# IMPACT OF SODIUM CHLORIDE ON WHEAT DOUGHS

**By**

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*And Jesus said to them,*

*"I am the bread of life. He who comes to Me shall never hunger, and he who believes in Me shall never thirst."*

*John 6:3*

# Abstract

The impact of salt (sodium chloride) on the wheat dough was studied, with a particular focus on the state and distribution of water and sodium in the dough system. In this study, dough samples were prepared using the same processing techniques as in commercial bakery (i.e. Chorleywood Bread Process) and were investigated simultaneously using molecular spectroscopy ($^{1}H$ and $^{23}Na$ NMR), deformation stress measurement (Kieffer test, Texture Profile Analysis and Chen-Hoseney test) and calorimetry (DSC). A progressive study of experimentation was carried out in which dough samples between zero and 5% added salt (on flour base) to exaggerate the effects of salt. Furthermore, test baking was also used to study the mechinability of doughs. All of the techniques studied enabled the construction of a complete picture of the sequential events occurring when salt is reduced.

Test baking confirmed that machine moulding of bread dough became more difficult at lower salt contents. This was more apparent when dough temperature was elevated, or when the delay time between mixing and moulding was increased. Laboratory measurements were not able to distinguish the increase of stickiness occurring in the low salt (1.4%) doughs and modifying the method also failed to establish variations in stickiness, although changes in the hardness of the dough at the different salt levels were detectable.

Measurements of the dough fluid phases were compared using three techniques: isolation of aqueous phase through ultracentrifugation, freezable water as measured by differential scanning calorimetry (DSC) and proton mobility using low field nuclear magnetic resonance ($^{1}H$ NMR). Salt increased the amount of dough liquor expressed on ultracentrifugation, however, the amount of freezable water and the molecular mobility of water ($T_2$) did not show significant changes. The findings suggest that the gluten-starch matrix is sensitive to salt in way that it affects the "drainage" properties and the capillarity of the dough matrix, but not the intrinsic levels of fluid in the dough.

The distribution of salt on the dough was also investigated using $^{23}Na$ NMR. A large proportion of the salt added was not detectable with this technique and could be thought of as immobile. Increasing the concentration of sodium in the dough gave the same proportion of "bound" sodium. It would seem to be the starch component in the flour that dominated the sodium binding in the dough samples. Salt may exert its effects through polymer-polymer interactions rather than polymer – water interactions and its exact influence on cereal products performance still needs to be established before the reduction of salt is a viable option for commercial bakers.

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# Abbreviations and Symbols

| | |
|---|---|
| a.u. | arbitrary unit |
| CBP | Chorleywood bread process |
| DATEM | diacetyl tartaric acid esters of mono and diglycerides |
| DSC | differential scanning calorimetry |
| Ext | distance at rupture point |
| $\hbar$ | Planck's constant ($6.6 \times 10^{-34}$Js) |
| HMW | high molecular weight |
| LMW | low molecular weight |
| M | net magnetisation |
| NMR | nuclear magnetic resonance |
| P180 | 180° pulse |
| P90 | 90° pulse |
| ppm | part per million |
| rf | radio frequency |
| Rmax | resistance of extension |
| SSL | sodium stearoyl lactylate |
| $T_{end}$ | end temperature |
| $T_o$ | onset temperature |
| $T_{peak}$ | peak temperature |
| $T_1$ | spin-lattice relaxation time |
| $T_2$ | spin-spin relaxation time |
| $T_2^*$ | spin-spin relaxation time decreased by field inhomogeneity |
| TA | texture analysis |
| TPA | texture profile analysis |
| WC | water content |
| $\gamma$ | gyromagnetic ratio |
| $\Delta H$ | enthalpy |
| $\nu$ | frequency |
| $\rho$ | spin angular momentum |
| $\tau$ | P90-P180 pulse spacing |
| $B_0$ | permanent magnetic field |

# CHAPTER 1. GENERAL INTRODUCTION

## 1.1. THE ISSUE OF SALT IN FOODS

### 1.1.1. What is salt?

Salt is the world's oldest know food additive and serves many purposes. Common salt (sodium chloride, NaCl) is composed of the element sodium and chloride through ionic bonding. It is a mineral that occurs naturally in many parts of the world with unlimited supply. Sodium chloride crystals are cubic in form and they are typically harvested from the mineral halite or by evaporation of sea water.

Besides contributing its own basic "salty" taste, salt has many other benefits. It "brings out" other flavours and makes foods acceptable, protects the safety of food by retarding the growth of spoilage and pathogenic microorganisms, influences the texture of processed foods, and participates in chemical reactions associated with colour and aroma.

### 1.1.2. Why is salt of concern?

Salt is the major source of sodium in the diet and it is an essential element for human life. It plays a diverse and important role in many physiological processes. Sodium is one of the most prevalent cations (positive ions) in extracellular fluids in human and decreases in sodium levels lead to decrease in blood volume and pressure that can be fatal. It is also necessary for regulation of blood and body fluids (Guyton et al., 1973; 1999), transmission of nerve impulses, heart activity, and certain metabolic functions (Kilcast et al., 2007). Furthermore, the chloride anion is required to maintain tissue osmolarity and the acid-base balance in blood. It is also essential to stomach enzymes and to form hydrochloric acid (HCl) in the stomach (Reddy et al., 1991). Thus, it is necessary for humans to be able to retain sodium in the body through reabsorption in the kidneys and other organs.

For thousands of years, people have observed that salt intake is associated with cardiovascular diseases such as stroke and hypertension. The earliest comment that relates dietary salt to blood pressure was recorded in the ancient Chinese medical

literature -- The Huang Ti Nei Ching Su Wein (the Yellow Emperor's Classic of Internal Medicine), probably dated about third century BC, described the "hard pulse" resulting from a high salt consumption (Danilczyk and Penninger, 2004).

In recent decades, epidemiological and clinical studies have examined the relationship between salt intake and blood pressure (for example: Xie et al., 1992; Sasaki et al., 1995; He et al., 1999; Chobanian, 2000; Tuomilehto et al., 2001; Nagata et al., 2004; Cohen, 2006). A recent study from Cook and colleagues (2007) reported long term effects of reduced dietary sodium on cardiovascular disease. More than 3000 participants without hypertension were randomised to a reduced sodium intake for 18 months or 36-48 months, or as control. The results show that people originally allocated to either sodium reduction group had a 30% lower incidence of cardiovascular events in the next 10-15 years, irrespective of sex, ethnic origin, age, body mass, and blood pressure.

Furthermore, increasing evidence is showing that high salt intake has other harmful effects on health (de Wardener and MacGregor, 2002), which may be independent of and additive to the effect of salt on blood pressure, including a direct effect on stroke (Perry and Beevers, 1992) , left ventricular hypertrophy (Kupari et al., 1994; Schmieder and Messerli, 2000), progression of renal disease and albuminuria (Heeg et al., 1989; Cianciaruso et al., 2004; Swift et al., 2005), stomach cancer (Joossens et al., 1996; Tsugane et al., 2004), and bone demineralization (Devine et al., 1995).

#### 1.1.2.1. The Pathophysiology of Hypertension

Blood pressure is a measurement of the force applied to the walls of the arteries as the heart pumps blood through the body. The recent Seventh Joint National Committee (JNC VII) defined individuals with blood pressure > 140 mmHg systolic or > 90 mmHg diastolic as hypertensive.

It is common knowledge that high salt intake contributes to high blood pressure, but the reason have not been clear. Studies in normal animals showed that, over the long term, blood pressure is controlled primarily by salt and water balance because of the infinite gain property of the kidneys to rapidly eliminate excess fluid and salt

(Guyton et al. 1969; 1973). When the renal function is reduced, a small increase in extra-cellular fluid volume inevitably causes the blood pressure to rise (Guyton et al. 1969). The hypertension that develops as a result of salt retention, as in mineral corticoid hypertension, is always preceded by increased plasma volume (Hamlyn et al, 1986). The high intake of sodium suppressed natriuresis (a process of sodium excretion in urea via the action of kidneys) to maintain a constant level of plasma fluid. This causes the re-absorption of sodium resulting in an increase of the extracellular fluid volume, which is mainly due to retaining an iso-osmotic balance of the plasma and eventually elevation of the blood pressure (Haddy, 2006).

Several indirect changes to biological pathways as a result of increasing blood pressure, due to high salt intake, have also been suggested:

- Increased dietary salt increases the renal excretion of potassium, resulting in a small fall in plasma potassium concentration. Reducing the plasma potassium concentration leads to vasoconstriction. Thus, it is possible that hypokalemia is in part responsible for the hypertension (Haddy et al., 1995; Pamnani et al., 2000).

- High salt intake increases the level of cardiotonic steroids such as ouabain or an isomer of ouabain in the plasma which inhibit the plasma membrane $Na^+$, $K^+$-ATPase, resulting in reduced activity of the $Na^+$-$K^+$ pump and therefore increase contractility of heart, arteries and veins leads to the increases of blood pressure (Danilczyk et al., 2004; Haddy et al., 2006).

- Higher local sodium concentration may induce calcium ion entry into vascular smooth muscle cells and produces electrogenic depolarisation of the vascular smooth muscle cells. It decreases the $Na^+$-$Ca^{2+}$ exchange across the plasma membrane of the cardiac and vascular smooth cells, resulting in more norepinephrine in the neuromuscular cleft. This mechanism therefore increases the contractile activity and hence elevates the blood pressure (Danilczyk et al., 2004; Haddy et al., 2006)

A summary of salt intake and blood pressure is demonstrated in Figure 1-1.

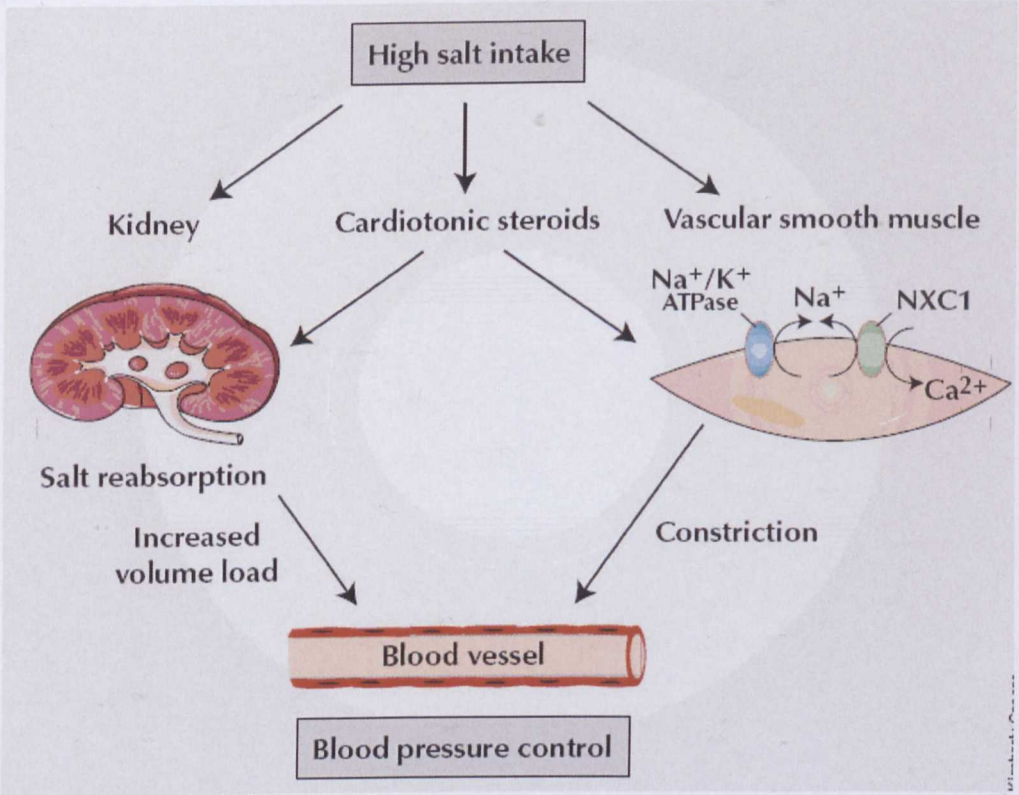



Figure 1-1. A schematic of the pathways linking salt intake and blood pressure (Danilczyk et al., 2004).

### 1.1.3. Current issue of salt in food

Cardiovascular disease (hypertension, strokes, and heart failure) is the leading cause of death and disability worldwide where they are extremely common in Western countries. In England, around 40% of the entire adult population is considered to be hypertensive (Primatesta et al., 2001). In 1985, the World Health Organisation recommended that the average salt intake should be reduced to 5g per day or less. However, until now, few countries have policies for targeted reduction in salt intake. Because of concerns over health-related issues, regarding high intake of salt by consumers, the reduction of salt in different food products has now come into focus. Since 1991, the UK government has been introducing recommended guidelines for sodium intake by setting the reference nutrient intake (RNI) for sodium at 1600 mg/day and advised a target reduction on average intake of salt from 9g to 6g a day (COMA, 1994, SACN, 2003). In 1994, the Food Standards Agency of UK has set a

target of reducing the average salt consumption of adults to 6 grams a day by 2010 in order to improve the health of the general public.

### 1.1.4. Bread and salt

In Westernized countries, people derive salt mostly from bread and processed food and only a small proportion comes from discretionary use. Since cereal and cereal products (bread, breakfast cereals, biscuits and pastries) are the main food group contributing to sodium intake (Figure 1-2), the Food Standards Agency has been co-operateing with food industries to help them reduce the sodium content in their products. A reduction of salt in cereal and cereal based products would make a significant contribution to the level of sodium chloride in the average diet.

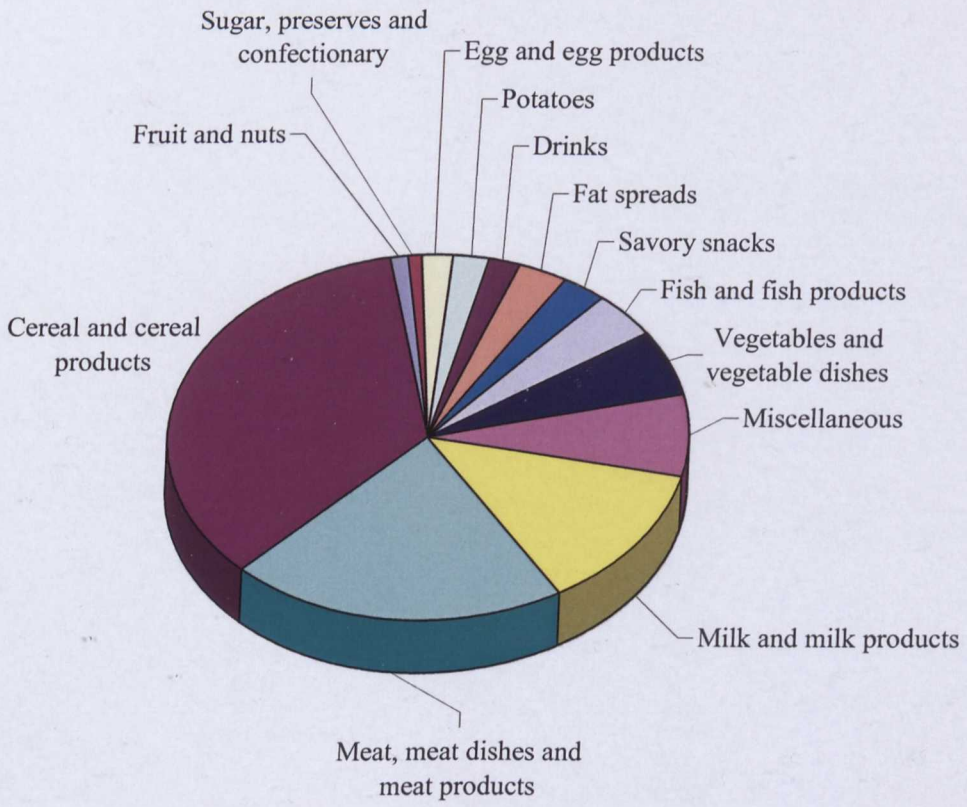



Figure 1-2. Percentage contribution of food types to average daily sodium consumption (Kilcast et al., 2007).

The salt levels in UK baked product formulations vary considerably depending on the product and the environment in which it is manufactured. The value given in Table 1-1 illustrates the salt content in various baked products.

Table 1-1. Sodium chloride levels in baked products (adapted from Kilcast et al. 2007).

| Product and manufacturing environment | Approximate level of sodium chloride (g) per 100g baked product | Typical level of sodium chloride in 100g dough or batter | Typical level of sodium chloride compared with 100g flour |
|---|---|---|---|
| UK pan bread | 1.15-1.25 | 1.08-1.19 | 1.8-2.0 |
| UK crusty breads | 1.15-1.34 | 1.08-1.27 | 1.8-2.1 |
| UK soft rolls | 1.00-1.12 | 0.94-1.11 | 1.6-1.9 |
| UK fruit buns | 0.72-0.86 | 0.68-0.80 | 1.6-1.9 |
| UK fruit cake | 0.39-0.52 | 0.37-0.49 | 1.5-2.0 |
| UK plain cake | 0.32-0.42 | 0.29-0.39 | 1.5-2.0 |
| UK pan bread (Cauvain, 1998) | 1.04 | 0.99 | 2.3 |
| French baguette (Calvel, 2001) | 1.23 | 1.17 | 2.0 |

The current target for salt levels in bread stands at 0.9g per 100g product by 2010 (Food Standards Agency, UK, 2005). Thus, there is still a gap between the proposed and the current levels of salt in bread. This has spurred interest in reducing the sodium chloride level or completely or partially replacing sodium chloride with alternative salts. In order to reach the target level of sodium in bread products without changing the quality of the final product, understanding of the underlying molecular mechanism is necessary.

## 1.2. AIM AND SCOPE OF THE THESIS

Salt (NaCl) is commonly added to bakery formulae at a level from 1 to 2.5% of the flour weight. Many studies have shown that sodium chloride has a significant impact on the physical properties of dough (Hibberd, 1970; Danno and Hoseney, 1982; Dreese et al., 1988; Kim & Bushuk, 1995; Larsson, 2002). In fact, large scale bakeries are finding it difficult to deliver further reductions in salt content because their processes become more sensitive at lower salt levels and plant waste increases

as salt level reduced. This is most apparent as sticky doughs, collapse of proved dough and open texture in the baked loaf. It is reported that these problems are more prevalent in the summer time as bakery temperatures are higher. While there are some ameliorative actions that can be taken, none are entirely satisfactory in solving the quality problem and achieving efficient bakery operation. However, all increase the cost of the ingredients or of the finished product.

The study reported in this thesis is aimed to deliver clarity of information on some aspects of the influence of sodium chloride and a better fundamental understanding of how salt (NaCl) level affects the physical and biochemical bases of wheat flour dough systems. In particular the work was to establish an understanding of the aqueous liquid phase in dough and its relationship with salt levels. The work was to be in partnership with commercial bread providers so that the information generated could help in finding better commercial ways of making bread down at salt levels commensurate with those recommended by the UK Food Standards Agency.

The investigations presented in this study relate to:

- The study of salt reduction on dough and bread quality.
- The nature of the aqueous layer in doughs and its relationship to the stability of the dough.
- The development of methods that may monitor quantitatively the effect of salt on dough/bread quality.
- The partition of sodium ions between each phase of the dough.

## 1.3. THESIS STRUCTURE

This thesis sets out the current knowledge relevant to the topic of salt and bread making and other chapters give the experimental details of the work undertaken. Chapter 2 of this thesis reviews the structural and properties of the various ingredients in a bread recipe and their relevance to bread making. Additionally, some information on the dough liquor hypothesis in the dough system is presented. Chapter 3 reports the materials and methods used in this study and a brief explanation of the concepts behind the techniques. Chapter 4 introduces three

developed methods to identify the fluidity in a wheat flour dough system. Chapter 5 reports a study of commercial bread doughs. A simplified commercial bread dough recipe was evaluated in Chapter 6. The possibility of using an objective technique to measure the surface properties of dough samples is introduced in Chapter 7 and Chapter 8 discusses the partition of sodium ions in the dough using $^{23}Na$ NMR. The final chapter, Chapter 9, gives a general conclusion of all the results obtained and recommendations are made for future work.

# CHAPTER 2. THE CHORLEYWOOD BREAD PROCESS

## 2.1. INTRODUCTION

Bread is a major nutritional component in the diet and bread making is one of the oldest processes known and practised for thousands of years, worldwide. Bread production can generally be classified into two main methods. Bulk fermentation which is the traditional method, where all the ingredients are mixed together to form a dough and are left to ferment for long hours before baking. Nowadays, the modern commercial process used in large bakeries in Britain and other countries is known as the Chorleywood bread process (CBP). The study presented in this thesis is based on the CBP bread production and the following sections provide an overview of the process.

## 2.2. DOUGH COMPOSITION

The structure of bread dough has been described as a bicontinuous medium of gluten-starch and water, including gas cells. To produce a well-expanded loaf of bread with a light and even crumb texture, the dough must be able to retain gas produced during fermentation and the baking stages. This section provides an overview of the key ingredients in a typical CBP dough recipe.

### 2.2.1. Wheat and Flour

Generally, flour from wheat, rather than from other cereal grains, is used for bread making because wheat flour is the only one which can produce a sponge-like bread with high volume (Baker and Mize, 1941). Wheat can be classified by the hardness of the kernel when it is milled. The "hardness" and "softness" are characterized by fragmentation of the endosperm. In hard wheat grains, the starch and protein tend to be more tightly bonded, and the milling process will cause some disruption of the starch granule structures. Thus, in hard wheat flour, a high level of starch damage is present and is desirable for bread production. In addition, "hard" wheat generally contains higher storage protein, and is thus able to produce a large loaf volume and good crumb structure. Since it forms strong gluten, hard wheat flour is considered

strong flour. On the other hand, flour milled from "soft" wheat kernels is called weak flour and it is low in storage protein, so produces a poor loaf volume and coarse crumb. Soft wheats are usually used for making biscuit and cake. The botanical structure of the wheat berry and the chemical composition of wheat and the individual mill fractions are given in Figure 2-1 and Table 2-1 respectively.

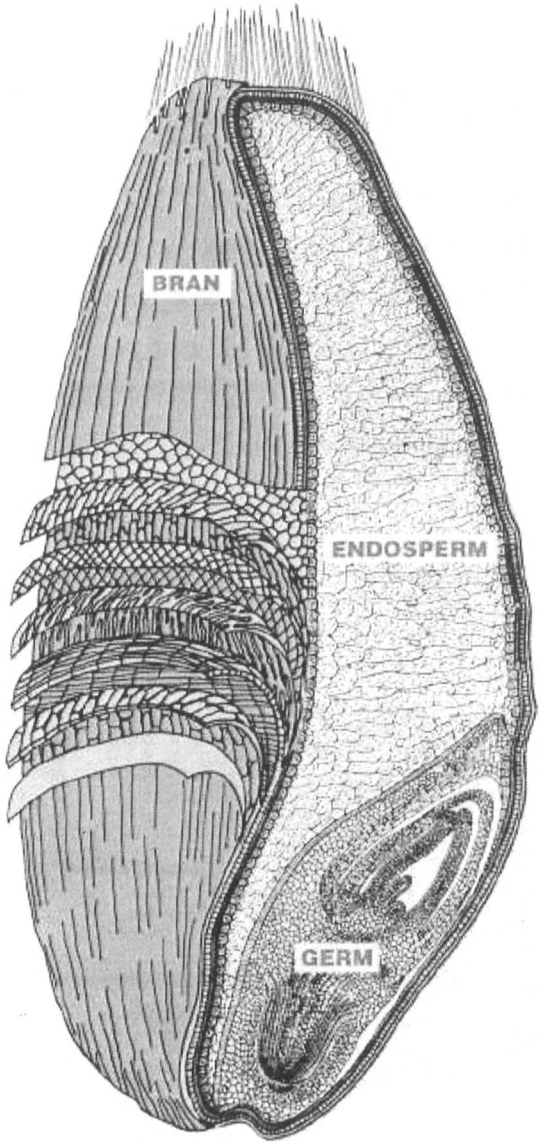



Figure 2-1. Longitudinal section of a wheat berry (Cauvin and Young (1998)).

Table 2-1. Chemical composition (dwb) of commercial wheat samples. Data from Dobraszczyk (2001), FSA (2002), Pomeranz (1988).

| | Whole grain | Endosperm | Bran | Germ |
|---|---|---|---|---|
| Proportion by weight (%) | 100 | 82-85 | 15 | 3 |
| Moisture (%) | 9-18 | 14 | 8.3 | 12 |
| Protein, N x 5.7 (%) | 13 | 8-13 | 7-8 | 27 |
| Lipids (%) | 1.1-2.5 | 1.2-1.4 | 1-5 | 9.2 |
| Carbohydrate (%) | 64 | 75 | 27 | 45 |
| Starch (%) | 62 | 74 | 23 | 29 |
| Sugar (%) | 2-3 | 1.4 | 3.8 | 16 |
| Chloride (%) | 0.04 | 0.05-0.08 | 0.06-0.09 | 0.08 |

Good bread-quality wheat flour is an optimum blend of components. Figure 2-2 illustrates the major components and the relative amounts of each in typical hard wheat flour. Such flour when hydrated forms dough, the basis of bread. The properties of dough are made possible due to the unique characteristics on wheat flour. Particularly important is the capability of wheat flour dough to retain the structure and gas during mixing, fermentation and baking.

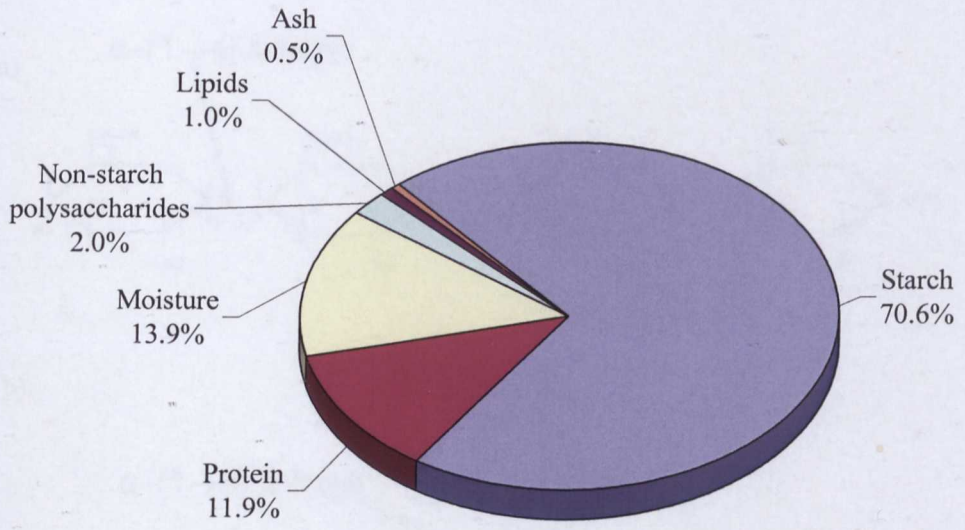



Figure 2-2. The composition of typical wheat flour.

### 2.2.1.1. Wheat Starch

Starch represents the largest portion of wheat flour, making up to 70-80% (dry basis) of ordinary flour and is composed of polymers of only one monosaccharide (glucose), protein components and minor lipid. The review by Buleon et al (1998) gave a good description of starch at the various level of organization: molecular, macromolecular, and granular.

Starch polymers are mixtures of mainly amylose, a linear chain of (1-4) linked α-D glucan, and high molecular weight amylopectin, which is a highly branched chain of linear (1-4) α-D-glucan chains connected through (1-6) α-linkages (Figure 2-3) (Buleon et al., 1998). The relative weight percentage of amylose and amylopectin in wheat starch are 27-31% and 69-73% respectively (Galliard and Bowler, 1987). Estimations of the polymerisation degree are between $10^5$ -$10^9$ monosaccharide units. Lipids, mainly lysophospholipids, represent the most important minor fraction and account for 88-94% of the total endosperm lipids. They are believed to form inclusion complexes with amylose in which the lipid fatty acid chain occupies the core of a helix containing six anhydroglucose units per turn.

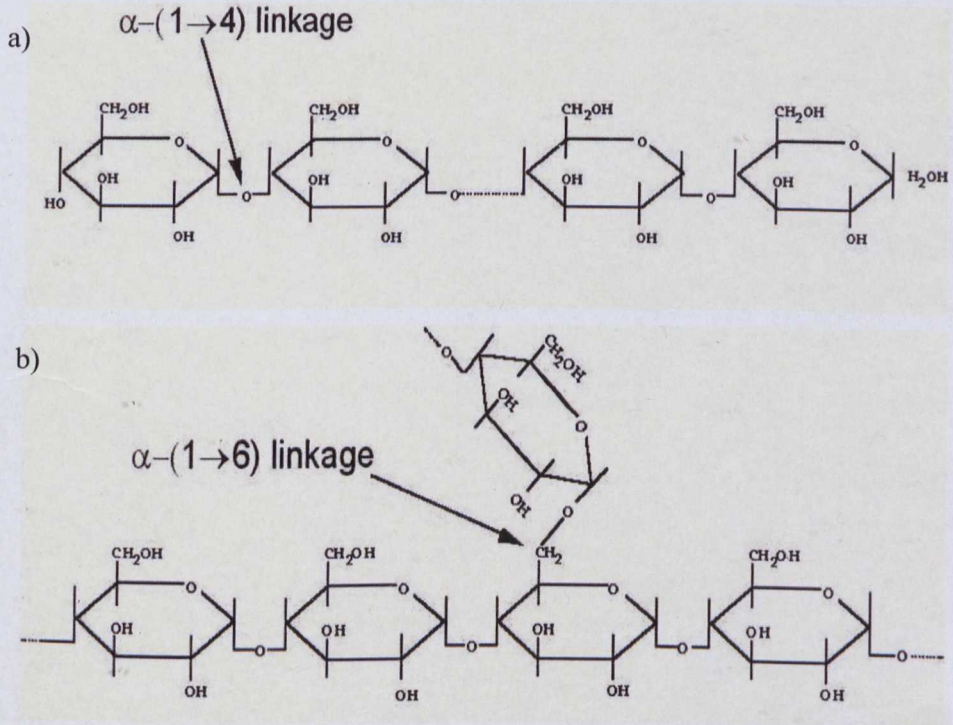



Figure 2-3. The chemical structures of (a) amylose and (b) amylopectin.

The basic crystalline packing/conformation organization of the starch chains are characterized by a multiplicity in branching. It was described by Peat et al. (1952) in term of A, B, and C chains (Figure 2-4). The external A-chains are connected to the B-chains. The B-chains are linked onto the C-chains that are characterised by one reducing terminal residue.

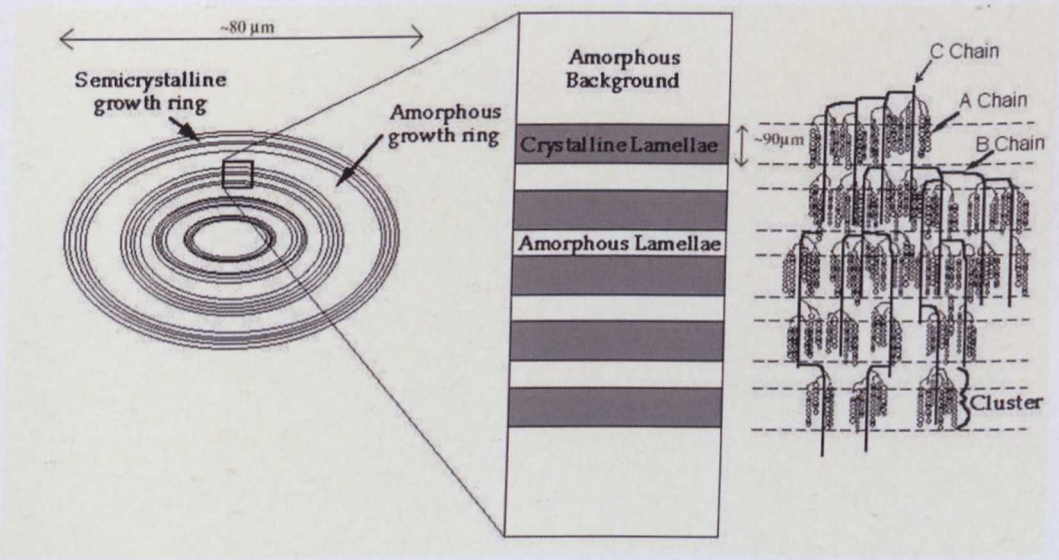



Figure 2-4. Structure of starch granule, with alternating amorphous and semi-crystalline zone constituting the growth rings.

It is widely recognised that starch plays an important role in bread making (Hibberd, 1970; Smith et al., 1970). Starch granules absorb approximately 10% by weight water during mixing and are evenly distributed throughout the dough acting as inert filler. The starch also interacts with gluten protein to effectively form cross linkages and reinforces the network as the starch begin to swell upon heating in the baking stage (Petrofsky and Honseney, 1995; Larsson and Eliasson., 1997; Edwards et al., 2002). Furthermore, starch provides yeast with fermentable sugar, and contributes towards the crumb texture and colour of the crust.

### 2.2.1.2. Wheat Proteins

Wheat proteins comprise about 12 to 18% of wheat flour on a dry basis, yet despite a relatively minor presence, compared to starch (~80%), this component is the most important in the bread making process as it provides an unique ability to retain gas during proof and form a stable aerated crumb structure (Ewart, 1989). Description of

wheat proteins are given in several reviews such as Schofield (1986) and Eliasson and Larsson (1993). One of the most significant means of classifying wheat proteins was proposed by Osborne (1907) based on its solubility. Five classes of proteins were observed: albumins (soluble in water), globulins (soluble in salt solution), gliadins (soluble in aqueous ethanol), glutenins (dispersed in diluted acidic or alkaline solutions) and insoluble residue.

The major storage protein in wheat endosperm is known as gluten and consists of two groups, gliadin and glutenin; together they give the viscoelasticity properties of dough. The baking quality (i.e. good volume and crumb structure) is largely determined by the quality and quantity of these proteins.

#### 2.2.1.3. Gliadins

Gliadins are predominantly monomeric and represent 30-40% of the total protein in the wheat flour (Eliasson and Larsson, 1993). These proteins are classified into four sub-categories ($\alpha$ $\beta$ $\gamma$ $\omega$) based on electrophoretic mobility methods (Woychick et al., 1961). The $\alpha$, $\beta$ and $\gamma$ gliadins are usually stabilised by covalent disulfide bonds and non covalent hydrogen bonds, whereas $\omega$ gliadins are stabilised by hydrophobic interactions.

Gliadins contain a large number of polypeptides, between 49 and 60 and have extremely high glutamine content and high proline content. The high proline content has an effect on the secondary structure of gliadin polypeptides because the proline side chains hinder the $\alpha$-helices formation. The low levels of lysine, arginine and histidine place the gliadins among the least charged proteins known. Earlier studies suggested that the molecular structure of the gliadins components is generally globular and generally have a relative molecular weight of 20,000 – 50,000 (Eliasson and Larsson, 1993).

Gliadins are generally considered to contribute to the viscosity and extensibility of gluten and allowing the dough to rise during fermentation, however, when isolated they are sticky. Studies shown that varying the gliadins fraction in the flour has a

minor effect on the baking performances. Therefore, gliadins could be of lesser importance in relation to baking performance than glutenins (MacRitchie, 1980).

### 2.2.1.4. Glutenins

Glutenins are recognized as being the most important component in the baking performance in flour as it is the major component associated with the viscoelastic properties of the dough structure. This property is important in retaining gas bubbles and hence development of crumb structure. They represent 40 - 50 % of the total protein in flour (Eliasson and Lansson, 1993). A review by Weegels et al. (1996) gave a detailed view of the current state of understanding of the functional properties of glutenins. It has two main unique characteristics: it is not soluble in dilute salt solutions and 70% ethanol, and the macromolecule is composed of polymeric chains bound by disulfide bonds, which are generally in linear form (Shewry et al., 1986). Glutenins have a slightly higher content of basic amino acids and a lower amount of glutamic acid and proline. The cysteine residues are mostly located on the ends of the subunits.

Belton et al. (1995) suggested that glutenins have a strong tendency to aggregate by forming both intra and intermolecular hydrogen and disulfide bonds. At each end of the glutenin chain are sulfur-containing amino acids that can form strong sulfur-sulfur bonds with the same amino acids at the end of other glutenin chains forming intra-molecular disulfide linkage. The main attributes for this structure-forming behaviour in the dough are due to an unusually high amount of glutamine, and potential for polar bonding of the many polar side chains and low ionic character of the gluten proteins. The low charge density of the gluten proteins, due to their low level of basic amino acids, prevents mutual charge repulsion between the wheat gluten protein, and as a result promotes association by non-covalent interactions.

According to Ewart (1979), glutenin subunits are joined by disulfide bonds into linear chains or concatenations of up to 50 molecules (sub-units). During mixing and moulding of the dough, these concatenations align, and the closeness of the alignment allows non-covalent bonding (e.g. hydrogen bonding, hydrophobic interactions, salt linkages and van der Waals). The aligned glutenin concatenations

are thus held strongly in relation to one another under deformation. The increased resistance to deformation on alignment is the cause of dough development. The glutenin is elastic, giving rise to unbonded mobile regions (loops) and bonded regions (trains). The loops can be stretched and then reformed when the stress is removed, which accounts for the restoring elastic force (Belton, 1999). This unique characteristic appears to be the major factor for the elasticity properties in the dough. Dough based solely on glutenin would be very stiff. The role of gliadins appears to be as plasticisers, imparting extensibility to the gluten structure by hindering interactions between concatenations. An optimum balance between the two proteins seems to be crucial for good bread making performance.

#### 2.2.1.4.1. Gluten Structure

The structure of gluten is still however under debate. Numerous models have been proposed to explain the unique visco-elastic properties of gluten. According to Eliasson and Larsson (1993), gluten can be considered as a continuous molecular weight distribution from 30,000 to 20 millions.

Grosskreutz (1960) proposed a lamellar structure related to glutenin polymerisation using x-ray diffraction. From the rheological behaviour of gluten, Mita and Bohlin (1983) suggested a lamellar supra-structure consisting of a non-close-packed fibrillar structure. Fibrils of hydrated protein can be observed under the light microscopy and are suggested to be part of a large sheeted structure (Bernardin and Kasarda 1973). Under stress, the gluten sheets are proposed to form a network structure organised in layers. It is possible to observe birefringence when gluten film is stretched which supports the organised structure (Eliasson and Larsson 1993). Glutenins have a structural role in the matrix while removing the gliadin fractions does not affect the layered structure (Amend and Belitz 1990). This model does not consider the relationship between gliadins and glutenins, where gliadins would only be considered as filler.

Furthermore, Tathan et al. (1987) and Miles et al. (1991) proposed that β-turn structures in glutenin seem responsible for the elasticity feature of the dough. This idea was supported with a model called the "linear glutenin hypothesis" reported by

Schofield (1986). The subunits were considered to have beta turn confirmations that may be extended when stretching and recover when the stress is released.

Another gluten model was proposed by Eliasson and Larsson (1993) with a model which is an amphiphile-water phase in the lipid-water system. Gluten has the ability to swell in water and coexist with an outside water phase. On observation using transmittance electron microscope (TEM), gluten exhibited a uniformly packed protein organization described as a globular structure with a hydrophobic core and a hydrophilic surface (Hermansson and Larsson, 1986). The presence of lipid does not seem to affect the structures. In the model, Eliasson and Larsson (1993) considered that the beta-turn structure formed the interior of the globular structure of gluten. Similar to Schofield (1986), under stress, the beta spirals would be stretched and reformed when the stress is removed. These cores would be associated with intermolecular disulfides bond to effectively increase of the molecular weight. Belton (1999) pointed out that intermolecular disulfide bonds can be used to explain the plasticity and resistance to extension of gluten, but not the elasticity properties of the dough. However, the β-turn structure can be used to explain the elasticity properties of the gluten matrix.

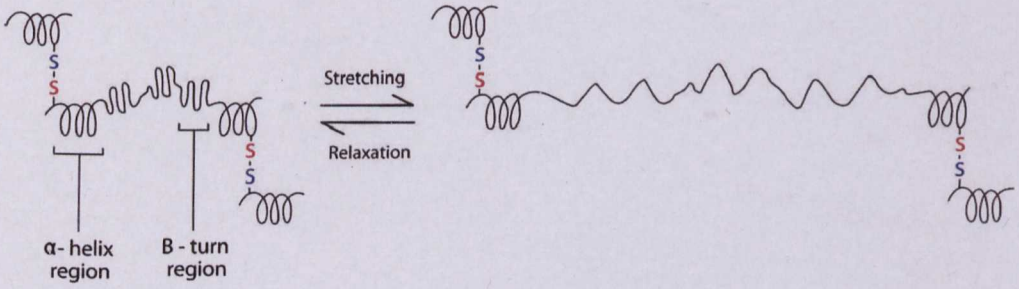



Figure 2-5. Schematic representation of glutenins model with the effect of elongation on B-turn (Schofield, 1986). Intermolecular disulfides bonds connect globular proteins.

Moreover, many authors have studied the functional characteristics and the structure of gluten (Pezolet et al 1992; Popineau et al 1994; Belton et al 1995). Popineau et al. (1994) observed the viscoelastic properties of gluten were related to interactions between glutenins subunits through aligned β-sheets. Using IR spectroscopy, Belton et al. (1995) proposed the co-existence of orderly intermolecular hydrogen bonding (β-sheets) regions and disordered unbonded regions. Belton (1999) later developed a

model to explain the elasticity of gluten based on the high molecular weight (HMW) subunits. It has suggested that hydrogen bonds between the repeat regions of the (HMW) subunits are responsible for the strength of the gluten (Ewart 1979). The model, shown in Figure 2-5, describes the formation of "loops and trains" with the "trains" associated with β-sheet formations. It involves both interaction between the polymer surface and the surrounding solvent. When stretching, the network would first deform the loops region and then the sliding of the chains occurs so that the trains are pulled apart. The structure will recover to the equilibrium position of loops and trains because of thermodynamic phenomenon. This model explains the involvement of gliadins in the resistance to extension by forming a viscous environment.

Disuflide bonds and thiol groups play an important role in determining gluten and dough properties. A study from Muller et al. (1998) showed that disulphide bonding is essential for the unique viscoelastic properties of dough. Although the intermolecular disulfide bonds play a major role in the structure and formation of the elastic protein network of dough, this has not been proven conclusively (Shewry and Tatham, 1996). The viscoelastic properties of gluten are not only required for the stability of the foam structure of bread dough, but also an optimum level of gluten elasticity is needed to maintain the gas-cell network. If the level of elasticity is too low, the bubble walls will rupture under the pressure of expansion towards the end of the proving stage and the early stages of baking, and the gas will escape. If the level of elasticity is too high, the gas cells will not be able to expand sufficiently, resulting in a loaf with poor height and volume. Recent studies have suggested that tyrosine cross-links would also provide a great influence to the gluten structure (Tilley et al. 2001). Their formation could explain partly the strengthening effect on gluten.

#### 2.2.1.5. Flour Lipids

Wheat flour contains about 2.5% lipids, a minor component in flour (Morrison, 1988). It consists of non-polar (or neutral lipids), glycolipid and polar lipids (Morrison, 1978). The non-polar lipid fraction comprises triglycerides, diglycerides and mono acyl glycerides and the other non-polar sterol lipids. Polar lipid fractions of wheat include glycolipids and phospholipids.

Although lipids appear to have little effect on mixing behaviour, they play an essential role in bread production and certain types of wheat lipids may contribute to the stability of the gas cell structure of dough during proving and the early stages of baking. Some classes of lipid fractions are thought to have good surfactant properties and are capable of stabilising interfaces. It has been shown that polar lipids such as phospholipids and glycolipids, in an optimal concentration, improve loaf volume and crumb texture (MacRitchie, 1977; Dubreil et al., 1997; 1988). On the other hand, non-polar lipids, tri-glycerides and free fatty acids in the wheat flour have poor foaming properties (Gan et al., 1995; Keller et al., 1997) and are detrimental to loaf volume (MacRitchie and Gras, 1973). The destabilising effects of cereal lipids to protein, stabilised foams have also been found in beer-foam studies (Cooper et al., 2002).

### 2.2.2. Water

Water is essential for dough development. It is necessary to mix the ingredients to form a homogenous mixture and the mixing plays an important role in all types of interactions and chemical reactions during different stage of bread processing. When water hydrates the flour, it allows the unfolding and mobilisation of the protein chains. The water content of bread dough is typically between 38 and 48% water (w.b.). The total amount of water added to the dough is related to the flour composition and the type of baking process and influences dough-handling characteristics. During the milling process, some disruption of the starch granule structure can occur. Damaged starch causes an increase in the water absorption capacity of flour (Tipples, 1969) and with more damaged starch in the dough, it is possible that water is drawn away from the gluten matrix by the starch granules.

### 2.2.3. Salt

Salt is generally used at levels of about 1 to 2% based on the flour weight. It is used to limit yeast activity during fermentation by controlling the rate at which the available substrate is digested. Salt also enhances the flavour of the final product and

shown to increase the strength of the gluten network (Preston, 1989), reduce stickiness of the dough and making it easier to handle.

#### 2.2.3.1. Salt and the development of gluten structure

As discussed earlier, gluten proteins are primarily responsible for determining the physical properties of wheat flour doughs (Bloksma, 1978). Numerous studies have shown that increasing concentrations of salts changes the structure and properties of the gluten probably due to the effect of electrostatic and hydrophobic interactions and the increase in the number of ionic bonds in the proteins (Preston, 1989).

In particular, at low salt concentrations (<0.15M) these effects are primarily due to the large changes in electrostatic free energy associated with the ionic shielding of charged amino acids that reside on the protein surface. These changes in electrostatic free energy can strongly affect interprotein interactions resulting in "salting in" or "salting out" of hydrated proteins. The magnitude of this effect is dependent upon the ionic strength and the charge density on the surface of the protein and is normally independent of ion type. The idea of salt induced aggregation of gliadins and glutenins is supported by Preston (1981), who found at low salt levels (0.05-0.1M) the solubility and aggregation of gluten were determined by ionic interactions, but at high salt levels (0.5-1.0M), solubility and aggregation depends on hydrophobic interactions. Furthermore, Preston (1981, 1984) found that gluten proteins varied widely in their hydrophobic properties and suggested that hydrophobic interactions are important in determining the physical properties of dough.

Studies also indicate the impact that salt-induced aggregation or disruption of gluten structure can have on bread properties (Greene and Kasarda, 1971; Caldwell, 1979; Chung and Pomeranz, 1979; Popineau and Pineau, 1987; Kobrehel, 1980; Huebner and Wall, 1980). Several authors have found differences in hydrophobicity or aggregation behaviour of gluten prepared from good or poor quality wheat (Huebner 1970; Arakawa and Yonezawa 1976; Chung and Pomeranz 1979), thus pointed to the potential importance of hydrophobic interactions in gluten and aggregation properties of gluten proteins. Further experiments showed that salt addition to dough also affected the distribution of the gluten fraction (Larsson, 2002). Adding salt

compound to the thiol radical causes the glutenin molecules to invert and become hydrophilic on the exterior and hydrophobic in the interior. That gives the dough a larger excess of water, hidden previously in the glutenin molecule. This excess of water results in a more liquid form of dough, with a lowering of the elasticity.

The effect of different salts, belonging to the Hofmeister series on physical properties of dough has been studied extensively (Preston, 1989; He et al., 1992; Butow et al., 2002). In the presence of salt, the quaternary structure of protein is stabilized and the lack of molecular expansion results in a less viscous protein system (Urbanski et al., 1982). This is generally described by bakers as a binding or tightening effect.

Numerous researches reported that addition of low concentrations (<0.10 M) of salt decreases water absorption, increases the time until optimum development, and stability of the dough as measured by the farinograph. The addition of salt may increase the possibility for ionic bonding in the dough and gluten molecules are bent and interlocked so that work in the form of additional mixing is required to unwind and unravel the gluten strands into more nearly linear alignments (Hlynka, 1962; Bennett and Ewart, 1965; Galal, et al, 1978; Preston 1989) .

The fundamental rheological properties analysed with rheomoters claimed that salt lowers the storage moduli G' and the loss moduli G" of dough. These reductions can be attributed to decreases in inter-protein hydrophobic interactions. The interactions decrease as a result of the water structuring events and is largely dependent on the content and quality of the proteins presents (Huebner, 1970; Arakawa and Yonezawa, 1975; Chung and Pomeranz, 1979; Preston 1984) as there is a reduced tendency for the proteins to aggregate, reducing elasticity. Similar results were also described by Salvador et al. (2003).

#### 2.2.3.2. Function of anions

Previous studies from Preston (1981 and 1984) showed that the extractability, turbidity, and gel filtration profiles of gluten proteins in simple neutral salts of the lyotropic series were very sensitive to anion type and concentration. Therefore, the

observed effects can not be attributed solely to ionic shielding of charged amino acids.

Nonchaotropic anions such as chloride ion ($Cl^-$), are also reported to increase dough strength properties and had little effect upon water absorption. Studies showed that nonchaotropic anions could increase dough extensibility. The general dough-strengthening effect at low salt concentrations, compared to water, can be attributed mainly to stronger interprotein interactions resulting from electrostatic shielding of charged amino acids on the surface of the gluten protein (Preston, 1989). Increasing concentrations of nonchaotropic anions increase the free energy of entropy associated with the exposure of apolar residues and thus increase inter- and intra-protein hydrophobic interactions and generally tend to stabilise native protein structure (Yoshino and Matsumoto, 1966, Bernardin, 1978).

#### 2.2.3.3. Salt and starch

The gelatinisation temperature of starch in a dough is an important characteristic in the baking process. The other components of the dough (proteins, sugar, salts, lipids etc) affect the gelatinisation temperature of starch, and thus the ultimate final structure of the baked product (Ghiasi et al., 1982). Added salt may have a plasticisation effect on wheat dough and thus lowers the glass transition temperature (Tg).

The effect of sodium chloride in delaying the starch gelatinisation has been reported (Chiotelli et al., 2002; Galal et al., 1978; Preston, 1989) and different explanations for this phenomenon proposed. The effect of adding salt has been described as an initial inhibition of gelatinisation up to a certain concentration (~ 2M) (Chiotelli et al., 2001). Ganz (1965) associated the presence of salt with enhancement of 'granule integrity' whereby greater swelling is experienced before fragmentation occurs. (Salvador 2003).

In addition, the structure of water and its modification in the presence of solutes has been suggested to be one of the most important factors in starch gelatinisation. When salt is added to dough, it lowers water activity and increases the energy necessary for

chemical and physical reactions involving water (Kim and Cornillon, 2001; Chiotelli et al., 2002).

### 2.2.3.4. Effect of salt on yeast fermentation

The impact of salt on yeast fermentation is well known (Williams and Pullen, 1998) and has been used by bakers for many years in the formulation of bread by balancing the levels of these two key functional ingredients.

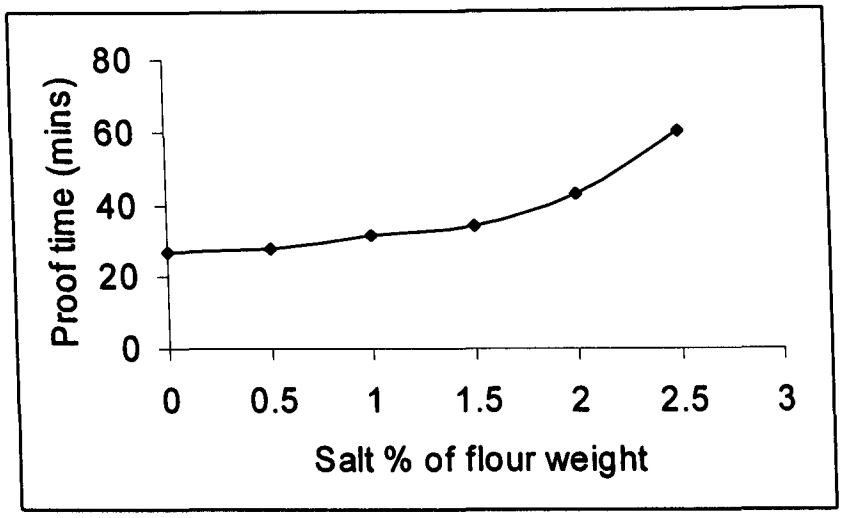



Figure 2-6. Effect of salt level on yeast activity (proof time to constant dough height in the pan) (Kilcast, 2007).

The overall effect of increasing levels of salt is to slow down the fermentation of yeast. This effect is critical in the closely timed fermentation processes that are used in modern bakeries. In fact, most bakeries work to a 'fixed' proof time that is a fixed fermentation period after the dough pieces have been moulded to shape and before they enter the oven. This period is important in bread making as it allows the dough piece to increase in volume which, along with modification of the rheological properties of the developed gluten network present, allows the dough piece to expand uniformly during the baking process in the oven. Once the appropriate proof time has been established it is important that the baker keeps to that time as under- and overproof leads to loss of product quality. The impact of changing salt levels on dough with a fixed level of yeast is illustrated in Figure 2-6. The variation in proving time, which is illustrated, indicates the length of time that the dough piece required in order to achieve a constant volume or height in the pan. The higher the level of salt

added to the recipe, longer the proof time needed for the yeast to produce the required level of gas to achieve a fixed volume or height.

### 2.2.4. Bakery Fat

Bakery fat, widely known as shortening, is usually a mixture of vegetable oil and solid fats. The level of fat used in bread making varies widely from zero up to 10% of flour weight. It is an essential ingredient for the CBP bread processing. Apart from stabilisation of gas cells during proving and baking (Brooker, 1996), it can improve the quality of baked loaves and soften the crumb (Baker and Mize, 1942; Daniels and Fisher, 1976; Elton and Fisher, 1966) and help to delay the staling of bread (Stauffer, 1998).

The effect of bakery fats depend on their amount, physical state, the solid-to-liquid ratio, crystal form and size, melting point and polarity (Baker and Mize, 1942; Baldwin et al, 1963, 1965; Carr et al., 1992; Chamberlain et al., 1965; Mahai et al., 1981; Pomeranz et al., 1966). Elton and Fisher (1968) concluded that the melting point of fats is important, but also suggested that the chain length, the mobility and the orientation of the chains at the melting point region are also important. Slade et al. (1989) related the increased loaf volumes with the plasticising effect of fats on the gluten. In addition, fats were found to interact with starch and affect the rheological and thermo-mechanical properties of dough such as viscosity peak and gelatinisation enthalpy of starch (De Stefanis and Ponte, 1976; Nierle et al., 1990; Slade and Levine, 1995).

Brooker (1996) investigated the effect of fats on both the proving and baking stage and found a positive effect of solid fats on bubble expansion in dough during mixing and baking. As mixing proceeds, gas cells and fat crystals come into contact, and the protein layers fuse so as to form a single continuous film surrounding both the gas cells and the fat crystals. It has been shown that fat crystals become aligned tangentially at the gas cell surface in cake batters and that this improves gas retention (Brooker, 1996). In general, small fat crystals of the $\beta'$ polymorph has a great ability to stabilize gas cells. Similar events may occur in bread doughs containing fat and may help to explain the role of fat in improving gas retention in bread doughs.

As the temperature of the dough increases, the fat crystals melt and the liquid oil then flows over the inner surface of the gas cells to form a hybrid interface comprising the oil layer in addition to the protein and/or polar lipid layer. The layer of oil helps to maintain the continuity of the gas/liquid interface in the expanding dough piece and thus aids gas retention during oven spring. A further advantage of the $\beta$' fat crystal polymorph over the $\beta$ polymorph in this respect may be that the former has a lower melting temperature and thus may be more readily available for contributing to gas cell surface integrity during baking than the latter.

In addition to the known advantages of using fat in bread doughs it has been found that fat can bind or sequester endogenous lipids shown to be detrimental to crumb score, and possibly, to loaf volume. The hypothesis that one of the role of added bakery fat is to bind endogenous lipid and reduce the amount which is free in the aqueous phase to occupy gas cell surfaces in the presence of protein, which is less surface-active, but can form stronger films.

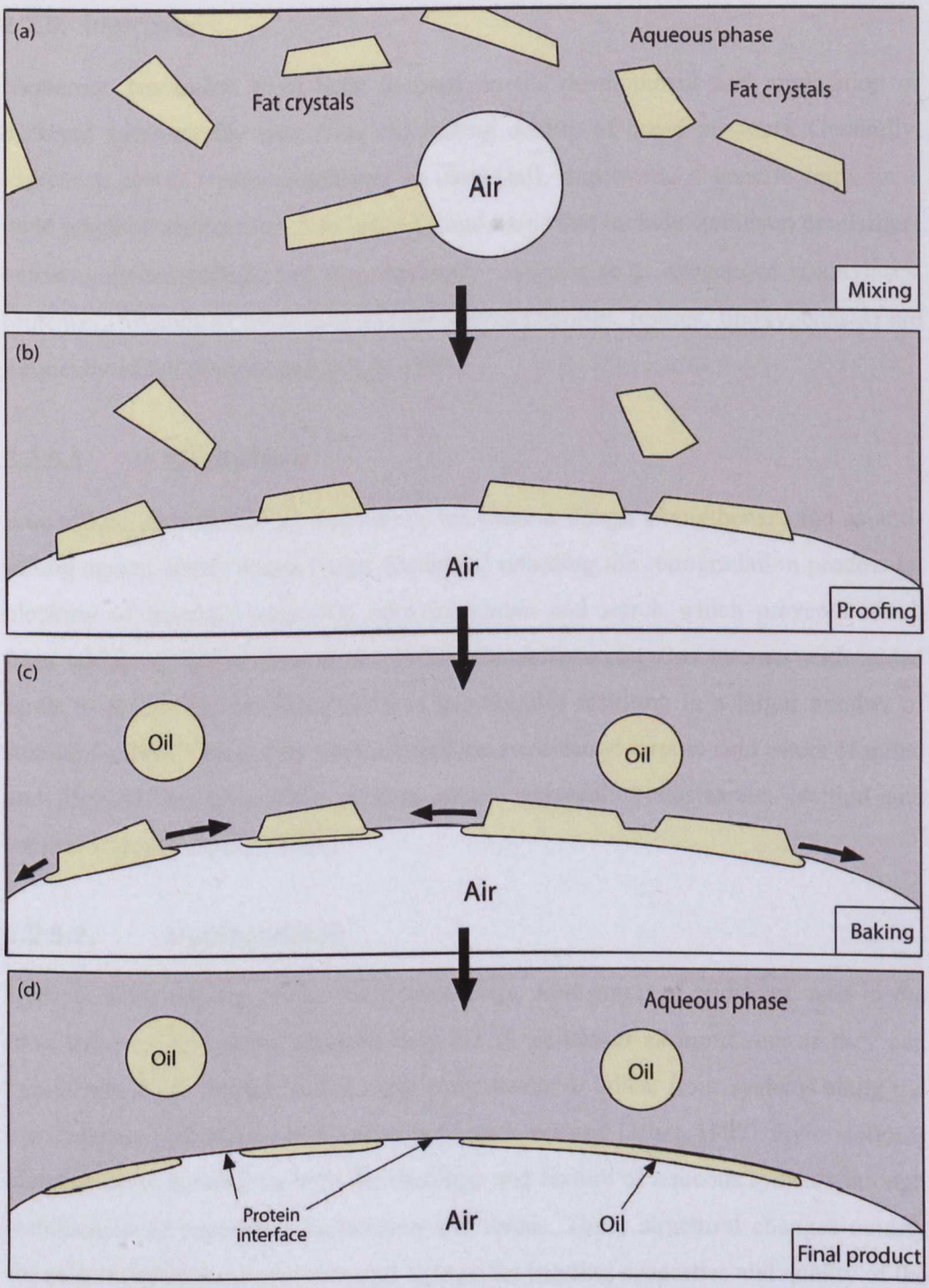



Figure 2-7. The role of fat in bubble growth in bread at different stages of bread baking. (a) Mixing stage showing fat crystals dispersed between yeast cells, starch granules, gluten and air; (b) proving stage showing the fat crystals adsorbed to the air cell. (c) The formed crystal-water interface is incorporated into the air bubble surface as it expands and (d) finished product showing that part of the fat crystallizes as the product was cooled, but part remains in the crystal-water interface and adsorbed to the air bubble surface (Brooker, 1996).

### 2.2.5. Improver

Numerous researches have been focused on the development and application of different additives for improving the baking quality of bread products. Generally, leavening agents (micro-organisms or chemical), improvers, a generic term for a wide range of additives used in bread formulations that include stabiliser, emulsifiers, oxidants, hydrocolloids and supplementary enzymes (e.g. exogenous α-amylases, proteases, hydrolases for non-cellulosic polysaccharides, lipases, lipoxygenases) are frequency added (Gujrail and Singh, 1999).

#### 2.2.5.1. Emulsifiers

Emulsifiers, also known as surfactants, are used as dough strengtheners and as anti-staling agents which interact with starch and retarding the retrogradation process by blocking of moisture migration between gluten and starch which prevents starch from taking up water (Rao et al., 1992). Emulsifiers can also interact with added lipids to reduce the surface tension in gas bubbles resulting in a larger number of smaller bubbles. Commonly used emulsifiers are diacetyl tartaric acid esters of mono and diglycerides (DATEM), sucrose esters, glycerol monostearate, lecithin and sodium steroyl lactylate (SSL).

#### 2.2.5.2. Hydrocolloids

Hydrocolloids are one of the most extensively used group of additives used in the food industry. In baking industry, they are as important as improvers as they can induce structural changes in the main components of wheat flour systems along the breadmaking process and bread storage (Appelqvist and Debet, 1997). Hydrocolloids are capable of controlling both the rheology and texture of aqueous systems through stabilisation of emulsions, suspension and foams. These structural changes modify the selectivity of some enzymes and change the handing properties and quality of the dough and bread (Armero and Collar, 1997). Hydrocolloids also affect the baking performance by influencing the melting, gelatinisation, fragmentation and retrogradation process of starch (Fanta and Christianson, 1996). These effects were shown to affect the pasting properties and rheological behaviour of dough (Rojas et al., 1999). These compounds also affect the shelf life of stored bread which form

complexes with the starch polymers and slow down the re-crystallisation of starch (Armero and Collar, 1997).

### 2.2.5.3. Reducing agents

Reducing agents such as L-cysteine are often added as the hydrochloride salt to weaken the gluten structure by reducing the disulfide (-S-S) cross-links to thiols, thereby improving the mixing and moulding properties of dough without structural damage. The maximum permissible level of L-cysteine hydrochloride is 300 mg $kg^{-1}$ (on flour weight) (SI 1998).

### 2.2.5.4. Oxidising agents

Oxidants are used to improve the structure and strength of the dough resulting in a better loaf volume and texture of the bread. At the molecular level, oxidizing improvers prevent thiols from splitting glutenin concatenations. Commonly used oxidants are ascorbic acids and lipoxygenase.

Ascorbic acid (AA) has been widely used as an oxidant in the baking industry. It is an oxidising agent that strengthens the gluten network by creating disulfide bonds (Nakamura and Kurata, 1997). Both ascorbic acid oxidase and oxygen are needed in this reaction. It also gives large increases in oven rise and bread score (Yamanda and Preston, 1992). The amount used for good dough processing is 10 – 200 ppm, based on flour weight, and it depends on the desired effects on the quality of baked goods (El-Hady et al., 1999). At present, the level of use of ascorbic acid is limited in the UK to 200 ppm by weight of flour (SI 1998).

Lipoxygenase occurs naturally in wheat, but it is usually supplemented in dough as enzyme-active soya which is a rich source of these enzymes. In the presence of oxygen, lipoxygenase catalyses the formation of hydroperoxides from polyunsaturated fatty acids with the pentadiene structure in the n-6 or n-3 position e.g. linoleate and linolenate in wheat. The bleaching and oxidising actions of hydroperoxides are exploited by bakers to remove the caroteniod pigments in flour and improve dough rheology through gluten modification (Marrison, 1988).

### 2.2.5.5. Enzymes

For decades, enzymes such as bacterial and fungal α-amylases have been extensively used in the bread-making industry. Amylases, which occur naturally in wheat, catalyse the hydrolysis of starch polysaccharides. They digest available starch (damaged and gelatinised) to form sugars that the yeast then converts into carbon dioxide: α-amylases produce dextrin oligosaccharides and a little maltose; β-amylase acts on α-1,4 glycosidic bonds near the non-reducing ends of amylose and amylopectin to produce maltose. In the case of the latter enzyme, the conversion rate is about 60%; the material remaining is referred to as "limit" dextrin (Cauvin and Young (1998).

Hemicellulases are used as baking enzyme for the enhancement of dough quality (mechanical handling and stability) and for bread optimisation (loaf volume, crumb softness and texture). Some of the mechanisms that contribute to these improvements include: release of water from non-starch polysaccharides and improvement of gluten hydration and solubilisation of insoluble arabinoxylans thereby removing them from the gluten network (Courtin and Delcour, 2002).

Beneficial effects of added lipases on bread quality have been reported. Lipases can be used to improves crumb softness of bread and retard bread staling by forming monoglycerides, which act as surfactants (Gil et al., 1999; Leon et al., 2002). However, lipases have been rarely used in bread making as its detrimental effects being observed due to the action of endogenous lipases liberating unsaturated free fatty acids into the dough (Gil et al., 1999).

