Investigation of the factors affecting the staling of Arabic flat bread

This item was submitted to Loughborough University's Institutional Repository by the/an author.

Additional Information:

- A Doctoral Thesis. Submitted in partial fulfillment of the requirements for the award of Doctor of Philosophy of Loughborough University.

Metadata Record: [https://dspace.lboro.ac.uk/2134/8768](https://dspace.lboro.ac.uk/2134/8768)

Publisher: © Latifah Abdullah Ali Alhajji

Please cite the published version.
This item was submitted to Loughborough’s Institutional Repository (https://dspace.lboro.ac.uk/) by the author and is made available under the following Creative Commons Licence conditions.

For the full text of this licence, please go to:
http://creativecommons.org/licenses/by-nc-nd/2.5/
INVESTIGATION OF THE FACTORS AFFECTING THE STALING OF ARABIC FLAT BREAD

by

LATIFAH ABDULLAH ALI ALHAJJII

A Doctoral Thesis submitted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy in Chemical Engineering

© by Latifah Abdullah Ali Alhajji (2011)
Abstract

ABSTRACT

The focus of this thesis is the area of staling of Arabic flat bread (or pita bread). The reductions in shelf life due to bread staling cause significant economic losses in the Middle East where preservatives are not permitted. The aim was to investigate whether processing solutions could solve this problem.

A commercial bread produced in Kuwait was initially studied. The industrial baking process was monitored closely, and the products analyzed for moisture content in Kuwait after storage at 4 and 20 °C. Representative samples were ground and freeze-dried in Kuwait, and transported to the UK for analysis by Differential Scanning Calorimetry (DSC). The findings from this study showed moisture content variations between different bread formulations after baking, however the DSC analysis was inconclusive due to possible changes in the freeze dried bread.

The industrial process was thus replicated as closely as possible at Loughborough University so that the bread could be analysed immediately after baking and after up to 3 days storage. It was also found that high pressure stainless steel pans produced better DSC baseline stability compared to aluminium pans due to suspected leakage of the latter (used in literature studies), and were thus used for the rest of the study. Attention was also paid to the possibility that different parts of the bread receive different radiant heating intensities during baking as evidenced by the different degrees of brown coloration. This showed that whilst almost complete gelatinisation initially occurs, the highest levels of subsequent retrogradation occurred in an area intermediate between the centre and outside of the pita bread. This coincided with the region with the highest moisture content immediately after processing (and likely to receive the least amount of heat). A parallel study using DSC which subjected dough samples to a temperature profile similar to that found in baking also found that lower heating rates (with albeit higher heat exposure) produced greater amounts of retrogradation. In each case moisture contents during storage were comparable between samples, thus indicating that heating rates during processing is a key parameter governing subsequent retrogradation, and also that future studies should identify the sampling position within the bread when performing analyses.

Thermocouple studies showed that the temperatures in the steam pocket that develops during puffing were close to that of the boiling point of water. Consequently, a further study was also performed in which Arabic flat bread samples were baked at different pressures (up to 2 bar) in a novel high pressure oven. The motivation was that changing pressure to increase baking temperatures could be a way of using processing rather than formulation methods to extend shelf life. Whilst applying pressure was found to retard subsequent retrogradation, it did result in breads becoming firmer during storage which was not the desired effect. However, this may be a consequence of the slightly lower moisture contents found in the bread baked at higher pressure.
ACKNOWLEDGEMENT

All praises are due to my Lord “Allah” the creator of everything; who gave me the power, knowledge and patience to overcome all difficulties.

“Who does not thank people, does not thank God” Prophet Mohammed (SAW).

I would like to express my deepest appreciation to my supervisors; Dr. Andrew Stapley and Prof. Vahid Nassehi. This dissertation could not have been written without them. I’m honoured to be supervised by them; they encouraged, support and challenged me through my academic program. They never accepted less than my best efforts. Thank you.

I would like also to sincerely thank Prof. M. I. Zaki (Egypt); who kept encouraging me all the way from my M.Sc. studies until now. I’m sure he will continue his support and encouragement for ever.

I would like to extend my acknowledge to Dr. Val Street (Food Sciences, Sutton Bonington, Nottingham University) for her kindness and help in texture analysis.

I would also like to sincerely thank Mr Mark Barron, Mrs Kim Robertshaw, Mr Tony Eyre, Mr Graham Moody, Mr Dave Smith, Mr Sean Creedon, and Mr Terry Neale for their technical support and assistance. Special thanks for Mrs Anna Temple for her permanent kindness.

A very special recognition needs to be given to all my friends in S158 (Bahareh, Hakem, Fahd, Matthew, Redah & Saleemi) and for Boom in room S 169 for their consideration, motivations and funny pizza times. Without them I could not have gotten the power to finish this work.

This recognition may also be extended to my friends in CAL, KISR for their “long distance” support.
Acknowledgements

Special thanks to people in I had the honour to know them in Loughborough; I had been overwhelmed with their kindness and love: Mrs Alsha’lan, Mrs Alkhabbaz, Mrs Altowajri, Mrs Alkandari, Mrs Alsebie, Mrs Al-enizi, Mrs Aloufi and Mrs Aljaff.

A very special thanks and love is dedicated to my grandmother “Omy Maryam”, my mother “mama Amina”, my lovely father “Abdullah”, two brothers and sisters for their unconditional love and support. Special thanks to “Khally Mohammed” and Mr and Mrs Alshatti for their unlimited support.

Last but not the least, special thanks to my beloved husband and children for supporting and helping me at all times.
TO

OSAMA

ABDULLAH, EBRADHEEM, AMENAH & FATMAH

"DREAMS COME TRUE

IF YOU BELIEVE IN"
# TABLE OF CONTENTS

**ABSTRACT** .................................................................................................................... I

**ACKNOWLEDGEMENT** .................................................................................................. II

**DIDICATION** ................................................................................................................ IV

**TABLE OF CONTENTS** ................................................................................................... V

**LIST OF FIGURES** ....................................................................................................... XI

**LIST OF TABLES** .......................................................................................................... VII

1 **INTRODUCTION** .......................................................................................................... 1

1.1 **BACKGROUND TO THIS WORK** ........................................................................... 1

1.2 **OBJECTIVES OF PRESENT STUDY** ...................................................................... 4

1.3 **ORGANISATION OF THE DISSERTATION** .......................................................... 4

2 **LITERATURE REVIEW** .............................................................................................. 7

2.1 **INTRODUCTION** .................................................................................................... 7

2.2 **BREADMAKING PROCESS** ..................................................................................... 8

2.2.1 **Bread main ingredients** ..................................................................................... 8

2.2.1.1 **Flour** .............................................................................................................. 8

2.2.1.2 **Water** ............................................................................................................ 12

2.2.1.3 **Salt** .............................................................................................................. 12

2.2.1.4 **Yeast** ............................................................................................................ 13

2.2.1.5 **Other additives** ............................................................................................ 13

2.2.1.6 **Conclusions** .................................................................................................. 13
# Table of Contents

2.3 BREADMAKING METHOD ................................................................. 14
  2.3.1 Mixing ....................................................................................... 15
  2.3.2 Bulk fermentation ...................................................................... 16
  2.3.3 Dividing and resting ................................................................. 17
  2.3.4 Moulding, sheeting, docking ..................................................... 17
  2.3.5 Final proof .................................................................................. 18
  2.3.6 Baking ....................................................................................... 18
  2.3.7 Cooling and packaging ............................................................... 20
  2.3.8 Conclusions ................................................................................ 20

2.4 TRANSFORMATION OF STARCH STRUCTURE DURING AND AFTER BAKING ........................................................................................................... 21
  2.4.1 Methods used to study starch gelatinisation and retrogradation .... 22
    2.4.1.1 Nuclear magnetic resonance (NMR) ........................................ 23
    2.4.1.2 Infra red (IR) ......................................................................... 23
    2.4.1.3 X-ray diffractometry (XRD) ................................................... 24
    2.4.1.4 Optical microscopy and scanning electron microscopy ......... 25
    2.4.1.5 Thermal analysis - differential scanning calorimetry (DSC) .... 26
    2.4.1.6 Texture analysis ...................................................................... 30
  2.4.2 Starch gelatinisation .................................................................... 31
  2.4.3 Starch retrogradation ................................................................... 36
    2.4.3.1 Factors affecting starch retrogradation ................................. 38
      2.4.3.1.1 Water content ................................................................. 39
      2.4.3.1.2 Temperature-time dependency ....................................... 40
      2.4.3.1.3 Amylose : amylopectin ratio ......................................... 41
      2.4.3.1.4 Processing techniques .................................................... 42
    2.4.4 Conclusions ................................................................................ 43

2.5 STALING ........................................................................................... 43
  2.5.1 Introduction .................................................................................. 43
  2.5.2 Evidence linking staling with starch retrogradation .................... 44
  2.5.3 Evidence linking staling with other bread constituents ............... 46
### Table of Contents

2.5.4 Effect of ingredients and process parameters on bread quality and starch state ................................................................. 48  
2.5.4.1 Starch damage ......................................................................................................................................................... 48  
2.5.4.2 Effect of wheat bran .............................................................................................................................................. 49  
2.5.4.3 Additives .................................................................................................................................................................. 50  
2.5.4.4 Modified breadmaking processes .............................................................................................................................. 52  
2.5.4.5 Nonconventional methods in bread baking .............................................................................................................. 53  
2.5.5 Conclusions ................................................................................................................................................................. 55  

3 EXPERIMENTAL METHODOLOGY ................................................................................................................................. 57  
3.1 INTRODUCTION .......................................................................................................................................................... 57  
3.2 MATERIALS .................................................................................................................................................................. 58  
3.2.1 KFMB bread ............................................................................................................................................................ 58  
3.2.2 Laboratory-baked bread ................................................................................................................................................ 58  
3.3 ARABIC FLAT BREAD INDUSTRIAL BAKING DESCRIPTION .................................................................................. 60  
3.4 ARABIC FLAT BREAD LABORATORY BAKING DESCRIPTION .................................................................................. 60  
3.4.1 Dough preparation ...................................................................................................................................................... 60  
3.4.2 Laboratory-oven-baking ............................................................................................................................................ 63  
3.4.3 High pressure oven (HPO) baking ........................................................................................................................................ 65  
3.4.3.1 HPO design ............................................................................................................................................................. 65  
3.4.3.2 HPO baking procedure ................................................................................................................................................ 71  
3.5 METHOD FOR MEASURING THE REAL TEMPERATURE INSIDE THE DOUGH SHEET DURING BAKING ................................................. 73  
3.6 STORAGE METHODS OF ARABIC FLAT BREAD ........................................................................................................ 73  
3.6.1 KFMB bread ............................................................................................................................................................. 73  
3.6.2 Laboratory baked bread ................................................................................................................................................ 75  
3.7 MOISTURE ANALYSIS ..................................................................................................................................................... 75  
3.7.1 KFMB bread ............................................................................................................................................................. 75
Table of Contents

3.7.2 Laboratory baked bread ................................................................. 75
3.7.3 HPO moisture loss ........................................................................ 77

3.8 DIFFERENTIAL SCANNING CALORIMETRY (DSC) .......................... 78
  3.8.1 Measurements of starch retrogradation in freeze-dried KFMB bread 79
  3.8.2 Pan types ..................................................................................... 80
  3.8.3 Measurements of starch retrogradation in laboratory-baked bread ... 81
  3.8.4 Thermal analysis of unbaked dough samples ................................. 82

3.9 TEXTURE EVALUATION OF ARABIC FLAT BREAD ................. 82

3.10 STATISTICAL ANALYSIS .................................................................... 83

4 PRELIMINARY STUDIES ON KFMB BREAD ........................................ 84
  4.1 INTRODUCTION .................................................................................. 84
  4.2 DETERMINATION OF STORAGE PERIOD ...................................... 84
  4.3 MOISTURE CONTENT ....................................................................... 85
  4.4 DIFFERENTIAL SCANNING CALORIMETRY .................................... 90
  4.5 CONCLUSIONS ................................................................................ 100

5 SPATIAL VARIATION OF STARCH RETROGRADATION IN ARABIC
FLAT BREAD DURING STORAGE .............................................................. 101
  5.1 INTRODUCTION ................................................................................. 101
  5.2 BREADMAKING DEFECTS ................................................................. 103
    5.2.1 Blisters ...................................................................................... 103
    5.2.2 Uneven crust colour ................................................................. 103
  5.3 MEASUREMENTS OF THE REAL TEMPERATURE INSIDE THE DOUGH
  SHEET DURING BAKING ................................................................. 105
  5.4 MOISTURE CONTENT ..................................................................... 109
# Table of Contents

5.5 DIFFERENTIAL SCANNING CALORIMETRY ......................................................... 111

5.5.1 DSC of unbaked dough samples and comparison of pan types ..... 111

5.5.2 Baked bread ......................................................................................... 113

5.5.3 "Simulated baking" in DSC ........................................................................ 119

5.5.3.1 Temperature/time profile................................................................... 119

5.5.3.2 Moisture loss results ......................................................................... 120

5.5.3.3 Immediate DSC rescans .................................................................... 121

5.5.3.4 DSC rescans after storing .................................................................. 123

5.6 CONCLUSIONS ......................................................................................... 125

6 THE EFFECT OF APPLIED PRESSURE DURING BAKING ON STARCH
RETROGRADATION AND BREAD FIRMNESS .............................................. 126

6.1 INTRODUCTION ............................................................................................ 126

6.2 MEASUREMENTS OF THE REAL TEMPERATURE INSIDE THE DOUGH
SHEET DURING BAKING UNDER PRESSURE .............................................. 128

6.3 MOISTURE LOSS ......................................................................................... 130

6.4 DIFFERENTIAL SCANNING CALORIMETRY .................................................. 134

6.4.1 Puffed bread ......................................................................................... 134

6.4.2 Unpuffed bread ..................................................................................... 139

6.5 TEXTURE ANALYSIS .................................................................................. 144

6.5.1 Puffed bread ......................................................................................... 144

6.5.2 Unpuffed bread ..................................................................................... 146

6.6 CONCLUSIONS ............................................................................................. 148

7 CONCLUSIONS AND FUTURE WORK ........................................................... 150

7.1 GENERAL CONCLUSIONS ...................................................................... 150

7.2 MAIN CONTRIBUTIONS OF THE STUDY ............................................... 153
# Table of Contents

7.3 SUGGESTIONS FOR FUTURE WORK ................................................. 154

REFERENCES .................................................................................. 156

APPENDIX A ................................................................................. 178

APPENDIX B ................................................................................. 196
LIST OF FIGURES

Figure 2.1 Breadmaking processes for all types of bread ................................. 14

Figure 2.2 Schematic diagram of DSC sample holder and heaters ..................... 28

Figure 2.3 Gelatinisation dependency on starch-water-temperature-time interaction.............................................................................................................................. 33

Figure 2.4 A model for amylopectin retrogradation showing the formation of double helices ......................................................................................................................... 37

Figure 2.5 Major factors affecting starch retrogradation ................................... 38

Figure 3.1 (a) KENWOOD mixer, (b) fermented dough in lined plastic bowl .... 61

Figure 3.2 Fermentation cabinet used to proof the dough at 30 ± 1 °C and 70 % RH ........................................................................................................................................ 62

Figure 3.3(a) Dough balls and resting box used for proofing in Arabic flat bread baking, (b) A hand-rolled dough ball to 2 mm sheet thickness with the circular mould. ................................................................................................................................. 63

Figure 3.4 Baking of Arabic flat bread in laboratory oven ................................. 64

Figure 3.5 Schematic diagram of high pressure oven ........................................ 66

Figure 3.6 Picture of high pressure oven (HPO) ............................................... 67

Figure 3.7 A closer picture to the baking chamber (a) front side, (b) back side 68

Figure 3.8 PID controllers which control the upper and bottom heaters of the HPO ........................................................................................................................................ 69

Figure 3.9 (a) Air compressor and the air tank, (b) Foot operated pneumatic positioned .......................................................................................................................... 70
List of Figures

Figure 3.10 (a) Domnick filter and 2-port valves (b) Three-way valve (on left) and pressure relief valve (on right). ................................................................. 70

Figure 3.11 Freeze-dried KFMB bread samples stored in air tight container. ... 74

Figure 3.12 Representative diagram of bread division; where C represents the centre, B is the intermediate and A characterises the outer zone. ................. 76

Figure 3.13 DSC instrument used in this study. .................................................. 79

Figure 3.14 Calculation of ΔH as the area enclosed by straight line and the endotherm curve. ........................................................................................................... 80

Figure 3.15 Types of DSC pans used: Aluminium pans (Al pan) and high pressure stainless steel pans (HHP). ................................................................. 81

Figure 4.1 Moisture content in bread stored in the refrigerator (FSW = soft white bread, FSB = soft brown bread, FNW = normal white bread, FNB = normal brown bread and F = refrigerator). ................................................................. 86

Figure 4.2 Moisture content in bread stored at room temperature (SW = soft white bread, SB = soft brown bread, NW = normal white bread, NB = normal brown bread and O = shelf storing at room temperature)................................. 87

Figure 4.3 Moisture content for soft white bread stored in refrigerator (FSW) and room temperature (OSW)................................................................. 88

Figure 4.4 Moisture content for soft brown bread stored in refrigerator (FSB) and room temperature (OSB)................................................................. 89

Figure 4.5 Moisture content for normal white bread stored in refrigerator (FNW) and room temperature (ONW)................................................................. 89

Figure 4.6 Moisture content for normal brown bread stored in refrigerator (FNB) and room temperature (ONB)................................................................. 90
List of Figures

Figure 4.7 DSC endotherms for freeze-dried fresh bread; where NW=normal white bread, SB=soft brown bread, SW=soft white bread and NB=normal brown bread. ...................................................................................................................... 92

Figure 4.8 DSC endotherms for freeze-dried soft white bread stored at 4 °C (FSW) from 0 to 4 days. ........................................................................................................................................ 95

Figure 4.9 DSC endotherms for freeze-dried soft white bread stored at 20 °C (OSW) from 0 to 4 days. ........................................................................................................................................ 95

Figure 4.10 DSC endotherms for freeze-dried soft brown bread stored at 4 °C (FSB) from 0 to 4 days. ........................................................................................................................................ 96

Figure 4.11 DSC endotherms for freeze-dried soft brown bread stored at 20 °C (OSB) from 0 to 3 days. ........................................................................................................................................ 96

Figure 4.12 DSC endotherms for freeze-dried normal white bread stored at 4 °C (FNW) from 0 to 4 days. ........................................................................................................................................ 97

Figure 4.13 DSC endotherms for freeze-dried normal white bread stored at 20 °C (ONW) from 0 to 4 days. ........................................................................................................................................ 97

Figure 4.14 DSC endotherms for freeze-dried normal brown bread stored at 4 °C (FNB) from 0 to 4 days. ........................................................................................................................................ 98

Figure 4.15 DSC endotherms for freeze-dried normal brown bread stored at 20 °C (ONB) from 0 to 4 days. ........................................................................................................................................ 98

Figure 4.16 DSC endotherms for soft white bread stored at 4 °C for 3 days showing different DSC responses. ......................................................................................................................... 99

Figure 5.1 Laboratory baked bread showing undesirable blister. ................. 104

Figure 5.2 Laboratory baked bread showing an uneven baking.................... 104

Figure 5.3 Thermocouple temperature/time profile for baking of Arabic flat bread of 2 mm thickness for 25 ± 1 second (oven temperature 600 ± 10°C). ........ 106
List of Figures

Figure 5.4 Arabic bread baking process .......................................................... 108

Figure 5.5 Moisture contents at different positions in the upper layer of baked bread after storing in sealed packaging for 0, 1, 24 and 96 hours (A = outer zone, B = intermediate zone and C = centre zone) ...................................................... 110

Figure 5.6 Comparison of DSC gelatinisation endotherms of dough using a high pressure pan (HPP) and aluminium pan (Al), both at a scanning rate of 3 °C min⁻¹. ......................................................................................................................... 112

Figure 5.7 DSC thermogram (HPP) of fresh bread crumb showing the melting of amylose-lipid complexes. .................................................................................................................. 114

Figure 5.8 DSC thermogram (HPP) of bread crumb stored at room temperature in outer zone (A) ...................................................................................................................... 116

Figure 5.9 DSC thermogram (HPP) of bread crumb stored at room temperature in intermediate zone (B) .................................................. 116

Figure 5.10 DSC thermogram (HPP) of bread crumb stored at room temperature in centre zone (C). ................................................................................................. 117

Figure 5.11 DSC peak enthalpy values (in temperature range 40-80 °C) for bread crumb stored in HPP at room temperature (20 °C) for 1, 2 and 3 days, where A=outer zone, B=intermediate zone and C=centre zone. .......... 118

Figure 5.12 DSC peak enthalpy values (in temperature range 40-80 °C) for bread crumb stored in HPP in refrigerator (4 °C) for 1, 2 and 3 days, where A=outer zone, B=intermediate zone and C=centre zone ................................................. 118

Figure 5.13 Temperature/time profiles of DSC at different heating rates (20 and 200 °C/min) held for 30 seconds and cooled down at 50 °C/min .................. 120

Figure 5.14 Moisture loss % of dough samples heated at different heating rate (20 and 200 °C/min) in DSC open pans. ........................................................................ 121
List of Figures

Figure 5.15 DSC thermograms (HPP) from immediate rescans at 3 °C min⁻¹ of dough samples previously heated from 30 to 105 °C at heating rates of 20 and 200 °C min⁻¹. .......................................................... 122

Figure 5.16 DSC thermograms from rescans at 3 °C min⁻¹ of dough samples previously heated from 30 to 105 °C (HPP) at heating rate of 20 °C min⁻¹, and then stored at 4 °C for 1, 3, 5 and 7 days.................................................. 123

Figure 5.17 DSC thermograms from rescans at 3 °C min⁻¹ of dough samples previously heated from 30 to 105 °C (HPP) at heating rate of 200 °C min⁻¹, and then stored at 4 °C for 1, 3, 5 and 7 days.................................................. 124

Figure 5.18 DSC peak enthalpy values (in temperature range 50-80 °C) for dough samples previously heated from 30 to 105 °C (HPP) at different heating rates (20 and 200 °C min⁻¹) and then stored at 4°C for 1, 3, 5 and 7 days..... 124

Figure 6.1 Temperature/time profile for baking of puffed Arabic flat bread of 2 mm thickness baked in HPO. .......................................................... 129

Figure 6.2 Temperature/time profile (duplicate) for baking of puffed Arabic flat bread of 2 mm thickness baked in HPO................................................. 129

Figure 6.3 Moisture loss in the puffed bread loaves baked at different pressures directly after baking. .......................................................... 131

Figure 6.4 Moisture loss in the unpuffed bread loaves baked at different pressures directly after baking. .......................................................... 131

Figure 6.5 Moisture loss in the unpuffed bread loaves baked at P = 0 bar for different baking times directly after baking. .................................................. 134

Figure 6.6 Development of crust colour in bread baked in HPO at different pressures. .......................................................... 135

Figure 6.7 DSC endotherms for puffed bread crumb (light part) baked under P=0 and stored at 4 °C.......................................................... 136
List of Figures

Figure 6.8 DSC endotherms for puffed bread crumb (light part) baked under P=1 and stored at 4 °C. ................................................................. 136

Figure 6.9 DSC endotherms for puffed bread crumb (light part) baked under P=2 and stored at 4 °C. ................................................................. 137

Figure 6.10 DSC peak enthalpy values (in temperature range 40 – 80 °C) for puffed bread crumb (light part) stored at 4 °C. ............................................. 137

Figure 6.11 DSC thermograms of unpuffed bread crumb baked at P=0 and stored at 4 °C. ......................................................................................... 140

Figure 6.12 DSC thermograms of unpuffed bread crumb baked at P=1 and stored at 4 °C. ......................................................................................... 140

Figure 6.13 DSC thermograms of unpuffed bread crumb baked at P=2 and stored at 4 °C. ......................................................................................... 141

Figure 6.14 DSC peak enthalpy values (in temperature range 40-80 °C) for unpuffed bread crumb stored at 4 °C. .......................................................... 141

Figure 6.15 Firmness of different bread parts baked at 0 bar during 0-4 days (puffed bread).................................................................................. 145

Figure 6.16 Firmness of different bread parts baked at 1 bar during 0-4 days (puffed bread).................................................................................. 145

Figure 6.17 Firmness of different bread parts baked at 2 bar during 0-4 days (puffed bread).................................................................................. 146

Figure 6.18 Firmness of unpuffed Arabic flat bread baked at 0, 1 and 2 bar during 0-4 days .................................................................................. 148
LIST OF TABLES

Table 3.1 KFMB bread ingredients ................................................................. 59

Table 3.2 General specifications of wheat white flour supplied by Kuwait Flour Mills and Bakeries (KFMB) ................................................................. 59

Table 3.3 Baking parameters: pressure in bar and time in seconds, used in HPO ................................................................................................. 72

Table 3.4 Symbols used to designate different types of bread .................... 74

Table 4.1 Freeze-dried samples average moisture content (MC) % ± standard deviation (STD) ........................................................................ 93

Table 4.2 DSC peak temperature (T_p) and the enthalpy values (ΔH_r, J/g) for KFMB bread stored in refrigerator (4 °C) and bread kept on shelf at room temperature (20 °C) (average ± standard deviation) ....................... 94

Table 6.1 Average moisture content of bread baked at different pressures in HPO (average ± standard deviation) ................................................. 132

Table 6.2 DSC onset temperature (T_o), peak temperature (T_p), offset temperature (T_c) and the enthalpy values (ΔH_r, J/g) for HPO puffed bread (Light zone) stored at 4 °C (average ± standard deviation) ........................................ 138

Table 6.3 DSC onset temperature (T_o), peak temperature (T_p), offset temperature (T_c) and the enthalpy values (ΔH_r, J/g) for unpuffed HPO bread stored at 4 °C (average ± standard deviation) ........................................ 143
1 INTRODUCTION

1.1 BACKGROUND TO THIS WORK

Bread is considered to be one of the oldest processed foods and most consumed by humanity. It is an essential part of the daily food menu, and is prepared by cooking dough made of flour, water and other ingredients. A cooking process may vary from baking to steaming and frying.

Breadmaking is a major industry in most countries around the world. Over the years, there are numerous ways in which the breadmaking process can be organized on the industrial scale. The choice of a particular process in a country is based on many factors such as tradition, cost, available source of energy, flour botanical source and bread type (Collado-Fernandez, 2003).

Bread is accepted as a general term that refers to a variety of products. The differences arise from the different ingredients used to prepare it, besides the environmental and cultural differences. These different bread types have different characteristics and quality attributes. Nevertheless, there is a primary objective for all these differences, which is good quality bread for the consumer with an acceptably long shelf life.

Accordingly, bread can be classified into categories:

- Pan bread; where the main characteristics of this type of bread is that it rises significantly and hence should be baked in pans
- French type bread (baguette) with medium volume, and
- Flat bread which barely rise

The most popular type in the United Kingdom is pan bread, whereas in France it is the baguette. In Germany there is rye bread, while in Mexico, the most popular is tortilla. In Kuwait and Middle East countries, flat bread is the most popular form of bread.
This type of bread can be described as a flat round loaf which varies in diameter and thickness. It can be used to wrap sandwiches or filled with various ingredients to form a sandwich, or simply broken apart and used to scoop sauces and dips. Flat bread can be divided further into two types: single-layered flat bread such as Mexican tortilla or Indian chapati. The other type is double-layered flat bread such as Egyptian flat bread (Balady) and Arabic flat bread; which is known in the Europe and USA as pita bread.

In order to improve bread quality, researchers extensively investigated the chemical, physical and rheological properties of flour, dough, and bread. The influence of different processing techniques on the breadmaking, dough components and additives on the quality and shelf life of bread are being widely considered. However, there is no single universal technique for the investigation of the influence of complex factors affecting bread quality and shelf life.

The roles of protein and starch in bread baking have been subjected to numerous investigations. Protein quality has a great effect on dough performance during processing, and hence, a significant effect on bread volume (Wilkström and Bohlin, 1999; Baardseth et al., 2000; Magnus et al., 2000). While starch represents the largest portion of flour and hence plays a significant role in bread quality and its shelf life (Goesaert et al., 2005). Starch is known to be very sensitive when subjected to different amounts of heat at different water levels. These differences will have a great effect on its thermal properties such as gelatinisation and retrogradation and accordingly will affect bread quality.

