO MY CREATOR  
TO MY SUSTAINER  
FOR GIVING ME KNOWLEDGE, PATIENCE, GOOD HEALTH,  
CARING PARENTS, WIFE AND FAMILY**

## ABSTRACT

Tannin binding of kafirin and its effects on kafirin films

By

Mohammad Naushad Emmambux

Supervisor: Prof J.R.N. Taylor

Co-Supervisor: Dr M. Stading

Kafirin, the prolamin protein of sorghum grain, could be extracted from the by-products of the sorghum processing industry and used to make films and coatings for food packaging, in particular to extend the shelf-life of fruits and nuts. Protein-based films can be an environment-friendly alternative to synthetic plastic packaging systems. However, the properties of protein-based films are generally inferior to those of synthetic plastics. Modification can alter the properties of protein-based films. In this project, the interaction between phenolic compounds and kafirin was investigated in relation to their potential to modify kafirin films.

A range of phenolic compounds was tested in terms of their ability to bind and complex with kafirin in an *in vitro* binding assay. The protein-phenolic compound interaction was quantified by haze formation and colorimetric determination of total polyphenol bound. Ferulic acid, catechin and extracted flavonoids from condensed tannin-free sorghum did not complex with kafirin. Tannic acid (TA) and sorghum condensed tannins (SCT) complexed kafirin and formed haze. Thus, TA and SCT were selected as potential modifying agents for kafirin films.

TA and SCT were added at up to 20% (w/w tannin to protein basis) during kafirin film casting. Both TA and SCT bound to kafirin in the film. Scanning electron microscopy showed that TA modified films were less porous; and the SCT modified films appeared more globular in structure than unmodified film. Modification with both tannins increased the tensile stress and Young's modulus and decreased the tensile strain of the kafirin films. Oxygen permeability of the modified films was decreased, but no change in the apparent water vapour permeability. The  $T_g$  of the films increased with increased modification level.

SDS-PAGE, FT-IR and Raman spectroscopy were used to study TA and SCT interaction with kafirin. SDS-PAGE revealed a high  $M_r$  band for kafirin-SCT complexes which did not enter the separating gel. FT-IR of kafirin complexed tannins and tannin modified films showed a decrease in the absorbance at the frequency of about  $1620\text{ cm}^{-1}$ , suggesting a decrease in  $\beta$ -sheet structures. FT-IR results also suggested that the  $\beta$ -sheets of kafirin in dry form were probably changed into random coils during kafirin dissolution to make films. Raman spectra showed a shift in the TA peak at about  $1710\text{ cm}^{-1}$  to about  $1728\text{ cm}^{-1}$  in the kafirin-TA complexes, suggesting participation of the carbonyl groups of TA in TA-kafirin interaction.

It is proposed that hydroxyl groups of tannin can form hydrogen bonds with carbonyl groups of random coils of kafirin during film casting. Thus, the carbonyl groups are probably not available to be reorganized into  $\beta$ -sheets. The other possible mode of interaction can be hydrophobic interaction between the aromatic rings of tannins and the pyrrolidine rings of proline. Because tannins have numerous aromatic rings with hydroxyl groups, it is also proposed that they can bind with more than one polypeptide chain at the same time to cross-link kafirin. This cross-linking probably produces a high  $M_r$  kafirin-tannin complex that leads to haze. The cross-linking would also lead to lower molecular mobility of modified kafirin films. This could decrease oxygen permeability, probably as a result of decreased free volume. Cross-linking could also be responsible for the increased tensile stress and decreased tensile strain of modified kafirin films.

The higher tensile stress of modified kafirin films suggests that they can have the potential to form stronger coatings around fruit such as litchi fruit to possibly reduce pericarp microcracking as an example, and thus may reduce the pericarp browning of litchi. The lower oxygen permeability of the modified films and the potential antioxidant activity of the tannins suggest that these films can be a good coating to prevent rancidity of nuts.

