INVESTIGATIONS INTO THE ANTISTALING EFFECT OF WAXY DURUM IN BREAD

LAURA BLAKE

A thesis submitted to the University of Adelaide in fulfilment of the requirements for the degree of Doctor of Philosophy

School of Agriculture, Food & Wine
The University of Adelaide
Adelaide, Australia

May 2015

TABLE OF CONTENTS

LIST OF FIGURES

List of Tables

ABSTRACT

Bread staling leads to substantial food wastage and hence economic and environmental burdens in the industrial world. Reduced amylose waxy wheat flour has shown potential as an antistaling additive in bread, although the causal mechanism at the molecular level is not well defined. The waxy durum under investigation in this study represents a novel substrate due to its breeding history, and has shown a strong antistaling effect in bread. Bread made from waxy durum flour was about half as firm initially and 25% less firm than conventional bakers' flour after a week's storage. The aim of this study was to comprehensively analyse the physicochemical and functional properties of this waxy durum flour and starch, to provide insights into its antistaling mechanism.

The swelling and gelatinisation properties of waxy durum were compared to those of a range of normal and waxy cereal starches. It was found that the waxy durum starch exhibited delayed swelling behaviour relative to other waxy cereal starches, most likely due to its higher relative amylose content. Surfactant treatment was found to eliminate diversity of swelling behaviour in normal, but not waxy starches. This suggested that factors other than starch surface proteins and lipids influence the swelling behaviour of waxy starches. As starch retrogradation was identified in the literature as a key factor in bread staling, flour and starch thermal properties were assessed at different water contents. The relationship between starch retrogradation and water content was also found to be different between the waxy and normal wheat starches studied, with retrogradation of waxy starches favoured by a more concentrated system.

Waxy durum starch and flour in isolation were found to exhibit a higher resistance to retrogradation than normal durum, and normal and waxy bread wheat starches. Both waxy flours were found to have a similar crumb softening effect after the first day of bread storage, however only the waxy durum flour produced a significant antistaling effect over longer term storage. It was postulated that the early stage crumb softening effect was related to the impact on rheological behaviour of the dough during the baking process, but that in the longer term the waxy durum reduced amylopectin retrogradation, suggesting that its unique thermal behaviour was the primary factor influencing its antistaling effect with a secondary contribution by minor flour components. The results of this study add to the breadth of current knowledge of the physicochemical and thermal properties of waxy durum starch and flour in particular and waxy starches and flours in general, with particular emphasis on mechanisms of antistaling functionality at the molecular level.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.
The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Laura Blake
May 2015

ACKNOWLEDGEMENTS

I would like to thank my supervisors for their support and guidance throughout the project. Dr Andrew Barber for introducing me to the world of food engineering and always focussing on the applied aspects of the research. Dr Colin Jenner for the interesting discussions, not just on waxy starches but also theatre and literature. Professors Bob Gibson and Brian O'Neill for their dry wit and cynicism. Finally, Professor Mike Gidley for coming aboard during the later stages and guiding the project to its completion.

I would like to acknowledge the assistance of the Grains Research and Development Corporation (GRDC) for providing the operating funds for this project. The Food and Beverage development fund of SA also provided financial support to attend training and conferences. Dr Hugh Wallwork and Dr Jim Lewis (SARDI) performed the advanced breeding and selection from the original waxy durum line. Australian Grain Technologies Pty Ltd (AGT) kindly provided the background parental germplasm and made the cross and early generation selection of the waxy bread wheat breeding line (CO6165). Laucke Flour mills kindly donated the commercial bakers' flour and test-milled the grain.

A big thanks must go to Dr John Carragher, not technically my supervisor but definitely my go-to person for when things went awry. Also all the staff and students at FOOD*plus* for the support and company along the PhD journey, particular Kanch who was able to share the waxy durum journey. The staff at Waite Analytical Services, Teresa, Lyndon and David for their help with the elemental and fatty acid analyses. The staff at the Waite campus, although in different areas of research, were always willing to assist. Dr Daniel Cozzolino introduced me to the world of ATR-MIR spectroscopy and multivariate data analysis. Professor Dzuy Nguyen for assisting in the dough rheology work. Professor Daryl Mares' lab for allowing me to use their facilities. Jelle Lahnstein at the Plant Cell Walls group for helping with the HPAEC-PAD. Dr Olena Kravchuk for the statistics consulting. The staff at Flinders for providing me access to their thermal analysis equipment.

Finally thanks to my family and friends for putting up with me during the past few years. I wouldn't have made it through without your support and love. Especially my mum and Stevie Bryan, who took the brunt of my PhD frustrations.