Bread staling has been a major concern in breadmaking industries due to the large economical losses reported. Bread that has hardened, dried, changed in taste and fragrance is said to be stale (Ghiasi et al. 1984; Gudmundsson and Eliasson, 1990; Zobel and Kulp, 1996; Sasaki, 2005). Therefore, staling is a term used to define the end of shelf life of bread.
Introduction

Staling has been the subject of a global interest. In Kuwait, according to the head of the Quality Assurance Bakeries Laboratory in Kuwait Flour Mills and Bakeries Company (KFMB), studying the staling and increasing shelf life of the company’s different products is an urgent demand. Arabic flat bread has been reported to have a very short shelf life (Sidhu et al., 1997b); causing economical problems for the producer, distributor, consumer and Kuwait economy in general, especially that the wheat prices have been doubled in the last few years.

Scientists have varied in explaining the aspects leading to end bread shelf life. Some believe that starch has a significant effect in bread firming; being the most abundant component of flour (Ghiasi et al. 1984; Gudmundsson and Elliasson, 1990; Zobel and Kulp, 1996; Sasaki, 2005). Others considered the role of protein or water mobility which had been also highly correlated to the firming process (Maleki et al., 1980; Martin et al., 1991). However, to date, the mechanism of bread staling is thought to be more complex process than it initially seems.

Scientists who believe that starch has a main role in bread staling, had utilized Differential Scanning Calorimetry (DSC) extensively to follow the increase in starch crystallinity associated with change in the thermal and texture properties of bread (Zeleznak and Hoseney, 1986; Sidhu et al., 1997b). Factors such as sample moisture content, type of pan, thermal history of starch are believed to affect the thermal analyses, but they also have been overlooked in the studies conducted on flat bread. For this reason, there is a need to develop techniques that can perform precise evaluations of the thermal analysis of flat bread.

Furthermore, it is well known that significant efforts have already been made by scientists to improve techniques of breadmaking and to extend shelf-life for pan bread and baguettes, but scientific analysis of the manufacturing of flat bread has not been carried out to such an extent. Researchers on bread, in general, and flat bread, in particular, studied the effect of altering dough components;
like starch substitution, changing the gluten percentage, water availability or adding additives to affect the staling rate. Additives such as enzymes, hydrocolloids and emulsifiers had been found to improve bread quality (Krog, 1981; Qarooni, 1988; Stampfli and Nersten, 1995; Guarda et al., 2004), however, it is not an option for Arabic flat bread produced by KFMB due to the health concerns.

Recently, significant efforts are directed toward studying the effect of breadmaking techniques’ modifications. The application of submitting starchy foods to high pressures has become a promising industrial reality, but, required to be investigated more on its potential in bread industry. However, the effect of high pressure on bread processing is still largely unknown.

Therefore, the aim of the present work is to apply a systematic analysis for the thermal analysis to be followed, which will consider the variations in heat, moisture content as well as the variation in sampling techniques. The effect of pressure on starch retrogradation and bread firming will also be studied establishing the first study on bread baking under pressure as far as we know.

1.2 OBJECTIVES OF PRESENT STUDY

The primary objective of this research is to develop and apply a systematic analysis method to study the effect of processing conditions on Arabic flat bread, and consequently to determine what processing parameters, if any, can be altered to extend shelf life of Arabic flat bread.

1.3 ORGANISATION OF THE DISSERTATION

The dissertation encompasses seven chapters, the content of which are presented in the following order:
Chapter 1. Introduces the thesis and explains the background of this work and the main objectives.

Chapter 2. Consists of a literature review of previous work covering these topics:
- Bread types, the main ingredients of bread and their function
- Functions of breadmaking processes
- Starch transformation during and after baking: gelatinisation and retrogradation, and techniques used to study starch retrogradation
- Proposed theories of bread staling mechanisms, covering all bread types studied to date.
- Effect of water content, temperature, flour starch constituents and processing techniques on starch retrogradation
- Evidences linking staling to starch retrogradation or other constituents
- Effect of pressure on starch transformation and bread quality

Chapter 3. Explains the experimental and analytical techniques utilized in this study. These include the analysis methods of Differential Scanning Calorimetry (DSC), Texture Analyzer (TA), and the specification of ovens used in this study. This chapter describes the techniques used in bread preparation, sampling and storing.

Chapter 4. Discusses the influence of storing temperature on starch retrogradation and moisture content of bread baked in Kuwait by KFMB
Chapter 5. Discusses the results of the study of the influence of location within the bread on starch retrogradation and moisture content of bread baked in conventional oven in Loughborough University Laboratories. The role of heating rate is also examined using “baking simulations” in DSC.

Chapter 6. Introduces a novel study on the effect of pressure on bread moisture content, starch retrogradation and texture firmness on bread baked in a specially constructed high pressure oven in Loughborough University laboratories.

Chapter 7. Provides a general conclusion on the whole research study contained within this study and provides recommendations for further work.
2 LITERATURE REVIEW

2.1 INTRODUCTION

Bread is an important part of diet and is prepared by cooking dough of flour, water and other ingredients. The cooking method may range from baking, steaming to frying. Consequently, many different types of bread may arise from these different processes beside the environmental and cultural factors. These different bread types have different characteristics and quality attributes. Nevertheless, there is a primary objective for all these differences, which is good quality bread with long shelf life.

The most important quality parameters required for pan bread are high specific volume, low crust : crumb ratio, spongy tender crumb and golden brown crust. On the other hand baguettes are characterised by crisp thin crust, an open crumb cell structure, open porosity and relatively higher crust : crumb ratio than those characterized for pan bread (Baardseth et al., 2000). Tortillas must be soft, dense, light coloured and flexible if rolled or folded (Waniska, 2004). While pocket formation and pliability are the most required characteristics in Arabic flat bread; other characteristics like golden brown crust, high crust : crumb ratio are of second order (Farvili et al., 1995). Virtually, all of these quality attributes are adversely affected by staling. Bread that has hardened or dried is said to be stale.

This review will first cover bread essential ingredients and processing techniques; which include mixing, bulk fermentation, dividing, moulding, proofing and baking.

Starch; as being the most important component in wheat flour, will be reviewed. Its structure had shown to be affected by heat or pressure, or a combination of both of them. Starch gelatinisation during baking had been reported to be a prominent process during baking; which later on will affect starch structure restoration during storing.
Staling is a term used to define the end of shelf life of bread. Many fresh bread characteristics are lost during the storing period. Scientists diverge in explaining the aspects leading to end bread shelf life. Part of them had considered the role of starch components; amylose and amylopectin (Ghiasi et al., 1984; Gudmundsson and Elliasson, 1990; Zobel and Kulp, 1996; Hug-Iten et al., 1999; Sasaki, 2005). Others believed that protein had a significant effect in this process (Maleki et al., 1980; Martin et al., 1991). Water mobility had been also highly correlated to the firming process (Ruan et al., 1996). Evidences linking these opinions to bread staling had been stated in addition to the factors affecting such process.

### 2.2 BREADMAKING PROCESS

#### 2.2.1 Bread main ingredients

All types of bread are prepared from four essential ingredients: flour, water and comparatively little amounts of yeast and salt. Optional additives can be added to enhance the nutrition value of bread, improve texture, flavour, quality and extend shelf-life.

##### 2.2.1.1 Flour

Flour is the most important ingredient in the breadmaking process and cannot be replaced or substituted by any other substance. The source and type of flour have the most important effect on the quality of bread. Many grains can be used to produce bread, but the most important of all these grains is wheat (Goesaert et al., 2005). This is simply because of the special protein content which is gluten which provides elasticity to the dough. The quantity and quality of gluten
has a great effect on mixing, aeration, water absorption and the final bread quality (Petrofsky and Hoseney, 1995; Pascut et al., 2004).

Many studies had been subjected on different wheat types to find the suitable flour for the production of a particular bread type (Faridi and Rubenthaler, 1983; Qarooni, 1988; Quail et al., 1991; Waniska et al., 2004). Quail et al. (1991) investigated different Australian hard and soft wheat, and their effect on flour characteristics and quality on pan bread and Arabic flat bread. The researchers reported that hard grain wheat produced higher quality bread; attributing that to the higher water absorption found in the flour of hard wheat. In the same study, a strong linear relationship was also reported for protein content and pan bread, while in the case of Arabic flat bread a parabolic relationship was found.

Similarly, Qarooni et al. (1994) on their study on American hard white wheat supported the previous protein effect on the quality of pan bread, tortilla and Arabic flat bread, and reported that flour suitable for pan bread is not necessarily suitable for the production of tortilla and Arabic flat bread.

**Protein**

The quality of bread is significantly governed by its gluten proteins. Both the quantity and quality of gluten fractions influence the breadmaking process and the resulting product (Dobraszczyk, 2004; Goesaert et al., 2005). These fractions are glutenin and gliadin. Gliadin provides viscosity and extensibility allowing the dough to rise during fermentation, while glutenin is responsible for the elasticity and the strength of dough. The contribution of both gliadin and glutenin in dough is unique for wheat flour, and gives the unique visco-elastic properties (Deshpande, 2003).

These proteins play a significant role during various breadmaking stages. In the mixing stage, the quality of these proteins determines the parameters required;
such as mixing time and work input. While in the fermentation stage, the quality of the protein network formed is vital to the gas retention properties, which in turn will affect bread volume and crumb structure during the baking stage.

Furthermore, it is worthwhile in this stage to consider the importance of gliadin-to-glutenin ratio in determining both dough and bread quality parameters. An appropriate balance between viscosity and elasticity is required for reaching an optimum quality for each type of bread (Toufeili et al., 1999b).

For pan bread and French baguettes, where the volume and open crumb structure are important, most researchers have shown a positive linear correlation between loaf volume and protein content (Quail et al., 1991; Qarooni et al., 1994; Wilkström and Bohlin, 1999; Baardseth et al., 2000; Magnus et al., 2000).

In contrast, high volume requirement is not necessary in flat bread, while elasticity is important in giving the final shape of the final product by sheeting the dough to a circular shape (Toufeili et al., 1999b). Higher proteins resulted in a smaller surface area due to the difficulty in sheeting stage; dough tends to shrink during and after sheeting (Quail et al., 1990). On the other hand, low protein contents resulted in thinner sheets of greater surface area. Lower proteins resulted in dry and poor quality Arabic flat bread (Qarooni, 1988).

Protein quality is important in flat bread industry since flat bread is baked at much higher temperatures and for a shorter time than pan bread. Toufeili and his co-workers (1999b) studied the role of protein fractions in baking of Arabic flat bread. High quality glutenin was reported to be inappropriate with flat bread baking conditions of high temperature and short baking time. Arabic flat bread became more rubbery after the addition of 1% glutenin, while the addition of gliadin increased bread resilience and improved its quality.

Generally, researchers on such bread had found a parabolic relationship between protein content and bread quality (Quail et al., 1991) and agreed on
that bread made from flour of moderate protein content (9 - 12.5 %) is optimal for flat bread manufacturing (Qarooni, 1988; Quail et al., 1991; Farvili and co-workers, 1997; Pascut et al., 2004; Waniska et al., 2004; Gocmen et al., 2009; Salehifar et al., 2010). Researchers on flat bread had agreed on that low protein flour (< 9 %) resulted dough which thinly sheeted to give larger surface area and a dry, lighter colour and poor quality bread, while high protein flours (14 %) resulted in dough that shrank after difficult sheeting, and when baked, a blistered poor shape bread of low surface area and dark crust colour was resulted.

**Starch**

The role of starch in breadmaking process has been one of the major areas of investigation, because starch represents the largest portion of flour, and thereby, plays a significant role in bread quality. The proper knowledge of starch transformation is really an essential requirement in food technology.

Starch’s role in the breadmaking process, quality of baked bread and staling has been extensively studied (Goesaert et al., 2005). Researchers found a strong relationship between starch degree of crystallinity and granular size with starch gelatinisation and retrogradation behaviour which accordingly will affect the physical and chemical properties of bread during processing and storing (Sahlström et al., 1998). The role of starch will be reviewed in detail in Section 2.4.
2.2.1.2 Water

Water is a necessary constituent of virtually any food recipe. It assists in dispersion and solubilisation of many food components, which once stabilized, takes role in the interaction with other molecules (Kilara, 2006).

In breadmaking, water is used to produce the dough. The dough consistency is dependent on the quantity of added water. This amount depends on many factors such as flour moisture content, amount of damaged starch and other physicochemical properties of the flour (Gil et al., 1997). Additionally, not only water content is significant, but also the water temperature is of great consequence in controlling dough temperature; in order for the yeast and the enzymes to be active.

Moreover, water acts as a medium for the distribution of all other ingredients in the formulation. Water is added as a plasticizer to hydrate both wheat protein and starch and to facilitate the formation of gluten matrix and starch swelling, respectively (Cesaro and Sussich, 2001).

2.2.1.3 Salt

Salt (Sodium Chloride, NaCl) is used to strengthen the flavour and to increase the shelf life of food products. It is believed to toughen the protein network. Salt is also added to bread recipes to decrease the stickiness of dough.

In spite of the fact that salt quantity is important in determining the bread flavour, Angioloni and Rosa (2005) investigated the significance of the amount of salt (NaCl) in modifying dough rheology, by comparing the thermo-mechanical properties of two different dough prepared in different conditions of mixing speed, mixer type and amount of NaCl added, and found that increased addition of salt can change the thermo-mechanical properties of dough by
delaying protein coagulation and starch gelatinisation. Accordingly, some bakers delay the addition of salt until the later stages of mixing to ensure a complete hydration of flour proteins as reported by Cauvain (2007).

Salt is also believed to affect the calorimetric behaviour of starch, however, its behaviour is agreed to be complex (Bello-Pérez, and Paredes-López, 1995 a, b). Researchers reported that the addition of NaCl in low concentration (up to 9%) caused elevation in the starch gelatinisation enthalpy value. Conversely, a reversed effect was observed when salt concentrations exceeded 16% (Chiotelli et al., 2002).

2.2.1.4 Yeast

Yeast is a leavening agent used to help dough rise as a result of production of carbon dioxide (gas) and ethanol in the fermentation stage.

2.2.1.5 Other additives

Other additives such as fat, shortenings, vegetable oils, eggs, ascorbic acid, sugar and bran can be added to enhance flavour, enrich dietary benefits or enhance the bread colour and texture (Qarooni, 1988; Toufeili et al., 1995; Demiralp et al., 2000).

2.2.1.6 Conclusions

The basic formulation of bread is wheat flour, water, salt and yeast, all of which play an important role. The protein content of the flour determines the bread type. This means that high protein content flour is suitable for high volume bread such as pan bread and baguette, whereas moderate protein content can be recommended for flat bread, in which the volume is not an essential criterion.
Starch type is also of great importance. It plays a significant role in determining the quality of baked products during baking and storage period.

Water is of great importance in any bread recipe. Dough consistency is dependent on its quantity and temperature. Salt is important to provide strength for the dough and flavour for the baked loaf, while yeast has a significant role in rheological properties of dough.

### 2.3 BREADMAKING METHOD

All types of bread around the world have to pass through certain essential steps. An overview of the operations is shown in Figure 2.1, and each operation is described in the following subsections.

![Breadmaking processes for all types of bread](image-url)
2.3.1 Mixing

Mixing is one of the most important steps in breadmaking process which is studied extensively by researchers. It is not only performed to mix the different ingredients, but also to ensure a uniform composition, properties and temperature of the bulk material (Marsh and Cauvain, 2007). Mixing is also essential to the dough hydration and aeration process, i.e. the incorporation of air and the formation of bubbles within the dough (Campbell et al., 2001; Chin and Campbell, 2005a, b). Cauvain (2007) summarized the sub-processes taking place during mixing: a uniform dispersion of the ingredients followed by hydration of flour proteins, which then develop into a gluten structure.

Mixing is also necessary for setting suitable rheological properties of the dough. Many researchers correlated mixing intensity with dough rheology and baking performance and hence the quality of the final product (Qarooni, 1988; Baardseth et al., 2000; Alava et al., 2001; Kim and Cornillon, 2001; Deshpande, 2003; Dobraszczyk and Morgenstern, 2003; Goesaert et al., 2005). This can be attributed to the formation of a gluten viscoelastic network during mixing.

Indeed, the changes occurring in dough during mixing had been widely investigated. Both mixing speed and time had a noticeable effect on dough consistency and rheology and hence final bread quality. However, dough development cannot be measured directly; the increase in dough resistance to deformation can be noticed by the increase in the mixer motor power (Zheng et al., 2000). Similarly, a strong correlation between mixing speed and the peak torque; in which consequently negatively associated to mixing time was reported (Chin and Campbell, 2005a, b).

Mixing time is also of great importance in flat bread processing. In a study on its effect on the quality of Arabic flat bread, it was reported that mixing affected mostly the dough sheeting stage, level of blistering and tearing quality of baked product (Qarooni, 1988). The researcher reported that short mixing time (2
minutes) resulted in poorly developed dough that was harder to be sheeted and yielded low quality bread. The researcher attributed this to the lack of support for starch granules by the gluten protein due to discontinuities occurred in protein matrix. While seven minutes of mixing resulted in a sticky dough that was difficult to be processed but yielded a superior quality product although a lack of symmetry was reported. The optimum mixing time (that was suitable for the flour used in Qarooni’s investigation) was suggested to be 4 minutes, which resulted good quality Arabic flat bread.

It is worth mentioning that absolute values of mixing time or speed cannot be directly compared between different types of bread, because other factors such as protein quality and water content should be considered.

### 2.3.2 Bulk fermentation

At this stage, dough undergoes many physical and chemical changes that prepare it for further processes. During this period, the enzymes convert the starch into sugars; providing food for the yeast (Wiggins and Cauvain, 2007). The yeast activity starts to produce carbon dioxide gas; which will then be retained in gluten network. Accordingly at the end of this stage, the dough volume is doubled. Temperature, time and humidity control are necessary (Collado-Fernández, 2003). Scientists found a negative correlation between fermentation time and temperature; lower temperatures required a longer fermentation time. Qarroni (1988) in his study on Arabic flat bread found that increasing the fermentation time will produce a lighter crust colour and decrease the level of blisters. Zehentbauer and Grosch (1998) were interested in studying the effect of changing the fermentation conditions by altering the amount of yeast and fermentation time. They reported that longer fermentation time resulted in more pleasant baguette odour; due to the increase in the production of aldehydes methylpropanal, and 2- and 3-methylbutanal. A more intense,
roasty odour was reported for baguette prepared with a higher amount of yeast due to the production of higher concentration of pyrroline.

2.3.3 Dividing and resting

The bulk dough must be divided into specific sized pieces and be allowed to relax before further processing. Gould (2007) stressed the importance of accurately dividing the bulk dough into a specific weight pieces without damaging the bubble structure of the dough. Resting is a necessary step at this stage as an intermediate proofing (at the same temperature and relative humidity as the bulk fermentation) in order to “produce a piece of dough which is sufficiently soft, extensible and relaxed to allow optimum performance in the moulding stage” as stated by Gould (2007).

2.3.4 Moulding, sheeting, docking

The purpose of moulding is to create a dough piece of the right shape and size while at the same time producing a dough structure that maintain its shape and texture for the final product (Gould, 2007).

On the other hand, sheeting means the transformation of the divided dough into flat round pieces of the desired thickness. The thickness of the sheeted dough has a significant effect on the quality of the Arabic flat bread (Qarooni, 1988; Quail et al. 1990).

In addition, docking is used in some countries in the production of single-layered flat bread, as a decorative process of making grooves or holes on the surface of the sheeted dough. Docking will also prevent the separation into upper and bottom layers of the bread (Qarooni, 1996; Farvili et al., 1997).
2.3.5 Final proof

With the exception of the single-layered flat bread, moulded or sheeted dough are allowed to rest again prior to baking (at the same assigned temperature and relative humidity of the bulk fermentation). This period is necessary to achieve the required shape and volume when baked. Both Qarooni (1996) and Gould (2007) emphasized on the importance of this step and suggested that this proofing period is essential for the dough to relax, aerate and form a thin skin which is transformed into a crust during baking.

This crust has a crucial role in breadmaking. In the case of Arabic bread processing; it will act as a barrier preventing the escape of the expanded steam and the carbon dioxide inside the dough, leading to an increase in the internal pressure which will allow the separation of the upper and the bottom crust forming the characteristic pocket of this type of bread.

2.3.6 Baking

Baking is obviously a critical stage in the breadmaking process. It contains many factors that influence the sensory quality of the final product (Kihlberg et al., 2004). During this stage, severe changes are occurring in the chemical and physical properties of dough; transferring raw dough into an edible tasty product.

An informative summary was reported by Mondal and Dutta (2008) cited the changes occurring during the baking stage into sub-stages including: evaporation of water, formation of a porous structure, volume expansion, protein coagulation, starch gelatinisation and crust formation.

Baking process varies for each type of bread. Baking temperature, time and modes remain the most prominent factors affecting the final crumb structure
and the bread quality (Faridi and Rubenthaler, 1984; Quail et al., 1990; Saxena and Rao, 1996; Hayman et al., 1998; Patel et al., 2005). The structural interactions between starch and protein are launched during the baking process as a function of those parameters (Patel et al., 2005), while their effect on starch was a crucial interest of many researchers (Faridi and Rubenthaler, 1984; Schiraldi et al., 1996; Giovanelli et al., 1997; Fessas and Schiraldi, 2000; Seetharaman et al., 2002; Shittu et al., 2007). For these reasons, different processing technologies result in products of different qualities (Martin et al., 1991; Patel et al., 2005; Yin and Walker, 1995; Li and Walker, 1996).

Finally, the influence of heating rate on the bread texture and shelf life of pan bread has been reported (Seetheraman et al., 2002; Patel et al., 2005; Patel and Seetheraman, 2006). These studies suggested that high heating rates would be likely to impact upon starch granule swelling, leaching of amylose and the reorganisation of amylose upon cooling, and consequently resulting in different bread texture.

Similarly, different heating rates have been implicitly used in some studies of flat bread (Quail et al., 1990; Qarooni, 1996; Saxena and Rao, 1996) in which different oven temperatures are used, however these have not included temperature information from within the bread and so an assessment of the impact of different rates is difficult. Thus, to our knowledge, no studies have been performed directly comparing heating rates at the heating rates typically encountered during the baking of Arabic flat bread.

On the other hand, the effect of baking time and temperature on the quality of flat bread had also been investigated (Faridi and Rubenthaler, 1984; Qarooni, 1988; Quail et al. 1990). In contrast to pan bread, flat bread was reported to have higher moisture content when baked at higher temperature for shorter time, i.e. higher heating rate (Faridi and Rubenthaler, 1984; Quail et al., 1990).
2.3.7 Cooling and packaging

All bread loaves must be cooled to the proper temperature before slicing or packaging. Gould (2007) gave an attention on avoiding excess moisture loss during this stage. He reported that "Not only will excess loss adversely affect the cost of manufacture but it will also accelerate the staling process". Insufficient cooling for Arabic flat bread leads to difficulty in separating the bread layers and moisture condensation within packaging material as reported by Qarooni (1996). On the other hand, packaging is an essential step to decrease water vapour escape, preserve the moisture content, and decrease the microbial attack.

2.3.8 Conclusions

The different processing steps in breadmaking are all considered to play a part in producing quality products. Mixing time and intensity have a great effect on dough rheology and bread quality. Long mixing times will give loose sticky over-developed dough which will yield bread of uncontrolled volume. Conversely, short mixing time will give hard under-developed dough and yield small volume bread with dense crumb. Researchers agreed on that achieving the optimum mixing time is necessary in order to reach an optimum bread quality. The same is true for mixing intensity or speed. Fermentation time, temperature and the relative humidity during this stage are also very important factors to achieve correct carbon dioxide production and bread volume. Baking is a critical stage in the breadmaking process. Baking time, temperature and mode all affect bread quality. Finally, cooling and packaging are also essential in order to preserve bread from microbial attack and decrease the moisture loss during storage period.

It is useful to summarize the similarities and differences between bread processing techniques. The flowchart in Figure 2.1 shows that mixing, bulk
fermentation, dividing and rounding, baking and cooling processes are universal. After the dividing and rounding process, the first difference occurs; where pan bread and baguette must be moulded or shaped to the final product shape.

The flat bread, on the other hand, must be sheeted or flattened to a desired thickness. This chart also emphasizes the main difference between the single-layered flat bread and the double-layered flat bread; where the former is docked and the latter type, the sheeted dough is given proof time prior baking. This time is necessary to allow the dough to relax, aerate and form a thin skin; which will become crust during baking.

2.4 TRANSFORMATION OF STARCH STRUCTURE DURING AND AFTER BAKING

Starch, as being the most abundant component in cereals, has a significant impact on bread quality. Starch is a polymer of glucose consisting of two structurally discrete polysaccharides: amylose and amylopectin (Buléon et al., 1998; Fredriksson et al., 1998; Goesaert et al., 2005). Both polysaccharides are based on chains of 1-4 linked α-D-glucose. Amylose is a linear amorphous polymer consisting of approximately 4000 glucose units, whereas amylopectin is partially crystalline and multi-branched polymer containing approximately 100,000 units of glucose. These distinct polymer chains can be held by crystalline junction points to form a rigid network. The content of amylose and amylopectin has a significant role in starch gelatinisation and retrogradation (Silverio et al., 1996; Zobel and Kulp, 1996; Buléon et al., 1998; Sasaki, 2005). The amylose : amylopectin ratio varies between starches, however, average levels of amylose and amylopectin are 25 - 28% and 72 - 75%, respectively (Colonna & Buléon, 1992).
The starch granule is a very complex macromolecular assembly whose exact structure has not been yet fully revealed. It is partially crystalline with a degree in crystallinity in the region of 20 - 40 % being reported (Hizukuri, 1996).

Crystallinity of wheat starch has been determined to 35.5 % (Morrison et al., 1994). Some of the crystalline forms are known. The A pattern; which is found in most cereal starches, including wheat, consists of starch double helices packed in a monoclinic array (Gudmundsson, 1994; Goesaert et al., 2005). The B pattern, which is found in tubers, high amylose and retrograded cereal starches, is a more highly hydrated and open structure, consisting of double helices packed in a hexagonal array (Gudmundsson, 1994; Goesaert et al., 2005). Waxy starches contain only amylopectin chains within the crystalline domain (Goesaert et al., 2005).

Starch undergoes a variety of transformations into different states. Various methods are used to probe these states and these are now described.

### 2.4.1 Methods used to study starch gelatinisation and retrogradation

Karim et al. (2000) classified the methods used to study starch retrogradation into (i) macroscopic and (ii) molecular techniques. In macroscopic techniques, certain mechanical or textural properties are observed. Rheological methods, sensory evaluation of texture and Differential Scanning Calorimetry (DSC) can be used. On the other hand, the molecular techniques are related to the changes in starch polymer conformation or water mobility. X-ray Diffracrometry (XRD), Nuclear Magnetic Resonance (NMR), Fourier Transform Infra-Red (FTIR), Near Infra-Red (NIR) can be utilized in this case.
2.4.1.1 Nuclear magnetic resonance (NMR)

This technique utilizes the magnetic properties of certain atomic nuclei to verify the physical and chemical properties of the molecules. When a sample is placed in a magnetic field, NMR active nuclei (such as $^1$H or $^{13}$C) absorb electromagnetic radiation at a frequency characteristic of the isotope. The resonant frequency, energy of the absorption and the intensity of the signal are comparative to the strength of the magnetic field (Keeler, 2010).

This technique is used to follow staling (Farhat et al., 2000; Ruan and Chin, 2001), because it can follow the mobility of protons connected to different molecules. Relaxation times: spin-lattice relaxation time ($T_1$) and spin-spin relaxation time ($T_2$) are used to designate the state of water in bread; where water is bound to different sites like solid site and liquid-phase site. Farhat et al. (2000) in their study on waxy maize starch found that both relaxation times decrease during storage period; attributing their results to the decrease in the molecular mobility of the starch as it retrograded, i.e. the mobility of gelatinized starch is more than the ordered crystalline fraction.