### 2.2.6. Yeast

Bakery yeast (*Saccharomyces cerevisia)* acts mainly as a leavening agent. The use of yeast in bread making has at least 6000 years of history, since fermentation of bread dough is thought to have started with the ancient Egyptians. Yeast can be compressed (28-30% dry matter) or, more typically, in a liquid (cream) form. The main role of yeast is to increase loaf volume by the production of carbon dioxide and to enhance flavour (Gassenmeier and Schieberle, 1995; Schieberle and Grosch, 1991).

The actions of yeast may be shown in a simplified form as follow:

| | | | | |
|---|---|---|---|---|
| Simple sugar | → | Ethyl alcohol | + | Carbon dioxide |
| $C_6H_{12}O_6$ | → | $2\ C_2H_5OH$ | + | $2\ CO_2$ |

Both salt (sodium chloride) and sugar can affect the activity of yeast. They can be used to aid the control of fermentation.

## 2.3. DOUGH PROCESSING

There are many bread making processes around the world and consequently a wide range of bread products. The modern commercial process used in large bakeries in Britain and elsewhere today is the Chorleywood Bread Process (CBP). This method was developed in 1961 by the Flour Milling and Baking Research Association at Chorleywood, and it produces bread without the need to ferment dough in bulk. Dough development in CBP is achieved during high-speed mixing by intense mechanical working of the dough in a few minutes. This method saves time and is cost-effective, but more importantly, it produces bread with better volume and colour that keeps for longer. The work presented in this thesis is based on the CBP and the following section provides an overview of the process.

The processing of bread making can be divided into three basic operations: mixing or dough formation, fermentation, and baking. This section provides an overview of the key stages of the CBP bread making process (Figure 2-8).

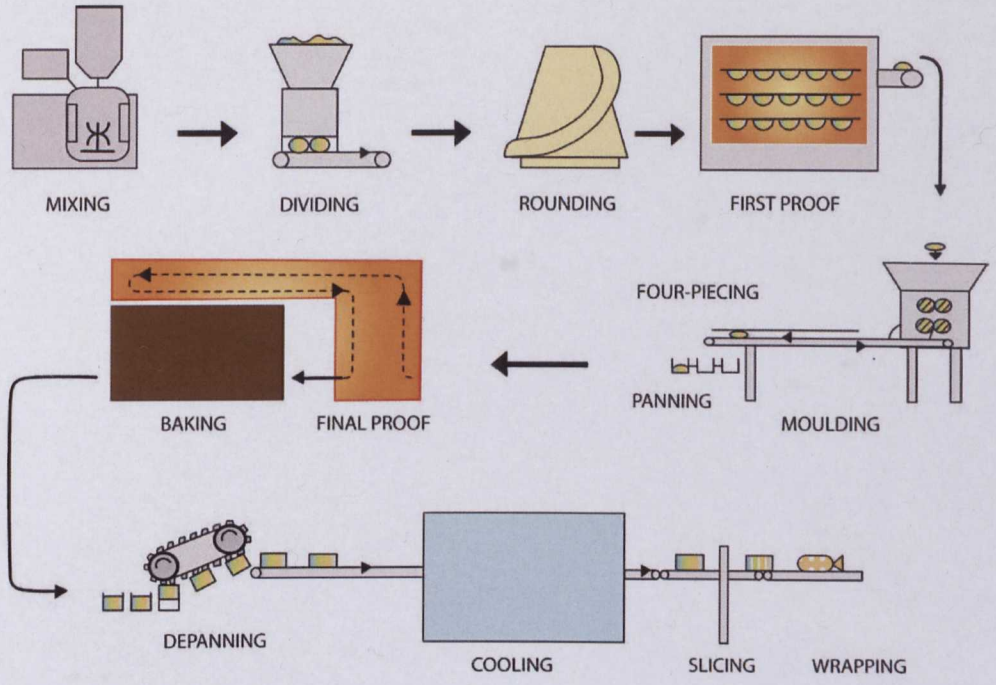



Figure 2-8. Steps in the Chorleywood Bread Process (adapted from Campbell 1991).

### 2.3.1. Dough Mixing

Dough mixing is the most critical step in the bread making process, and is responsible for several functions:

- Hydrate and develop the wheat flour protein (gluten)
- Incorporate air bubbles in the dough (also known as aeration)
- Bleach the yellow pigments in the dough.

#### 2.3.1.1. Dough development

During the early stage of mixing, all the ingredients of dough are being blended to give a homogeneous dough mass. When flour meets water, several processes begin. The mixer produces shear and extensional forces bring the glutenin concatenations into alignment in a cooperative way for dough development. Meanwhile, broken starch granules absorb water and enzymes digest their exposed starch into sugars. The yeast cells use the sugars to produce carbon dioxide and alcohol. Lipids (from flour and added fat) are uniformly distributed and brought into contact with the protein fibers, and soluble materials are fully dissolved and distributed in the aqueous matrix. The final structure of dough involving complex interactions of two

continuous phases co-exist: the hydrated gluten network and the water phase with dispersed starch granules.

In the CBP, dough development is typically achieved by high speed mixing. A considerable amount of energy input is required, usually about 36 kJ $kg^{-1}$ of dough in less than three minutes. To ensure consistency, large commercial mixers calculate the energy imparted to the dough from the electrical power required to run the mixer. The final temperature of the dough, typically 29°C, is important for subsequent yeast activity.

#### 2.3.1.2. Air incorporation

During the mixing stage, air is incorporated into the dough matrix. The incorporated air bubbles are then further broken down during mixing, reducing the mean bubble size and increasing the number of bubbles. After mixing, the gas cells are spherical with an estimated diameter between 10 and 100 μm (Bloksma, 1990). These small bubbles provide nucleation sites for diffusion of carbon dioxide and bubble growth during proving. The number of nuclei is controlled by applying partial vacuum during mixing, typically 318 mm Hg (or 0.50 atm) as atmospheric oxygen is also necessary for dough development and colour formation.

#### 2.3.1.3. Colour formation

The yellow pigments from the wheat flour can be removed by the presence of oxygen (and lipoxygenase). For this reason, delay in creating the partial vacuum is generally used to ensure adequate blenching on the dough mass.

### 2.3.2. Proving

Proving is the stage during which the dough is set aside for the yeast cells to produce carbon dioxide, which diffuses into the air cells, slowly inflates them, and thus raise the dough. It takes place in a controlled environment, typically at 45°C and 80% relative humidity (RH) to prevent dough surface drying. This temperature is above the maximum gassing temperature for yeast of 40°C to allow for the temperature lag associated with the large dough mass. During proving, the enzymes from the flour

convert starch to dextrins and sugars. The yeast contained in the aqueous phase ferments sugar to produce carbon dioxide. It saturates the aqueous phase and diffuses into the atmosphere or into pre-existing air cells inflates the bubbles in the dough and eventually leads to dough expansion due to increasing pressure in the gas cells. Gas cell stabilisation and retention at this stage is critical, since it will determine the crumb structure and volume in wheat bread.

In the later stage of proving, bread dough starts to show small splits in the viscoelastic walls between the gas bubbles. Gan et al. (1995) proposed a model in which, during the end of the fermentation and early baking, the gas cell would changes from independent gas cells to gas cells with discontinuities filled by a liquid film rich in surface active materials. The surface area of the liquid films would increase with more expansion as discontinuities become more frequent. These liquids films would eventually fail leading to the sponge structure of bread. The role of this liquid film is further discussed in section 2.4.

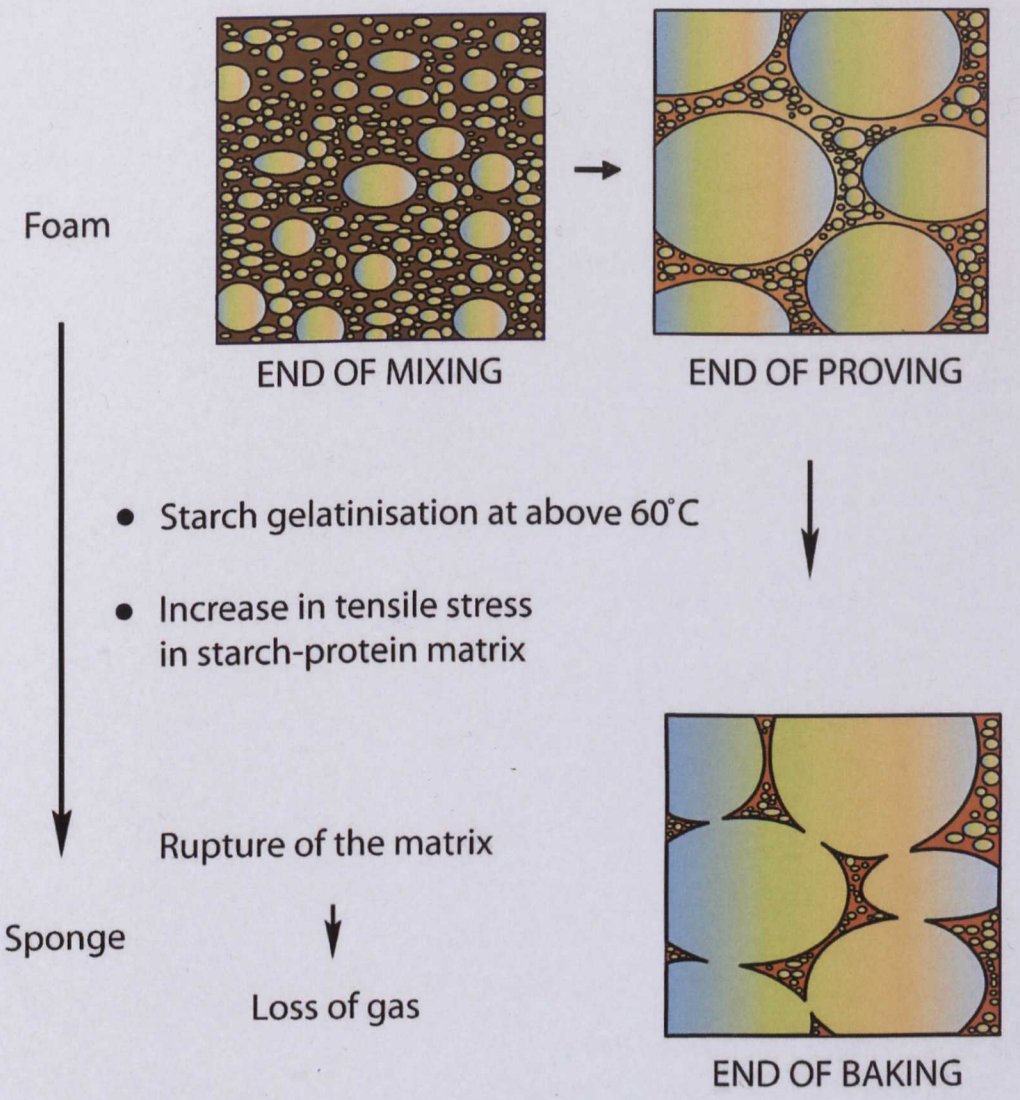



Figure 2-9. Gan et al. (1995) proposed a model of dough expansion. After mixing, the dough consists of discrete gas cells lined with liquid films and embedded in a continuous starch-protein matrix. The matrix fails to enclose the gas cells completely at advanced stages of fermentation, leaving area that contain only a thin liquid lamella. Baking increases the rate of expansion until the lamellar film is incapable of meeting the demand for new surface area generation, thus converting the foam structure of dough into an open sponge. The loss of gas retentions is caused, therefore, by the rupture of the liquid film, not that of the starch-protein matrix.

### 2.3.3. Baking

During the baking process, high temperature triggered a series of physical and chemical changes of the dough to form a sponge-like structure named bread. Yeast activity starts to decline from 43°C and ceases at 55°C. Heating during baking causes expansion of gas cells and further stretching and thinning of the protein sheet. At the

early stage of baking, flexibility of the gluten matrix allows trapped gas cells to expand and the carbon dioxide remains in the dough helps to produce a large loaf of bread at the end. Furthermore, starch granules, entirely enrobed by protein, are gelatinise at about 60°C reinforcing the gluten system. The extent of gelatinisation depends on the available water in the dough and temperature during baking. Under these conditions, α-amylase converts the starch into dextrins and sugars (maximum α-amylase activity occurs between 60-70°C).

At the later stage of baking, the expansion of gas cells lead into an open network of pores. As the lamella between gas cells ruptures, the cells become inter-connected, forming a sponge structure.

## 2.4. THE DOUGH LIQUOR FILM HYPOTHESIS

Wheat flour dough contains typically 0.6-0.8 g water/g of dry flour, of which approximately half is thought to be "bound" or unfreezable (Davis et al., 1969; Bushuk et al., 1977). The presence of "free" or freezable water can be detected only when the water content exceeds about 30- 35% by weight (MacRitchie, 1976). A liquid phase may be separated by centrifuging the dough at very high centrifugal fields and this was first described by Baker et al. in 1946 (Ablett et al., 1986; MacRitchie, 1976). This aqueous phase was thought to be necessary for dissolving soluble flour components and in providing the medium for reactions to take place within the dough (Bake et al., 1946; Gan et al., 1995). The high electrical conductivity measurement of a developed dough confirmed the presence of the continuity of this aqueous phase (Eliasson and Larsson, 1993).

Numerous studies have proposed that an aqueous phase exists in dough and is beneficial to the baking process (MacRitchie, 1976; Gan et al., 1990, 1995; Sahi, 1994, 2003; Ornebro et al., 2000). It has been proposed that this aqueous layer is important in maintaining gas-cell integrity when gluten network ruptures. Recent studies using scanning electron microscopy (SEM) have highlighted the significance of a continuous lamellar film in gas cell stabilization and gas retention (Gan et al., 1990; 1995). Studies show discontinuities do occur in the starch gluten-matrix during

proving, indicating the possible loss of gas from the dough. However, dough increases in size during this stage suggesting that these discontinuities in gas cells become sealed. It is possible that liquid lamellae might continue to separate the gas cells, maintaining the bubble network in dough. In the absence of this aqueous phase, bread dough could lose its capacity for gas retention (Gan et al., 1990; 1991).

The properties and composition of the dough aqueous phase have been described by a number of workers (MacRitchie, 1976; Sahi, 1994, 2003; Dubriel et al., 1998; Gan et al., 1999; Mills et al., 2003; Primo-Martin et al., 2006; Salt et al., 2006) and it is thought to be comprised of mixture of lipids (phospho- and galacto-lipid), soluble proteins and non-starch polysaccharides (Baker, 1946; MacRitchie, 1976, Sahi, 1994, Gan et al., 1995; Salt, 2004). All these components are known to be surface active molecules and have all been shown to increase gas-cell stability. Generally, the two main functions of a surfactant and/or stabiliser are to lower the interfacial free energy in order to facilitate the formation of the dispersion and to stabilise the resulting dispersed system.

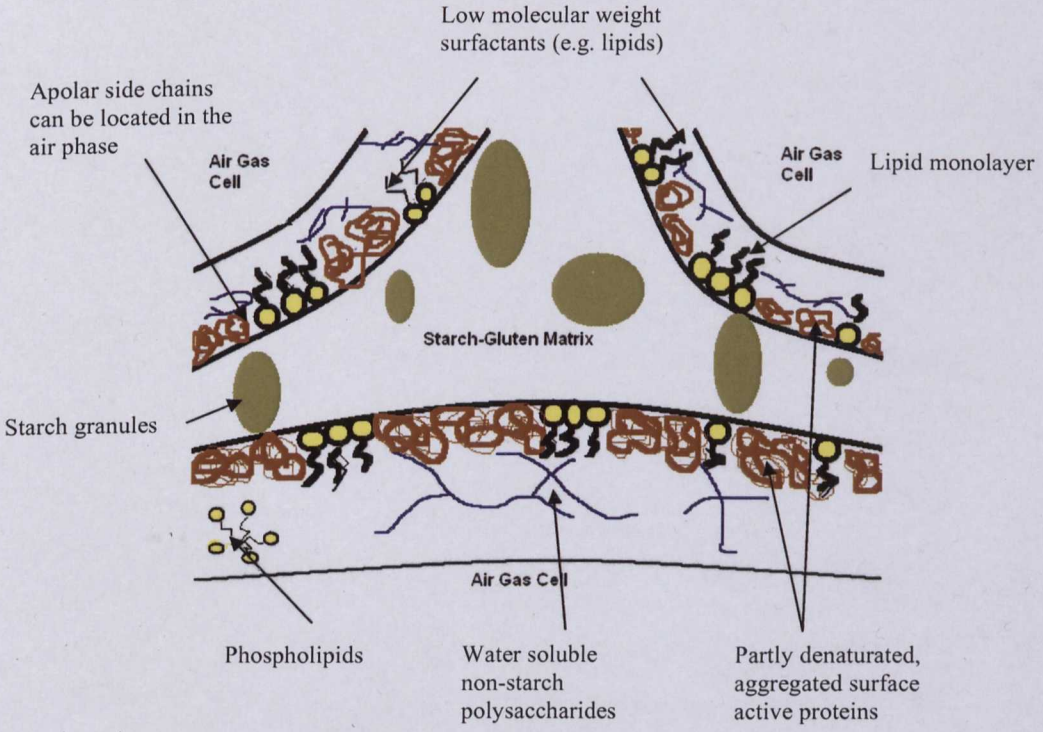



Figure 2-10. Components involved in thin films and interfaces formed at the gas-cell surface (Adapted from Mill et al., 2003). As wheat starch granules are ~5 um for B starch granule and ~25 for A granules (Dai et al., 2009) an approximate scale can be deduced.

### 2.4.1. Surface Active Proteins

Proteins, being amphipathic, in nature are one of the important surface-active components in food systems (Wilde, 2000), as they can form a strong, elastic interface by unfolding at the interface and thus increasing the surface viscosity. Sahi (1994) stated that the levels of proteins soluble in the aqueous phase of dough were found not to be related to the protein content of the flour (or the bread making quality).

The variation of protein present in the aqueous phase is believed to be determined by its compactness, rigidity, and its surface hydrophobicity (Bigelow et al., 1967). More flexible proteins can rearrange their tertiary structures to minimize the free energy and induce the absorption at the air/water interface. The more hydrophilic residues being located in the water phase further stabilise the liquid film.

However, little is known about the detailed protein composition of dough liquor or the proteins which may form an interface lining the bubble walls in bread doughs. Certain salt-soluble and interfacially active proteins have been implicated in determining the surface properties of the aqueous phase, including nonspecific lipid transfer protein (nsLTPs), puroindolines (Douliez, 2000) and certain members of the α-amylase-trypsin inhibitor family such as CM3 (Gilbert et al., 2003). Therefore they might be expected to find their way into dough liquors and the thin films lining the bubbles.

Indeed, it is been shown that puroindolines, can also act to improve the foaming properties of lipid damaged foam. Its addition to dough having an effect on the crumb structure of bread (Dubriel, 1998) and may enhance foam stability by sequestering lipids, preventing them from going to the interface. Furthermore, the surface properties of wheat prolamines, which form gluten, have been studied since they may also play an important role at the gas/liquid interface (Paternotte et al., 1994; Wannerberger et al., 1997).

### 2.4.2. Surface Active Lipids

The polar fraction of lipids, particularly, are capable of orienting themselves at the gas/liquid interface, thereby are thought to assist in the foamability of dough by forming a lipid monolayer at the gas/liquid interface (Gan et al., 1995; Keller et al., 1997; Dubriel et al., 1998). The spreading pressure of the film provides a force that counteracts the interfacial tension between the gas and the liquid phases, and, thus, stabilises the gas cells.

The role of lipids in determining baking quality of wheat flour has been debated (Baker and Mize, 1942; Leissner, 1998) for many years. There is evidence that polar lipids can be both detrimental and beneficial to loaf volume, depending on their concentration, whilst non-polar lipids seem to have only a detrimental effect on loaf volume.

Gan et al. (1995) stated that at low concentrations, the interface of the aqueous phase comprises primarily of proteins, but as the proportion of lipid present increases, the lipids breakdown the viscoelastic protein network, reducing the film stability. The interplay between the properties and composition of the proteins and lipids is clearly very important in determining the surface properties of the aqueous phase and hence gas cell stability. The thin aqueous film can be stabilised with protein-lipid mixtures up to a certain critical ratio. Above this ratio, the protein molecules become mobile and de-stabilise the film.

Another class of lipid-like components important to bread making are the surface active synthetic emulsifiers which are routinely included as ingredients in order to improve loaf volume and to stabilise the fragile foam structure developed following proving (Carlson and Sun, 2000; Mettler and Seibel, 1993). These include DATEM (di-acyl tartaric esters of monoglycerides) and SSL (sodium stearoyl lactylate), although little is known about the mechanism whereby they improve baking performance. They may act by replacing the lipids, and perhaps even the protein, adsorbed at the surface. As DATEM and SSL are highly surface active, they will

stabilise the bubbles against coalescence, and hence improve dough stability during proving (Eliasson and Tjerneld, 1990).

### 2.4.3. Involvement of Bakery fat

Bakery fat is an optional ingredient for "traditional" long or bulk fermentation bread making processes. For "no time" mechanical dough development process, such as the CBP, however, it is an obligatory ingredient for production of bread for acceptable quality. Furthermore, not only must fat be included, but a proportion (5%) of that fat must be solid (i.e. in crystalline form) at proving temperature (Baker et al., 1946; Baldwin et al., 1945; Chamberlain et al., 1965a, 1965b). Reasons for this are described in section 2.2.4.

### 2.4.4. Other surface active compounds

Another component likely to be soluble in the aqueous phase to some degree is the non-starch polysaccharide, arabinoxylan. This may also play a role in determining the surface properties of the aqueous phase, but not by virtue of its intrinsic surface activity (Izydorczyk et al., 1991a, b). Rather the arabinoxylans can mediate interactions and cross-links between proteins in an adsorbed layer, increasing the viscosity of thin liquid films, thereby enhancing the stability of any foam structures formed (Sarker et al., 1998).

### 2.4.5. The role of starch

Starch is one factor that might affect the stability of gas cells in bread dough and the subsequent crumb quality of the baked dough system. The effect of starch on dough film stability has been studied in terms of starch granule sizes by Van Vliet et al. (1992). The presence of starch granules that are larger than the thickness of a gas cell wall in dough is thought to induce instability in gas cell wall (Van Vliet et al., 1992). When the gas cell wall is thick compared with the size of large wheat starch granules (25 - 40 um), the wall will be stabilized against coalescence. Hayman et al. (1998) also observed that the presence of a great proportion of large starch granules in wheat dough affected the expansion due to an increase of gas cell coalescence.

### 2.4.6. The role of salt

Evident has shown that certain interfacially active proteins such as non-specific lipid transfer proteins (nsLTPs), puroindolines (Douliez et al., 2000) and certain members of the α-amylase-trypsin inhibitor family such as CM3 (Gilbert et al., 2003), are soluble in dilute salt solutions. Therefore they might be expected to find their way into dough liquors and the thin films lining the bubbles with in dough.

Mills et al. (2004) analyzed dough liquor proteins by 2D-PAGE and revealed that there was a complex mixture of proteins with a large number of polypeptides present. The addition of salt and ascorbate to the dough caused some changes to the protein profiles, with regards both the abundance and appearance and loss of particular polypeptides in certain regions of the gels. Addition of salt increased the abundance of a group of proteins running at neutral pI and with molecular weights ($M_r$) of 55-60,000, with a new cluster appearing and running at a slightly higher molecular weight, compared with the flour-water only dough samples (Mill et al., 2004).

Addition of salt also increased the abundance of proteins of molecular weight 13-14,000, which probably correspond to members of the α-amylase/trypsin inhibitor family. A major protein of $M_r$ 9,000 and basic pI found in flour water dough liquor disappeared when salt was added to the dough mix.

## 2.5. SUMMARY OF CBP OPERATIONS

Table 2-2. Summary of major operations in CBP. Adapted from Cauvin and Young (1998) and Campbell (1991).

| Operation | Functions | Details Description |
|---|---|---|
| Mixing | Mix and hydrate ingredients.<br>Develop dough.<br>Incorporate gas cell nuclei. | Intense batch mix, 12Wh imparted in < 3 min.<br>Final temperature of 29C.<br>Delayed vacuum. |
| Dividing | To divide dough mass into approximately 900g piece. | A volume of dough is sucked into a chamber and ejected onto a conveyor. |
| Rounding | Shape the dough piece. | A rotating cone carries the dough in a spiral fashion along a curved channel. |
| First proof | To allow stresses in dough to relax, improving handling. | Dough piece travel through a cabinet in individual pockets for < 10 min. |
| Sheeting | To convert dough ball to a flat sheet and then roll it up like a Swiss roll. | Sets of rotating rollers of gradually decreasing separation squeeze dough flat. Chain and pressure board roll the sheet up and stretch it out. |
| Four piecing and panning | To orientate elongate bubbles giving a finer firmer structure. | Roll of dough is divided into four, turned through 90° and deposited into baking pans. |
| Second proof | Allow dough to rise by the action of $CO_2$ from yeast. | Tins travel through cabinet maintained at 45°C, 80% RH, for 45-50 min. |
| Baking | To set the loaf structure and develop colour and flavour. | Loaves baked in continuous oven at 200°C for 20-25 min. |
| Depanning | To remove the baked loaves from tins. | Loaves are lifted out of tins by rubber suckers onto a conveyor belt. |
| Cooling | To cool the loaves for slicing and prevent moisture migration on wrapping. | Loaves cooled at a centre temperature of < 27°C in cold air, residence time ~ 2 hours. |
| Slicing | To slice loaf. | A frame of high speed reciprocating blades slices the loaf. Internal crumb must not be too sticky. |
| Wrapping | Hygienic and aesthetic protection of loaf. | Automated wrapping of sliced loaves into polythene bags. |

## 2.6. CURRENT KNOWLEDGE

Bread making is a complicated process and sodium chloride seems to influence many factors. After discussions with the commercial bread bakers, it was evident to the UK Food Standards Agency that reducing the sodium levels in plant manufactured breads would be commercially difficult due to the lack of understanding of the role of salt (NaCl) had during bread production.

To gain a better understanding of the role of sodium played in bread manufacturing, it was necessary to:

1. Establish and quantify the effects of changes in salt level (over the relevant range) on commercial bread manufacture and the final quality of the bread.
2. To establish a testable hypothesis that could explain the range of quality issues observed during the manufacture of low salt (<0.8%) bread.

Test baking of bread was undertaken by CCFRA and testing the hypothesis that salt changed the "free" fluid content in bread dough was the major objective of the work carried out within this thesis. The methods used for the work are described in the next chapter.

# CHAPTER 3. MATERIALS AND METHODS

## 3.1 INTRODUCTION

This chapter describes the materials that were used to prepare the various samples investigated. It will also introduce the preparation techniques that were used to make the bread dough from the CPB and mini-pin methods. Finally, the chapter will provide a description of the various techniques that were used to analyse the samples.

## 3.2 MATERIALS

Much of the work discussed in this thesis used Viking flour obtained from Whitworth Bro. Ltd. (Northants, UK) and the specifications are given in Table 3-1. Flours were stored at -18°C and were warmed to the required temperature a night before they were used. Other flours were obtained from the consortium and used flours of different quality and milled to different levels of starch damage.

Table 3-1. Wheat flour used in this project and its specification (% dwb)

| Sample | Moisture (%) | Nitrogen (%) | Water Absorption (%) |
|---|---|---|---|
| Viking Flour [a] | 13.5 | 11.5 | 58.0 |
| Resolute Flour[b] | 14.5 | 9 | 54 |
| Canadian Flour | 14.9 | 13.5 | 58.6 |
| Solstice Flour | 15.1 | 11.3 | 59.6 |

[a] adapted from Whitworth Bro. Ltd., UK.
[b] adapted from Smiths Flour Mills, UK.

Bakery fat, improver (composition of improver is shown in Table 3-2) and fresh compacted yeast were kindly supplied by CCFRA. Cooking salt (sodium chloride) was a locally obtained commercial sample.

Table 3-2. The composition of improvers used in this thesis.

| Basic improver formula | | Compound improver formula | |
|---|---|---|---|
| Ascorbic acid | 200 ppm | Ascorbic acid | 40 ppm |
| Alpha amylase | 0.5 % | Fungal alpha amylase | 10 ppm |
| | | Soya flour | 1500 ppm |
| | | DATEM | 1500 ppm |
| | | SSL | 3000 ppm |
| | | Hemicellulase | 12 ppm |

## 3.3 METHODS

### 3.3.1 Samples preparation

#### 3.3.1.1 CBP dough preparation

The control of the final dough temperature is vital to the quality of dough and bread. Preliminary work established the mixing time to achieve a consistent work input and final dough temperature.

The temperature of both water and flour was calculated from the following formula (Cauvin and Young, 1998):

$$T.raise = (T.dough \times 2) - T.flour - T.water \qquad \text{Equation 3-1}$$

where T.raise is the temperature raise, T.dough is the temperature of the dough, T.flour is the temperature of the flour and T.water is the temperature of the water.

The standard recipe used in the work described in this thesis is given in Table 3-2; individual recipes are detailed in the results chapters. Samples of bread dough were prepared using a Tweedy 10 Mixer (Tweedy of Burnley Ltd., Burley, UK) with 3 kg flour per batch (Figure 3-1). Salt was dissolved in the water before adding to the flour. Water temperature was adjusted to 12 + 1°C to achieve final dough temperature of 29 $\pm$ 1°C. Mixing time was adjusted to provide a work input of 11Wh for Viking flour. Vacuum was induced to the mix after 60 seconds of mixing at 0.5 Bar. The total mixing time was 2 min 10 sec. The final dough temperature was

recorded. Sub samples were taken immediately after the mixing stage and used within 1 hour of mixing.

Table 3-3. CBP dough recipes (% on flour weight) used in this thesis.

| Ingredients | Full Recipe Baker's Percentage (%) | Simplified Recipe Baker's Percentage (%) |
|---|---|---|
| Flour | 100 | 100 |
| Salt | 0-2 | 0-5 |
| Water | 62.3 | 62.3 |
| Improver | 1 | 1 |
| Fat | 1 | 1 |
| Yeast | 1.7-2.0 | -- |

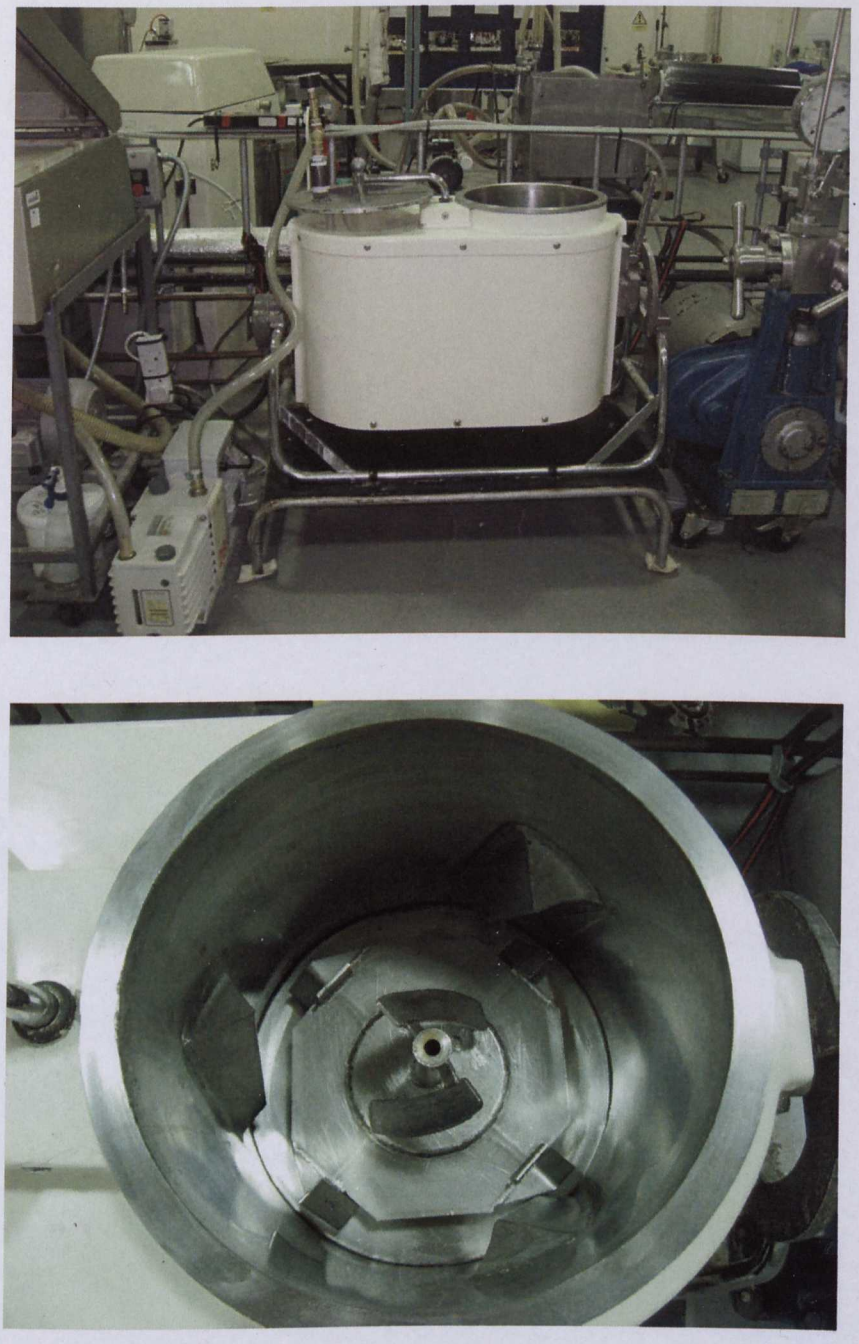


Figure 3-1. Tweedy 10 Mixer (Tweedy of Burnley Ltd., Burley, UK).

### 3.3.1.2 Minor-pin dough preparation

The 50-g scale pin mixer (Henry Simon Ltd., UK) was used to reproduce the conditions of commercial high speed mixing, necessary for dough development in the CPB. This minor-pin mixer achieves dough development by means of extension using a series of counter rotating pins.


Figure 3-2. Minor-pin Mixer (Henry Simon Ltd., UK).

#### 3.3.1.3 Dough liquor isolation

After mixing, dough samples were weighed (approximately 17 g) into polycarbonate ultracentrifuge tubes (25 x 89 mm) and centrifuged (Kontron Instrument Centrikon T-1065, UK) in a 20° fixed angle rotor at 35,000 rpm (126,000 g) for 1 hour at 15°C. The resulting supernatant ("dough liquor") was then separated. The pellet and the dough liquor were isolated and stored at -18°C for further experiment.

## 3.4 ANALYTICAL METHODS

### 3.4.1 Moisture content determination

The moisture content of cereal samples was determined gravimetrically in triplicate. Samples (4-6 g) were weighed accurately into disposable aluminium pans and heated at 105 °C in a laboratory fan oven (LTE, UK) for 24 hours. The percentage moisture was calculated from the loss in weight expressed as a mean of triplicate determinations (wwb).

### 3.4.2 Protein Content Determination

The protein concentration of aqueous extracts was determined by using the Biuret protein assay. The Biuret reagent is made of potassium hydrogen (KOH) and copper (II) sulfate ($CuSO_4$), together with potassium sodium tratrate ($KNaC_4H_4O_6H_2O$) and supplied by Scientific Laboratory Ltd (U.K.). A standard protein solution of 20mg/ml was prepared from bovine serum albumin (BSA). The BSA solution was serially diluted with $H_2O$ to give a range of standards from 1.0 mg/ml to 20.0 mg/ml protein. Biuret reagent (4 ml) was mixed with 1 ml of dough liquor sample. The solution is allowed to stand at room temperature for 30 minutes. After the incubation period, the solution with protein present became purple in colour. This colour change is due to the protein reducing the copper (II) ions in strongly alkaline conditions. At high concentrations of protein the purple colour of the reagent becomes more intense. The colour density was quantified by measuring the absorbance 540 nm against the reagent blank using PU 8720 UV/VIS Scanning Spectrophotometer (Philips, UK).

### 3.4.3 Differential scanning calorimetry (DSC)

Differential scanning calorimetry has become valuable tool and is one of the most widely used techniques for studying the phase transition properties of starch systems.

#### 3.4.3.1 Theory of differential scanning analysis

Differential scanning calorimetry (DSC) is a thermo-analytical technique that measures the temperatures and heat flows associated with the physical changes in materials often as a function of time and temperature in a controlled atmosphere. Most DSC equipment belongs to one of the following two groups: power-compensated DSC or heat flux DSC for the work described in this thesis, the latter was used and hence will be described in more detail here.

With a heat flux DSC, the sample and reference pans are enclosed in a single furnace and are connected by a low resistance heat flow path (a metal disc) (Figure 3-3). The sample and reference pans are subjected to the same time-temperature regime. When the sample undergoes a transition where heat is either being released (exothermic event) or gained (endothermic event), the temperature difference between the sample and reference pan is recorded.

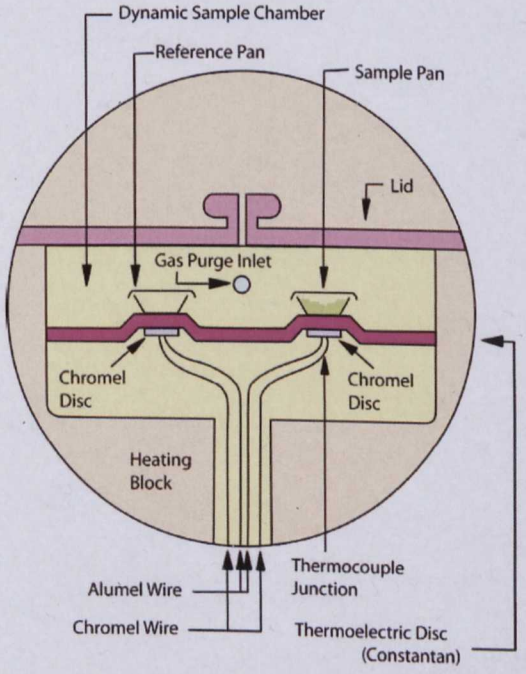



Figure 3-3. Schematic representation of the heat flux DSC with the sample holder on the right and the reference holders on the left.

### 3.4.3.2 Gelatinisation Temperature

The enthalpy and temperature of gelatinisation of the endotherms obtained with DSC have been used to study the effect of processing and composition of starch during heating. The gelatinisation temperature of starch in a dough is an important characteristic in the baking process. Other components of the dough (protein, sugar, salts, lipids etc.) also affect the gelatinisation temperature of starch, and thus the ultimate final structure of the baked product.

A typical starch melting endotherm observed in dough sample is shown in Figure 3-4. The typical variables that are of interest for such a melting transition are the onset temperature ($T_o$), the peak temperature ($T_{peak}$) and the final temperature ($T_{end}$). The enthalpy of the transition ($\Delta H$) is calculated from the area underneath the peak (represented by the shaded area). The onset and final temperatures are classified as the intersection between the extrapolated baseline (dotted line) and the tangent of the side of the peak, as illustrated.

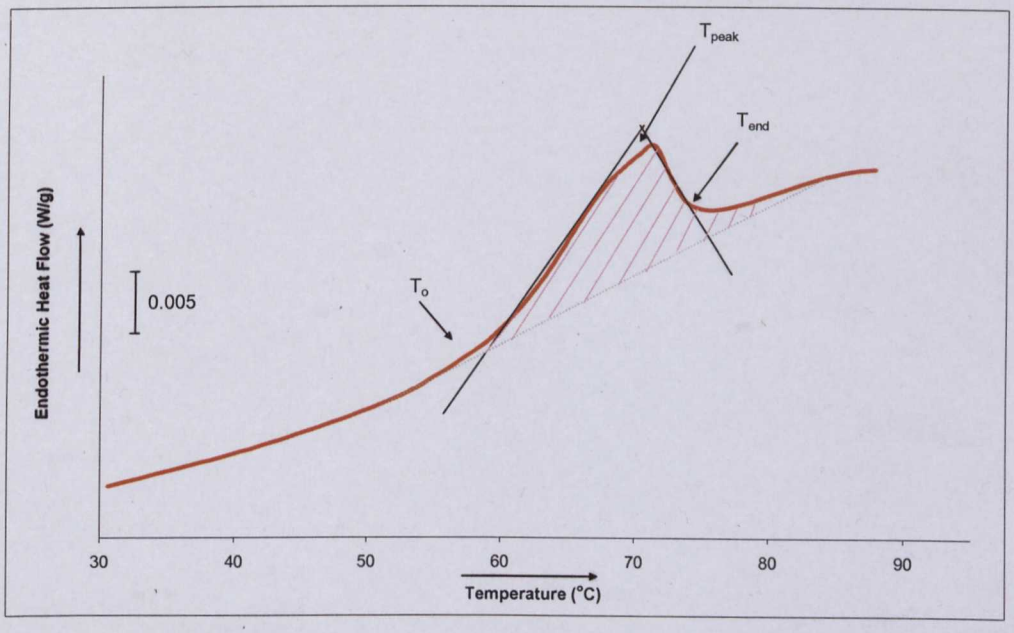



Figure 3-4. A typical endotherm observed on heating starch in dough system.

#### 3.4.3.2.1 Experimental procedure

The endotherms of starch gelatinisation in samples were carried out with a Mettler Toledo DSC 823e differential scanning calorimeter (UK). The DSC was calibrated

with indium standard to check machine calibration and reproducibility. Initial studies using fresh flour-water dough without salt were used to ascertain suitable running condition. A small piece of dough samples (~30mg) was weighed and hermetically sealed in an aluminium DSC pan. The samples were heated from 5 to 95°C at a heating rate of 5.0°C /min, using an empty aluminium pan as reference. Duplicate measurements were carried out for each sample. In order to obtain $T_o$, $T_{peak}$, $T_{end}$, and $\Delta H$, the accompanying Mettler Toledo software (STARe version 9.10) was used.

#### 3.4.3.3 Ice formation

Water is an important chemical and structural component in the food system, and it plays a significant role in the final quality. The amount of water that can be form ice crystals is related to water levels in the dough system. In this study, the bread dough's thermo-physical properties of interest are the amount of freezable water, enthalpy of fusion, onset temperature ($T_o$), the peak temperature ($T_{peak}$) and the final temperature ($T_{end}$).

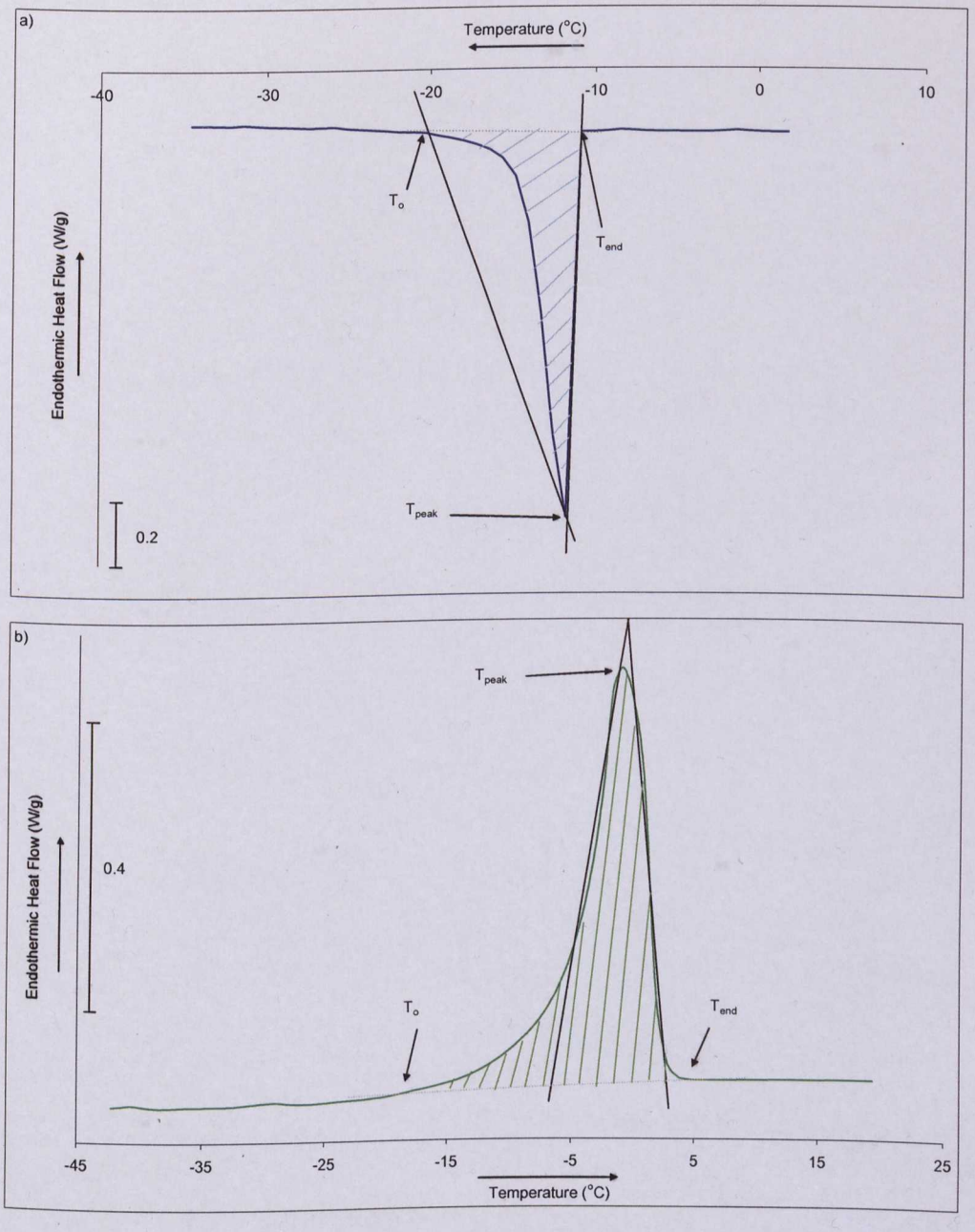



Figure 3-5. A typical DSC thermograms: (a) exothermic event and (b) endothermic event of water in the wheat dough.

#### 3.4.3.3.1 Experimental procedure

The measurement of the thermal events with fresh dough samples was carried out in a Mettler Toledo DSC 823e differential scanning calorimeter (UK). The DSC was calibrated with ___ and an empty aluminium pan was used as reference. Control measurements with fresh flour-water dough without salt were used as reference. A small piece of dough (~30mg) was weighed and hermetically sealed in aluminium DSC pan. The samples were cooled from 20 to -50°C at a rate of 2.5°C/min. The samples were held at -50°C for 5 min and then heated at a rate of 2.5°C/min from -50 to 20°C in order to measure the melting of the ice formed at the low temperature in the dough. For each exotherm and endotherm, the $T_{onset}$, $T_{peak}$, $T_{end}$, and $\Delta H$ (in °C and J/g respectively) were obtained using Mettler Toledo software (STARe version 9.10). All experimental tests were performed at least in duplicate.

### 3.4.4 Low resolution time domain $^1$H nuclear magnetic resonance ($^1$H NMR)

Nuclear magnetic resonance is a technique concerned of the transitions between energy levels of nuclei placed in an external magnetic field. The energy involved in these transitions lies in the radio frequency region of the electromagnetic spectrum. It has become one of the most widely used spectroscopy techniques to obtain information at the molecular level. This technique is commonly used to determine the chemical structure of molecules in the liquid and solid states; however, NMR has now split into many specialist areas for example structural determination, multi-dimensional NMR, solid state NMR, chemometrics and imaging NMR. However, besides all these techniques, it can also yield important dynamic information about the molecular mobility within a sample through relaxation measurement. It is this aspect of NMR that was used in this thesis and therefore will be discussed in the following sections.

#### 3.4.4.1 Theory of NMR (Harris, 1983; Ruan and Chen, 1998)

Each elementary particle has an inherent angular momentum, known as spin. NMR deals with the nuclear spin of an atom, which is labelled by the spin-quantum number (*I*). Atoms with an odd mass number (such as $^1$H, $^{13}$C, $^{15}$N, etc.), have a nuclear spin

of a half-integer. A nucleus that has a spin, also has a magnetic moment ($\mu$), calculated as follows:

$$\mu = \gamma p = \gamma \hbar \sqrt{I(I+1)} \qquad \text{Equation 3-2}$$

where $\gamma$ is the magnetogyric ratio of the specific nucleus; $p$ is the spin angular momentum and $\hbar$ is the Planck's constant ($6.626 \times 10^{-34}$ J.s.).

In the absence of a magnetic field, the energy of a nucleus is independent of the quantum number $m_I$. However, when a nucleus is placed in a static magnetic field ($B_0$), the energy ($E$) of the magnetic moment can be calculated as follows:

$$E = \mu B_0 \qquad \text{Equation 3-3}$$

There then exists $2I + 1$ energy levels of a nucleus. In case of the hydrogen atom, $I = 1/2$ generates two energy levels, denoted by the nuclear spin quantum numbers +1/2 and -1/2. In order for a particle to undergo a transition from the lower energy state ($m_I = +1/2$), where the nuclear magnetic moment is parallel to $B_0$, to the higher energy state ($m_I = -1/2$), where the nuclear magnetic moment is anti-parallel to $B_0$, energy must be absorbed. The absorbed energy must match exactly the difference between the two energy states, and is related to its frequency ($\nu$), also known as resonance or Larmor frequency:

$$E = \hbar\nu = \hbar\gamma B_0 \qquad \text{Equation 3-4}$$

The Larmor frequency can also be calculated from the following relationship between the magnetogyric ratio and the magnetic field:

$$\nu = \frac{\gamma B_0}{2\pi} \qquad \text{Equation 3-5}$$

When many nuclei are present, the total magnetic moment of the nuclei is called magnetisation *(M)*, The net magnetization is the sum of the individual nuclear magnetic moments ($\mu$). It is usually represented by a vector to simplify the

explanations. The net magnetisation will equal zero when all nuclear magnetic moments of the nuclei are randomly oriented with no magnetic field applied. However, when a magnetic field is being applied, the nuclei will adopt the "parallel" (low energy) and the "anti-parallel" position as described earlier. The difference between these two populations and the ratio of the two can be calculated using the Boltzmann equation:

$$\frac{N_{1/2}}{N_{-1/2}} = \exp^{\frac{-\Delta E}{kT}}$$

Equation 3-6

where $k$ = Boltzmann constant ($1.38 \times 10^{-23}$ $JK^{-1}$) and $T$ = temperature (in K).

As the magnetic field exerts a torque on the magnetic momentum of a nucleus perpendicular to its direction ($z$), the magnetic momentum will precess around the magnetic field in two energy states, as shown in Figure 6, shaped into two cones. This is known as Larmor precession. The net magnetisation *(M)* of all the nuclei is indicated.

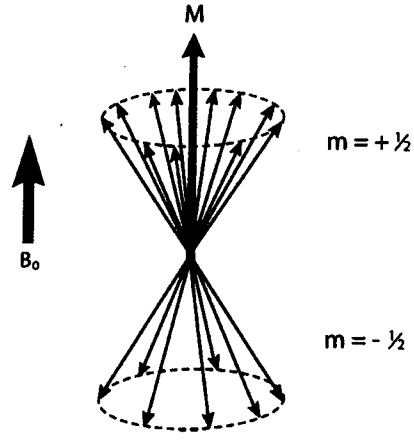



Figure 3-6. Precession of nuclei where $I$ = ½ about a magnetic field $B_0$ (Ruan and Chen, 1998).

### 3.4.4.1.1 Applying a radio frequency pulse

Since the net magnetisation is precessing, it is more convenient to visualise in the classical mechanics context by using a rotating frame that rotates about the $z$-axis at

the Larmor frequency. Thus, a magnetisation vector ($M$) that is precessing at the Larmor frequency will appear to be stationary in the rotating frame.

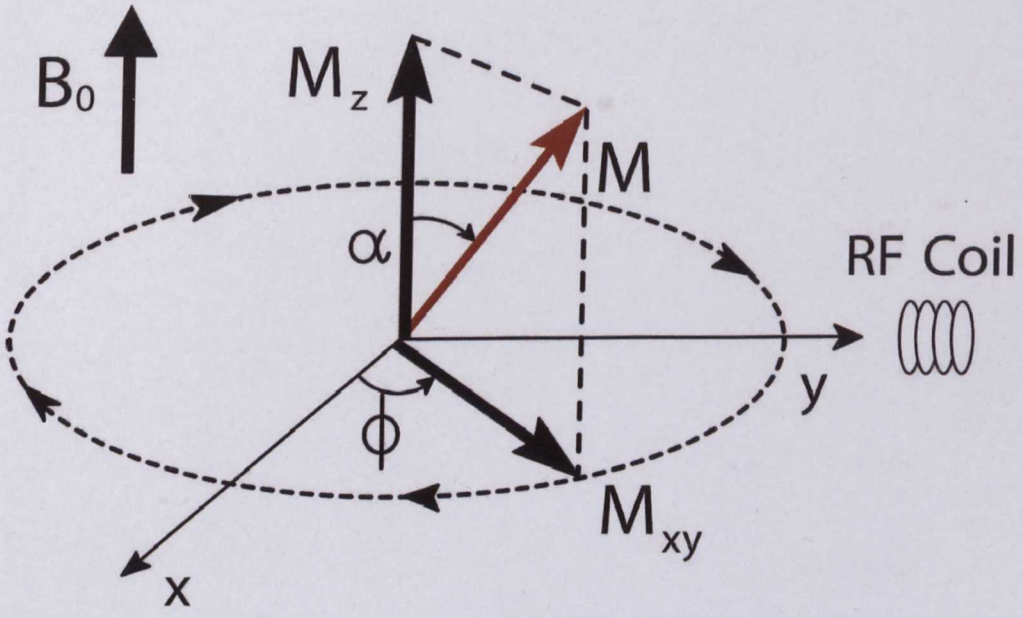



Figure 3-7. The net magnetisation $M$ in the rotating frame.

When the nuclei are placed in the magnetic field, they will align with the field ($z$-axis) (Figure3-7) and the net magnetisation will remain there unless an additional magnetic field is applied. By applying a second oscillating magnetic field ($B_1$) of the correct radio frequency ($rf$) perpendicular to $B_0$, would produce a torque which moves the magnetisation toward the $xy$ plane. The final position of the magnetisation will depend upon the length of time for which the radio frequency is applied (Figure 3-8).

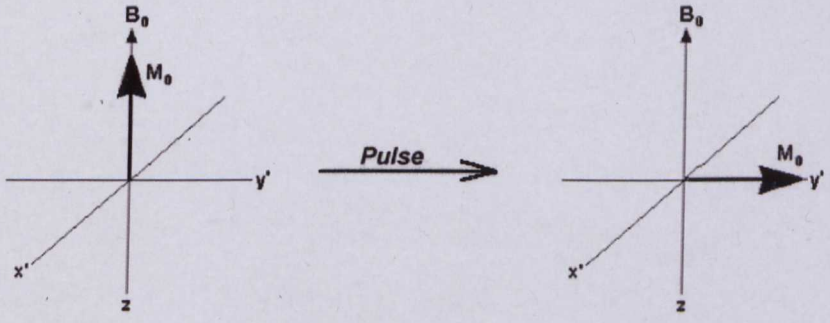



Figure 3-8. The magnetisation vector M in a static magnetic field ($B_0$) and after applying a 90° pulse in the rotating frame.

#### 3.4.4.1.2 Spin relaxation

By applying a short *r.f.* pulse for a few microseconds in any chosen direction, the net magnetisation $M$ to be rotated 90° to that direction. The magnetisation vector will then return to its original position along the static magnetic field, $B_0$, through a relaxation processes. There are two kinds of relaxation processes: spin-lattice (longitudinal) relaxation and spin-spin (transverse) relaxation. The time constants that describe these exponential relaxation processes are known as relaxation times. The spin-lattice relaxation time is denoted by $T_1$ and the spin-spin relaxation time by $T_2$. As illustrated in Figure 3-9, the longitudinal relaxation process follows a recovery or growth curve and the transverse relaxation process follows a decay curve. Both relaxation processes return to their equilibrium state after five repetitions of their relaxation time constants.

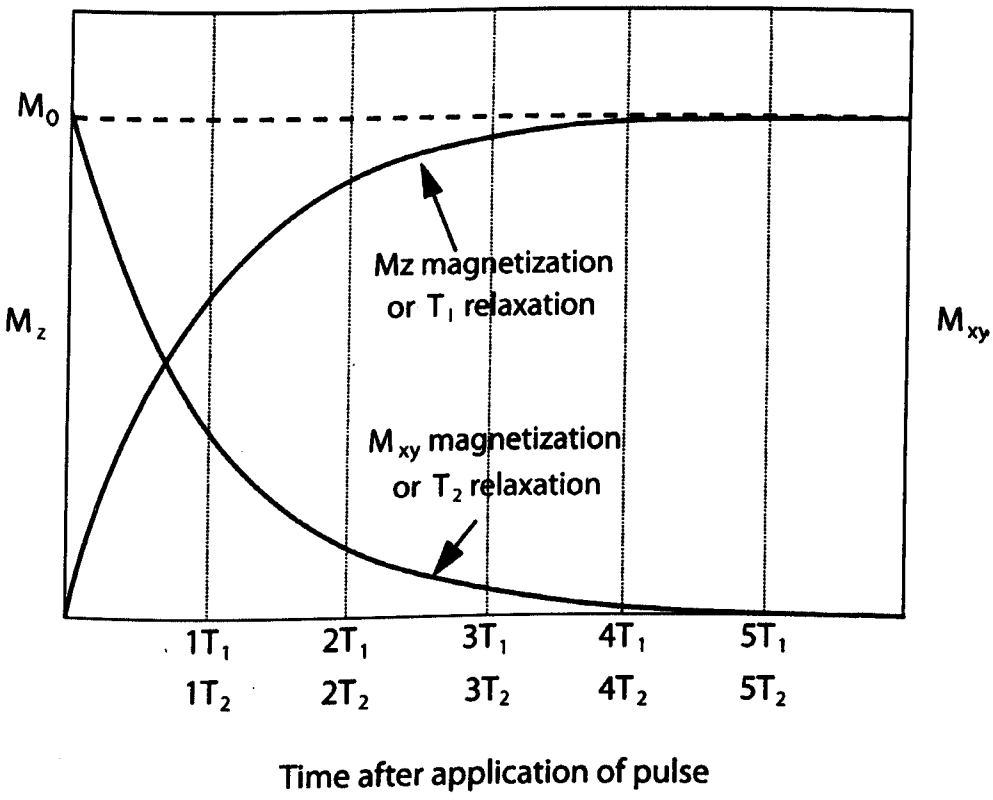



Figure 3-9. Relaxation of NMR signals after a 90 RF pulse. From Ruan and Chen (1998).

Relaxation time is a function of the spin species and the chemical and physical environments surrounding the spins. The relaxation time constants are a fundamental property of the chemical and physical environment. The relaxation rate is related to the physical state of the polymers. Therefore, analysis of $T_1$ and $T_2$ of a sample permits the study of chemical and physical properties of food polymers. The relationship between relaxation rate R and time constant takes the form of

$$R\,l = 1/T_l \qquad \text{Equation 3-7}$$

where $l$ takes the value of 1 and 2, for spin-lattice relaxation and spin-spin relaxation, respectively.

#### 3.4.4.1.3 Spin-lattice relaxation

The relaxation mechanism following a 180° pulse (along the $z$-axis) is known as spin-lattice or longitudinal relaxation. A common pulse sequence for $T_1$ measurement is the inverse –recovery pulse sequence. The relationship between the $T_1$ and time can be established through the following equation:

$$M_z(t) - M_0 = [M_z(0) - M_0] \exp^{-\frac{t}{T_1}} \qquad \text{Equation 3-8}$$

where $M_z$ = $z$-axis component of magnetisation $M$; $M_0$ = magnetisation when system is at equilibrium and no pulse is applied; $t$ = time; $T_1$ = spin-lattice relaxation time constant.

Within a sample there will exist many nuclear spin dipoles that will be varying in the sample at random. These nuclei will have components that are fluctuating at the Larmor frequency. Spin-lattice relaxation involves the interaction of the nuclear spin dipole with these fluctuating magnetic fields. Energy can then be transferred from the excited nucleus to the lattice in three different forms until thermal equilibrium has been reached. These forms are rotational, translational or vibrational energy.

#### 3.4.4.1.4 Spin-spin relaxation

Spin-spin or transverse relaxation describes the decay of transverse signals, and is readily detected by applying a 90° pulse to rotate the net magnetisation from along the z-axis into the *xy* plane. The relaxation of magnetization is then measured and can be described by the following equation

$$M_{xy}(t) = M_0 \exp^{-\frac{t}{T_2}}$$ Equation 3-9

where $M_{xy}$ = component of magnetisation $M$ in the *xy* plane; $T_2$ = spin-spin relaxation time constant.

Spin-spin relaxation, like spin-lattice relaxation, is sensitive to magnetic field fluctuations at the Larmor frequency. However, in addition to this, spin-spin relaxation is also sensitive to magnetic field fluctuations at lower frequencies up to tens of kHz. Spin-spin relaxation is brought about by several events, such as magnetic field dephasing, magnetic field inhomogeneity, cross relaxation and chemical exchange of protons. The following equation indicates that spin-lattice relaxation processes also affect spin-spin relaxation:

$$\frac{1}{T_2} = \frac{1}{T_1} + \gamma \Delta B$$ Equation 3-10

where $\Delta B$ = magnetic field dephasing.

A typical decay of transverse magnetisation can be seen in Figure 3-10. For this project, only the spin-spin relaxation parameter of different samples was studied in depth. This parameter was of major interest as it provided a very useful means for investigating molecular motions (Derbyshire, 1982).

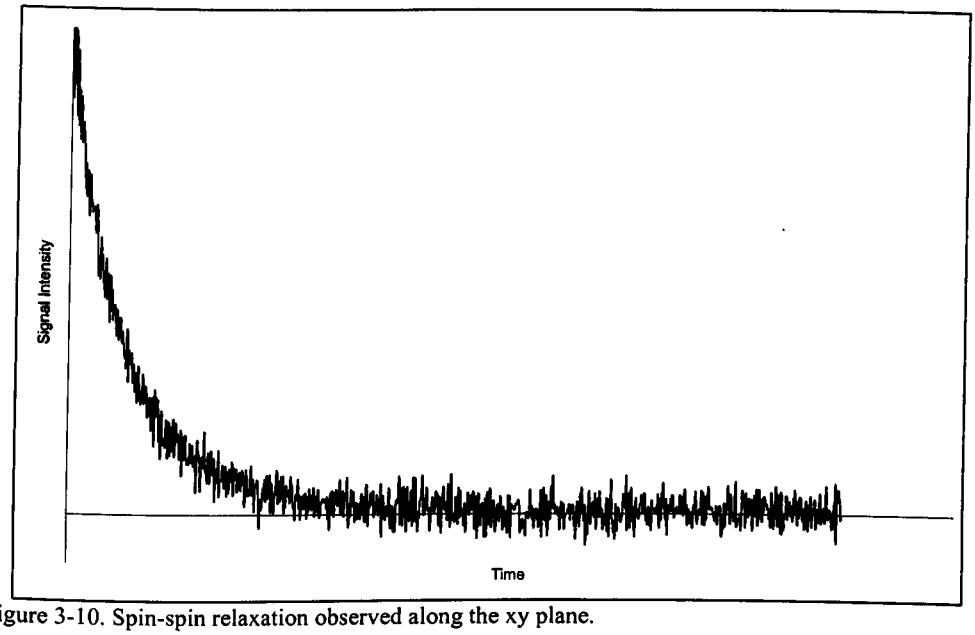



Figure 3-10. Spin-spin relaxation observed along the xy plane.

The mobility of the resonant nuclei has an effect on both the spin-lattice and spin-spin relaxations. The following Figure (Figure 3-11) illustrates, for isotropic motion characterised by a correlation time, (the average time required by a molecule to proceed through 1 radian), the effect on the relaxation time $T_2$.

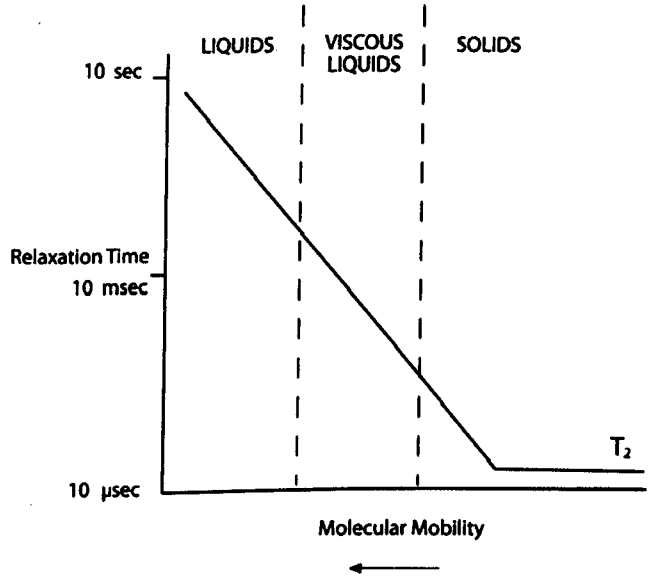



Figure 3-11. Schematic diagram of the dependence of $T_2$ on the correlation time ($\tau_c$) (Ablett et al., 1993).

### 3.4.4.2 Experimental procedures

Proton relaxation NMR experiments were performed using a Maran benchtop NMR spectrometer (Resonance Instruments Oxford, U.K.) operating at a resonance frequency of 22.9 MHz and a temperature of 29°C with a magnet temperature of 35°C.