## **ACKNOWLEDGEMENTS**

My sincere gratitude goes to my supervisor, Prof JRN Taylor for his expertise guidance, challenging ideas, constructive criticism, keen interest and cheerful encouragement throughout the course of this work. I am very thankful to Dr M Stading as my co-supervisor, from the Swedish Institute for Food and Biotechnology, Sweden for all his help and great contribution towards this project. My special thanks also go to Prof A Minnaar, head of the Department of Food Science, University of Pretoria for her contribution in the initial phase of this project as a co-supervisor.

I gratefully acknowledged the financial supporters of this project. This work was conducted within European Commission INCO-DEV contract ICA4-CT-2001-10062. The National Research Foundation, South Africa and the Mellon Foundation Mentoring Program from the University of Pretoria also financially sponsored me during the project.

I am thankful to the SIK institute, Sweden to give me the opportunity to do some of the rheology works on the modified kafirin films. I thank Prof P Belton from the University of East Anglia, UK; and Dr C Gao and Dr N Wellner at the Institute of Food Research (IFR), Norwich, UK for helping me in the spectroscopy work during this project. Mr A Hall and Mr C Van der Merwe from the laboratory for microscopy and microanalysis, University of Pretoria, are acknowledged for their contribution in microscopy analysis of the films.

My special thanks go to the members of the academic and non-academic staff and the postgraduate students of the department of Food Science, University of Pretoria for their support in fulfilling the research project.

A great thanks to the colleagues of this EU projects, Mrs Janet Taylor, Mrs L Da Silva, Miss H M Van Eck, and Mr Y Byaruhanga for their team spirit.

Finally, my special thanks go to my parents, wife, brothers and sisters for their moral support.

## TABLE OF CONTENTS

<b>ABSTRACT</b>	<b>III</b>
<b>ACKNOWLEDGEMENTS</b>	<b>V</b>
<b>LIST OF TABLES</b>	<b>IX</b>
<b>LIST OF FIGURES</b>	<b>X</b>
<b>LIST OF ABBREVIATIONS</b>	<b>XIII</b>
<b>1. INTRODUCTION</b>	<b>1</b>
<b>1.1 Statement of the problem</b>	<b>1</b>
<b>1.2 Literature review</b>	<b>3</b>
1.2.1 Sorghum kafirin	3
1.2.1.1 Chemistry of kafirin	3
1.2.2 Protein-based films	6
1.2.2.1 Formation of protein-based films	7
1.2.2.2 Mechanical and barrier properties of protein-based films	9
1.2.2.3 Modification of protein-based films	12
1.2.2.3.1 Plasticization	12
1.2.2.3.2 Compositing films	13
1.2.2.3.3 Enzymatic modification	14
1.2.2.3.4 Physical treatments	14
1.2.2.3.5 Chemical modification	16
1.2.3 Phenolic compounds	19
1.2.3.1 Chemistry of phenolic compounds	19
1.2.3.2.1 Mechanisms of phenolic-protein interaction	24
1.2.3.2.2 Chemical interactions between protein and phenolic compounds	29
1.2.3.2.3 Factors affecting protein-phenolic interaction	30
1.2.4 Phenolic compounds and sorghum proteins	33
1.2.5 Conclusions	34
<b>1.3 Objectives and hypotheses</b>	<b>35</b>
1.3.1 Objectives	35
1.3.2 Hypotheses	35