ABBREVIATIONS & SYMBOLS

Abbreviation	Description
A_0	Avrami equation; initial value of parameter
AACC	American Association of Cereal Chemists
A_f	Avrami equation; final or limiting value of parameter
AGT	Australian Grain Technologies
ANOVA	Analysis of variance
AP	Amylopectin
ATR	Attenuated total reflectance
CLD	Chain length distribution
DP	Degree of polymerisation (number of glycosyl units)
DSC	Differential scanning calorimetry
FAME	Fatty acid methyl ester
G	Lower temperature amylopectin gelatinisation endotherm
GBBSI	Granule Bound Starch Synthase I
GC	Gas chromatography
GOPOD	Glucose oxidase peroxidase
G	Lower temperature amylopectin gelatinisation endotherm
G_R	Lower temperature amylopectin retrogradation endotherm
HPAEC	High performance anion-exchange chromatography
ICPOES	Inductively coupled plasma optical emission spectrometry
k	Avrami equation; rate constant
LSD	Least significant difference
M1	Higher temperature amylopectin gelatinisation endotherm
$M1_R$	Higher temperature amylopectin retrogradation endotherm
M2	Amylose-lipid complex melting endotherm
mb	Moisture basis (%)
MIR & NIR	Mid infrared and near infrared (spectroscopy)
MVA	Multivariate data analysis
n	Avrami equation; Avrami exponent
PAD	Pulsed amperometric detection
P content	Phosphorus content
PMP	1-phenyl-3-methyl-5-pyrazolone
P-value	Statistical significance
RACI	Royal Australian Chemical Institute
rpm & rcf	Speed of centrifugation; in revolutions per minute (rpm) and relative centrifugal force (rcf)
RVA	Rapid visco analyser or analysis
S content	Sulphur content
SDS	Sodium dodecyl sulphate
SRC	Solvent Retention Capacity
$t_{0.5}$	Avrami equation; half-time (time to reach half the final value)
TLC	Thin layer chromatography
Tonset	Onset temperature (DSC)
Tpeak	Peak temperature (DSC)
WAS	Waite Analytical Services
wx	Waxy
v/v, v/w, w/v & w/w	Solute concentration in solution, specified with respect to weight (w) or volume (v).

PUBLICATIONS ARISING FROM THIS THESIS

The list of publications associated with this work is provided below. These include journal articles and conference proceedings. The published journal articles are provided at the end of the thesis. Whilst I participated in the writing of the Lafaye et al. (2013) publication, the experimental work was completed by the French exchange students Julie Lafaye and Heiterani Lee, and as such is not included as a chapter in this thesis.

- Blake, L.H., Jenner, C.F., Gidley, M.J & Cozzolino, D. 2015. Effect of surfactant treatment on swelling behaviour of normal and waxy cereal starches. *Carbohydrate Polymers* **125**, 265-271.
- Blake, L., Jenner, C.F., Barber, A.R., Gibson, R.A., O'Neill, B.K. & Nguyen, Q.D. 2015. Effect of waxy flour blends on dough rheology and bread quality. *International Journal of Food Science & Technology* **50**, 926-933.
- Lafaye, J. Lee, H. Blake, L. & Jenner, C. 2013. Waxy durum acts as a unique form of crumb softener in bread. *Journal of Cereal Science* **58**, 393-399
- Blake, L., Jenner, C. F., Barber, A. & O'Neill, B. Waxy durum: novel starch with antistaling effects. Cereals 2013. Proceedings of the 63nd Australian Cereal Chemistry Conference in association with International Association for Cereal Science and Technology, 25th-28th August 2013, Freemantle, WA, Australia.
- Blake, L., Jenner, C. F., Barber, A. & O'Neill, B. Waxy durum slows staling in bread: mechanism of action. Cereals 2012. Proceedings of the 62nd Australian Cereal Chemistry Conference, 26th-29th August 2012, Gold Coast, QLD, Australia.
- Blake, L., Jenner, C. F., Barber, A. & O'Neill, B. Waxy durum flour as an antistaling agent in baked goods. AIFST 2012. Proceedings of the 45th Annual AIFST Convention, 15th- 17th July 2012, Adelaide, SA, Australia.
- Blake, L., Jenner, C. F., Barber, A. & O'Neill, B. Antistaling effects of waxy durum addition in sponge cakes. Cereals 2011. Proceedings of the 61st Australian Cereal Chemistry Conference, 4th-9th September 2011, Coolangatta- Tweed Heads, N.S.W. Australia.

STRUCTURE OF THIS THESIS

This thesis is presented as a combination of papers that have been published, as well as chapters that have not yet been submitted for publication. The published papers have been formatted to be consistent with the other results chapters and the acknowledgements sections removed. However, these chapters were prepared as standalone for publication. Therefore, this style of presentation results in some areas of repetition, particularly in the introductions and methods sections. The final journal publications as they appear in print are provided at the end of the thesis for reference. A single reference list (formatted in the style for *International Journal of Food Science & Technology*) is provided at the end of the thesis.

Chapter 1 provides an overview of the literature and the objectives of this research. Each chapter also includes a brief introduction of the literature and concepts relative to that piece of work. Chapter 2 details the material and methods used throughout the thesis. The published articles also contain materials and methods sections. However, Chapter 2 contains the most detailed description of experimental methodology, as published work was abridged due to space and word limitations. The other results chapters, not yet published, also include a short section entitled experimental methodology, which details which methods were used in that chapter and a brief discussion of the experimental design.

Chapter 3 comprises a paper published in *Carbohydrate Polymers*, entitled 'Effect of surfactant treatment on swelling behaviour of normal and waxy cereal starches'. It describes the effect of a common surfactant treatment on the swelling behaviour of four normal and waxy cereals; durum, bread wheat, barley and maize. In this chapter, the behaviour of the waxy durum material is compared to these other starches. Unpublished results from this study that were peripheral to the aims of the paper, but were of importance to the broader aims of this thesis are included in Appendix A.

Chapter 4 describes the thermal properties of the waxy durum in a concentrated system. This chapter follows on from Appendix A of Chapter 3, which details the thermal behaviour of waxy durum starch in a dilute system. These results have not yet been submitted for publication. The waxy durum starch was found to contain a relatively high amylose content (8.8%), compared to both literature values for waxy wheat starches and the previous harvest of this cultivar. Chapter 5 investigates potential causes of this high amylose content, and its impact on the physicochemical behaviour of the waxy durum starch.

Chapter 6 comprises a paper published in the *International Journal of Food Science & Technology*. It primarily describes the dough rheological behaviour of waxy flour blends and the correlations to baking performance. Chapter 7 outlines the bread staling experiments, including combined texture analysis and differential scanning calorimetry analyses. These results have not yet been submitted for publication. Chapter 8 provides a synthesis of the findings contained in this thesis and includes recommendations for future work.