The spectrometer was set up for experiments by optimising firstly the resonance condition i.e. choosing a value for 01 – the frequency offset such that the system was on resonance. This meant that a signal recorded in quadrature – as all experiments were decayed monotonically towards the x axis. Secondly, the optimum 90° pulse to gain the maximum signal and hence the best signal to noise ration was measured using the minimum signal from a 180° pulse and dividing this pulse length by 2. The receiver gain RG was also optimized at this point by ensures that regards filled the receiver buffer to about the 80-90% level. This ensured that no signal suffered clipping in the electronics.

The samples were sealed inside 8mm diameter tubes. A glass rod was placed into the tube to prevent moisture loss from the sample. A PC-based NMR software, provided by the instrument manufacturer, was used for pulse sequencing and data acquisition. The NMR system was equipped with a temperature control device allowing temperature regulation at $\pm$0.5°C. Sixteen scans were accumulated with a recycle delay (delay between 2 successive scans to allow complete relaxation) of 3 sec. Data was processed using Resonance Instruments exponential filled software and a version of CONTIN which gave continuous fits to data. Both amplitude and decay time information was obtained.

The magnetisation was recorded in quadrate and consisted of both real and imaginary components along the *x*- and *y*-axes. Both components were recorded and the component $M_{xy}$ was calculated as follows:

$$M_{xy} = \sqrt{(M_x^2 + M_y^2)} \qquad \text{Equation 3-11}$$

In this study, both the Free Induction Decay (FID) and Carr-Purcell_Meiboom-Gill (CPMG) pulse sequence were used and the details of each sequence will be discussed in the relevant results chapters in the thesis.

#### 3.4.4.2.1 Free Induction Decay (FID)

FID (Free Induction Decay) pulse sequence is one of the most commonly and simplest pulse sequences used for acquiring data about the relaxation properties of a material. It is recorded after applying a 90° *r.f.* pulse on the sample before acquiring data. However, immediately after applying the pulse, no data can be recorded due to the probe and receiver dead times of the instrument. Therefore, the first data point can only be measured after ~9μs. This delay means that some of the useful information about the rapidly decaying solid-like component in the samples has been lost, as illustrated in Figure 3-12. For this reason it is better to use the solid-echo pulse sequence to collect the FID decay, as it can overcome the dead time problem of the instrument.

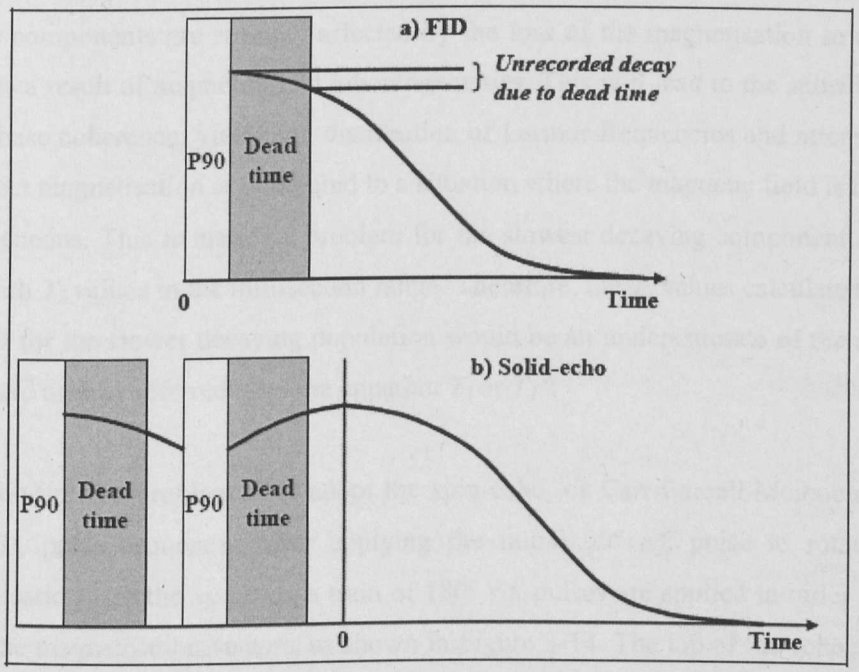



Figure 3-12. Schematic diagrams of the (a) FID and (b) solid-echo pulse sequences and their recorded NMR signals.

The spin-spin relaxation parameter for the solid-like component within the system can be obtained from the solid-echo pulse sequence, acquired after applying two 90°

radio frequency pulses using a pulse gap of 10μsec. The FID decay was analysed using data fitting software (WinFit, Resonance Instruments Ltd., UK) by fitting the curves with 2 exponentials (Equation 3-12) and a Gaussian (Equation 3-13). This was performed by minimising the sum of the squared difference between the experimental and fitted data. The fitting will be explained in more detail in chapter 7. Duplicate runs were carried out for each sample.

$$M_{xy}(t) = \sum M_{0i} \exp\left[-\left(\frac{t}{T_{2i}}\right)\right] \qquad \text{Equation 3-12}$$

$$M_{xy}(t) = \sum M_{0i} \left[-\left(\frac{t}{T_{2i}}\right)^2\right] \qquad \text{Equation 3-13}$$

#### 3.4.4.2.2 Spin-echo decay

One of the limitations of measuring molecular mobility of liquid-like components using the FID pulse sequence is that the spin-spin relaxation processes of highly mobile components are strongly affected by the loss of the magnetisation in the *xy* plane as a result of magnetic field inhomogeneities. This will lead to the spins losing their phase coherence, yielding a distribution of Larmor frequencies and attenuation of the net magnetisation as compared to a situation where the magnetic field is highly homogeneous. This is mainly a problem for the slowest decaying component of the FID, with $T_2$ values in the millisecond range. Therefore, the $T_2$ values calculated from the FID for the slower decaying population would be an underestimate of the actual $T_2$ and are usually referred to as the apparent $T_2$ or $T_2$*.

A solution to the problem is to adopt the spin-echo, or Carr-Purcell-Meiboom-Gill (CPMG), pulse sequence. After applying the initial 90° *r.f.* pulse to rotate the magnetisation into the *xy* plane, a train of 180° *r.f.* pulses are applied in order to re-phase the magnetisation vectors, as shown in Figure 3-14. The top of the echoes will then yield an envelope reflecting the 'true' spin-spin relaxation decay. This decay was then fitted to a series of exponential components as described below.

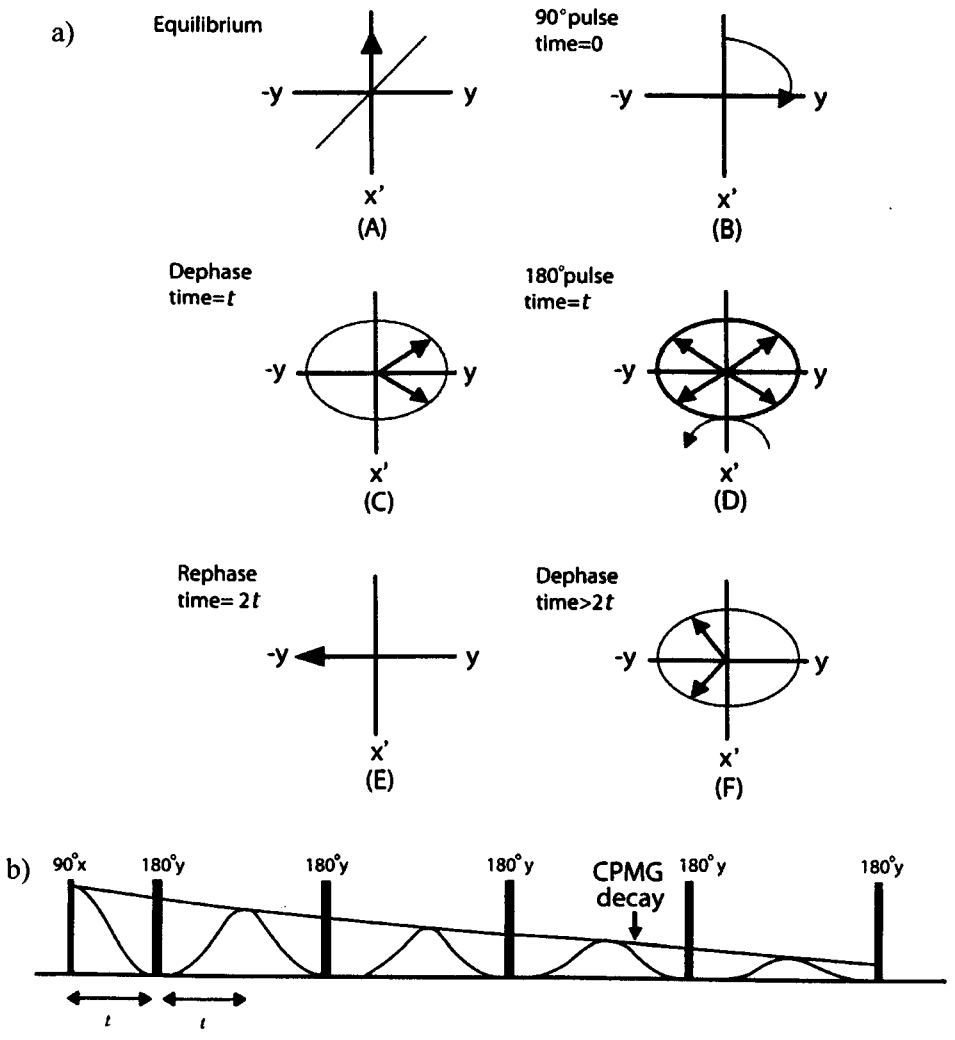



Figure 3-13. (a) The principle behind the spin-echo experiment (Ruan and Chen, 1998) and (b) the CPMG pulse sequence and the recorded signal (adapted from Farhat (1996)).

The $T_2$ relaxation parameters for the mobile components were obtained from the CPMG decays recorded using $\tau$ spacings of 0.05-1 msec. The CPMG data was analysed using the software RI WinDXP (Resonance Instruments Ltd., U.K.), which fitted a continuous distribution of exponentials to the data shown in Figure 3-14. This type of fitting has advantages over the conventional fitting, as in the latter case the decay is fitted to a limited number of exponential components and it is necessary to pre-estimate the number of exponential components required. With the continuous distribution this is not necessary and it is possible to see the distribution of mobilities within a population. The following were the parameters used: 512 exponentials were fitted; data pruning was logarithmic; the low value of the time constant was set to $2\tau$

and the high value was set to 140 msec. Duplicate runs were carried out for each sample.

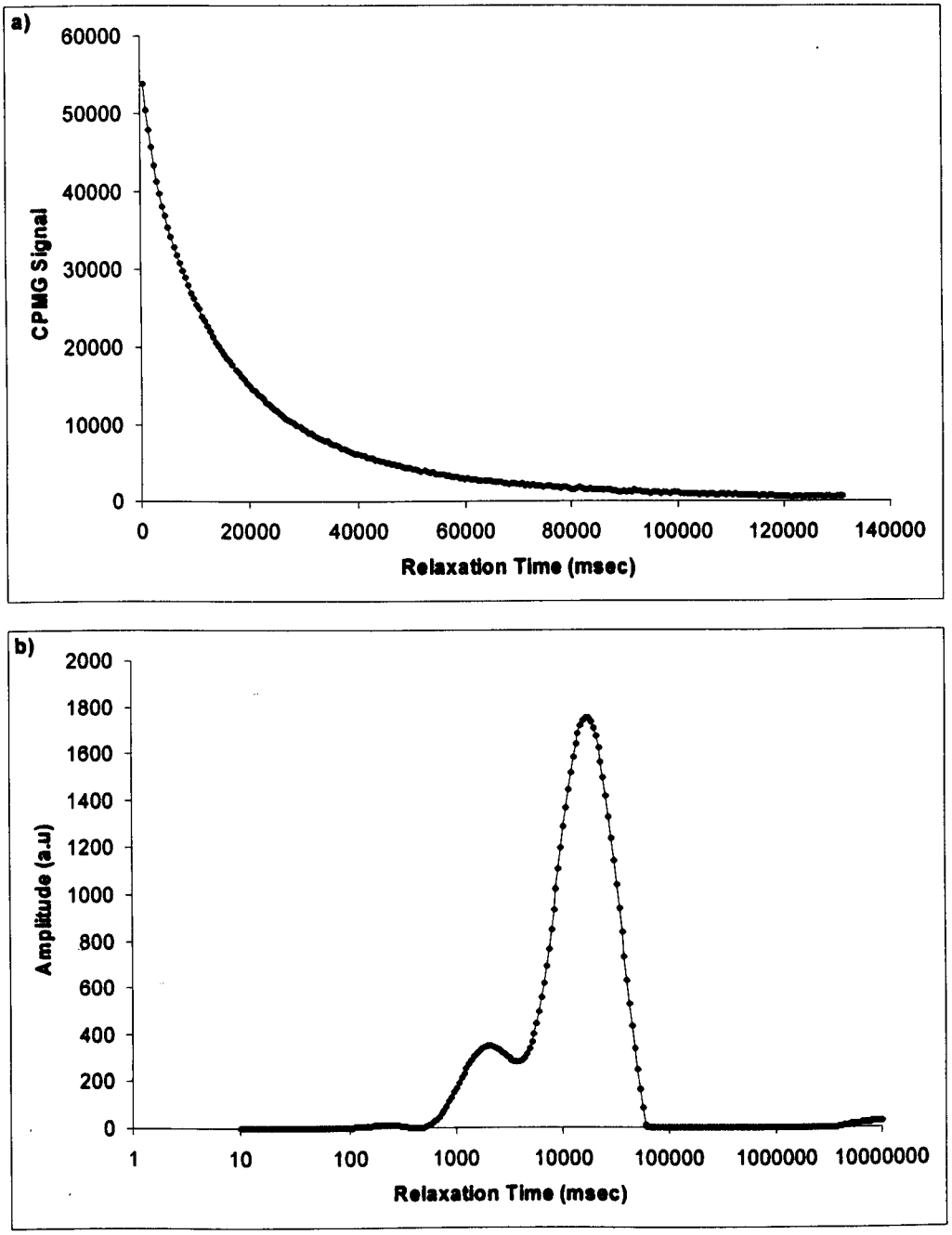



Figure 3-14. (a) NMR CPMG signal for dough sample obtained from RI WinDxp software, (b) $T_2$ distribution from the fit.

### 3.4.4.3 Sodium nuclear magnetic resonance ($^{23}$Na NMR)

The interaction of the sodium ion ($Na^+$) with other food components needs to be considered when modifying formulations to produce low sodium products. The physical state of the sodium in foods may have a pronounced influence on their quality and stability. It has been recognized that the sodium ion in a food system is somehow bound to different sites or acting as free sodium. Bound sodium is the portion that is closely associated with the host material and thus shows physical properties different than those of free or bulk sodium. By adopting the theory behind $^1$H NMR, one can study the binding of sodium ions in food system. It can be used to measure the longitudinal ($T_1$) and transverse ($T_2$) relaxation times of sodium which are characteristics of the states of sodium. When sodium is associated tightly with the substrate (e.g. starch and gluten), it is highly immobilised and shows reduced $T_2$; whereas sodium dissolved in aqueous phase is mobile and has a relatively long $T_2$. Thus, useful information on the strength or degree of sodium binding can be obtained. This experimental programme is a first attempt, at measuring the mobile sodium with in University of Nottingham Food Sciences.

#### 3.4.4.3.1 Experimental Procedure

The sodium probe was installed in a bench top Resonance Instrument Maran NMR spectrometer. Essentially, calibration of $^{23}$Na NMR followed the methods described in section 3.4.4.2. The LARMOR frequency of operation of this probe was 6 MHz. However, the dead times were also larger being typically μsec due to the longer ringdown time and electrical characteristics of the probe.

To make the sodium measurement, a small piece of dough samples (~ 0.85g) was placed in the 8 mm diameter test tube with minimum manipulation. The tube was then sealed (see section 3.4.4.2). An absolute method of measurement was adopted, where the sample was completely enclosed in the coil, and the sample weight noted and used for the purpose of normalization. Both the FID and CPMG sequences were recorded, however, due to the long dead time of the probe and hence it is almost impossible to record any solid or immobile component, it was then decided to concentrate on the CPMG measurement.

The dwell time, time between each data point (DW), used was 30 μsec and a total of 2048 points (SI) were collected. Averaging was carried out over a total 1024 scans (NS), with a repeat time (RD) of 0.2 sec, making a total of about 14 minutes acquisition time. The $T_1$ for the salt standards was determined as ~50msec, using the rule of thumb that RD ~ x 5 $T_1$ of ~ 200msec (Hore, 1995).

$$Y = A\, e^{\left(-\frac{t}{T_2}\right)} + offset$$

Equation 3-14

Curve fitting was carried out in the region of the CPMG where there were no distortions due to ring down of the probe. Single exponential decays were observed from most of the $^{23}Na$ experiments conducted on dough samples. The concentration of mobile sodium standard curve was constructed by using the known concentrations of reference sodium chloride water solutions. The *A* or intensity value of the samples were determined from the standard curve.

### 3.4.5 Extensibility analysis

Texture is one of the most important attributes that strongly influence dough machinability and the quality of the finished product. The gluten proteins have unique properties as a number of proteins interact by disulphide bonds, hydrogen bonds, entanglement and hydrophobic interactions (MacRitchie, 1992) to form a large continuous network. These macropolymers are broken down during mixing and reformed during resting of dough (Weegels et al., 1996, 1997). The protein-protein interactions help to explain the basis for the dough development and gas retention. In this respect of texture anaylsis, which evaluates the effect of an applied force on the deformation of a sample, is a good method for evaluating the macroscopic changes that a sample undergoes during dough development, which could be related to the machinability of dough. For this reason, texture measurements were carried out on the fresh dough samples to quantify the strength of the dough structure and could be compared with the dough development and bread making performance.

### 3.4.5.1 Kieffer gluten extensibility test

By mixing, flour and water are transformed into cohesive dough with viscoelastic properties. With the Kieffer rig, large deformation stress measurements were performed on the fresh dough. The dough samples were held securely in the rig, as illustrated in Figure 3-15. The test hook was placed underneath the sample, such that the arm of the texture anaylser moved upward, and the hook would move vertically. Neither temperature nor relatively humidity control being available, the tests were carried out as quickly as possible (test time was <10 sec). The sample was deformed for a specific distance, and the force required for the sample to be ruptured was recorded. A typical result obtained is shown in Figure 3-16. The values of major importance are the maximum force, i.e. resistance to extension (Rmax), and the distance at which is maximum force occurs (at the extension limit, Ext), which is the measurement of extensibility of the dough sample.

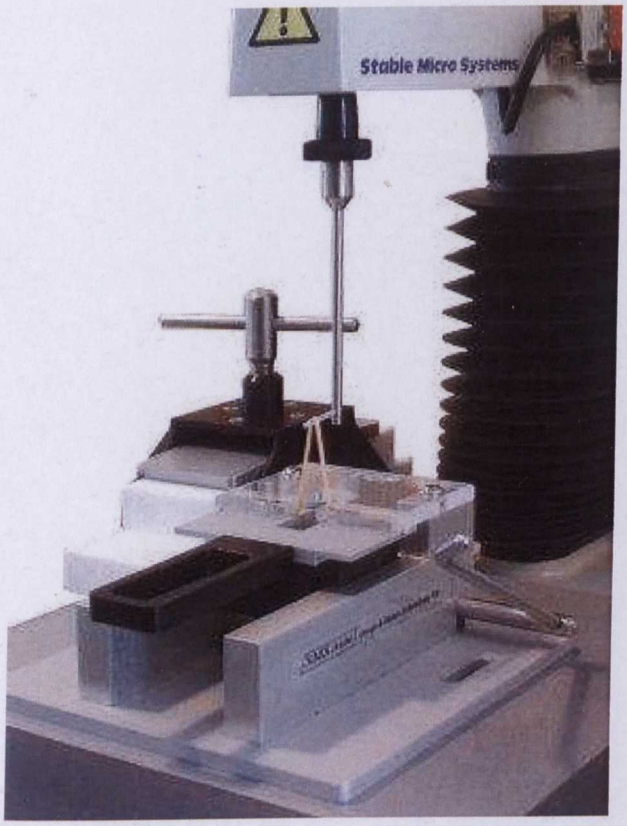



Figure 3-15. Photograph of the Kieffer dough and gluten extensibility rig.

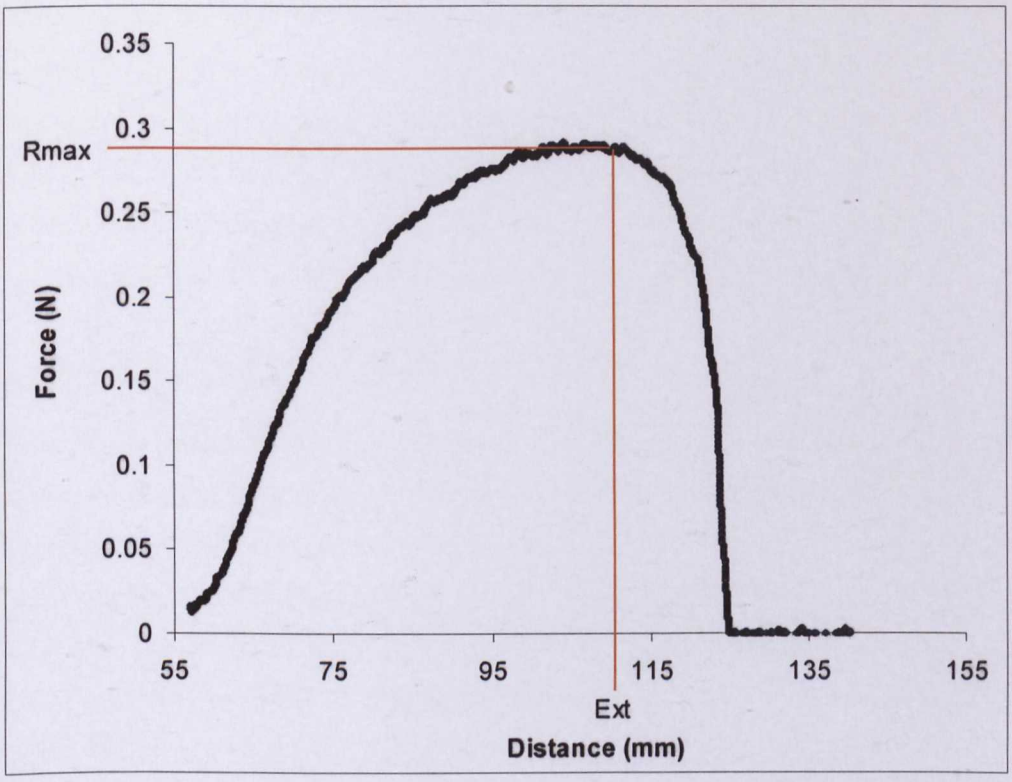



Figure 3-16. The result obtained from a large deformation stress measurement of dough sample.

### 3.4.5.2 Experimental procedure

Large deformation extensibility measurements were performed using SMS/Kieffer dough and gluten extensibility rig with a TA-XT Plus (Stable Micro Systems, U.K.) texture analyser equipped with a 25 kg load cell. A piece of test dough was made into a roll and rested for 5 minutes, then put on the lubricated (paraffin oil) lower plate of the Teflon mould and compressed with the lubricated top with a clamp (Kieffer et al., 1998). The doughs were covered and left resting at room temperature for 30 minutes before measurement. The sample was deformed to 150 mm at a speed of 2.0 mm/sec. For each sample, a minimum of 10 replicate measurements were performed.

### 3.4.6 Surface stickiness test

Stickiness is a common problem in the food industry, especially in the baking industry, and it causes a major problem during processing. The major problem in determining the cause of dough stickiness is measuring the degree of stickiness.

There is no one universal standard measurement of stickiness within the food industry. The measurement and description of stickiness is generally based on subjective assessments, usually by manual handling or visual inspection, and little is known about the factors which control stickiness in dough system. For this reason, adhesiveness measurements were carried out on fresh dough samples to quantify the stickiness properties. Details of different methods are given in chapter 6.

### 3.4.6.1 Sampling method – The texture profile analysis

One of the measures used to ascertain the surface adhesiveness properties were performed on the dough surface. After mixing, dough samples were transferred into a large container with an area of 21 x 30 $cm^2$ and a thickness of 4.5 cm covered and stored at 4 °C for 15 minutes. The prepared dough was allowed to rest for 20 minutes to release the stress produced by handling the sample. The cover was then removed, and the texture analyser probe was driven to contact the dough surface. A typical result is shown in Figure 3-17, where the hardness, adhesiveness and cohesiveness were recorded.

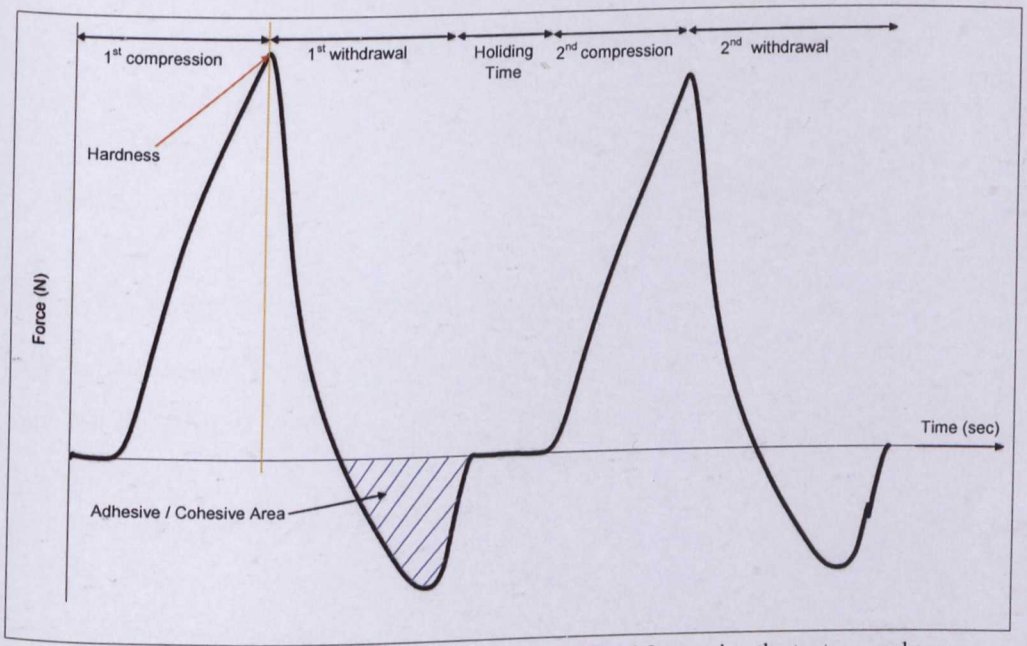



Figure 3-17. Typical plot of the texture profile analysis obtained from using the texture analyser.

### 3.4.6.2 Experimental procedure

The forces required for compressing and separation the probe from the dough surface was recorded using a texture analyser (TA.XT. Plus, Stable Micro Systems, U.K.) to provide a constant compression force and to measure the tension force. The Texture Profile Analysis (TPA) was used from the TA.XT library. The compression travel speed selected was 5.0 mm/sec. The probe travel distance was selected as 10 mm distance and the holding time was 5 seconds. The probe reversing speed was 5mm/sec. The test was performed with a 25 mm Perspex cylinder probe (P/25P). The dough samples were compressed twice to give a TPA graph from which the three primary textural parameters were obtained: hardness, cohesiveness and adhesiveness, as calculated by the provided software. The test was performed 3 times on the dough surface randomly.

## 3.4.7 Flame Photometry

Flame photometry, also called atomic emission spectroscopy (AES), has become a widely used method to determine elements because it is sensitive, rapid and does not produce hazardous waste. Flame photometry is suitable for qualitative and quantitative determination of several cations, especially for metals that are easily excited to higher energy levels at a relatively low flame temperature mainly, sodium, potassium and calcium, particularly in biological fluids and tissues.

### 3.4.7.1 Theory of Flame Photometry

Flame photometry is an atomic emission method for the routine detection of metal salts. In this project, sodium was the focus. The assay is performed by measuring the flame emission of solutions containing the metal salts. Solutions are aspirated into the flame. The hot flame evaporates the solvent, atomizes the metal, and excites a valence electron to an upper state. Light is emitted at different wavelengths for each metal as the electron returns to the ground state. For sodium, a bright orange flame is produced. Optical filters are used to select the emission wavelength. A photocell detects the emitted light and converts it to a voltage, which can be recorded.

The intensity of the light emitted could be described by the Scheibe-Lomakin equation:

$$I = k \times c^{n} \qquad \text{Equation 3-15}$$

where c = the concentration of the element; k = constant of proportionality; n ~1 (at the linear part of the calibration curve), therefore the intensity of emitted light is directly proportional to the concentration of the sample.

Comparison of emission intensities of unknowns to standard solutions allows quantitative analysis of the analyte metal in the sample solution.

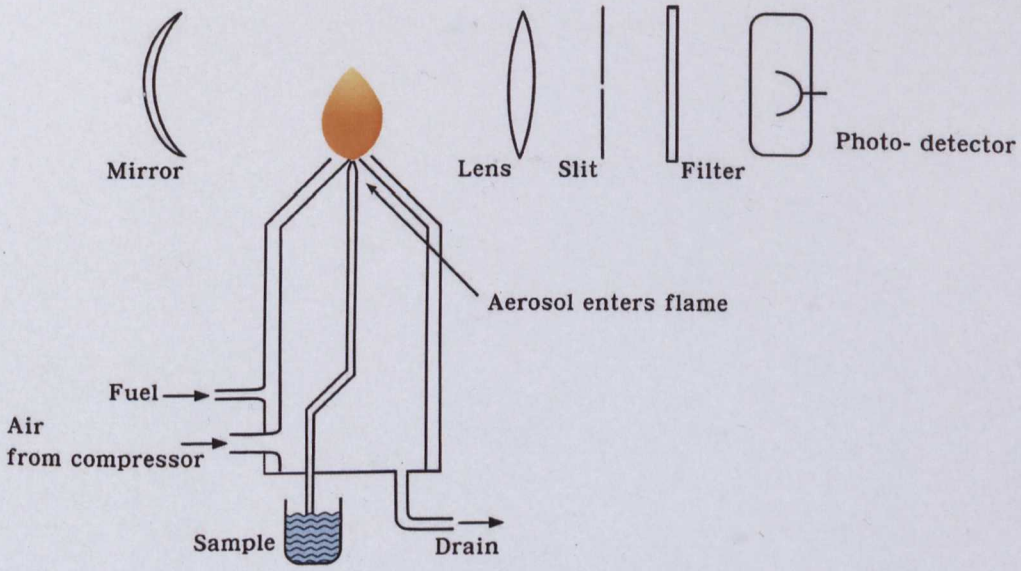



Figure 3-18. Principle of flame photometers.

### 3.4.7.2 Experimental procedure

Flame photometer 410e (Scientific Laboratory Supply, UK) was used to determine the sodium concentration in the sample. Sodium standard solutions were prepared by the stock solution (range from 1-10 ppm) and were used to calibrate the instrument. Dough liquor samples (~ 1ppm of sodium) were weighed into a plastic test tube and deionised-distilled water was added to the test tube to make a 1000-factor dilution. Samples were put into the sample tube of the flame photometer for 15 sec and values were recorded. Diluent solution was used between samples to clean out the sample

tube and the aspirator. For each sample, a minimum of 3 replicate measurements were performed.

### 3.4.8 Total Lipid Content

To determine the total lipid content, 1 ml of dough liquor was pre-weighed to the MMB tube and freeze dried. Isooctane (500 µl) were added to the MMB and mixed with mini bead beater for 30 seconds and centrifuged at 13,000g for 5 minutes. 450 µl solution was collected and repeated for total 3 times. All the collected solution was centrifuged at 13,000g for 10 minutes to remove any pellet. The solution was transferred to the glass bottle and was dried with nitrogen gas and the dried left-over lipid sediment was weighed to determine the total lipid content.

### 3.4.9 Statistical Analysis

Typically all the experiments were performed at least with five dough and each dough was tested at least 5 times, although not always were the same number of replications made for each dough . The mean value from each dough was taken and were analysed using the SPSS v.16 statistical software (U.K). A one-way between group analysis of variance (ANOVA) was conducted to explore the impact of salt levels at 0.05 significant level.

If the analysis of variance test show significance, the Post-Hoc test was used to further investigate the relation between each depend salt level. The Tukey's HSD (honestly significant difference) was used to determine the significant groups. In the figures, a letter is assigned to each result indicating there is a significant difference between each group. In addition, $\pm$ 1 standard deviation (SD) of each group is also indicated. In some case the trend of the salt effect on the measured parameter was also calculated.

# CHAPTER 4. ESTABLISHING METHODS FOR MEASURING FLUIDITY IN DOUGH SYSTEMS

## 4.1 INTRODUCTION

The physical state of the water in foods may have a pronounced influence on their quality and stability. It has long been recognized that the water in a material exists at different levels of mobility; often extremes of mobility are referred to as bound and free. Bound water is the portion that is closely associated with the host material and thus shows physical properties different than those of free or bulk water (Kuprianoff, 1958).

Wheat flour dough contains typically 0.6-0.8 g water/g of dry flour, of which approximately half is thought to be "bound" or unfreezable (Davies et al., 1969; Bushuk et al., 1977). Further addition of water (> 30 % by weight) forms a second aqueous phase in the dough matrix (MacRitchie, 1976). This water phase is said to be "free" or freezable. Numerous studies have proposed that an aqueous phase exists in dough and is beneficial to the baking process (MacRitchie, 1976; Gan et al., 1990, 1995; Sahi, 1994, 2003; Ornebro et al., 2000). It has also proposed that this aqueous layer is important in maintaining gas-cell integrity when gluten network ruptures. Studies, using scanning electron microscopy (SEM), have highlighted the significance of a continuous lamellar film in gas cell stabilisation and gas retention (Gan et al., 1990; 1991). The high electrical conductivity measurement of a developed dough also confirmed the presence of the continuity of this aqueous phase (MacRitchie, 1976). This liquid phase, that may be separated by centrifuging the dough in very high centrifugal fields, and was first described by Baker et al. in 1946 (Ablett et al., 1986; MacRitchie, 1976). This aqueous phase is thought necessary in dissolving soluble flour components and in providing the medium for reactions to take place in the dough (Bake et al., 1946; Gan et al., 1995).

For many years, the study of water binding capacity in dough relied on the farinograph. This instrument is useful in order to determine of the dough mixing characteristics; such as water absorption, mixing time, resistance to mixing, viscosity

and stability, which are closely related to the baking performance of the dough. However, these techniques do not provide any direct information on the physical state of water when mixed with the flour. Recently, a number of independent physical methods have been used to explore the concept of molecular mobility or degree of "boundness" of water in a dough system. These methods included freezing-point depression, NMR, calorimetric determination of freezable water, equilibrium uptake of water by flour components in a humidity-controlled environment, and extrapolation of farinograph measurement to zero mobility.

In bread making, water is necessary for gluten development and plays an important role in all types of interactions and chemical reactions. It is common practice in the commercial bread-making industry to add water at a somewhat higher level than the measured water absorption for a particular flour. Understanding the properties and functions of water in dough is of great interest and vital to the bakery industry. It was suggested that even minor changes in salt could alter the quantities of "free" fluid in a dough and this would then cause a range of different behaviours that was compatible with the observations made by the bakers on salt reduction in bread doughs.

This chapter shows the method development in order to investigate the mobility and distribution of water in the flour-water dough prepared at different water contents through ultracentrifugation, differential scanning calorimetry (DSC) and low field time domain proton nuclear magnetic resonance ($^1$H NMR). The analytical conditions are also established for interpreting the dough behaviour using these three methods. In addition, preliminary studies were also conducted to observe the effect of bakery ingredients such as bakery fat and salt levels influencing the dough aqueous phase using the established techniques.

## 4.2 MATERIALS

The work discussed in this chapter mainly used Viking flour obtained from Whitworth Bro. Ltd. (Northants, UK). Flour-water dough with 30 to 80% of water content (flour base) was prepared with mini-pin mixer following the method given in 3.3.1.2. The moisture content of the Viking flour was 13.77% (± 0.88).

## 4.3 METHODS DEVELOPMENT

### 4.3.1 Dough liquor isolation

A number of researchers described that an aqueous layer can be separated from dough using high centrifugal force and this phase can be used to estimate the amount of 'free' water in wheat-flour dough systems (Baker et al., 1946; MacRitchie, 1976; Sahi, 1994; Larsson et al., 1996). Therefore flour-water dough of 60% water content (flour base) was mixed (see method 3.3.1.2) and centrifuged for 1 hour (see method 3.3.1.3). Two main phases were observed: the straw-colour dough aqueous phase and the sediment phase that consisted of a gluten-starch mixture (Figure 4-1).

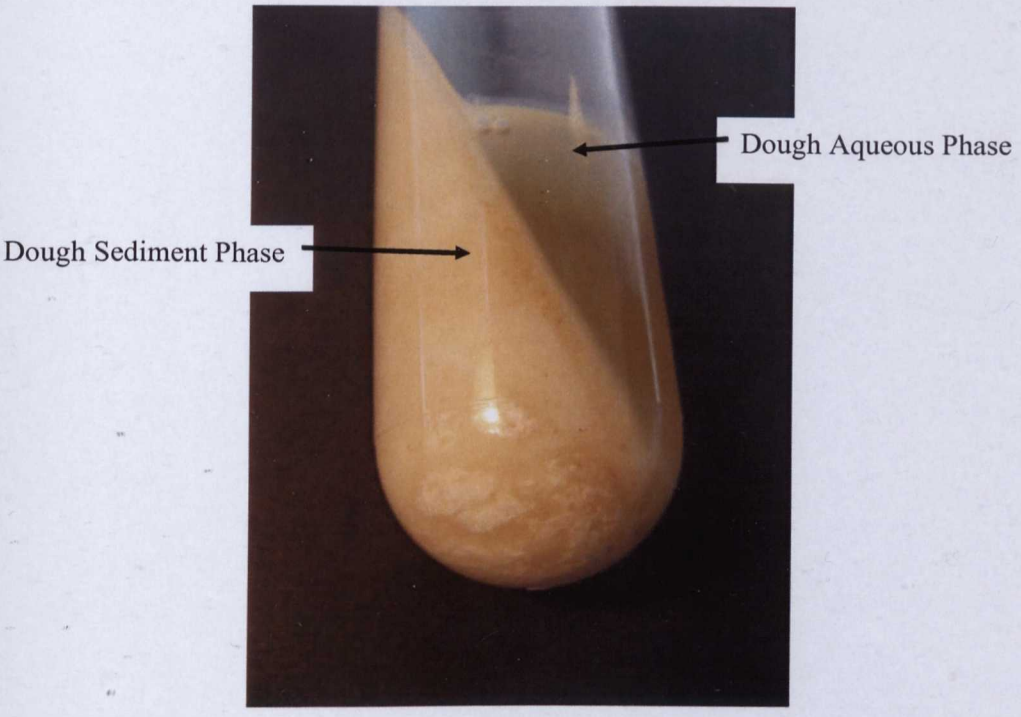



Figure 4-1. Flour-water dough at 60% water (flour base) after centrifugation at 126,000 g for 1 hour.

#### 4.3.1.1 Calculation of dough liquor recovery

The aqueous phase recovered from ultracentrifugation was calculated using Equation 4-1:

$$\text{Dough liquor recovery (\%)} = \frac{\text{g of dough liquor isolated}}{\text{g of water added}} \times 100\%$$

Equation 4-1

with

$$\text{grams of water added} = W_{total} \times \text{grams of dough sample}$$

Equation 4-2

where $W_{total}$ is the amount of water added to the recipe. Note that moisture content of the flours was not used in the calculations.

#### 4.3.1.2 Different centrifugation force

In the early stage of the study, the amount of the aqueous phase isolated by centrifugation was studied. Batters, of 75% water content (300% on flour base), were made by mixing with soft wheat flour and the liquid yield obtained plotted against centrifugal field. Results are shown in Figure 4-2. The liquid recovered from flour-water system increased with higher centrifugation forces.

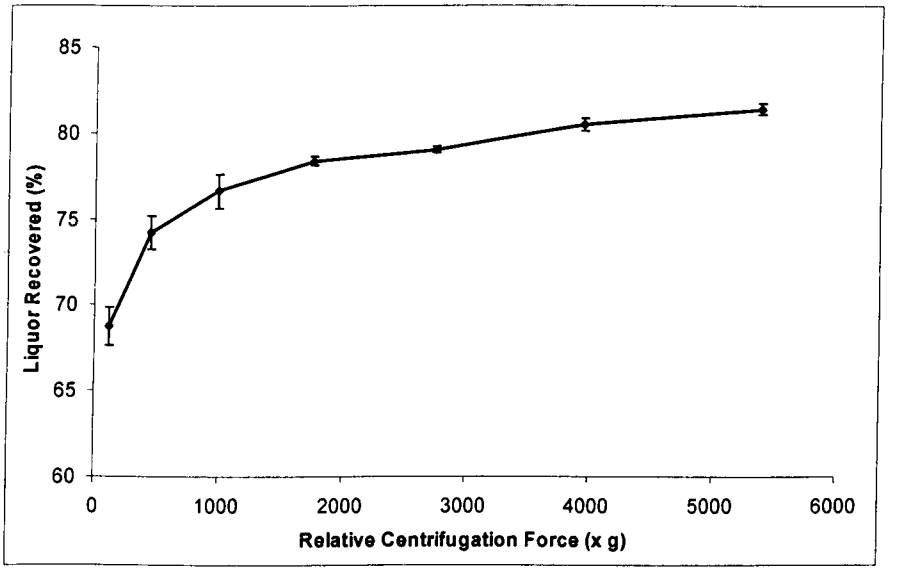



Figure 4-2. Liquor yield of flour-water system (75% water content) at different centrifugation forces applied for 30 minutes at 15°C. n = 5 (each dough with 8 replications) with ± 1 standard deviation.

A similar experiment was conducted with flour-water dough at ~62% water content (flour base). Above 100,000 g, a limiting value is found for the amount of dough liquor which can be separated. A centrifugal force of 126,000 g was adopted as the standard procedure for dough liquid phase isolation and has been used for all the work described throughout the rest of this thesis.

#### 4.3.1.3 Different water content

Flour-water dough samples with water contents of 30 to 80% (flour base) were centrifuged at 126,000g and the amount of the aqueous phase isolated is shown in Figure 4-3.

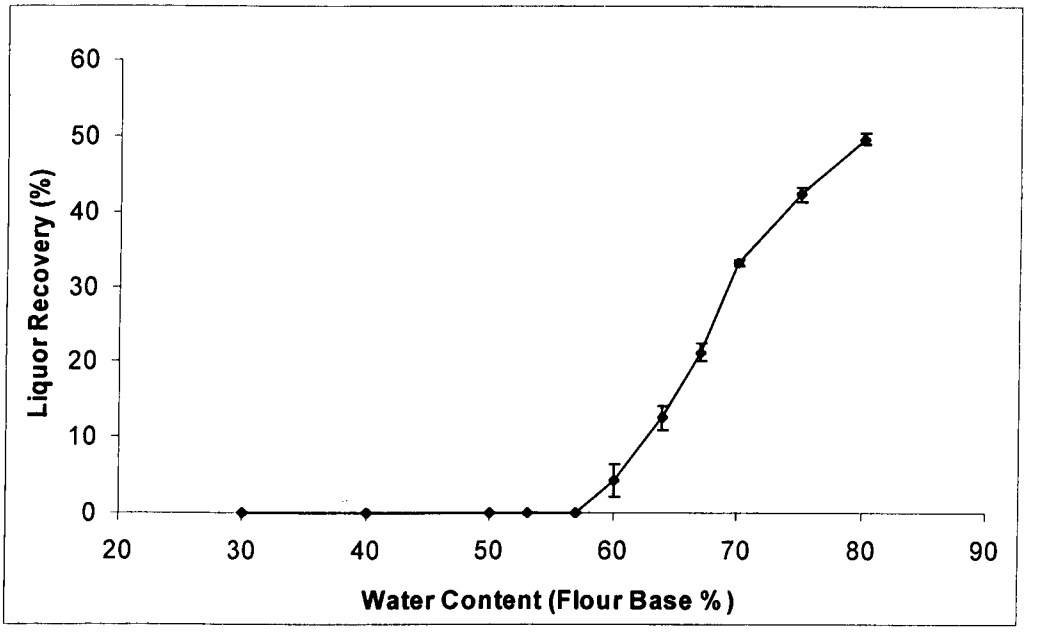



Figure 4-3. Flour-water dough liquor recovered at a range of water contents between 30% to 80% (flour base). n = 5 (each dough with 8 replications) with ± 1 standard deviation.

At lower water content, no separation was obtained at all. The separation started somewhere after the water content corresponding to the farinograph water absorption given by the manufacture. This was 58% water absorption as shown in Table 3-1. Studies have shown that the critical water content of dough of about 0.3g/g dry flour, above this value "free" water exists, although there has been some variability and a value as high as 0.7g/g dry flour has been observed (Toledo et al., 1968; Davies,

1969). The sample flour used in this work described in the thesis showed a value of ~ 0.5 g/g dry flour.

The total liquid yield was less than the amount of extra recipe water added to the dough, suggesting that surplus recipe water is needed to hydrate the dry components of flour (protein, starch, etc.) during mixing. The maximum liquid layer was only ~ 50% of the total liquid added to the system (see Figure 4-3). These results indicated that a flour-water system exists as one phase below a critical water content, and at higher water contents a second liquid phase is present.

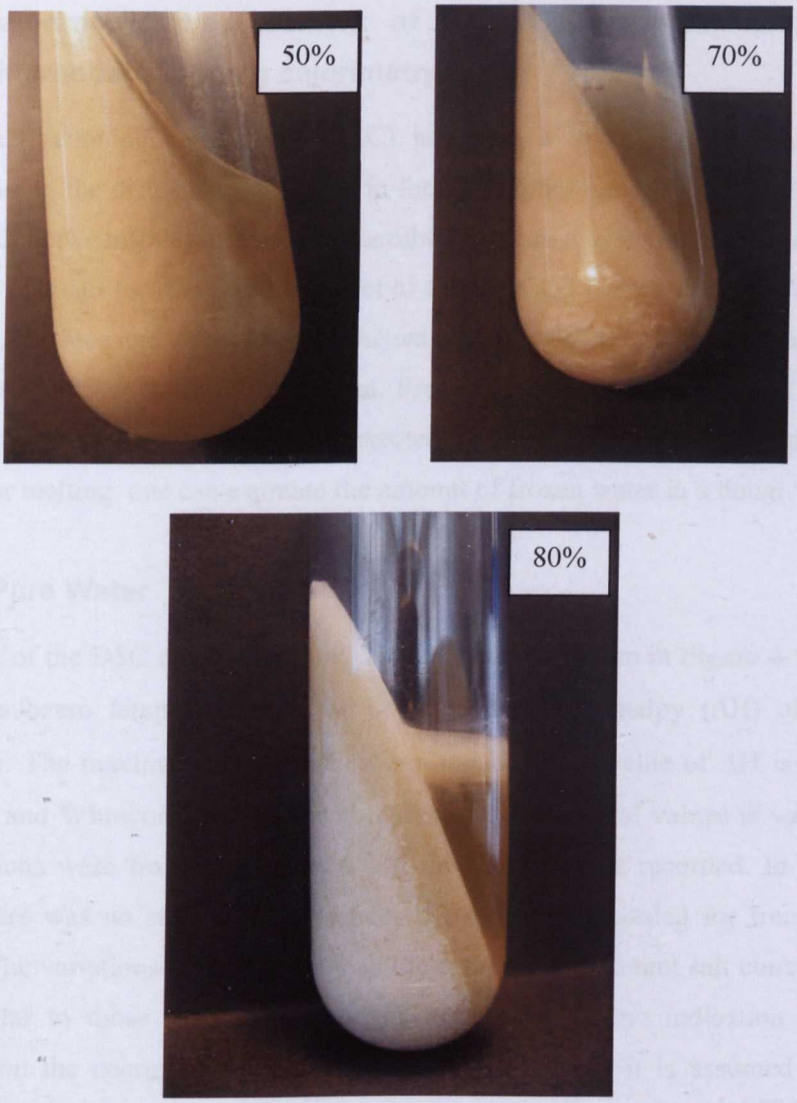



Figure 4-4. Flour-water dough separation at different water contents on flour base.

At higher water contents, the pellet of the centrifuged dough was not homogenous. Figure 4-4 shows that with the different water content levels the number of phases that can be separated varies. When the amount of water increased, separation into four or five phases was observed. It is suggested that with 80% water content, the dough has separated phases that may be lipid, liquid, gel, gluten and starch (Larrson, 2002; Primo-Martin et al., 2006). Depending on the efficiency of the separation, more or less unseparated dough was found at the bottom of the test tube. Below the 60% water content samples, some unseparated dough was seen, but at the highest water level (80% flour base), this unseparated fraction more or less disappeared.

### 4.3.2 Calorimetric measurement of freezable water in dough with differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) has been a widely applied method for understanding the distribution of water in food and other materials. It can be used to distinguish between freezable and unfreezable water in a system. Freezable water is the water that can form ice when subject to freezing and frozen storage (Rasanen et al., 1998). Unfreezable water refers to the water that is bound with other material and does not act as free water in the system. Freezable water can be detected by DSC, however, unfreezable water is not detected. By measuring the enthalpy of ice freezing or melting, one can estimate the amount of frozen water in a dough system.

#### 4.3.2.1 Pure Water

The shape of the DSC curve with pure distilled water is shown in Figure 4-5. Frozen water at subzero temperature can be detected by the enthalpy ($\Delta H$) of the ice crystalline. The maximal difference between the literature value of $\Delta H$ is 333.5J/g (Petrenko and Whitworth, 1999) and the present experimental values is within 5%. Salt solutions were frozen and melted and the thermograms recorded. In terms of energy there was no statistical differences between that recorded for freezing and melting. The variations observed between the samples of different salt concentration were similar to those seen in the doughs and there was no indication that salt impacted on the energy required. Therefore for this work, it is assumed that the freezing and melting enthalpy of ice always has the same value and depends neither

on biopolymer contents of dough nor on depressed freezable/ melting temperature (Baier-Schenk et al., 2005).

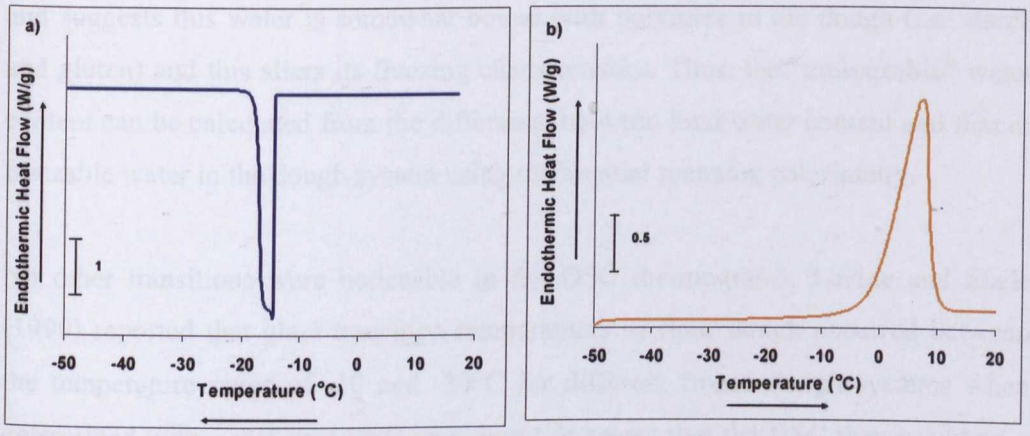



Figure 4-5. DSC thermogram from the (a) freezing mode and (b) melting mode of pure water.

### 4.3.2.2 Basic flour-water dough

A typical DSC curve from freezing and melting of flour-water dough at 60% (flour base) is shown in Figure 4-6. One crystallisation and melting peak were observed from the experiment. The endothermic melting peak of ice was found to have an onset temperature at around -10°C. This low onset value of ice melting was probably due to the presence of solute in the aqueous phase of the dough such as protein, lipid and soluble polysaccharides.

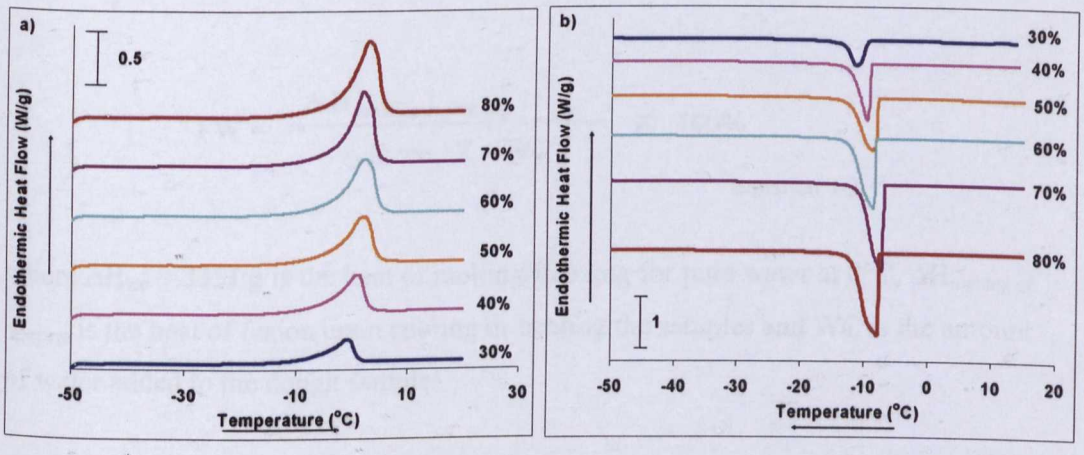



Figure 4-6. The DSC (a) melting and (b) freezing thermograms for flour-water dough sample with 60% (flour basis) water content a scanning rate of 2.5°C/min.

A smaller enthalpy (ΔH) of the freezable water was observed compared with the pure water implies part of the water did not behave as "normal" water, which freezes at subzero temperatures. This undetected water is known as the "unfreezable" water and suggests this water is somewhat bound with polymers in the dough (i.e. starch and gluten) and this alters its freezing characteristics. Thus, the "unfreezable" water content can be calculated from the difference between total water content and that of freezable water in the dough system using differential scanning calorimetry.

No other transitions were noticeable in the DSC thermograms. Levine and Slade (1990) reported that glass transition temperatures of flour dough occurred between the temperature range of -10 and -30°C for different frozen dough systems when determined with a different DSC instrument. It seems that the DSC they used had a higher sensitivity. However, using more sensitive methods of analysis to observe state transition, such as glass transition in the frozen dough, was not needed as it was not in the scope of this study.

#### 4.3.2.3 Determine the amount of freezable water

The enthalpy of ice from the dough sample can be closely associated with the amount of "free" or mobile water in the system. The freezable water content (FW) is determined from the relationship between latent heat of ice melting/freezing and water content in the sample calculated using Equations 4-3:

$$FW = \frac{\Delta H_{\text{melting or freezing}}}{\Delta H_{\text{ice}} \times WC} \times 100\%$$

Equation 4-3

where $\Delta H_{ice} = 335 J/g$ is the heat of melting/freezing for pure water at 0°C, $\Delta H_{melting\ or\ freezing}$ is the heat of fusion upon cooling or heating the samples and WC is the amount of water added to the dough sample.

It is recognised that many investigators have chosen enthalpy of melting in the heating mode to calculate for the freezable water fraction, although the cooling mode may sometimes be used in comparison with other techniques [e.g. Korber et al.

(1991)]. For this particular study, the melting and cooling results did not show a significant difference ($p > 0.05$). Thus, the average of the heat of melting upon heating and the heat of cooling upon freezing were used.

It should be kept in mind that the approximate amount of unfreezable water was calculated using $\Delta H_{ice}$ for pure water at 0°C and may be subject to some error since heat of fusion varies with temperature, e.g. $\Delta H$ -20°C = 287.6 J/g. It has been pointed out by Hatley et al. (1991) that the calculated unfrozen water using the conventional means ($\Delta H_{ice}$ of pure ice at 0°C) may be subject to error when the value is divided by a small value of solids (to calculate grams of unfrozen water on a solid weight basis).

Because crystallisation is a kinetically controlled process, the observed freezing was likely to be specific to the experimental condition used. Studies had shown that variation in the cooling rates affected the onset temperature of crystallisation, rather than the enthalpy. Lind (1998) has also reported that the amount of ice in dough varies at different subzero storage temperature.

### 4.3.2.4 Difference in Water Content

Figure 4-7 shows the DSC cooling and melting thermograms of flour-water dough prepared at different water content between 30 to 80% (flour base) and Figure 4-8 shows the fractions of freezable water as a function of water content.

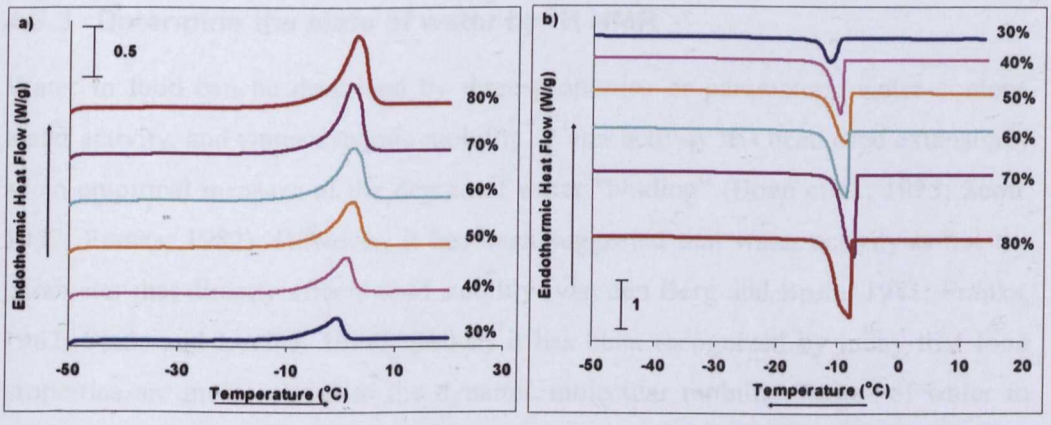



Figure 4-7. DSC (a) cooling and (b) melting thermograms at different water content between 30 to 80% water content at scanning rate of 2.5°C/min.

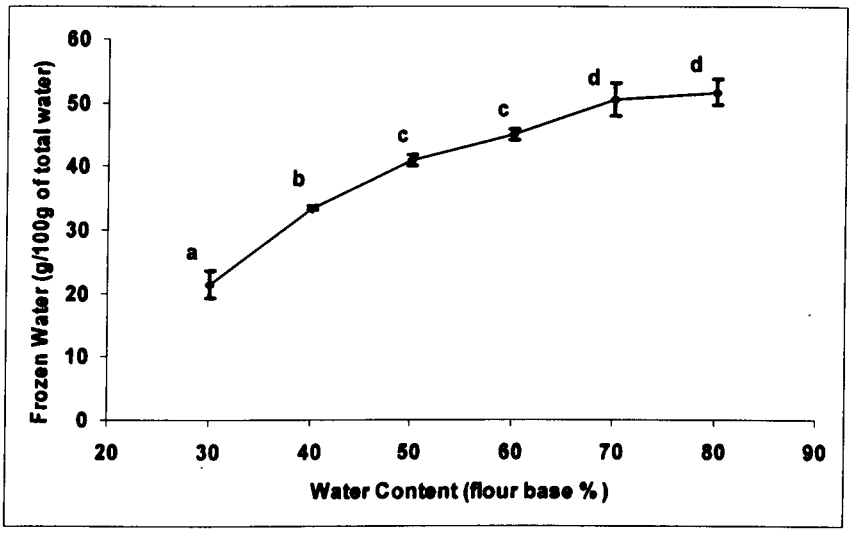



Figure 4-8. Change in freezable water in dough samples over a range of water contents as measured by DSC (n = 5 with ± 1 standard deviation). a-d: different letters in chart show significant difference of $p \leq 0.05$.

Crystallisation of water in the flour only sample (<14% moisture content) was not detectable upon cooling and melting using the DSC, and water present in this sample has been classified as unfreezable water (Slade & Levine, 1991; Hatley et al., 1991). At higher water contents, enthalpies, from cooling and melting, gradually increased with increasing water content, suggesting that calculation of the ΔH can be a sensible measurement for the "free" water in the dough system.

### 4.3.3 Determine the state of water by $^1$H NMR

Water in food can be described by three properties or parameters: water content, water activity, and water dynamic mobility. Water activity has been used extensively as an empirical measure of the degree of water "binding" (Bone et al., 1975; Scott, 1957; Franks, 1982). However, it has been suggested that water activity is not the parameter that directly affects food stability (van den Berg and Bruin, 1981; Franks, 1982; Slade and Levine, 1991). Rather, it has been recognized by many that food properties are more related to the dynamic molecular mobility "state" of water in foods (e.g. van den Berg and Bruin, 1981). On the basis of the dynamic behaviour of water in food polymers, bound water, also referred to as "unfreezable" water, was suggested not to be energetically bound to polymer chains in any equilibrium sense,

but rather as the result of kinetically retarded diffusion of water molecules in the solid state (Levine et al., 1988).

In recent years, the distribution of water molecules in biological system has been studied extensively using nuclear magnetic resonance (NMR) (e.g., Hills et al., 1989; Yakubu et al., 1991; Chinachoti, 1993; Belton et al., 1997; Botlan et al., 1998; Li et al., 1998). The longitudinal ($T_1$) and transverse ($T_2$) relaxation times of proton are usually used to characterize the molecular mobility of the system. In the samples used in this study changes in mobility can be assumed to be due to protons associated with water. Although it is recognised that proton exchange etc may influence the results. When water is bound tightly to the substrate (e.g. flour), it is highly immobilised and shows a reduced $T_2$; whereas free water is mobile and has relatively long $T_2$. Thus, useful information can be obtained from NMR to understand the strength and degree of water binding. Furthermore, the distribution of water can also be obtained based on the molecular mobility of protons in the system. The following section describes studies on the mobility or binding of water in flour dough by measuring the transverse ($T_2$) relaxation times of water protons.

#### 4.3.3.1 Flour- water dough with $^1$H NMR

The 90° pulse (One pulse) sequence and the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence were used for acquisition of the free induction decay (FID) data for the spin-spin relaxation times, $T_{2(1)}$ and $T_{2(2)}$, respectively. The $^1$H NMR was calibrate with copper sulfide solution and flour-water with no salt was used as control. The FID obtained from a 90° pulse sequence ($T_{21}$) and the CPMG pulse sequence ($T_{22}$) were analysed as a multi-exponential and continuous distribution of exponentials using the RI Winfit and WinDXP software, respectively (see section 3.4).

##### 4.3.3.1.1 Multi-exponential analysis

Spin-spin relaxation times in the range of 11.5-1000 μsec were determined by a single-pulse experiment (FID). The typical $T_2$ relaxation time graph obtained from a single pulse (FID), at 29°C, for flour dough at 60% of water (flour base) is shown in Figure 4-9. The FID data were fitted using a discrete method, based on a Gaussian

function for the less mobile phase, and a multi-exponential function for the mobile phase (Le Botlan and Ougueram, 1997; Ruan et al., 1998). Hence, relaxation times and intensity of the less mobile and mobile phase can be obtained.

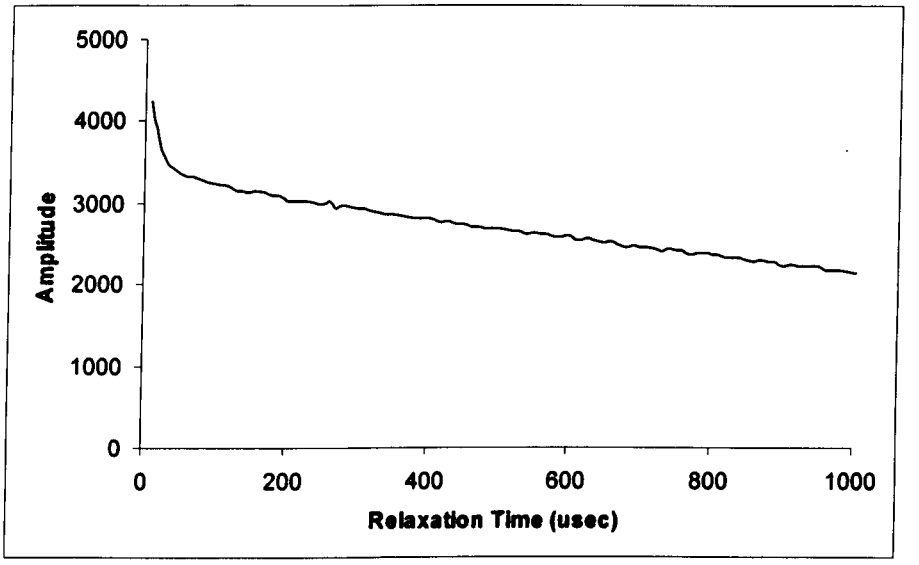



Figure 4-9. The Free induction decay (FID) relaxation of flour-water dough at 60% water content (flour base) ranged from 10 to 1000 μsec.

Two main proton populations were shown, including one very fast component (having a relaxation time less than 25 μsec) and one or two slower components. The population associated with the less mobile region may correspond to protons in solid-like components, such as starch, proteins and water molecules tightly associated with those solids (Kim & Cornillon, 2001; Ruan et al., 1999). The second population may correspond to all the more mobile protons in the flour dough. It is know that FID signal is limited for measuring higher mobility. In fact, the FID signal contains not only the spin-spin relaxation but also a decay in intensity, due to the inhomogeneous local magnetic field (Hore, 1995). Therefore the slower decay time constants measured using FID are not the true spin-spin relaxation times, and caution must be taken when comparing and using the FID data. Higher value of spin-spin relaxation times can be better determined using a spin-echo sequence (CPMG).