In earlier study, Chen et al. (1997) studied the mobility of water in pan bread stored at different temperatures: some are stored in refrigerator and others are kept at room temperature. The researchers also attributed the decrease in $T_1$ to the decrease in water mobility in aged bread.

2.4.1.2 Infra red (IR)

Vibrational spectra-structure correlations of an empirical nature have shown great use in the structure diagnosis of unknown molecules (Degen, 1997). Infrared spectroscopy has become of paramount importance when dealing with amorphous materials for which X-ray crystallographic data are tenuous (Gadsen, 1975). Upon the interaction with the IR radiation, portions of incident
radiation are absorbed at particular wavelengths, which may lead to highly complex absorption spectra being uniquely characteristic of molecular vibrational modes. This helps the identification of an unknown via comparison with spectra of pure compounds. IR is also considered to be rapid, sensitive, cheap and non-destructive analytical tool (Cocchi et al. 2005).

On the other hand, Fourier Transform Infra-Red Spectroscopy (FTIR) can be used to observe the conformational changes due to crystallisation during bread aging (Karim et al., 2000; Smits et al., 1998). Near Infra-Red (NIR) was used to study the effect of starch, protein and storing temperature on bread staling (Xie et al., 2004; Abu-Ghoush et al., 2008a, b).

2.4.1.3 X-ray diffractometry (XRD)

X-ray diffractometry (XRD) is a powerful technique used to determine the structure of crystalline solids, the precise atomic position, the bond length and angles of the molecule within a single crystal. If such a sample is placed in the path of a monochromatic X-ray beam, diffraction will occur from planes in those crystallites, which happen to be oriented at the correct angle to fulfil the Bragg condition (Smart and Moore, 1995). The relationship between the wave length of the X-ray beam “λ”, the angle of diffraction “θ” and the distance between each set of atomic planes “d”, of the crystal lattice is given by the Bragg equation (Smart and Moore, 1995):

\[ m\lambda = 2d \sin \theta \]  \hspace{1cm} (2.1)

(Where “m” represents the order of diffraction)
That is why this technique has been utilized to follow the changes in the degree of crystallinity of starches during storage. X-ray was the first technique that has been used for the investigation of starch recrystallisation as a sign of staling (Zobel and Kulp, 1996; Pateras, 2007).

XRD had been used to study starch crystallinity changes with aging in bread (Dragsdorf et al., 1980; León et al., 1997; Sidhu et al., 1997b; Hug-Iten et al., 1999; Ribotta et al., 2004). Researchers were able to identify a V-type structure related to the first signs of firming in fresh bread. This structure is related to the complex formed by amylose and the fatty acids to form a helical complex. V-type structure extent did not change after 24 hours. The A-pattern is lost during gelatinisation, while with aging, a B-type structure appeared and increased over time until it approach a limiting value (Gudmundsson, 1994).

Farhat et al. (2000) in their study on effect of moisture content in waxy maize starch found that A-pattern was obtained in samples stored at low moisture content and at high temperature. B-pattern was formed when samples were stored at higher moisture content and at lower storage temperature.

2.4.1.4 Optical microscopy and scanning electron microscopy

Microscopy is the most appropriate technique that can recognize the shape, size, texture and topochemical relationships. Use of electron microscopy provides a great focus and allows higher magnifications than other optical methods (Galwey and Brown, 1999). In scanning electron microscopy an electron beam is focused by lenses paced before specimen to obtain a very small electron probe directed onto the specimen (Bergert et al., 1997). Emitted signals such as backscattered and secondary electrons are detected and used to form images by remoduling the brightness on the CRT (cathode ray tube) (Bergert et al., 1997). The number of electrons emitted depends markedly on the angle of the specimen surface with respect to the electron beam and
detector. Therefore, each point on the screen corresponds to a point on the sample and images are obtained sequentially by continuous scanning.

Optical microscopy can be used to recognize the size, texture and surface of pan bread, and can follow the reorganisation changes of amyllose and amyllopectin during aging, providing depth of field in studying bread staling (Hug-Iten et al., 1999). While, scanning electron microscopy (SEM) is used to study the morphological changes that take place in starch granules. Sidhu et al. (1997b) followed these changes in Arabic flat bread during storage period. The researchers reported that SEM showed large gas cells presented in the crumb and concluded that although that measuring firming rate in Arabic flat bread was not achieved by SEM, it provided useful information about the different extent of gelatinized starch.

2.4.1.5 Thermal analysis - differential scanning calorimetry (DSC)

Thermal analysis is a group of techniques that are used to observe the physical and chemical changes of a sample when subjected to temperature change (Cammenga and Epple, 1995). Differential scanning calorimetry (DSC) is a thermal analysis technique that monitors the thermal changes between a sample and a reference cell. In Flux DSC (Figure 2.2), both cells are subjected to the same programmed temperature profile heating, and the heat flow is then plotted against the temperature. The temperature difference between the sample and reference is converted to differential thermal power \((dq/dt)\). Since the pressure is constant in DSC, then the heat flow is equivalent to the enthalpy changes \((dH/dt)\) as follows:

\[
\left(\frac{dq}{dt}\right)_p = \frac{dH}{dt}
\]  

(2.2)
If heat flows associated with reference cell are subtracted from those in from the sample pan then the resulting heat flow must be due to thermal events taking place within the sample pan.

\[
\Delta \frac{dH}{dt} = \left( \frac{dH}{dt} \right)_{\text{sample}} - \left( \frac{dH}{dt} \right)_{\text{reference}}
\]  

The peak temperature \((T_p)\) and the energy of reaction \((\Delta H)\) are important parameters obtained from DSC and used to compare different samples. Other parameters can be also deduced such as glass transition temperature \((T_g)\), onset temperature \((T_o)\) and offset temperature \((T_c)\). When the sample experiences a physical change, more (exothermic process) or less (endothermic process) heat will be required to flow to it than the reference to maintain both at the same temperature. When a solid sample melts to a liquid, it will absorb heat as it undergoes endothermic phase transition, while crystallisation is an exothermic process.
Karim et al. (2000) mentioned that the DSC endotherms describe the presence of ordered structure, and can show, for example, the development of amylopectin crystallinity during storage. The enthalpy values (ΔHr) give a quantitative measure of the energy transformation that takes place during the melting of retrograded amylopectin. Zobel and Kulp (1996) described these thermograms as “staling endotherms” which can be considered as a measure of amylopectin retrogradation. Most of the researchers used DSC results data to corroborate the results from other techniques.

Furthermore, DSC has been used to study the effect of starch type, protein and storing temperature on pan bread staling (Xie et al., 2004). DSC has also been used to study the effect of additives like malto-dextrins on crumb firming during aging as a result of amylopectin retrogradation (Rojas et al., 2001).

It is worth mentioning that this technique had been utilized to follow starch retrogradation in starch water mixtures (Zeleznak and Hoseney, 1986), fresh...
bread samples (Schiraldi et al., 1996; Shaikh et al., 2007) and freeze-dried samples (León et al., 1997; Sidhu et al., 1997b; Indrani et al., 2000).

Moreover, DSC had been used as an oven to simulate baking of bread (León et al., 1997). Similarly, Bárcenas et al. (2003) used DSC to simulate the part-baking process. Baking simulation by DSC is considered to be an insitu sampling which allows a better control and a direct observation of starch behaviour during the baking process and storage (Fessas and Schiraldi, 2000).

Bread dough was heated in a DSC pan at similar temperatures to that of the centre of the crumb during baking.

One of the key factors in DSC analysis that is often overlooked is the type of the pan used in studying starch transformations. Aluminium pans are used widely in DSC for their cheap price and reasonable thermal conduction although that low leaking temperatures have been reported (Yu and Christie, 2001). The leaking may affect the thermal contact between the pan and the furnace, but more likely it induces further vaporisation of water which consequently affects the measured heat flow to the pan (Schiraldi et al., 1996). On the other hand, high-pressure stainless steel pan had been known to be thermally stable; preventing leaking at low temperatures, however, its lower thermal conductivity may be considered (Yu and Christie, 2001). Stainless steel pans have higher mass and heat capacity; which results in a slower thermal response.

DSC, like all other techniques, has its limitations. The poor DSC reproducibility can be due to the “unavoidable variability in dough preparation and bread condition” as stated by Schiraldi and his group (1996). The researchers applied a statistical treatment to overcome DSC problem for food samples compared to samples from pure compounds or physically homogeneous systems.
2.4.1.6 Texture analysis

Sensory or organoleptic evaluations of texture are carried out by trained panel. This panel score the bread depending on specific required characteristics. This type of analysis is carried out by lots of researchers due to its simplicity, low cost and direct relationship to the extent of firming of bread (Qarooni, 1988; Toufeili et al., 1995; Farvili et al., 1997; Sidhu et al., 1997a; Fiszman et al., 2005). But, it is worth saying that this type of analysis cannot be considered as a reliable trusted analysis, because it differs from person to another and from culture to another.

Rheological techniques are compatible techniques that had been used to mimic sensory evaluation and to build a sensible idea about the development of bread firming as aging (Gudmundsson, 1994; Karim et al., 2000; Wilkinson et al., 2000). Texture analyses are important in determining and identifying the quality and shelf life of bread and might be considered as a direct measurement of bread firmness assigned for staling. Compression test measures the force required to compress bread using a flat Aluminium disc, specific test speed and trigger force to calculate the hardness of bread (Callejo et al., 1999; Rojas et al., 2001; Fiszman et al., 2005; Gómez et al., 2008). Puncture tests had the same format. On the other hand, bending tests use a continuous force required to bend a slice of bread (Gil et al., 1997; Callejo et al., 1999).

Additionally, researchers on flat bread used a variety of texture analysis techniques as an indication of bread toughness and the increase in firmness during storage period. Penetration (Puncture) tests were used to determine the force required to puncture a hole in the bread (Sidhu et al., 1997a; Başman and Köksel, 1999), while tensile test were used to determine the shearing force required to tear this type of bread (Ucles, 2003; Ghodke Shalini and Laxmi, 2007; Abu Ghoush et al., 2008a, b; Sheikh et al., 2007). It was also reported by the same researchers that an incline in the penetration forces and a decline in shearing force with time were an indication of bread quality deterioration.
Stress-relaxation requires a longer time to run as reported by Ucles (2003) in his research on the effect of additives on tortilla texture. This will affect the moisture content of the sample; which in turn will affect bread firmness results. Stress relaxation technique is used to determine the changes on final bread stiffness.

On the other hand, Salehifar and his Co-workers (2010) utilized a uniaxial compression test to follow firmness in Taftoon bread; which is flat bread produced in Iran. The researchers found that the increased compression force correlated significantly with bread firmness, and reported that bread firmness increased with time as measured by the increase of the sample resistance to compression force.

2.4.2 Starch gelatinisation

When starch is heated in the presence of water, it undergoes an irreversible transition process called gelatinisation. Starch gelatinisation is a significant process in many types of food products, and has been extensively studied due its significant contribution to product’s quality.

Starch gelatinisation is a more complex process than it might initially appear. A lot of researchers suggested many mechanisms that described this process. However, the process complexity arises from the fact that many factors must be considered and well understood. This process includes water diffusion into the granules, which lose their crystallinity, and swell to several times their original size and sometimes burst. The viscosity of mixture will increase and amylose will leach from the granules. Thermal gelatinisation is well accepted and can be described as a transition of starch granules from an ordered state to a disordered state (Lelièvre, 1973; Primo-Martin et al., 2007).
Furthermore, starch gelatinisation is not a random process; it is controlled by temperature and the available water. Gelatinisation is dependent on a 4-way starch-water-temperature-time interaction (Figure 2.3); where starch behaves differently when heated in different water levels (Donovan, 1979; Lelièvre and Liu, 1994; Fukuoka et al., 2002).

The differential scanning calorimetry (DSC) enthalpies correspond to all endothermic reactions that happen within the temperature range (Ratnayake, and Jackson, 2007). Donovan (1979) studied the hydrothermal properties of potato starch and reported a single endotherm at 66 °C as a result of heating starch in excess water. The researcher attributed his result to the disordering of the starch chains by the swelling action of water. The gelatinisation endotherms obtained by DSC have been interrupted differently (Zobel et al., 1988); likely because it is affected by starch botanical source (Orford et al., 1987). The gelatinisation temperature range of amylopectin was reported to be in the ranges 70 - 82 °C for amylopectin derived from wheat (Lelièvre, 1973) or 65 °C as reported by Seetharaman et al. (2002). Even lower gelatinisation temperature was reported at 60 °C (Douzals et al., 1998). However, this single peak will not appear in bakery products due to their limited water content (Münzing, 1991).

At lower water contents, a biphasic endotherm becomes evident. The first peak is attributed to starch gelatinized with the available water, whereas the second peak is due to melting of remaining crystallites (Donovan, 1979, Liu et al., 2009). Then, when moisture levels are even reduced further, the first peak diminishes gradually whilst the second peak increases in magnitude until it dominates and only this peak is apparent at higher temperatures. The position of this peak is strongly affected by moisture content (Donovan, 1979; Lelièvre and Liu, 1994).
For wheat starch, Kugimiya et al. (1980) and Biliaderis et al. (1983, 1985) reported a peak at even higher temperature (110 – 120 °C) related to the melting of amylose-lipid complexes. The gelatinisation of wheat starch is considered to be more complex than that of other starches, since the wheat starch granules’ particle size can vary greatly, and accordingly behaves differently during gelatinisation (Chiotelli and Le Meste, 2002) and is affected by the presence of protein. In a previous study, Olkku and Rha (1978) summarized the main factors affecting starch gelatinisation. These factors are: the type and size of starch granules, their age and previous treatments, the temperature-time profile, the mechanical treatments during heating process, the thermal conditions after heating; i.e. the time and temperature during storage period, and the types and amounts of added ingredients.

The effect of heating rate on starch morphology had been investigated. Different heating rates are believed to affect the product’s texture and shelf life (Seetharaman et al., 2002; Patel et al., 2005; Patel and Seetharaman, 2006).
Both studies suggested that high heating rates would be likely to impact upon starch granule swelling, leaching of amylose and the reorganisation of amylose upon cooling. Moderate changes in heating rate (up to 10 °C/min) affect the DSC endotherms as reported by Donovan (1979) and Lelièvre and Liu (1994) but this is largely an artefact of the technique (signal to noise and thermal lag effects) rather than due to changes in the sample. However, when heating rates were raised to 32 °C/min by Wootton and Bamunarachchi (1979) a significant decrease in enthalpy was observed.

In bakery products, a gradient in the degree of starch gelatinisation within the loaf was reported (Yasunaga et al., 1968; Faridi and Rubenthaler, 1984). Both researchers found the centre of the bread to be less gelatinized than other parts of the bread. They attributed their results to different reasons, the most obvious being the different moisture and temperature levels in different parts of bread. In the study on pan bread, Yasunaga et al. (1968) agreed that the crust had less moisture content and less extent of gelatinisation, but they found that the centre crumb was of higher moisture content but lower extent of starch gelatinisation. Later, Faridi and Rubenthaler (1984) reported higher degree of gelatinisation had been found in Balady bread (double layer flat bread produced in Egypt) compared to pan bread. Differences in starch gelatinisatin within different areas of the investigated flat bread were reported; where the centre was less gelatinized than the edges. Likely because the former was subjected to more heat intense and lost more moisture during baking than the edges.

However, these researchers’ results are not conflicting with each other. In fact, these differences are expected if these factors are considered:

- The difference in crust : crumb ratio; in which it is higher in the case of flat bread. This will lead to that the crumb of the later bread will be subjected to more intense heat than that of pan bread, causing a higher loss in moisture content; in which starch gelatinisation will be affected.
Both types of bread had a common result; which is that the extent of gelatinisation was less at the centre. But these results can be attributed to different reasons. In the case of flat bread; the less gelatinisation degree can be attributed to the shortage of moisture required for the starch gelatinisation. But in the case of pan bread, the moisture content was more in the centre of the loaf; however, the heat required for the gelatinisation was not enough.

In addition to thermal gelatinisation, pressure-gelatinisation has become a focal point of interest for many researchers (Muhr and Blanshard, 1982; Ezaki and Hayashi, 1992; Stute et al., 1996; Douzals et al., 1998; Douzals et al., 2001; Stolt et al., 2001; Katopo et al., 2002; Baks et al., 2008; King and Kaletunç, 2009; Liu et al., 2009; Bárcenas et al., 2010). Gelatinisation can be induced at room temperature or even at zero temperature if the applied pressure is high enough (Muhr and Blanshard, 1982; Stolt et al., 2001). High pressure treatments could also cause granule structure destruction, but in a different mechanism from heat induced gelatinisation. However, it was reported (Knorr et al., 2006) that high pressure (up to 650 MPa) treatments at ambient temperature caused irreversible destruction, but in contrast to thermally-gelatinization starch, some starch samples did not show any extensive swelling and kept granular character after pressurization treatments, which resulted in a weaker starch gel (Douzals et al., 1998; Stolt et al., 2001). For this reason, the gelatinisation enthalpy was decreased under pressure as reported by Liu et al. (2009). The researchers also attributed their finding to the destruction of some starch weak structures. On the other hand, the effect of pressure on gelatinisation temperature was a controversial point. A decrease in gelatinisation temperature was reported (Knorr et al., 2006) when starch was exposed to higher pressure. On the other hand, an increase in gelatinisation temperature had been found by other researchers (Muhr et al., 1982; Liu et al., 2009).
Similarly to thermal gelatinisation, the water availability is critical for pressure gelatinisation and, besides that, the extent of pressure-gelatinisation depends on pressure level, treatment temperature and processing time (Stolt et al., 2001).

Furthermore, pressure/temperature phase diagrams were generated to identify the start of the starch gelatinisation at different conditions as measured by microscopic measurements following the loss of birefringence (Douzals et al., 2001; Smeller, 2002). These diagrams can be used to speculate the effect of combined pressure and temperature treatments on gelatinisation of starch.

High pressure technology is very promising industry and the understanding of pressure-gelatinisation is an essential requirement. Also a deeper knowledge on the action of the combined pressure and temperature treatments is vital for future researches.

2.4.3 Starch retrogradation

Although that starch retrogradation or in other words recrystallisation is not the only cause of bread staling, many studies insist that it is the main factor and it is believed that it is responsible for the texture changes that take place directly after baking and during the storage of bread (Schiraldi et al., 1996; Zobel and Kulp, 1996; Farhat et al., 2000).

Starch retrogradation is not a simple process. It consists of two processes; the rapid amylose gelation directly after the loaves taken out of the oven and the slower amylopectin retrogradation. The first process; which is responsible for short term changes (Gudmundsson, 1994), cannot be reversed by normal reheating (Miles et al., 1985; Ring et al., 1987; Eerlingen et al., 1994; Lelièvre and Liu, 1994; Seetharaman et al., 2002), while the re-crystallised amylopectin is responsible for long-term structural changes (Gudmundsson, 1994).
Amylopectin recrystallisation from completely amorphous state directly after baking to partially crystalline state in aged bread is time-temperature dependent (Pateras, 2007). The gelatinized amylopectin re-associate to form a double helix crystalline structure as shown in Figure 2.4.

The endothermic transition temperature associated with the melting of retrograded amylopectin is reported in the range between 45 - 64 °C as reported by Ottenhof et al. (2005) and Gavilighi and his Co-researchers (2006). A melting temperature range of 50 – 89 °C was reported by other researchers (Sahlström and Bråthen, 1997; León et al., 1997; Sidhu et al., 1997b; Jagannath et al., 1999; Indrani et al., 2000; Seetharamn et al., 2002; Ucles, 2003; Patel et al., 2005). Zeleznak and Hoseney (1986) reported the temperature range 30 - 80 °C for the amylopectin recrystallisation.

![A model for amylopectin retrogradation showing the formation of double helices](image)

Figure 2.4 A model for amylopectin retrogradation showing the formation of double helices

In the present study, the temperature range followed by Schiraldi and his Co-researchers (1996) of 40 - 80 °C will be applied. These differences in retrograded amylopectin are expected and can be attributed to the fact that the
whole retrogradation process is affected by many factors such as added ingredients, storing temperature and water content during aging.

Furthermore, the application of pressure on food especially starches during processing is of great interest. Significant efforts have already been made by scientists to study the effect of pressure on starch gelatinisation, but scientific research on its effect on starch retrogradation has not been carried out to such an extent.

As in gelatinisation, starch retrogradation is also affected by the pressure applied during gelatinisation. Researchers had shown that pressure had a significant effect on starch retrogradation (Ezaki and Hayashi, 1992). And because pressure had been known to destroy the structure of the starches in a different way than that of thermal destruction (Douzals et al., 1998; Stolt et al., 2001); the restoration to its natural structure during aging was less and through different mechanism (Ezaki and Hayashi, 1992).

2.4.3.1 Factors affecting starch retrogradation

There are several factors that affect starch retrogradation. The most important factors are shown in Figure 2.5.

Figure 2.5 Major factors affecting starch retrogradation
2.4.3.1.1 Water content

Scientists diverge in estimating the role of water during aging (Gil et al., 1997; Zeleznak and Hoseney, 1986; Farhat et al., 2000). Gil and Co-workers (1997) reported that water did not affect firmness caused by starch retrogradation unless the flour was enriched with gluten. However, the study of Zeleznak and Hoseney (1986) is the most authoritative. Water remains the most prominent factor that affects starch retrogradation. In order to investigate if the amount of water during DSC heating can influence the value of enthalpy of the melting of aged starch, the researchers (Zeleznak and Hoseney, 1986) prepared different ratios of water to bread crumb (aged and freeze-dried) and scanned them by DSC. They reported that the retrograded starch melting enthalpy was not considerably changed as the water content changed. Then, to ensure if water presented during gelatinisation or aging can affect the value of enthalpy, their research diverted in two ways: investigating the effect of moisture presented during starch gelatinization and that presented during aging of starch gels. They found that the enthalpy of melting of the retrograded starch was controlled by water presented during aging, and that presented during gelatinisation had no significant effect on starch retrogradation. However, their study was subjected on samples of moisture content from 30 to 70 %.

Researches of the effect of more concentrated systems are rare (Farhat et al., 2000). That is why those researchers studied the effect of moisture content between 19 – 38 % (wet weight basis) on starch retrogradation in waxy maize starch extrudates. From XRD spectra, the researchers found that not only the starch crystallinity was affected with moisture content, but also the crystalline forms; A-pattern was obtained in samples stored at low moisture content and at high temperature. B-pattern was formed when samples were stored at higher moisture content and at lower storage temperature.
For this reason, an important feature of bread baking that should be taken into account in baking studies is that differences in starch state and moisture content often exist between different parts of the bread. For example, it has long been established in pan bread that moisture content is highest in the centre of the bread than the edges (Yasunaga et al., 1968), but this is not the case for Arabic flat bread (Faridi and Rubenthaler, 1984). It is known that differences in moisture content can result in different degrees of retrogradation during aging (Zeleznak and Hoseney, 1986). However, sometimes this spatial variation factor has been overlooked. For example, Sidhu et al. (1997b) analyzed powdered freeze dried samples of flat bread, and thus undoubtedly represented an ensemble average of the whole loaf. Using DSC Aluminium pans, Sidhu and his co-workers (1997b) claimed that measured amyllopectin retrogradation increases with time. However, they have failed to back this claim by providing a thermogram or a table of experimental data. The study also did not consider the effects of liberal amounts of flour that they used to prevent dough sticking to the cutting and sheeting tools. This flour becomes gelatinised if heated in the presence of water affecting the experimental results. Therefore results obtained using DSC for their study of starch retrogradation in Arabic flat bread should be treated with care.

2.4.3.1.2 Temperature-time dependency

The magnitude of the retrogradation enthalpy ($\Delta H_r$) is a function of aging time and temperature. The rate of retrogradation of gelatinized waxy starch (Farhat et al., 2000) shows a “bell-shape” dependence on storage temperature. This agrees with the crystallisation general theory; where the effect of temperature on the rate of crystallisation is the result of its net affect on the nucleation and propagation rates, as reviewed by Levine and Slade (1990).
Starch retrogradation is highly influenced by storing temperature during aging (Farhat et al., 2000). Schiraldi, Piazza and Riva (1996) suggested that amylopectin melting enthalpy was inversely related to storing temperature in their calorimetric study on bread staling. Earlier studies had suggested that starch recrystallisation achieved its limiting value faster when bread was stored at lower temperatures than 21 °C, compared to bread stored at higher temperatures than 21 °C and up to 43 °C (Colwell et al., 1969). Recently, a storing temperature of 4 °C was regarded as the optimum temperature for retrogradation (Seetharaman et al., 2002). In other words, bread stales at a slower rate when stored at room temperature (25 °C) than when refrigerated (4 °C).

The dependency of starch retrogradation on time is reported by many researchers (Zobel and Kulp, 1996; Farhat et al., 2000). This pattern of dependency is found in starch gels (Farhat et al., 2000; Ottenhof et al., 2005) and bread (Patel et al., 2005).

2.4.3.1.3 Amylose : amylopectin ratio

The amylose-to-amylopectin ratio is believed to affect starch retrogradation. The effect of varying amylose : amylopectin ratio on bread staling was studied by Ghiasi and co-workers (1984). Three types of bread of different amylose : amylopectin ratios were under investigation; compression tests were applied to measure and compare firmness of the prepared and stored loaves, while DSC was utilized to follow amylopectin retrogradation. The researchers found that bread prepared from waxy barley were softer in the first day of storage period than other prepared loaves. But equal firmness was reported for all types after a few days of storage. A conclusion was derived that amylose was involved in the earlier stages of staling and was responsible for setting bread structure, and
hence its absence can lead to the collapse of bread loaves prepared from 100% waxy barley starch after baking.

Later, Gudmundsson and Eliasson (1990) reported that amylose has an indirect influence on amylopectin recrystallisation. Lower rates of recrystallisation were recorded for samples with waxy wheat up to 30%, but when 100% waxy wheat was used the enthalpy of the recrystallized amylopectin was highest. Therefore, the researchers concluded that with higher amylopectin ratios amylose does not influence amylopectin recrystallisation to a great extent.

2.4.3.1.4 Processing techniques

Giovanelli et al. (1997) studied the effect of baking temperature range varied from 90 to 110 °C on starch retrogradation on pan bread. The study showed that baking temperature significantly affected the starch retrogradation rate; likely because crumb baked at higher temperatures would show a greater extent of starch gelatinisation and during storing would cause more starch retrogradation.

On the other hand, an influence of heating rate on the bread texture and shelf life of pan bread compared to tortilla has been reported (Patel et al., 2005). This study suggested that baking heating rate and processing techniques would be likely to impact upon starch granule swelling, leaching of amylose and the reorganisation of amylose upon cooling. Different heating rates have been implicitly used in some studies of flat bread in which different oven temperatures are used, however these have not included temperature information from within the bread and so an assessment of the impact of different rates is difficult. Thus, to our knowledge, no studies have been performed directly comparing heating rates at the heating rates typically encountered during the baking of Arabic flat bread.
2.4.4 Conclusions

The function of starch in food, in general, and in breadmaking process, in particular, is vital. Starch as a polymeric compound of high molecular weight is sensitive to many factors, such as the water availability and the thermal treatments. Both starch gelatinisation and retrogradation are important stages in starch transformation life. They determine the product’s texture, quality and its shelf life. Researchers diverge on how some factors are affecting the starch transformations. However, their dissension can be attributed to the differences in material under investigation.

Different bread types cannot be compared with each other, or even be examined by the same techniques, without full understanding of the whole process as the gradient in degree of starch gelatinisation within bread loaf was studied, while its effect on the starch retrogradation was overlooked.

Moreover, thermal results integrated by DSC may be affected by factors that had been overlooked. Sample type, analysis heating rate and pan type may have a significant effect on results.

2.5 STALING

2.5.1 Introduction

Bread staling has been a major concern in breadmaking industries due to the large economical losses reported. Staling leads to a loss of consumer acceptance and saleability. It is different than bread spoilage; which is caused by microbial attack. After baking and during storing, there are many changes that are related to each other and lead to a stale flavour; the bread crumb firmness increases while the crispness of the crust decreases and the fresh fragrance of bread is lost. Researchers are actively investigating the factors governing bread staling. Different reports and different opinions have been
suggested with very few firm conclusions for the staling of bread. However, the most important judge on bread remains the bread texture and the consumer acceptance.

