UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

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 permeabilty. 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 cm^{-1}, suggesting a decrease in β-sheet structures. FT-IR results also suggested that the β-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 cm^{-1} to about 1728 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 β-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

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ASBC, American Society of Brewing Chemists

ASTM, American Society for Testing and Materials

σ_b, stress at break

σ_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

ϵ_b, strain at break