A typical CPMG plot of determining $T_2$ for water protons in flour-water dough is shown in Figure 4-10. The two-exponential decay of the signal indicated the existence of two or more populations of water molecules on the NMR time scale,

characterised by a short and a long relaxation times, $T_{2a}$ and $T_{2b}$ respectively. The $T_{2a}$ component represents the less mobile, or more tightly bound water fractions (from macromolecules), while $T_{2b}$ component represents the more mobile water fraction (Wu et al., 1992). Similar phenomenon of multi-phase behaviour in water proton relaxation has also been observed in other systems such as hydrated starches (Le Botlan, 1998), polysaccharide gels (Belton, 1997) and biscuit dough (Assifaoui et al., 2006). As shown in the present study, the mobile water fraction is much shorter than free or bulk water. This implies that even the very loosely held water in the dough is relatively immobile when compared to free water.

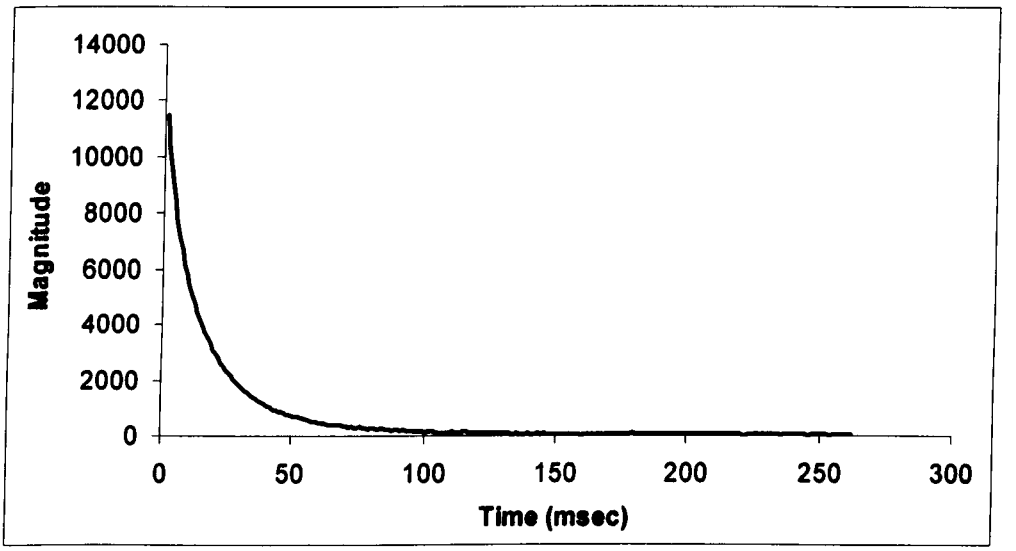



Figure 4-10. The Carr-Purcell-Meiboom-Gill (CPMG) $T_2$ relaxation of flour-water dough at 60% water content (flour base).

#### 4.3.3.1.2 Continuous distribution analysis

Apart from using the multi-exponential model to analyse the NMR relaxation data, many researchers suggested that continuous distribution analysis of relaxation times could be a better representation of the information especially in a heterogeneous food system (Assifaoui et al., 2006; Doona et al., 2007).

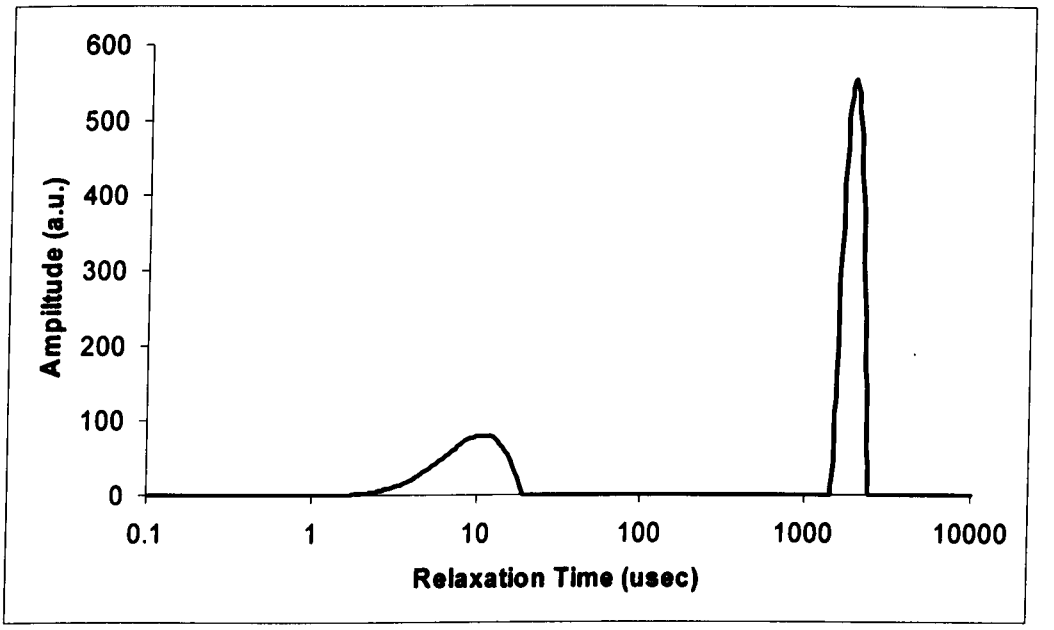



Figure 4-11. Continuous distribution of spin-spin relaxation times ($T_2$ obtained from FID) of flour-water dough sample at 60% water content (flour base) at 29°C.

Figure 4-11 illustrates the continuous distribution of spin-spin relaxation time (obtained from FID) of flour-water dough at 60% water content (flour base) at 29°C. It showed two peaks and implies that there are two groups exhibiting distinctively different molecular mobilities which are correlated to the results obtained from multi-exponential analysis. Because these two groups of $T_2$ values are distributed in the microsecond range, it suggested that the population associated with the less mobile region may correspond to protons in solid like components, such as starch, protons and water molecules tightly associated with those components. The second population may correspond to more mobile protons in the dough sample (Ruan et al., 1999; Donna et al., 2007). Two proton populations were also observed from a single pulse experiment for biscuit dough at 19.4% moisture content (at 25°C) (Assifaoui et al., 2006).

The transverse relaxation curves obtained from a CPMG sequence (Figure 4-12) can also be fitted with continuous distribution of exponentials. The distribution of relaxation times in flour dough at 60% water content (on flour base) showed three populations from a range of 0.5 to 500 msec (Figure 4-12).

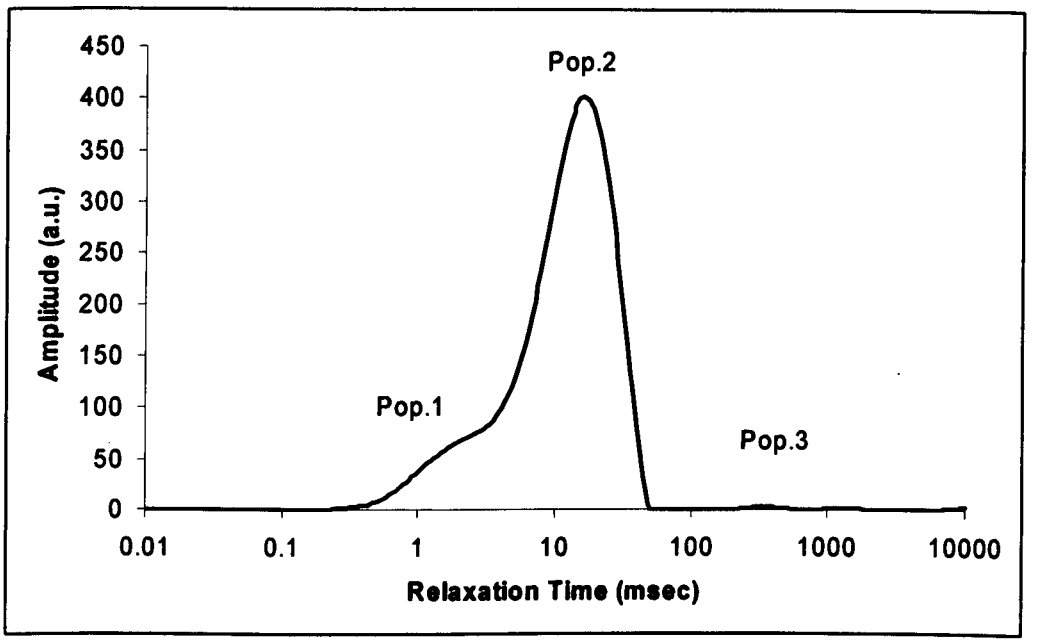



Figure 4-12. A typical continuous distribution of $T_2$ relaxation time from CPMG sequence for flour dough at 60% water content (flour base) obtained from WinDxp software.

### 4.3.3.2 Effect of water content

The $^1H$ NMR $T_2$ relaxation behaviour of the flour-water dough was studied at different water contents between 30 to 80% (flour base). Figure 4-13 shows typical FID relaxation curves of flour-water dough as a function of water content from 30 to 80% (flour base). The decay times of the FID resulted in 2 components. The decay time was the lowest for the solid component (around 25 μsec at 29°C) and the higher for the liquid component (around 400 μsec at 29°C). As the water content of the flour-water dough sample increases, there is a decrease in the signals from the solid component, as expected.

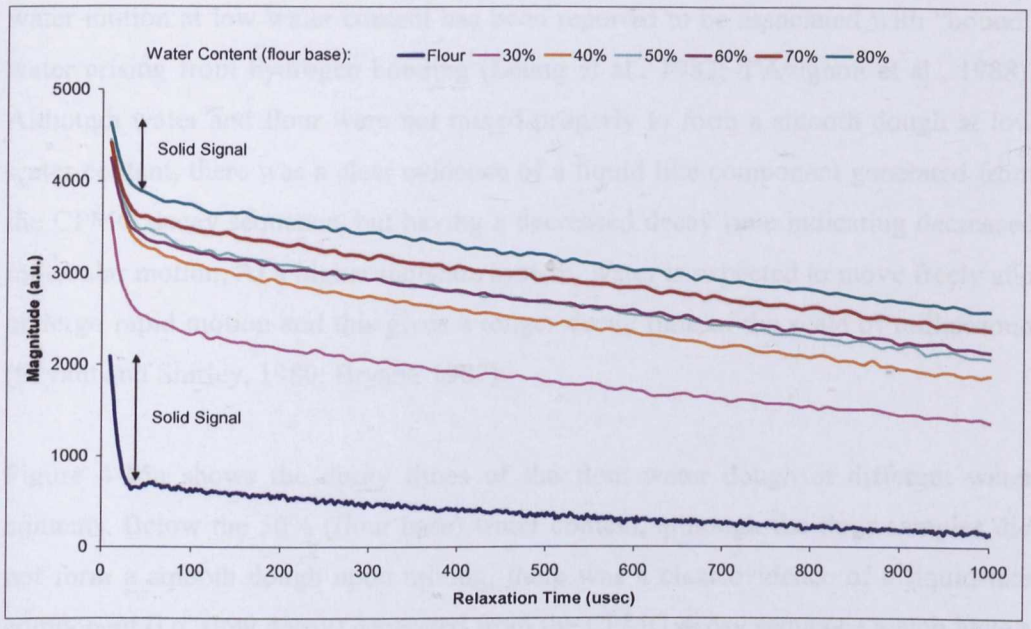



Figure 4-13. FID signal of flour-water dough at different water contents between 30% and 80% (flour base) (n = 5).

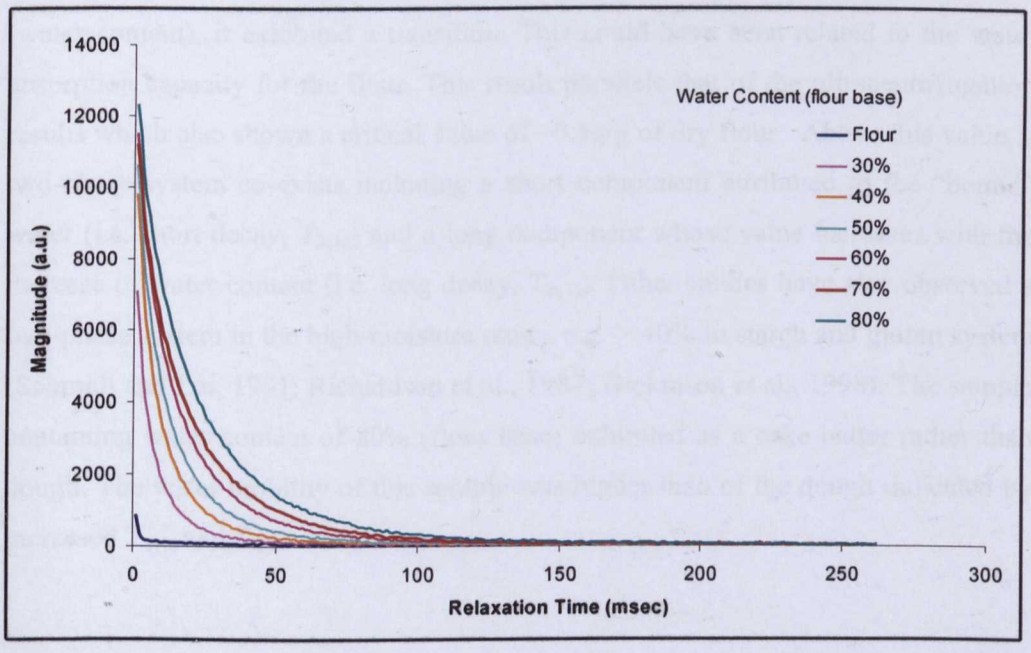



Figure 4-14. CPMG signals of flour-water dough at different water contents between 30% and 80% (flour base) (n = 5).

Figure 4-14 shows the relaxation signals from the CPMG sequence revealed that changes occurred in the water content range 30 to 80% (flour base). When the water content decreased, the magnitude of the CPMG signal was observed to be decreased. This was consequence of the limited free water in the system. The slowing down of

water motion at low water content has been reported to be associated with "bound" water arising from hydrogen bonding (Leung et al., 1983; d'Avignon et al., 1988). Although water and flour were not mixed properly to form a smooth dough at low water content, there was a clear evidence of a liquid like component generated from the CPMG decay sequence, but having a decreased decay time indicating decreased molecular motion. At a higher moisture system, water is expected to move freely and undergo rapid motion and this gives a longer decay time of the scale of millisecond (Bryant and Shirley, 1980; Bryant, 1987).

Figure 4-15a shows the decay times of the flour-water dough at different water contents. Below the 50% (flour base) water content, although the flour samples did not form a smooth dough upon mixing, there was a clear evidence of a liquid-like component (i.e. slow decay) generated from the CPMG decay sequence which have a shorter decay time indicates decreased molecular motion. This small amount of water in the mobile state was probably due to incomplete mixing. Between 40 and 50% (water content), it exhibited a transition. This could have been related to the water absorption capacity for the flour. This result parallels that of the ultracentrifugation results which also shown a critical value of ~0.5g/g of dry flour. Above this value, a two-phase system co-exists including a short component attributed to the "bound" water (i.e. short decay, $T_{2(1)}$) and a long component whose value increases with the increase of water content (i.e. long decay, $T_{2(2)}$). Other studies have also observed a two-phase system in the high-moisture range, e.g. > 40% in starch and gluten system (Schmidt and Lai, 1991; Richardson et al., 1987; Dickinson et al., 1998). The sample containing water content of 80% (flour base) exhibited as a cake batter rather than dough. The water mobility of this sample was higher than of the dough indicated by increased $T_{2(1)}$ and $T_{2(2)}$ values.

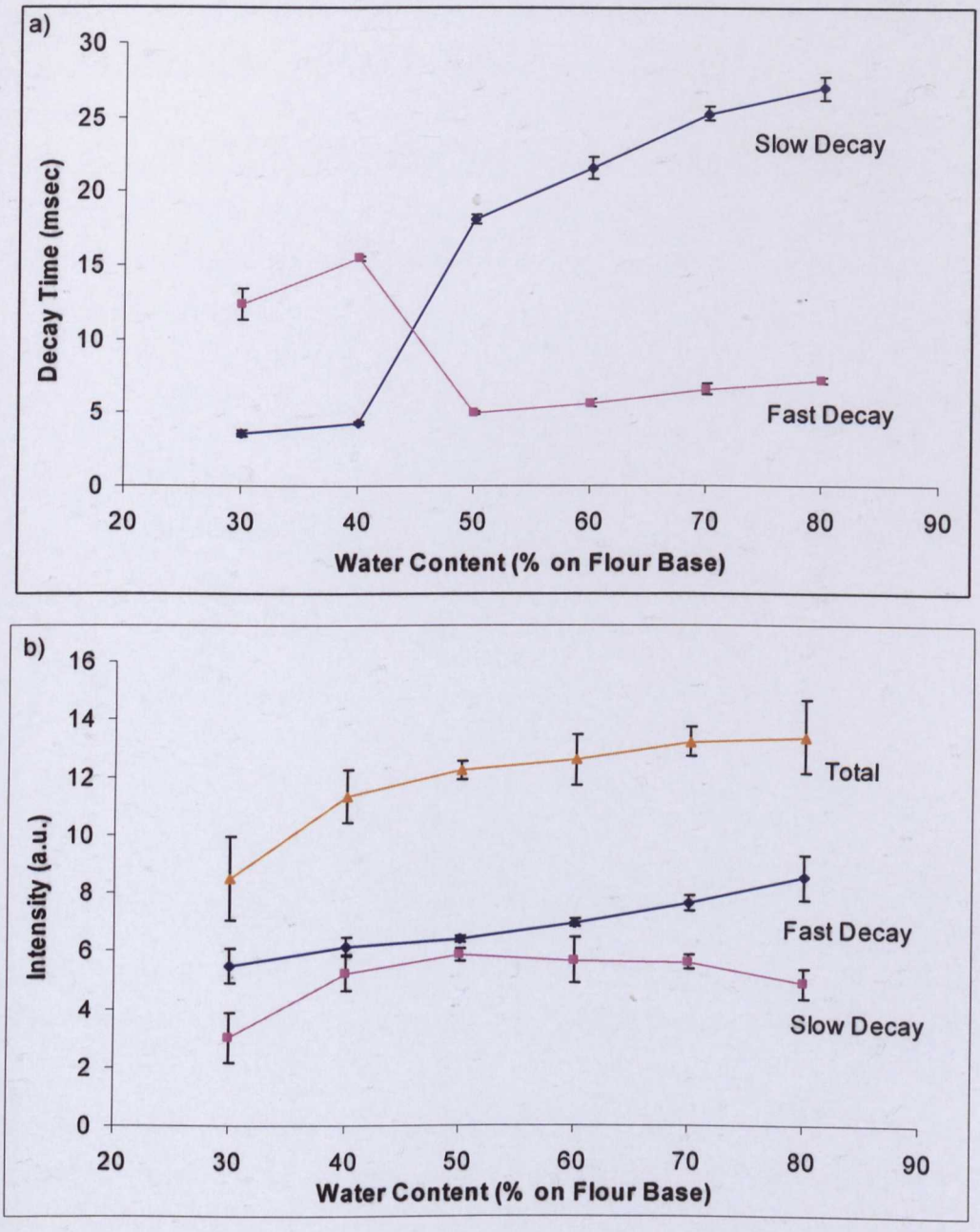



Figure 4-15. Effect of water content on the (a) decay time and (b) intensity of the CPMG sequence (n = 5 with $\pm$ 1 standard deviation).

The effect of water content on continuous distribution of spin-spin relaxation time of flour-water dough obtained from FID experiment is shown in Figure 4-16. At water content of 30 to 80%, two peaks appear on each distribution, indicating different water mobilities. As mentioned earlier, this $T_2$ distribution would represent the proton signals from solids or water molecules closely associated with the solids. There is an initial shift from the proton signals associated with solids, however, as water content of the dough increased, there is no significant difference ($p > 0.05$). The second peak correlated to the mobile water shifted from 600 μsec to 2000 μsec.

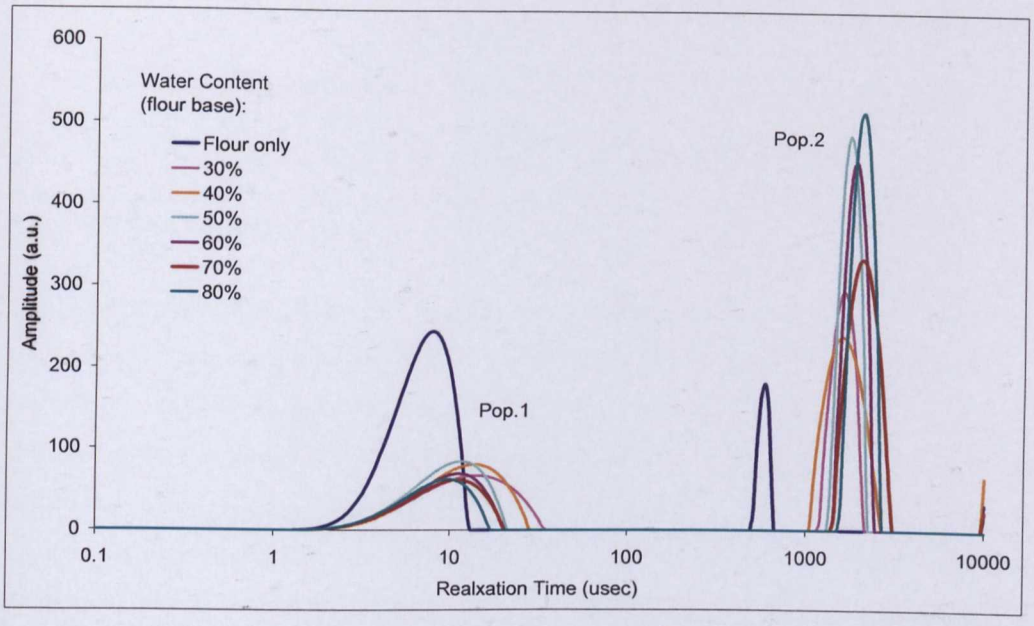



Figure 4-16. Continuous distribution of spin-spin relaxation times ($T_2$) obtained from FID experiment at different water contents (n = 5).

It is known that FID signal is limited for measuring high mobility. High spin-spin relaxation times can be determined by using the CPMG sequence. Thus, the continuous distribution of relaxation times determined by CPMG sequence was also studied on flour-water dough at different water content between 30 to 80% (flour base) and showed three populations (Figure 4-17).

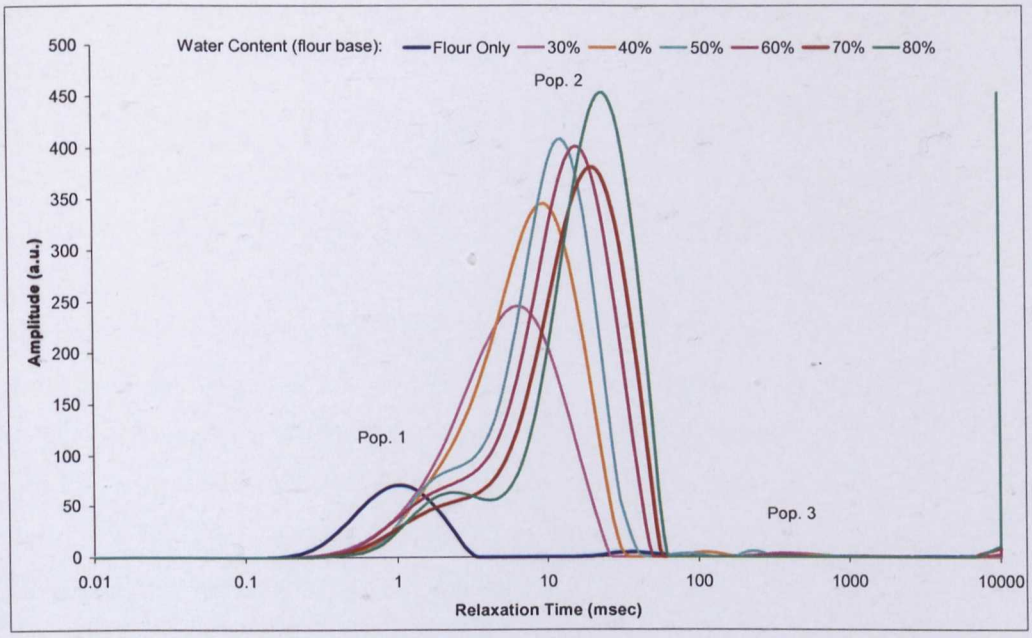



Figure 4-17. Continue distribution of spin-spin relaxation times ($T_2$) obtained from CPMG experiment at different water content (n = 5).

It is generally recongised that starch and gluten have different water binding capacities. However, the location and behaviour of water during dough preparation is still contraoversial. On group suggested that there was more water associated with the gluten than with the starch in the dough and water moves from the gluten to the starch during conventional baking (Willhoft, 1971; Umbach et al., 1992). The other group proposed that more water is associated with the starch than with the protein, thus there would be more hydrogen atoms associataed with starch-interacting with water than protein interacting with water (Bushuk and Hlynka, 1964). According to those report, water mobility in wheat flour dough would not be solely manifested by one component because both water-starch and water-gluten interactions would influence the overall water mobility in a dough system. It should exhibits a mixed spectra repesentative of the different components (Doona, 2007).

In Figure 4-17, three different relaxing components were identified from continuous distrubution of spin-spin relaxation times obtained from CPMG, which were relative to low mobility (Pop.1), intermediate mobility (Pop.2) and high mobility (Pop.3) components. Since the shortest time is constant was above 1 msec, all the obtained NMR signals is considered to come from liquid-like protons. Assuming that each component reflected the mobility of water protons, the different time constants

represented to different degree of molecular interaction of water molecules with other macromolecules.

The change of water content in flour-water dough has an effect on the number of proton populations. Only one broad population (Pop.1) was observed at 30% water content (flour base). This population appeared to be between $T_{2(1)}$ and $T_{2(2)}$. Above the water content of 50% (flour base), the broad population in flour-water dough samples is divided into two populations at $T_{2(1)} \sim$ 2msec and $T_{2(2)} \sim$ 7-23 msec, respectively. At the higher water content, there is a clear proton populations of Pop.1 and Pop.2. In addition, the broadening of the more mobile fraction (Pop.2) suggested that there is an increase of variation in the chemical and physical states of water molecules in wheat flour dough. This was in accordance with Doona (2007). Water content variation had no significant effect on Pop.3.

Figure 4-18 shown a significant effect of water content on the relaxation time values and the amount of protons increased linearly as a function of water content for the intermediate population (Pop.2). This can be related to the increase of free water in the dough system, in which additional water goes into a more mobile state. Therefore, the increase of water greatly influenced the intermediate fraction ( pop.2) of wheat flour dough.

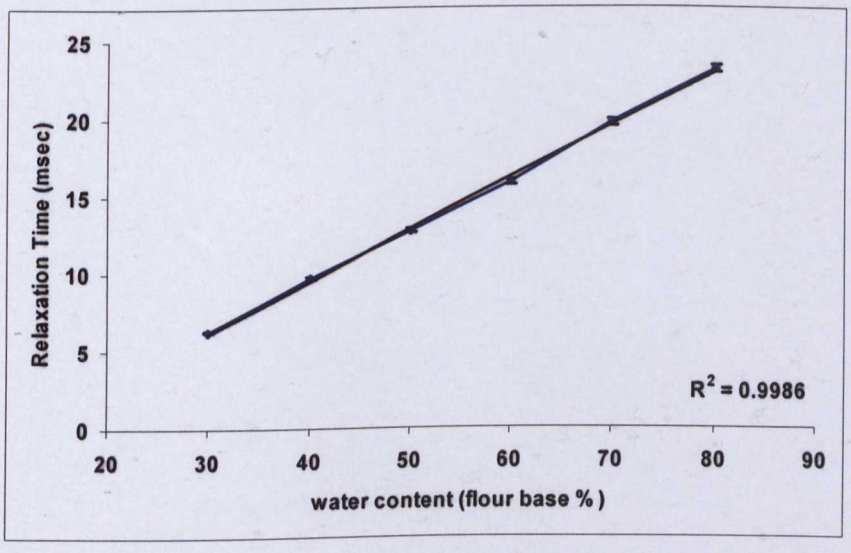



Figure 4-18. Effect of water content on $T_2$ relaxation time for the population (Pop.2) in flour-water doughs at 29 °C (Values represent the mean ± 1 standard deviation of five doughs, each replicated three times).

## 4.4 EFFECT OF BAKERY INGREDIENTS

Preliminary studies were conducted to understand the effect of bakery ingredients such as bakery fat and salt levels on the aqueous phase using the above established techniques.

### 4.4.1 Effect of sugar and bakery fat

In the early stages of study, a soft wheat flour-water batter recipe (Table 4-1) was used to study the effect of different ingredients on the amount of the aqueous phase isolated by ultracentrifugation.

Table 4-1. Flour-water batter recipe.

| Ingredients | Flour-Water Recipe | Flour-Water-Oil Recipe |
|---|---|---|
| Flour | Soft Wheat | Soft Wheat |
| Water Addition (on total base) | 75.00% | 64.56% |
| Other Bulk Materials | No | Sugar |
| Minor Ingredients | No | Lecithin, Sunflower Oil |

Figure 4-19 shows that using a batter with additional ingredients (such as lipid and sugar) significantly reduced the total liquor recovered. This suggested that the water surplus in the flour-water system is directly influenced by the ingredients added into the system.

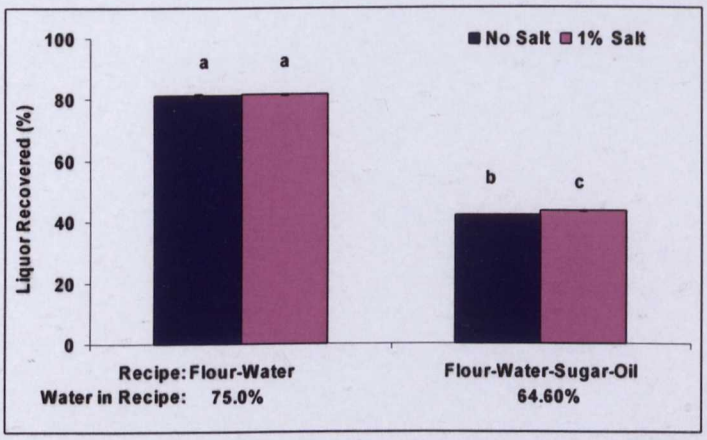



Figure 4-19. Liquor yield from different soft wheat flour batter recipe using ultracentrifugation. n = 5 dough samples (8 replications were carried out for each dough). a-c: different letters in chart show significant difference at $p \leq 0.05$.

Bakery shortening and improvers are regularly used in commercial bread making to produce acceptable bread. Bakery shortening is generally added to bread dough to stabilize the gas cells during heating in oven. The role of improver also is said to help to hold water into the dough matrix. Figure 4-20 shows the amount of freezable water in a dough samples with bakery fat and improver addition.

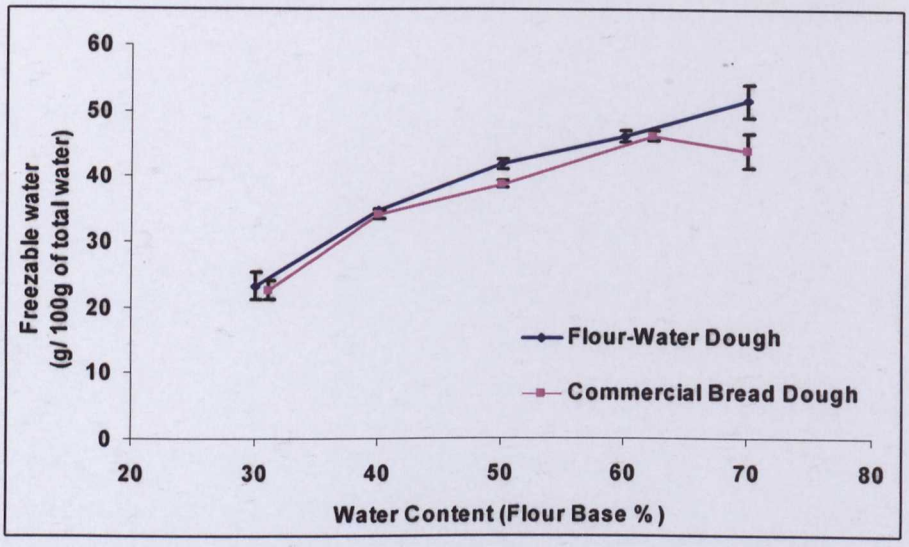



Figure 4-20. Change in freezable water in flour-water dough over a range of water content with added fat and improver by DSC (values represent the mean ± 1 standard deviation of five doughs, each replicated three times).

### 4.4.2 Salt Content

Figure 4-21 shows dough liquor recovered from flour-water dough samples with 0% and 2% salt (on flour base). It shows an increase as water content increases. In addition, the added salt sample shows a significant increase in the amount of liquor recovered compared to the no salt sample at each level of water content.

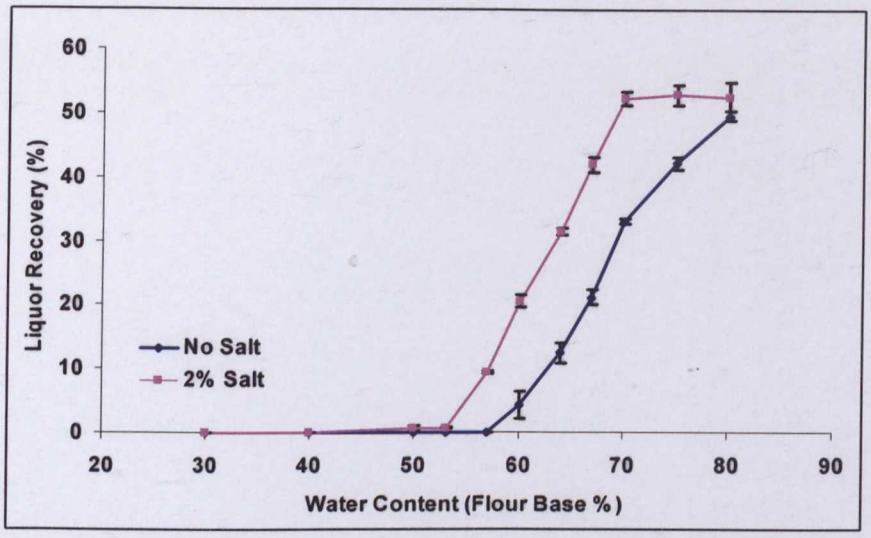



Figure 4-21. Liquor recovered from flour-water dough samples at different water content between 30 to 80% (on flour base) (values represent the mean ± 1 standard deviation of five doughs, each replicated four times).

Experiments were also conducted to observe the effect of salt (sodium chloride) concentration, in a range of 0 – 5% (on flour base), on the amount of aqueous phase recovered. Hard wheat flour were mixed at water content of 40% and 75% (total base) and the liquor recovery is given in Figure 4-21. The dough recipe, with a limited amount of surplus water content (40% on flour base) showed an increase in the amount of aqueous phase isolated by centrifugation when salt was present.

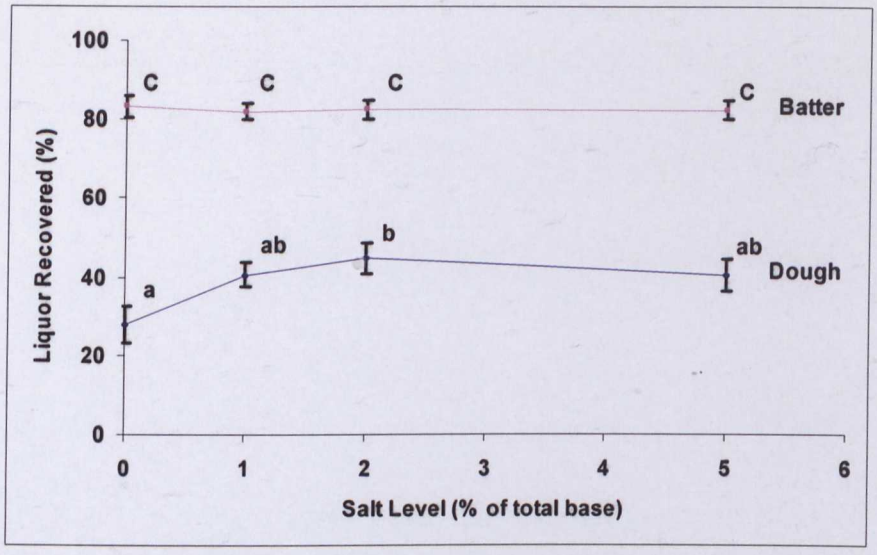



Figure 4-22. Liquid recovered from added recipe water between hard wheat flour dough with 67% water content and batter with 300% water content (on flour base) at different added salt levels (values represent the mean $\pm$ 1 standard deviation of five doughs, each replicated eight times). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$ within each flour type (a,b,c,d for hard flour dough and A,B,C, D, E for soft flour dough)

At low concentration of salts (1% and 2%, total base), addition of salt (NaCl) to the dough caused an increase of liquor recovery compared with pure water. However, at the higher concentration (i.e. 5% salt), there is no significant difference on dough liquor recovery compared to the lower salt concentrations. On the other hand, interestingly, there is no significant difference between the liquor recovery and addition of salt on batter with 300% water content (flour base).

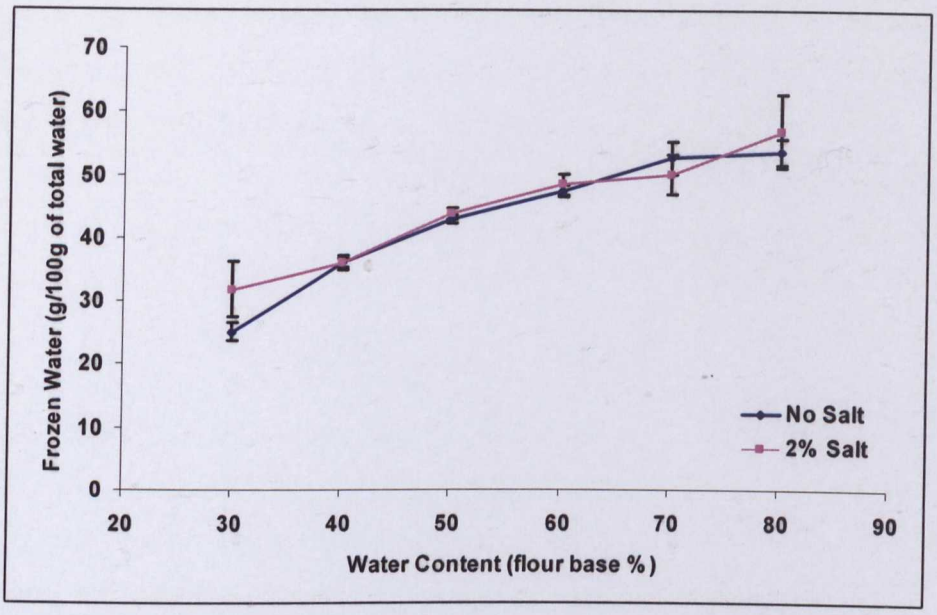



Figure 4-23. Change in freezable water of flour-water dough at salt level of 0% and 2% (flour base) over a range of water contents (values represent the mean $\pm$ 1 standard deviation of five doughs, each replicated three times)

The salt levels in flour-water dough at different water content was investigated using differential scanning calorimetry in order to identify the freezable water (Figure 4-22). Flour-water doughs were prepared with different water contents (30 to 80% flour base) and with two levels of salt (0 and 2% flour base). The amount of freezable water correlated well with the water levels, but addition of salt did not show a significant difference on the amount of freezable water ($p \geq 0.05$).

## 4.5 CONCLUSIONS

The distribution of water is of paramount importance in dough because it has direct influence on the gas cells stability, rheological properties of the dough, gelatinisation properties of the starch, colour reactions, etc. The results suggested that the state of water in flour-water dough exists in different states. The bound water is the portion of water associated closely with the flour components (gluten and starch) and other added ingredients.

The studies with different water content present strong evidence that ultracentrifugation, differential scanning calorimetry and low field $^{1}H$ NMR could be used to characterise the distribution of water in bread dough. The main advantage of all three techniques is that fresh dough samples can be used. Additional advantages such as low field $^{1}H$ NMR studies require no heating and cooling of the samples like DSC and therefore it is a non-destructive technique to study the mobility or binding of water in flour dough. It also provides a rapid, sensitive, and non-destructive determination of not only the quantity of water present, but also the structure and dynamic characteristic of water in the dough samples.

The results presented here set the scene for the following three results chapters, as they will involve the techniques developed in here, as a tool to observe the state of water in full commercial dough system.

# CHAPTER 5. COMMERICAL BREAD DOUGH

## 5.1 INTRODUCTION

The Food Standards Agency has an objective to reduce the salt intake of the UK diet and has identified bread as the major contributor of salt in the average diet. However, members of the Federation of Bakers encounter difficulties in the production of premium bread at lower salt content, the most significant of which is a loss of tolerance during manufacture. The importance of salt on the formation of dough and in the bread making process has been long recognised, but while many studies illustrated the effects of salt on dough mixing and loaf quality, there is little work which explains the effects seen in fundamental physical and molecular terms. A better fundamental understanding of the effects of salt is needed to progress beyond the present status of an average of 1.25g salt / 100g of bread in UK.

In spring 2007, a co-operative research group was funded by the Food Standards Agency with an objective of increasing the fundamental understanding of the effect of salt on Chorleywood Bread Process bread dough and baked white bread. The project consortium included the Federation of Bakers, Food Processing Knowledge Transfer Network, Campden BRI (named Campden and Chorleywood Food Research Association at the start of this work and will be referred to as CCFRA in this thesis) and the University of Nottingham. It was hoped that an increase in understanding could lead to new ways of manufacturing bread at lower salt levels with high efficiency and acceptability.

This chapter summarises the key findings of this experimental work which was carried out in three phases by CCFRA, with work described in this thesis as supporting data. In the initial stage a wide range of parameters were evaluated in a statistical manner (see Table 5.1). The range of variables was then decreased and one type of flour, one form of starch damage, one level of water content and the simple improver was used. Two salt levels 1.4 and 2.0% (flour basis) were used as they represented the present and targeted salt levels in the dough, respectively. Conclusions for the test baking and results for the measures of the physicochemical

behaviour of the doughs are presented in this chapter. Results incorporated into this thesis, but not carried out by the author will be clearly defined.

## 5.2 PROJECT OBJECTIVE

The project sought to answer some fundamental questions of how salt level affects the physical and biochemical bases on dough and bread. The findings could then be used to predict how the major components of dough (starch, protein and lipid) will respond to the changes in salt level, and therefore what compensatory mechanisms (at a molecular level) are needed to improve the process tolerance of bread with reduced salt content. This information would help to find better commercial ways of making bread at salt levels consistent with to the FSA model level of 1.4% (salt on flour basis; equates to 350mg of sodium or 0.875g of salt per 100g of bread).

It was recognised that there were other issues with commercial manufacture of bread with reduced salt content that were discussed amongst the project group, but are not included in this study. These include collapse and oven spring for bread that is not baked in a tin, runaway gassing if proof is prolonged, differences in yeast activity between block and cream yeast when salt level is reduced, reduced mould-free shelf life, changes in staling behaviour and flavour aspects.

## 5.3 METHODS AND MATERIALS

The consortium identified a large number of factors which can influence the dough handling properties and bread quality when varying the salt level. Thus, a 2-level fractional factorial designed experiment was carried out at CCFRA and University of Nottingham covering a wide range of ingredients shown in Table 5-1. In total, 48 samples were carried out (see Appendix A1).

Table 5-1. Ingredients used in the project.

| Ingredient | Levels used | | | |
|---|---|---|---|---|
| **Wheat flour** | Solstice (UK Breadmaking) | | Canadian Breadmaking | |
| **Starch Damage** | Approximately 35 unit | | Approximately 25 unit | |
| **Improver** | Basic improver (enzyme active soy flour (0.5%), ascorbic acid (200ppm)), commercial breadmaking fat (1%) | | Compound improver (soy flour, ascorbic acid, DATEM/SSL mix, fungal amylase) supplied by Fermex | |
| **Salt level** | 1.4% (flour base) | | 2.0% (flour base) | |
| **Water addition (flour base)** | 59.6% | 62.3% | 58.6% | 61.8% |

Wheat samples (Solstice and Canadian) were harvested and milled from the same specific batch specific for this part of the study and flour analysis was carried out by CCFRA to confirm that they have the characteristics expected, before commencing the experiment. All flours were stored frozen and were warmed to 18 $\pm$ 1°C the night before they were used. CCFRA also carried out an initial test baking to define the yeast addition level needed to deliver target proof height of 12 cm at different added salt levels.

The project used the standard Chorleywood Bread Process for dough preparation (see section 3.3.1.1) and test baking procedures (see Appendix A3). In this particular project, mixing time was adjusted to provide a work input of 11Wh for Solstice flour and 13Wh for Canadian flour. Vacuum was introduced to the mix after 60 seconds of mixing. All tests were carried out on yeasted dough. This gave confidence to the consortium members that the technical phenomena observed mirror the experience in commercial bakeries and in–house test baking.

In University of Nottingham, mixed dough samples were put immediately to -18°C for less than 10 minutes and then kept at 4°C before experiment to prevent dough expansion due to yeast. Portions of the mixed doughs were sealed and immediately frozen with liquid nitrogen for future experiment at usage.

All salt addition levels in the following chapters are expressed as "grams of sodium chloride per 100 grams of flour in the recipe". This convention is common in the baking community and recognises that other ingredients, including water are used in variable proportions. In this chapter, a paired-sample t-test was conducted to evaluate the impact of salt on the commercial dough samples.

## 5.4 RESULTS AND DISCUSSIONS

This project was separated into two main parts. Test baking was mainly carried out by CCFRA and they were responsible for identifying the dough handling and baking properties in order to understand the relationship between the effect of salt levels, dough temperature and delay time. Work at Nottingham mainly focused on the relationship between isolation of dough liquor from ultracentrifugation, the mobility of water in the dough system using $^{1}H$ nuclear magnetic resonance ($^{1}H$ NMR) and differential scanning calorimetry (DSC). In addition, $^{23}Na$ NMR was used to gain information on the partition of salt between the phases of the dough. Rheological tests including Kieffer dough extensibility test and texture profile analysis (TPA) test were also carried out on the dough samples.

### 5.4.1 Laboratory analysis from University of Nottingham

#### 5.4.1.1 Dough liquor isolation

When dough is subject to a high gravitational field for a relatively long period of time, it separates into a liquor phase and one or more solid phases. The amount of dough liquor is thought to be a reflection of the availability of water in the dough. The composition of the dough liquor is taken to represent the composition of the liquid phase surrounding protein and starch which are phase separated in dough.

Dough liquor was separated by using the standardised ultracentrifugation protocol described in chapter 3.3.1.3. After centrifugation, 2 layers were observed. Figure 5-1 shows that the average amount of dough liquor isolated at salt content of 1.4% and 2% was approximately 20% of samples (which ranged between 59.6 to 61.8% added water in the dough). There is no statistical significant difference with this level of salt

using paired t-tests when only considering salt as the only variable factor. Previous experimental work from Salt et al. (2006) suggested that higher salt addition would result in greater dough liquor recovery.

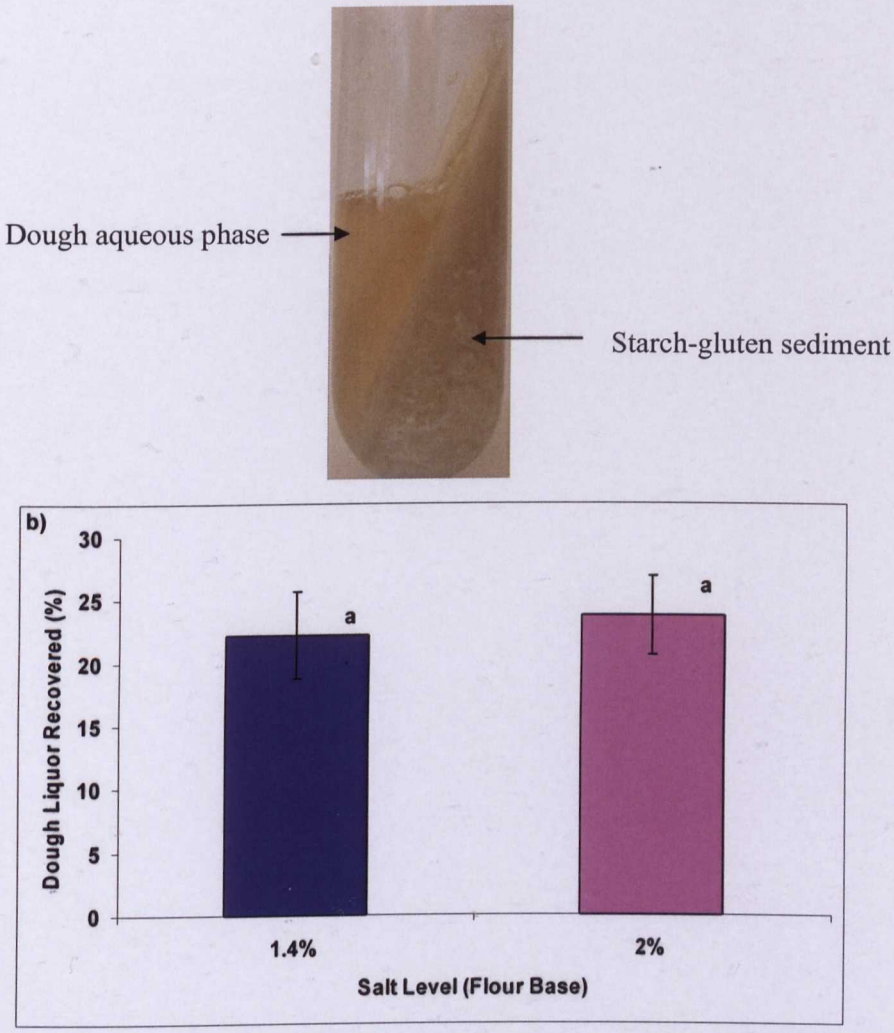



Figure 5-1. (a) Dough liquor yield from ultracentrifugation of CBP dough with compound improver and (b) the dough liquor recovered from 1.4% and 2% salt including $\pm$ 1 standard deviation. a: same letter in chart shows no significant difference ($p \geq 0.05$).

Analysis showed that improver type and the level of added water affected the liquor recovered from the ultracentrifugation test, as expected. The yield of the aqueous phase also appeared to be related to the flour type, which in turn related to the protein content in the flour. Wheat flour that contains higher protein content (i.e. Canadian flour) may need more water to give the same strength of dough compared to the weaker flour (Solstice). This implies more water is needed to interact with the gluten-starch matrix thus less "free" water is available in the system and lowers the

liquor yield. The water absorption capacity of flour can also be influenced by the level of damaged starch. However, result from this experiment did not show that the level of starch damage had any an effect on the liquor yield.

Type of the improvers may alter the amount of dough liquor extracted from centrifugation (Figure 5-2). Basic improver produced more liquid than the comparable doughs made with the compound improver (paired t-test $p \leq 0.05$). It was observed that dough liquor prepared with compound improver produced slightly more viscous and sticky to the touch than dough liquor from basic improver recipe, which was less viscous and poured more easily.

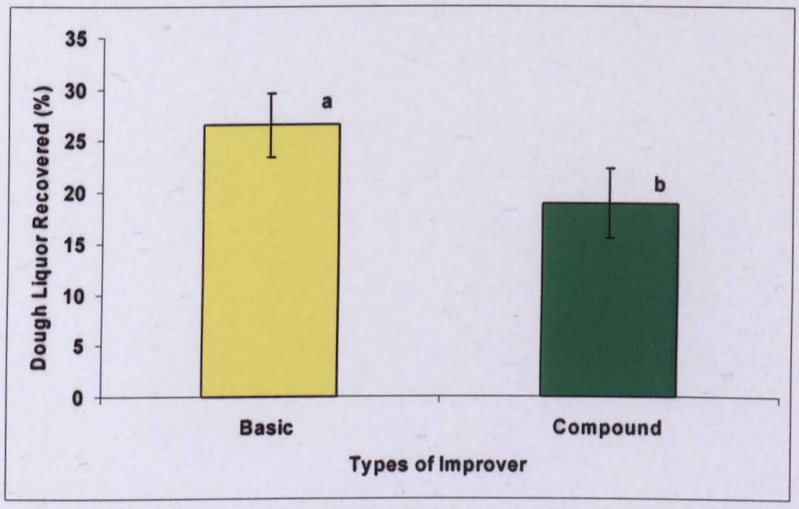



Figure 5-2. Dough liquor yield from ultracentrifugation of CBP dough with basic and compound improvers ± 1 standard deviation ($p<0.05$). a - b: different letters in chart show significant difference at $p \leq 0.05$.

### 5.4.1.2 Mobility of Water in Dough

#### 5.4.1.2.1 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) is a method for measuring the change in specific heat capacity of a sample as the temperature of the sample is changed by addition or removal of heat. The method developed here designed is to measure the amount of freezable water in dough. The hypothesis was that if salt affected the amount of water that behaved like bulk water, it would result in a change in the

amount of freezable water which can be detected through freezing or melting of the dough using DSC.

Figure 5-3 shows a cooling scan of dough samples as the temperature changes from 20°C to -50°C at a cooling rate of 2.5°C/min. DSC analysis of the frozen water peak shows that the area of the peak (ΔH, the total enthalpy of freezing) was not affected by the amount of salt in the dough ($p > 0.05$).

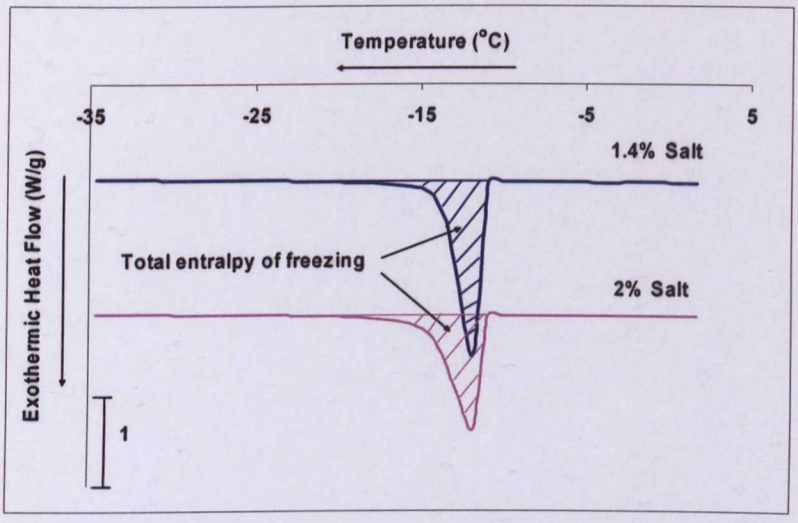



Figure 5-3. A typical DSC trace of dough with 1.4% and 2% salt (on flour base) when cooled from 20°C to -50°C at 2.5°C/min at reference temperature.

Table 5-2 shows the temperature measured during cooling of dough samples with compound improver and yeast. $\Delta H_{ice}$ is the energy removed to form ice in the dough sample and the exotherm temperature describes the temperature ranges over which ice formation occurred. The ΔH did not show an effect on the dough samples with 1.4 and 2% salt levels, however, the melting temperature of ice in the system showed an expected decrease as salt concentration increased due to the freezing point depression which is a colligative property for the sodium chloride.

Table 5-2. DSC cooling of dough samples with compound improver and yeast. (values represent the mean ± 1 standard deviation of 48 doughs, each replicated two times). $\Delta H_{sample}$ represents the total enthalpy of the dough sample and $\Delta H_{water}$ represents the total enthalpy of water in the dough sample through calculation.

| Salt Concentration | ΔH (J/g) (sample) | ΔH (J/g) (water) | Exotherm Temperature (°C) | | |
|---|---|---|---|---|---|
| | | | $T_o$ | $T_{peak}$ | $T_{end}$ |
| 1.4% Salt | -71.8 (± 3.3) | -158.7 (± 42.0) | -11.36 (± 0.8) | -9.72 (± 1.0) | -13.8 (± 2.2) |
| 2% Salt | -70.79 (± 7.9) | -155.2 (± 43.3) | -12.49 (± 1.23) | -11.00 (± 1.2) | -14.98 (± 1.4) |

The exact values for ice creation in these samples may be difficult to ascertain. For example, the sample may supercool and ice could suddenly form which would lead to a large release of heat in a short period of time. This causes the temperature of sample to increase as the heat is conducted away relatively slowly through the various thermal resistances in the furnace assembly.

Similar results were observed when the frozen dough sample was reheated from -50°C to 20°C at a relative slow rate of 2.5°C /min shown in Table 5-3. The enthalpy of ice in the dough samples ($\Delta H_{sample}$) was not affected by the salt content (either in cooling or reheating modes). This implies that the amount of freezable water was not influenced by the added salt content in the range of 1.4 to 2%.

Table 5-3. DSC melting of ice in dough samples with compound improver and yeast (values represent the mean ± 1 standard deviation of 48 doughs, each replicated three times). $\Delta H_{sample}$ represents the total enthalpy of the dough sample and $\Delta H_{water}$ represents the total enthalpy of water in the dough sample.

| Salt Concentration | ΔH (J/g) (sample) | ΔH (J/g) (water) | Endotherm Temperature (°C) | | |
|---|---|---|---|---|---|
| | | | $T_o$ | $T_{peak}$ | $T_{end}$ |
| 1.4% Salt | 75.03 (± 4.4) | 166.7 (± 47.4) | -9.3 (± 1.7) | -4.28 (± 1.2) | -1.3 (± 1.5) |
| 2% Salt | 76.53 (± 3.3) | 168.4 (± 44.0) | -10.3 (±1.6) | -4.89 (± 1.0) | -1.77 (± 1.5) |

One can explain the term unfreezable water (as discussed by Belton 1997) by considering that it is not a water fraction that is "bound" in any way, but it is a result of the fact that biopolymer systems cannot crystallise in a conventional eutectic sense. This can is due to the system containing entangled polymer networks, and therefore cools with the formation of ice, but this is still associated with a fraction of the water. At equilibrium, where there is no driving force to produce more ice, the activity of the water phase must be reduced to equal that of the ice. This liquid water is therefore the unfreezable fraction, but the arguments are thermodynamic in nature and do not involve a bound fraction.

DSC was also used to measure the gelatinisation temperature of the dough samples. The total enthalpy ($\Delta H$) for the gelatinisation process remained almost constant between 1.4% and 2% salt levels (Table 5-4). Although there is no statistical difference on gelatinisation temperature between the matched samples, the trend showed a slight increase of the gelatinisation temperature, which occurred at about 65°C for the doughs, as salt was added to the dough sample as expected. Since various ingredients were added to the dough sample, the main factor responsible for the starch gelatinisation changes was not able to be identified.

Table 5-4. DSC heating of starch gelatinisation in the dough samples with compound improver and yeast. a: same letter in chart shows no significant difference ($p \geq 0.05$).

| Salt level in dough (flour base) | Gelatinisation temperature $T_{peak}$ (°C) |
|---|---|
| 1.4% | 64.57 (±2.0) [a] |
| 2.0% | 64.98 (±2.8) [a] |

While the DSC measurement does not provide information on the water distribution in the dough system, it does show that the temperature at which the starch crystallite losses order is affected by the salt content.

#### 5.4.1.2.2 $^1$H Nuclear Magnetic Resonance

The low field time domain proton nuclear magnetic resonance ($^1$H NMR) has been used to study the mobility of protons and is used to determine the distribution of

water in the samples. Pure water has a long decay time or $T_2$ of 2 seconds, whereas protons in an immobile material have a short decay time. If materials are mixed with water, resultant decay times are shorter and may be close to the values of the immobile material. This means that either the mobility of the water has been massively restricted or that protons can exchange between the two sites and produce an "averaged" decay.

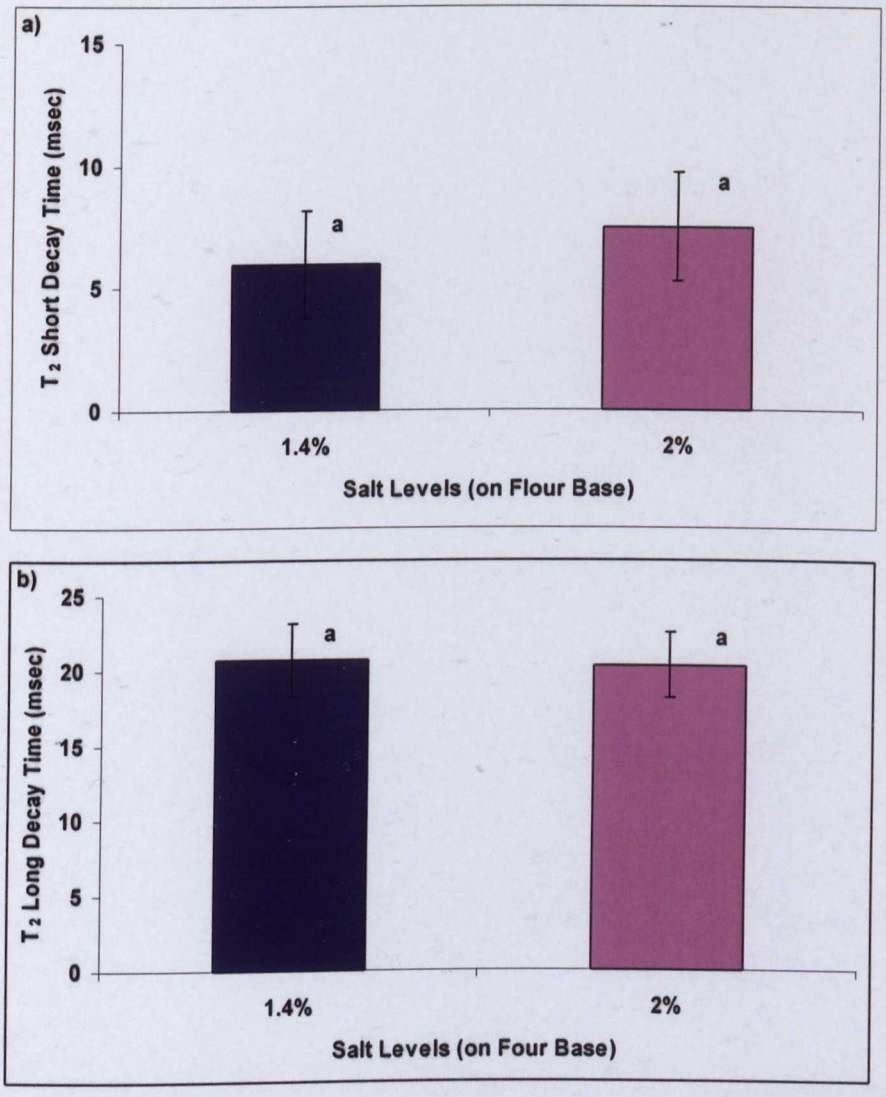
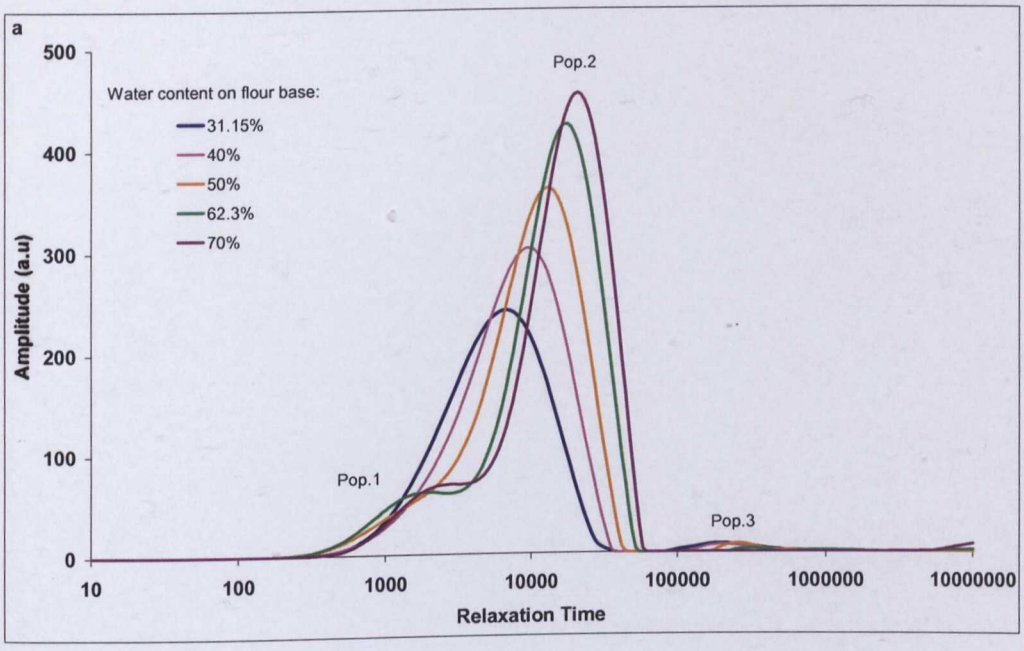



Figure 5-4. The CPMG transverse relaxation times (a) short decay ($T_{2(1)}$) and (b) long decay ($T_{2(2)}$) for dough with compound improver and yeast at 1.4 and 2% salt levels (flour basis). a: different letters in chart show significant difference at $p \leq 0.05$.