All researchers had agreed on that firmness was a major sign that can be used to monitor bread staling; however, they diverge on the origin of this firmness. Many researchers suggested that starch retrogradation is a major factor in bread firmness since the starch is considered as a major fraction in bread flour, and consequently followed amylopectin recrystallisation in aged bread. Other researchers have found that bread firming is not synonymous with amylopectin recrystallisation in bread and suggested different mechanisms for bread firming showing the role of other starch constituents. Water migration and redistribution were also involved in such studies on firmness.

2.5.2 Evidence linking staling with starch retrogradation

Starch represents the largest portion of flour. It plays a significant role in bread quality and shelf life. Pateras (2007) exalted the efforts of J. R. Katz, who was the pioneer in studying bread staling and focusing on the significant role of starch and water. Katz as reported by Zobel and Kulp (1996) utilized X-ray to study fresh and aged bread crumb and starch gels, and observed that during bread aging, crystallinity increased, and the solubility of starch decreased while protein solubility did not change. Katz had been mentioned to be the first researcher who pointed to the role of starch in bread staling.

Furthermore, Zobel and Kulp (1996) considered starch retrogradation as an “integral part of any discussion on bread staling”. The supporters of this theory believe that starch has the major role in firming of bread since it represents the largest portion of flours. Protein role is negligible and water acts as a plasticizer (Schiraldi and Fessas, 2001). Some researchers focused on the role of amylose in the gelation and retrogradation of starch (Ghiasi et al. 1984; Gudmundsson
Literature Review

and Elliasson, 1990; Sasaki, 2005), while others examined the role of amyllopectin in retrogradation process (Zobel and Kulp, 1996; Hug-Itten et al., 1999; Osella et al, 2005).

The strongest defence was given by Zobel and Kulp (1996). The researchers revised the role of starch, gluten and other flour constituents on bread firming and suggested that the major role of firming was afforded by starch, while the gluten role was limited to granule shielding and bridging. X-ray patterns for starch slurry, fresh bread crumb and aged bread crumb were studied. Three main stages were observed.

The dough stage; where amyllopectin aggregates into crystalline regions into a helical molecular conformation, and the amorphous amylose is depicted as a single-helical conformation laying freely beside the native polar lipids exist naturally in wheat starch granules.

Then, the bread stage starts when starch granules in the presence of water and heat are swollen and gelatinized. Amylose diffuses out of the granules due to its comparatively small size, forming double helices in the inter-granular space. Then these helices are joined through a juncture points forming a gel as the baking stage ends. This gelation gives the first signs of loaf firming and is considered to be the first stage of starch retrogradation. However, not all the amylose can diffuse out of the granules; because some have been blocked by the polar lipids forming a helical complex. This complexation decreases the amount of free amylose leaching out of granules. On the other hand, the granule swelling can be attributed to amyllopectin, which expands and its branches are exuded out of the granule. At this stage, the crystallinity of amyllopectin is lost.

The third stage commences when bread ages, the crystallinity of amyllopectin is developed, increasing the firming of the bread. However, since amyllopectin is
more sensitive to heat than amylose; the staled bread can be refreshed to some extent upon reheating (which melts the amylopectin crystallites).

Many researchers’ results’ were in agreement with Zobel and Kulp model such as Ribotta et al. (2004) in their study on starch crystallinity changes with aging in pan bread, Indrani and his co-workers (2000) in their study on Parotta flat bread, Sidhu and his group of researchers (1997b) work conducted on the Arabic flat bread produced by Kuwait Flour Mills and Bakeries.

On the other hand, Toufeili and his group (1999a) examined the role of amylopectin in the staling of bread by substituting waxy barley starch for wheat starch. They found that the added amylopectin rich flours firmed faster that regular wheat flour; owing to the higher amylopectin content which will lead to higher retrogradation rate for substituted bread.

### 2.5.3 Evidence linking staling with other bread constituents

Recently, several researchers have suggested that mechanisms other than amylopectin recrystallisation are implicated in crumb firming of aged bread. Bread firming and starch recrystallisation were found not to be synonymous as reported by others although that both processes occur directly after baking and during bread storage (Dragsdorf and Varriano-Marston, 1980; Ghiasi et al., 1984, Roger et al., 1988; Martin et al., 1991). Even when bread was stored at modified atmosphere (Rasmussen and Hansen, 2001) or baked at lower temperature (Giovanelli et al., 1997), starch retrogradation came to an end limiting value as measured by the melting enthalpy, while crumb hardening continues to increase during the storage period.

There are some arguments among the researchers about of the gluten role in the staling process. The supporters’ evidence is that bread with high protein content tends to have high specific volume, softer crumb and lower firming rate.
Maleki et al. (1980) suggested that the rate of firming was connected and positively correlated to the protein quality of the flour. A later paper by Martin et al. (1991) suggested that bread firmness might be caused by starch gluten interactions; where hydrogen bonds are formed between gluten and starch granules. Protein was found to affect loaves volume and softness in the early period of storage. Similarly, Gil et al. (1997) and Callejo et al. (1999) reported that adding gluten decreased both bending and compression forces, and hence reported a reduction of bread firmness during the storage period. A comparable conclusion was derived by Xie and his co-researchers (2004) in their study on protein-free bread and protein-rich bread as they attributed their findings to the protein role in starch dilution that similarly was reported earlier by Kim and D’Applonia (1977a, b).

On the contrary, the study of Morgan (1997) had reported equal rate of firmness for gluten-free bread and gluten-rich bread. Zobel and Kulp (1996) attributed for the conflict between protein role and bread firmness in that higher gluten breads were found to have higher specific volumes compare to lower gluten loaves and this observation may affect the firmness measurements as the lower gluten loaves tend to be more dense.

Recently, Ottenhof and Farhat (2004) reviewed the gluten role. They investigated the effect of gluten on starch retrogradation using XRD and DSC, and found no significant effect for gluten on amylopectin retrogradation during storage.

On the other hand, moisture changes play a significant factor in staling through evaporation and water redistribution (Baik and Chinachoti, 2000; Bollain et al., 2005). It is known that differences in moisture content can result in different degrees of retrogradation during aging (Zeleznak and Hoseney, 1986; Liu and Thompson, 1998) (Section 2.4.3.1.1). In fact, water has a significant part in the firming process, either by enhancing the molecular mobility of polymer chains or by acting as a coordination agent between them (Schiraldi and Fessas, 2001).
The same authors focus their review on water content, activity and migration within different phases. They concluded that water works as a plasticizer that enhanced and direct moisture migration from crumb to crust.

2.5.4 Effect of ingredients and process parameters on bread quality and starch state

2.5.4.1 Starch damage

The research on the role of starch in bread also needs to take into account two starch quality parameters that may have a great effect on bread quality. These quality parameters are falling number and starch damage. Falling number relates to the $\alpha$-amylase activity in the flour, which is an enzyme that occurs naturally in cereal flours, and it is necessary for proper fermentation and softening effects. $\alpha$-amylase is believed also to retard crumb firming by hydrolyzing starch to smaller dextrins. The lower the falling number, the more enzymatically active is the flour (Cauvain, 2007), the more sticky will be dough and the baked bread will collapse when slicing.

On the other hand starch damage is an indication of the mechanical damage of starch during milling. A significant fraction of the starch granules is damaged during milling. The level of damaged starch affect flour properties, high level require more water absorption and hence longer baking time (Goesaert et al., 2005). Soft wheat grains were reported to give flours with lower starch damage, while harder grains milling resulted in flours with higher starch damage (Quail et al., 1991). It tends to be the case that amylases are capable of attacking only the damaged starch (Goesaert et al., 2005).

The effect of starch damage on the quality of pan bread was studied by Every and his co-workers (2002). They reported a strong negative relationship between starch content and $\alpha$-amylase activity for strong flours (as would be
expected). In contrast, they found a positive correlation between protein content and α-amylase activity. They attributed their results to the enzyme extraction process, which removed less starch-bound α-amylase for higher starch content flour. Quail et al. (1991) reported that levels of starch damage (6 - 9 %) resulting from the milling of hard wheat grains were found suitable for the production of Arabic flat bread; attributing that to the increase in water absorption of flour. However, higher levels resulted in sticky and poor sheeting quality dough and a poor quality bread with poor separation of layer as reported by Qarooni (1988).

Thus, the effect of damaged starch level on bread firming and hence, bread staling has been studied extensively (León et al., 2006). A positive correlation between damaged starch and amount of amylopectin retrogradation and bread firmness was found. High contents of damaged starch were reported to have a negative influence of the quality of bread and its shelf life.

Earlier, higher levels of damaged starch content in flour were reported to give good quality Roti (Saxena et al., 2000). Also, Arora (2003) suggested that a moderate levels of damaged starch improved shelf life of tortillas.

However, these researchers’ findings are not as contradictory as would first appear; León et al. (2006) damaged starch content of their study ranged from 8.4 to 17.7%, while Saxena (2000) and Arora (2003) studied damaged starch content of (5.65 - 8.01%) and (5.4 - 12.6%), respectively. The lowest content of damaged starch considered by León is highest for Saxena and moderate for Arora.

2.5.4.2 Effect of wheat bran

The consumption of whole wheat bread has been rising in the last few years due to their significant health advantages. However the addition of cereal bran
caused problems to bread quality such as increase in dough stickiness and the shrinking in the loaf volume (Katina, 2003).

Sidhu and co-workers (1997a, b) reported a difference in firming rate between white and extra bran Arabic flat bread. They observed that the extra bran bread showed a higher degree of amylopectin crystallinity as observed by DSC and XRD in the first two days of storage than white Arabic flat bread and thus firmed faster. Later on, the increase of crystallinity of white bread was sharper and its firmness, as measured by the force required to puncture the flat bread, was greater than that observed for the other type after four days. Unfortunately and up to our knowledge, no study has been found to explain this observation.

2.5.4.3 Additives

Food additives are substances not normally consumed as a food, but added intentionally to preserve or improve its appearance, texture, flavour or expand its shelf-life (Deshpande, 2002). The effects of hydrocolloids (Guarda et al., 2004), and emulsifiers (Stampfli and Nersten, 1995) were reviewed extensively, and the researchers were able to classify them into different classes on the basis of origin, solubility and the functional group: emulsifiers that can act as dough strengtheners if they interact with gluten protein, or act as crumb softener if they formed a complex with the gelatinized starch.

In order to understand the function of emulsifiers, Krog (1981) studied the theories that described the function of dough strengtheners and crumb softeners. The researcher reported that the dough strengtheners such as Sodium-stearoyl-2-lactylate (SSL) and diacetyl tartaric acid ester of monoglycerides (DATEM) are capable of forming liquid films of a special structure in the inter-phase between the starch and gluten strands, and they are able to enhance the ability of gluten to form a film which retains gas produced by the yeast during fermentation and proofing stages. On the other hand, the
crumb softener emulsifiers such as SSL and Distilled Monodiglycerides (DMG) work as starch-complexing agents which delay starch gelatinisation.

Qarooni (1988) studied the effect of adding shortening and SSL to the formula of Arabic flat bread, and reported that addition of SSL increases the softness of dough and increased the keeping quality of bread although of the larger cracks on bread surface. Similar conclusions were reported for the addition of shortening up to 1%. At higher levels of shortening (2%) bread lost its tearing quality and became fragile.

Similarly, Toufeili and his group (1995) studied the effect of shortening on the quality and shelf-life of this type of bread, and reported that in spite of the fact that these additives increased the whiteness and softness of bread crumb, they didn't affect the shelf life of Arabic flat bread to any extent.

Then, Farvili et al. (1995) investigated the inclusion of SSL, DATEM and glycerol mono stearate (GMS) in the formula of Arabic flat bread. Three types of flour with different protein content were used to prepare bread. Two important flat bread characteristics were used to evaluate the shelf life: pliability and tearing quality. The researchers reported that the effects of emulsifier on flours with different protein contents were varied.

Furthermore, the influence of added enzymes on quality and shelf life of bread was studied comprehensively. Enzymes are defined as sensitive organic catalysts that are used to hydrolyze starches or proteins in bread converting them into smaller units (Cauvain, 2007; Bowles, 1996; Zobel and Kulp, 1996). α-amylase is one of the recently permitted enzymes to be used in breadmaking process as reported by Cauvain (2007), α-amylase, as reported earlier (Section 2.5.4.1) is believed to retard crumb firming by hydrolyzing starch to smaller dextrin.

However, with the shift in consumption of wheat bread, public health concerns had been raised. α-amylase is reported to induce allergic symptoms (Baur and
Literature Review

Czuppon, 1995; Moreno-Ancillo et al., 2004). Therefore, they were restricted in the production of Arabic flat bread in Kuwait.

2.5.4.4 Modified breadmaking processes

In order to reduce the economic losses as a consequence of bread staling and to reduce health concerns for additives; modified bread making techniques have arisen. Several efforts were attempted to study the effect of altering processing techniques on the quality of bread. The study of Seetharaman et al. (2002) comparing processing techniques used for buns and tortilla production had proved that different processing conditions can affect the product quality.

Gómez et al. (2008) examined the effect of changing fermentation parameters such as time, temperature and yeast dose on white and whole-meal pan bread. Their study follow the changes in crumb firmness as an initial sign of bread staling. A positive correlation between fermentation time bread firmness was reported, while fermentation temperature was found to affect the early stages of firmness only in whole-meal bread. In the case of Arabic flat bread, it was reported that increasing mixing time resulted in a better bread keeping quality although the severely increase in dough stickiness (Qaroooni, 1988).

Several efforts were also made to study the effect of altering dough sheet thickness and baking temperature/time on the quality of Arabic flat bread (Faridi and Rubenthaler, 1984; Qaroooni, 1988; Quail et al. 1990). These researchers agreed on that Arabic flat bread baked at higher temperature for a shorter time were of superior quality; likely because of the higher moisture content as the researchers reported.

In order to provide fresh bread for consumers, a part-baking technique was created. In the part-baking technique (Bárcenas et al., 2003; Vulicevic et al., 2004; Bárcenas and Rossel, 2005; Bárcenas and Rossel, 2006; Karaoğlu et al., 2006, Bárcenas and Rossel, 2007), the baking process is divided into three
stages. The first stage is that when dough is baked and until crumb is partially formed but before the crust is formed. Then, the part-baked bread is refrigerated, frozen or even kept at room temperature. After that, the consumer continues baking at home, and enjoys fresh bread out of the oven.

Regarding packaging, a number of studies have been developed for investigating the effect of Modified Atmospheric Packaging (MAP) on shelf life and staling rate of bread (Rasmussen and Hansen, 2001; Kotsianis et al., 2002). However, while it has been reported that MAP is effective to inhibit microbial contamination, there is no evidence for any effect on bread firming.

Hallberg and Chinachoti (2002) examined the effect of different packaging types on amylopectin recrystallisation and firming of pan bread over an extended storage time. They found that hermetically sealed packaging helps to retain moisture but did not retard amylopectin recrystallisation.

2.5.4.5 Nonconventional methods in bread baking

Over the last decade, significant progress have been made to find alternative and non conventional methods for food processing; such as using microwave heating or high pressure processing. These alternative technologies are aimed to save energy, preserve quality, enhance microbiological safety and improve shelf life of food.

Microwaves are electromagnetic waves in the range of 300 - 300,000 MHz. The polar components in food are oriented rapidly if exposed to these waves causing heat elevation (Palav and Setharaman, 2006). Microwave heating has been used in baking bread (Içöz et al., 2004). However, a low quality baked product was reported.
High pressure processing, as an alternative to the thermal processing, is a developing part of the food processing industry and has met the requirements of safer and more natural products without the need of chemical preservatives or additives. This technology kills pathogens by submitting food to various hydrostatic pressures (150 - 1000 MPa) at lower temperature than that had been used in thermal processing and with various exposure times.

The application of submitting foods to high pressures has shown impressive potential and has become a promising industrial reality (Norton and Sun, 2008; Bermúdez-Aguirre and Barbosa-Cánovas, 2011) rising due to its significant effect in obstructing microorganisms causing spoilage and ending shelf life of the food products (Knorr et al., 2006; Bárcenas et al., 2010). Today, the quality of pressurized products has been shown a high consumer acceptance. High pressure technologies have been used widely in many food industries such as wine making, meat, poultry, and fish industries, dairy, grain and egg products and also packaging for high-pressure products.

It was mentioned that increasing treatment pressures will increase microbial inactivation, but may also cause greater levels of prospective detrimental changes in food quality when compared to the unpressurized product such as protein denaturation and starch structure modifications (Smelt, 1998; Norton and Sun, 2008; Bermúdez-Aguirre and Barbosa-Cánovas, 2011).

As mentioned earlier (Section 2.4.2), the application of high pressure on starch is believed to induce starch gelatinisation in a different mechanism than that induced by thermal treatments (Ezaki and Hayashi, 1992; Stute et al., 1996; Douzals et al., 1998; Douzals et al., 2001; Stolt et al., 2001; Katopo et al., 2002; Baks et al., 2008; King and Kaletunç, 2009; Liu et al., 2009). Studies had shown that pressure treatments causes destruction of the natural structure of starches in a different manner to than that caused by thermal treatments. The effect of high pressure was mostly investigated on starch gelatinisation but less on starch retrogradation, and is even rare in cereal based products (Bárcenas et
al., 2010). Even more, there is no study found in the literature on the application of pressure higher than the atmospheric pressure while baking bread. This study will establish the first step in the studies of the effect of pressure on bread baking process.

Furthermore, high pressure techniques was applied during the fermentation process when Bárcenas et al. (2010) studied wheat dough for microbial, physicochemical, and structural characteristics after pressurization at 50 - 250 MPa for 1 - 4 min. After pressurizing, microbial counts were reduced significantly in the dough, while dough stickiness conversely affected treatment time. After conventional baking, changes in colour and crumb structure, and gas cells distribution were reported. The researchers also expected to find a significant reduction in dough expansion and bread volume as a function of pressure. They attributed the higher crumb hardness to modifications in the protein network. The moisture content of treated bread was found to increase as the applied pressure increased; as a result of an increase in crumb water holding capacity.

2.5.5 Conclusions

Bread staling is a significant challenge for breadmaking industries. Scientists diverge in their opinions and evidences relating different factors and constituents to the staling process.

Bread firmness was followed as the most obvious change that occurs in the bread after baking and which is noticed by consumers. Many researchers suggested that starch retrogradation is behind this firmness, however, others have found that bread firming is not synonymous with amylopectin recrystallisation in bread and suggested different mechanisms for bread firming showing the role of other starch constituents.
Literature Review

Therefore, many efforts have been focused on how to increase shelf life. Some researchers studied how some of flour properties effects on staling rate. Others investigated the influence of additives on bread crumb firming during aging. Additives had been reported to affect bread softness and improve the quality of bread in general and Arabic flat bread in particular (Qarooni, 1988) but they are restricted in the production of later bread in Kuwait for the health concerns. Therefore, there are growing attempts toward studying the effect of breadmaking processing modifications and their effect on bread shelf life. These modifications are still unknown.

The application of submitting foods to high pressures has shown impressive potential in killing microorganisms and has become a promising industrial reality, but, this technique had not been applied in breadmaking industry to that extent and required to be investigated more on its potential in such industry. The effect of high pressure on bread processing is still unexplored.
3 EXPERIMENTAL METHODOLOGY

3.1 INTRODUCTION

This research was conducted to determine the factors behind the staling of Arabic flat bread. In order to achieve this goal, preliminary tests were carried out on flat bread produced by Kuwait Flour Mills and Bakeries, Kuwait (KFMB) to find out the general characteristics of this type of bread. Both moisture content variations and the starch recrystallisation process; as measured by the increase in the enthalpy ($\Delta H_r$) of the retrograded starch were monitored. For deeper investigations, bread was baked at laboratory scale in Loughborough University Laboratories, taking into consideration that there are many differences between the industrial-scale and laboratory-scale bread making processes. These differences arise from the fact that larger amounts of raw materials and larger equipments are used at the industrial bakeries. The industrial mixer requirements were found to be more for adequate dough development (Wilson et al., 1997). Seeking to diminish the differences between the two processes and to achieve the best possible performance for laboratory-scale bread (lab-bread), Qaroooni’s (1988) and Quail’s et al. (1990) laboratory baking methods were both merged with the method used in KFMB. The essential steps of the baking Arabic flat bread will be followed and will differ only in baking modes whether it is via laboratory oven or the designed high pressure oven.

For bread baked in Loughborough University by the laboratory oven, a systematic analysis was applied to both moisture content and DSC techniques, where the upper layer of Arabic flat bread was divided into different zones. The use of this novel approach is to investigate the spatial variation of moisture content and thermal response as measured by DSC in order to find out how different parts of the bread are affected during its processing and aging.

Furthermore, “baking simulation” studies using DSC to create the temperature-time profile that starch sample experiences during baking were conducted on dough samples for deeper understanding of the whole starch retrogradation process and the factors affecting it.
An experimental oven was designed and constructed in Loughborough University to study the effect of pressure on Arabic flat bread baking. In high pressure oven (HPO), pressure was applied on bread dough while baking using compressed air. HPO products were analyzed for moisture loss during baking, measurements of starch retrogradation melting enthalpy and bread firmness during storing period.

### 3.2 MATERIALS

#### 3.2.1 KFMB bread

Four types of Arabic flat bread, made of wheat, were produced by Kuwait Flour Mills and Bakeries (KFMB). This classification was established depending on the type of wheat flour used and the thickness of the sheeted dough: soft white, soft brown, normal white and normal brown. The term brown bread was used to define bread made of whole wheat flour, whereas the terms soft and normal were used to describe the presence and absence of sugar, which also corresponded to thicknesses of the dough sheets of 1.5 and 3 mm, respectively. It is worth noting that the wheat flour used to prepare white bread is fortified by a variety of B vitamins to compensate the vitamin loss from wheat flour upon the removal of wheat fibres. The ingredients used by KFMB to prepare each type of bread are listed in Table 3.1.

#### 3.2.2 Labatory-baked bread

Pita bread dough was prepared using the following composition based on normal white KFMB bread: 100 g of white flour, water (58 wt % of flour basis), yeast (1 wt % of flour basis), (Nevada Instant Yeast, Lesaffre Group, Turkey) and salt (1.5 wt % of flour basis) (Saxa Table Salt, Premier Food Group, Spalding, UK). The flour was provided by Kuwait Flour Mills and Bakeries
Experimental Methodology

Company (KFMB) (Table 3.2). Dough moisture content was calculated to produce a wet basis to be 44.7%.

Table 3.1 KFMB bread ingredients

<table>
<thead>
<tr>
<th>Bread Type</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal White Bread</td>
<td>Fortified wheat flour with iron and folic acid, water, salt and yeast</td>
</tr>
<tr>
<td>Normal Brown Bread</td>
<td>Wholewheat flour, water, bran, salt and yeast</td>
</tr>
<tr>
<td>Soft White Bread</td>
<td>Fortified wheat flour with iron and vitamins (B1, B2, B3, B9), water, salt, sugar and yeast</td>
</tr>
<tr>
<td>Soft Brown Bread</td>
<td>Wholewheat flour, water, salt, sugar and yeast</td>
</tr>
</tbody>
</table>

Table 3.2 General specifications of wheat white flour supplied by Kuwait Flour Mills and Bakeries (KFMB)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour source</td>
<td>Multi source, mainly from Australia</td>
</tr>
<tr>
<td>Protein content (dry gluten)</td>
<td>8.5-10%</td>
</tr>
<tr>
<td>Moisture content</td>
<td>13-14 %</td>
</tr>
<tr>
<td>Ash content</td>
<td>0.6-0.65%</td>
</tr>
<tr>
<td>Falling number</td>
<td>450-550</td>
</tr>
<tr>
<td>Iron</td>
<td>60 mg/kg</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>15 mg/kg</td>
</tr>
</tbody>
</table>
3.3 ARABIC FLAT BREAD INDUSTRIAL BAKING DESCRIPTION

In KFMB, flour water absorption and dough resistance for mixing were routinely measured using a Brabender Farinograph (Germany). The amount of water was adjusted to provide a dough consistency of 500 F.U. Water is added and mixed slowly for 4 minutes. The dry ingredients (see Table 3.1) were mixed for 1 minute. Mixing was continued at a faster speed for 6 minutes. The dough was left to rest in the fermentation room for 45 - 50 minutes at 30 °C. The bulk dough was then divided into balls of specific weight (60 and 90g for soft and normal bread, respectively). These balls were allowed to rest on the belt conveyers for 7-13 minute at 32 °C and 70 % RH. The dough balls were then passed under pressing rollers, to form oval sheets. The sheeting stage was performed in 2 to 4 stages (depending on the production line) to provide a circular shape of specific thickness (1.5 and 3mm for soft and normal bread, respectively). These sheets were allowed to final proof at 34 °C and 70 – 80 % RH for 17-27 minutes. It is worth saying at this stage that the resting time depends on the conveyer belts length. Baking was carried out at 700 °C for 74 seconds. Immediately after baking, bread was finely sprayed with water. This step will cool bread down, add glaze on the bread surface and remove extra flour from bread crust as a result of dusting with liberal amounts of flour during processing to prevent stickiness. The bread loaves were allowed to cool for sufficient time prior to packaging (5 - 7 minutes in winter and 9 - 11 minutes in summer).

3.4 ARABIC FLAT BREAD LABORATORY BAKING DESCRIPTION

3.4.1 Dough preparation

The ingredients mentioned in section 3.2 were mixed using 100 g of flour (with the other ingredients scaled according to the stated ratios) for 4.5 minutes at
Experimental Methodology

speed 4 in a Kenwood mixer (Chef Classic, Model KM 400, Havant, UK) as shown in Figure 3.1a. The settings of Kenwood mixer were adjusted in KFMB laboratories to mimic the performance of the industrial scale mixer at KFMB. A Brabender Farinograph (Germany) was used to determine the mixing time and speed, and adjust the amount of water to provide a dough consistency of 850 Farinograph units (F.U.). The mixture was left to rest in a sealed plastic bowl (Figure 3.1b) for 60 minutes at 30 ± 1 °C and 70 % RH in a fermentation cabinet (Figure 3.2). The bulk dough was then divided, rounded and allowed to rest for another 10 minutes under similar conditions (Figure 3.3a).

Figure 3.1 (a) KENWOOD mixer, (b) fermented dough in lined plastic bowl.
Finally, dough balls were sheeted to a round shape of 2 mm thickness and 14 cm diameter, and allowed to rest for the final proof period of 30 minutes under similar conditions (Figure 3.3b). Pita bread is traditionally made in a circular shape. Recently, oval shapes started to appear. For these experiments it was decided to make in a circular shape to simplify the geometry, a circular moulder was used to cut the sheets to the desired shape.

It is worthwhile to note that in some previous studies (e.g. Sidhu et al., 1997b) it was reported that the equipment and dough were dusted with flour during cutting, rounding and sheeting to prevent the bread sticking to the equipment surfaces. However, this flour may affect the DSC endotherms. In order to avoid the need of dusting in this study, the plastic bowl and the sheeting area were instead lined with grease-proof paper.
Figure 3.3(a) Dough balls and resting box used for proofing in Arabic flat bread baking, (b) A hand-rolled dough ball to 2 mm sheet thickness with the circular mould.

Dough samples for DSC pan selection experiments and baking simulation experiments were prepared in the same way as for the pita dough but without adding the yeast. They were mixed at the same speed and allowed to rest for 1 hour at 30 ± 1 °C and 70 % RH before loading into DSC pans.

3.4.2 Laboratory-oven-baking

The dough sheets were subsequently baked at an oven temperature of 600 ± 10 °C for 25 ± 1 second in a high temperature oven (maximum temperature 1100 °C, Carbolite Type BWF 11/13, Sheffield, UK). A type K thermocouple was used to measure the temperature inside the dough during baking. The
The thermocouple was placed in the centre of the baking tray in the oven with the tip angled upwards before placing the dough sample so that it penetrated the dough sheet when the dough was placed on to the tray. The oven was also instrumented with a thermocouple positioned at the upper left corner of the back wall. A photograph of the oven showing pita bread at the end of a baking period is shown in Figure 3.4.