1.3.2 Hypotheses	35
<b>2. RESEARCH</b>	<b>37</b>
<b>2.1 Sorghum kafirin interaction with various phenolic compounds</b>	<b>38</b>
2.1.1 Abstract	38
2.1.2 Introduction	39
2.1.3 Experimental	40
2.1.3.1 Materials	40
2.1.3.2 Analyses	41
2.1.3.2.1 Binding Assay	41
2.1.3.2.2 Determination of Haze	42
2.1.3.2.3 Determination of total polyphenols bound to protein	42
2.1.3.2.4 Statistical analyses	43
2.1.4 Results and discussion	43
2.1.5 Conclusions	57
2.1.6 References	58
<b>2.2 Sorghum kafirin film property modification with hydrolysable and condensed tannins</b>	<b>63</b>
2.2.1 Abstract	63
2.2.2 Introduction	64
2.2.3 Experimental	65
2.2.3.1 Materials	65
2.2.3.2 Film preparation	65
2.2.3.3 Analyses	66
2.2.3.3.1 Tannin bound by kafirin	66
2.2.3.3.2 Scanning electron microscopy (SEM)	66
2.2.3.3.3 Tensile properties of films	66
2.2.3.3.4 Water uptake by films	67
2.2.3.3.5 Barrier properties of films	67
2.2.3.3.6 Dynamic mechanical analysis (DMA)	68
2.2.3.3.7 Statistical analysis of data	68
2.2.4 Results and discussion	69
2.2.5 Conclusions	85
2.2.6 References	85



<b>2.3 Effects of tannins on the secondary structure of kafirin and kafirin films</b>	<b>92</b>
2.3.1 Abstract	92
2.3.2 Introduction	93
2.3.3 Materials and methods	94
2.3.3.1 Materials	94
2.3.3.2 Preparation of kafirin complexed with tannins and kafirin films	94
2.3.3.3 Analyses	95
2.3.3.3.1 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS PAGE)	95
2.3.3.3.2 FT-IR spectroscopy	95
2.3.3.3.3 Raman spectroscopy	96
2.3.4 Results and discussion	96
2.3.5 Conclusions	108
2.3.6 References	109
<b>3. GENERAL DISCUSSION</b>	<b>112</b>
3.1 Methodological considerations	112
3.2 Kafirin interaction with phenolic compounds	119
3.3 Modification of kafirin films with tannins	132
<b>4. CONCLUSIONS AND RECOMMENDATIONS</b>	<b>139</b>
<b>5. REFERENCES</b>	<b>141</b>
<b>SCIENTIFIC CONTRIBUTIONS</b>	<b>165</b>



## LIST OF TABLES

Table 1.1 Partial amino acid composition of total, $\alpha$ -, $\beta$ -, and $\gamma$ - kafirin _____	4
Table 1.2 Mechanical properties of protein-based and synthetic films _____	10
Table 1.3 Water vapour permeability (WVP) and oxygen permeability (OP) of protein-based films and synthetic films _____	11
Table 2.1 Haze of kafirin protein as affected by sorghum condensed tannins at 4 °C and 30 °C _____	54
Table 2.2 Mole ratio of proline from kafirin and bovine serum albumin bound by tannic acid or sorghum condensed tannin as determined from the total polyphenol binding assay _____	57
Table 2. 3 Effect of tannic acid (TA) and sorghum condensed tannin (SCT) modification on the water absorbed (%) by kafirin films _____	80
Table 3.1 Mechanical and barrier properties of kafirin films modified with tannins and other protein-based films modified by various methods, and synthetic film. _____	133

## LIST OF FIGURES

Figure 1.1 A structural model of zein	5
Figure 1.2 Schematic representation of film formation from protein by the wet and dry process	8
Figure 1.3 Possible reaction of an aldehyde with an amino group in the presence or absence of a reducing agent. The side R can be an aliphatic chain (attaching hydrophobic groups) or an aliphatic chain end capped by another functional group (cross-linking)	17
Figure 1.4 Basic structure of phenolic acids	20
Figure 1.5 Flavonoid type phenolics (a) The basic structure of flavonoids and (b) some examples	21
Figure 1.6 The chemical structure of gallotannins	22
Figure 1.7 Chemical structure of condensed tannins	23
Figure 1.8 Schematic representation of polyphenol complexation and co-precipitation mechanism	25
Figure 1.9 Cross-linking mechanism of the interaction between polyphenol and gelatin at different ratios	26
Figure 1.10 Three stages in the cross-linking mechanism between salivary proline rich protein and phenolic compounds	27
Figure 1.11 Proposed mechanisms of phenolic and protein interaction	28
Figure 2.1 Schematic representation of the research parts	37
Figure 2. 2 Haze formation of kafirin (■) and bovine serum albumin (▲) as affected by phenolic compounds	
a) Ferulic acid	45
b) Catechin	46
c) Sorghum flavonoids	47
d) Tannic acid	48
e) Sorghum condensed tannin	49
Figure 2.3 Quantity (—) and percentage (-----) phenolic compounds bound to kafirin (■) and bovine serum albumin (▲)	
a) Catechin	50
b) Sorghum flavonoids	51