The NMR relaxation curve from CPMG sequence was analysed with two exponential fits grouped into $T_{2(1)}$ and $T_{2(2)}$. $T_{2(1)}$ would represent the shorter decay time

components in the system where the protons are less mobile, or are the more tightly bound water faction and can be referred to as the immobile fraction. The $T_{2(2)}$ component represents the more mobile water fraction (Leung et al., 1976). Despite the large changes in the amount of dough liquor, as assessed using ultracentrifugation (see section 5.4.1.1) arising as a result of salt addition, no changes were observed to the proton mobility measured by $^{1}H$ NMR (Figure 5-4). In this data set, $T_2$ values show no statistically significant differences when comparing with values based on any of the 5 variables used in the dough preparation (see Table 5-1). The standard deviations are higher for the values shown in Figure 5-4, but they present all the data from the experiments. However, there were still no significant differences when paired t-tests were performed, with salt levels being the only variable. The presence of numerous ingredients and their competition, in respect of water, make the study of water mobility particular complex. For example, the compound improver may mask the effect on the distribution of water in the dough system.

The distribution of the relaxation times determined by CPMG sequence was studied on commercial bread dough at 2% salt levels with different water contents in the range of 31.5% to 62.3% (flour base). The evolution of the continuous distribution is presented in Figure 5-5a. There is a significant effect of water content on the peak relaxation time which was observed at the intermediate population (Pop.2). There is a linear increase on the relaxation times as water content increase in Pop.2 (Figure 5-5b). This finding parallels with the results published by Assifaoui et al. (2006) on biscuit dough. The figure also shows that the measurement of relaxation time can resolve differences in water levels of about 2-3% (on flour base). This implies that the limit of resolution that can be expected for dough samples varying with salt levels is equivalent to more than a 3% increase in moisture.



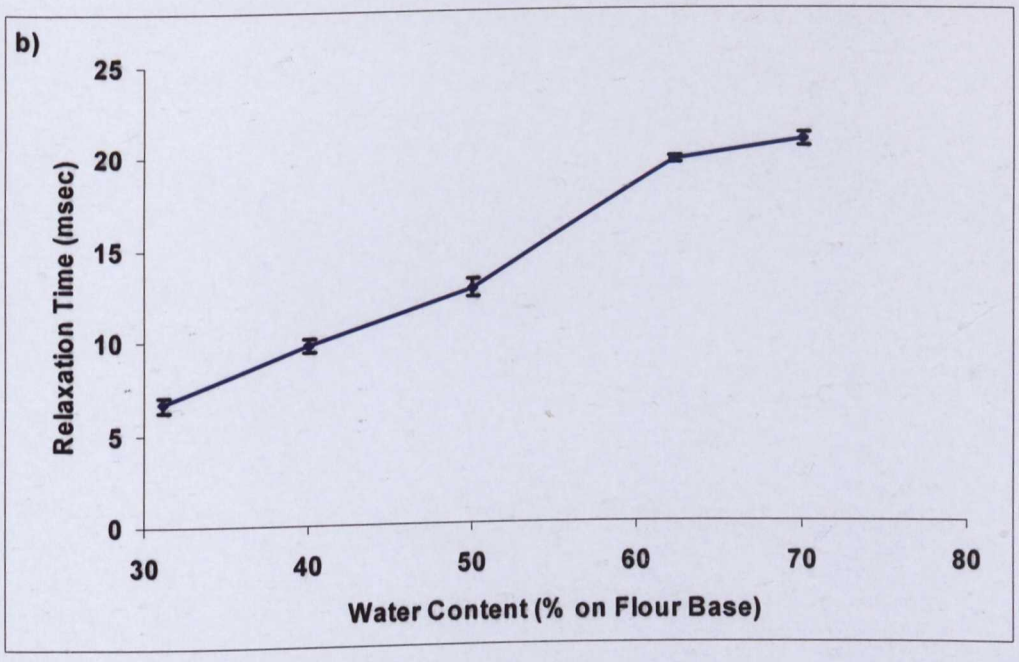




Figure 5-5. (a) Relaxation time distribution ($T_2$) for commercial bread dough with improver prepared with different water contents measured at 29°C. (b) Effect of water content on $T_2$ relaxation time for population 2 in commercial bread doughs with improver at 29°C (Values represent the mean $\pm$ 1 standard deviation of five doughs, each replicated three times).

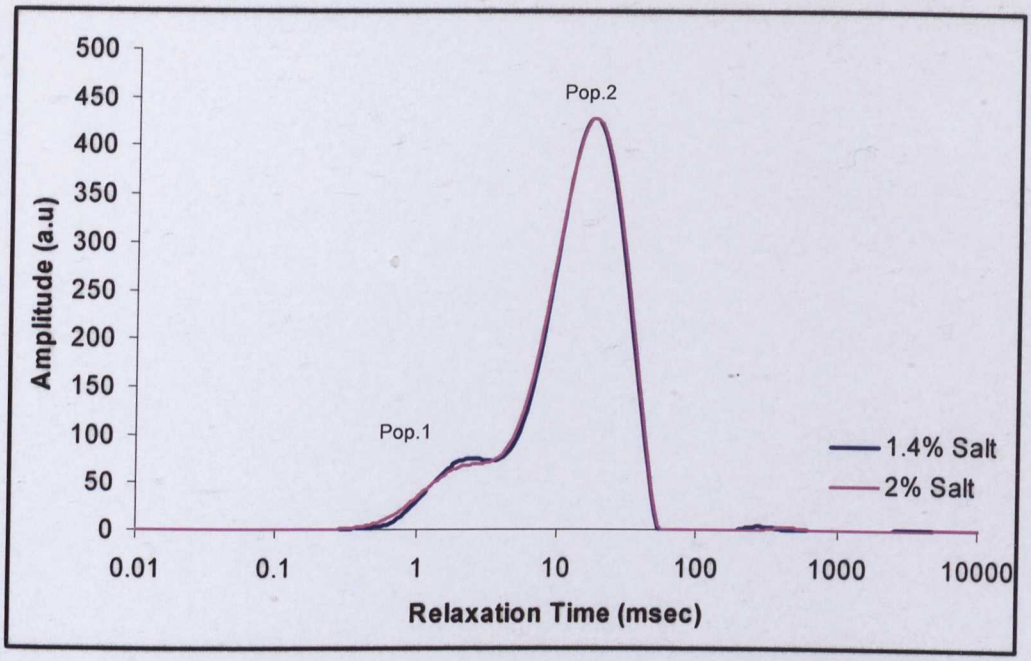



Figure 5-6. The continuous distribution of commercial bread dough with improver and yeast at 1.4% and 2% salt levels.

In the range of salt between 1.4% and 2%, Pop.1 and Pop.2 were approximate between 2 msec and 20 msec, respectively. There was a rather small variation in Pop.1 and Pop.2 at this salt content indicating that the mobility of each water fraction, as measured by NMR, did not change appreciably with addition of salt. This suggested that the extra liquid isolated from ultracentrifugation experiment is not categorised as "free" or mobile water and it is still somehow "bound" within the system. This will be discussed in more detail in the conclusion chapter.

### 5.4.1.3 Dough liquor composition

#### 5.4.1.3.1 Total protein content

A number of studies have proposed that the aqueous phase extracted by centrifugation has a beneficial effect on the baking process. The properties and composition of this dough aqueous phase have been described by many workers (MacRitchie, 1976; Sahi, 2003; Mills et al., 2003; Salt et al., 2006), when it has been reported to contains lipids, soluble proteins and non-starch polysaccharides (Sahi,

1994; Mills et al., 2003, Salt, 2004) and are all known to be surface active molecules which may help the gas-cell stability within the dough structure.

Proteins, being amphiphilic, in nature are one of the important surface-active components in food systems (Wilde, 2000), as they can form a strong, elastic interface by unfolding at the interface and thus increase the surface viscosity. Preliminary studies showed that as increasing the salt concentration in the flour-water dough increased the amount of protein presented in the isolated dough liquid phase increased, as shown in Table 5-5. Salt et al. (2006) had also measured an increase in dough liquor protein content and total dough liquor protein as a result of salt addition to dough (2% flour basis).

Table 5-5. Protein content of dough liquor isolated from flour-water dough systems with salt levels between 0 and 5% (flour base). n = 5 with ± 1 standard deivation. a-b: different letters in chart show significant difference ($p \leq 0.05$).

| Salt concentration (%) | Protein concentration (mg/ml) | Average dough liquor weight (g) | Total protein in dough liquor * (mg) |
|---|---|---|---|
| 0 | 9.81a | 2.00 (± 0.34) | 19.62 |
| 1 | 15.26b | 2.92 (± 0.34) | 44.56 |
| 2 | 17.02b | 3.22 (± 0.42) | 54.80 |
| 5 | 18.12b | 2.92 (± 0.61) | 52.91 |

* assume 1g = 1ml of dough liquor

However, from the data set from the trails with multiple variables (Table 5-1), there was no significant effect of salt content (over the range 1.4g/100g flour to 2.0g/100g flour) on the concentration of protein in the dough liquor (Table 5-6) when compared using paired t-tests of matched samples.

Table 5-6. Effect of salt level on the protein concentration in dough liquor isolated from CBP dough. a: same letters in chart show no significant difference ($p \geq 0.05$).

| Salt Level (flour base) | Protein Content (mg/ml) |
|---|---|
| 1.4% | 21.95 (± 7.28) [a] |
| 2.0% | 22.79 (± 9.18) [a] |

#### 5.4.1.3.2 Total lipid content

The role of lipids in determining the baking quality of wheat flour has been debated for many years (Baker and Mize, 1942; Leissner, 1998). Evidence suggested that polar lipids can be both detrimental and beneficial to loaf volume, depending on their concentration, while non-polar lipids seem to have only a detrimental effect on loaf volume.

Salt content (over the range 1.4g/100g flour to 2.0g/100g flour) had no measurable significant effect on the concentration of lipid in the dough liquor which was about 0.48 mg/ml for liquor extracted from dough which contains compound improver and yeast. One assumption is that the DATA ester (in the compound improver) binds to gluten protein (Selomulyo et al., 2007) and this association prevents them from entering the aqueous phase of the dough. The DATA esters may also be sequestering endogenous lipids and preventing them entering the dough aqueous phase. This may mask the salt effect on the lipid content.

### 5.4.1.4 Dough rheology

The mechanical properties of wheat flour doughs are important in determining both the handling properties of the dough during processing and the quality of the finished product. Large scale bakeries are finding it difficult to further reduce salt in bread as it produces unacceptable dough during process handling. Rheological measurements were made to quantify the dough samples using 3 methods:

- Chen – Hoseney test cell to measure adhesiveness
- Kieffer rig to measure the extensibility under tension force
- Texture Profile Analysis test to measure stiffness and stickiness on dough surface

Dough stickiness and dough extension tests were carried out at both CCFRA and Nottingham to allow comparison between experimentation at each site and to allow conclusions to be drawn about the effect of salt levels on the variables under test. Results from each test are discussed separately below.

#### 5.4.1.4.1 Chen – Hoseney test (CCFRA)

Measurements of dough stickiness were made using the Chen-Hoseney cell at CCFRA and it showed a statistically significant difference in stickiness. Results concluded that dough samples with higher salt level (2%) were more sticky (higher adhension) than the lower salt (1.4%) dough samples. This finding was not considered to be in accord with the experience of commercial bakers. It was also shown that there was a high variability using the Chen-Hoseney method. Thus, with the agreement of the consortium, dough stickiness was assessed only by expert baker score in the later stages of the work.

#### 5.4.1.4.2 Kieffer rig dough extensibility test

Kieffer rig test is a method assessing dough extensibility with two parameters: extensibility and resistance to extension at the time of dough rupture and are related to dough handling and gas bubble stability properties.

The Kieffer test was conducted using two different conditions between the sites, however, because of the protocol variations and this means that it was not practical to compare the numerical values obtained. However, from the results, there were no significant effects seen from the measurement of dough extensibility on dough samples that included compound improver and yeast at both sites. The results may be masked by the DATEM present in the compound improver as it helps to strengthen the dough by forming hydrogen bonds with starch and glutenins (Haehnel et al, 1995).

#### 5.4.1.4.3 Texture Profiling Analysis (TPA)

A simple and rapid experiment was adopted from the texture profile analysis try to understand the surface properties of the dough samples. Three dough surface properties were identified: (1) hardness of the dough, (2) relaxation of stress, and (3) surface adhesion to the probe. A typical plot is shown in Figure 5-7. Results from the experimental set show there was no significant different on the texture of the dough

samples between 2% and 1.4% salt levels (flour base), which was opposite to the baker's experience.

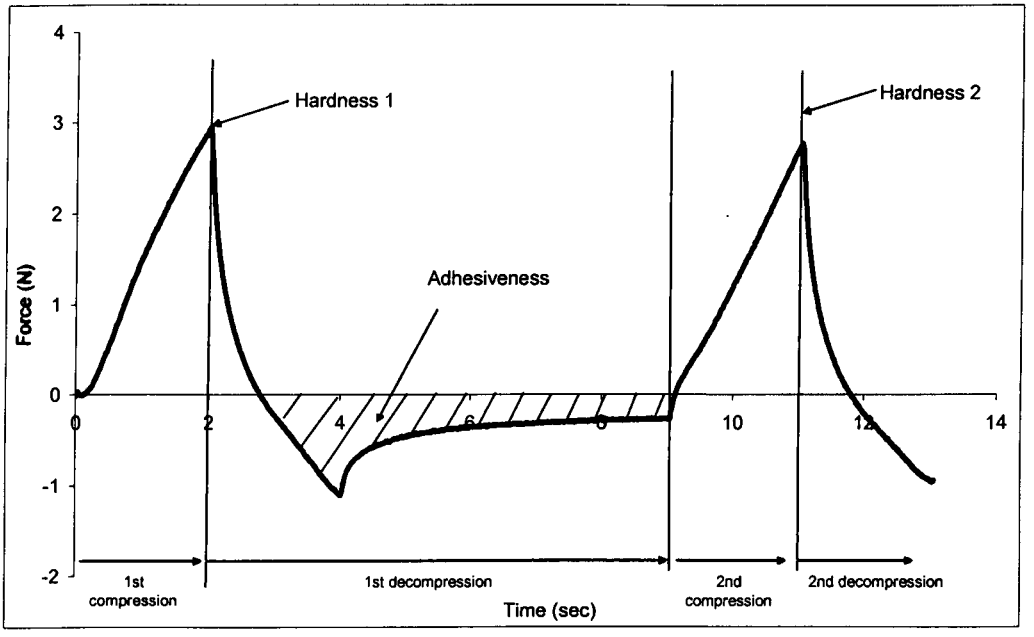



Figure 5-7. A typical plot of texture profiling analysis on CBP dough surface.

At the later stage of the project, modified texture analysis test was developed and thus will be discussed in more detail in Chapter 7.

### 5.4.1.5 Sodium partition in dough using $^{23}$Na NMR

Analogous to proton mobility assessment by NMR in the dough system, it is also believed that sodium ions in the dough system are constrained in mobility at different sites of the gluten-starch matrix or remain mobile when dissolved in the aqueous phase. Therefore $^{23}$Na NMR was used to identify the state and concentration of sodium within the wheat flour dough system. Under appropriate conditions, the re-orientation of $^{23}$Na nuclei can be followed in a nuclear magnetic resonance instrument. The measurement is technically more difficult than measurement of protons because of a weaker resonance signal compared to that for proton. The work now reported was the first attempt in the laboratories within Food Sciences, Nottingham to detect the mobility of sodium ions and details of the method developed are given in Chapter 8. In this particular study, full dough recipe which

include yeast were prepared according to the standard CBP procedure (from section 3.3.1.1).

Table 5-7. Percentage of sodium presented in each phase measured by $^{23}Na$ NMR.

| | Salt Level | |
|---|---|---|
| Recipe salt addition | 1.4g salt/100g flour | 2.0g salt/100g flour |
| Recipe water addition | 62.3ml water/100g flour | 62.3ml water/100g flour |
| Concentration of salt in dough (g salt/100g dough) | 0.84g/100g dough | 1.20g/100g dough |
| Apparent concentration of salt in dough (by NMR) | 0.458 (± 0.085)g salt/100g dough | 0.604 (± 0.093)g salt/100g dough |
| Apparent concentration of salt in dough liquor (by NMR) | 1.309 (± 0.190) g salt/100ml dough liquor | 1.702 (± 0.188) g salt/100ml dough liquor |
| Apparent concentration of salt in sedimented dough (by NMR) | 0.413 (± 0.139) g salt/100g sediment | 0.483 (± 0.086) g salt/100g sediment |

Sodium NMR measurements on the whole dough showed that a percentage (between 45-55%) of the added salt is dissolved in the water phase and was detected as mobile sodium in the dough system. The remaining salt (between 55- 45%) was not detected as their mobility was restricted. Under these particular experimental conditions, it was concluded that the percentage of salt with restricted mobility is not greatly affected by total added salt level in the range 1.4g salt/100g flour to 2g salt/100g flour.

Different phases of the dough (isolated dough liquor and the sediment) were carried out to investigate the partition of sodium between each phase. The results showed that the percentage of the added salt which is recovered in dough liquor is not affected by the amount of added salt within the range 1.4 – 2.0g salt/100g flour (Table 5-7). The sediment (that contains the solid material and remaining part of the added water) contains the majority of the sodium. This is expected as this sediment represents about 88% of the dough weight.

The pie chart in Figure 5-8 gives a generalised schematic of the relevant proporption of salt in the dough, where a large proportion cannot be detected by the NMR.

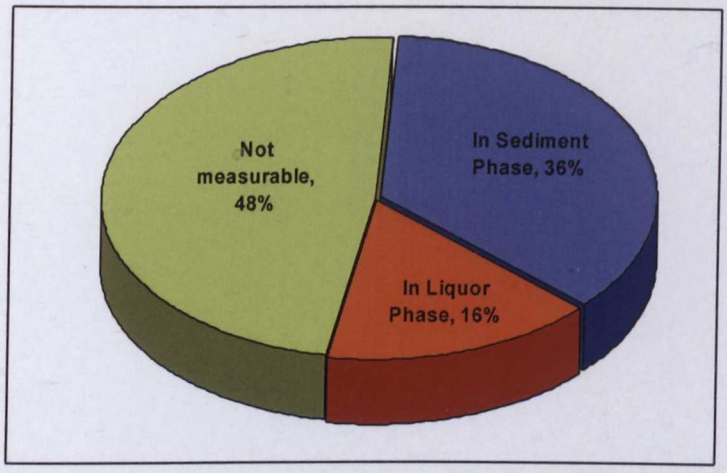



Figure 5-8. Partition of salt between dough liquor, dough sediment and "invisible" phases as calculated from 2% salt (flour base) whole dough and ultracentrifuged dough fractions using $^{23}Na$ NMR measurements.

It must be borne in mind that, as interpreted above, dough liquor is the material released from the solid under the effect of high gravitational field, but is not illustrative of the mobility of water within dough. The importance of dough liquor composition, particularly with respect to mobile species such as sodium ions, is questionable for the understanding of dough behaviour.

### 5.4.2 Test baking from CCFRA

#### 5.4.2.1 Yeast gassing rate and salt addition

Generally, the rate of gas production by yeast increases when salt level is reduced. In order to maintain a constant rate of gassing and dough gas content, the yeast level was adjusted according to the salt content thereby delivering a fixed proof height of 12 centimetres after 50 ± 5 minutes. This was achieved for all doughs irrespective of dough temperature. The yeast levels added to the recipe are given in Table 5-8.

Table 5-8. The amount of yeast added to CBP dough at different salt levels.

| Salt level (flour basis) | Yeast level (flour basis) |
|---|---|
| 1.4% | 1.7% |
| 2.0% | 2.0% |

### 5.4.2.2 Dough handling

The Baker's Score of Dough Handling is a subjective measurement to quantify the properties of freshly-mixed bread dough and it was performed with experienced and well-trained bakers. The dough samples were scored for the consistency and stickiness.

The incidences of failure at the second moulder showed a pattern that was consistent with the industrial baker's experience. From observation, an increase of the dough temperature after mixing and lowering the level of salt made the dough samples more difficult to handle.

In addition, the machineability score, commonly used among bakers, was also used to measure the number of failed doughs between 15 and 30 minutes rest after mixing. Result (Table 5-9) showed that the number of failed doughs increased when salt level in the dough decreased and the results became more distinctive when increasing the resting time from 15 to 30 min. These findings imply that changes with salt level, dough temperature and resting time affected the quality parameters of stickiness and machineability.

The consistency and machineability of 4-pieced dough was also tested. They were more difficult to mould than single-pieced dough because it required a thinner sheeting height and additional contact of the 4-piecing knives.

Table 5-9. The percentage of dough pieces failed at the second moulder from 1-piece and 4-piece dough samples. Results adapted from CCFRA internal report).

| **Single piece** | Percentage of doughpieces failing at moulder | |
|---|---|---|
| | 15 minutes bulk rest | 30 minutes bulk rest |
| **2% salt** | 0 | 0 |
| **1.4% salt** | 0 | 0 |
| **0.8% salt** | 0 | 25 |

| **4-piece** | Percentage of doughpieces failing at moulder | |
|---|---|---|
| | 15 minutes bulk rest | 30 minutes bulk rest |
| **2% salt** | 0 | 17 |
| **1.4% salt** | 0 | 42 |
| **0.8% salt** | 25 | 67 |

In the moulding stage, a dough piece is shaped into required form for bread production. It has been identified as a critical stage of bread production and this stage is thought to be sensitive to the stickiness of the dough. The operating conditions (deformation rate and the extent of deformation) in the moulder can change the properties of the dough stickiness. Small changes in the dough properties could affect the operation and processing by stopping the equipment and the need to clean before more dough can pass through the moulder. Therefore, several factors affect the satisfactory performance of the dough samples and these include the moulder design, the setting of the moulder (both the machine settings, ancillary services such as inflow and throughput rate) and the environmental conditions (temperature and humidity).

Table 5-10. Effect of salt content, dough temperature and rest time on ease of handling at second moulder with a 4-piece dough. Results adapted from CCFRA internal report and indicate the amount of dough pieces that failed to pass through the moulder.

| % of failure | 0.8% salt | 1.4% salt | 2.0% salt |
|---|---|---|---|
| 29°C dough | 0 | 0 | 0 |
| 33°C dough | 6.7 | 0 | 0 |

| % of failure | 0.8% salt | 1.4% salt | 2.0% salt |
|---|---|---|---|
| 15 minutes dough to moulder | 25 | 0 | 0 |
| 30 minutes dough to moulder | 66.6 | 41.6 | 16.8 |

In addition, two levels of dough temperature (29°C and 33°C) were investigated. The Baker's Score of Dough Handling and the incidence of failure at the second moulder showed a pattern that was consistent with the industrial baker's experience (Table 5-10). The difference in machineability score (number of failed doughs) between 15 and 30 minutes rest became greater as the salt content of the dough decreased.

### 5.4.2.3 Loaf properties

Test baking was carried out by CCFRA to gain a better understanding of the effect of salt on the final bread product (baking conditions were given in Appendix B). Dough samples were baked at 244°C for 30 to 35 minutes in a reel oven.

Figure 5-9. Baked bread ready for analysis.

Several tests were performed on the baked bread at different salt levels including the measurement of oven spring height and specific volume. In addition, crumb texture and butterability tests were performed using Texture Analyser and the crumb structure was determined by C-cell.

A selection of photos of the baked bread is shown in Figures 5-10 and 5-11. None of the bread showed defects comparable with the bakers complaints.


Figure 5-10. Photo of bread samples prepared with Canadian flour (low starch damage level), low water content at 1.4% and 2.0% salt levels.


Figure 5-11. Photo of bread samples prepared with English flour (low starch damage level), low water content at 1.4% and 2.0% salt levels.

Oven spring is the sudden increase in the volume of the dough during the first 10 to 15 minutes of baking. Generally, it is due to the increased rate of fermentation and expansion of gases under high temperature in the oven. The oven spring height of the baked loaf can be used to understand the strength of the gluten-starch matrix in the dough and thus the quality of the final bread product. The oven spring recorded from test baking was not consistently related to final loaf volume, or to salt content, as shown in Figure 5-12 for single piece loaves.

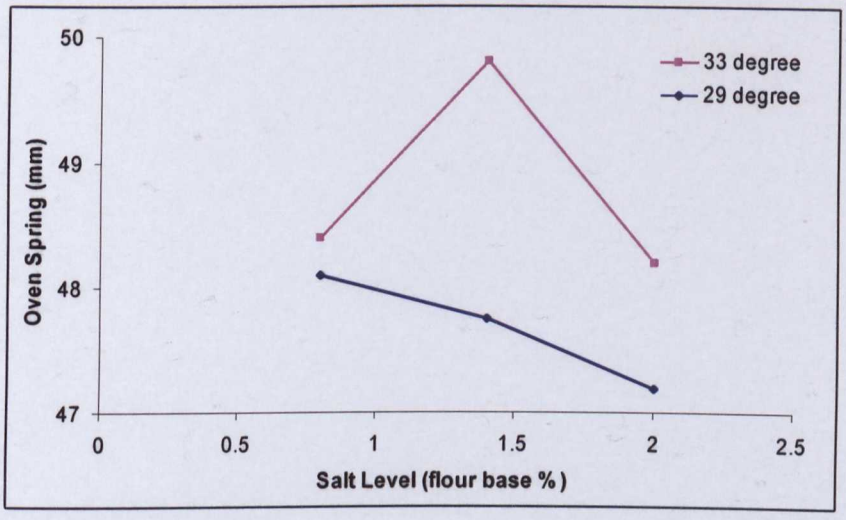



Figure 5-12. Effect of salt content and dough temperature on oven spring of single piece bread (n = 32). Results adapted from CCFRA report.

Bread was cooled at a controlled temperature (20°C) and humidity (50% RH) and the specific volume of the bread was measured. Figure 5-13 shows the specific volume of the single piece loaf increased with addition of salt and bulk resting time.

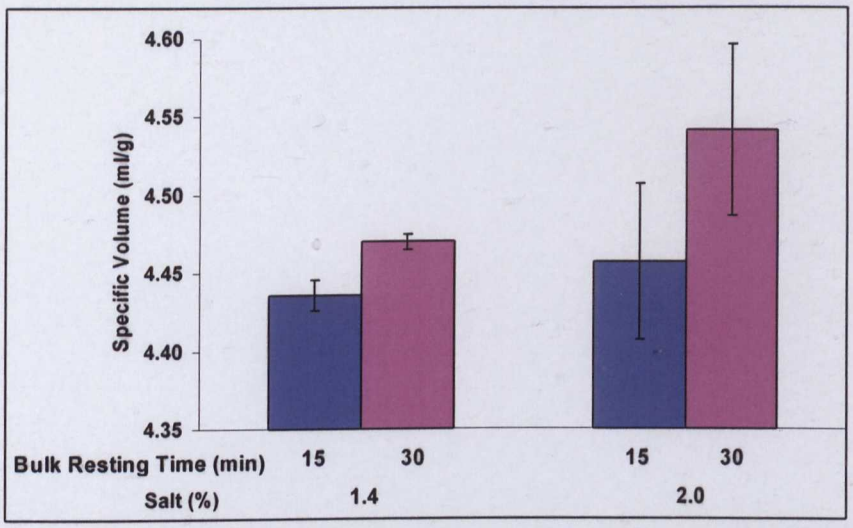



Figure 5-13. The effect of salt content and bulk rest time on the specific volume of single piece bread (n = 32). Results adapted from CCFRA report.

One interpretation of the changes in the specific volume may due to the relationship between salt levels and a delay in the transition from a closed gas system to an open sponge structure. Bread dough exhibits the viscoelastic behaviour combining the properties of both viscous and elastic components, which is a key factor associated with a good oven rise when baking. The viscous components are responsible for the flow of the dough, while the elastic components are responsible for the extensibility of the network. The rheology of the dough comes mainly from gluten component. DSC measurements (from section 5.4.1.2) showed the starch gelatinisation temperature increases as salt content increases. Although the measures on the doughs used for this work did not show significant differences (Table 5.4). Once the starch gelatinises the viscosity of the matrix changes dramatically to become much more viscous and the gluten has much less influence over the structure. If there is a delay in the changes of the starch, the flow of the dough network can continue and there will be greater expansion before the creation of the open sponge structure due to the gas bubble wall no longer expanding, but breaking (Sevenou, 2002). Although starch gelatinisation is one of the possible factors, there are other factors that affect the expansion capability of dough. The properties and composition of the aqueous phase may also be affected by the levels of salt in the dough system. Salt may also impact on the levels of protein that become soluble in the aqueous phase of the dough and therefore alter the gluten strength. From the Kieffer test (see section 5.4.1.4.2), the dough extensibility was affected by the level of salt. This alters the resistance to

deformation of the dough. This effect could be more apparent at higher temperatures. The gluten also undergoes changes at high temperature and there may be a relationship between the thermal transition of gluten and salt concentration (Salvador et al., 2006).

Furthermore, the final volume of the loves could have been affected by the salt due to the biochemistry of the system. Yeast levels were adjusted to give the specific height after the proving stage (from section 5.4.2.1), however, the performance of the yeast (i.e. the gassing levels) was not investigated. Generally, yeast continue to produce gas in the dough piece in the early stage of baking, until the temperature rises to a point that kills the yeast cells, however no measure of yeast gassing at the beginning of baking was measured. The amount of carbon dioxide produced in the oven may differ between doughs of different salt contents leading to different final heights and volumes of the baked bread. Changes in salt levels could also influence other enzymatic reactions within the dough (e.g. amylases and glutathione) and these factors could change the expansion of the dough and formation of bread in the baking stage (Kilcast et al., 1997).

Some dough pieces were subjected to physical shock (dropping from a standard height) after being fully proved. This was to assess the insusceptibility to collapse in prover – oven transfer. This had a limited effect on the specific volume of the baked bread and there was no strong evidence of salt level effecting collapse.

The crumb texture of the bread was assessed by the C-Cell instrumental method, Expert Baker Scoring by CCFRA staff and was also ranked by representatives of the Federation of Bakers. No evidence was show that the crumb quality was affected by the salt level. The crumb texture (1.4% and 2% salt levels) was generally in the borderline of being acceptable.

Crumb compression tests were carried out with the Texture Analyser. There was no significant difference ($p \geq 0.05$) on the firmness of the bread and its resilience at the different salt levels. A crumb stretch test was also carried out using a spherical probe to identify the "butterability" of the bread. While 4-pieced bread showed to be stronger, there was no significant effect due to the differences in salt content.

Figure 5-14. The "butterability" test with Texture Analyser (adapted from CCFRA report).

### 5.4.2.4 Summary of the CCFRA results

The test baking results from CCFRA is summarized in Table 5-11.

Table 5-11. Summary of test baking results from CCFRA (adapted from CCFRA internal report).

| Experiment | Increase salt level gives rise to: | Comment |
|---|---|---|
| Dough pH | Lower pH | Although it shows statistical difference, this small change is believed not significant to ultimately alter the dough. |
| Dough Density | Higher density | Related to air incorporation and retention. |
| Kieffer Extensibility Test | More extension | Distance of the dough can be stretch further before it fails. |
| Chen-Hoseney Test | More adhesive dough | Dough sticks to machinery either because it is too adhesive or it is not sufficiently cohesive to be pulled off. This finding is contrary to the baker's view as low salt dough is stickier. |
| Bread Volume (specific volume) | More volume (higher specific volume) | Expected by bakers. Salt strengthen the gluten aid the integrity of the cell wall structure for gas retention |
| Bread Volume (dropped) | More volume | Opposite with the baker's expectation. Reflects reduced tolerance of the dough at the lower salt level. |
| TPA Bread Firmness | Softer texture | Texture becomes softer as loaf volume increases, so this finding may be more about loaf volume than salt effect per se. |
| Bread Crumbs Moisture | No Effect | Water levels and starch damage levels influence the moisture content of the bread. |
| C-Cell Number | No Effect | Key factors affect the cells number is the hydration rate and protein content of the flour. |

## 5.5 CONCLUSION

Commercial bread dough, including improver and yeast, was investigated to understand the effect of salt levels on the dough and bread properties. Laboratory analysis and test baking were performed at University of Nottingham and CCFRA. This chapter summarised the findings observed in the project which was sponsored by the Food Standards Agency (UK). Despite the very large numbers of dough samples prepared and measured by CCFRA there was very little evidence that changing the salt content of the doughs influenced the baking performance or the final quality of the breads.

The most noticeable factor was that doughs compromised by low salt and at higher temperature (33°C) tended to stick to the dough moulder. Dough samples going through the moulder showed a clear effect of the salt level on the degree of stickiness. This trend was consistent with industrial experience where any dough that sticks is unprocessable because of the high plant throughout rate. This loss of processability could only be quantified in terms of the number of doughs sticking to the equipment but the "stickiness values" obtained by objective measurement, including Chen-Hoseney test, Kieffer extensibility and texture profiling analysis (TPA) did not show the expected rheological effect on the dough samples and did not reflect the stickiness observed during the processing.

Under certain circumstances, salt reduction did lead to a reduction in the specific volume of the baked loaf. From the test bakery, problems of collapse and open texture were not observed at any of the salt levels used. Even with a complete test bakery description and measurement of relevant dough handling properties, it was not possible to give any indication of the direct effects of salt reduction on dough/bread quality.

Laboratory analyses were carried out on the dough samples both at CCFRA and Nottingham and the doughs care was taken to ensure the doughs were comparable between the two sites and with the doughs used by CCFRA to bake bread for the investigation. Measures of the dough rheology and some other measures were difficult because of the time required to carry out the assessments. During this time

delay the action of the yeast changed the samples and often made it difficult to handle.

The hypothesis that salt affected the distribution of water in the dough, using ultracentrifugation, nuclear magnetic resonance ($^{1}$H NMR) and Differential Scanning Calorimetry (DSC) was tested.

$^{1}$H NMR (Nuclear Magnetic Resonance) and DSC (Differential Scanning Calorimetry) was used to identify the state the water in the dough system, it was concluded that the mobility of water and its partition between "solid" and "liquid" phases is not affected by salt content. Although the yield of dough liquor can be shown to increase with salt addition, the parallel measurements of NMR and DSC would indicate that this finding cannot be related to the mobility of water in the system. The interpretation offered is that the properties of the solid dough matrix are sensitive to salt concentration in a way that affects the drainage of liquid from the dough under high gravitational field. The effect is probably mediated by the capillarity of the dough matrix or by the surface tension of the dough liquor phase.

$^{23}$Na NMR measurements were carried out and it was found that approximately 50% of the added sodium (over the addition range 1.4 – 2.0g salt/100g flour) showed the same mobility as salt dissolved in water, however the remaining sodium was "invisible" to the $^{23}$Na NMR probe under the conditions of the experiment. The "invisible" sodium seems to be closely associated with the solid material in the system.

After discussions with the consortium, it was agreed that yeast would be omitted from the mixture for a better understanding of the action of salt in the dough system. Although salt interaction with yeast, the by-products of its metabolism and the interaction between all these factors, are recognised as not trivial, the gaseous expansion of the doughs makes its assessment too difficult for accurate interpretation for the measures required to test the hypothesis put forward.

# CHAPTER 6. SIMPLIFIED COMMERICAL BREAD DOUGH

## 6.1 INTRODUCTION

The physical and biochemical bases of dough have been widely studied with a simple recipe consisting of flour-water and its constituents (gluten and starch). However, only limited works have been done within complex systems such as bread dough. While the effect on flour-water dough is well documented, the understanding of a multi-component mixture remains sketchy.

In this chapter, work utilising bread doughs, complete with improver and fat, but not containing yeast, is discussed. To investigate the role of salt on the physical properties of commercial bread dough and to gain insight into the molecular water dynamics and its mobility in the commercial bread dough, the range of salt content was extended. Typical salt levels, on a flour basis, were 0, 0.4, 0.8, 1.4, 2 and 5%. In addition to increase the salt levels, a simplified commercial bread dough recipe consists of one flour type (Viking), water level (62.3%), basic improver and bakery fat was used shown in Table 6-1.

Table 6-1. Simplified CBP dough recipe.

| Ingredients | Percentage on flour base (%) |
|---|---|
| Flour | 100 |
| Water | 62.3 |
| Improver | 1 |
| Fat | 1 |
| Salt levels | 0-5 |
| Yeast | 0 |

A higher amount of water (62.3%) was added to the recipe compared to the water absorption percentage (58.0%) given by the flour manufacturer in order to exaggerate the effect on the dough properties. All the dough samples (~50g) were mixed with the minor-pin mixer (see section 3.3.1.2). In order to understand the effect of salt on dough properties, soft wheat flour of 9% protein content was also used in some parts of the study for comparison.

## 6.2 RESULTS AND DISCUSSION

### 6.2.1 Availability of free water in the dough system

Water is essential for the transformation of flour to dough. Water is also responsible for the establishment of a viscoelastic structure in dough and may directly be involved in the structure by providing a medium for interactions among flour constituents. It has been recognised that the water in a dough system exists in states often called bound and free. Bound water is the portion that is closely associated with the host material (i.e. flour components) and thus shows physical properties different than those of free or bulk water in the system (Kuprianoff, 1958). The quantity of water bound to the dough system is an important parameter since it directly influences the rheological properties of the dough and the quality of final product. Three techniques have been identified to quantify the state of water in the dough system. They were ultracentrifugation, differential scanning calorimetry (DSC) and low field proton nuclear magnetic resonance ($^1$H NMR).

#### 6.2.1.1 Dough liquor isolation

CPB dough samples with basic improver and different salt levels (0-5% flour base) were prepared from two types of flours (hard wheat and soft wheat) and centrifuged following the standard procedure given in section 3.3.1.3. Figure 6-1 shows the yield from both flour dough samples showed less than the amount of recipe water added to the dough, suggesting that there was excess water binding capacity, beyond the water requirement in the flour, as determined by the water absorption test. The water absorption capacity of flour can be influenced by the level of damaged starch, hard wheat flour generally will have a higher level of damaged starch.

The soft wheat flour dough showed a higher amount of dough liquor compared to hard wheat flour dough samples. This higher dough liquor recovery of soft wheat dough may be related to the weaker gluten network, which leads to easy physically puncture of structure in dough under high centrifugation force and form channels aid for liquid flow. The fact that the sample has less starch damage is also likely to be very important in the free liquid measured by this technique. However, in the work described in Chapter 5, flours of different starch damage were assessed and there was

no statistical variation due to the starch damage level and the amount of liquid obtained by ultracentrifugation, when all the other factors were taken into account. Although the values of free liquor were less for the highly damaged starch samples (marginal means were 30 for the low starch damaged and 22 ml for the high starch damaged samples). In these earlier studies, the Solstice flour had a higher liquor yield compared to the Canadian flour ($p \leq 0.05$). Therefore, it is likely that protein quality and starch absorbance influences the liquor yield on centrifugation. The compound improver also reduced ($p \leq 0.001$) the amount of liquor yield.

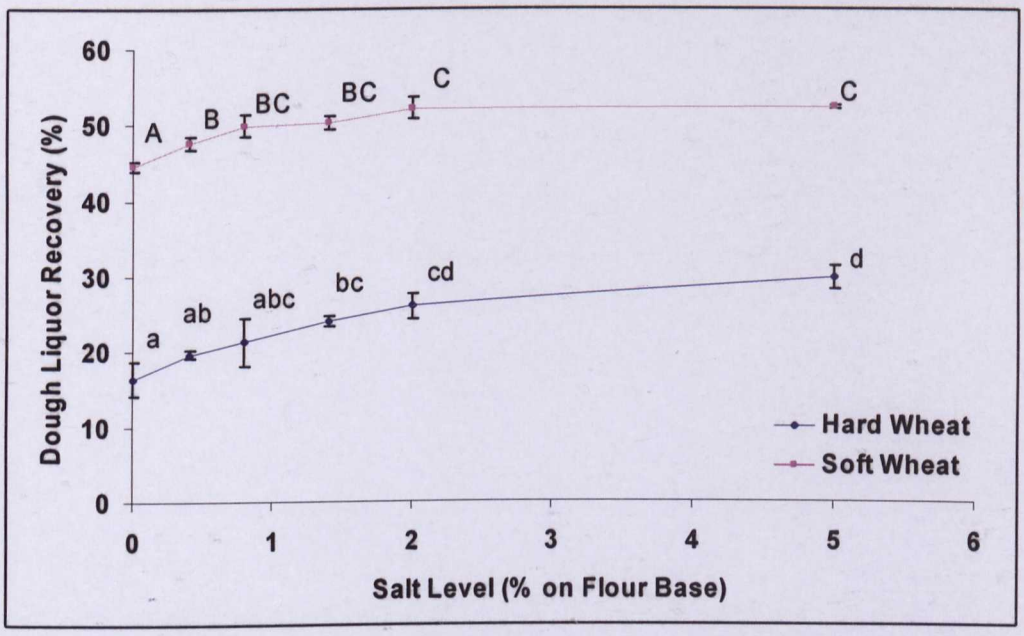



Figure 6-1. Dough liquor recovery rate with hard wheat and soft wheat flour at salt level between 0 to 5% (flour base) (values are the means $\pm$ 1 standard deviation from the 5 doughs. Each mean value was derived from four replicates). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$. (a,b,c,d for hard wheats and A,B,C, D, E for soft wheats)

Soft wheat flour of 9% protein content was used in order to observe if the difference in flour composition will influence the yield of dough liquor. For both types of flour, the amount of aqueous phase isolated by centrifugation increased with addition of salt, as shown in Figure 6-1. This could be due to altering the gluten structure in such a way that the salt occupies the sites once occupied by the bound water (Galal et al., 1978; Preston, 1989). This may help to explain why liquor yield increased when salt was added.

### 6.2.1.2 Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was used to study the water distribution in dough system through heating and cooling the dough samples.

#### 6.2.1.2.1 Starch gelatinisation

The gelatinisation properties of starch are of paramount importance in bread. The setting of the product and its texture are determined by the temperature at which starch gelatinises and by the degree of gelatinisation. Other components of the dough (protein, sugar, salts, lipid etc) also affect the gelatinisation temperature of starch, and thus the ultimate final structure of the baked product. Both water and energy are necessary for starch gelatinisation.

Figure 6-2 shows the DSC thermogram for CBP dough samples prepared with hard wheat flour at different salt levels. Soft wheat flour also shows a similar effect on salt addition (Figure 6-3).

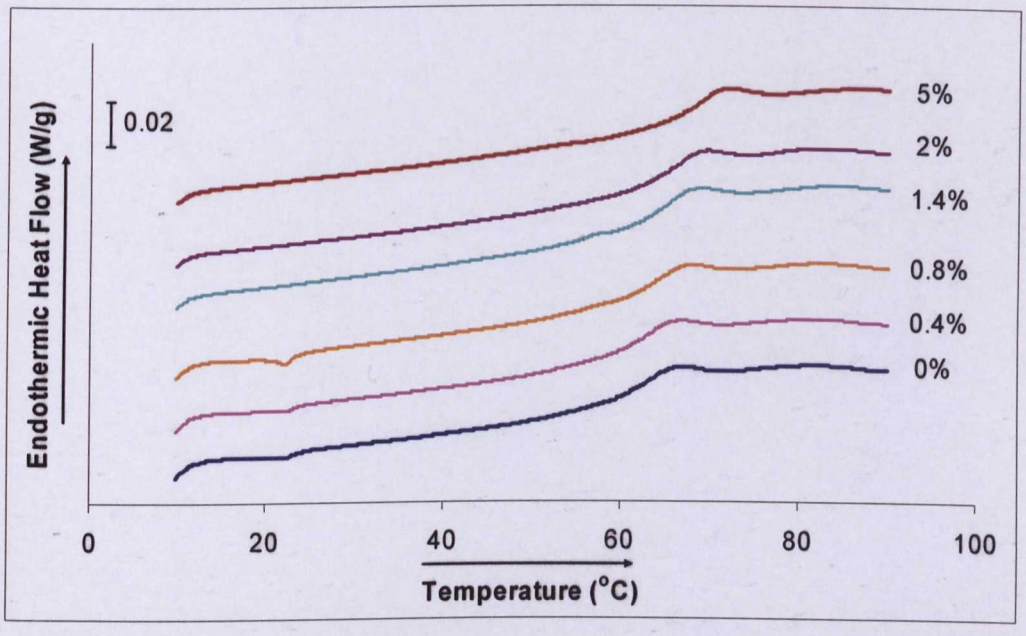



Figure 6-2. The DSC heating thermograms of hard wheat CBP dough at different levels of salt content between 0 and 5% (flour base).

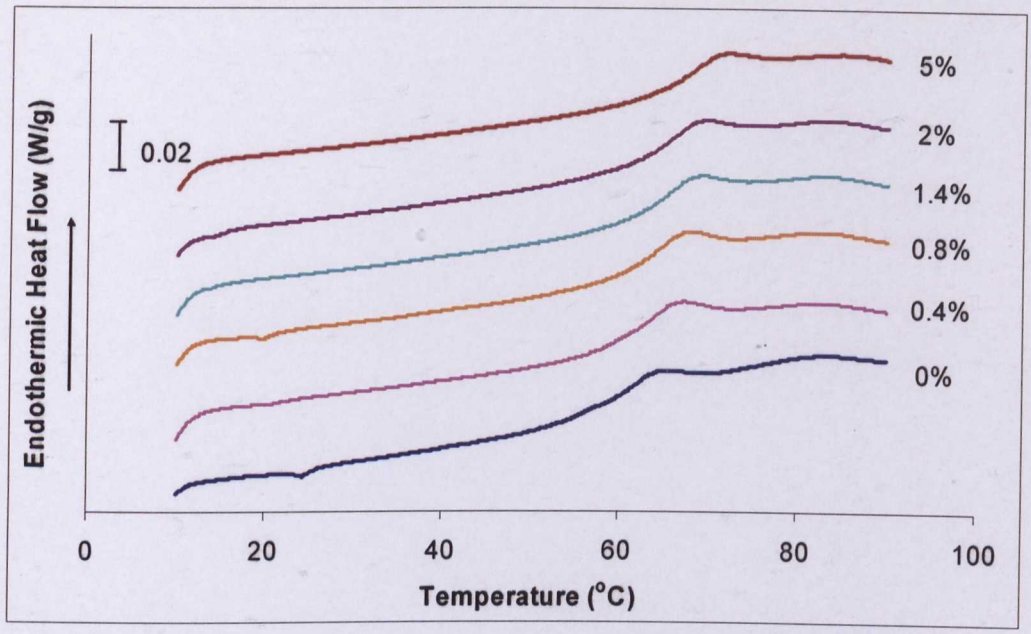



Figure 6-3. The DSC heating thermograms of soft wheat CBP dough at different levels of salt content between 0% to 5% (flour base).

The gelatinisation endotherm occurred at higher temperature for hard wheat dough (64.8°C) compared with soft wheat dough (63.5°C). An explanation of the observation may be found from the consideration of the protein content in the different flour type. Early studies (Bagley et al., 1982; Bernardin et al., 1973; Champenois et al., 1998) had shown that starch and protein compete for the availability of water. Soft wheat flour contains less protein and less water is needed to bind with gluten, thus more "free" water is available in the system to aid starch gelatinisation and this water result in a lower mid-point temperature ($T_{peak}$). Donovan (1979) noted the amount of water has a great effect on the gelatinisation temperature.

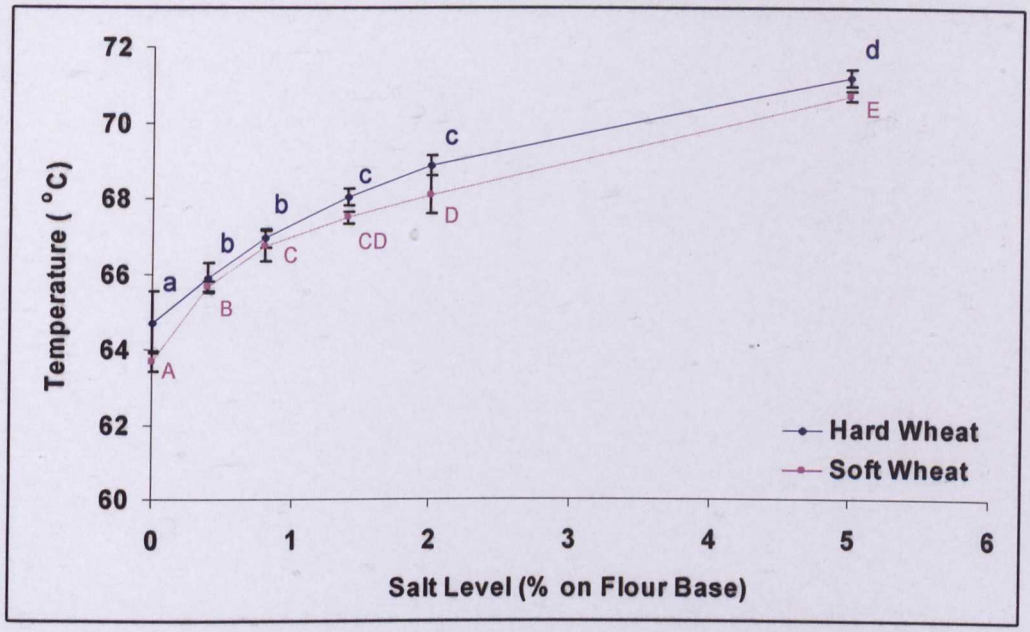



Figure 6-4. Effect of salt on the peak temperature of gelatinisation ($T_{peak}$) of hard wheat and soft wheat CBP dough (values represent the mean $\pm$ 1 standard deviation of five doughs, each replicated three times). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$ within each flour type (a,b,c,d for hard wheats and A,B,C, D, E for soft wheats)

Figure 6-4 shows the gelatinisation temperature ($T_{peak}$) of both flour types with different salt content were all higher than that obtained in the control sample (0% salt). This is in agreement with the results from Chiotelli et al. (2000) on wheat starch. The effect of adding salt to starch has been described as an initial inhibition of gelatinisation up to a certain concentration (~2M) (Chiotelli et al., 2002). Ganz (1965) also associated the presence of sodium chloride with enhancement of 'granule intergrity' whereby greater swelling is experienced before fragmentation occurs (Salvador et al., 2006).

Another possible explanation is that small solutes such as sugar and salt could lower water activity (Labuza, 1975). Sodium chloride may influence the ions present on the water, starch and the interaction between these two components. Competition between starch and sodium chloride for available water, thus, decreases the available water due to moisture binding by sodium chloride and inhibit the granular hydration for the suppression of starch gelatinisation by sodium chloride (Wootton et al., 1980).

**Interestingly, for both wheat flour doughs, the total enthalpy (ΔH) for** gelatinisation process in the presence of salt remained almost constant (Figure 6-5). This is in agreement with the results of Chiotelli et al. (2000) on wheat starch at low salt concentrations.

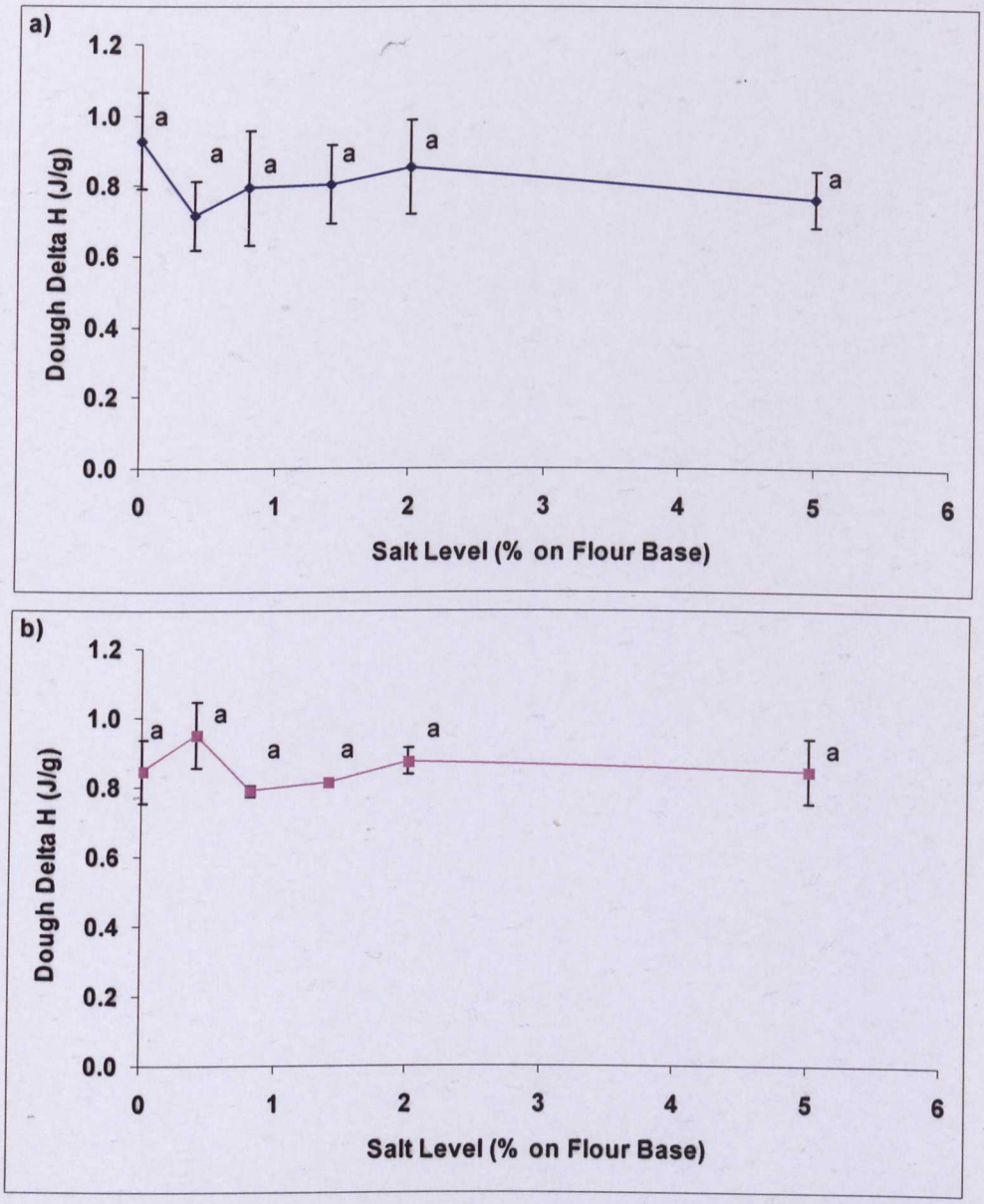



Figure 6-5. Total enthalpy for gelatinisation process of (a) hard wheat and (b) soft wheat CBP dough in the presence of salt levels between 0 to 5% (flour base) (values are the means ± 1 standard deviation from the three doughs. Each mean value was derived from four replicate). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$.

#### 6.2.1.2.2 Freezable water

Some of the water in the dough can be described as "unfreezable" water. This is considered to be "bound" water, and the free water is that which can be frozen at subzero temperatures. Freezable water can be detected by DSC, while unfreezable water can not be detected.

The enthalpy values of ice formation and melting in dough prepared from hard wheat and soft wheat flour are presented in Figure 6-6 and 6-7, respectively. The calculated frozen water detected by DSC was less than the water added to the recipe. In addition, both hard wheat and soft wheat dough samples showed a significant reduction of total enthalpy ($\Delta H_{sample}$) as salt content increased in freezing and melting experiment.

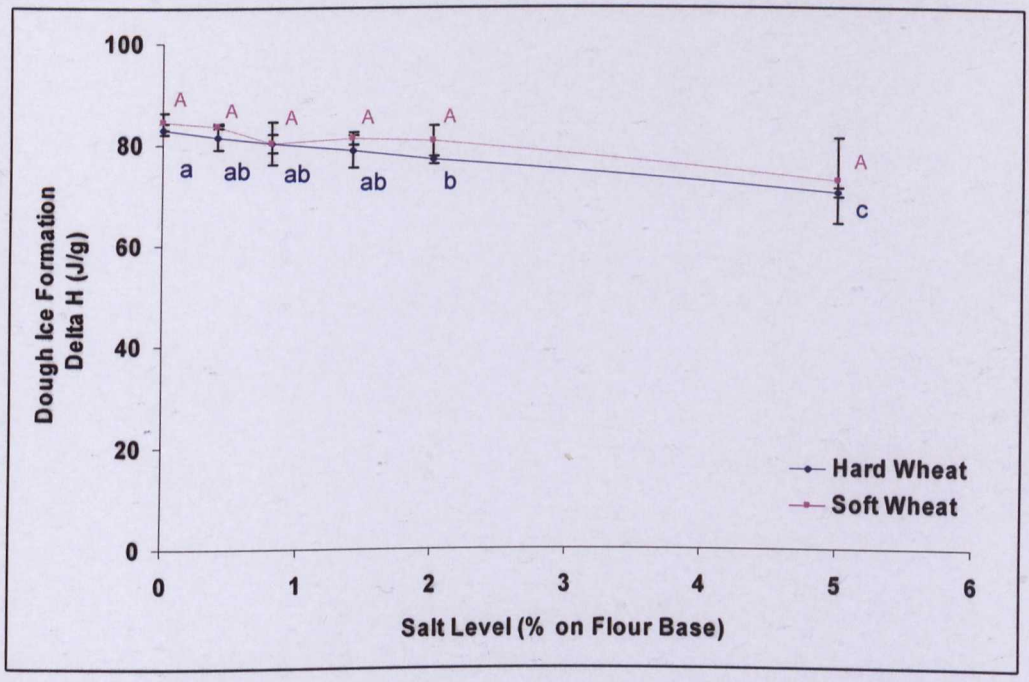



Figure 6-6. Enthalpy values of ice formation on CBP dough with salt content between 0 to 5% (flour base) (values are the means $\pm$ 1 standard deviation from the 5 doughs. Each mean value was derived from three replicate). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$ within each flour type (a,b,c,d for hard wheats and A,B,C, D, E for soft wheats)

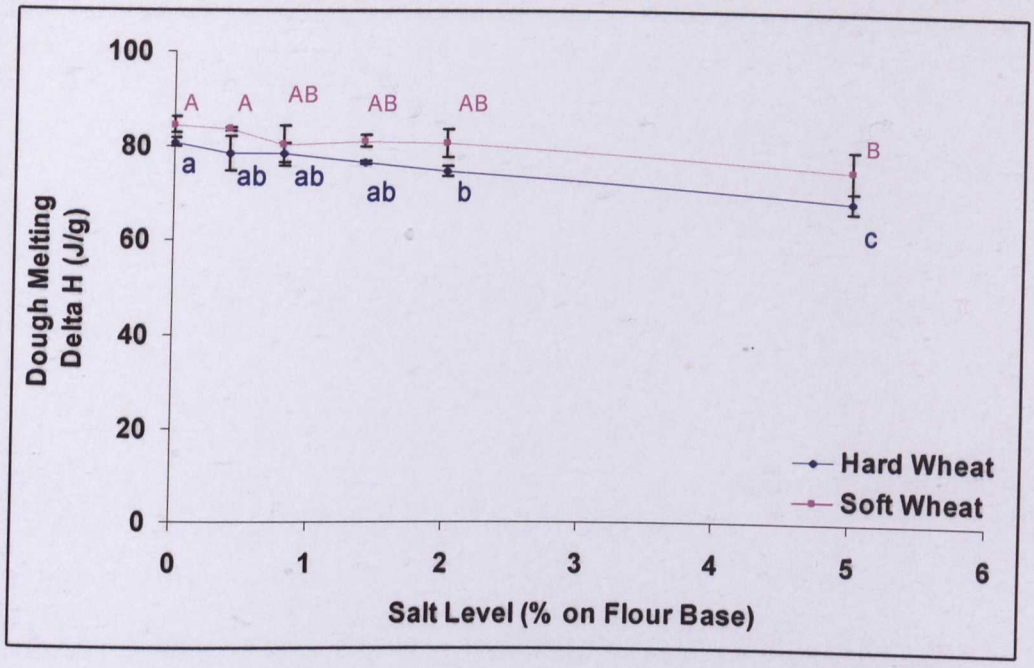



Figure 6-7. Enthalpy values of ice melting on CBP dough with salt content between 0 to 5% (flour base) (values represent the mean $\pm$ 1 standard deviation of five doughs, each replicated three times). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$ within each flour type (a,b,c,d for hard wheats and A,B,C, D, E for soft wheats)

In hard wheat dough samples, there is an expected shift in the onset, mid-peak and end temperature for freezing and melting of water as salt content changes as shown in Table 6-2a. Soft wheat dough also showed a similar trend of changes as salt concentration increased (Table 6-2b). The depression of freezing and melting point would be expected due to colligative properties of the sodium chloride.

Table 6-2. Effect of salt content of (a) hard wheat and (b) soft wheat doughs on various parameters of differential scanning calorimetry on the average melting and freezing of the sample (Values represent the mean ± 1 standard deviation of three doughs, each replicated three times).

a) Hard wheat CBP dough

| Salt Concentration (%) | Endotherm Temperature (°C) | | |
|---|---|---|---|
| | $T_{onset}$ | $T_{peak}$ | $T_{end}$ |
| 0 | -4.61 (±0.45) | -1.42 (±0.22) | 2.23 (±0.42) |
| 0.4 | -5.42 (±0.46) | -2.01 (±0.30) | 1.25 (±0.75) |
| 0.8 | -6.48 (±0.7) | -2.52 (±0.45) | 0.82 (±0.81) |
| 1.4 | -6.76 (±0.21) | -2.66 (±0.15) | 0.81 (±0.45) |
| 2 | -7.55 (±0.18) | -3.12 (±0.15) | 0.24 (±0.24) |
| 5 | -11.53 (±0.36) | -5.76 (±0.08) | -3.17 (±0.61) |

b) Soft wheat CBP dough

| Salt Concentration (%) | Endotherm Temperature (°C) | | |
|---|---|---|---|
| | $T_{onset}$ | $T_{peak}$ | $T_{end}$ |
| 0 | -4.23 (±0.21) | -1.03 (±0.18) | 2.98 (±0.45) |
| 0.4 | -5.11 (±0.18) | -1.43 (±0.20) | 2.76 (±0.39) |
| 0.8 | -5.73 (±0.39) | -1.88 (±0.25) | 1.83 (±0.30) |
| 1.4 | -6.50 (±0.16) | -2.19 (±0.25) | 1.41 (±0.45) |
| 2 | -7.25 (±0.17) | -2.71 (±0.13) | 0.83 (±0.32) |
| 5 | -11.82 (±0.83) | -5.47 (±0.57) | -1.29 (±1.84) |

The amount of frozen water in the dough system was calculated knowing that the enthalpy of fusion of ice was 335J/g (Petrenko, 1999) and the measured enthalpy of the sample ($\Delta H_{sample}$). Interestingly, the amount of detectable frozen water decreased as salt content increased and the flour quality seemed to have only a small effect on the amount of frozen water (Figure 6-8). This suggested that the amount of frozen water did not show the extra dough liquor yield as seen by ultracentrifugation as salt content increased (from section 6.2.1.1).

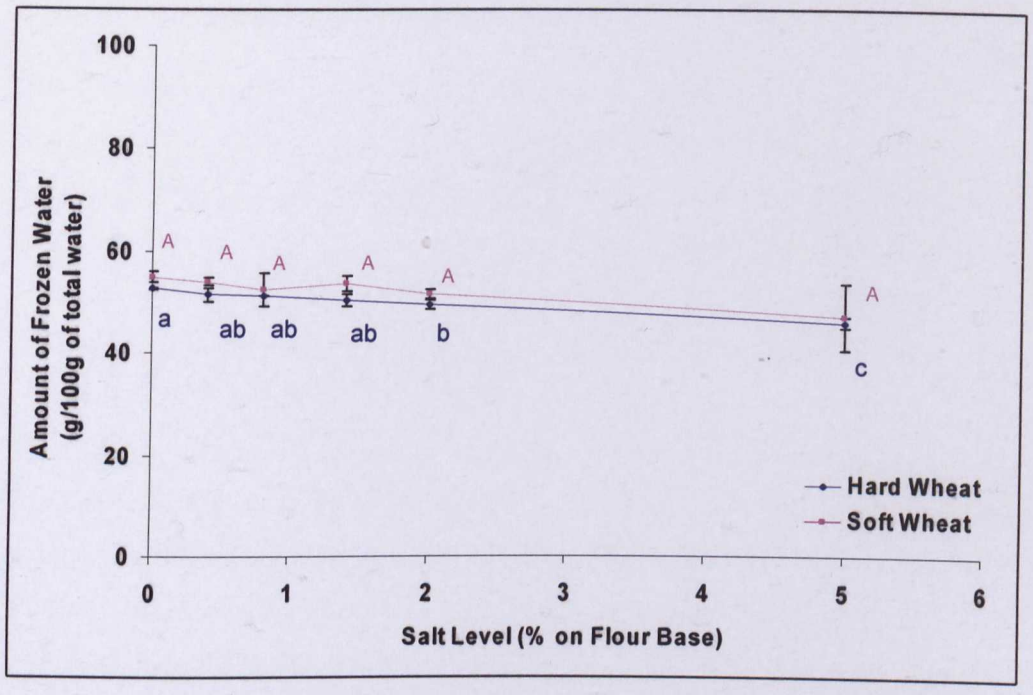



Figure 6-8. Amount of frozen water in the CBP dough with salt level between 0 to 5% (flour basis) (Values represent the mean $\pm$ 1 standard deviation of five doughs, each replicated three times). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$ within each flour type (a,b,c,d for hard wheats and A,B,C, D, E for soft wheats)

Use of DSC as a measurement of frozen water can be subjected to some error. In general, for calculation of the frozen water, it has been assumed that the enthalpy of ice melting is constant, though it changes with temperature (Petrenko, 1999). This error is negligible, if a constant enthalpy of ice is also assumed for calibration of the instrument, provided ice melting/freezing occurs in the same temperature range. Furthermore, neither the enthalpy of the dissolution of the freshly melted water into the concentrated solution (Schenz et al., 1991) nor the contribution of ingredients, were taken into account. The small sample size for DSC measurements (~ 20mg) may need to be considered, in relation to the pronounced inhomogeneity of the given dough, in particular regarding water distribution. In addition, the ice crystallisation is a kinetically controlled process, the observed freezing is likely to be specific to the experimental conditions used. Therefore the amount of frozen ice as measured by this method may not truly reflect the "free" water in the dough system. However, it does appear that added salt does not increase the fluid available for freezing.