Figure 3.4 Baking of Arabic flat bread in laboratory oven.
3.4.3 High pressure oven (HPO) baking

3.4.3.1 HPO design

An experimental oven was designed and constructed to study the effect of pressure on Arabic flat bread baking. Figure 3.5 depicts the schematic diagram of the oven while Figure 3.6 shows the picture of full equipment. This oven is equipped with:

**Baking chamber**; this oven was made in two halves, an upper and lower half (see Figure 3.7a and b). The upper half contained a circular heating element the temperature of which was measured and controlled via a PID controller. The lower half consisted of a similar heater which received the same power as the upper heater. A ceramic plate was positioned above the lower heater (but not contacting) upon which the bread would be placed. The top half of the oven could be moved up and down using a foot operated pneumatic positioned to “open” or “close” the oven, respectively. Once closed, the oven would be immediately pressurised (if desired) with air from a compressed air-line, via a 3 way valve which could also be set to closed (to isolate the oven) or open to ambient (to rapidly vent the oven). The operating pressure in the oven was controlled using a pressure relief valve which would vent if the pressure in the oven exceeded a preset limit. The pressure in the oven was determined from a manual pressure gauge.

**Temperature/Process Controller**; which consists of a PID controller (OMEGA Model CN7500) (Figure 3.8) and a type K thermocouples attached to the upper heaters. PID system is equipped to facilitate oven temperature measurements and control. The upper temperature limit was set on 450 °C. The bottom heater can be switch on only if the upper heater was switched on. Type K thermocouples were equipped to measure the temperature of air inside the oven and the temperature inside the pita bread.
Figure 3.5 Schematic diagram of high pressure oven.
Experimental Methodology

Figure 3.6 Picture of high pressure oven (HPO).
Figure 3.7 A closer picture to the baking chamber (a) front side, (b) back side.
Pressurizing system; which contains the air compressor (Figure 3.9a), the pipelines, the valves, the pressure gauges and the pneumatic positioner (Figure 3.9b). (CLARKE STRONG-ARM) The air compressor (HYDROVANE 15, The Hydrovane Compressor Co., LTD. REDDITCH, ENGLAND) is a machine that uses an electric motor to power a device that sucks air from the atmosphere, compresses it in a confined place to increase its pressure by making the volume smaller, and then transfers the high-pressure air to a receiver tank (WELDED PRESSURE VESSEL, REDNAL PNUMATICS, Oswestry, Shropshire, ENGLAND). Then, the compressed air will move through a main pipeline to a Domnick filter (E 006AA, DOMNICK HUNTER FILTERS, ENGLAND) which is a moisture trap filter (Figure 3.10a). This purifier will remove water vapour from the compressed air. The main pipeline will be split into two sub lines: the first line will be connected directly to the oven and will supply the air required to increase the pressure inside the oven during baking.
Experimental Methodology

Figure 3.9 (a) Air compressor and the air tank, (b) Foot operated pneumatic positioned.

Figure 3.10 (a) Domnick filter and 2-port valves (b) Three-way valve (on left) and pressure relief valve (on right).
The other sub line will be connected directly to the foot pump and will control the opening and closure of the oven using pneumatic positioner.

All these pipe lines are equipped with valves; which can be categorized into three types:

- Two-port valves; which can be either closed so that no flow at all goes through, fully open for maximum flow or sometimes partially open to any degree in between (see Figure 3.10a).

- Pressure valves or balanced relief valves are designed to manage and minimize the effect of back pressure. These valves are equipped with a pressure gauge and if these valves are not open directly to the atmosphere, pressure will build at its outlet (Figure 3.10b on right).

- Three-way valve (Figure 3.10b on left); which is used to permit to set the mode of the baking:
  - Fill mode: filling the oven cavity with compressed air to increase the pressure inside the oven
  - Shut mode: to maintain the pressure inside the oven cavity and isolate it
  - Vent mode: which is designed to discharge the air to the atmosphere

3.4.3.2 HPO baking procedure

Bread was baked under three different pressures (reading was taken from the pressure gauge attached to the oven): 0, 1 and 2 bar (all pressures quoted in
Experimental Methodology

this thesis are given as gauge pressures). The general method was as follows. The pressure relief valve was preset for the desired operating pressure and the 3-way valve closed. The oven was then preheated such that the top heating element reached 400 °C. For experiments at 1 and 2 bar the 3 way valve was set to “fill” to allow a flow of compressed air into the oven. The bread sample was then inserted into the oven and placed on the ceramic plate with a metal foil sheet to initially shield it from the top heater. The oven is then closed using the pneumatic positioner. This would typically take seconds. Just before closing the metal sheet is pulled away. The closure of the oven then immediately results in the oven pressure rising (for experiments at 1 and 2 bar). After the heating period the 3-way valve is set to “vent” and allowed to vent for a “vent time” which was dependent on the pressure (see Table 3.3), the oven opened, and the bread removed using a fish-slice. The total baking time (from insertion of the bread to removal of the bread was maintained at 50 seconds across all experiments.

Table 3.3 Baking parameters: pressure in bar and time in seconds, used in HPO

<table>
<thead>
<tr>
<th>Pressure (bar)</th>
<th>Vent time (seconds)</th>
<th>Total time inside oven (Seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>50</td>
</tr>
</tbody>
</table>
3.5 METHOD FOR MEASURING THE REAL TEMPERATURE INSIDE THE DOUGH SHEET DURING BAKING

A type K thermocouple was used to measure the temperature inside the dough during baking. The thermocouple was placed in the centre of the baking tray in both ovens with the tip angled upwards before placing the dough sample so that it penetrated the dough sheet when the dough was placed on to the tray.

3.6 STORAGE METHODS OF ARABIC FLAT BREAD

3.6.1 KFMB bread

The four types of bread were brought from the bakeries and transported directly to Central Analytical Laboratories (CAL) in Kuwait Institute of Scientific Research (KISR). The samples were then marked for 0, 1, 2, 3 and 4 days storage at room temperature or stored in the refrigerator. The temperature in the KISR’s laboratory during the entire 4-days storage period was 20 ± 2 °C, while the refrigerator temperature was 4 ± 1 °C.

For convenience, the samples were denoted by a combination of the initials of the parameters applied which are shown in Table 3.4. For instance, OSB-3 is designated for soft brown bread samples which have been kept outside the refrigerator at room temperature and stored for 3 days, whereas, FNW-2 is assigned for normal white bread kept in the refrigerator for 2 days. On the other hand, NW0 is designated for fresh normal white bread.

Moisture content analysis was measured in CAL (Kuwait). For DSC, a representative sample of each storage treatment group was ground and freeze-dried. The freeze-dried bread samples were stored in air tight containers and kept in a freezer in Kuwait (Figure 3.11). Then these samples were transported to the United Kingdom. By the time the samples had reached the UK, they had thawed. They were thus re-frozen again.
Table 3.4 Symbols used to designate different types of bread

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW</td>
<td>Soft White bread</td>
</tr>
<tr>
<td>SB</td>
<td>Soft Brown bread</td>
</tr>
<tr>
<td>NW</td>
<td>Normal White bread</td>
</tr>
<tr>
<td>NB</td>
<td>Normal Brown bread</td>
</tr>
<tr>
<td>0,1,2,3,4</td>
<td>Storage period in days, where 0 denotes for fresh bread</td>
</tr>
<tr>
<td>F and O</td>
<td>F denotes storing in refrigerator</td>
</tr>
<tr>
<td></td>
<td>O denotes storing at room temperature</td>
</tr>
</tbody>
</table>

Figure 3.11 Freeze-dried KFMB bread samples stored in air tight container.
3.6.2 Laboratory baked bread

After baking, the loaf was removed, left to cool for about 5 minutes, placed in a zip-lock plastic bag. The cooling time was determined as that required to cool the interior of the loaf to 38 °C as measured by a thermocouple (nominated temperature was recommended by KFMB). This temperature measurement would only be performed once (for the first loaf) in a series of experiments, with the same time being used for further trials. The samples were kept either complete in a zip-lock plastic bag, or small samples taken from them and stored in DSC pans to avoid moisture redistribution during the storage period. The storing techniques and temperature will be stated where necessary.

3.7 MOISTURE ANALYSIS

3.7.1 KFMB bread

Bread samples were analysed for moisture content by following AACC 44-40 method (AACC, 2000) which involved taking a bread sample of approximately 2g, tearing and placing it in an aluminium dish which was weighed before and after drying in an oven for 24 hours at 100 ± 2 °C. The moisture content was calculated by subtracting the weight of the aluminium dish with the sample after oven drying from its previous weight. The net weight was divided by the sample weight to produce the wet basis moisture content. On each day of storage period, KFMB bread samples were analyzed for moisture content (in CAL, KISR).

3.7.2 Laboratory baked bread

To assess the possible spatial variation of moisture (and the thermal behaviour of starch), the upper layer of laboratory bread (crust and crumb) was divided
into three zones: A, B and C. Zone C covered the central part of the loaf within a radius of 2 cm, zone A refers to the region outside a radius of 4 cm and zone B is the region in between zones A and C. Figure 3.12 shows a representative diagram of the sampling positions. Reported moisture contents are the average of two measurements from the same zone of five baked bread samples. Moisture content of bread was calculated to produce the wet basis \( \text{(MC}_w\text{)} \) using this equation:

\[
\text{MC}_w = \frac{M_1 - M_2}{M_1}
\]  

(3.1)

where

\( M_1 \) is bread weight before drying,

\( M_2 \) is bread weight after drying.

Figure 3.12 Representative diagram of bread division; where C represents the centre, B is the intermediate and A characterises the outer zone.
3.7.3 HPO moisture loss

For bread baked by HPO; moisture loss (\(\varnothing\)) during baking was also measured. Moisture loss was calculated using this equation:

\[
\varnothing = \frac{M_{\text{dough}} - M_{\text{bread}}}{M_{\text{dough}}} \tag{3.2}
\]

where

\(M_{\text{dough}} = \) dough weight before baking,

\(M_{\text{bread}} = \) bread weight after baking.

Moisture content (\(MC_{\text{HPO}}\)) of the bread was then calculated to produce wet basis using the following equation:

\[
MC_{\text{HPO}} = \frac{MC_{\text{dough}} - \varnothing}{1 - \varnothing} \tag{3.3}
\]

where

\(MC_{\text{dough}}\) is the moisture content of the dough (on wet basis) calculated from its recipe; which is equal to 0.447 (section 3.2.2).
3.8 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

A heat flux differential scanning calorimetry (DSC) (model DSC Q10, TA Instruments, Crawley, UK) (Figure 3.13) equipped with a thermal analysis data station was utilised to determine the onset temperature ($T_o$), the peak temperature ($T_p$), the offset temperature ($T_c$) and the enthalpy of starch gelatinisation ($\Delta H_g$) of unbaked dough samples and to follow the change in enthalpy of starch retrogradation ($\Delta H_r$) of bread samples during storage period at the selected conditions. Nitrogen (99.999% purity) was used as a purge gas and flowed at approximately 50 mL/min. The DSC instrument was calibrated for temperature and enthalpy using an indium standard. An empty reference cell was used which is common in the literature (Liu and Thompson, 1998; Indirani et al., 2000; Ottenhof and Farhat, 2004; Ottenhof et al., 2005). An isothermal period for 2 minutes was performed immediately preceding and following the scan so that a zero power baseline could be deduced. All thermal analysis data were recorded by the manufacturer’s software (TA Instruments Universal Analysis, TA Instruments, Crawley, UK). All samples were tested at least in duplicate and sometimes in triplicate. The enthalpy ($\Delta H$) was taken as the area enclosed by a straight line and the amylopectin endotherm curve in the region of 40-80 °C as shown in Figure 3.14. Since sample moisture content may affect the calculation, the starch retrogradation enthalpy ($\Delta H_r$) was calculated using this equation:

$$\Delta H_r = \frac{\Delta H}{1 - MC_w}$$  \hspace{1cm} (3.4)

where

$MC_w$ is the sample moisture content calculated from equation 3.1. (in the case of HPO bread $MC_w = MC_{HPO}$).
Experimental Methodology

Three different types of experiment were performed using DSC (all measurements made at least in duplicate):

3.8.1 Measurements of starch retrogradation in freeze-dried KFMB bread

The freeze-dried samples were analysed for moisture content in order to aid interpretation of DSC endotherms and to ensure that no moisture was gained during the process of transportation.

The choice of freeze-drying the KFMB samples for DSC analysis was based on two main reasons:

1. Difficulty of keeping the sample fresh and moulds free until thermally analysed at Loughborough University laboratories.
2. Following Sidhu’s method (Sidhu, et al., 1997b) in following up starch retrogradation in the same bread type, except that no water was added to the freeze-dried samples for reason that will be discussed later.
An aluminium sample pan (approximately 5 mg) and empty reference pan were used. Samples were held at 20 °C for 2 minutes and then heated to 160 °C at a heating rate of 5 °C/min, and held for 2 minutes.

Figure 3.14 Calculation of ΔH as the area enclosed by straight line and the endotherm curve.

3.8.2 Pan types

Two types of pans from TA were used: aluminium pans (Al pan) and high-pressure stainless steel pans (HPP) (Figure 3.15). Dough samples were weighed into the two different pans and were hermetically sealed to avoid moisture leakage. An empty pan of each type was used as a reference pan. Samples were placed in a DSC microfurnace and were subjected to the above method to measure the starch gelatinisation enthalpy.
Experimental Methodology

Figure 3.15 Types of DSC pans used: Aluminium pans (Al pan) and high pressure stainless steel pans (HPP).

3.8.3 Measurements of starch retrogradation in laboratory-baked bread

Preliminary studies had shown that storing bread crumbs inside pans gave better DSC results than that kept in bread. Scanning of laboratory-baked bread samples was performed after storing in HPP at 20 °C for 1 hour, 1 day, 2 days and 3 days after baking. Samples were taken from the crumb only (not exterior crust) from zones A, B and C after storing the whole bread sample in sealed packaging for one hour. Another set of samples were stored at 4 °C to confirm the starch retrogradation trend.

For HPO bread, samples were taken from puffed bread from dark and light coloured part. For unpuffed bread, samples were taken from bread crumb avoiding the edges. HPO samples were stored at 4 °C (all measurements made in triplicate).

A sample pan and an empty reference pan were subjected to the following temperature programme: isothermal at 20 °C for 2 minutes and heated to 160 °C at a rate of 3 °C/min. They were held at this temperature for 2 minutes.
3.8.4 Thermal analysis of unbaked dough samples

Dough samples kept in HPP were heated in DSC from 30 to 105 °C at scan rates of 20 or 200 °C/min. The pans were either scanned immediately to measure the residual enthalpy or stored for up to 7 days at 4 °C and rescanned. The rescan heating was from 20 °C to 160 °C at 3 °C/min. On the other hand, and for the sake of investigating the moisture loss during baking at different rates, DSC pans were used without sealing and subjected to the previous baking programme. Moisture loss was calculated using equation 3.2.

3.9 TEXTURE EVALUATION OF ARABIC FLAT BREAD

Bread firmness was determined according to the AACC approved method 74-09 (AACC, 2000) using a texture analyzer (TAXT2i, Stable Micro Systems, Godalming, Surrey, UK) at 20 °C. The texture analysis was performed at the Division of Food Sciences in University of Nottingham. Bread was compressed with a 25 mm flat aluminium plunger up to 40 % maximum strain at a speed of 1.7 mm/second. Pre-test speed and post-test speed were 1.0 mm/second and 10.0 mm/second, respectively. The bread was laid on the Texture analyzer platform, and the distance between the platform and the plunger was set to 5 cm. Once the run is completed, the plunger would return to the start position. Although that compression tests are usually conducted on crust-less high or medium volume bread, the crust in the case of flat bread is very thin and difficult to be removed without taking parts of the crumb. For this reason, the first 25 % of the analysis was discarded. The bread firmness parameters were defined as the force at 40 % strain minus the force at 25 % strain. This was implemented using a user defined MACRO in the texture analyses software (Exponent version 5.0.7.0).
Measurements of all the above quality parameters were performed for three replicate bread samples. For unpuffed samples four measurements were made on each bread sample. For puffed samples four measurements were made from both “light” and “dark” regions.

3.10 STATISTICAL ANALYSIS

Analyses were performed using Sigma Plot 11.0 to evaluate whether changes in sample location and storage time had a statistically significant effect (above that caused by sample on sample variation), following a similar methodology to that used by Rasmuseen and Hansen (2001). Data were analysed by either one-way or two-way analysis of variance (ANOVA) followed by paired comparisons (t-tests) of the means when the ANOVA analysis indicated significant differences. A 95% confidence level (P < 0.05) is used throughout to determine significance. Bar charts show mean values with error bars denoting standard deviations.
4 PRELIMINARY STUDIES ON KFMB BREAD

4.1 INTRODUCTION

This study is considered as a key stage to identify closely the breadmaking process and the main conditions that may affect the whole process. This field work helped in transferring the Arabic flat breadmaking process from the large industrial scale to a smaller laboratory scale; which will be discussed comprehensively in Chapter 5.

Furthermore, this study was conducted in order to investigate the moisture content and the retrogradation as measured by DSC of Arabic flat bread produced by Kuwait Flour Mills and Bakeries (KFMB). These preliminary tests helped to find the general characteristics and the proper methodology and techniques to be followed in thermal analysis for this type of bread. Bread moisture content and freeze drying were done in Central Analytical Laboratories (CAL) in Kuwait Institute for Scientific research (KISR, Kuwait), while the thermal analysis was conducted in Loughborough University.

4.2 DETERMINATION OF STORAGE PERIOD

The establishment of an appropriate storage period for the analysis of Arabic flat bread is of great importance in planning and designing the structure of the experiments. In fact, there are two major factors that must be considered in estimating the storage period; time and temperature of storing.

Although that the shelf life of Arabic flat bread was reported to be very short (Sidhu et al., 1997b). In this study the basic storage period was adjusted on the time required for the appearance of moulds in any type of Arabic flat bread produced by KFMB. Moulds were detected first in the soft brown bread after 4 days of storage at room temperature. No moulds were found in bread stored in the refrigerator even when the storage period was extended to 10 days. Therefore, it might be suggested that the storage period would be adjusted to 4
days during the current study for all types of bread whether if it was produced by KFMB or baked in Loughborough University.

4.3 MOISTURE CONTENT

The results of moisture contents for samples stored at refrigerator at 4 °C and those kept at room temperature at 20 °C are depicted in Figure 4.14.1 and Figure 4.2. The analysis was performed in Kuwait using the method described in Section 3.7.1. Differences in moisture content which were observed between the different types of bread were expected and can be attributed to the different water content and ingredients used to produce each type of KFMB flat bread. The highest moisture content was recorded for normal brown bread (30.45%) and the lowest was recorded for soft white bread (24.74%). The moisture content was also detected to be higher in the brown bread than white bread during the storage period; which is due to the fact that extra bran absorbs more water (Kihlberg, et al., 2004; Onwulata, et al., 2008; Seyer and Gélinas, 2009). While, the moisture content in both soft bread types were less than that those detected for corresponding normal types; which can be attributed to the higher crust : crumb ratio which leads to higher water evaporation during baking. Sugar content in the soft bread was the other reason; sugar is known to inhibit swelling of starch granules during baking (Olkku and Rha, 1978). In a previous study on the same type of bread (Sidhu et al., 1997a), the researchers reported that the moisture content of the normal white bread and brown bread to be 28.54 % and 28.26 %, respectively. These results are lower than the results presented here, however, the tendency of brown bread to be of higher moisture content was shown. On the other hand, Abu Ghoush (Abu Ghoush et al., 2008a and b) reported moisture content of 32 % for fresh white Arabic flat bread produced in Jordan.
On the fourth day of storage period, significant moisture loss was found for all types of bread; whether it was stored at room temperature (P{sub}\text{SW}=0.015, P{sub}\text{SB}≤0.001, P{sub}\text{NW}=0.001, P{sub}\text{NB}≤0.001), or it was stored in refrigerator (P{sub}\text{SW}=0.018, P{sub}\text{SB}≤0.001, P{sub}\text{NW}=0.001, P{sub}\text{NB}≤0.001). Moisture loss was found to be higher in brown bread, and found to be also higher in the normal types compared to the soft ones. Sidhu and his Co-Workers (Sidhu et al., 1997a) did not report any significant moisture loss during storage for the normal bread types and they attributed their results for the packaging type, although that the packaging type used in this study was the same (Neck-lock plastic bag).

Figure 4.1 Moisture content in bread stored in the refrigerator (FSW = soft white bread, FSB = soft brown bread, FNW = normal white bread, FNB = normal brown bread and F = refrigerator).
A study on the effect of storing temperature was depicted in Figure 4.3 to Figure 4.6. A statistically significant interaction between storage temperature and period was only found in white bread ($P_{SW}=0.012$, $P_{NW}=0.021$); where a higher decrease was found for bread stored at room temperature. Similarly, Sheikh et al. (2007) found a decrease in moisture content in Chapatti (Indian single-layered flat bread) stored at room temperature, however, the reduction in moisture content was examined over a longer storage period of one month. These researchers also recorded a decrease from 32.35% to 24.19% on the fourth day of storage at room temperature, while no significant decrease was reported for the same samples stored in a refrigerator.

Moisture loss from samples during storage can occur for two reasons. Firstly, the packaging may be permeable to water (even plastic is slightly permeable) or
poorly sealed. Secondly, moisture may leave the bread and condense on the inside of the packaging. Although the moisture contents did not fall appreciably during storage, these decreases were not large compared to others and were deemed acceptable.

Figure 4.3 Moisture content for soft white bread stored in refrigerator (FSW) and room temperature (OSW).
Figure 4.4 Moisture content for soft brown bread stored in refrigerator (FSB) and room temperature (OSB).

Figure 4.5 Moisture content for normal white bread stored in refrigerator (FNW) and room temperature (ONW).
Figure 4.6 Moisture content for normal brown bread stored in refrigerator (FNB) and room temperature (ONB).

4.4 DIFFERENTIAL SCANNING CALORIMETRY

DSC was utilized to determine the peak temperature ($T_p$) and to study the change in enthalpy of starch retrogradation ($\Delta H_r$) of bread samples during storage period at room temperature (20 °C) or in refrigerator (4 °C). Analysis was carried out in triplicate and the starch retrogradation enthalpy was taken as an average calculation of the area enclosed by a straight line and the amylopectin endotherm curve of the first peak (40-80 °C). The freeze-dried samples were loaded into empty Aluminium pans (Al), placed in DSC microfurnace and heated from 20 to 160 °C at a heating rate of 5 °C/min.

The recorded results for fresh bread sample (Figure 4.7) had shown a peak in the range of 40-65 °C. Several possibilities emerge to explain this peak; the first possibility might suggest that amylopectin was not fully gelatinised during baking, while the second possibility suggested that this peak was due to a rapid
recrystallisation of amylopectin after baking. The third possibility considered the effect of liberal amounts of flour which are used to prevent dough sticking to the cutting and sheeting tools. Flour becomes gelatinised if heated in the presence of water and this would affect the experimental results (This was the reason for lining with grease proof paper). For this reason, the moisture contents of freeze-dried samples were measured again using AACC 44-40 and the results listed in Table 4.1 had shown minimal amounts of water hidden in the freeze dried samples, which discounts this possibility. The endotherms are thus likely to be due to retrogradation which must have occurred during the freeze drying process. On the other hand, these endotherms may not represent the enthalpy of amylopectin since the samples were freeze dried. It was reported previously the presence of excess water (water/starch ≥ 3) aids the measurements of DSC in order to bring the temperature of amylopectin melting below 100 °C (Ottenhof et al. 2005). The average enthalpy ($\Delta H_r$) and average peak temperature ($T_p$) values during the storage period are listed in Table 4.2 and shown in Figure 4.8 to Figure 4.15. They did not seem to give a clear trend for amylopectin recrystallisation ($R^2 << 0.8$ for all samples except for SB kept at low temperature $R^2 = 0.897$).
Figure 4.7 DSC endotherms for freeze-dried fresh bread; where NW=normal white bread, SB=soft brown bread, SW=soft white bread and NB=normal brown bread.
## Table 4.1 Freeze-dried samples average moisture content (MC) % ± standard deviation (STD)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day</th>
<th>Average Moisture Content % ± STD At 4 °C</th>
<th>At 20 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft White: SW</td>
<td>0</td>
<td>1.88±9.3E-03</td>
<td>1.88±9.3E-03</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3.44±1.7E-02</td>
<td>1.83±1.4E-03</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.53±1.4E-03</td>
<td>2.37±5.7E-03</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.12±3.6E-02</td>
<td>2.08±1.4E-03</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.53±6.4E-03</td>
<td>2.32±3.5E-02</td>
</tr>
<tr>
<td>Soft Brown: SB</td>
<td>0</td>
<td>1.63±1.8E-03</td>
<td>1.63±1.8E-03</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3.87±1.2E-02</td>
<td>2.67±2.1E-03</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.30±1.2E-03</td>
<td>2.40±6.8E-03</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.28±3.7E-03</td>
<td>2.72±1.5E-03</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.95±3.4E-02</td>
<td>ND</td>
</tr>
<tr>
<td>Normal White: NW</td>
<td>0</td>
<td>2.88±1.1E-02</td>
<td>2.88±1.1E-02</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.21±2.8E-04</td>
<td>2.81±2.1E-03</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.30±1.4E-02</td>
<td>2.77±1.6E-03</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.67±2.1E-02</td>
<td>2.82±2.8E-02</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4.68±2.1E-02</td>
<td>0.29±7.6E-04</td>
</tr>
<tr>
<td>Normal Brown: NB</td>
<td>0</td>
<td>0.02±4.9E-04</td>
<td>0.02±4.9E-04</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2.18±3.9E-03</td>
<td>0.77±2.8E-03</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.47±2.1E-03</td>
<td>2.50±6.9E-03</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.37±6.9E-02</td>
<td>2.82±6.6E-03</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.70±6.3E-02</td>
<td>4.57±2.2E-04</td>
</tr>
</tbody>
</table>
Table 4.2 DSC peak temperature ($T_p$) and the enthalpy values ($\Delta H$, J/g) for KFMB bread stored in refrigerator (4 °C) and bread kept on shelf at room temperature (20 °C) (average ± standard deviation)

<table>
<thead>
<tr>
<th>Bread Type</th>
<th>Day</th>
<th>Bread kept at 4 °C</th>
<th>Bread kept at 20 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ave $T_p$ °C</td>
<td>Ave $\Delta H_r$ J/g</td>
<td>Ave $T_p$ °C</td>
</tr>
<tr>
<td>Soft White: SW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>58.7±6</td>
<td>0.126±0.07</td>
<td>58.7±6</td>
</tr>
<tr>
<td>1</td>
<td>60.1±7</td>
<td>0.365±0.10</td>
<td>58.4±2</td>
</tr>
<tr>
<td>2</td>
<td>60.8±1</td>
<td>0.372±0.03</td>
<td>58.2±12</td>
</tr>
<tr>
<td>3</td>
<td>52.9±11</td>
<td>0.631±0.09</td>
<td>59.9±3</td>
</tr>
<tr>
<td>4</td>
<td>61.9±8</td>
<td>0.387±0.471</td>
<td>51.4±4</td>
</tr>
<tr>
<td>Soft Brown: SB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>59.6±3</td>
<td>0.055±0.028</td>
<td>59.6±3</td>
</tr>
<tr>
<td>1</td>
<td>54.8±4</td>
<td>0.273±0.23</td>
<td>55.2±10</td>
</tr>
<tr>
<td>2</td>
<td>54.2±4</td>
<td>0.497±0.12</td>
<td>48.6±9</td>
</tr>
<tr>
<td>3</td>
<td>54.2±5</td>
<td>0.552±0.08</td>
<td>60.1±1</td>
</tr>
<tr>
<td>4</td>
<td>53.3±5</td>
<td>1.176±0.2</td>
<td>ND</td>
</tr>
<tr>
<td>Normal White: NW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>50.4±7</td>
<td>0.497±0.09</td>
<td>50.4±7</td>
</tr>
<tr>
<td>1</td>
<td>49.8±8</td>
<td>0.711±0.25</td>
<td>46.1±3</td>
</tr>
<tr>
<td>2</td>
<td>63.3±8</td>
<td>0.363±0.32</td>
<td>46.6±1</td>
</tr>
<tr>
<td>3</td>
<td>59.9±5</td>
<td>0.511±0.15</td>
<td>58.2±3</td>
</tr>
<tr>
<td>4</td>
<td>52.7±5</td>
<td>1.025±0.26</td>
<td>48.8±7</td>
</tr>
<tr>
<td>Normal Brown: NB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>57.3±8</td>
<td>0.826±0.10</td>
<td>57.3±8</td>
</tr>
<tr>
<td>1</td>
<td>53.7±10</td>
<td>0.308±0.27</td>
<td>47.2±12</td>
</tr>
<tr>
<td>2</td>
<td>60.6±4</td>
<td>0.128±0.13</td>
<td>41.9±4</td>
</tr>
<tr>
<td>3</td>
<td>46.3±4</td>
<td>1.144±0.21</td>
<td>49.0±8</td>
</tr>
<tr>
<td>4</td>
<td>56.2±4</td>
<td>1.166±0.13</td>
<td>46.8±3</td>
</tr>
</tbody>
</table>
Figure 4.8 DSC endotherms for freeze-dried soft white bread stored at 4 °C (FSW) from 0 to 4 days.