c) Tannic acid _____	52
d) Sorghum condensed tannin _____	53
Figure 2.4 Quantity (—) and percentage (----) of tannin bound to kafirin film	
a) Tannic acid _____	70
b) Sorghum condensed tannin _____	71
Figure 2.5 Scanning electron micrographs of freeze fracture surfaces of unmodified and modified kafirin film _____	72
Figure 2.6 Effect of tannic acid (■) and sorghum condensed tannin (●) on tensile properties of kafirin films	
a) Stress at maximum force _____	74
b) Stress at break _____	75
c) Strain at break _____	76
d) Young's modulus _____	77
Figure 2.7 Effect of tannic acid (■) and sorghum condensed tannin (●) on the oxygen permeability (—) and apparent water vapour permeability (----) of kafirin films _____	81
Figure 2.8 Effect of tannic acid (■) and sorghum condensed tannin (●) on the glass transition temperature, $T_g$ (—) and moisture content (----) of kafirin films _____	82
Figure 2.9 Storage modulus $E'$ of unmodified (control) and modified kafirin films with tannic acid (TA) and sorghum condensed tannins (SCT) at 10% and 20% (w/w of protein) during DMA under changing RH _____	84
Figure 2.10 SDS-PAGE under non-reducing condition of kafirin and complexed kafirin _____	97
Figure 2.11 FT-IR spectra of kafirin in dry form (a), tannic acid (b), sorghum condensed tannins (c) and kafirin solution (d) _____	98
Figure 2.12 FT-IR spectra of kafirin-tannin complexes	
a) Tannic acid _____	100
b) Sorghum condensed tannin _____	101
Figure 2.13 FT-IR spectra of kafirin films modified with tannins	
a) Tannic acid _____	103
b) Sorghum condensed tannin _____	104

Figure 2.14 Raman spectra of tannic acid and kafirin-tannic acid complexes	106
Figure 2.15 Raman spectra of sorghum condensed tannin and kafirin- sorghum condensed tannin complexes	107
Figure 3.1 Potential molecular interactions between the tannins and kafirin polypeptide chains to show the possible binding sites	122
Figure 3.2 Cross-linking of two polypeptide chains of kafirin by tannic acid through hydrogen bonds	124
Figure 3.3 Hydrophobic interactions between galloyl rings of tannic acid and proline residues of different polypeptide chains of kafirin	125
Figure 3.4 Schematic model for kafirin monomers and oligomers from the Argos et al. (1982) model showing the proposed form of kafirin in dry state and kafirin in solution	128
Figure 3.5 Cross-linking of the kafirin molecules by tannins	130

## LIST OF ABBREVIATIONS

ASBC, American Society of Brewing Chemists

ASTM, American Society for Testing and Materials

$\sigma_b$ , stress at break

$\sigma_y$ , stress at maximum force

BSA, Bovine Serum Albumin

DM, dry mass

DMA, dynamic mechanical analysis

$E'$ , storage modulus

$E$ , Young's modulus

FT-IR, Fourier transform infrared

FTU, Formazin Turbidity Units

OP, Oxygen permeability

PEG, polyethylene glycol

PRP, proline rich protein

PVPP, polyvinyl polypyrrolidone

RH, relative humidity

SCT, sorghum condensed tannins

SDS-PAGE, Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

SEM, Scanning electron microscopy

TA, tannic acid

$T_g$ , glass transition temperature

WVP, water vapour permeability

$\epsilon_b$ , strain at break