### 6.2.1.3 $^1$H Nuclear magnetic resonance

The low field proton nuclear magnetic resonance ($^1$H NMR) was used for dough to understand the changes in the molecular dynamics and behaviour of water, which may have a pronounced influence on the quality and stability of the final bread. $^1$H NMR was chosen for the experiment because the analysis time for one sample is relatively quick (< 5 min), which is important when studying dough as the rate of changes of water distribution and hydration of gluten-starch, appears to be very fast.

Generally, water gives a decay of ~ 2 sec and it is known that FID signal is limited for measuring higher mobility. In addition, the FID signal contains not only the spin-spin relaxation but also the lost signal due to inhomogeneous local magnetic field (Hore, 1995). So higher time constant values, such as water mobility, measured using FID does not reflect the true spin-spin relaxation times, and caution must be taken when comparing and using FID data. Thus, high spin-spin relaxation times ($T_2$) can be better determined by using a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. In the following section, only results from the CPMG sequence will be discussed.

#### 6.2.1.3.1 The effect of salt concentration

Previous sections have shown that salt has an effect on the liquid yield by centrifuging the dough at very high centrifugal fields, however, the partition of water in the dough system was not understood. CPMG sequence was used to help elucidate the molecular dynamics and behaviour of the water in the dough and the effect of salt concentration.

Figure 6-9 indicates that the CPMG decay signals of the dough samples showed no difference between salt levels from 0 to 5% (on flour base). The curves indicated the existence of two groups of water molecules on the NMR time scale. It can be resolved into 2 components characterised by the short and long relaxation times, $T_{2(1)}$ and $T_{2(2)}$. The less mobile region ($T_{2(1)}$) may correspond to protons in solid-like components, such as starch, proteins and water molecules tightly associated with those solids (Kim and Cornillon, 2001; Ruan et al., 1999). The second population ($T_{2(2)}$) may correspond to more mobile protons or free water in the dough system.

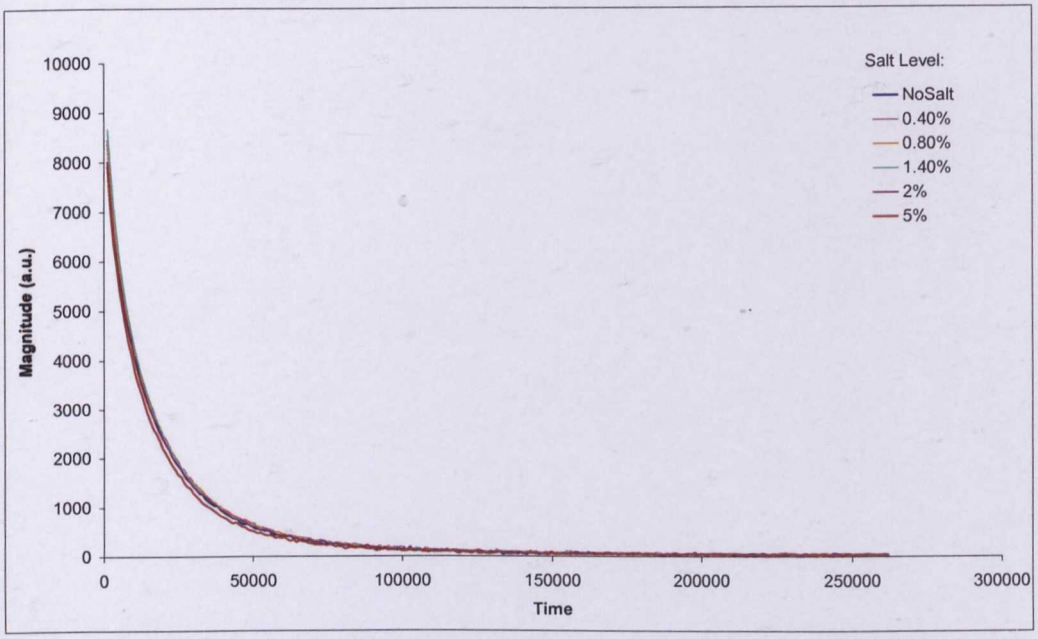



Figure 6-9. CPMG decay signals of dough samples with salt concentrations between 0 and 5% (n = 5).

The relaxation curves obtained from a CPMG sequence (Figure 6-9) were fitted as a 2-exponential component with Winfit software from Resonance Instruments (Oxford, UK). As all the time constants were above 1 msec, all the obtained NMR signals were considered to come from the liquid-like protons. As each component reflected the mobility of water protons, the different time constants represented the different degree of molecular interaction between water molecules and other macromolecules. The $T_2$ relaxation time for hard wheat CBP dough did not show a significant difference between salt content of zero and 5%. If there is any trend in the $T_2$ values it is a slight decrease in the mobile component (slow decay) and would imply there is a decrease of water mobility in the system as salt level increases.

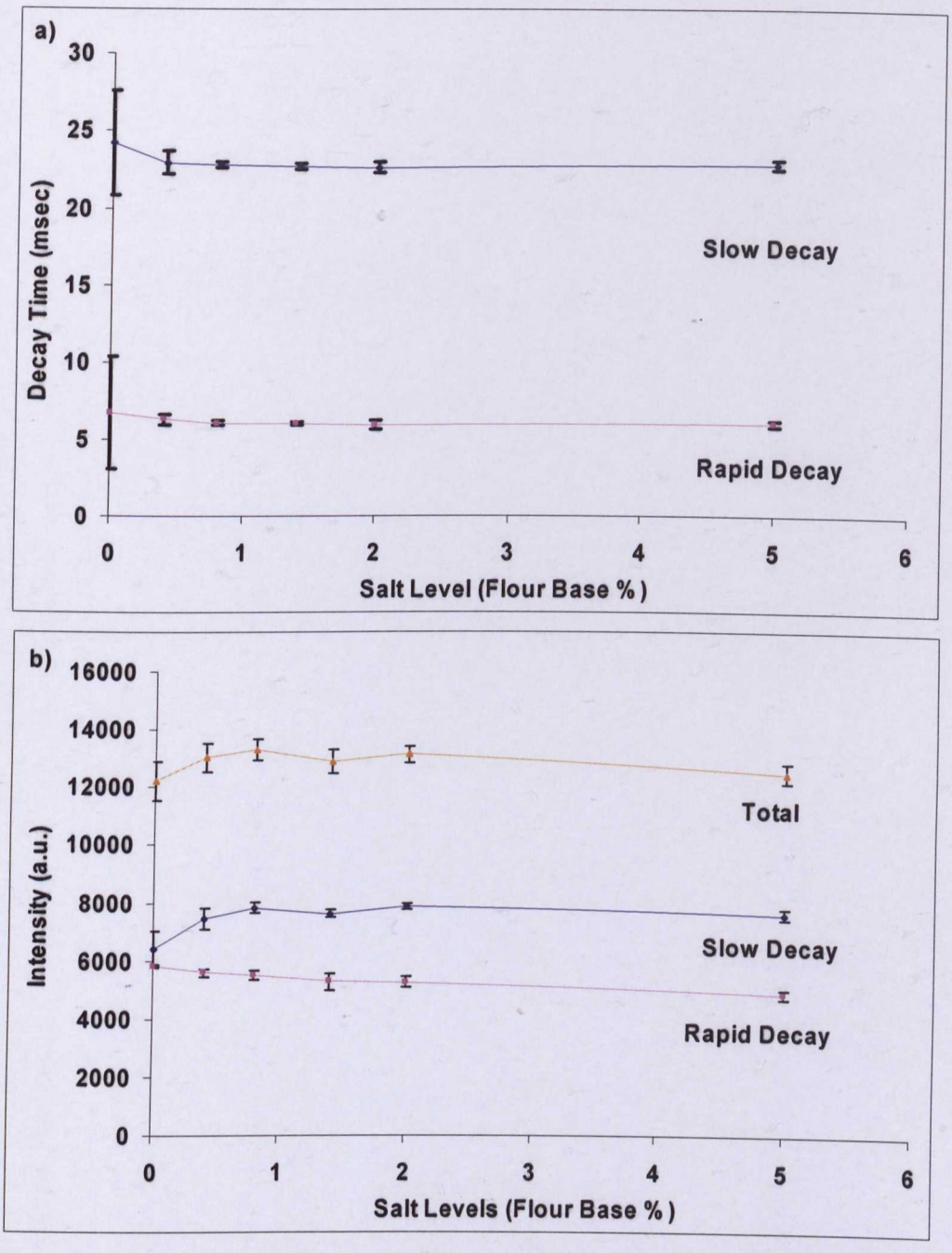



Figure 6-10. $T_2$ relaxation time of (a) decay time and (b) intensity obtained from 2 exponential components at salt level of 0 to 5% (flour base) from hard wheat CBP dough (Values represent the mean $\pm$ 1 standard deviation of five doughs).

In order to understand the distribution of the protons in the dough system, continuous distribution of exponentials with WinDxp software was used. Figure 6-11 shows the $T_2$ distribution of hard wheat CBP dough at different salt levels. Although there is no significant difference in the experimental range of salt levels on the peak relaxation

from population 2 (pop.2), a trend showed an increase of salt prolonged the relaxation time as shown in Figure 6-11b. This may in accordance with the centrifugation results as salt increased so did the dough liquor yield. However, the changes in relaxation time on population 2 are lesser than the results observed from ultracentrifugation, which is thought to be the mobile water in the system.

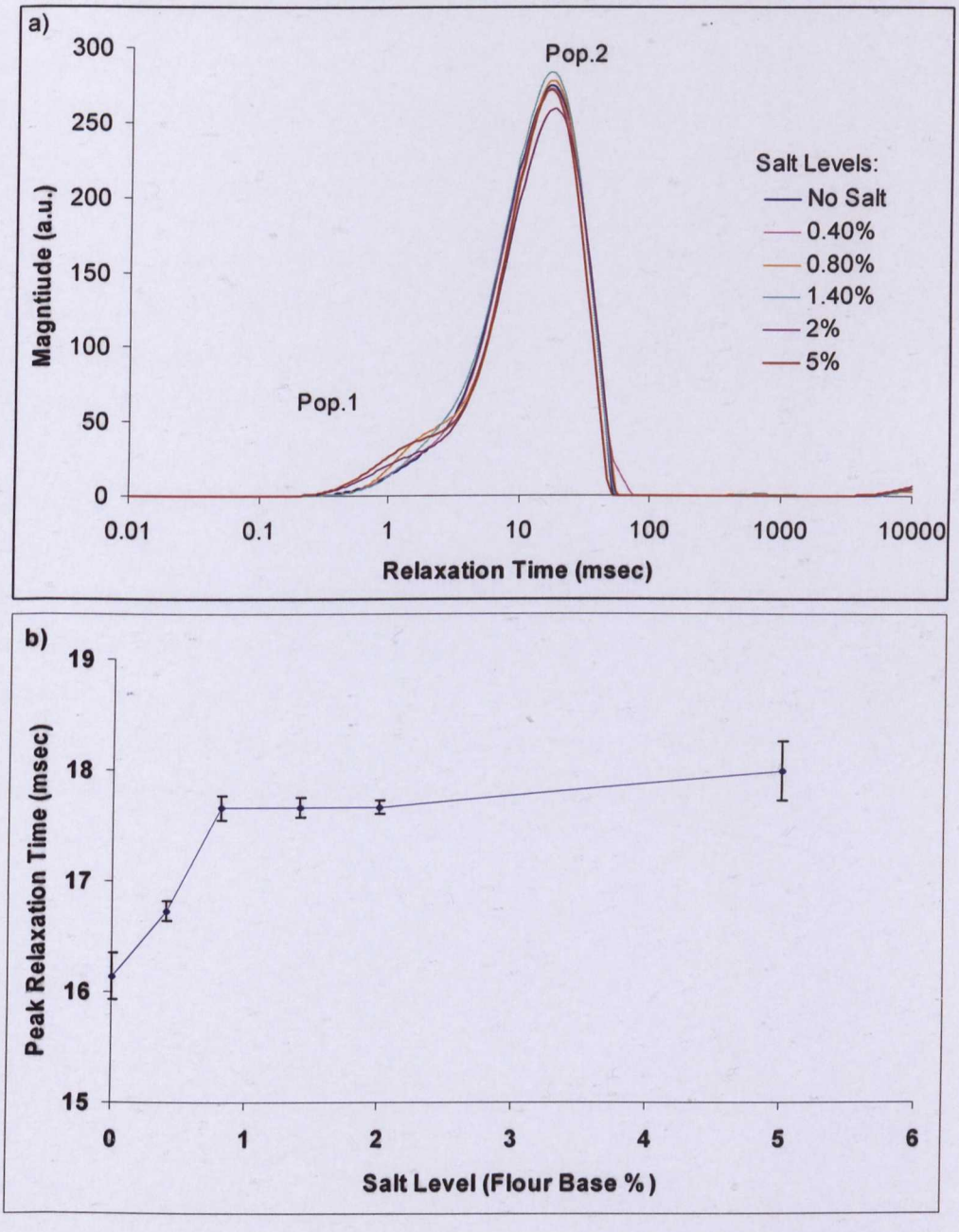



Figure 6-11. (a) $T_2$ continuous distribution obtained from $^1$H NMR CPMG signals for hard wheat CBP dough at different salt levels; and (b) shows the peak relaxation time of population 2 from (a) (values represent the mean $\pm$ 1 standard deviation of five doughs, each replicated three times).

In wheat flour, at 38% w.b. of water, Kim and Cornillon (2001) found three water population: $T_{2(1)}$ = 1.5 msec, $T_{2(2)}$ = 6-7 msec and $T_{2(3)}$ = 40-60 msec. Similarly, Engelsen et al. (2001) have also found three water populations in bread (45.3% w.b. of water). These populations have been attributed to water in association with gluten (population 1) and starch (population 2). The third population may be due to a diffusive exchange of water between starch and protein fractions. Figure 6-11 shows two populations rather than three as other authors have mentioned. This may be due to the difference between the sample dough ingredients. For example, the improver has a function of holding water within dough system which may alter the distribution of water.

CBP dough prepared with soft wheat flour was also tested. Similar results were observed as for the hard wheat flour and a slight decrease (not significant, $p \geq 0.05$) in the mobile component (slow decay) was noted by changing the salt concentration in the dough system (Figure 6-12).

Studies (Li et al., 1996; Umbach et al., 1992) have shown that the more mobile population ($T_{2(2)}$) can be affected by gluten content. Li et al. (1996) have also found that wheat gluten has a stronger affinity for water molecules since water in gluten remained immobile, while water in starch was more mobile. Thus, the water self-diffusion, measured by pulsed gradient spin-echo NMR, decreased when the amount of gluten added to starch-water mixture increased (Umbach et al., 1992). This indicated that gluten binds or entraps water more effectively than starch.

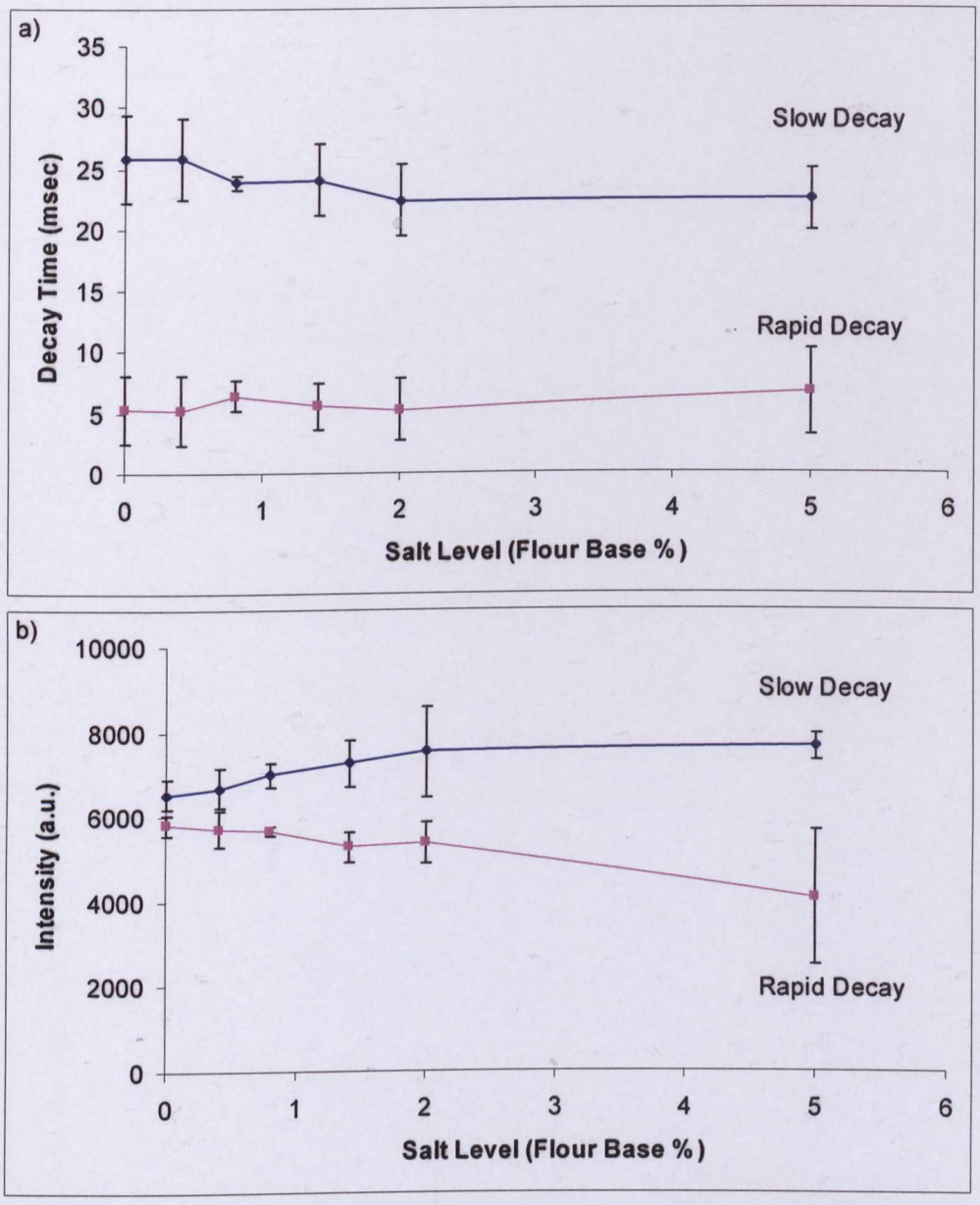



Figure 6-12. The $T_2$ relaxation time of (a) decay time and (b) intensity obtained from 2 exponential components at salt level of 0 to 5% (flour base) from soft wheat CBP dough (values represent the mean $\pm$ 1 standard deviation of five doughs, each replicated three times).

However, there was a slight increase in peak value of relaxation time in Pop.2 in the continuous distribution curves compared with hard and soft wheat dough samples (Figure 6-13). This maybe due to the differences in protein content or starch damage rather than the effect of salt. Soft wheat flour generally contains a lessen amount of protein and damaged starch. These two factors affect the water absorption rate of the flour. In soft wheat dough samples, less water is needed to hydrate the protein and

starch, more "free" water is therefore in the dough system. These results would be in agreement with the observed results from ultracentrifugation (Figure 6-1).

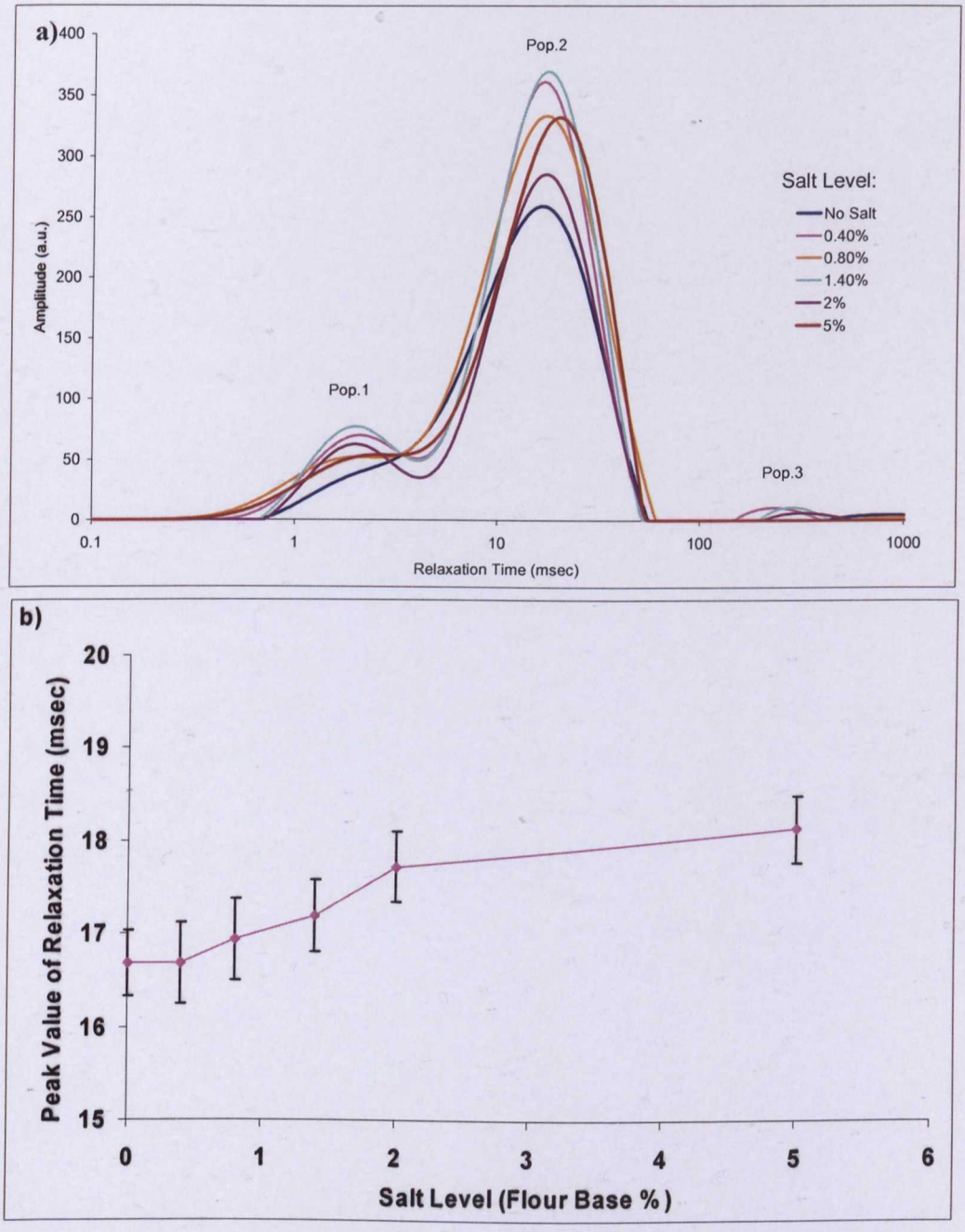



Figure 6-13. The continuous distribution curves (a) and the relaxation time of the peak value (Pop.2) (b) from soft wheat CBP dough at different salt levels (values represent the mean ± 1 standard deviation of five doughs, each replicated three times).

#### 6.2.1.3.2 The effect of resting time

NMR was also used to investigate the effects of time on water mobility in the doughs. Dough rested on the process line at ambient temperature for over 30 minutes is not used for baking, since it is not able to bake high quality bread. It is recognised that the dough system changes continuously and could influence the rheology and then the machinability of dough. This continuous change is thought to be related to the redistribution of water, continuing hydration of flour components and enzymatic reactions (Petrofsky et al., 1995). $^{1}$H NMR was used to follow the changes of water in the dough system over time. For the purpose of this study, dough samples were immediately tested after mixing and a 2-hour experimental time was chosen in order to follow the mobility of water in the dough system. The transverse relaxation time ($T_2$) was determined at 29°C using Carr-Purcell-Meiboom-Gill (CPMG) sequence.

The evolution of proton decay time and intensity of a 2% salt d dough as a function of resting time are shown in Figure 6-14. The proton intensities are relative measures of water in to the lesser mobile fraction and greater mobile states is shown in Figure 6-14b. The amount of water in the rapid decay component increased over the resting time, while water in the slow decay component decreased during storage. A decrease of water in slow decay component results in an increase of water in the rapid decay component, indicating a shift of water molecules from mobile to less mobile fraction. The redistribution of water in the dough system may influence the handling properties of dough (i.e. sticky dough).

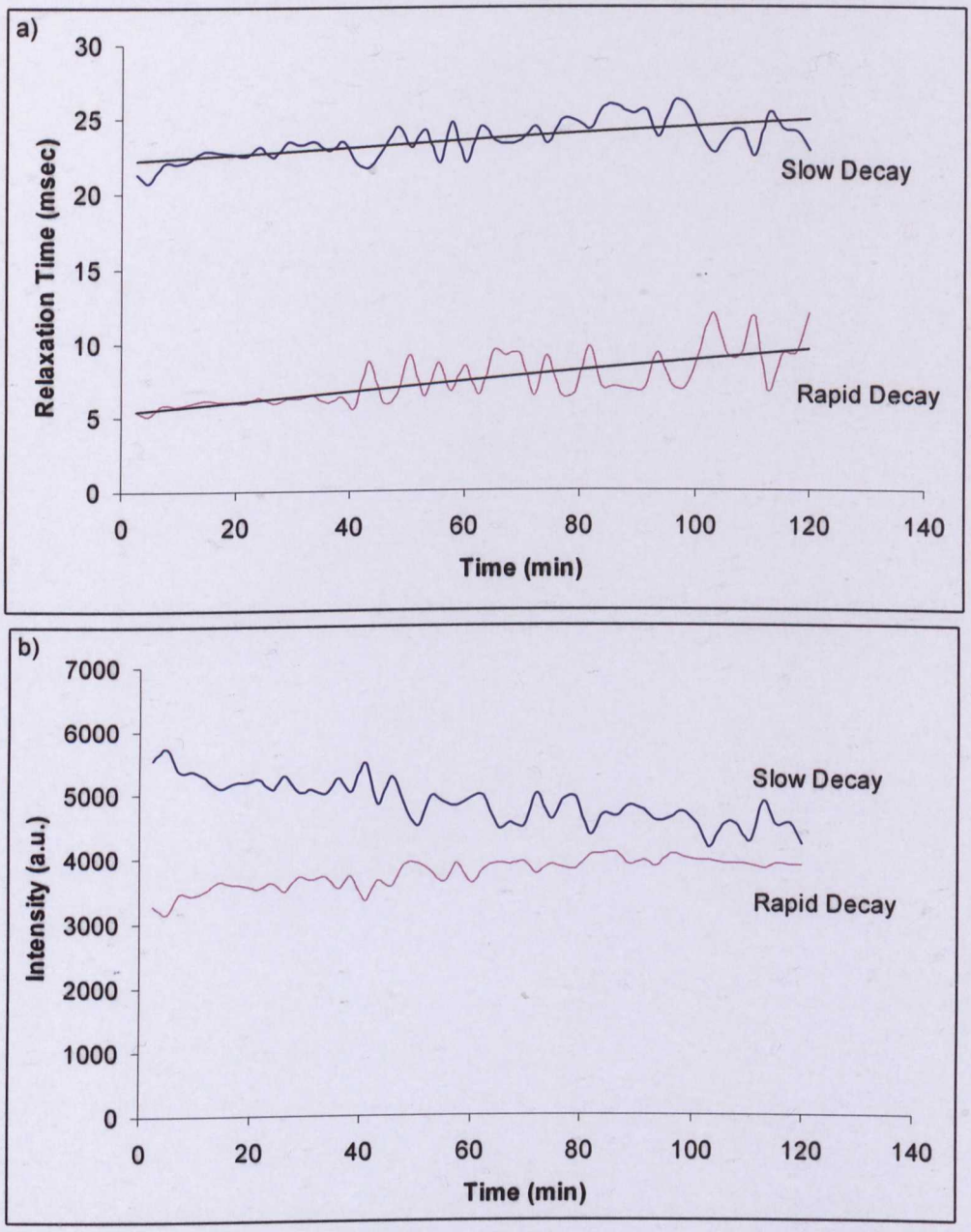



Figure 6-14. Evolution in the CPMG transverse time constants ($T_2$) of (a) decay time and (b) the intensity during resting of the dough with 2% salt level at 29°C (Values represent the mean ± 1 standard deviation of five doughs, each replicated three times).

Figure 6-15 shows the effect of resting time (4 hours) on the evolution in CPMG transverse time constants ($T_2$) on CBP dough sample with no added salt. It clearly shows systematic changes in proton mobility in the dough system, however on a minor scale. Thus, increasing the resting time showed a small but measurable

increase in water mobility. One possibility of the observed changes maybe due to the rearrangement of protein leading to a decreasing amount of "bound" water. Complementary with this, the amount of mobile water therefore increases, thus the fluidity of the dough would increases.

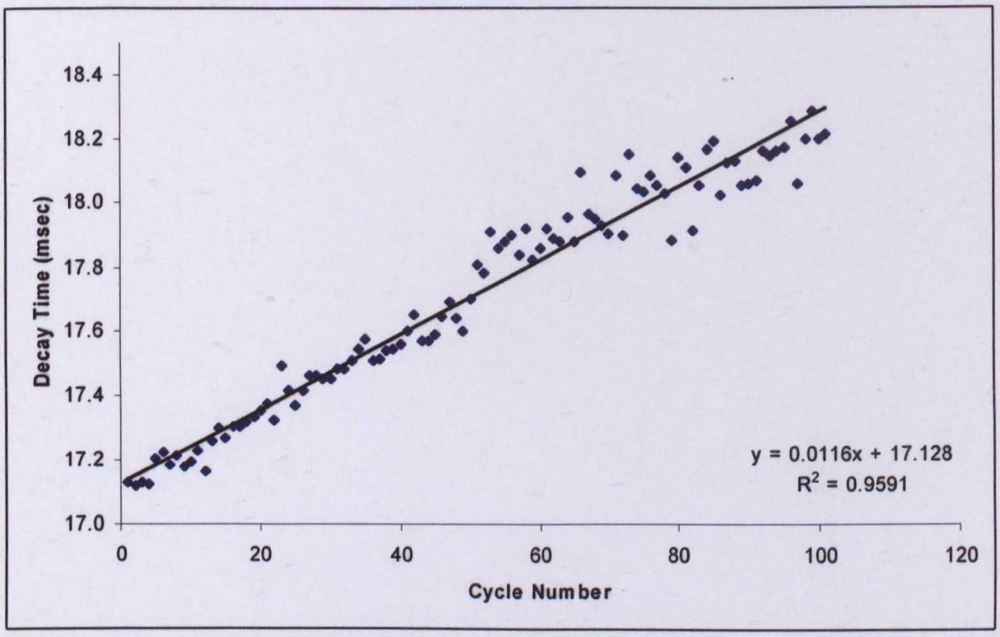



Figure 6-15. The 1-expontential relaxation time ($T_2$) obtained from CPMG sequence with time on hard wheat CBP dough (Values represent the mean $\pm$ 1 standard deviation of five doughs).

Figure 6-16 shows the effect of resting time on continuous distribution of spin-spin relaxation time of hard wheat CBP dough obtained from a CPMG experiment. At a resting time of 2 hours, two or three peaks appear on each distribution, which indicates they have distinctively different mobilities. As mentioned before, population 1 represents the proton signals from solid or water molecules closely associated with the solids.

There is a slight difference between the relaxation time of population 2, which is evidence for a minor water redistribution in the dough system during the 2-hour experimental time scale. As resting time is longer, the more mobile fraction becomes broader whereas the less mobile fraction did not change with resting time. The broadening of the more mobile fraction suggests that there is an increase of in the chemical and physical states of the water molecules in the dough sample. No change

in the less mobile fraction might indicate that these water molecules remain closely associated with the solids in dough.

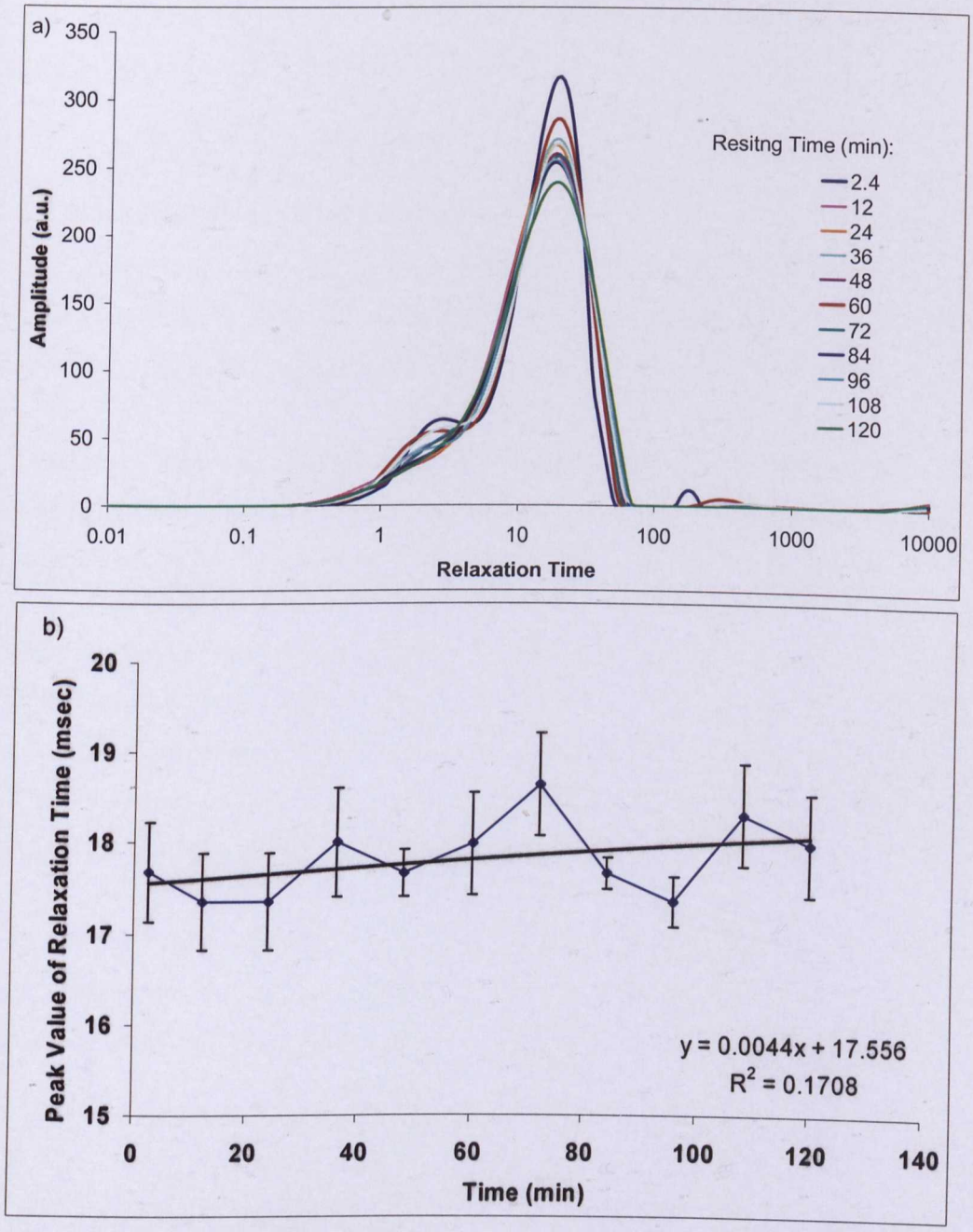



Figure 6-16. Continuous distribution of $T_2$ and peak values of relaxation time determined by the CPMG experiment with resting time (values represent the mean $\pm$ 1 standard deviation of five doughs).

The $T_2$ relaxation values obtained from resting of dough samples were not statistically significant different. However, the proton relaxation time increased with resting time is an indication of the increased mobility of water protons and it is more likely due to redistribution of water within the dough system. Therefore, prolonging the resting time influenced the more mobile fraction of the dough samples in both peak shape and the range of relaxation times.

#### 6.2.1.3.3 The effect of temperature

The properties of the dough can be influenced by many factors. Temperature has been one of the critical factors affecting the properties of the dough, which is commonly considered as a problem in the bakery during the summer months.

Changes in the dough properties more commonly observed as "sticky dough". Thus the $^1H$ NMR experiment was used to follow the changes of the protons in the dough system at different temperatures.

CBP dough samples prepared with hard wheat flour was mixed according to the methods given chapter 3.3.1.2. Mixed doughs were put into the water bath at a pre-set temperature until it reached to the required temperature (usually within 10 min). The temperature of the NMR machines was also set to the same temperature and spin-spin relaxation times ($T_2$) recorded.

Figure 6-17 shows the effect of temperature on the signal curves from a CPMG experiment. As temperature increases, surprisingly, a sequential shift to the right in the relaxation curves occurs that implies the system is becoming more solid-like.

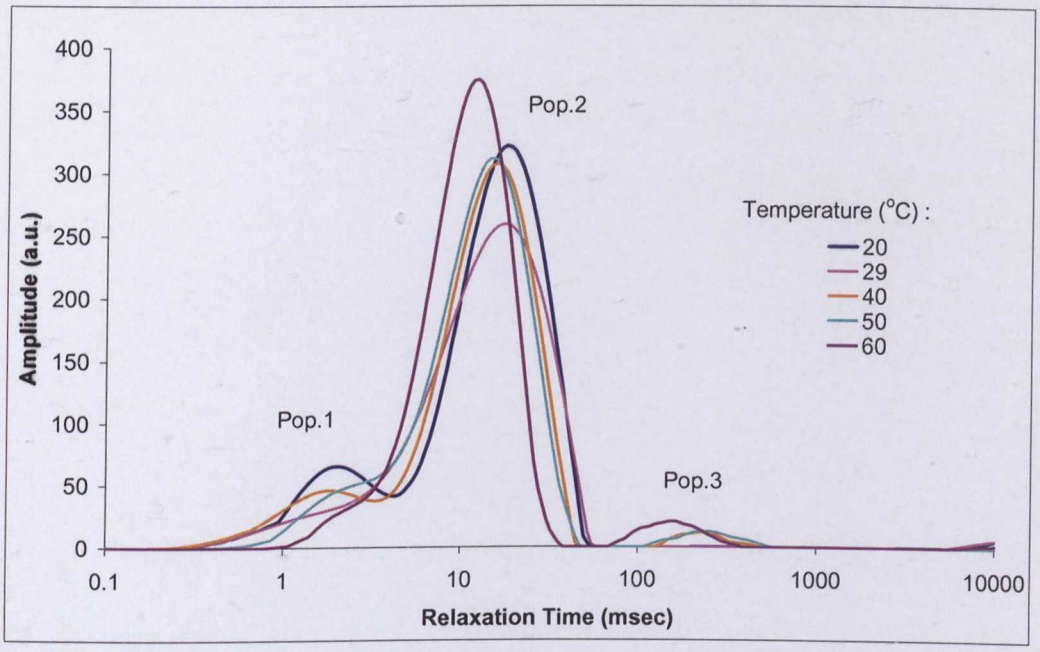



Figure 6-17. CBP dough samples relaxation signals obtained from CPMG pulse sequence experiment at different experimental temperature (n = 5).

Several factors may affect the water mobility and distribution in the dough system. According to Belton et al. (1998) with starch-water suspension, the mechanisms of this phenomenon may be related to the occurrence of an exchange phenomenon between the "weakly bound" and "bound" water. They observed the $T_2$ peak of "weakly bound" water and "bound" water decreases as the temperature increases. This indicated an exchange phenomenon. The effect of an increase in the exchange rate is to average the two $T_2$ relaxation time to give a signal peak. The $T_2$ of the "weakly bound" water peak decreases, although the single effect of temperature without exchange phenomenon would have been to increase it; the behaviour of the "weakly bound" water peak is thus compatible with an exchange phenomenon. Other explanation may be due to the granule swelling as temperature increased. However, population 3 did not show any predictable trend.

Figure 6-18 shows the variation of the spin-spin relaxation times ($T_2$) on dough samples at different temperatures ranging from 20 to 60°C. Two different relaxing components were successively isolated which were characterised as rapid decay and slow decay components. The time constant is an indication of the mobility of the spins and the amplitude of the signal is proportional to the amount of protons present in the sample. As hydration of different components proceeded, more and more

protons belonging to the liquid-like protons (slow decay component) became migrated to the solid-like phase. Thus the mobility of the liquid-like proton decreased due to increasing interactions with macromolecules (Figure 6-18b). However, as shown in the amplitude of the signals, the mobile component being the majority. Along the whole temperature range, the amplitude of each component did not change significant (Figure 6-18a).

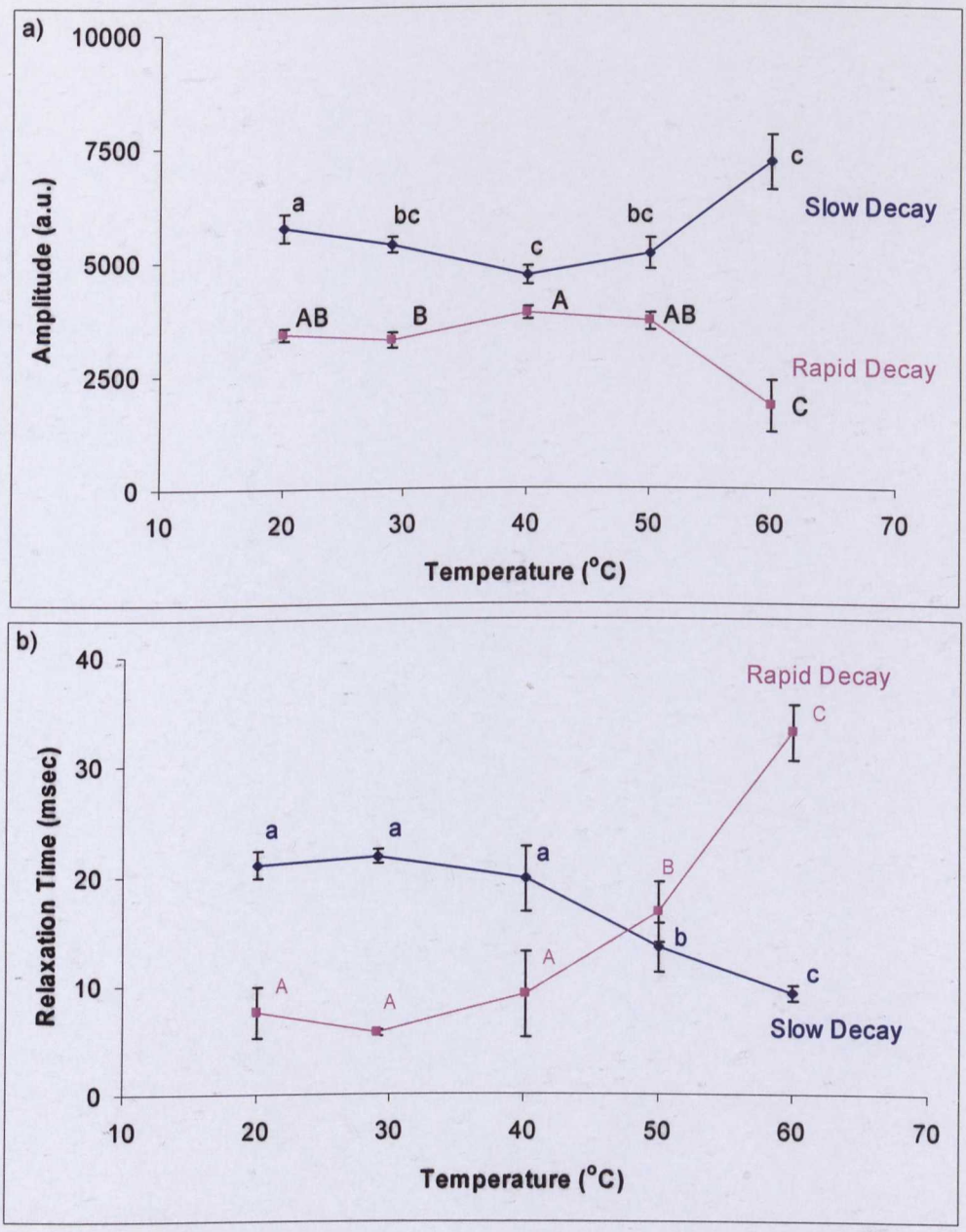



Figure 6-18. Evolution of the NMR (a) signal amplitude and (b) time constant of two components in spin-spin relaxation curves ($T_2$) of hard wheat dough samples (values represent the mean $\pm$ 1 standard deviation of five doughs) (a,b,c,d for slow decay and A,B,C, D, E for rapid decay).

The continuous distribution of spin-spin relaxation time ($T_2$) obtained from CPMG experiments on hard wheat CBP dough (2% salt) with varying temperature is shown in Figure 6-19. Three different relaxation components were identified, which were relatively to low mobility (Pop.1), intermediate mobility (Pop.2) and high mobility (Pop.3) components. Since the shortest time constant was above 1 msec, all the obtained NMR signals are considered to come from liquid-like protons. Assuming that each component reflected the mobility of water protons, the different time constants represented to different degree of molecular interaction of water molecules with other macromolecules. Studies have shown that the more mobile population (Pop.2) is very sensitive to temperature variations (Kim and Cornillon, 2001; Ruan et al., 1999). As temperature increases, the peak value of Pop.2 decreases from 17 msec to 12 msec (Figure 6-20). However, the less mobile fraction (Pop.1) disappeared when temperature increased, this might indicate a redistribution of water within the dough and the water molecules closely associated with solids in the dough system were much more mobile at the higher temperatures. However, there was no observed trend for pop.3.

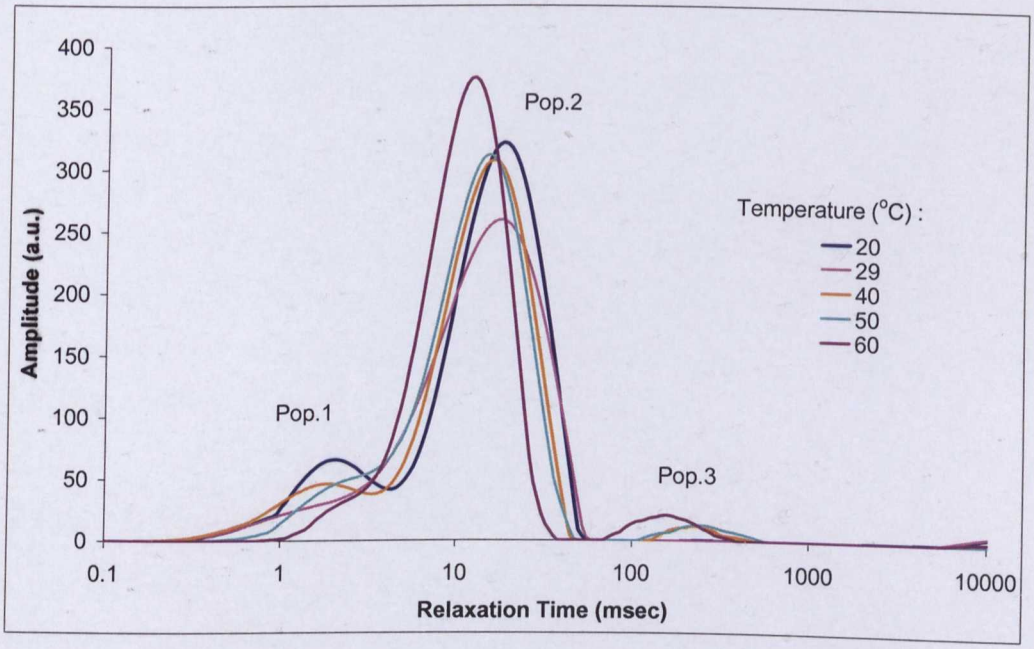



Figure 6-19. Continuous distribution obtained from T2 relaxation data of hard wheat CBP dough (2% salt) at different temperature (n = 5).

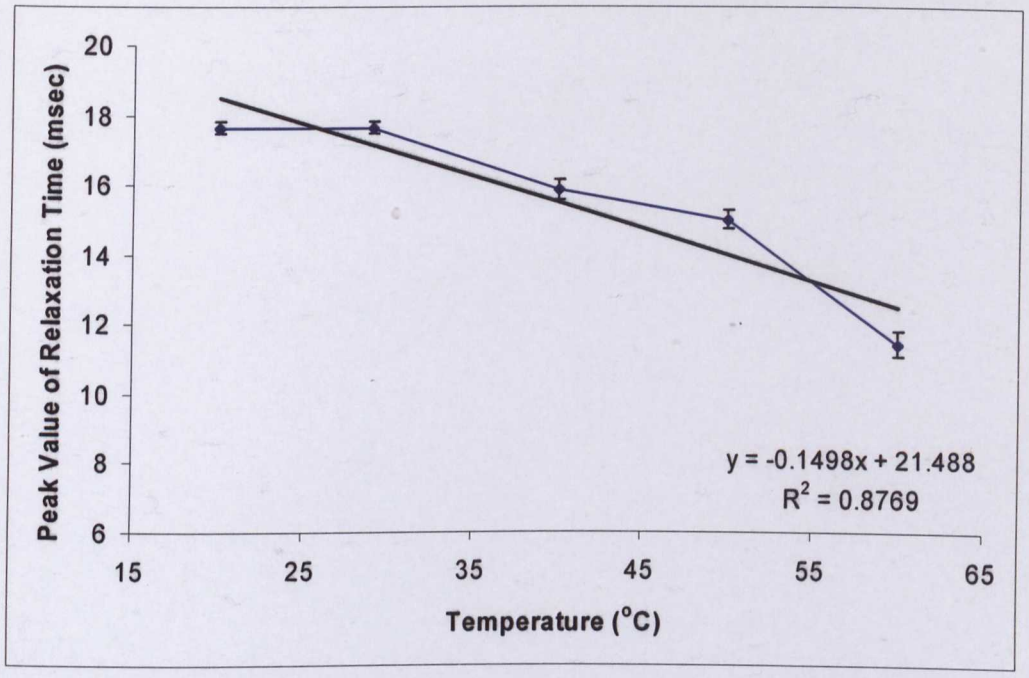



Figure 6-20. The effect of temperature on the $T_2$ relaxation time for the population (Pop.2) in hard wheat CBP dough sample at 2% salt (on flour base) (values represent the mean $\pm$ 1 standard deviation of five doughs).

### 6.2.2 Rheology Properties

The rheological properties of the dough play an important role in the bread making process. The viscoelastic properties of gluten are required for the stability of the foam structure of bread dough and an optimum level of gluten elasticity is required to maintain the gas-cell network. If the level of elasticity is too low, the bubble walls will rupture under the pressure of expansion towards the end of the proving stage and the early stages of baking, and the gas will escape. If the level of elasticity is too high, the gas cells will not be able to expand sufficiently, resulting in a loaf with poor height and volume.

#### 6.2.2.1 Kieffer Rig

Large deformation testing, using the Kieffer extensibility rig is a common method of assessing dough and gluten extensibility. Parameters including extensibility (distance) and resistance to extension (force) at the time of the dough rupture can be assessed. This data can be related to the dough handling and gas bubble stability properties.

#### 6.2.2.1.1 Method Development from flour-water system

During the early stages of method development for dough extensibility, the effect of testing speed on dough was tested. Flour-water dough with 1% salt was prepared and assessments were performed at various testing speeds as shown in Figure 6-21.

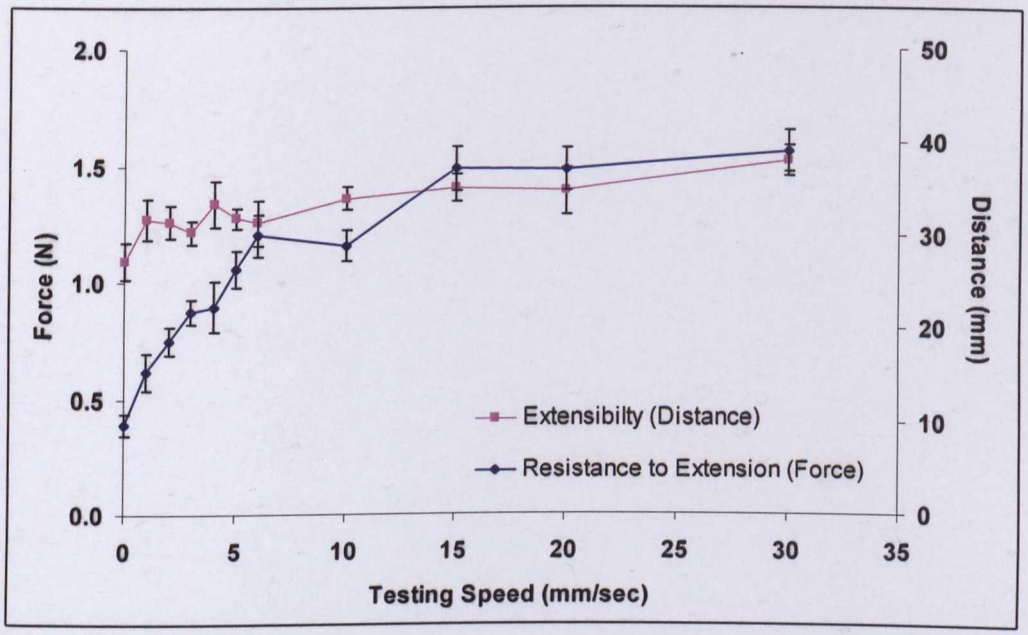



Figure 6-21. Flour-water dough (40% water content) with 1% salt at various tension speeds (0.01 to 30 mm/sec) with Texture Analyser (Values represent the mean ± 1 standard deviation of five doughs, each replicated fifteen times).

Lower testing speeds are used to estimate the stress applied to the dough at the proving and baking stage, which may be important for gas retention during fermentation and oven rise (Cauvin and Young, 1998; Anderssen et al. 2004). On the other hand, higher testing speed can be used to indicate how well the dough would respond to sudden shock during dough transportation.

#### 6.2.2.1.2 Commercial Bread Dough

Optimum dough extensibility is needed for proper dough handling and baking performance. Generally, "good" dough would exhibit value of around 0.588N for resistance to extension. Figure 6-22 shows the resistance to extension (force) obtained from extensibility testing of hard wheat CBP dough at different salt

contents at the speed of 2 mm/sec. When the salt content is increased in the dough, the force (i.e. energy) needed to rupture the samples increased, as anticipated.

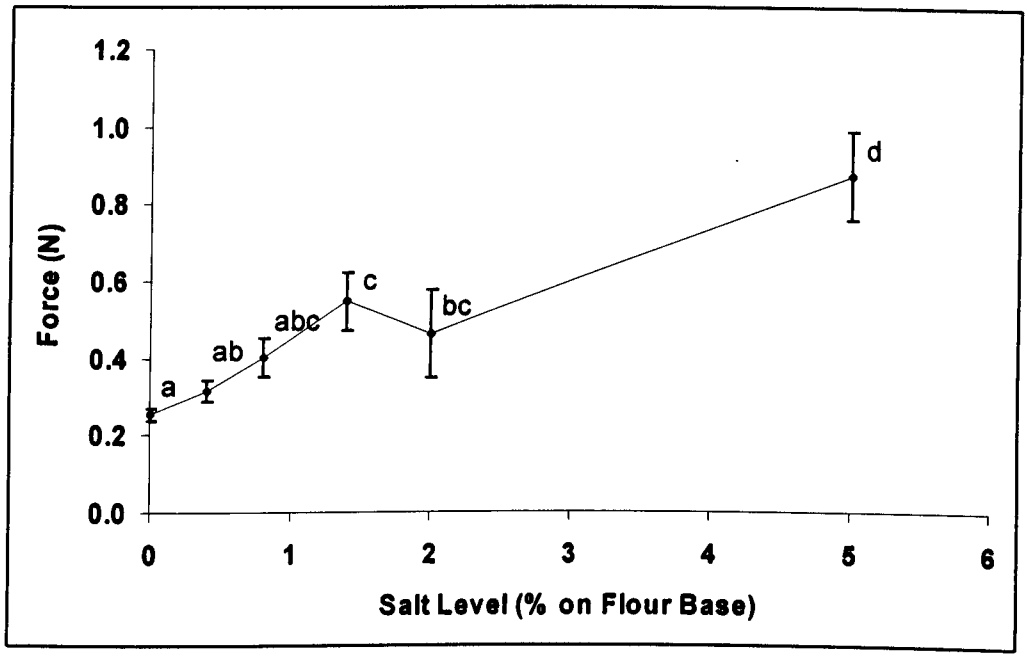



Figure 6-22. Effect of CBP dough (hard wheat flour) with different salt content on resistance to extension (force) using the Kieffer dough extension test (values represent the mean $\pm$ 1 standard deviation of five doughs, each replicated fifteen times). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$.

Several studies have shown salt has a modifying effect on the physical properties of gluten. In general, gluten film stability increased when the salt concentration increased where as the elasticity decreased (Butow et al., 2002). Belitz et al. (1986) stated that addition of low molecular weight divalent ions changes the rheological properties of the gluten film by strengthening the disulfide bonds, which are of great importance to gluten protein elasticity. This is the reason why bakers are thought to routinely add a small amount of salt to wheat flour dough (~2% w/w) to improve the dough rheology.

The effects of salt at low concentrations would result in a general increase in the intramolecular interactions within and among the polypeptide chains of the gluten proteins. This may induce gluten protein aggregation by suppression of the intramolecular repulsions of the gluten positive charges (Gatal et al., 1978). Kim and

Bushuk (1995) stated that salt strengthens dough by promoting the aggregation of glutenin and gliadins. Indeed, water molecules are drawn away from the polypeptide chain structure to interaction with the salt. This could induce more compact gluten protein molecules compared to the spread with pure water.

Preston (1989) showed that salts (such as NaCl) influence the hydrophobic and elastrostatic interactions causing conformational changes in gluten proteins. Salts induce an electrostatic shielding of ionic amino acids on the surface of the gluten proteins, which normally have an excess of positively charged basic amino acids (Yoshino & Matsumoto, 1966), thus reducing electrostatic repulsion or attraction of the proteins and inducing hydrophobic aggregation (Bernardin 1978; Preston 1981; 1984, 1989). Ultimately, these cohesive forces determine the physical dough properties of wheat flours and are dependent on the concentration and type of salt anion added. Thus, addition of low salt level (0.05-0.1M) may contribute to an increase of dough strength.

However, dough samples prepared with soft wheat responded to salt differently (Figure 6-23). Although there is no statistically significant difference at the $p < 0.05$ level for difference salt content in the dough samples for resistance to extension, there is a strong trend of decreasing the resistance of force when salt is added ($R^2 = 0.93$). The differences in response to salt addition may be related to the flour quality. Environmental factors such as relative humidity and the ambient temperature in the lab may also influence the results.

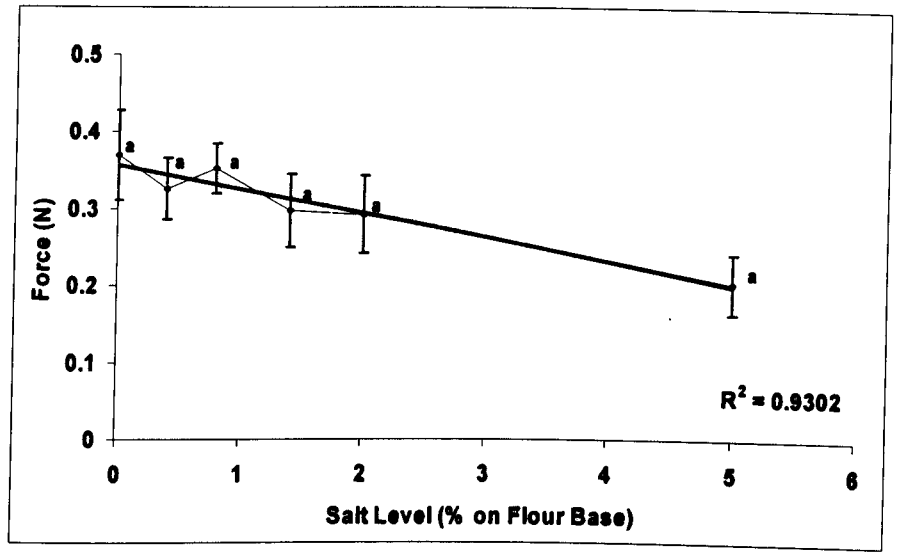



Figure 6-23. Effect of CBP dough prepared from soft wheat flour at different salt content on the resistance to extension (force) using the Kieffer dough extension test (Values represent the mean ± 1 standard deviation of five doughs, each replicated three times). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$.

Figure 6-24 shows the extensibility of the hard wheat dough samples in relation with salt concentration. There was no significant difference between extensibility and salt addition, which is opposite to the results observed by others such as Tronsmo et al. (2003) who concluded that the extensibility was significantly affected by the flour type, salt addition and interaction between the two factors.

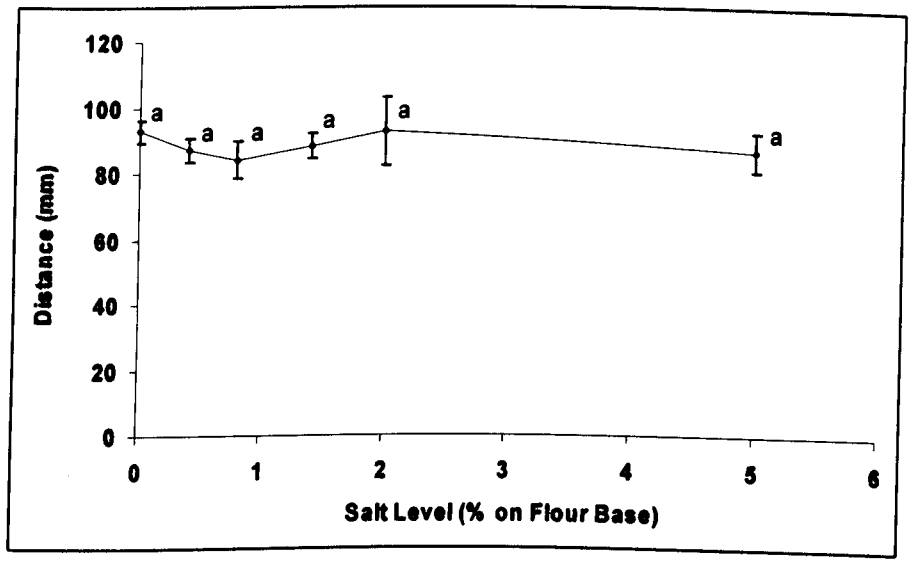



Figure 6-24. Effect of added salt (hard wheat CBP dough) on distance to failure during the Kieffer dough extension test (Values represent the mean ± 1 standard deviation of five doughs, each replicated fifteen times). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$.

The effect of salt concentration on the extensibility of soft wheat flour dough was similar to the hard wheat dough samples, but the values were much higher (Figure 6-25). This is mainly due to the difference between the flour types. Gluten proteins are primarily responsible for determining the strength of the dough.

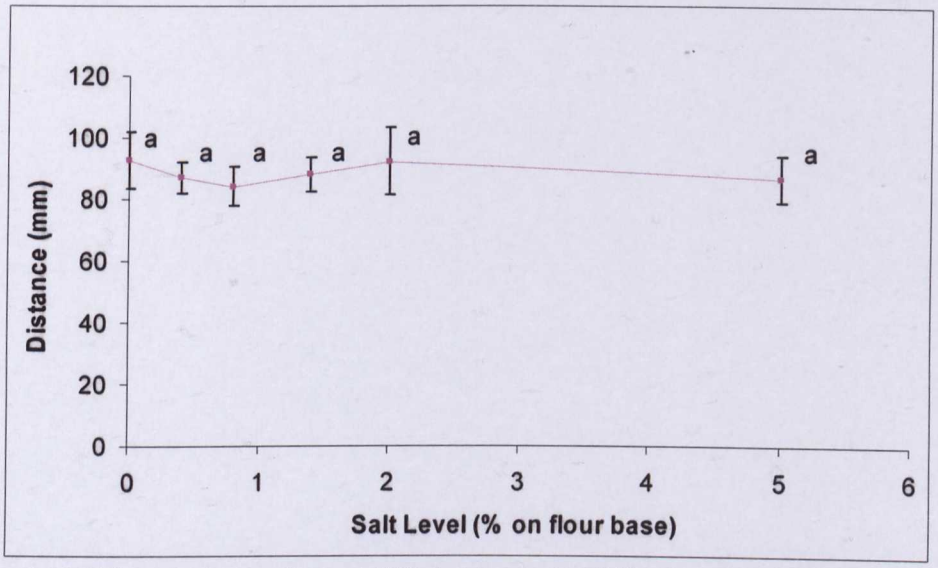



Figure 6-25. Effect of salt on soft wheat CBP dough on extensibility (values represent the mean $\pm$ 1 standard deviation of five doughs, each replicated fifteen times). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$.