Figure 4.9 DSC endotherms for freeze-dried soft white bread stored at 20 °C (OSW) from 0 to 4 days.
Figure 4.10 DSC endotherms for freeze-dried soft brown bread stored at 4 °C (FSB) from 0 to 4 days.

Figure 4.11 DSC endotherms for freeze-dried soft brown bread stored at 20 °C (OSB) from 0 to 3 days.
Figure 4.12 DSC endotherms for freeze-dried normal white bread stored at 4 °C (FNW) from 0 to 4 days.

Figure 4.13 DSC endotherms for freeze-dried normal white bread stored at 20 °C (ONW) from 0 to 4 days.
Figure 4.14 DSC endotherms for freeze-dried normal brown bread stored at 4 °C (FNB) from 0 to 4 days.

Figure 4.15 DSC endotherms for freeze-dried normal brown bread stored at 20 °C (ONB) from 0 to 4 days.
However, the variations in $\Delta H_r$ values for the same sample were unexpected; since that the samples were all freeze-dried using the same method. An example of these variations is depicted in Figure 4.16 for soft white bread stored at 4 °C.

Although the enthalpy values rise during storage, there were significant problems with reproducibility which meant that it was impossible to establish any trends. Some of the repeatability issues were suspected to be due to the use of aluminium pans which showed evidence of leaking. A further possible cause was that changes may occur within the bread during freeze drying (or even after that). Finally, it was also suspected that the variations in the thermal treatment may occur on different parts of the bread as a result of different thermal treatments.

Figure 4.16 DSC endotherms for soft white bread stored at 4 °C for 3 days showing different DSC responses.
4.5 CONCLUSIONS

This chapter provided a detailed analysis of the commercially produced bread in Kuwait. The industrial baking process was closely monitored to understand the nature of all baking steps. Another aim was to scale down the industrial process to laboratory scale process. Products were analyzed for moisture content in Kuwait after storage at 4 and 20 °C. Representative samples were ground and freeze-dried in Kuwait, and transported to the UK for analysis by Differential Scanning Calorimetry (DSC). The findings from this study showed moisture content variations between different bread formulations after baking; however the DSC analysis was inconclusive due to possible changes in the freeze dried bread.
5 SPATIAL VARIATION OF STARCH RETROGRADATION IN ARABIC FLAT BREAD DURING STORAGE

5.1 INTRODUCTION

The study of the staling of Arabic flat bread as followed by starch retrogradation required the analysis of bread characteristics directly after baking. This is due to the fact that Arabic flat bread is reported to lose its freshness after 6 hours of baking for normal brown bread, and less than 24 hours in the case of normal white bread (Sidhu et al.; 1997b). As concluded from the previous chapter, the freeze-dried samples could not give a reliable picture of this phenomenon. Therefore, the first task was to transfer the breadmaking process from the industrial-scale in Kuwait to the laboratory-scale in Loughborough. The work input level to the dough is decisive to the quality of the finished product. Studies have shown that bread quality is dependent on the amount of work given to the dough (Wilson et al., 1997). Olatunji et al. (1992) found that the quantity of water needed for mixing in the commercial bakery in Nigeria was about 10% less than on laboratory scale. Experimental parameters (ingredients weights, mixing speed and time, proofing time, temperature and relative humidity) were determined in KFMB laboratories. The settings of Kenwood mixer were adjusted also there to mimic the performance of the industrial mixing with larger amounts of ingredients. A Brabender Farinograph (Germany) was used to determine the mixing time and speed, and adjust the amount of water to provide a dough consistency of 850 Farinograph units (F.U.), although that lower consistency (500 F.U.) was used for the industrial scale to ease the transferring operation of the dough between different tools (cutting, rounding, sheeting) to be smoother and slippery. Other parts of the preparation process were also adjusted so that they matched the industrial process as closely as possible. The laboratory bread composition was chosen to be the same used for the production of normal white bread (flour, water, yeast and salt). This type of bread was selected due to its simple composition and to avoid complexity by reducing the number of variables that can affect flour constituents' behaviour such as bran
and sugar. Many initial baking trials were performed adjusting the oven temperature to achieve an acceptable bread product.

In this chapter a number of issues are explored.

1. The relative performance of Aluminium pans (Al pans) compared to high pressure stainless steel pans (HPP) to determine thermograms of freshly baked and stored bread.

2. Trial baking studies at Loughborough showed that different parts of the bread appear to receive different amounts of heat (as judged by different shades of colour) which will be likely to affect the state of the starch in the bread. Thus it was decided to divide the bread into zones to assess the effect of spatial variation on subsequent retrogradation. This does not appear to have been studied by previous researchers.

3. Due to the potential variability in results in baking experiments, further experiments were performed in which “baking simulations” were conducted in DSC pans. These tried to mimic the temperature-time profile encountered by the bread in a controlled environment, and to eliminate moisture content changes during retrogradation.

4. In order to conduct the “baking simulation” it is necessary to know the actual temperature profile of the bread during baking. To this end, an experiment was performed in which the temperature within the bread was monitored by a thermocouple.
5.2 BREADMAKING DEFECTS

“There is no single ideal loaf of Arabic bread” (Quail, 1996). Arabic flat bread has two types of defects: major and minor. Major defects include insufficient separation of bread layers, cracks on the surface of the crust and burned bread surface. On the other hand, existence of blisters or uneven crust colour, may be considered as minor defects (Qarooni, 1988). Almost all the bread made in Loughborough laboratories meets the main requirements of Arabic flat bread; which are the separation to two layers and the rollability. However some of those loaves suffered from the minor defects which may affect the reproducibility of the analysis. Loaves with such defects were discarded. The minor defects are:

5.2.1 Blisters

The existence of blisters in the small size laboratory baked bread (Figure 5.1) is undesirable and can also affect the thermal analysis results. Blisters were reported to occur as a result of moisture trapped in the dough during the final proofing, insufficient fermentation or the surface roughness caused during sheeting process (Quail, 1996).

5.2.2 Uneven crust colour

This can be shown clearly in Figure 5.2, and can lead to a conclusion of uneven baking conditions for the individual bread loaf. This figure had also elucidated that the door-side half of the loaf was subjected to less heat and thereby showed a lighter crust colour. The uneven crust colour was a sign of the heterogeneity in the amount of heat received by the different parts of the crust and this will lead to differences in the moisture content distribution and the extent of starch gelatinisation (Yasunaga et al., 1968; Faridi and Rubenthaler, 1986).
Spatial Variation of Starch Retrogradation in Arabic Flat Bread during Storage

Figure 5.1 Laboratory baked bread showing undesirable blister.

Figure 5.2 Laboratory baked bread showing an uneven baking.
In a recent study on flat bread, uneven crust colour was reported (Banoooni *et al.*, 2008). The researchers also reported that the surface colour was unique for each individual loaf. They attributed their findings to the difference in the heat transfer distribution within the crust of flat bread.

The variations between the baked loaves are familiar phenomena even in the large scale bakeries. This can be due to the special baking process of Arabic flat bread; in which the dough sheets are not fixed in pans and can push each other on the baking conveyers after puffing leading to differences in the amount of heat received; loaves at the centre of the oven will be subjected to more even heat intensity than that at the edges of the oven conveyers.

In the present study, although every effort was made to overcome any difference in baking procedures, the baked loaves were often different in appearance from each others. These differences can be expected to arise because only one bread can be baked at a time; letting the other loaves have a longer final proof time. The unavoidable variability in dough preparation and bread condition was previously reported to affect the thermal analysis (Schiraldi *et al.*, 1996).

**5.3 MEASUREMENTS OF THE REAL TEMPERATURE INSIDE THE DOUGH SHEET DURING BAKING**

Unlike pan bread; where thermocouples can easily follow temperature variations during the baking process (León *et al.*, 1997), no literature data were found regarding temperatures in Arabic flat bread. Besides the baking short time (25 sec in the present study), Quail (1996) attributed the scarcity in such information to the difficulty of monitoring the dough baking conditions under extremely high oven temperatures. Also, the physical contact of these thermocouples may damage the surface of the bread loaf.
Results depicted in Figure 5.3 show an initially high temperature as the thermocouple was fixed to the baking tray inside the hot oven before the dough was introduced. Introducing the dough (at 4 seconds) then cools the thermocouple, which stabilises initially at around 89 °C. During this time the bread will be warming from room temperature to approximately the recorded thermocouple temperature. It then encounters a small step change to 100.4 °C before slowly rising to 103.4 °C at which point the pita bread is removed from the oven.

During pita bread baking a crust forms which prevents steam escaping from the dough and leads to the well known phenomenon of pita bread inflation due to the internal generation of steam. It can be surmised that the initial trapping of steam caused by the formation of the crust produces the step change in temperature from 88.6 to 100.4 °C as steam pervades the interior structure. As
the pressure slowly increases inside the pita this then leads to a boiling point rise of the water. Assuming water is exhibiting its full saturated vapour pressure the internal pressure rise can be estimated to be 0.11 bar (values interpolated from steam tables from Haywood, 1972), and this drives the puffing of the bread. The full baking process is shown in Figure 5.4. These pictures were taken for inflation demonstration while the oven door was opened. Therefore, no analysis was carried on those bread samples.

There are also significant temperature gradients in the oven, where the oven temperature was recorded as 600 °C and the thermocouple reading before laying the dough was below that (485 °C). This may be attributed to the different positions of the two thermocouples which confirm that the upper layer of the bread was subjected to higher temperature than other parts of the bread.
Figure 5.4 Arabic bread baking process.
5.4 **MOISTURE CONTENT**

The moisture analysis results presented in Figure 5.5 (section 3.7.2) had shown heterogeneity in the distribution of moisture across the bread loaf immediately after baking. The intermediate zone B (Figure 3.12) has a higher moisture content than the other zones and a statistically significant interaction between time and position of the sample was found (P < 0.001). The explanation for the outer zone A being drier than zone B is most likely caused by it being closest to the side walls of the oven (where the heater elements are positioned) and directly facing the wall during puffing. An explanation for the central zone C being drier than zone B is less apparent, as it does not appear to receive more radiant heat. One possible explanation is that the central region may puff first, and the separation of the layers means that the top layer is insulated to some extent from the bottom layer (which receives all its heat via the top layer). Thus if less heat is transmitted to the bottom layer then there is more heat available for evaporation of water from the top layer. This explanation would require further verification in future studies. The other explanation is that the central zone C receives more radiant heat than zone B being subjected to heat radiant from two sides, or being the highest part in the loaf allows it to receive more intense heat as a result of heat transfer by convection. This convection occurs due to significant temperature gradients in the oven, where the oven temperature was recorded as 600 °C and the thermocouple reading before laying the dough was below that (485 °C). This may be attributed to the different positions of the two thermocouples and this may suggest that the upper layer of the bread was subjected to higher temperature than other parts of the bread.
Figure 5.5 Moisture contents at different positions in the upper layer of baked bread after storing in sealed packaging for 0, 1, 24 and 96 hours (A = outer zone, B = intermediate zone and C = centre zone).

Thus when studying pita bread baking (whether in a laboratory or industrial context), attention should be paid to the position of radiant heaters and the strong likelihood of a variation in radiant heat flux incident upon different parts of the bread, and the role that changes to the bread shape during puffing play in heat transfer.

After storing the bread for 24 hours, the moisture contents for all 3 zones were found to be almost the same (P > 0.05); indicating an almost complete equilibration. No significant change was recorded in the moisture content after the fourth day of storage (P > 0.05), which was expected as the bread loaf was packaged in a sealed plastic bag. The moisture content of commercial Arabic flat bread produced in Kuwait is 28.5% (Sidhu et al., 1997a), which is comparable to the results presented here.
The results obtained in this study are in agreement with moisture distribution results reported by Faridi and Rubenthaler (1984) relating to their work on Balady bread. These researchers also noted the importance of baking conditions which also affected the degree of starch gelatinisation. Similarly, the described results corroborate the results of moisture studies on Arabic flat bread carried out by Sidhu (Sidhu et al., 1997a) who reported an insignificant moisture loss after a storage period lasting four days. On the contrary, Shaikh et al. (2007) found a decrease in moisture content in Chapatti (Indian single-layered flat bread) stored at room temperature although it was kept packaged in sealed plastic bag. However, the reduction in moisture content was examined over a longer storage period of one month.

5.5 DIFFERENTIAL SCANNING CALORIMETRY

5.5.1 DSC of unbaked dough samples and comparison of pan types

As previous experiments (see chapter 4) showed evidence of possible leaks it was decided to trial the use of more expensive stainless steel pans. These have a higher thermal mass and so potentially slower thermal response but are designed to withstand higher pressures than aluminium pans (such as generated by evaporating water at high temperatures). Figure 5.6 shows DSC gelatinisation endotherms of dough samples (without baseline correction) using two different types of pans (Al pans & high pressure stainless steel pans (HPP)). Results obtained using both types of pans involve three peaks. According to previous reports (Donovan, 1979; Lelièvre and Liu, 1994), the first peak can be attributed to amylopectin gelatinisation with the available water, whereas the second peak is due to melting of remaining crystallites. The third peak is believed to be due to the melting of amylose-lipid complexes (Biliaderis et al., 1985). As the peaks overlap it is difficult to deconvolute the individual peak enthalpies (ΔHₚ), however the combined enthalpies of the first two peaks attributed to amylopectin gelatinisation were 4.7 and 7.4 J/g for Al pans and
HPP, respectively, but no significant difference between the peak temperatures ($T_p$) in their corresponding endotherms was observed.

Above 130 °C differences can be seen between the endotherms from the two pan types and this is attributed to the bursting of Al pans (which are not as mechanically strong as HPP) and subsequent leak of leaking of water vapour. Some Al pans (endotherms not shown) also appeared to leak at temperatures as low as 90 °C. Even low leaking temperatures have been reported by Yu and Christie (2001) for this type of pan. HPP ultimately leaked at a significantly higher temperature than 200 °C. The leaking may affect the thermal contact between the pan and the furnace, but more likely it induces further vaporisation of water which consequently affects the measured heat flow to the pan (Schiraldi et al., 1996). We strongly suspect that the “Peak III” enthalpy values

![Figure 5.6 Comparison of DSC gelatinisation endotherms of dough using a high pressure pan (HPP) and aluminium pan (Al), both at a scanning rate of 3 °C min⁻¹.](image)
reported by Sidhu et al. (1997b) from experiments using Aluminium pans are in fact due to water evaporation. Although the actual endotherms were not shown in their paper, this explanation is supported by the order of magnitude larger enthalpies reported for this peak (40 J/g) and the fact that the peaks consistently occur close to 100 °C. Consequently, further experiments after this point only used HPP.

5.5.2 Baked bread

Arabic flat bread was baked as described in section 3.3. An example endotherm using HPP for a fresh bread sample is shown in Figure 5.7. The sample was taken from the crumb in zone C but identical endotherms were found for samples from zones A and B. The endotherm suggest almost complete starch gelatinisation during the 25 seconds of baking as is evidenced by the lack of enthalpy peak in the range of 40 - 80 °C. A small peak was recorded at temperature ~ 100 °C. The detected peak at 120 °C can be attributed to the melting of amylose-lipid complexes which tend to reform rapidly upon cooling. Amylose is important in setting the primary structure of bread (Seetharaman et al., 2002), and this peak was consistently present during the storage period without a significant change.
Spatial Variation of Starch Retrogradation in Arabic Flat Bread during Storage

Investigation of the Factors Affecting the Staling of Arabic Flat Bread

Figure 5.7 DSC thermogram (HPP) of fresh bread crumb showing the melting of amylose-lipid complexes.

This result is in agreement with previously published results (Biliaderis et al., 1985; Gudmundsson, 1994; Fessas and Schiraldi, 2001; Hug-Iten et al., 2003). Crust samples were also analysed by DSC (using Al pans) but they showed no evidence of enthalpic peaks.

The thermograms from each of the three zones over 3 days of storage at room temperature (Figure 5.8 to Figure 5.10) show bimodal endotherms with peaks temperatures approximately around 62 and 98 °C. However only the first peak appears to grow over time, indicating that retrogradation (probably of amylopectin) continues on the second day after baking. The second peak remains more or less unchanged. Similar peaks were reported by other researchers (Zobel and Kulp, 1996; Sidhu et al., 1997b). It was also observed that the temperature ($T_p = 62 ^\circ C$) corresponding to the first peak is lower than
the temperature of the first gelatinisation peak shown in Figure 5.6 ($T_p = 69 \, ^\circ C$). A similar result was reported by León and his Co-workers (León et al., 1997).

These results were expected based on previous studies; a rapid gelation of amylose after baking, and a slower amylopectin recrystallisation which is expected to govern the retrogradation process during days of storage, although the second peak attributed to amylopectin was not reported previously in the studies on starch retrogradation in pan bread or buns.

Figure 5.11 shows the enthalpy values (the first peak in the range of 40-80 °C) of bread samples during storage were calculated using equation 3.4 (section 3.8). These results ($P < 0.001$) not only show an increase in the values of $\Delta H_r$ for all samples they also show that such an increase occurs at different rates. The increase was found to be highest in the intermediate zone (sample $MC_w = 29.4 \%$) followed by the value for the centre zone (sample $MC_w = 27.6 \%$). The rate of increase during the storage period at the outer zone was found to be lowest (sample $MC_w = 27.1 \%$). It had been reported previously that the moisture content is an important factor affecting the retrogradation of starch (Lelièvre, 1974; Donovan, 1979; Zeleznak and Hoseney, 1986; Lelièvre and Liu, 1994; Fukuoka et al., 2002). When samples were stored at lower temperature (4 °C), the same retrogradation trend was found (Figure 5.12), however, $\Delta H_r$ values were greater due to the effect of storage temperature in consistent with other studies (Schiraldi et al., 1996; Farhat et al., 2000).
Figure 5.8 DSC thermogram (HPP) of bread crumb stored at room temperature in outer zone (A).

Figure 5.9 DSC thermogram (HPP) of bread crumb stored at room temperature in intermediate zone (B).
Figure 5.10 DSC thermogram (HPP) of bread crumb stored at room temperature in centre zone (C).

The results for retrogradation mirror those for moisture content presented in Figure 5.5. This might be attributed to the difference in moisture content (although these are equilibrated after 24 hours) of these zones or to the different exposure to heat. To further explore this, experiments were performed in which dough samples were “baked” in a DSC with conditions of constant moisture content but different heating rates.
Figure 5.11 DSC peak enthalpy values (in temperature range 40-80 °C) for bread crumb stored in HPP at room temperature (20 °C) for 1, 2 and 3 days, where A=outer zone, B=intermediate zone and C=centre zone.

Figure 5.12 DSC peak enthalpy values (in temperature range 40-80 °C) for bread crumb stored in HPP in refrigerator (4 °C) for 1, 2 and 3 days, where A=outer zone, B=intermediate zone and C=centre zone.
5.5.3 "Simulated baking" in DSC

To achieve more controlled conditions with which to study the subsequent retrogradation of starch it was decided to perform experiments in which the temperature-time profile during baking was simulated in a DSC pan for a small dough sample of constant (unchanging) moisture content.

Two different heating rates 20 and 200 °C min\(^{-1}\) were selected in this study, whereby samples were heated to 105 °C, and held for 30 seconds. The pans were then cooled down at a rate of 50 °C min\(^{-1}\). The lower rate of 20 °C min\(^{-1}\) represents typical heating rates when baking pan bread and buns. León and his co-workers had reported a heating rate of 11.7 °C min\(^{-1}\) to simulate the baking of pan bread crumb (León et al., 1997). However, flat bread must be baked at much higher heating rates to achieve an acceptable product (Faridi and Rubenthaler, 1984; Quail et al., 1990). For example, Quail et al. (1990) baked Arabic flat bread in 21 seconds, giving an apparent heating rate of 200 °C min\(^{-1}\). The temperature-time data presented in Figure 5.3 suggest a temperature rise of approximately 80 °C in 20 seconds, corresponding to a heating rate of 240 °C/min. In this simulated baking, a heating rate of 200 °C min\(^{-1}\) was selected as being the maximum nominal heating rate that can be achieved by DSC.

5.5.3.1 Temperature/time profile

Results depicted in Figure 5.13 shows that sample heated at lower heating rate did not only take longer time in the whole baking simulation but also took longer time to reach the maximum temperature. This figure also shows that the measured pan temperature only reaches 97.6 °C at the end of the isothermal period in the case of the 200 °C min\(^{-1}\) heating rate, whereas 103.5 °C was reached in the case of the 20 °C min\(^{-1}\) heating rate.
Figure 5.13 Temperature/time profiles of DSC at different heating rates (20 and 200 °C/min) held for 30 seconds and cooled down at 50 °C/min

5.5.3.2 Moisture loss results

Moisture loss analysis results for samples heated at different heating rates in open DSC pans (section 3.8.4) are shown in Figure 5.14. As demonstrated from this figure, a significant decrease in moisture loss as function of heating rate was found. Samples heated at 20 °C/min had lower moisture contents (higher moisture loss) than those samples heated at 200 °C/min. This is likely, because samples heated at lower heating rates were subjected to a longer period of heating and pan temperature reached higher temperature (103 °C) (more intense heat). The pans also were held for 30 seconds at temperature higher than those in the case of 200 °C/min.
Figure 5.14 Moisture loss % of dough samples heated at different heating rate (20 and 200 °C/min) in DSC open pans.

This result came in agreement with the results reported on pan bread made of composite cassava-wheat flour by Shittu et al. (2007) who reported a significant effect of temperature on bread moisture content. These results also agreed with previous studies on double layered flat bread (Balady and Arabic flat bread) (Faridi and Rubenthaler, 1984; Qaroo, 1987; Quail et al., 1991). The researchers found that bread baked for longer times were drier due to the increased loss in bread moisture.

5.5.3.3 Immediate DSC rescans

Thermograms from the immediate rescans of samples are shown in Figure 5.15. Both samples show amylose-lipid melting occurring at just over 115 °C, but there appears to be an indication of incomplete gelatinisation in the
endotherms immediately preceding the amylose-lipid complex peak, the apparent area of which increases with heating rate. Some of this is due to thermal lag in the DSC as the measured pan temperature only reaches 97.6 °C at the end of the isothermal period in the case of the 200 °C min⁻¹ heating rate, whereas 103.5 °C was reached in the case of the 20 °C min⁻¹ heating rate (Figure 5.13).

Figure 5.15 DSC thermograms (HPP) from immediate rescans at 3 °C min⁻¹ of dough samples previously heated from 30 to 105 °C at heating rates of 20 and 200 °C min⁻¹.
5.5.3.4 DSC rescans after storing

When held in storage over 7 days at 4 °C, however, a significant endotherm appeared in the temperature range between 40 and 80 °C (Figure 5.16 and Figure 5.17). The enthalpy values are shown in Figure 5.18 and are much larger than those observed for conventionally baked pita bread. This may be due to the lower storage temperature and also the higher moisture content within the DSC pans (assumed to be the same as the initial dough moisture content $MC_{dough} = 44.7\%$ on wet basis). The increase in the enthalpy value with time was significant for both treatments ($P<0.001$). It appears that the lower heating rate samples show a slightly greater amount of retrogradation even though more starch was initially gelatinised.

![DSC thermograms from rescans at 3 °C min$^{-1}$ of dough samples previously heated from 30 to 105 °C (HPP) at heating rate of 20 °C min$^{-1}$, and then stored at 4 °C for 1, 3, 5 and 7 days.](image)

Figure 5.16 DSC thermograms from rescans at 3 °C min$^{-1}$ of dough samples previously heated from 30 to 105 °C (HPP) at heating rate of 20 °C min$^{-1}$, and then stored at 4 °C for 1, 3, 5 and 7 days.
Figure 5.17 DSC thermograms from rescans at 3 °C min⁻¹ of dough samples previously heated from 30 to 105 °C (HPP) at heating rate of 200 °C min⁻¹, and then stored at 4 °C for 1, 3, 5 and 7 days.

Figure 5.18 DSC peak enthalpy values (in temperature range 50-80 °C) for dough samples previously heated from 30 to 105°C (HPP) at different heating rates (20 and 200 °C min⁻¹) and then stored at 4°C for 1, 3, 5 and 7 days.
5.6 CONCLUSIONS

Despite the high oven temperatures used, thermocouple data indicate that temperatures within Arabic flat bread during baking do not rise more than a few degrees above the normal boiling point of water. This is enough, however, to provide sufficient internal pressure to drive the characteristic puffing that occurs in successful pita bread baking. Although after baking it would be expected that differences in moisture content will occur between the crust and crumb (interior) parts of Arabic flat bread, this study has also shown that there are noticeable variations in moisture content across the bread which is likely to arise in part from the different intensities of radiant heat reaching different parts. We thus recommend that the location of sampling should always be recorded when taking samples from pita bread for analysis. In this case it appears that a region halfway from the centre to the edge of the pita bread (zone B) retained the most amount of moisture. After one hour of storage the moisture gradients had largely disappeared (almost completely disappearing after 24 hours), but the intermediate zone then also produced the most retrogradation as measured by DSC over 3 days of storage at room temperature. This is presumably not a storage moisture content effect (which was almost the same for all parts of the bread during storage) but may be linked to the lower heating rate or evaporation rate during processing. When compared with retrogradation studies of samples heated at different rates in a DSC to simulate baking it was found that higher amounts of retrogradation were found in the samples heated at lower heating rates (but higher overall heat exposure). So it would appear that the only conclusion that would be consistent across both baking and simulated baking studies is that higher heating rates during processing reduce the amount of subsequent retrogradation. Heating rate has previously been found to influence starch granule swelling which is a kinetically constrained process (Patel & Seetharaman, 2006), and thus more experiments are required to explain the effect of heating rate on starch retrogradation of Arabic flat bread.
6 THE EFFECT OF APPLIED PRESSURE DURING BAKING ON STARCH RETROGRADATION AND BREAD FIRMNESS

6.1 INTRODUCTION

Arabic flat bread is reported to stale rapidly (Qarooni, 1988; Quail et al. 1991; Sidhu et al., 1997b) compared to pan bread. The use of additives such as enzymes and emulsifiers has shown a significant influence on pan bread firmness and shelf-life (Stampfli and Nersten, 1995) and even in flat bread such as Chapatti (Sidhu et al., 1989); which is single layer flat bread produced in India or even Arabic flat bread (Qarooni, 1988; Toufeili et al. 1995). However, it is the policy of KFMB to prevent any additive in the processing of this type of bread. Therefore, there is a demand to study the effect of breadmaking processing modifications on Arabic flat bread shelf life.

Several efforts were attempted to study the effect of altering processing techniques on the quality of Arabic flat bread; such as altering temperature/time combinations, changing mixing time or speed or even changing dough sheet thickness (Faridi and Rubenthaler, 1984; Qarooni, 1988; Quail et al. 1990). Recently, the application of submitting foods to high pressures has been rising due to its significant effect in obstructing microorganisms causing spoilage and reducing shelf life of the food products (Knorr et al., 2006). The application of high pressure on starch is believed to induce starch gelatinisation in a different mechanism than that induced by thermal treatments although high pressures must be used (> 300 MPa). However, such researches are rare in cereal based products (Bárcenas et al., 2010). Even more, there is no study found in the literature on the application of pressure higher than the atmospheric pressure in flat bread processing. This study will establish the first step in the studies of the effect of pressure on the baking of such bread.