### 6.2.3 Dough liquor composition

In dough, the potential role of an aqueous liquid phase is to maintain the integrity of gas bubbles and promoting gas retention. The aqueous liquid phase is thought to be stabilized by surface-active material such as lipids, soluble proteins and non-starch polysaccharides at the gas-liquid interface, which is shown to increase the gas-cell stability in dough. Two main components were measured in this study to get quantitative values for the amount of sodium and protein.

#### 6.2.3.1 Sodium content

It is believed that sodium ions in dough are somehow bound to different sites of the gluten-starch matrix or dissolved in the aqueous phase. A flame photometer was used to determine the concentration of sodium in the dough liquid phase (see section 3.4.7.2).

Figure 6-26 shows the sodium concentration in the dough liquor phase prepared from hard and soft wheat CBP dough. There is an increase of sodium concentration in the dough liquor as salt levels increased in dough prepared with both types of flour. Interestingly, a higher concentration of sodium is measured from the hard wheat flour dough liquor than soft wheat dough liquor at lower concentrations of added salt until 1.4% salt (on flour base), while the soft wheat dough liquor contained more sodium at higher salt levels. Interestingly, as salt concentration increases, a decreasing amount of the total sodium content is presenting in the dough liquor phase as measured in ppm.

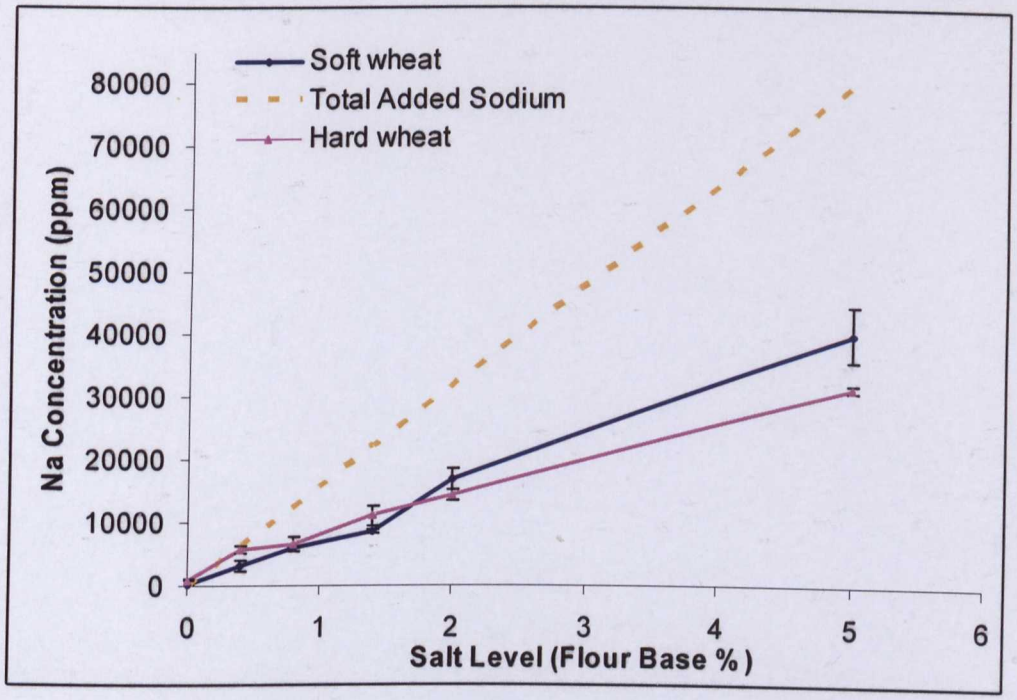



Figure 6-26. Sodium concentration in dough liquor phase prepared from hard and soft wheat CBP dough (values represent the mean ± 1 standard deviation of five doughs, each replicated five times). Total added sodium refers to the expected sodium dissolved in the water.

Figure 6-27 shows the percentage of measured sodium recovered in the centrifuged dough liquor by assuming all the added salt was dissolved into the aqueous phase and the volume of the aqueous phase was measured by the ultracentrifugation recovered (see Figure 6-1). Soft wheat and hard wheat samples seemed to behave differently as salt concentration increased. In hard wheat dough liquor, introducing the salt to the dough system decreases proportionally the amount of sodium that can be recovered in the dough liquor. This could be due to altering the gluten-starch

matrix when salt is added which then leads to more potential binding sites for sodium in the dough system. On the other hand, increasing salt level with soft wheat samples showed a slight proportional increase in the amount of sodium recovered in the dough liquor. This may be due to the difference of the flour composition as soft wheat flour generally contains lower protein content. When salt level increases, the binding site for sodium in the matrix decreases to a certain level. Thus the extra salt may be dissolved into the aqueous phase.

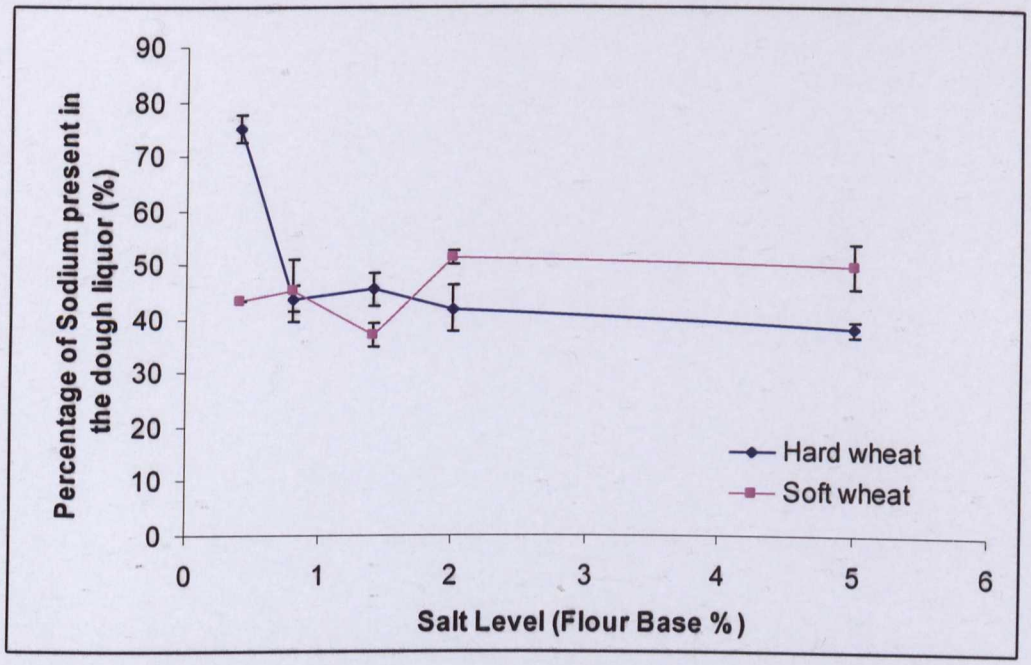



Figure 6-27. Percentage of sodium presented in the dough liquor at different salt levels (Values represent the mean $\pm$ 1 standard deviation of five doughs, each replicated three times).

### 6.2.3.2 Protein content

Preliminary studies (from section 5.4.1.3.1) showed that increasing the salt concentration in the flour-water recipe increased the amount of protein present in the dough aqueous phase. However in the study using hard wheat CBP dough, increasing the salt level showed a decrease in the protein concentration (Figure 6-28). These findings is opposite to other studies (Sahi, 1994, 2003; Mills et al, 2004; Salt, 2004; Primo-Martin, 2006) as they showed increasing salt generally increased the protein concentration in the dough aqueous phase.

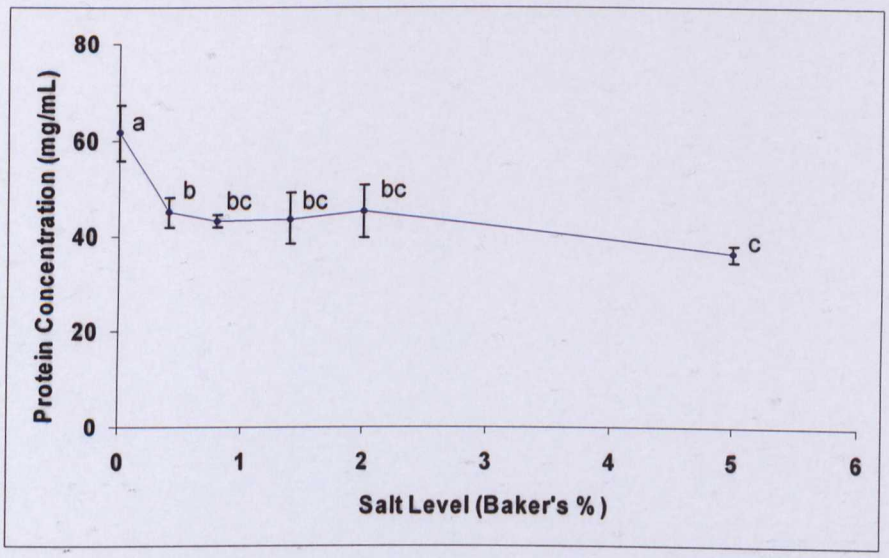



Figure 6-28. Protein concentration from CBP hard wheat dough liquor (values represent the mean ± 1 standard deviation of five doughs, each replicated five times). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$.

Figure 6-29 shows soft wheat CBP dough gave a higher protein concentration in dough liquor phase compared with hard wheat dough results and there is no statistical difference with increasing salt content on the protein concentration. Sahi (1994) stated that the levels of proteins soluble in the aqueous phases of dough were found not to be related to protein content of the flour. This may due to the mixing time with dough preparation. As same mixing time was applied to both wheat flour types, soft wheat dough is more suspect to over mixing and breakage of the disulfide bonds which hold the polypeptide subunit together. The glutenin protein is partially depolymised and produce small molecules (Letang et al., 1999). These small protein molecules could soluble in the liquor phase and be extracted.

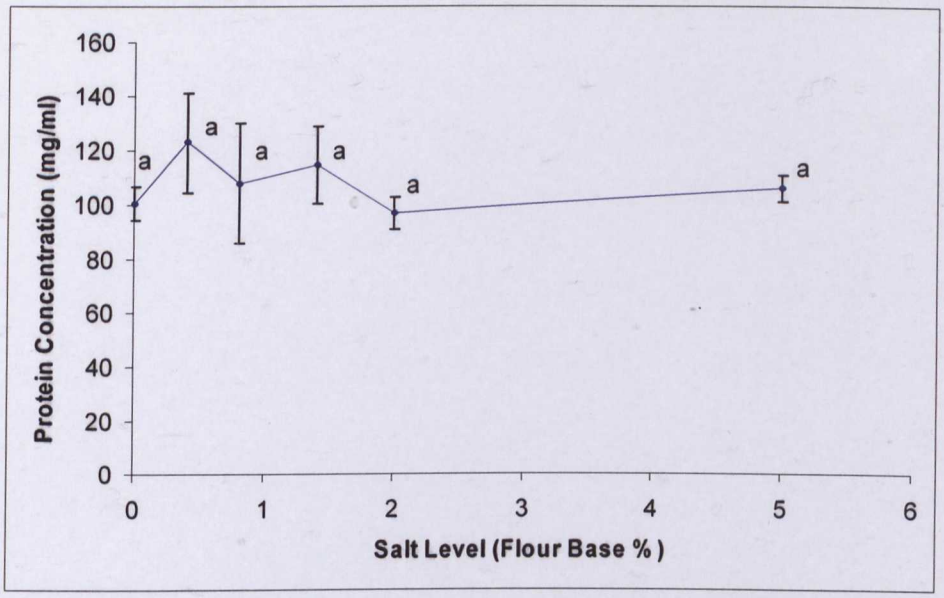



Figure 6-29. Protein concentration from CBP soft wheat dough liquor (values represent the mean ± 1 standard deviation of five doughs, each replicated five times). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$.

The total amount of protein in the aqueous liquid phase relates to the amount of dough liquor extracted from the dough sample. Figure 6-30 shows the total amount of protein in the dough liquor was not affected by the salt levels from soft wheat dough sample, but there maybe an increase of total protein in dough liquor from this hard wheat dough sample.

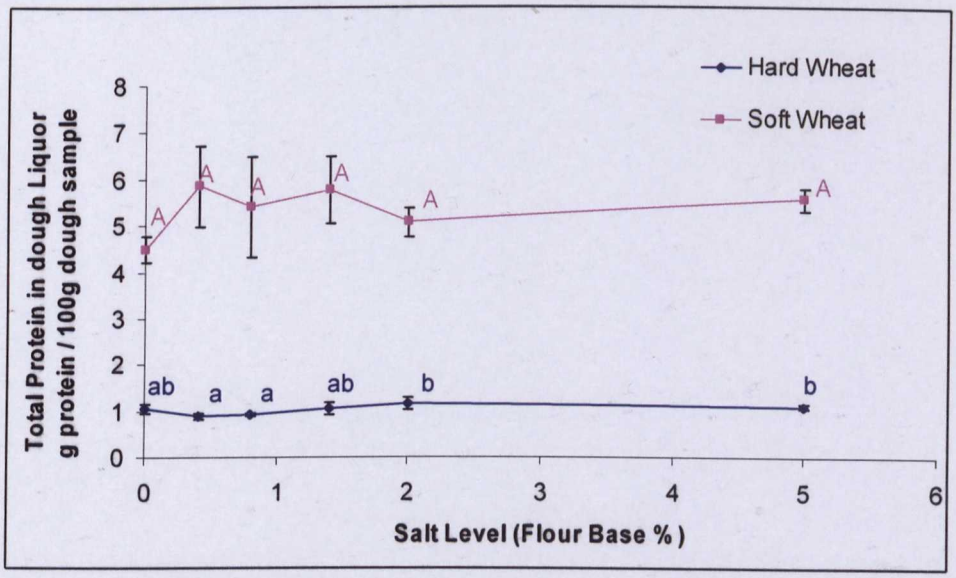



Figure 6-30. Total protein content in dough liquor (values represent the mean ± 1 standard deviation of five doughs, each replicated three times). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$ within each flour type (a,b,c,d for hard wheats and A,B,C, D, E for soft wheats)

## 6.3 CONCLUSION

The use of the non-yeasted doughs supported some of the earlier findings in that:

1. Salt increased the amount of liquid on centrifugation, but did not alter/or decreased the amount of freezable water or the mobility of the protons, as measured by DSC and NMR.
2. Increasing salt increased the force required to stretch the dough, but the distance before breaking of the dough strand was not affected by the salt content of the dough.
3. Salt content in the dough liquor phase, as measured by flame photometry, indicated that salt addition in the recipe increased the sodium concentration in the dough aqueous phase; however, the amount recovered from the fluid phase is less then the amount added. This indicated that part of the salt is "trapped" in the gluten-starch matrix.
4. Protein concentration in the fluid phase did not increase with salt levels in the dough. This finding was opposite to the results found in preliminary work and reported by others (Sahi, 2000; Salt, 2006) who show that the addition of salt to the dough modified and increased the protein composition of the dough liquor.

# CHAPTER 7. BREAD DOUGH STICKNESS TEST

## 7.1 INTRODUCTION

Dough stickiness is a major problem for large mechanised bakeries as it leads to costly disruptions to production schedules and loss of product quality. The major observation from the baking trials described in this thesis was the sticking of doughs to the moulder. However, objective measures of dough stickiness did not reflect the observations from the baking line. In this chapter stickiness and how it may be measured is described in some detail.

The quality of bread dough is influenced by several factors, for example the type and level of ingredients used, processing conditions; such as mixing, resting and moulding of the dough, and baking and cooling of the breads. Sticky doughs can be created by over-mixing or the use of excess water, which can be avoided by careful control. Among the ingredients, the quality of flour, fat and additives greatly influence the quality of dough and bread.

Since stickiness is influenced by interactions between several factors, test baking has been found to be the method of choice when predicting the suitability of flour for bread making. However, the test is time consuming and requires specialised equipment and trained personnel. Moreover, moderate yet unacceptable levels of dough stickiness, which could create problems in a modern automated bakery are often not expressed in laboratory test baking. There are several tests for different ingredients like protein quality and quantity of flour, solid fat index for fat. However no single test could predict the quality of dough and bread in terms of stickiness.

Dough is the intermediate product between flour and bread. Dough rheology is of considerable importance in bread manufacture as it influences the machinability of dough and the quality of the breads. Dough which is too firm or too soft, will not process satisfactory using appropriate dough forming equipment and will not yield a satisfactory product (Wade, 1988). Manley (1983) reported that dough consistency influences the quality of bread.

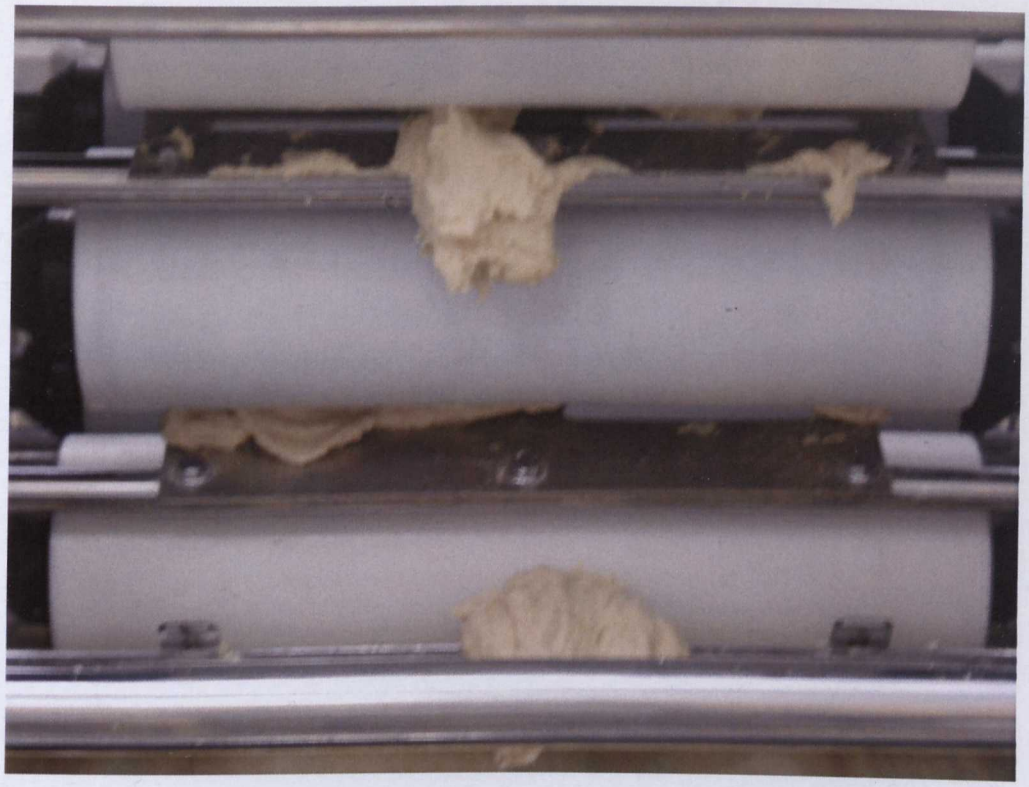


Figure 7-1. Dough piece which has been stuck in the final moulder, losing integrity and providing a dough surface to which the next dough piece will most likely to adhere. (Photo taken from CCFRA pilot plant)

Traditionally, dough stickiness has been assessed subjectively by skilled technologists. Pena and coworkers (1990) designated doughs as nonsticky or sticky based on the number of times the dough could be hand-kneaded and pressed before it stuck to the hands of the tester. Martin and Stewart (1989, 1990) categorised commercial cultivars and bread making of wheat lines as nonsticky, based on degree of adherence to palm and fingers when handled. MacRitchie and coworkers (1986) and Dhaliwal and MacRitchie (1990) scored doughs for their adhesiveness to hands (two tests), a mixing bowl, paper and a Teflon surface. These methods are time consuming, and could also lead to variation depending on the panel members. Recently, a number of studies have tried to quantify the surface stickiness of dough using instrumental tests, but these methods have suffered from poor reproducibility or have lacked standardisation against quantitative measurements. Furthermore, these methods require lots of manipulation of the dough samples before testing. Therefore, there is a need for simple and objective methods to assess the stickiness of bread dough.

The aim of this chapter is to evaluate a potential method to quantify the surface stickiness of dough under various conditions to study the effects of salt reduction on dough stickiness. The method, which is named "dough stickiness", uses an adaptation of the traditional two bite "texture profiling analysis" (TPA) described by Friedman et al. (1963) and Szczesniak et al. (1963) and modified by Henry and Katz (1969) and Bourne (1978), to measure viscoelastic characteristics of dough. While the profiling technique is not new, the design of the measuring parameter, the introduction of a relaxation phase, the mathematical data analysis for the compression relaxation-tension cycles, and the application to dough properties are novel.

## 7.2 METHODS DEVELOPMENT

### 7.2.1 Understanding dough stickiness

Stickiness is considered a physical viscoelastic property of a material. The properties of stickiness can be divided into adhesion and cohesion. Adhesion is the binding force between two different materials, whereas cohesion is the binding force between two similar materials.

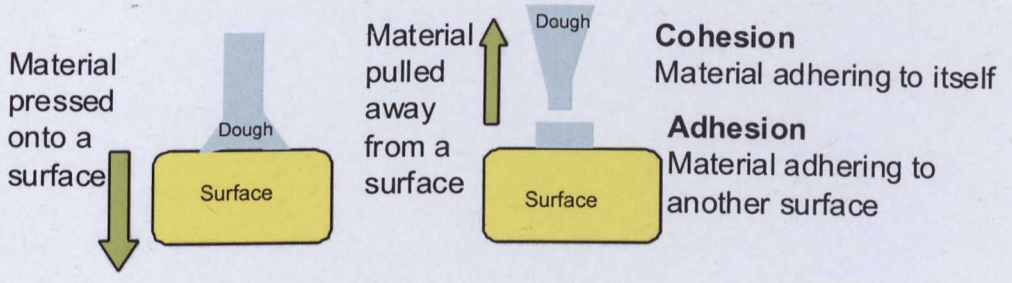



Figure 7-2. Defining cohesion and adhesion.

Adhesion is dependent on the flow of material onto the surface and this depends on the difference between the surface energy of the material and the surface energy of the adhesive. The cohesive force results from the molecular structures resisting separation from each other. Therefore cohesion can be thought of in terms of the ability to resist extension. Force to break in extension represents the energy requirement to sever the connectivity within the structure and the distance represents

the ability of the molecules within the system to slide past one another without loss of connectivity. Both adhesion and cohesion are very sensitive to rates of deformation (Dobraszczyk, 1997) and gap sizes (Couch and Binding, 2003). Little is understood about the rates and forces occurring during the bread making processes that might be relevant to the stickiness. It is unlikely that the rates used in the Kieffer tests are directly comparable.

### 7.2.2 Materials and Methods

#### 7.2.2.1 Design on the method

Three critical factors were examined when designing a method to quantitatively measure the stickiness of flour dough (hardness, cohesion and adhesion).

##### 7.2.2.1.1 Selection of probe surface material

Different types of material have different degrees of adhesion to the surface. In preliminary trails a number of surfaces, including stainless steel and plastic, were studied. Probes were found to have similar characteristics the stickinesss of dough. Since most of the studies on stickiness used Perspex as their probe material, it was selected for dough stickiness measurement.

##### 7.2.2.1.2 Selection of probe geometry

A cylinder geometry is used in many studies to characterise stickiness of food samples such as the TPA and Chen-Hoseney tests. The flattened surface of the cylinder probe is typically in contact with the surface of the dough samples.

In the moulding stage, dough is subject to high compression forces in order to flatten the sample. When dough is compressed, it is extended and new dough surface is created. It was thought that this freshly made surface was "sticky" and thus adhered to the equipment surfaces which lead to process breakdown. In order to increase the creation of new surfaces during testing, a 1 inch spherical probe was used in the later stages of the stickiness testing.

Figure 7-3. 25mm Perspex cylinder probe (P/25P).

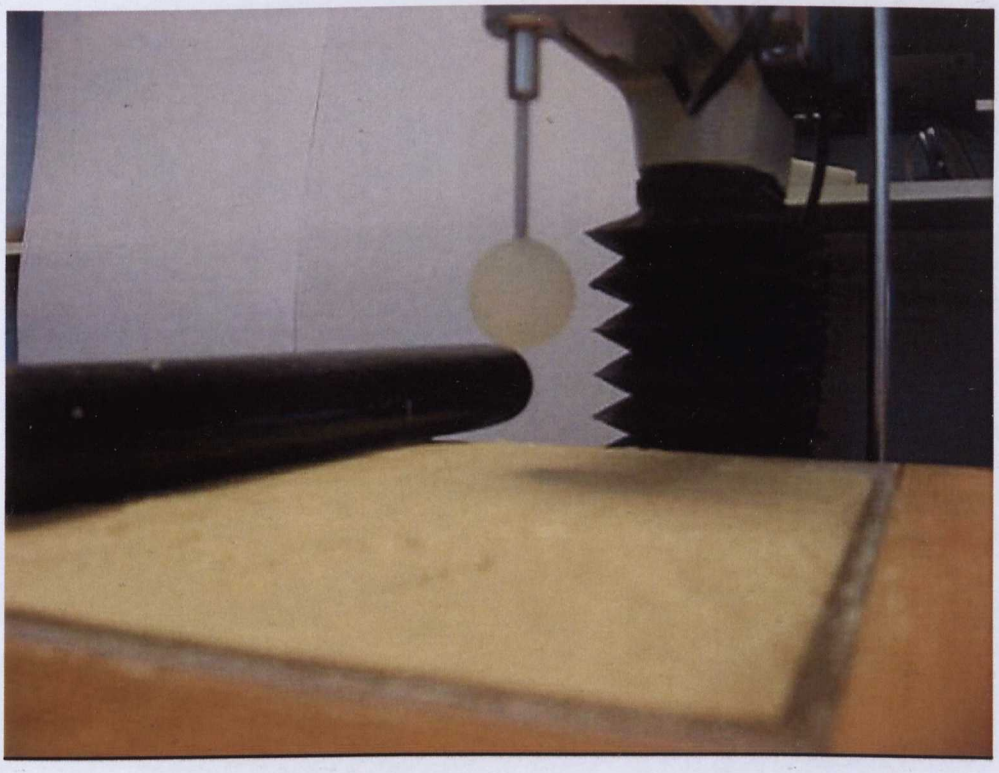


Figure 7-4. 1 inch Perspex spherical probe (S/S1).

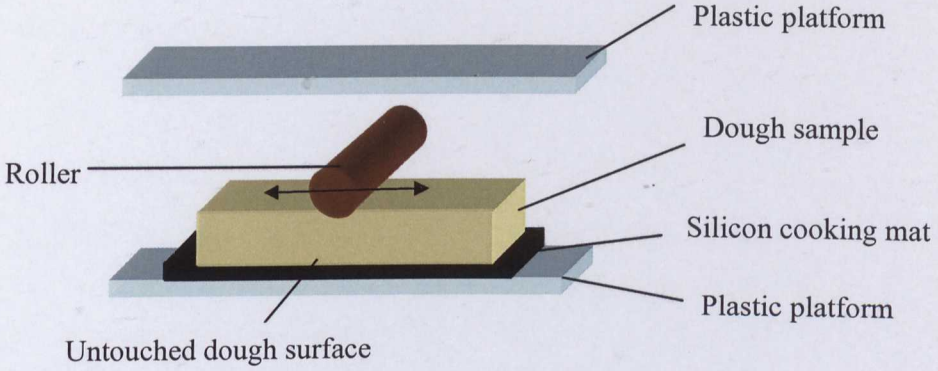
#### 7.2.2.1.3 Instrument

Stickiness evaluation is influenced by the force of adhesion and cohesion (Sherman 1979). Control of compression force exerted on the dough and the contact area or interface area between the dough and the probe are critical for dough stickiness measurement. To compare stickiness values of different doughs, these two parameters must be constant. The most common used instrument for many food technologists to understand the food texture is the Texture Analysis (TA). It can provide a constant compression force to a dough surface for each experimental test. In this study, several parameters were of particular interest including hardness (also known as firmness), adhesiveness, and work of adhesion.

### 7.2.2.2 Dough Preparation and Handling

Simplified commercial bread dough (non-yeasted) samples were prepared according to section 3.3.1.1. with salt levels range from 0% to 5%. A carefully standardised procedure for dough sampling and handling was followed to maximize reproducibility. Dough was sheeted using a rolling pin over a rectangular platform and frame with a height of 2 cm to get a sheet of 2 cm thickness. Skinning of the dough surface was minimised by using a large dough sample (~ 2 kg). A silicon cooking mat (Du Port$^{TM}$ Teflon) was placed at the bottom of the frame to avoid the sample surface sticking to the plastic platform. The sheeted dough was reversed (turned over so the bottom surface was now at the top) and rested for 5 minutes before testing. The matting was carefully taken off before experiment started. Dough profiles were determined using the Texture Analyser (Stable Micro System, UK) with a 25 mm Perspex cylinder probe (P/25P) or 1 inch Perspex spherical probe (S/S1). At each salt level, five dough samples were made and for each dough 12 readings were taken randomly across the sample. Replicates were measured in rapid succession and there was no consistent trend in the measured parameters from first replicate to last. All sampling was completed in 15 minutes. The mean values from five doughs were analysis by one-way between-group analysis of variance (differences between the doughs) and Post-Hoc Tukey HSD was used to identify the subgroups.

a)

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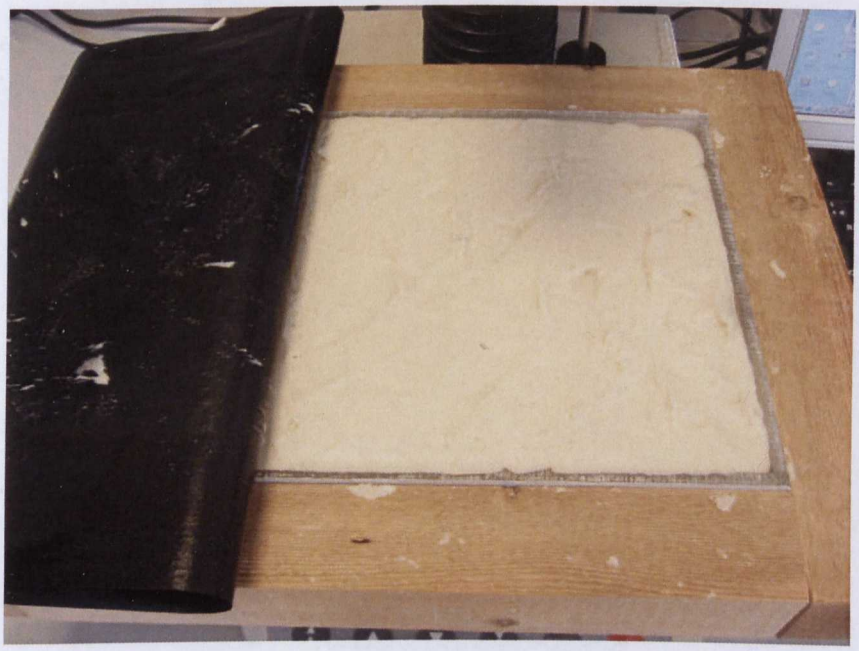

Figure 7-5. (a) A schematic diagram of the dough sample preparation and (b) sample dough block.

Two probes (cylinder and sphere) were evaluated. They were used to penetrate into the dough blocks to study the amount of adhesion/cohesion of the dough samples. The dough sample was compressed with a cylinder probe for 2 mm distance and 12.7 mm for the spherical probe at 1 mm/sec, a 10-sec time elapsed before withdraw at of the probe at 0.5 mm/sec. The trigger force was set at 5 g-force. Force on the probe was measured throughout the test. The surface of dough samples were compressed 6 times randomly within the block of dough and the force time data was collected as shown in Figure 7-6. Three dough properties were measured sequentially using the modified texture analysis test. They were:

- Hardness of the dough
- Relaxation of stress (decline force)
- Surface adhesion to the probe

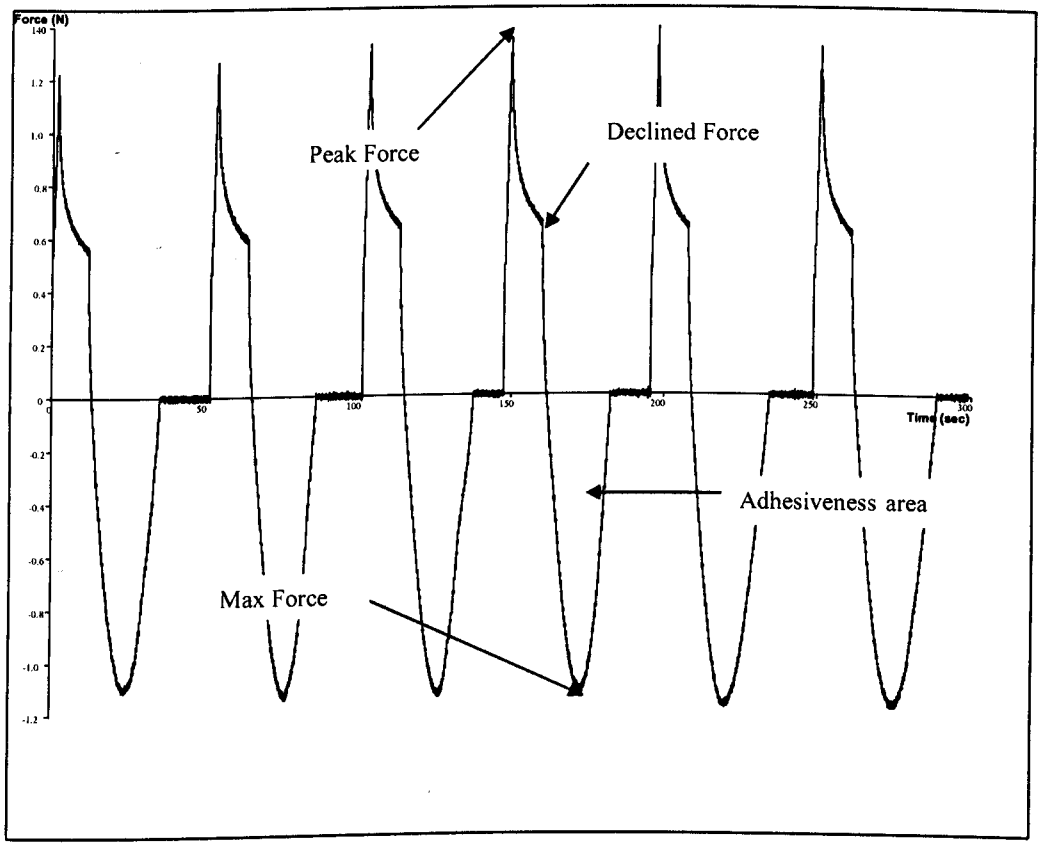


Figure 7-6. A typical plot of the results from stickiness experiment performed with the Texture Analyser.

The percentage of decline was calculated using the Equation 7-1. This value helps to understand the strength of the bonds (i.e. covalent bonds) as well as the elasticity of the dough samples. When the value of the percentage of decline is small, it implies that the sample is elastic. In contrast, if the value of the percentage of decline is large, it means the sample behave more liquid-like and can flow easily.

$$\text{Percentage of decline} = \frac{\text{Peak force} - \text{Declined force}}{\text{Peak force}} \times 100\%$$

Equation 7-1

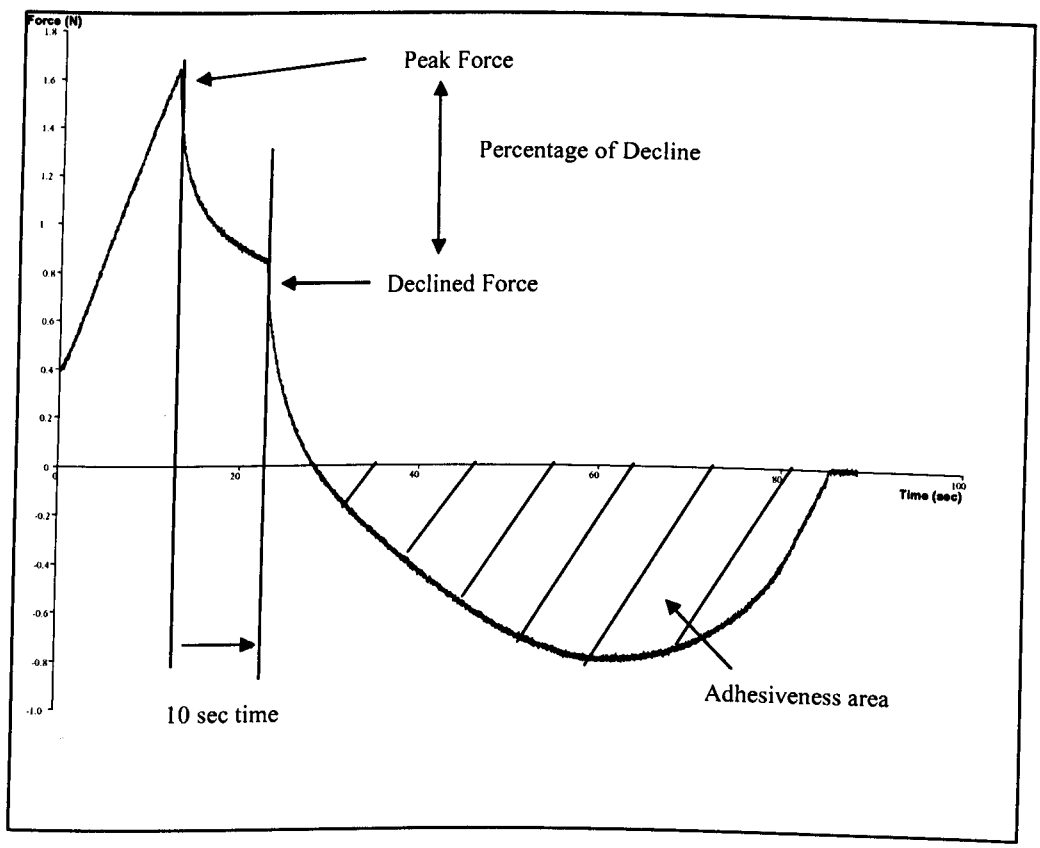


Figure 7-7. A typical plot of the stickiness experiment performed with the texture profile analyser.

## 7.3 RESULTS AND DISCUSSION

### 7.3.1 Effect of salt on dough properties

The experimental values of the peak force obtained with two probes are shown in Figure 7-8.

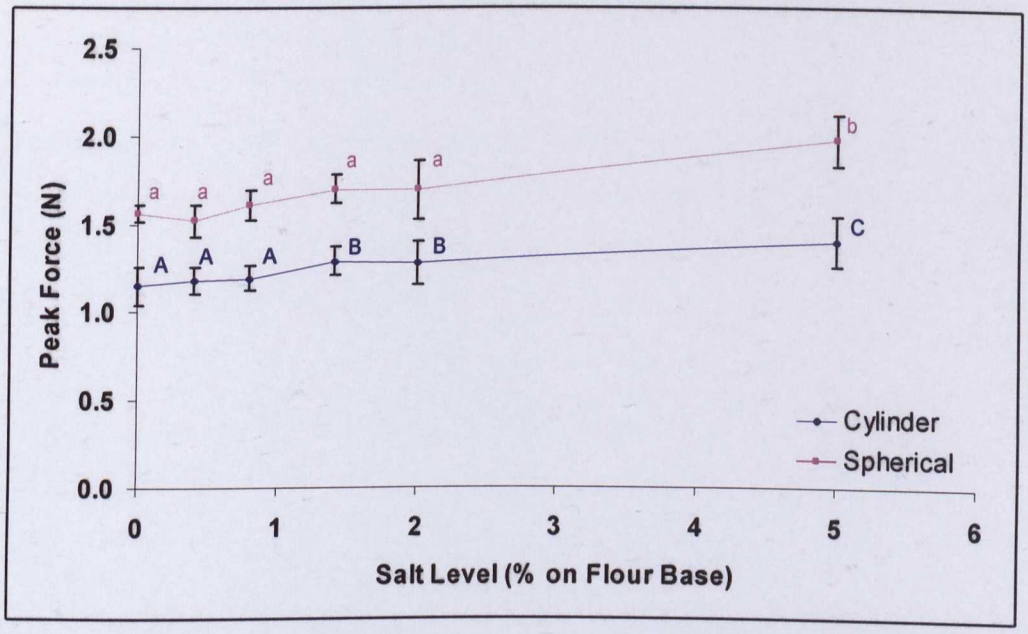


Figure 7-8. Effect of salt levels on peak force in dough texture profiling test using cylindrical and spherical probes (values are the means $\pm$ 1 standard deviation from the 5 doughs. Each mean value was derived from 12 replicates). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$. (a,b,c,d for spherical probe and A,B,C, D, E for cylinder probe)

The force from the spherical geometry showed a higher value than the cylinder geometry. This is as expected and is due to the differences in the amount of penetration and the amount of dough subjected to compression. For the cylinder 490.87 $mm^2$ of dough surface was in contact with the probe, while for the spherical probe the surface area was 1013.41 $mm^2$. Not only are the contact areas different, but also the movements within the dough as the different shaped probes penetrate, are not be comparable. Both geometries showed a dependency of surface hardness with salt level from ANOVA ($p \leq 0.05$). The results agree with the Kieffer dough extensibility test (as described in Chapter 6), as salt is added to the recipe, it strengthens the gluten network with $R^2 = 0.96$ for spherical probe measurement and $R^2 = 0.95$ for cylinder probe measurement. Several studies have shown salt has a

modifying effect on the physical properties of gluten. In general, gluten film stability increased when salt concentration increased while the elasticity decreased (Butow et al., 2002). Belitz et al. (1986) also stated that the rheological properties of the gluten film can be changed by adding low molecular weight divalent ions. This strengthening the disulfide bonds which seem to have a great importance on gluten protein elasticity, this is the reason why bakers routinely add a small amount of cooking salt (NaCl) to wheat flour dough (~ 2% w/w) to improve the dough rheology.

The decline force and the percentage of declined force are shown in Figure 7-9 and 7-10, respectively. The decrease in force during the rest period can be interpreted as an ability of the system to relax due to rearrangement of the forces (often associated with hydrogen bonds) within the network.

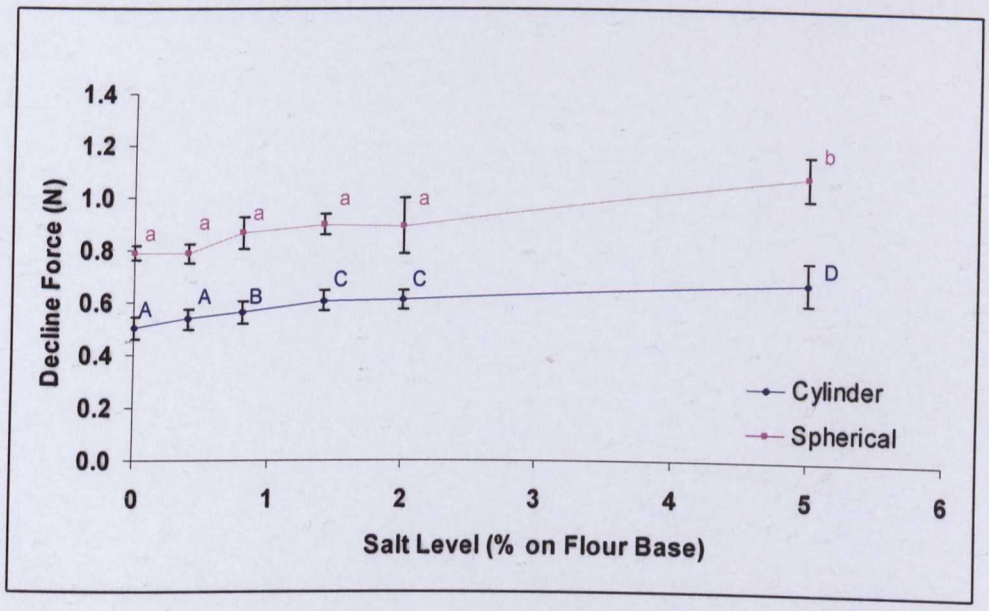


Figure 7-9. Effect of added salt on decline force in dough texture profiling test using cylindrical and spherical probes (values are the means $\pm$ 1 standard deviation from the 5 doughs. Each mean value was derived from 12 replicates). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$. (a,b,c,d for spherical probe and A,B,C, D, E for cylinder probe)

From Figure 7-9, one could interpret the findings as the high salt samples behaving in a less elastic (i.e. more viscous) manner than the no/low salt samples, as the decline forces are greater. However, the forces required initially to compress the high salt samples was significantly greater (see Figure 7-8). Therefore, the percentage of

decline (see Equation 7-1) may be a more appropriate value to understand the relaxation of the system under stress.

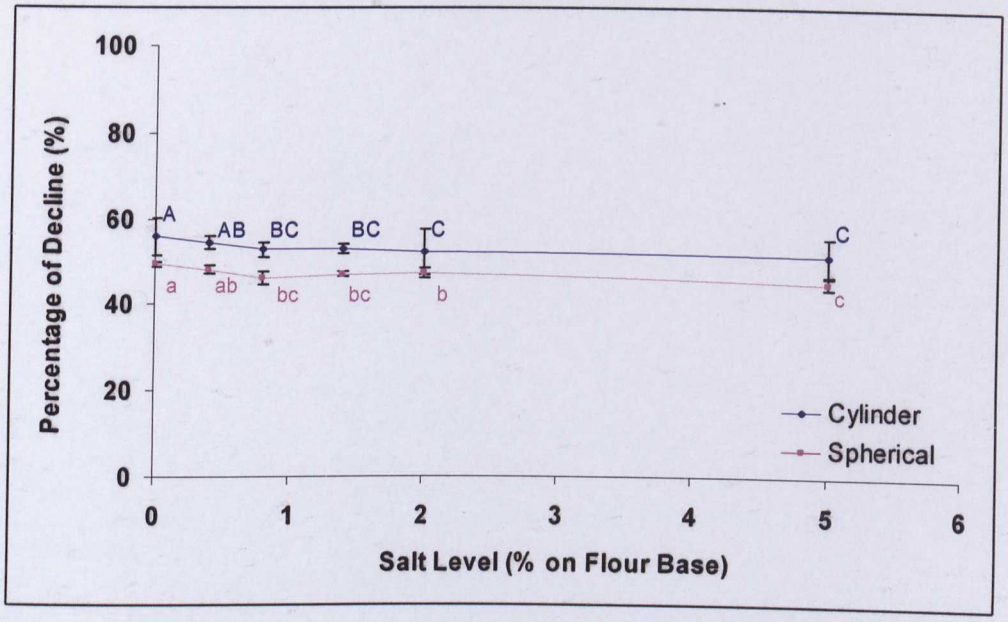


Figure 7-10. Effect of added salt on the percentage of decline in dough profiling test using cylindrical and spherical probes (values are the means $\pm$ 1 standard deviation from the 5 doughs. Each mean value was derived from 12 replicates). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$. (a,b,c,d for spherical probe and A,B,C, D, E for cylinder probe)

Figure 7-10 indicates that the samples relax proportionally less for the low salt samples. A small decrease in the forces required to compress the material reflects an elastic character and that the forces holding the network are likely to be co-valent (Peleg, 1979). Further work should be carried out on the doughs to see if at the same initial forces the relaxation values are indicative of different behaviour. However, the current test seems to show rheological differences for the samples using a simple test method. This could well be due to more disulfide linkages within the gluten structure. Salt has also been reported to increase dough "strength" as measured by recording mixers (Miller and Hoseney, 2007).

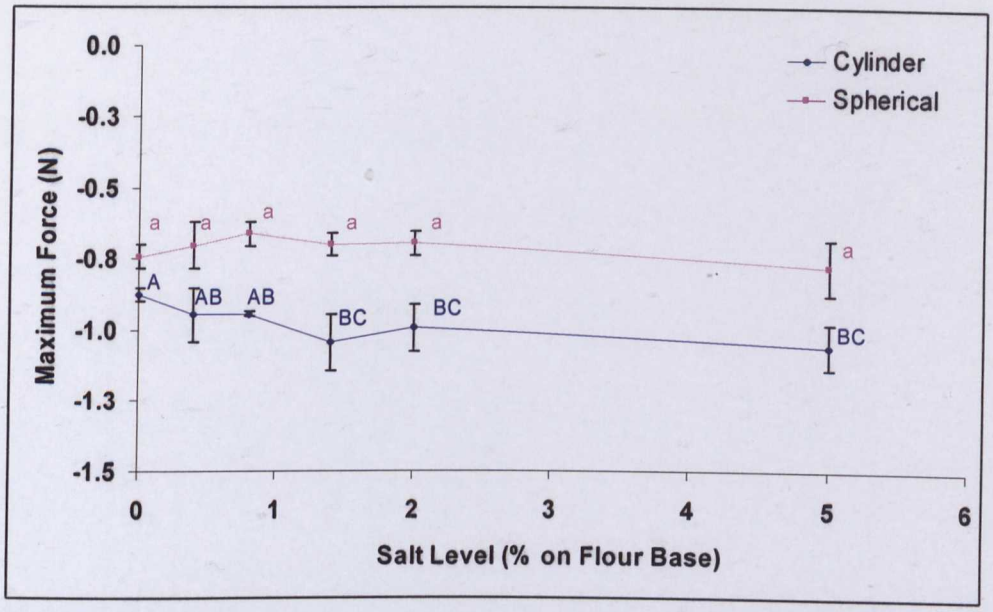


Figure 7-11. Effect of added salt on maximum force in dough texture profiling test using cylindrical and spherical probes (values are the means ± 1 standard deviation from the 5 doughs. Each mean value was derived from 12 replicates). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$. (a,b,c,d for spherical probe and A,B,C, D, E for cylinder probe)

The more difficult features of dough to quantify are their adhesion/cohesion values. This was attempted by looking at the removal of the probe from the dough. The maximum force can be an indication of the force (i.e. energy) needed to pull the probe out of the dough samples as the texture analyser records these as negative values as the direction of travel of the probe is reversed. The higher the numerical value of the max force (i.e. more negative), the more energy is required to pull the probe out of the sample. The effect of salt on the maximum force is presented in Figure 7-11.

An increase in the amount of energy required to remove the probe when salt was added to the dough samples was detected using different probes. However, the spherical probe did not show any correlations on the force or work required to withdraw the probe from the dough samples at different salt levels.

Figure 7-12 shows no influence of salt content on surface adhesion when measured as the work required to withdraw the cylinder probe from the dough, although the data are reproducible. Some statistical differences were observed for the spherical

probe, but the effects if present, are not linearly proportional to salt levels. The standard deviations for these assessment were all high (CV = 0.17) and there would need to be addressed if this assay was to yield relevant information.

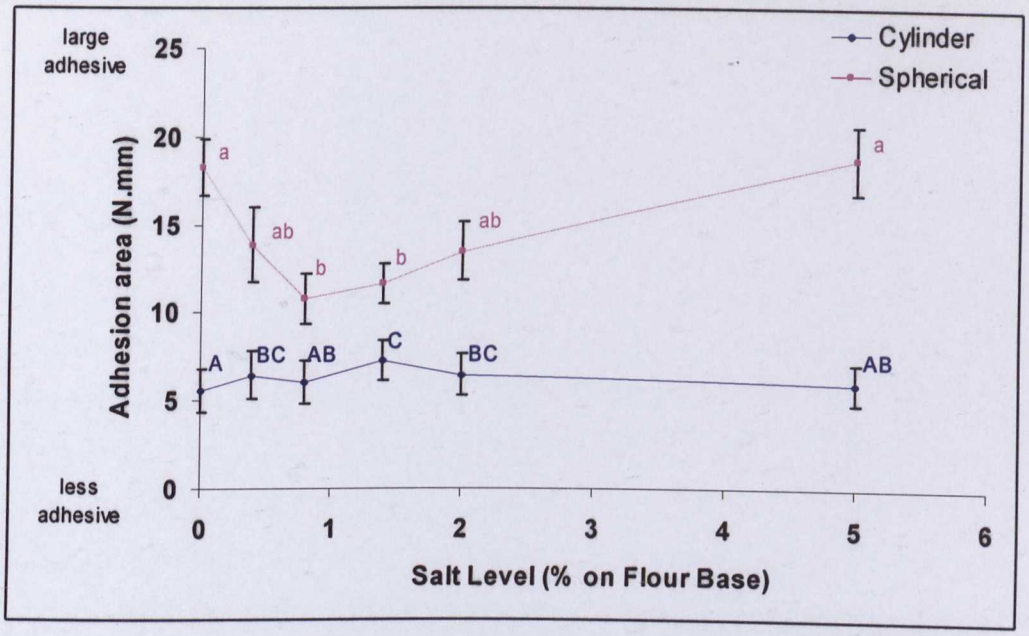


Figure 7-12. Effect of added salt on work to withdraw probe in dough texture profiling test using cylindrical and spherical probes (values are the means $\pm$ 1 standard deviation from the 5 doughs. Each mean value was derived from 12 replicates). Tukey honest differences were used to compare the mean values and the different letters in chart show significant difference at $p \leq 0.05$. (a,b for spherical probe and A,B,C for cylinder probe)

### 7.3.2 Effect of temperature on dough properties

Large bakeries are finding it difficult to handle the reduced salt dough especially in summer time. In order to identify the problem, dough samples at 2% salt (flour base) were mixed to two final dough temperatures (25°C and 30°C). The sample dough was divided into two blocks. One of the blocks was tested immediately and the other block sample was sealed and placed into a conventional oven at 26°C for 20 minutes. Dough samples were tested with the modified texture analysis profile using a spherical probe (see methods in 7.2.2.2) and the dough properties were analysed with paired-sample t-test to evaluate the impact of the temperature changes on the textural properties of the dough sample at 2% salt level. Although there is a coefficient of variation between the dough of 7%, the statistical analysis indicated significant differences between the treatments.

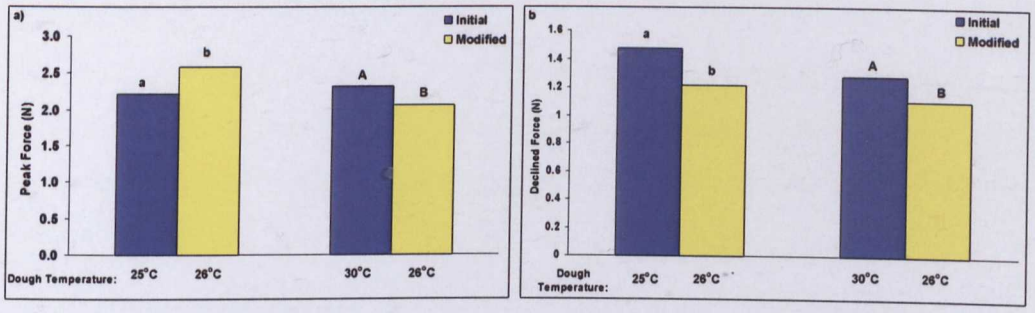


Figure 7-13. (a) Peak force and (b) decline force of CBP dough at 2% salt level (on flour base) mixed at 25°C and 30°C and modified to 26°C (values are the means ± 1 standard deviation from the 5 doughs. Each mean value was derived from 12 replicates). a,b for sample mixed at 25 °C and A,B for sample mixed 30 °C.

Figure 7-13 shows the peak force and decline force of the dough samples mixed at 25°C or 30°C and modified to 26°C. It was noted that the values from this set of data were higher than the previous results in Figure 7-8. This may due to the different batch of flour used. There was a statistically significant increase in the peak force from 25°C to 26°C ($p < 0.05$) indicating that the dough became harder. However, dough samples mixed at higher temperature (30°C) and cooled to 26°C showed an opposite effect ($p < 0.05$). Both direction of modifying the temperature in the dough samples show a statistically decrease in the decline force ($p < 0.05$) from Figure 7-13b. This may due to the redistribution of water and the continuous hydration of the components in the dough system.

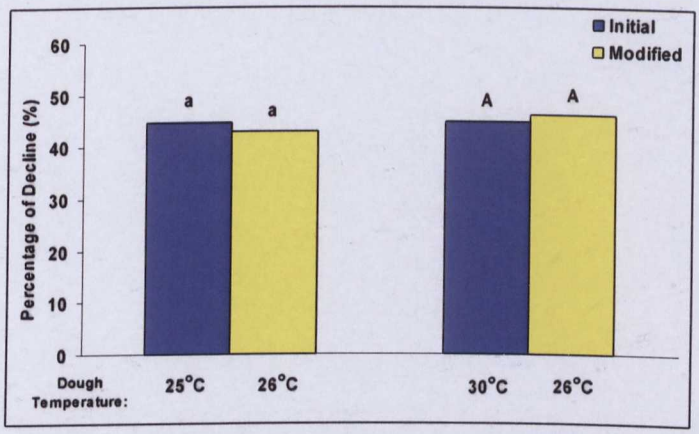


Figure 7-14. The percentage of decline of dough samples mixed at 25°C and 30°C and modified to 26°C (values are the means ± 1 standard deviation from the 5 doughs. Each mean value was derived from 12 replicates). a,b for sample mixed at 25 °C and A,B for sample mixed at 30 °C.

The percentage of decline was calculated from the initial peak force required to compress the dough and the declined force which the samples were relaxed on holding and is presented in Figure 7-14. Although there was a noticeable difference to the touch from subjective evaluation (softer touch with higher temperature dough), there was no statistically difference on temperature modification ($p > 0.05$).

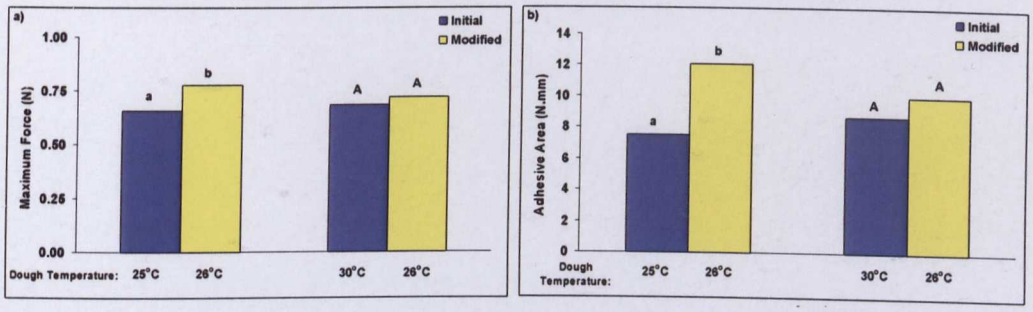


Figure 7-15. The (a) maximum force and (b) adhesion area of the dough samples at 25°C and 30°C and modified to 26°C (values are the means $\pm$ 1 standard deviation from the 5 doughs. Each mean value was derived from 12 replicates). a,b for sample mixed at 25 °C and A,B for sample mixed at 30 °C.

The adhesion properties determined by maximum force and adhesion area are shown in Figure 7-15. When the dough temperature decreased from 30°C to 26°C, there was no statistically difference on both maximum force values and adhesion area ($p > 0.05$). However, there was a statistically significant increase on the maximum force to withdraw and the adhesion area when the dough was changed from 25 °C to 26°C. This suggests that it requires more energy for the probe and the dough sample to be separated resulting in a more sticky dough effect. However, apart from temperature factors, the holding time is also a variable that occurs during manufacture, which may alter the degree of stickiness on the dough surface.

## 7.4 CONCLUSIONS

Surface-related properties of doughs, such as adhesiveness and/or stickiness, affect the dough machinability. Sticky doughs are of particular concern during high speed mixing, which is commonplace in modern mechanised bakeries. Costly disruptions to production schedules and loss of product quality can be the result.

Stickiness of dough involves various factors. One of the tests for measuring the degree of stickiness on the dough sample is Chen-Hoseney test. Chen and Hoseney (1995) devised a cell that caused the dough to separate from the probe surface under tensile. Previous study (see Chapter 5) showed that Chen-Hoseney test is not an ideal test for this particular set of samples. This maybe due to several factors such as the dry dough surface produced by vacuum mixing, the rate of rotation on the internal screw to extrude the sample from the cell, and the small amount of dough sample may not represent the whole dough sample. Thus, in this chapter, the modified texture analysis profiling using a spherical geometry was developed to measure dough stickiness and has the advantage of creation of new dough surfaces for testing. This test can give a measure of stickiness and can be used to identify the quality of the doughs within the time scales relevant to dough age.

As the salt content increased from 0 to 5%, the peak force, decline force and the percentage of decline indicated an increase in dough strength and elastic properties. Although different batch of flour may produce different results, however, within the same batch, different salt levels showed a significant difference. It was also showed that temperature changes the surface adhesion/cohesion properties of the dough, as lowering the temperature reduces the stickiness properties. This may be related to the stability of the dough samples. Increasing the temperature of the dough samples may weaken the protein network leading to sticky dough.

Spherical probe could provide some advantage compared to the cylinder probe. When the spherical probe is compressed into the dough, fresh dough surface is made to reduce the effect of rapid drying of the dough surface. An additional practical advantage of using a spherical probe is that the flatness of the dough samples will not be a critical factor when testing. Although this test was not an ideal experiment, it provides an insight to measure the degree of stickiness in a more simple and direct way.

# CHAPTER 8. SODIUM NUCLEAR MAGNETIC RESONANCE

## 8.1 INTRODUCTION

Salt (sodium chloride) is one of the most important ingredients in baked products. It is commonly added to bakery formulae at a level from 1 to 2.5% of the flour weight. Salt performs a multi-purpose role in bread, for example: it imparts direct flavour to the product, enhances perception of other flavours, controls yeast growth and fermentation rate, assists product texture by strengthening the gluten structure and other factors so far poorly defined. By altering the amount of salt in the dough system changes in textural properties are possible could affect the final bread quality.

Traditionally, salt content in foods has been measured by chemical methods which are tedious and time-consuming. These methods also typically only indicate the amount of salt, not their location. Recently, NMR experiments have been shown to be very effective tools for quantifying levels of total and "bound" sodium ions in complex food system such as iota-carrageenan (Gobet et al., 2009), salmon fillets (Aursand et al., 2009) and snow crabs (Nagata et al., 2000). However, no literature was found that discussed the salt distribution in systems such as starch, gluten and flour doughs.

In a flour dough system, salt can associate with flour constituents such as starch, gluten, water and with other added ingredients. Therefore the study of salt in bread dough is very complex and a preliminary study of the mobility of salt in flour and its constituents (gluten and starch) was necessary. The objective of the work described in this chapter was to characterise salt distribution in a multi-component mixture (CBP dough) and to correlate it with what was observed in a more simple system including starch-water, gluten-water, and flour-water mixtures.

## 8.2 METHOD DEVELOPMENT

### 8.2.1 Sample preparations

Native wheat starch was prepared with 62.3% water (by starch weight) at 0.4, 2 and 5% salt level (by starch weight).

Wheat gluten (Sigma, UK) and water were mixed in 2:3 ratios at 1, 5, and 12.5% salt level (on gluten base).

Doughs made from starch and gluten at a 89:11 ratios with 62.3% water (on starch-gluten weight) at 0.4, 0.8, 1.4, 2, and 5% (on starch-gluten weight) were also prepared.

A set of flour-water dough samples was prepared at 62.3% water (by flour weight) at 0.4, 0.8, 1.4, 2, and 5% salt (on flour weight).

CBP dough prepared with hard and soft wheat was made according to the standard small sample mixing procedure (see section 3.3.1.2).

Table 8-1. Sodium chloride levels in the dough samples.

| Level of salt compared with 100g flour Flour Base (%) | Level of salt in 100g dough | | |
|---|---|---|---|
| | Whole Dough Base (%) | Number of Mole (N) | Molarity[a] (M) |
| 0.0 | 0.000 | 0.000 | 0.000 |
| 0.4 | 0.243 | 0.003 | 0.090 |
| 0.8 | 0.485 | 0.009 | 0.227 |
| 1.4 | 0.845 | 0.014 | 0.364 |
| 2.0 | 1.203 | 0.021 | 0.548 |
| 5.0 | 2.953 | 0.051 | 1.395 |

[a] Level of salt in added water (62.3% on flour base)

### 8.2.2 Theory of $^{23}$Na NMR

Nuclear magnetic resonance (NMR) is one of the most powerful tools for determining the changes in mobility and the dynamic molecular interaction of nuclei. With a correct electromagnetic radio frequency (r.f.) pulse, one can follow the relaxation spins of a specific nucleus. The quadrupolar $^{23}$Na nucleus (spin $I = 3/2$), with its 9.27% receptivity relative to $^{1}$H nucleus and its short recycle time is an ideal method, using low field NMR, for non-invasive measurement of sodium ion motional states in a complex systems (Gobet et al., 2009).