As the inflation process which leads to layers separation is considered to be a required characteristic in the Arabic flat bread, care must be taken when applying pressure during processing. Therefore, the pressures used in this
study are limited to 2 bar. Pressure of 0, 1 and 2 bar were used (all pressures are quoted as gauge pressures).

The main effect of the application of pressure will be to raise the boiling point of water and thus increase the temperatures experienced by the bread as evaporative cooling appears to maintain the bread at the boiling point of water (see Figure 5.3). To verify the temperatures experienced by the bread, thermocouple measurements have thus been made and these are first presented in section 6.2.

Further additions were made to the experimental programme as follows. Further experiments were performed with “docked” bread, i.e. bread that was punctured with holes to avoid puffing (steam being allowed to escape through the holes). The lack of puffing would provide a more uniform and consistent heat treatment (avoid spatial variations of heat treatment) and also allow the effect of puffing to be investigated.

Additionally, texture analyses are important in determining and identifying the quality and shelf life of bread and might be considered as a direct measurement of bread firmness associated with staling. Researchers on flat bread used different texture analysis techniques as indication of bread toughness and the increase in firmness during storage period; such as penetration tests (Sidhu et al., 1997a; Başman and Köksel, 1999), and tensile test (Ghodke Shalini and Laxmi, 2007; Abu Ghoush et al., 2008a, b; Sheikh et al., 2007). Compression tests were utilized to follow firmness in Taftoon bread (Salehifar et al., 2010). Furthermore, stress-relaxation was also reported to be useful in texture studies (Ucles, 2003); however, it required longer time. This will affect the moisture content of the sample; which in turn will affect the results.

Nevertheless, all these methods are considered to be destructive to the bread sample. With the limitation of our bread samples and the knowledge of that
each bread sample is considered as a unique product (Quail, 1996), a new texture analysis method was investigated and applied.

The sections of this chapter will be:

- Measurements of oven temperature profile (Section 6.2)
- Measurements of moisture loss during baking (Section 6.3)
- Measurements of the enthalpic parameters (Peak temperature and melting enthalpy) of retrograded starch (Section 6.4), and finally
- Measurements of the firmness of the baked loaves during the storage period (Section 6.5).

6.2 MEASUREMENTS OF THE REAL TEMPERATURE INSIDE THE DOUGH SHEET DURING BAKING UNDER PRESSURE

Temperature measurements for puffed bread were performed using a type K thermocouple using a similar method that used in Chapter 5. Results depicted in Figure 6.1 to Figure 6.2 show initially high temperature as the thermocouple was fixed inside the oven before the dough was introduced. The thermocouple stabilized at around 118 °C when pressure was set on 0 bar. No step change in temperature was noticed in baking in HPO compared to the previous baking oven (Figure 5.3); owing that to the faster inflation process; which commences while the thermocouple is cooling down. When pita bread was baked at higher pressures (1 and 2 bar), the temperature inside the bread had elevated to higher values. This can be attributed to the increase in the water boiling point temperature as the pressure increased.
Figure 6.1 Temperature/time profile for baking of puffed Arabic flat bread of 2 mm thickness baked in HPO.

Figure 6.2 Temperature/time profile (duplicate) for baking of puffed Arabic flat bread of 2 mm thickness baked in HPO.
6.3 MOISTURE LOSS

Moisture loss analysis results (calculated using equation 3.2) for both puffed and unpuffed bread are shown in Figure 6.3 and Figure 6.4, respectively. As demonstrated from these figures, a significant increase in moisture loss as function of pressure was found for both types of bread ($R^2 = 0.9931$, $P \leq 0.001$ and $R^2 = 0.9982$, $P = 0.02$, respectively). This is likely, because increasing pressure leads to a higher evaporation rate due to baking temperature (as revealed from measurements of temperature profile), and some flashing off of moisture will also occur on depressurisation. No significant differences in moisture loss were found between the two types of bread ($P = 0.13$), despite the differences in interior temperatures recorded. This may be attributed to the formation of a similar crust in both types. Accordingly, it seems that bread puffing per se has no major effect on moisture loss of bread during baking. Bread moisture contents were also calculated according to moisture loss results. Table 6.1 had shown that bread with highest moisture loss is the one with least moisture content (as expected).
Figure 6.3 Moisture loss in the puffed bread loaves baked at different pressures directly after baking (average ± standard deviation).

Figure 6.4 Moisture loss in the unpuffed bread loaves baked at different pressures directly after baking (average ± standard deviation).
Table 6.1 Average moisture content of bread baked at different pressures in HPO (average ± standard deviation).

<table>
<thead>
<tr>
<th>Pressure (bar)</th>
<th>Moisture Content % ± STD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Puffed bread</td>
</tr>
<tr>
<td>0</td>
<td>34.12 ± 0.95</td>
</tr>
<tr>
<td>1</td>
<td>33.01 ± 1.2</td>
</tr>
<tr>
<td>2</td>
<td>31.44 ± 1.3</td>
</tr>
</tbody>
</table>

The results of moisture loss of HPO bread are in agreement with the results reported on pan bread made of composite cassava-wheat flour by Shittu et al. (2007) who reported a significant effect of temperature on bread moisture content. These results do not contradict with the results in Section 5.5.3 (Figure 5.14); where samples heated at higher heating rates (200 °C/min) lost less moisture than those heated at lower heating rates (20 °C/min). This is because in DSC “baking simulation” studies time was variable; lower heating rates runs were longer than those of higher heating rates, making the moisture loss a function of both temperature and time. The time was fixed to 50 seconds for baking bread in HPO at all pressure treatments, so there is only the effect of temperature change.

Similarly, previous reports on the same type of bread reported an increase in bread moisture content as the baking temperature increase (Faridi and Rubenthaler, 1984; Quail et al., 1990), however, the increase in baking
temperature was accompanied with a decline in baking time, in which their conclusions are associated to the effect of two combined parameters change in baking temperature and time rather than the effect of absolute baking temperature as in this study; where the baking time was set on a fixed value.

The effect of baking time on moisture loss is depicted in Figure 6.5. Unpuffed bread was baked at P=0 for various baking times (50, 60 and 70 Seconds). As revealed, a linear relationship was found between baking time and moisture loss; as baking time increased, the bread moisture loss during baking increased. Crust colour was also affected by increasing baking time; crust colour was darker for bread baked for longer time. Longer baking times were reported previously to give a lower moisture content and lower Arabic flat bread quality (Faridi and Rubenthaler, 1984; Quail et al. 1990).

On the contrary, Bárcenas et al. (2010) reported a permanent increase in moisture content for bread fermented at higher pressure; owing their finding to the increase in crumb water holding capacity at higher pressures. However, these researchers’ finding is not as contradictory as would first appear; Bárcenas study was conducted at pressures of 500 bar (50 MPa) and higher applied during fermentation stage, whereas the present study was conducted at absolute pressures between 1 and 3 bar applied during baking stage. No further analysis was applied on bread baked at different baking times.

Moisture content is a critical factor affects subsequent starch retrogradation and causes texture changes (Zeleznak and Hoseney, 1986; Farhat et al., 2000).
Figure 6.5 Moisture loss in the unpuffed bread loaves baked at P = 0 bar for different baking times directly after baking

6.4 DIFFERENTIAL SCANNING CALORIMETRY

6.4.1 Puffed bread

As a result of the inflation process, bread was divided into light and dark zones, and samples were taken from both zones. The development of dark colour increased with pressure as shown in Figure 6.6. Fresh samples of both areas did not show an endotherm peak in the temperature range of 40 - 80 °C suggesting an almost complete starch gelatinisation during the baking process. During the storage period, only light coloured areas showed growing of an endotherm in the assigned temperature range (Figure 6.7 to Figure 6.9), while for dark areas, a small endotherm had only been shown in the fourth day for bread baked at 0 bar. This may be attributed to that darker zones were baked at higher rates than lighter zones (being closer the upper heaters), and as suggested from DSC “baking simulation” studies (Chapter 5) that higher heating rates resulted in lowest retrogradation.
As observed from Figure 6.10, the increase in the value of melting enthalpy ($\Delta H_r$) as a function of time in light coloured areas suggests an increase in starch retrogradation. This pattern of dependency of starch retrogradation on storage time agrees with other researchers on starch and bread in general (Zobel and Kulp, 1996; Sidhu et al., 1997b; Seetharaman et al., 2002).

The data in Table 6.2 also show differences in the melting enthalpy as a function of pressure. Enthalpy values declined as pressure was increased. No significant differences were found between the enthalpies of bread baked at 0 and 1 pressure. However, the enthalpy values for bread at 2 bar had shown significant differences than other treatments. These differences can be attributed in a part to the differences in moisture contents between loaves as a result of elevation in pita temperature as reported previously (Zeleznak and Hoseney, 1986; Farhat et al., 2000), and the indirect effect of pressure on the baking heating rate; which will consequently affect starch retrogradation as revealed from baking simulation studies (Section 5.5.3).
Figure 6.7 DSC endotherms for puffed bread crumb (light part) baked under P=0 and stored at 4 °C.

Figure 6.8 DSC endotherms for puffed bread crumb (light part) baked under P=1 and stored at 4 °C.
Figure 6.9 DSC endotherms for puffed bread crumb (light part) baked under P=2 and stored at 4 °C.

Figure 6.10 DSC peak enthalpy values (in temperature range 40 – 80 °C) for puffed bread crumb (light part) stored at 4 °C.
Table 6.2 DSC onset temperature ($T_o$), peak temperature ($T_p$), offset temperature ($T_c$) and the enthalpy values ($\Delta H_r$ J/g) for HPO puffed bread (Light zone) stored at 4 °C (average ± standard deviation)

<table>
<thead>
<tr>
<th>Pressure bar</th>
<th>Day</th>
<th>Bread kept at 4 °C</th>
<th>$T_o$ °C</th>
<th>$T_p$ °C</th>
<th>$T_c$ °C</th>
<th>$\Delta H_r$ J/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 bar</td>
<td>0</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>43.2±2</td>
<td>58.6±1</td>
<td>74.2±3</td>
<td>0.944±0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>44.7±1</td>
<td>58.1±1</td>
<td>79.5±1</td>
<td>1.241±0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>47.4±1</td>
<td>59.1±1</td>
<td>74.8±2</td>
<td>1.735±0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>47.5±1</td>
<td>59.1±1</td>
<td>75±3</td>
<td>2.146±0.16</td>
<td></td>
</tr>
<tr>
<td>1 bar</td>
<td>0</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>47.2±1</td>
<td>60.3±1</td>
<td>79.8±0.3</td>
<td>1.087±0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>47.9±1</td>
<td>60.1±1</td>
<td>77.9±2</td>
<td>1.277±0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>47.8±1</td>
<td>59.8±1</td>
<td>77.0±1</td>
<td>1.600±0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>47.9±1</td>
<td>59.5±1</td>
<td>77.2±3</td>
<td>1.852±0.17</td>
<td></td>
</tr>
<tr>
<td>2 bar</td>
<td>0</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>50.1±1</td>
<td>61.1±1</td>
<td>78.7±2</td>
<td>0.456±0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>48.9±1</td>
<td>61.2±1</td>
<td>78.3±0.2</td>
<td>0.753±0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>45.1±2</td>
<td>58.3±2</td>
<td>78.7±1</td>
<td>1.027±0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>47.8±1</td>
<td>59.5±1</td>
<td>77.2±3</td>
<td>1.187±0.12</td>
<td></td>
</tr>
</tbody>
</table>
6.4.2 Unpuffed bread

To eliminate the variability caused by different thermal treatments associated with different parts of the bread during puffing as revealed from Chapter 5, a second set of experiments were performed which avoided bread puffing by making holes in the dough sheets. This means that the bread layers do not separate while baking and cause changes in the amounts of radiant heat being received in different parts of the bread. Thus each part of the bread receives a similar amount of heat and strong variation in dark or light regions does not exist.

DSC endotherms of unpuffed bread baked under different pressures and stored at 4 °C are shown in Figure 6.11 to Figure 6.13. The average melting enthalpy ($\Delta H_r$) of the peak in the range of 40 - 80 °C accompanied with their onset, peak and offset temperature are shown in Table 6.3. As noticed, increasing pressure resulted in an observed decline in peak temperature from 62.66 ± 1.99 °C at 0 bar to 57.56 ± 0.81 °C at 2 bar. For all three treatments, endotherms of fresh samples suggest almost complete starch gelatinisation during the baking process as is evidenced by the lack of enthalpy peak in the range of 40 - 80 °C, while the increase in the value of melting enthalpy as a function of time (Figure 6.14) suggests an increase in starch retrogradation in a similar pattern to that increase of the lighter zone in puffed bread.

Results in Table 6.3 also showed differences in $\Delta H_r$ as a function of pressure. Enthalpy values declined as pressure was increased. The significant differences were only found in bread baked at 2 bar. These differences can be attributed in a part to the differences in moisture contents between loaves as a result of elevation in pita temperature, and the indirect effect of pressure on the baking heating rate; which will consequently affect starch retrogradation (Section 5.5.3).
Figure 6.11 DSC thermograms of unpuffed bread crumb baked at P=0 and stored at 4 °C.

Figure 6.12 DSC thermograms of unpuffed bread crumb baked at P=1 and stored at 4 °C.
Figure 6.13 DSC thermograms of unpuffed bread crumb baked at P=2 and stored at 4 °C.

Figure 6.14 DSC peak enthalpy values (in temperature range 40-80 °C) for unpuffed bread crumb stored at 4 °C (average ± standard deviation).
Generally, the results of both studies (puffed and unpuffed breads) may suggest that pressure treatments during baking may lead to a decline in starch retrogradation or suppress starch recrystallisation during the period of storage. Previous studies had shown that high pressure processing has been known to affect high molecular weight molecules such as polymers and starches, causing phase changes and lower melting enthalpies (Ezaki and Hayashi, 1992; King and Kaletinç, 2009). However pressures used in this study are not high enough to destroy the crystalline structure. Based on the pressure-temperature (P-T) gelatinization phase diagram of wheat starch reported by Douzals et al. (2001) and other researchers work on the effect of pressure on starch gelatinization (King and Kaletunç, 2009) provided evidences that starch gelatinisation by pressure only starts at 300 MPa (3000 bar), and a complete gelatinisation at 600-700 MPa (6000-7000 bar) at 25 °C (Douzals et al., 1998; Douzals et al., 2001; Baks et al., 2008; King and Kaletunç, 2009). The extent of starch gelatinisation during baking is believed to affect subsequent starch retrogradation (Martin et al., 1991).

It is therefore probable that in our experiments the pressure indirectly affects the starch by altering the temperatures found within the bread (in much the same way as a domestic pressure cooker does). Highest pressure also (which presumably gives highest heating rate) gave the lowest retrogradation. This matches with the results of “baking simulation” by DSC; in which lower heating rate baking resulted in the highest retrogradation.

In addition, and as revealed from the temperature measurements inside the pita bread; higher pressure had a significant effect on bread moisture content; which will consequently influence the rate of starch retrogradation as reported earlier (Zeleznak and Hoseney, 1986). In contrast, Giovanelli et al. (1997) had reported a faster recrystallisation during storage for pan bread cooked at higher temperature owing their results to higher degree of gelatinisation found for bread baked at higher temperature. However, a direct comparison between different types of bread is difficult due to the differences between their characteristics.
Table 6.3 DSC onset temperature ($T_o$), peak temperature ($T_p$), offset temperature ($T_c$) and the enthalpy values ($\Delta H_r$ J/g) for unpuffed HPO bread stored at 4 °C (average ± standard deviation)

<table>
<thead>
<tr>
<th>Pressure bar</th>
<th>Day</th>
<th>Bread kept at 4 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$T_o$ °C</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>47.6±4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>52.0±1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>50.3±3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>47.5±3</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>46.2±3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>51.0±5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>48.1±2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>50.6±2</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>46.4±1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>49.3±3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>46.9±1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>47.1±1</td>
</tr>
</tbody>
</table>
6.5 TEXTURE ANALYSIS

Although that compression tests are usually conducted on crust-less high or medium volume bread, the crust in the case of flat bread is very thin and difficult to be removed without taking parts of the crumb. For this reason, the first 25% of the analysis will be discarded. Once the tests have been performed, data analysis should be treated differently than that used for pan bread. Therefore, a new MACRO was established for this type of bread. Values of the maximum force at 40% strain and the values of the force at 25% strain can be automatically obtained by this MACRO. The bread firmness will then be defined as the maximum force (at 40% strain) minus the force at 25% strain and a curve of this calculated force vs. time will be established.

6.5.1 Puffed bread

Figure 6.15 to Figure 6.17 show the change in firmness of different bread parts during the storage period. Significant differences between dark and light areas of bread were found. These differences could be attributed, at least in part, to moisture variations within the loaf. Dark areas (bread centre) tend to be stiffer during the first hours after baking for all pressure treatments. This can be attributed to lesser moisture content in that area compared to other areas since it faces directly the oven heaters. Firmness results of both parts were less for bread baked at lower pressure. Bread baked at higher pressure showed initially highest firmness in both dark and light part. This may be attributed to the lower moisture content in bread baked at higher pressure as revealed from Section 6.3.

However, after day 1, it is generally found that the light areas have a greater firmness. The switch in behaviour is likely to be due to moisture equilibration in the bread as it has been found (Al-Hajji et al., 2011) that moisture contents tends to equilibrate within 24 hours after baking, and it appears that the lighter regions are inherently firmer as storage progresses than the darker areas.
Figure 6.15 Firmness of different bread parts baked at 0 bar during 0-4 days (puffed bread) (average ± standard deviation).

Figure 6.16 Firmness of different bread parts baked at 1 bar during 0-4 days (puffed bread) (average ± standard deviation).
A statistically analysis of the firmness results confirmed that all three experimental parameters: pressure, position (light or dark areas) and storing day were significant ($P \leq 0.001$). But the effect of one factor is not clear at all combinations of the two other factors; and therefore an unambiguous interpretation of the main effects is difficult.

### 6.5.2 Unpuffed bread

In this set of experiments, inflation process was eliminated to reduce the variability between different parts of bread as a result of different thermal treatments which will consequently vary the moisture distribution within different parts of bread. The strong variation in dark or light regions does not exist. As observed from Figure 6.18, firmness of bread baked at $P = 0$ was initially lower than that of higher pressure baked bread. This may be attributed in part to the formation of thicker and darker crust colour for bread baked at higher pressure.
as revealed from their pictures in Figure 6.6. Significant changes in bread firmness were detected during storage period for all samples baked at different pressures. Both pressure and storage period are significant parameters (P ≤ 0.001). This figure also suggests that that the firmness of bread baked at pressure 1 and 2 bar significantly decrease after 1 day, attributing that to the moisture transfer for the crumb to the crust. Earlier study for Piazza and Masi (1995) had reported moisture redistribution in pan bread from the crumb to the crust.

For bread baked at 0 bar, a significant increase in the firmness rate was only found between day 1 and day 2. No significant change was found before or after that period.

After day 1, bread firmness increased at different rates, and was highest for bread baked at 2 bar, while no significant difference in firmness was recorded between bread baked at 0 and 1 bar. Bread firmness continued to increase and a significant change in firmness was recorded among the different baking pressures at the third day, in which after that no significant increase in the firmness for all loaves. These results may suggest that pressure may affect the firming rate of flat bread by increasing the baking temperature; which will result in a higher moisture loss. Firmness is believed to be affected by bread moisture content (Faridi and Rubenthaler, 1984).

These results were inconsistent with the results on the effect of baking temperature reported earlier, that lower baking temperatures gave softer crumb (Giovanelli et al., 1997; Shittu et al., 2007). However sensory tests on Arabic flat bread demonstrated that the first perception of hardness by a trained panel corresponds to bread baked at lower temperatures (Faridi and Rubenthaler, 1984; Quail et al., 1990). These findings did not conflict with the results reported in this study since that baking times were altered in the other studies; making Arabic flat bread baked at lower temperature and for longer time of lower moisture content.
These results apparently contradict the thermal analysis results which favoured higher pressures to reduce retrogradation. However, other studies have indicated (Dragsdorf and Varriano-Marston, 1980; Ghiasi et al., 1984, Roger et al., 1988; Martin et al., 1991; Giovanelli et al., 1997; Rasmussen and Hansen, 2001) that bread firming is not synonymous with starch retrogradation, and so other factors must be at play.

6.6 CONCLUSIONS

While bread was baked in HPO at different absolute pressures ranging from 1 to 3 bar, the temperature inside pita bread generally elevated as pressure increased. This elevation in pita temperature directed toward higher moisture loss, which in turn is believed to affect the starch retrogradation during storage time as well as the bread firmness.
Thermocouple data indicate that temperatures within Arabic flat bread during baking increased as a function of pressure leading to more water evaporation and moisture loss.

There was no significant effect of bread puffing on moisture loss during baking. In both puffed and unpuffed bread, moisture loss was found to increase significantly as a function of pressure.

The thermal analysis of puffed bread had shown that retrogradation mostly found in light areas; likely because these areas were subjected to lower heating rate baking compared to the darker areas. Similar trend had been found for unpuffed bread. The thermal analysis had shown that bread baked at higher pressure resulted in the lowest retrogradation. The pressure indirectly affects the starch by altering the temperatures found within the bread. Highest pressure also (which presumably gives highest heating rate) gave the lowest retrogradation. This matches with the results of “baking simulation” by DSC; in which lower heating rate baking resulted in the highest retrogradation.

Inflation process during baking affected not only the moisture variations within the bread and the thermal analysis as suggested from Chapter 5, but also affected the firmness force. Parts of the bread that received most radiant heat (as determined by their darker colour) showed less development in firmness during storage (whilst being firmer immediately after baking) than the less heated regions (of lighter colour). The firmnesses found with lighter regions of the puffed bread corresponded relatively well with those from the unpuffed bread. Pressure did not affect the firmness in the early stages of storing, but significant changes in bread firmness were detected later.

This study was in consent with other studies (Zobel and Senti 1959, Dragsdorf and Varriano-Marston 1980, Ghiasi et al. 1984, Levine and Slade; 1988, Rogers et al.; 1988, He and Hoseney; 1990, Martin and Hoseney; 1991, Martin et al.; 1991, Giovanelli et al., 1997) in that starch retrogradation is not the only factor in bread staling and the development of bread firmness. Other factors and mechanisms must be involved.
7 CONCLUSIONS AND FUTURE WORK

7.1 GENERAL CONCLUSIONS

Bread is considered to be one of the oldest processed foods and most consumed by humanity. Bread is accepted as a general term that refers to variety of products. These variations resulted from the different ingredients used to prepare them, besides the different cooking methods. The environmental and cultural differences may also play a significant role in determining preferences. These different bread types have different characteristics and quality attributes, however, bread remains to be a good source of carbohydrates which provides energy for the body.

In the last few years, the price of bread has continuously increased; however, its shelf-life is still limited by changes in its texture leading to loss of its freshness. These changes can be encompassed in a single word, “staling”. Delaying staling is of great industrial and academic significance. As revealed from the literature review (Chapter 2), the staling mechanism is now still not fully understood. Different opinions have been suggested with very few concrete conclusions for the staling of bread. All researchers had agreed on that firmness was a major sign that can be used to monitor bread staling; however, they diverge on the origin of this firmness. Since the starch is found to be a major constituent in bread flour; many believed that starch retrogradation is a major factor in bread firmness, and consequently followed amylopectin recrystallisation in aged bread (Hug-Iten et al., 1999; Ribotta et al., 2004). Other researchers have found that bread firming is not synonymous with amylopectin recrystallisation in bread and suggested different mechanisms for bread firming showing the role of other starch constituents (Maleki et al., 1980; Martin et al., 1991).

Previous studies on bread staling were conducted mainly on pan bread. Studies of the staling of flat bread have been limited and mostly concentrating on sensory evaluations. Variations between the characteristics of each type of
bread were overlooked. These variations may have an effect on the bread constituents’ behaviour while processing and storing. For this reason, there is a need to develop techniques that can perform precise evaluations of the analysis of flat bread.

The study on KFMB flat bread (Chapter 4) was conducted on flat bread that differs in thickness and composition. It showed that different moisture contents occur with different formulations with lower moisture contents arising in soft bread (containing sugar) compared to normal bread (not containing sugar) and lower moisture contents also arising in white bread compared to brown bread. However, studying the staling process via DSC of freeze drying samples and analysing them after many days transport did not give conclusive results. So the process was replicated in the UK to give the same dough consistency and proofing times as used in the KFMB trials. An optimum thickness (2 mm) of bread was determined and it was also decided to stick to the simplest composition, which was normal and white (i.e. without sugar and bran) for further trials.

Some differences in method from that used by Sidhu (1997b) were implanted also. Firstly, the raw dough was not coated with flour to avoid the dough sticking to surfaces, but rather greaseproof paper was used. The reason for this was to avoid the surface flour contaminating the DSC response. Secondly, high pressure stainless steel pans (HPP) were used instead of aluminium pans (as used by Sidhu et al., 1997b) as these were found to provide better baseline stability due to lower suspected amounts of leakage. It is suspected that many of Sidhu’s DSC results are confused by the evaporation of water as the enthalpies reported are of the order of magnitude expected for water evaporation.

In Chapter 5, it was shown that the sample position within Arabic flat bread has a significant effect on the DSC response. The region halfway from centre to the edge of Arabic flat bread had shown most retrogradation. This region was also
the highest in moisture content directly after baking. Retrogradation continued
to increase over the storage period with the same trend, even though moisture
content (which affects retrogradation rates) equilibrated within 24 hours of
baking.

Heating rates of have been found in the literature to influence starch
gelatinisation and retrogradation (Chapter 2). These experiments used a DSC
as a "baking simulator"; however, these studies used heating rates appropriate
to pan bread baking and not the high heating rates used in the baking of flat
bread. When samples were heated at different heating rates and with equal
moisture contents in HHP (Chapter 5), higher amounts of retrogradation were
found in samples heated at lower heating rates. As the pans were closed then
this could not have been caused by differences in moisture contents.

This study is the first to report temperature measurements within pita bread
during baking. Temperature measurements in a conventional oven (Chapter 5)
showed temperatures similar to the expected boiling point of water. This was
expected as the driving force for puffing of flat bread is internally generated
steam pressure. The measured temperature experienced a small step change
during the latter stages of cooking which corresponded to the beginning of
inflation as the pressure increases from ambient due to the formation of a crust.

This finding suggested a way of influencing internal baking temperatures by
regulating the applied pressure. This was achieved using a specially designed
high pressure oven (Chapter 6). This produced higher internal temperatures as
expected. Experiments with docked samples (which were consequently unable
to puff) showed much higher temperatures (of the order of 100 °C compared to
the puffed temperatures) and these were attributed to the thermocouples
measuring the actual bread temperature as there was no steam pocket. The
moisture contents of the docked (unpuffed) samples were very similar to the
puffed samples which suggests the crust formation is similar in both and that
the temperatures experienced on the crust are probably quite similar in the two cases.

The effect of pressure on starch retrogradation and bread firmness during subsequent storage was also then examined to assess whether high pressure processing might have a beneficial effect. It was found that whilst higher pressures gave less retrogradation (as might be expected given the results on Chapter 5) the texture measurements did not bear this out. Indeed the samples processed at ambient pressure showed lower increases in firmness during storage. Higher pressures also corresponded to greater moisture loss which may contribute to this. So (unfortunately) the use of high pressure does not appear to offer any benefit.

From the results of the effect of pressure on starch retrogradation and bread firmness; which show different directions, it might be suggested that starch retrogradation is not the only factor affecting the staling of Arabic flat bread. A focussed study on gel firming theories may be a more fruitful line of investigation.

7.2 MAIN CONTRIBUTIONS OF THE STUDY

1. Different heat treatments are encountered in different parts of the bread during pita bread baking due to variations in incident radiation. Thus the precise location of samples in the bread should be recorded when analysing pita bread samples.

2. In this study, it would be the first time that temperatures of pita bread during baking have been presented, showing the significant effect that evaporation plays in maintaining internal temperatures within a few degrees of the boiling point of water. It also shows the slight rise in temperature that accompanies inflation.
Conclusions and Future Work

3. This study had shown that the use of aluminium pans is unreliable above 100°C in DSC studies of flat bread (as was performed by Sidhu and co-workers), and that high pressure stainless steel pans should be used instead.

4. The study demonstrates via experiments in both baking and “simulated baking” in DSC pans that heating rate during baking is a critical variable affecting subsequent starch retrogradation.

5. This study is the first time that the effect of pressure during baking on bread moisture, starch retrogradation and bread firmness has been studied. Higher pressures produced less retrogradation, but firmer breads. However, this may be due to lower moisture contents in the samples baked at higher pressures.

6. The effect of the inflation process was also studied. Its effect on bread moisture, thermal response and bread firmness have been shown.

7.3 SUGGESTIONS FOR FUTURE WORK

Since that it was found that higher pressures and heating rates can positively affect starch retrogradation, the work in this dissertation can be extended in future works which are suggested as follows:

1. This study confirms that higher baking heating rate is a key factor in starch retrogradation in Arabic flat bread. This study also showed that higher pressures during baking positively affected starch retrogradation in Arabic flat bread during storage. Therefore, further studies can examine these effects in more depth using X-ray diffractometry (XRD).
Conclusions and Future Work

2. Applying pressure while baking increased the baking temperature of Arabic flat bread. The samples baked at higher pressures produced firmer samples but this may be due to a lower moisture product. It would be interesting to use shorter times at the higher pressures to maintain a similar final moisture content product across in which samples can be compared.

3. A relationship can be developed to correlate the effect of pressure on starch retrogradation in Arabic flat bread. Higher pressures than 3 bar could be investigated with considering its effect on quality of layer separation (inflation process). Also baking under partial vacuum is an interesting issue, although that would be difficult to implement in practice.

4. The present study on the effect of pressure had been subjected on Arabic flat bread made of basic ingredients. The effect of pressure on bread containing bran, sugar or other additives may be of great interest.
REFERENCES


References


References


References


References


References


References


References


References


References


References


NORTON, T. and SUN, D.W., 2008. Recent advances in the use of high pressure as an effective processing technique in the food industry. Food and *Bioprocess Technology*, **1**(1), pp. 2-34.
References


References


References


References


References


References


References


References


APPENDIX A

This appendix will contain the statistical analysis for DSC data (Enthalpy) for both puffed (light) and unpuffed bread baked at different pressures (Chapter 6).

**Puffed Bread**

<table>
<thead>
<tr>
<th>P (bar)</th>
<th>Day</th>
<th>$T_a$ (°C)</th>
<th>$T_p$ (°C)</th>
<th>$T_c$ (°C)</th>
<th>$\Delta H$ (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>42.9</td>
<td>45.5</td>
<td>41.1</td>
<td>60.1</td>
</tr>
<tr>
<td>2</td>
<td>44.8</td>
<td>44.7</td>
<td>46.8</td>
<td>57.3</td>
<td>57.5</td>
</tr>
<tr>
<td>3</td>
<td>46.4</td>
<td>47.0</td>
<td>48.7</td>
<td>58.2</td>
<td>58.9</td>
</tr>
<tr>
<td>4</td>
<td>46.5</td>
<td>47.8</td>
<td>48.3</td>
<td>58.2</td>
<td>58.7</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>46.4</td>
<td>47.6</td>
<td>47.7</td>
<td>59.3</td>
</tr>
<tr>
<td>2</td>
<td>48.8</td>
<td>46.9</td>
<td>48.0</td>
<td>61.0</td>
<td>59.0</td>
</tr>
<tr>
<td>3</td>
<td>46.9</td>
<td>47.9</td>
<td>48.6</td>
<td>58.9</td>
<td>60.1</td>
</tr>
<tr>
<td>4</td>
<td>47.2</td>
<td>48.8</td>
<td>47.65</td>
<td>58.6</td>
<td>60.3</td>
</tr>
</tbody>
</table>
Appendix A

Investigation of the Factors Affecting the Staling of Arabic Flat Bread

<table>
<thead>
<tr>
<th>P (bar)</th>
<th>Day</th>
<th>$T_o$ (°C)</th>
<th>$T_p$ (°C)</th>
<th>$T_e$ (°C)</th>
<th>$\Delta H$ (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>50.18</td>
<td>49.3</td>
<td>50.8</td>
<td>61.2</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>47.9</td>
<td>49.6</td>
<td>49.1</td>
<td>60.4</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>47.2</td>
<td>44.5</td>
<td>43.6</td>
<td>60.3</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>43.4</td>
<td>42.8</td>
<td>43.1</td>
<td>55.9</td>
</tr>
</tbody>
</table>

One Way Analysis of Variance (Enthalpy vs. Pressure)

One Way Analysis of Variance

Dependent Variable: Enthalpy

Normality Test: Passed ($P = 0.411$)

Equal Variance Test: Failed ($P < 0.050$)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Thursday, August 18, 2011, 23:59:03

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Missing</th>
<th>Median</th>
<th>25%</th>
<th>75%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>12</td>
<td>0</td>
<td>1.487</td>
<td>1.076</td>
<td>1.907</td>
</tr>
<tr>
<td>1.000</td>
<td>12</td>
<td>0</td>
<td>1.433</td>
<td>1.160</td>
<td>1.670</td>
</tr>
<tr>
<td>2.000</td>
<td>12</td>
<td>0</td>
<td>0.881</td>
<td>0.599</td>
<td>1.084</td>
</tr>
</tbody>
</table>
Appendix A

H = 14.637 with 2 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Ranks</th>
<th>q</th>
<th>P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs 2</td>
<td>172.000</td>
<td>4.713</td>
<td>Yes</td>
</tr>
<tr>
<td>1 vs 0</td>
<td>2.000</td>
<td>0.0548</td>
<td>No</td>
</tr>
<tr>
<td>0 vs 2</td>
<td>170.000</td>
<td>4.658</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Note: The multiple comparisons on ranks do not include an adjustment for ties.

Two Way Analysis of Variance

Dependent Variable: Enthalpy

**Normality Test:** Passed (P = 0.336)

**Equal Variance Test:** Passed (P = 0.630)
Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of Pressure depends on what level of Day is present. There is a statistically significant interaction between Pressure and Day. (P = 0.003)

Power of performed test with alpha = 0.0500: for Pressure : 1.000
Power of performed test with alpha = 0.0500: for Day : 1.000
Power of performed test with alpha = 0.0500: for Pressure x Day : 0.905

Least square means for Pressure :

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>1.517</td>
</tr>
<tr>
<td>1.000</td>
<td>1.454</td>
</tr>
<tr>
<td>2.000</td>
<td>0.855</td>
</tr>
</tbody>
</table>

Std Err of LS Mean = 0.0283
### Appendix A

Investigation of the Factors Affecting the Staling of Arabic Flat Bread

#### Least square means for Day:

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.000</td>
<td>0.829</td>
</tr>
<tr>
<td>2.000</td>
<td>1.090</td>
</tr>
<tr>
<td>3.000</td>
<td>1.454</td>
</tr>
<tr>
<td>4.000</td>
<td>1.728</td>
</tr>
</tbody>
</table>

Std Err of LS Mean = 0.0327

#### Least square means for Pressure x Day:

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 x 1.000</td>
<td>0.944</td>
</tr>
<tr>
<td>0.000 x 2.000</td>
<td>1.241</td>
</tr>
<tr>
<td>0.000 x 3.000</td>
<td>1.735</td>
</tr>
<tr>
<td>0.000 x 4.000</td>
<td>2.146</td>
</tr>
<tr>
<td>1.000 x 1.000</td>
<td>1.087</td>
</tr>
<tr>
<td>1.000 x 2.000</td>
<td>1.277</td>
</tr>
<tr>
<td>1.000 x 3.000</td>
<td>1.600</td>
</tr>
<tr>
<td>1.000 x 4.000</td>
<td>1.852</td>
</tr>
<tr>
<td>2.000 x 1.000</td>
<td>0.456</td>
</tr>
<tr>
<td>2.000 x 2.000</td>
<td>0.753</td>
</tr>
<tr>
<td>2.000 x 3.000</td>
<td>1.027</td>
</tr>
<tr>
<td>2.000 x 4.000</td>
<td>1.187</td>
</tr>
</tbody>
</table>

Std Err of LS Mean = 0.0566
All Pairwise Multiple Comparison Procedures (Holm-Sidak method):

Overall significance level = 0.05

Comparisons for factor: Pressure

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 vs. 2.000</td>
<td>0.661</td>
<td>16.515</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>1.000 vs. 2.000</td>
<td>0.599</td>
<td>14.953</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>0.000 vs. 1.000</td>
<td>0.0625</td>
<td>1.562</td>
<td>0.131</td>
<td>0.050</td>
<td>No</td>
</tr>
</tbody>
</table>

Comparisons for factor: Day

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.000 vs. 1.000</td>
<td>0.899</td>
<td>19.457</td>
<td>&lt;0.001</td>
<td>0.009</td>
<td>Yes</td>
</tr>
<tr>
<td>4.000 vs. 2.000</td>
<td>0.638</td>
<td>13.806</td>
<td>&lt;0.001</td>
<td>0.010</td>
<td>Yes</td>
</tr>
<tr>
<td>3.000 vs. 1.000</td>
<td>0.625</td>
<td>13.524</td>
<td>&lt;0.001</td>
<td>0.013</td>
<td>Yes</td>
</tr>
<tr>
<td>3.000 vs. 2.000</td>
<td>0.364</td>
<td>7.873</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>4.000 vs. 3.000</td>
<td>0.274</td>
<td>5.933</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>2.000 vs. 1.000</td>
<td>0.261</td>
<td>5.651</td>
<td>&lt;0.001</td>
<td>0.050</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Comparisons for factor: **Day within 0**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.000 vs. 1.000</td>
<td>1.202</td>
<td>15.016</td>
<td>&lt;0.001</td>
<td>0.009</td>
<td>Yes</td>
</tr>
<tr>
<td>4.000 vs. 2.000</td>
<td>0.906</td>
<td>11.313</td>
<td>&lt;0.001</td>
<td>0.010</td>
<td>Yes</td>
</tr>
<tr>
<td>3.000 vs. 1.000</td>
<td>0.791</td>
<td>9.878</td>
<td>&lt;0.001</td>
<td>0.013</td>
<td>Yes</td>
</tr>
<tr>
<td>3.000 vs. 2.000</td>
<td>0.494</td>
<td>6.175</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>4.000 vs. 3.000</td>
<td>0.411</td>
<td>5.138</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>2.000 vs. 1.000</td>
<td>0.296</td>
<td>3.703</td>
<td>0.001</td>
<td>0.050</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Comparisons for factor: **Day within 1**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.000 vs. 1.000</td>
<td>0.765</td>
<td>9.555</td>
<td>&lt;0.001</td>
<td>0.009</td>
<td>Yes</td>
</tr>
<tr>
<td>4.000 vs. 2.000</td>
<td>0.575</td>
<td>7.183</td>
<td>&lt;0.001</td>
<td>0.010</td>
<td>Yes</td>
</tr>
<tr>
<td>3.000 vs. 1.000</td>
<td>0.513</td>
<td>6.410</td>
<td>&lt;0.001</td>
<td>0.013</td>
<td>Yes</td>
</tr>
<tr>
<td>3.000 vs. 2.000</td>
<td>0.323</td>
<td>4.038</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>4.000 vs. 3.000</td>
<td>0.252</td>
<td>3.145</td>
<td>0.004</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>2.000 vs. 1.000</td>
<td>0.190</td>
<td>2.372</td>
<td>0.026</td>
<td>0.050</td>
<td>Yes</td>
</tr>
</tbody>
</table>
## Appendix A

Comparisons for factor: **Day within 2**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.000 vs. 1.000</td>
<td>0.731</td>
<td>9.130</td>
<td>&lt;0.001</td>
<td>0.009</td>
<td>Yes</td>
</tr>
<tr>
<td>3.000 vs. 1.000</td>
<td>0.571</td>
<td>7.136</td>
<td>&lt;0.001</td>
<td>0.010</td>
<td>Yes</td>
</tr>
<tr>
<td>4.000 vs. 2.000</td>
<td>0.434</td>
<td>5.417</td>
<td>&lt;0.001</td>
<td>0.013</td>
<td>Yes</td>
</tr>
<tr>
<td>2.000 vs. 1.000</td>
<td>0.297</td>
<td>3.713</td>
<td>0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>3.000 vs. 2.000</td>
<td>0.274</td>
<td>3.423</td>
<td>0.002</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>4.000 vs. 3.000</td>
<td>0.160</td>
<td>1.994</td>
<td>0.058</td>
<td>0.050</td>
<td>No</td>
</tr>
</tbody>
</table>

Comparisons for factor: **Pressure within 1**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.000 vs. 2.000</td>
<td>0.631</td>
<td>7.887</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>0.000 vs. 2.000</td>
<td>0.489</td>
<td>6.103</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>1.000 vs. 0.000</td>
<td>0.143</td>
<td>1.784</td>
<td>0.087</td>
<td>0.050</td>
<td>No</td>
</tr>
</tbody>
</table>
Comparisons for factor: **Pressure within 2**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.000 vs. 2.000</td>
<td>0.524</td>
<td>6.546</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>0.000 vs. 2.000</td>
<td>0.488</td>
<td>6.093</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>1.000 vs. 0.000</td>
<td>0.0363</td>
<td>0.453</td>
<td>0.654</td>
<td>0.050</td>
<td>No</td>
</tr>
</tbody>
</table>

Comparisons for factor: **Pressure within 3**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 vs. 2.000</td>
<td>0.708</td>
<td>8.844</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>1.000 vs. 2.000</td>
<td>0.573</td>
<td>7.161</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>0.000 vs. 1.000</td>
<td>0.135</td>
<td>1.683</td>
<td>0.105</td>
<td>0.050</td>
<td>No</td>
</tr>
</tbody>
</table>

Comparisons for factor: **Pressure within 4**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 vs. 2.000</td>
<td>0.960</td>
<td>11.989</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>1.000 vs. 2.000</td>
<td>0.665</td>
<td>8.312</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>0.000 vs. 1.000</td>
<td>0.294</td>
<td>3.677</td>
<td>0.001</td>
<td>0.050</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Unpuffed bread:

<table>
<thead>
<tr>
<th>P (bar)</th>
<th>Day</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T₀ (°C)</td>
<td>T₀ (°C)</td>
<td>T₀ (°C)</td>
<td>ΔH (J/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>44.02</td>
<td>52.39</td>
<td>46.30</td>
<td>61.09</td>
<td>63.93</td>
<td>63.81</td>
<td>78.02</td>
<td>77.53</td>
<td>77.78</td>
<td>0.198</td>
<td>0.242</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>52.10</td>
<td>50.63</td>
<td>53.37</td>
<td>62.28</td>
<td>61.31</td>
<td>64.31</td>
<td>79.81</td>
<td>72.84</td>
<td>79.0</td>
<td>0.436</td>
<td>0.342</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>50.99</td>
<td>47.24</td>
<td>52.74</td>
<td>65.09</td>
<td>64.97</td>
<td>64.78</td>
<td>75.0</td>
<td>80.0</td>
<td>77.49</td>
<td>0.580</td>
<td>0.494</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>46.23</td>
<td>45.34</td>
<td>50.84</td>
<td>63.2</td>
<td>58.7</td>
<td>58.42</td>
<td>74.5</td>
<td>80.0</td>
<td>77.86</td>
<td>0.441</td>
<td>0.464</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>48.49</td>
<td>43.18</td>
<td>46.86</td>
<td>64</td>
<td>62.95</td>
<td>59.12</td>
<td>76.34</td>
<td>80.0</td>
<td>73.36</td>
<td>0.279</td>
<td>0.317</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>50.03</td>
<td>46.38</td>
<td>56.45</td>
<td>59.24</td>
<td>57.97</td>
<td>64.25</td>
<td>77.25</td>
<td>72.5</td>
<td>79.61</td>
<td>0.392</td>
<td>0.345</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>48.60</td>
<td>45.94</td>
<td>49.66</td>
<td>60.09</td>
<td>59.23</td>
<td>64.07</td>
<td>80.0</td>
<td>77.5</td>
<td>80.0</td>
<td>0.484</td>
<td>0.485</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>49.04</td>
<td>52.10</td>
<td>50.76</td>
<td>59.42</td>
<td>62.62</td>
<td>61.34</td>
<td>77.51</td>
<td>79.5</td>
<td>79.86</td>
<td>0.383</td>
<td>0.391</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>45.56</td>
<td>47.23</td>
<td>46.39</td>
<td>53.25</td>
<td>56.72</td>
<td>59.25</td>
<td>69.61</td>
<td>69.0</td>
<td>71.51</td>
<td>0.082</td>
<td>0.113</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>47.91</td>
<td>47.9</td>
<td>52.14</td>
<td>57.9</td>
<td>56.88</td>
<td>60.06</td>
<td>71.59</td>
<td>71.57</td>
<td>75.0</td>
<td>0.187</td>
<td>0.178</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>46.79</td>
<td>46.21</td>
<td>47.57</td>
<td>57.12</td>
<td>57.2</td>
<td>58.56</td>
<td>67.5</td>
<td>72.5</td>
<td>70.98</td>
<td>0.324</td>
<td>0.381</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>48.01</td>
<td>46.79</td>
<td>46.24</td>
<td>58.09</td>
<td>58.77</td>
<td>56.9</td>
<td>72.5</td>
<td>77.26</td>
<td>70.02</td>
<td>0.366</td>
<td>0.299</td>
</tr>
</tbody>
</table>
One Way Analysis of Variance (Enthalpy vs. Pressure)

Dependent Variable: Pressure

Normality Test: Passed (P = 0.489)

Equal Variance Test: Passed (P = 0.077)

<table>
<thead>
<tr>
<th>Group Name</th>
<th>N</th>
<th>Missing</th>
<th>Mean</th>
<th>Std Dev</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>12</td>
<td>0</td>
<td>0.410</td>
<td>0.119</td>
<td>0.0344</td>
</tr>
<tr>
<td>1.000</td>
<td>12</td>
<td>0</td>
<td>0.387</td>
<td>0.0638</td>
<td>0.0184</td>
</tr>
<tr>
<td>2.000</td>
<td>12</td>
<td>0</td>
<td>0.237</td>
<td>0.112</td>
<td>0.0324</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>2</td>
<td>0.212</td>
<td>0.106</td>
<td>10.299</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>33</td>
<td>0.340</td>
<td>0.0103</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>0.552</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.975
Appendix A

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):

Overall significance level = 0.05

Comparisons for factor: Pressure

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 vs. 2.000</td>
<td>0.173</td>
<td>4.178</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>1.000 vs. 2.000</td>
<td>0.150</td>
<td>3.624</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>0.000 vs. 1.000</td>
<td>0.0230</td>
<td>0.555</td>
<td>0.583</td>
<td>0.050</td>
<td>No</td>
</tr>
</tbody>
</table>

Two Way Analysis of Variance

Balanced Design

Dependent Variable: Pressure

**Normality Test:** Passed (P = 0.370)

**Equal Variance Test:** Passed (P = 0.886)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure</td>
<td>2</td>
<td>0.212</td>
<td>0.106</td>
<td>91.073</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Day</td>
<td>3</td>
<td>0.280</td>
<td>0.0934</td>
<td>80.274</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pressure x Day</td>
<td>6</td>
<td>0.0314</td>
<td>0.00523</td>
<td>4.497</td>
<td>0.003</td>
</tr>
<tr>
<td>Residual</td>
<td>24</td>
<td>0.0279</td>
<td>0.00116</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>0.552</td>
<td>0.0158</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of Pressure depends on what level of Day is present. There is a statistically significant interaction between Pressure and Day. \((P = 0.003)\)

Power of performed test with alpha = 0.0500: for Pressure : 1.000

Power of performed test with alpha = 0.0500: for Day : 1.000

Power of performed test with alpha = 0.0500: for Pressure x Day : 0.880

Least square means for Pressure :

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>0.410</td>
</tr>
<tr>
<td>1.000</td>
<td>0.387</td>
</tr>
<tr>
<td>2.000</td>
<td>0.237</td>
</tr>
</tbody>
</table>

Std Err of LS Mean = 0.00985

Least square means for Day :

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.000</td>
<td>0.215</td>
</tr>
<tr>
<td>2.000</td>
<td>0.323</td>
</tr>
<tr>
<td>3.000</td>
<td>0.454</td>
</tr>
<tr>
<td>4.000</td>
<td>0.387</td>
</tr>
</tbody>
</table>

Std Err of LS Mean = 0.0114
### Appendix A

Least square means for Pressure x Day:

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 x 1.000</td>
<td>0.243</td>
</tr>
<tr>
<td>0.000 x 2.000</td>
<td>0.410</td>
</tr>
<tr>
<td>0.000 x 3.000</td>
<td>0.543</td>
</tr>
<tr>
<td>0.000 x 4.000</td>
<td>0.443</td>
</tr>
<tr>
<td>1.000 x 1.000</td>
<td>0.305</td>
</tr>
<tr>
<td>1.000 x 2.000</td>
<td>0.382</td>
</tr>
<tr>
<td>1.000 x 3.000</td>
<td>0.464</td>
</tr>
<tr>
<td>1.000 x 4.000</td>
<td>0.398</td>
</tr>
<tr>
<td>2.000 x 1.000</td>
<td>0.0956</td>
</tr>
<tr>
<td>2.000 x 2.000</td>
<td>0.177</td>
</tr>
<tr>
<td>2.000 x 3.000</td>
<td>0.355</td>
</tr>
<tr>
<td>2.000 x 4.000</td>
<td>0.320</td>
</tr>
</tbody>
</table>

Std Err of LS Mean = 0.0197

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):

Overall significance level = 0.05

Comparisons for factor: **Pressure**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 vs. 2.000</td>
<td>0.173</td>
<td>12.425</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>1.000 vs. 2.000</td>
<td>0.150</td>
<td>10.776</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>0.000 vs. 1.000</td>
<td>0.0230</td>
<td>1.649</td>
<td>0.112</td>
<td>0.050</td>
<td>No</td>
</tr>
</tbody>
</table>
Comparisons for factor: **Day**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.000 vs. 1.000</td>
<td>0.239</td>
<td>14.883</td>
<td>&lt;0.001</td>
<td>0.009</td>
<td>Yes</td>
</tr>
<tr>
<td>4.000 vs. 1.000</td>
<td>0.173</td>
<td>10.734</td>
<td>&lt;0.001</td>
<td>0.010</td>
<td>Yes</td>
</tr>
<tr>
<td>3.000 vs. 2.000</td>
<td>0.131</td>
<td>8.147</td>
<td>&lt;0.001</td>
<td>0.013</td>
<td>Yes</td>
</tr>
<tr>
<td>2.000 vs. 1.000</td>
<td>0.108</td>
<td>6.736</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>3.000 vs. 4.000</td>
<td>0.0667</td>
<td>4.148</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>4.000 vs. 2.000</td>
<td>0.0643</td>
<td>3.998</td>
<td>&lt;0.001</td>
<td>0.050</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Comparisons for factor: **Day within 0**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.000 vs. 1.000</td>
<td>0.300</td>
<td>10.782</td>
<td>&lt;0.001</td>
<td>0.009</td>
<td>Yes</td>
</tr>
<tr>
<td>4.000 vs. 1.000</td>
<td>0.200</td>
<td>7.187</td>
<td>&lt;0.001</td>
<td>0.010</td>
<td>Yes</td>
</tr>
<tr>
<td>2.000 vs. 1.000</td>
<td>0.167</td>
<td>5.997</td>
<td>&lt;0.001</td>
<td>0.013</td>
<td>Yes</td>
</tr>
<tr>
<td>3.000 vs. 2.000</td>
<td>0.133</td>
<td>4.784</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>3.000 vs. 4.000</td>
<td>0.100</td>
<td>3.595</td>
<td>0.001</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>4.000 vs. 2.000</td>
<td>0.0331</td>
<td>1.189</td>
<td>0.246</td>
<td>0.050</td>
<td>No</td>
</tr>
</tbody>
</table>
Appendix A

Comparisons for factor: **Day within 1**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.000 vs. 1.000</td>
<td>0.159</td>
<td>5.692</td>
<td>&lt;0.001</td>
<td>0.009</td>
<td>Yes</td>
</tr>
<tr>
<td>4.000 vs. 1.000</td>
<td>0.0931</td>
<td>3.341</td>
<td>0.003</td>
<td>0.010</td>
<td>Yes</td>
</tr>
<tr>
<td>3.000 vs. 2.000</td>
<td>0.0820</td>
<td>2.942</td>
<td>0.007</td>
<td>0.013</td>
<td>Yes</td>
</tr>
<tr>
<td>2.000 vs. 1.000</td>
<td>0.0766</td>
<td>2.750</td>
<td>0.011</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>3.000 vs. 4.000</td>
<td>0.0655</td>
<td>2.351</td>
<td>0.027</td>
<td>0.025</td>
<td>No</td>
</tr>
<tr>
<td>4.000 vs. 2.000</td>
<td>0.0165</td>
<td>0.591</td>
<td>0.560</td>
<td>0.050</td>
<td>No</td>
</tr>
</tbody>
</table>

Comparisons for factor: **Day within 2**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.000 vs. 1.000</td>
<td>0.259</td>
<td>9.303</td>
<td>&lt;0.001</td>
<td>0.009</td>
<td>Yes</td>
</tr>
<tr>
<td>4.000 vs. 1.000</td>
<td>0.225</td>
<td>8.064</td>
<td>&lt;0.001</td>
<td>0.010</td>
<td>Yes</td>
</tr>
<tr>
<td>3.000 vs. 2.000</td>
<td>0.178</td>
<td>6.384</td>
<td>&lt;0.001</td>
<td>0.013</td>
<td>Yes</td>
</tr>
<tr>
<td>4.000 vs. 2.000</td>
<td>0.143</td>
<td>5.145</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>2.000 vs. 1.000</td>
<td>0.0813</td>
<td>2.919</td>
<td>0.008</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>3.000 vs. 4.000</td>
<td>0.0345</td>
<td>1.239</td>
<td>0.227</td>
<td>0.050</td>
<td>No</td>
</tr>
</tbody>
</table>
Comparisons for factor: **Pressure within 1**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.000 vs. 2.000</td>
<td>0.209</td>
<td>7.514</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>0.000 vs. 2.000</td>
<td>0.147</td>
<td>5.293</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>1.000 vs. 0.000</td>
<td>0.0619</td>
<td>2.221</td>
<td>0.036</td>
<td>0.050</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Comparisons for factor: **Pressure within 2**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 vs. 2.000</td>
<td>0.233</td>
<td>8.371</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>1.000 vs. 2.000</td>
<td>0.205</td>
<td>7.344</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>0.000 vs. 1.000</td>
<td>0.0286</td>
<td>1.026</td>
<td>0.315</td>
<td>0.050</td>
<td>No</td>
</tr>
</tbody>
</table>

Comparisons for factor: **Pressure within 3**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 vs. 2.000</td>
<td>0.189</td>
<td>6.771</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>1.000 vs. 2.000</td>
<td>0.109</td>
<td>3.903</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>0.000 vs. 1.000</td>
<td>0.0799</td>
<td>2.869</td>
<td>0.008</td>
<td>0.050</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Comparisons for factor: **Pressure within 4**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 vs. 2.000</td>
<td>0.123</td>
<td>4.415</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>1.000 vs. 2.000</td>
<td>0.0777</td>
<td>2.791</td>
<td>0.010</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>0.000 vs. 1.000</td>
<td>0.0453</td>
<td>1.625</td>
<td>0.117</td>
<td>0.050</td>
<td>No</td>
</tr>
</tbody>
</table>
APPENDIX B

List of Publications

L. Al-Hajji, V. Nassehi & Andrew Stapley. *Spatial Variation of Starch Retrogradation in Arabic Flat Bread during Storage*. Food Engineering.(Submitted)

Conference

L. Al-Hajji, V. Nassehi & Andrew Stapley. *The Effect of Pressure during Baking on Starch Retrogradation and Firmness of Arabic Flat Bread*. 5th Saudi International Conference (SIC 2011), University of Warwick, Coventry, UK.