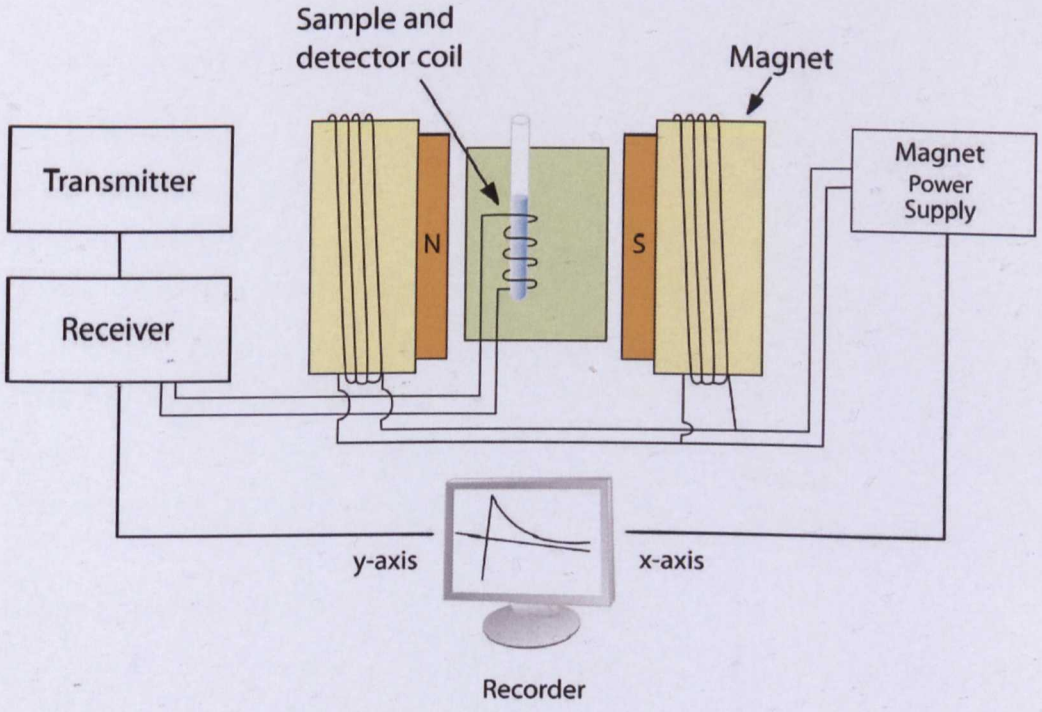


Figure 8-1. A schematic diagram of the $^{23}$Na NMR.

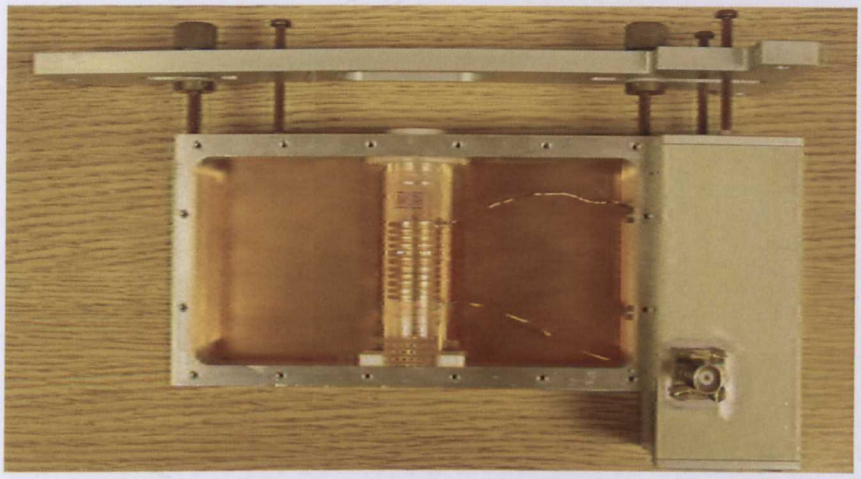

Figure 8-2. Prototype of a 10-mm $^{23}$Na NMR probe (built in house).

All the experiment in this chapter was conducted with an 8-mm $^{23}$Na NMR probe modified from the 10-mm prototype (Figure 8-2) and was fitted to the RI low field NMR spectrometer (Oxford, UK). The low field NMR spectrometer aligns the nuclear spins with the applied magnetic field. This spins are then manipulated by an r.f. pulse to produce transverse nuclear magnetisation. By detecting the small oscillating electric currents induced by the precessing transverse spin magnetisation, a plot of signal intensity against time can be obtained. This decay can be characterised by an exponential decay or relaxation time.

### 8.2.3 $^{23}$Na NMR experiment and data treatment

Dough samples were mixed according to the standard procedure mentioned in chapter 3. After mixing, a dough sample of ~ 1.85g was immediately introduced into an 8mm NMR glass tube and sealed. Transverse relaxation ($T_2$) was measured using the Carr-Purcell-Meiboom-Gill pulse sequence (CPMG) and the $^{23}$Na NMR conditions are presented in Table 8-1.

Table 8-2. $^{23}$Na NMR parameters for CPMG experiment.

| | | |
|---|---|---|
| Tau = 100 | Necho = 2000 | RG = 400 |
| NS = 1024 | P90 = 12 μsec | P180 = 24 μsec |
| SF = 6.04 | RD = 400 msec | DT = 75 |
| DW = 10 | SI = 1 | |

RD is the recycle decay between scans(s), P90 and P180 are the pulse width (μsec), DT is the dead time before and after applying the pulse (μsec), DW is the dwell time or Tau delay (μsec), which is the interval between two neighbouring data points, SI and Necho are the number of data points to be acquired and the number of echoes for CPMG sequence and NS is the number of scans.

The NMR transverse relaxation data ($T_2$) were analysed by muti-exponential analysis. A bi-exponential fitting of the data set was also tried, but this fitting seemed to "overfit" the data, thus 1-exponental analysis of $T_2$ relaxation data was deemed adequate and performed by fitting the absolute value of the CPMG, using the Winfit software (version 2.4., Resonance Instruments Ltd., 2004).

### 8.2.4 Standard salt-water solution

For the initial experiment, sodium chloride was dissolved into distilled water, at a concentration between 0.5 and 4%. Same volume of the NaCl solution (1.85g) was transferred to the 8mm test tube and sealed. Figure 8-3 shows the intensity of the CPMG signals increases with salt addition. This indicated that mobile sodium ion present in the aqueous solution can be detected under the present experimental conditions.

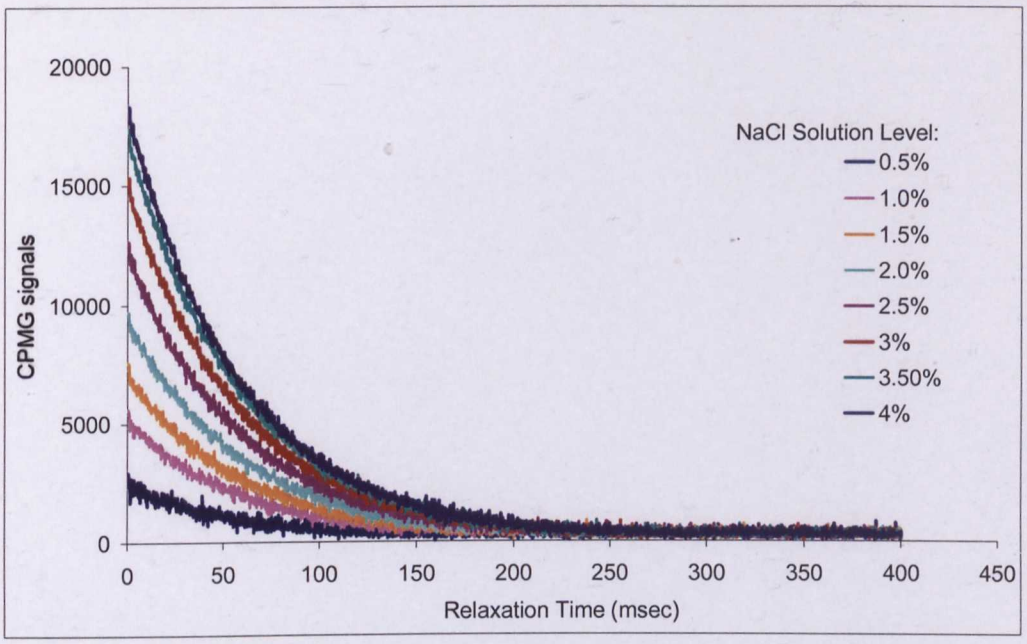


Figure 8-3. CPMG signals of sodium ions signals in the NaCl solution (values are the means from the 3 samples. Each sample was derived from five replicates).

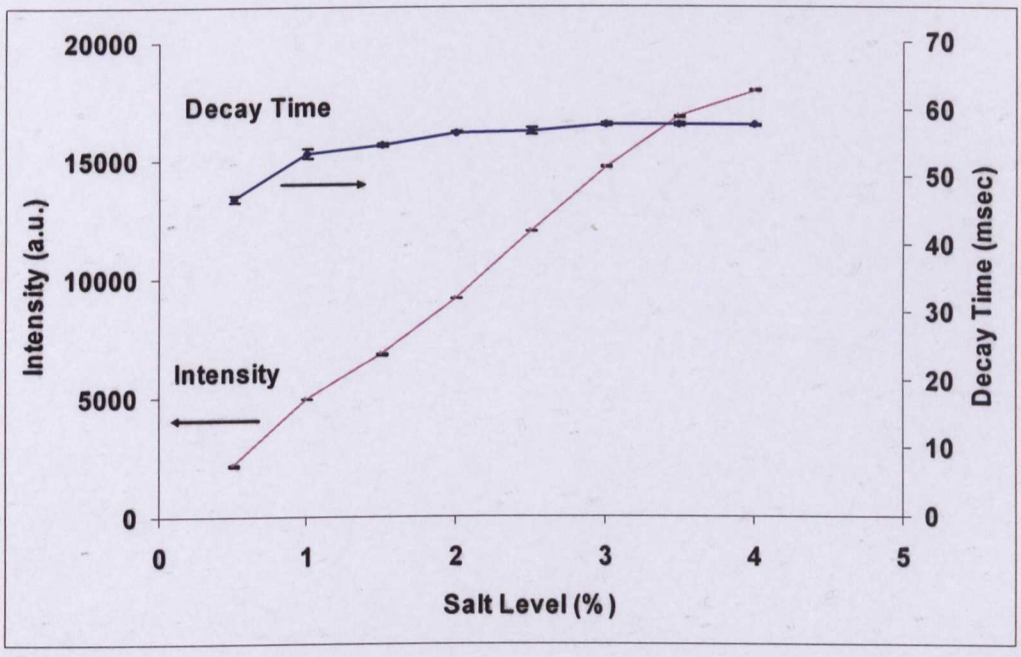


Figure 8-4. 1-exponential analysis obtained from a CPMG experiments of salt-water values from 0.5 to 4% salt (values are the means from the 5 samples $\pm$ 1 standard deviation. Each sample was derived from 3 replicates).

The 1-exponential analysis of CPMG sequence is presented in Figure 8-4. The $T_2$ decay time increased up to 2% salt content and then began to level off reaching a maximum relaxation time of about 58 msec. Since the experimental conditions were

set to measure only the sodium ions, the relaxation time should be the same at all the concentrations. The signal intensities increase with the salt concentration. The decay time is an indication of the mobility of the spins and the amplitude of the signal is proportional to the amount of sodium ions present in the samples. Thus the signal intensity can be used to quantify the amount of sodium in the dough system.

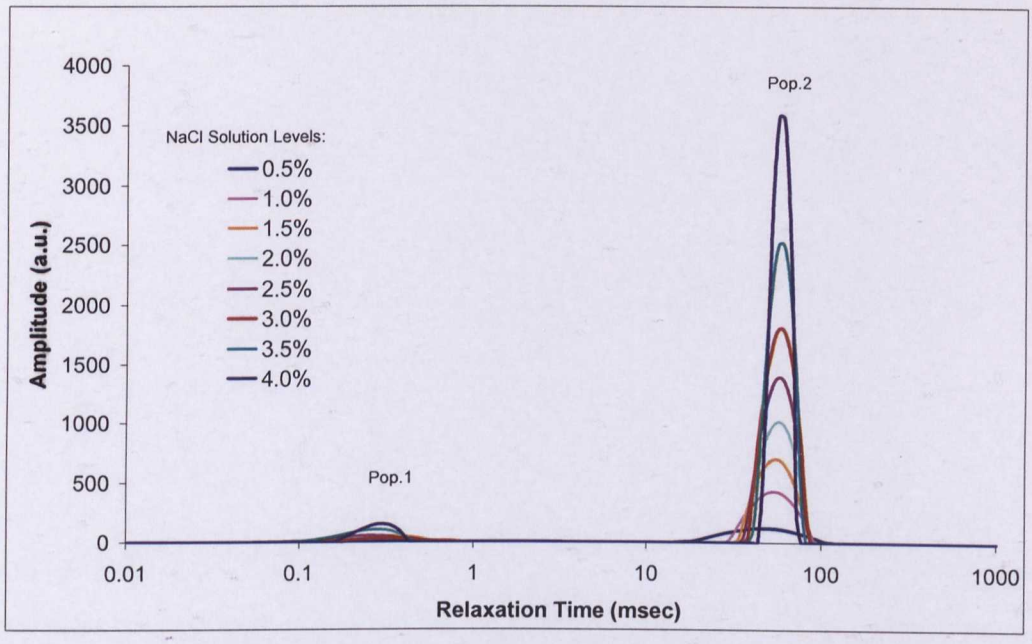


Figure 8-5. Continuous distribution obtained from CPMG experiment of sodium chloride water solution at different salt concentration between 0 and 4% (values are the means from the 5 samples ± 1 standard deviation. Each sample was derived from 3 replicates).

Figure 8-5 shows the continuous $T_2$ distributed curves obtained in different salt concentration. One main peak was detected at 30 – 90 msec. The peak magnitude is sensitive to the different salt concentrations, however, the relaxation time of the peak did not show a significant shift as salt concentration increases. A shorter relaxation component was also observed in the range of 100 – 1000 μsec. This component is expressed by only the first few data points in the raw $T_2$ relaxation curves, and it is not always reproducible in terms of its position and amplitude. This may be related to the impurity of the sample or instability of the NMR hardware such as coil ring down effect and thus reflects a processing artefact.

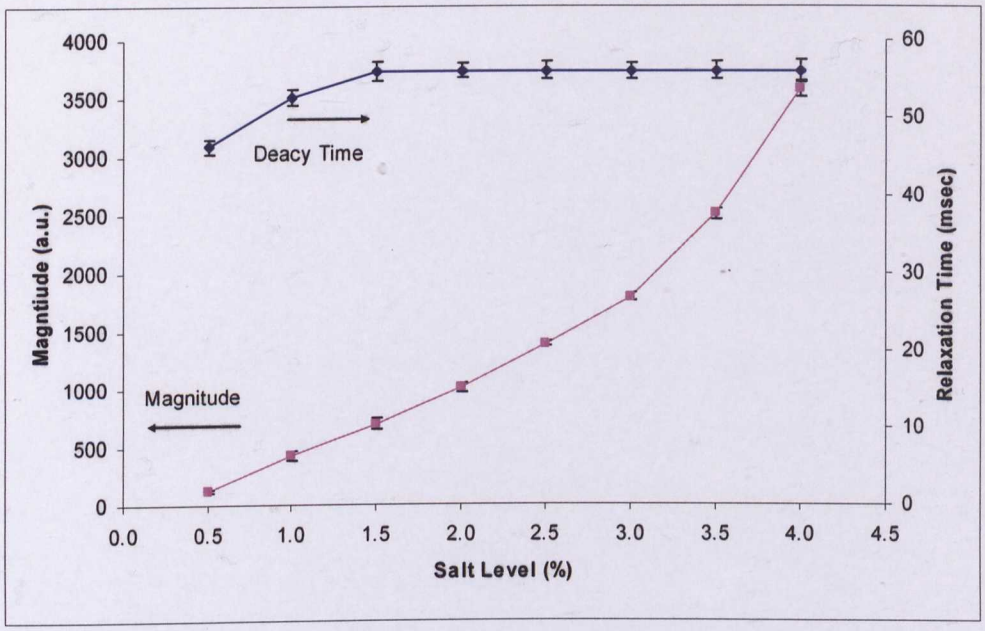


Figure 8-6. Value from the peak population (Pop.2) obtained from continuous distribution analysis. (n=5)

Figure 8-6 shows the relaxation time and magnitude of peak population (Pop.2). It showed a similar result as observed from the 1-exponential component analysis, where the magnitude of the peak is sensitive to the salt content in the system. This is because all the added salt can be considered as mobile sodium in this salt-water system.

### 8.2.5 Tweedy vs. Mini-pin mixing

Of primary importance to the work of this thesis is to ensure that the technical phenomena which are observed in the lab and mirror the experience in commercial bakeries. Hence doughs were prepared using the tweedy mixer (3 kg) and the minor-pin mixing system of (50 g) (see section 3.3.1 for details). The samples were made at 2% salt (flour base) and measured using the $^{23}$Na NMR over a number of cycles (equivalent to 2-hour time). The data obtained using the two mixing methods gave very similar results. Since, there was no difference in relaxation time ($T_2$) and intensity values (Figure 8-7) between the two types of mixing, only the minor-pin mixing results will be shown in the rest of this chapter.

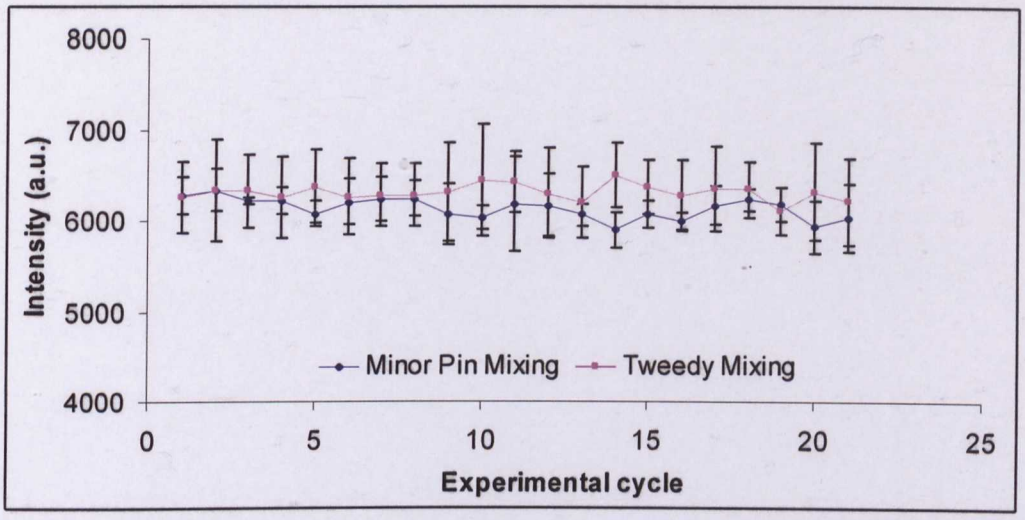


Figure 8-7. $T_2$ relaxation curves of CBP dough with 2% salt (on flour base) mixed with tweedy and mini pin mixer. (n = 3 with $\pm$ 1 standard deviation)

## 8.3 SODIUM MEASUREMENT IN DOUGH SYSTEM

Having shown that the $^{23}$Na NMR could be used to measure relaxation time for sodium in a quantitative way, the developed method was used to look at sodium mobility in a range of model systems relating to bread doughs.

### 8.3.1 Characterisation of sodium mobility in flour components

Figure 8-8 shows the $T_2$ relaxation time of gluten, starch, starch-gluten and flour mixtures at different salt concentrations. The $T_2$ relaxation times of all the samples were much lower than the salt-water standard and this implies part of the added salt is "bound" with other components in the sample. All three samples that contain starch, showed relatively lower $T_2$ values and may imply that starch may give a higher binding capacity than gluten.

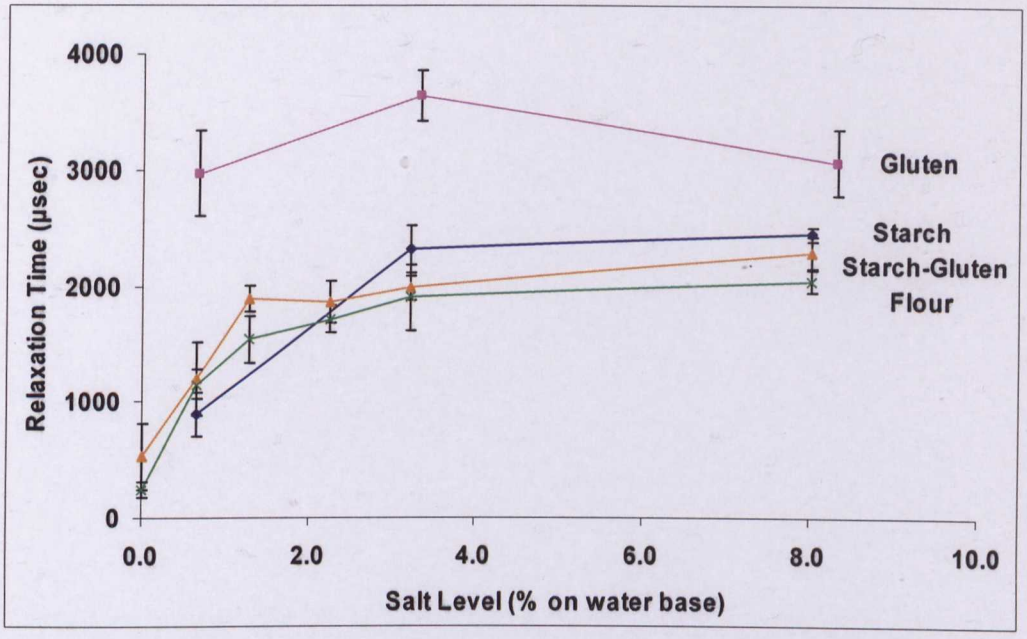


Figure 8-8. $T_2$ values obtained from CPMG sequence from 1-exponential analysis of the starch, gluten, starch-gluten and flour samples at different salt concentration (values are the means $\pm$ 1 standard deviation from the five doughs. Each mean value was derived from three replicate).

Starch, starch-gluten and flour mixtures show an initial increase of $T_2$ relaxation time until they reach a constant value at ~ 2 msec ($p \leq 0.05$). This indicated a progressive increase of the mobility of sodium ions in the aqueous phase. Although the relaxation time for gluten mixture show no significant difference between the salt levels ($p \geq 0.05$), all the $T_2$ values were higher then others mixtures. This implies more sodium ions are in the aqueous phase or there is a lower binding capacity for sodium ion to bind with the gluten matrix. However, water levels, as well as starch and mixing procedure would need to be understood to get a real understanding of the loss of mobility of sodium in the system.

Starch-gluten and flour model system generally followed the same trends indicating that starch and gluten are the main components influencing the mobility of sodium. Apart from starch and gluten, other constituents or starch damage in the flour may also influence the binding of sodium, as the flour samples have the lowest $T_2$ values.

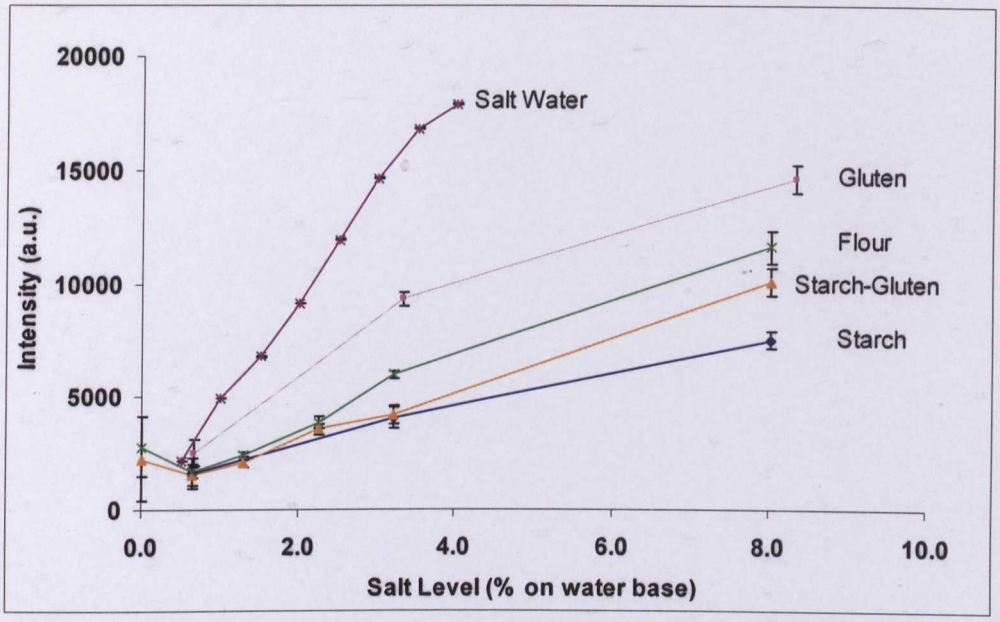


Figure 8-9. Changes in sodium ion intensity for salt water, starch, flour, starch-gluten and starch samples as a function of salt levels. All model system is in 100:62.3 solid: water ratio (values are the means $\pm$ 1 standard deviation from the five doughs. Each mean value was derived from three replicate).

The changes of $T_2$ intensities in the samples, as a function of salt content are shown in Figure 8-9 ($p \leq 0.05$). All the curves show an increase of sodium mobility as salt concentration increased. Salt-water samples show the highest intensity values, as all the added salt will be in the mobile state. The intensity for gluten samples showed significantly higher values than starch containing samples. At low salt concentrations, there was no significant difference between flour, starch-gluten and starch samples, however at 8% salt level, the difference in the intensity values became significant ($p \leq 0.05$).

### 8.3.2 CBP bread dough

CBP bread doughs, prepared with hard and soft wheat flour, were mixed at salt content between 0 to 5% (on flour base). Figure 8-10 shows the dependence of relaxation time and intensity values of the CBP dough samples on salt concentration. The Figure 8-10a shows an initial increase of $T_2$ relaxation time values until a constant value is reached. This increase could be a consequence of a progressive increase of the mobility of the sodium ion. At the low concentration of salt, increasing salt content increased the measured mobile salt present in the liquid phase

until the values plateaued at 1.5 - 2%. The salt may have altered the structure of gluten and increased the binding site for sodium ion at the higher concentration. This may indicate some change in the gluten to allow an increase of binding sites for sodium ion binding with the gluten matrix at these high sodium levels. The gluten proteins, especially the gliadins, may change their solubility (Larsson, 2002). However, at the lower salt levels, the binding of salt to the wheat flour seems less effective than that from the gluten mixture.

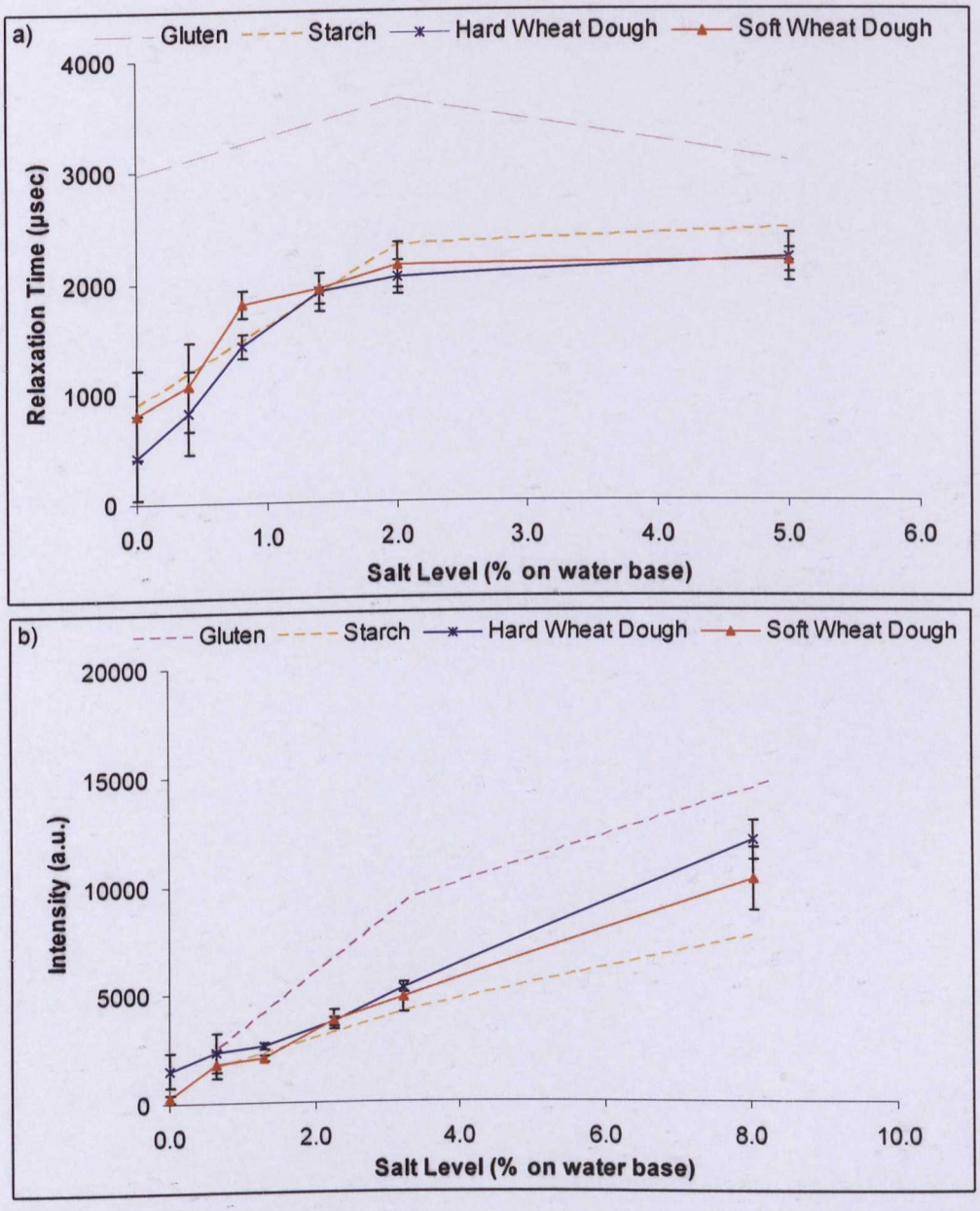


Figure 8-10. The (a) $T_2$ relaxation times and (b) intensity obtained from CBP hard and soft wheat dough (values are the means $\pm$ 1 standard deviation from the five doughs. Each mean value was derived from three replicate).

Both relaxation time and intensity values were lower than the salt water solution which implies a portion of the sodium ions interact or are tightly associated with the flour components and thus have restricted motion. It is clearly shown in this experiment that increasing the salt concentration increased the mobile sodium in the system for both hard and soft wheat dough samples (Figure 8-10). However, there are no significant difference between the hard wheat and soft wheat dough sample at any salt levels ($p \geq 0.05$). It is thought that the protein content and starch damaged level may influence the binding sites for sodium.

### 8.3.3 Sodium partition in dough system

It has been shown in Chapter 7 that a fluid phase can be spun out at high centrifugal forces. It was possible to measure the salt content in this fluid and sediment phases. Values from these two phases could then be used to calculate the amount of sodium ions, as detected by the $^{23}$Na NMR method within each phase.

Two methods can be used to calculate the amount of mobile sodium ions in the total dough system. One method is through direct measurement of the mobility of sodium in the whole dough. The second is to reconstitute the sodium ion concentration in each phase of the dough sample after centrifugation as a weighed proportion of the liquor and sediment phases.

Dough samples at different salt content were centrifuged (according to section 3.3.1.3) and each phase was measured with $^{23}$Na NMR. The sodium concentration was calculated assuming the intensity of the unbound sodium ions to be the same as in NaCl solutions and using the salt-water as a calibration. In the dough liquor phase, the dissolved sodium ions would be expected to be totally mobile and act as a free tumbling mobile fraction that can be detected by using $^{23}$Na NMR. Previous chapters mentioned that part of the water can be physically trapped in the gluten-starch matrix, but would still retain properties of mobile water. Thus sodium ions can be dissolved in this physically trapped water in the sediment phase and therefore would have the same mobility as free tumbling state. Other sodium ions may exchange with those entrapped in the matrix macromolecules and have a different measured mobility.

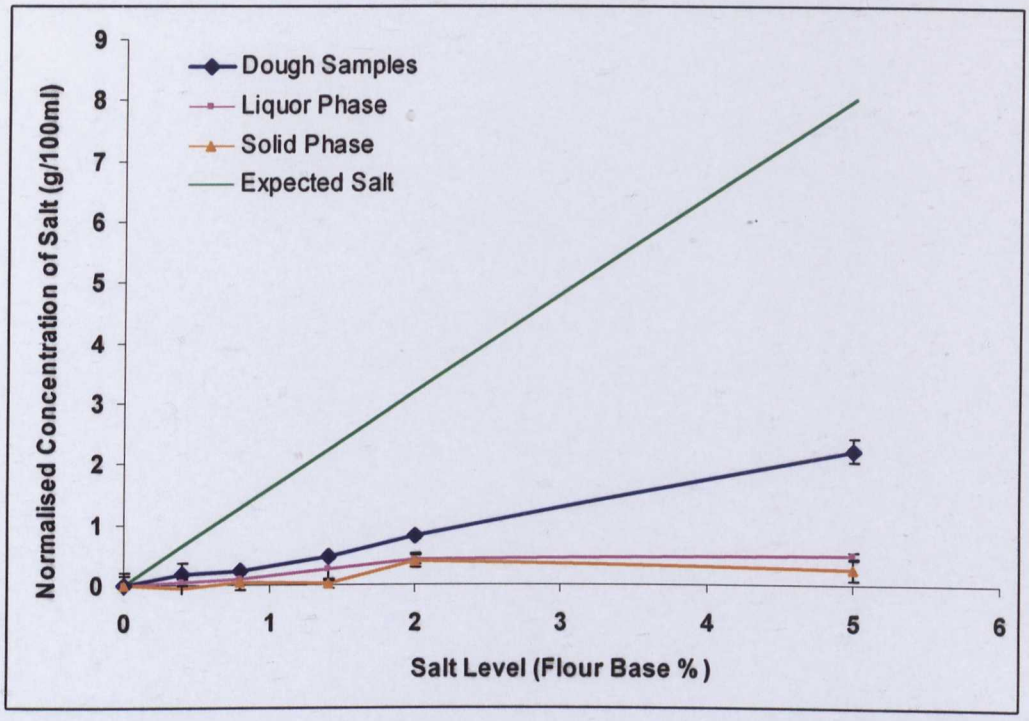


Figure 8-11. Concentration of salt in different phases of the CBP hard wheat dough at salt levels between 0 – 5% (on flour base) (values are the means ± 1 standard deviation from the five doughs. Each mean value was derived from three replicate). "Expected salt" represents the concentration of salt dissolved in water phase.

Figure 8-11 shows the salt concentration, as measured using salt-water calibration, of the dough samples, its resulting supernatant (dough liquor) and sediment phase. Dough liquor contains more dissolved sodium while the sediment phase contains the least mobile sodium, as expected. Both calculations showed that the measured sodium ions were less than that added to the formulation for the dough. In this context, the non-detectable sodium ions are thought to be in restricted in their motion and would indicate the presence of "bound" sodium ions.

The same set of experiments was carried out with soft wheat flour (Figure 8-12). From measuring the sodium ion in a whole dough system, similar results were observed, however the partition of sodium in different phase were different. Dough liquor phase from soft wheat samples contains a higher amount of detectable sodium then sample prepared with hard wheat flour and the sediment phase showed only a small portion of the sodium ions are in immobile in terms of the estimations used.

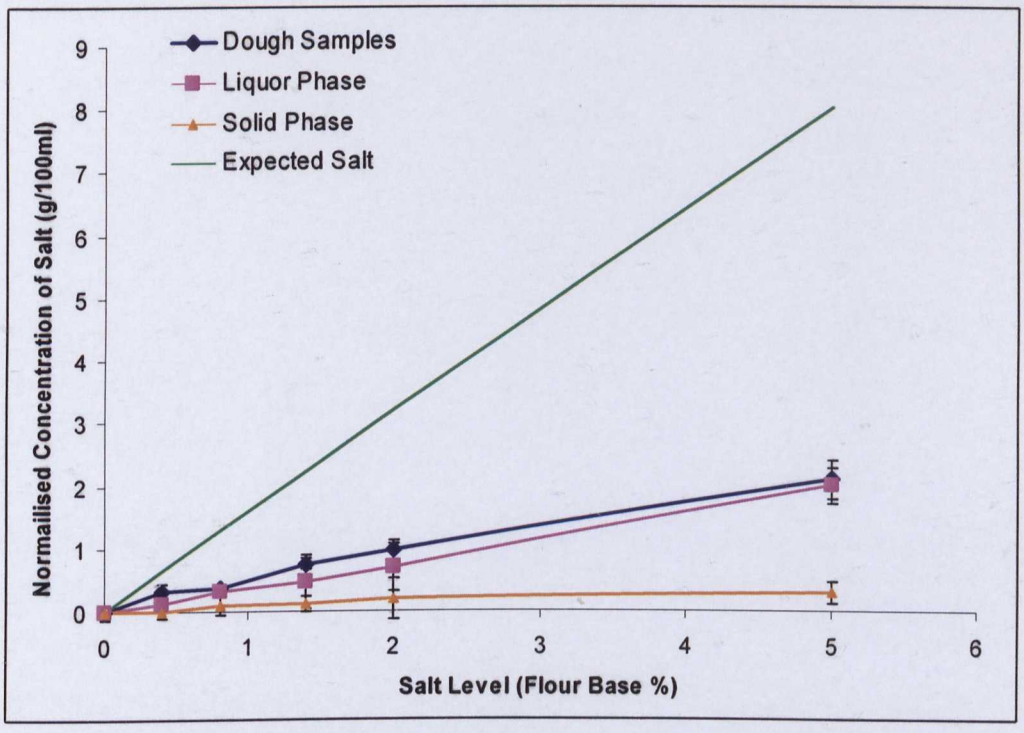


Figure 8-12. Concentration of salt in different phases of the CBP soft wheat dough at salt levels between 0 – 5% (on flour base) (values are the means ± 1 standard deviation from the five doughs. Each mean value was derived from three replicate).

In order to calculate the levels of sodium ions detectable in the dough sample, two concepts were considered:

1. The dough is regarded as a two-phase system which consists of the fluid phase (added water) and the inert filler (starch component). The added recipe salt is dissolved in the water phase and only these sodium ions can be detected by $^{23}$Na NMR.
2. The dough regard as one-phase system as the sodium ions are equally mobile in the water phase and the flour fraction.

By using the standard curve obtained from the salt (NaCl) solution, the amount of detected sodium ions can be predicted. The sodium detected in the dough and dough fractions can be used to see if either hypothesis can explain the sodium partition in the dough system.

Figure 8-13 showed the percentage of visible sodium detected by the present $^{23}$Na NMR experimental conditions by assuming all the added recipe salt is dissolved in the water phase and only these sodium ions can be detected.

Soft wheat flour samples show a decrease in detectable sodium ions as salt levels increased in the dough samples indicate a decrease of the sodium ion mobility. However, hard wheat flour dough samples show an initial decrease of mobile sodium ions then gradually there is an increase as salt concentration increase in the dough sample. Surprisingly, the sodium concentration from reconstituting the dough phases showed a different trend.

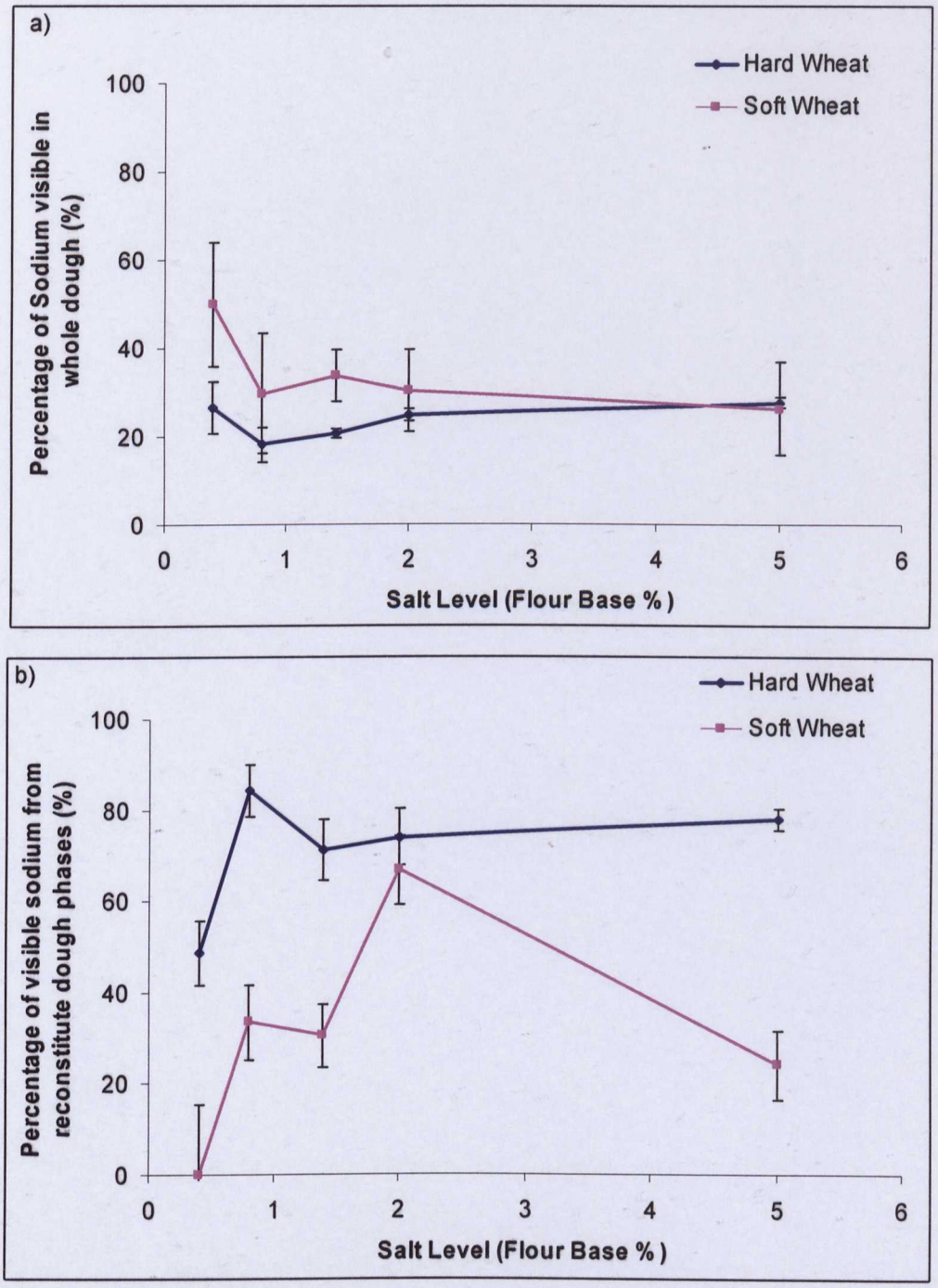


Figure 8-13. Percentage of visible sodium ion can be detected in the (a) whole dough and (b) reconstitute of the dough phase by assuming all the sodium is dissolved into the water phase.

Another method of calculating the percentage of visible sodium in the dough system is to assume all the sodium is equally partitioned within the dough samples. Thus, the sodium ions would be equally mobile in the added water and flour fraction. Figure 8-14 shows similar trends were observed for both the whole sample and the reconstituted samples.

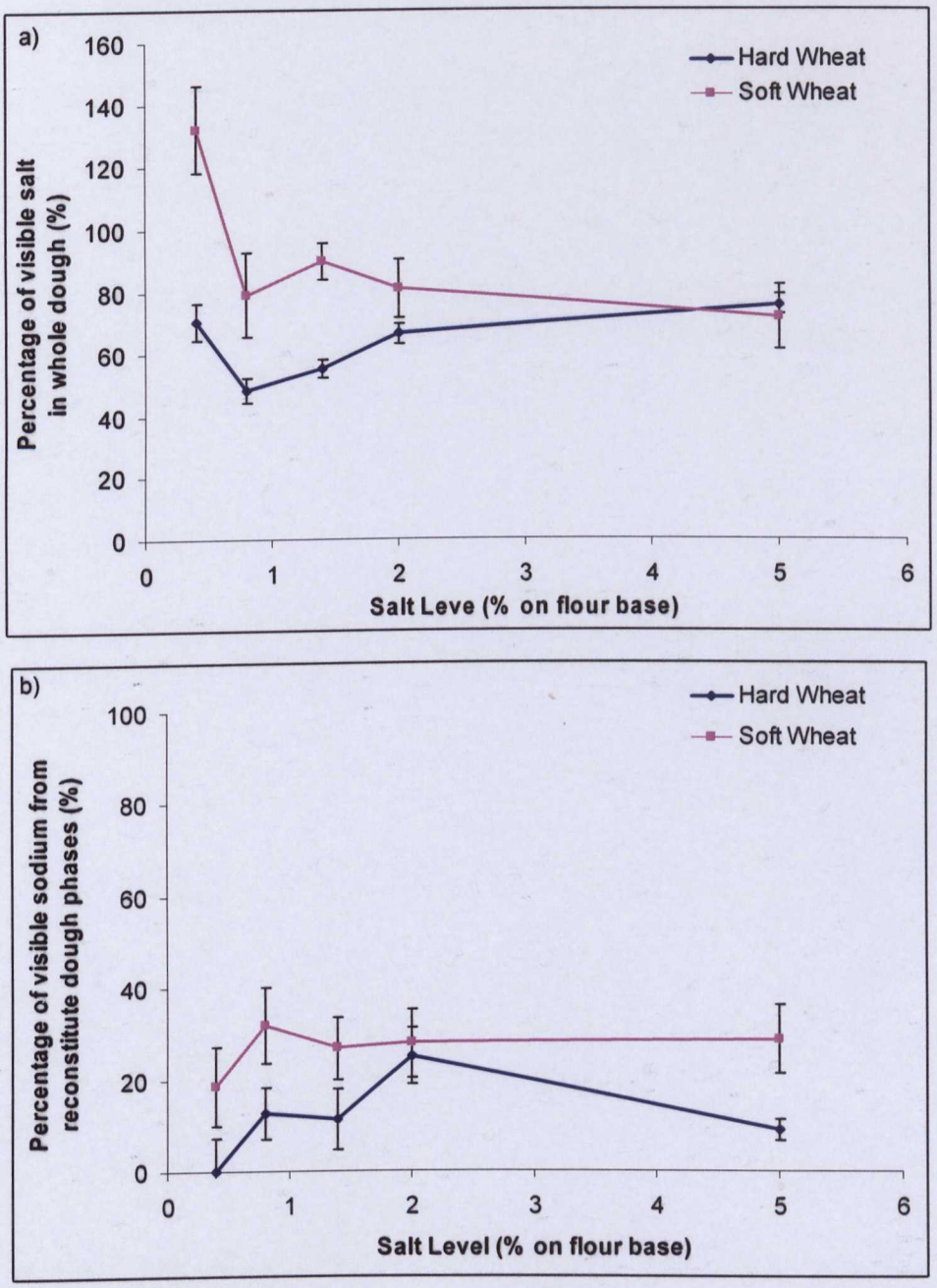


Figure 8-14. Percentage of visible sodium ion can be detected in the (a) whole dough and (b) reconstitute of the dough phase by assuming all the sodium ions are equally partition in the whole dough.

Both calculations indicate that a large portion of sodium ions were not detected and can be considered to be tightly associated with the gluten-starch matrix in the dough system. In this range of salt addition, the normalised "bound" sodium fraction increases with increasing salt content.

### 8.3.4 Effect of Resting Time

A factor considered by the commercial bakers to be relevant to sodium chloride levels is the resting time. It was possible that the mobility of the sodium may change of the dough relaxes. This could be due to exchange phenomena at vary prolonged time scales, changes in solubility or fluidity changes. Therefore $T_2$ values were recorded for doughs over extended time scales. One experimental cycle for the recording of the CPMG sequence took two minutes and 20 cycles were recorded. This time scale is relevant to the process of bread making (~40 minutes). The values for intensity are given in Figure 8-15. However, the possibility of salt redistribution would seem be negligible, since $T_2$ intensity of sodium ions underwent almost no change during storage (Figure 8-15).

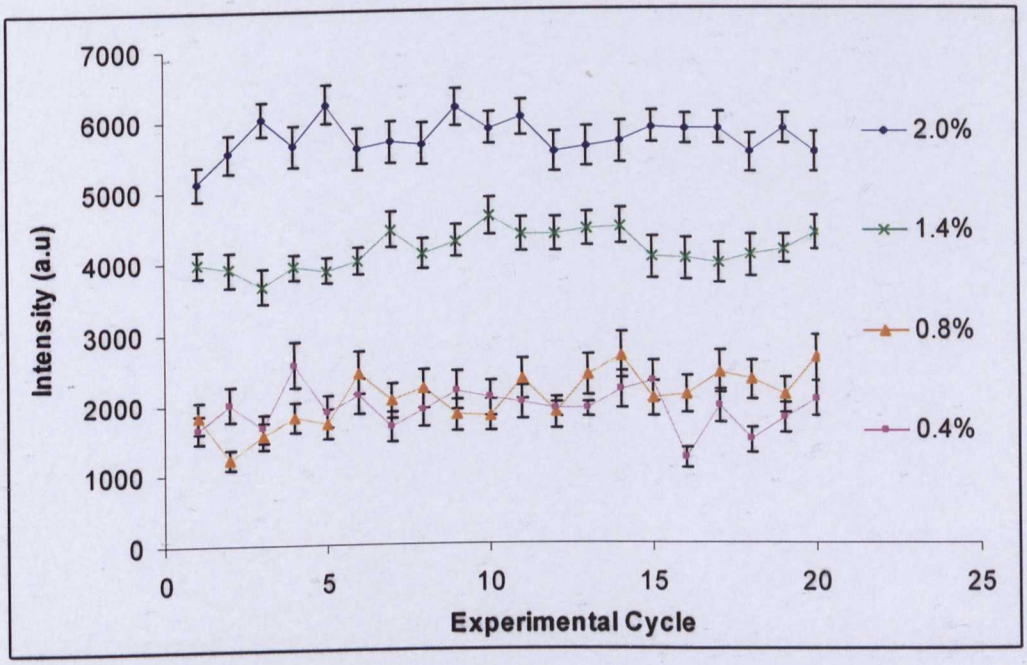


Figure 8-15. Intensity values of CBP dough obtained from CPMG sequence and analysed through 1- exponential component at different salt concentration. (values are the means ± 1 standard deviation from the five doughs. Each mean value was derived from three replicate).

## 8.4 CONCLUSION

A method has been developed that can indicate the amount of "mobile" sodium in starch based systems. The results from this chapter also showed that low field $^{23}$Na nuclear magnetic resonance ($^{23}$Na NMR) could be used as a rapid and non-destructive technique which offers a unique opportunity to visualise salt distribution in bread and dough, and might be a useful tool for optimising the use of salt in food processing. Although the state of sodium is governed by many interactions, starch and protein interactions with sodium would be the main components influencing the binding capacity in the dough system. Interestingly hard wheat and soft wheat flour have a similar binding capacity. It is therefore not just protein that influences sodium-macromolecular interactions. Although it is simplistic to think of the only differences between strong and weak flours to be the protein content, it does appear that gluten may not be the major component binding the sodium ions, on the other hand, the starch component reduces its mobility to the major extent.

The concentration of sodium in the dough samples were calculated from a calibration based on the NaCl solutions. The dough samples and the reconstituted phases were calculated to have less than the added recipe salt. This implies that part of the sodium is under restricted motion and could be considered to be tightly bound with the gluten-starch matrix in the dough system.

# CHAPTER 9. GENERAL CONCLUSION AND DISCUSSION

On discussion with the commercial bakers, many different quality changes were given as occurring when salt levels were reduced in bread formulations.
These included, in addition to lack of flavour,:

- More sticky during processing
- Less elastic doughs
- Yeast gassing changes
- Poor stability if proved doughs are knocked
- Change in volume of final breads
- Poor colour development of crust and crumb
- Open texture of the final bread crumb

Generally all these changes to the bread are combined into the term "loss of process tolerance" and results in an increase in waste products. The loss of process tolerance is thought to be exacerbated by high ambient temperatures in the bakery and prolonged storage time of the doughs.

Currently the typical salt level in UK plant white bread is 1.25g/100 g of bread (Kilcast et al., 2007). This equates to 2% salt (based on flour weight) in the dough and it was one of the major formulations used throughout the work on the breads and doughs. The other main formulation was 1.4% salt that is the level required in the FSA's 2003 salt model, which would equate to 350mg sodium or 0.875g salt per 100g bread.

**Dough stickiness**

Despite more than 60 dough preparations, skilled workers and controlled bakery condition comparisons between the breads showed few striking differences due to the variation in the two salt levels examined, 2 and 1.4%. In phases two and three of the bread making programme, the formulation utilised basic improver, none of the breads were of the final quality that the bread manufactures would routinely sell, however, failures specifically due to the salt levels were difficult to define. The one area that was identified with certainty was that machine moulding of the bread dough

became more difficult at lower salt contents. This was especially noticeable when dough temperature was elevated, or when the delay time between mixing and moulding was increased. These same doughs had been tested for the degree of stickiness using the Chen Hoseney cell at CCFRA, which demonstrated the opposite effect (i.e. less sticky) at lower salt levels. Thus, a quantitative laboratory test is required to match the stickiness of doughs, as seen during processing, if formulations are to be readily modified to ameliorate the low sodium levels required in the dough.

Work in Chapter 7 shows some attempt to use large blocks of dough to measure stickiness of newly created surfaces. However, this method like the Chen-Hoseney and the TPA methods, did not show the expected correlation between the degree of stickiness and salt levels. The question should be asked of why the salt may impact on stickiness. Stickiness itself is a difficult parameter to define in fundamental terms. It can be considered that it is due to cohesive forces within the matrix being less than the adhesive forces between the matrix and the material surface/probe. The rate at which the surface is peeled away from the material is also one of the critical factors. There has been some work on peel tests for doughs, but this in relation to stickiness and salt could be a fruitful area for further work, as it was the one area that was obvious when using the low salt breads.

**Oven spring**

Another feature of the test breads showing a significant difference with the different salt levels was their final volume. As the breads were proved to the same height, the difference in their volumes could arise due to the amount of dough expansion in the oven. There could be several reasons why the sponge foam transition may be dependent on the salt concentration. Even at the relatively low level of salt present in the dough systems (<0.6Mol), the gelatinisation temperature of the starch in the doughs was affected. The mid-point of the gelatinisation for doughs with no added salt was about 64.5°C, while it was ~69°C when the salt levels were 2% (flour basis). The delay in gelatinisation therefore allowing gas expansion for a longer period in the oven which could explain the observed differences in volume. However, other factors may also influence the final bread volume: for example, higher gassing rates in the yeast at elevated temperature, or differences in the available water in the system.

**Testable hypotheses**

Much of the work discussed in this thesis was designed to establish whether there was a relationship between the aqueous liquid phase in dough and salt levels. Changes in the fluidity of the dough, and the concomitant changes due to solubility, viscosity etc. could explain the spectrum of phenomena (structural, physical, chemical and sensory properties) said to alter in bread doughs due to changing salt levels.

Three techniques were developed to assist in the understanding of the physical state of water in the bread doughs. To investigate these techniques, doughs were prepared with different levels of water and the fluid properties measured.

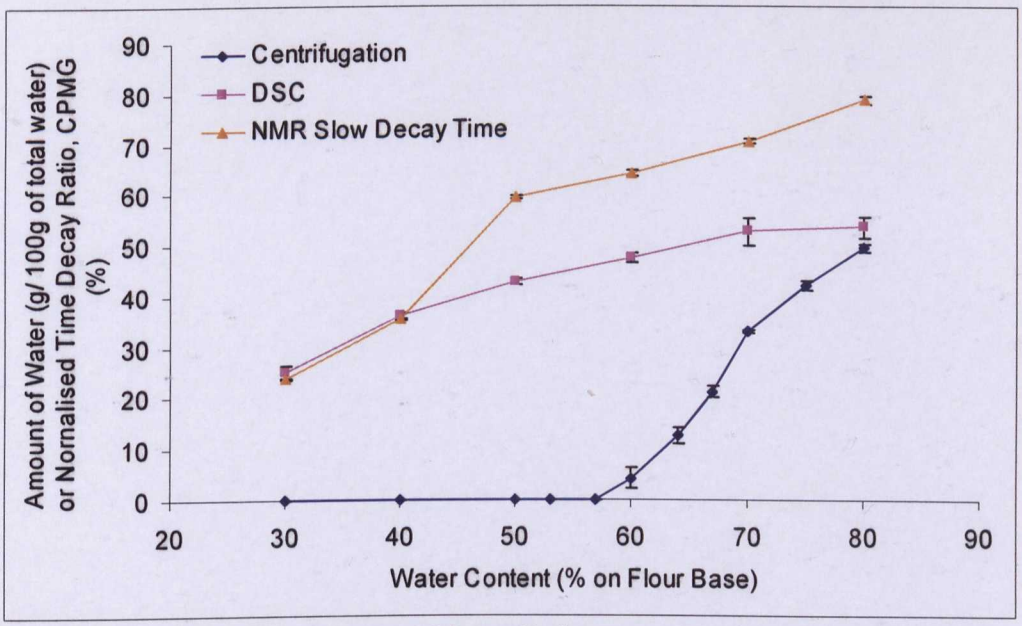


Figure 9-1. A summary of the percentage of detected bulk water at various water content between 30% and 80% (flour base) from three different techniques.

Figure 9-1 shows the combined results from ultracentrifugation, differential scanning calorimetry and $^{1}H$ nuclear magnetic resonance on the detection of bulk water in the dough system. Results are presented in gram of detected fluid per 100g of total added water, or for the NMR result, the values are the percentage of the normalised slow decay signal. Two techniques, NMR and DSC freezable water, show that added water to a level > 30% of the formulation increased the detectable water content.

However, results from ultracentrifugation showed that free liquid could only be isolated when it mixture is above 60% water content.

A higher percentage of detected bulk water in the dough was observed from $^{1}$H NMR and DSC compare with dough liquor recovered from ultracentrifugation. In addition, both the $^{1}$H NMR and DSC results showed a similar trend with respect to the detection of bulk water. The differences between the observed bulk water in the dough system can be explained as the three techniques measure very different fluid properties.


Figure 9-2. Simplistic schematic of the measures of fluidity by the three methods used for detection. (a) proton NMR showing proton motion; (b) represents ice formation within the polymer network system; (c) represents drainage of liquid water from the polymeric system due to centrifugal forces.

Figure 9-2 attempt to show the different mechanisms by which fluid measures were assessed in the different techniques. $^{1}$H NMR measures the mobility of water molecules and the distance for self diffusion. DSC measures the amount of water by freezing the "free water" in the dough sample. In order to grow ice crystal, a significant amount of space is needed. Ultracentrifugation uses high force to induce the flow of water from the dough matrix.

In all these systems the water/fluid is influenced by the matrix and water molecules will exchange from being associated with the fluid phase to being less mobile and associated with the rest of the polymer. It seems that $^{1}$H NMR measured the total responses of protons motion within the dough system. Tang et al. (2000) identified three water populations inside potato starch granules. These were assigned to water in the amorphous growth rings as well as water in the semi-crystalline lamellae and

in the hexagonal channels of B-type amylopectin crystals. The water present at different sites may change the motion and may show same properties as bulk water as characterised by isotropic rotation and a diffusion coefficient identical to that of bulk water. Relaxation can be dominated by the short $T_2$ of the exchangeable protons on the biopolymer (Hills, 1998). Therefore starch as well as protein and other materials will influence the detection of the mobility of the protons in the dough.

DSC measures the amount of water by freezing the "free water" in the dough sample. In order to grow ice crystal, a significant amount of space is needed. NMR results from other studies show that unfreezable water and even water in glasses is very mobile; not too dissimilar from "free"water (Li et al., 1998). Li et al. (1998) showed high mobility of water was observed even for supposed "bound" or non-freezable water fraction.

One explanation on unfreezable water detected with DSC is that it is not the fraction of water that is "bound" in any way, but it is a result of the biopolymer system cannot crystallise in a conventional eutectic sense (lowest possible temperature of solidification). Belton (1997) suggested that this may due to the system containing entangled polymer networks, and therefore cool with the formation of ice but still associated with a fraction of water. At equilibrium, where there is no driving force to produce more ice, the activity of the water phase must be reduced to equal that of the ice. This liquid water is therefore the unfreezable fraction, but the arguments are thermodynamic in nature and do not involve a bound fraction (Belton, 1997). This occurs by the reduction of activity by the dissolved solute (see red arrow on Figure 9-3).

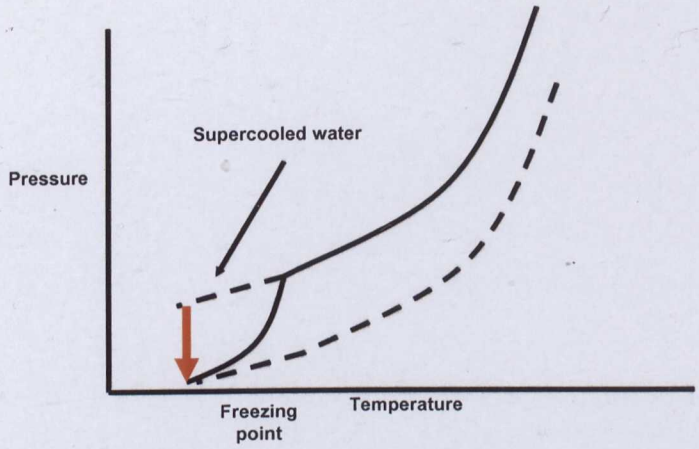


Figure 9-3. A schematic phase diagram of water at low pressures (not to scale).

Ultracentrifugation uses high forces to induce the flow of water from the dough matrix. The uncooked dough is a mixture of a foamed gluten matrix in which native starch granules are embedded. Partitioning of ingredients occurs as gas bubbles are formed in the dough and new hydrophobic surfaces are created.

Much of the starch is distributed as long fibrous strands interwoven with the protein network. The gluten network would not allow the easy flow of water through the structure and therefore the strength of the dough could be expected to reduce the ability of water to pass out of the system. It could be envisaged that the water would flow through capillaries within the structure and changes in the size of these pores and/or the surface tension of the fluid would influence the amount of bulk water collected by centrifugation.

The three methods developed for the detection of fluid levels are sensitive to about 5% changes in the water levels (flour weight basis) and therefore if salt alters the fluidity of the doughs by this amount it should be detectable.

**Effect of salt on the water distribution in the flour dough system**

Flour type, starch damage and improver all influence the amount of water that drains from the sample during ultracentrifugation. By changing from a hard flour to a soft flour, the amount of added water recovered by centrifugation changes from about 18% to 45%. In addition, the amount of water does increase with salt concentration,

but the amounts are small, on average 4% more water at 2% salt levels compared to a non salt control.

However, there was no comparable increase in the amount of mobile or freezable water when salt was added. At the length scales of proton mobility or for the amount of ice formation, the salt did not seem to impact on the flour dough system.

Preston (1989) stated that when salt is added to the gluten dough, it modifies the physical properties of the gluten by changing the electrostatic shielding of ionic amino acids on the surface of the gluten proteins, thus lead to the reduction of water absorption in the dough system. This non-associated water in the matrix is thought to be "mobile" and can be detected by $^{1}H$ NMR. However, for the $T_2$ data, the noted changes probably would be evident as decreases in the $T_{21}$ population (less mobile water) and coupled with an increase in the $T_{22}$ population (more mobile water). From our study, $^{1}H$ NMR experiments did not show any significant changes in the water mobility of both populations in the dough samples. In addition, the "free" water is thought to have the same freezing properties as bulk water, however, from DSC results, no significant increase was observed when the salt level increased. It can thus be concluded that the observed phase from centrifugation suggests that the extra dough liquor did not represent the true water around the gluten-starch matrix by addition of salt to the dough samples as the net enthalpy ($\Delta H$) of the water was not affected.

It could be concluded that the salt is therefore only influencing the matrix to the extent of the network formation, and that the strengthening of the gluten structure due to the presence of salt therefore allows for greater drainage of the fluid from the system.

Salt can modify the dough matrix in two ways. The general tendency of salts to reduce the water absorption of protein also adds to the electrostatic shielding of ionic acids on the surface of the gluten proteins and thus induces stronger inter-protein hydrophobic and hydrophilic interactions. This was confirmed by a rheological experiment (Kieffer extensibility test). In addition, with the presence of salt, water absorption into the starch granules would be reduced due to increase competition for

binding sites. These two effects increase the available free water in the dough matrix. Thus, free water could lubricate particles and enhance flow. As a result, more dough liquor can be centrifuged under high force. However, the results show that ultracentrifugation did not reflect the true distribution of water in the dough system. This phenomenon could not be attributed solely to ionic shielding effect, it is more likely to be due to the "drainage" properties from the gluten-starch matrix, and is not the consequence of the "free" water in the system which is controlled by properties of the gluten-starch matrix rather than the consequence of the mobility of water in the system. One mechanism to explain the effect of release of liquid from the gluten-starch matrix is the capillarity phenomenon. Generally, lower capillary forces would permit greater drainage and cause by larger diameter capillaries and lower surface tension. In this case, one might hypothesis that salt may affect the bridges between the polymers in the gluten-starch matrix, or the extraction of surface active compounds in the liquid phase. Glutenins increased the inter-disulfide bonds which strengthen the matrix. Rheological results confirmed that salt strengthen the properties of the dough can be caused by the electrostatic shielding of ionic amino acids on the surface of the gluten proteins (Preston, 1989). By strengthening the gluten network, more liquid can be isolated before the collapse of the matrix when the dough sample is under high centrifugation force. In addition, results showed that salt affects the presence of proteins and lipids appear to have an impact on the proposed drainage phenomenon. Some proteins are salt soluble and can easily extracted into the aqueous phase, act at interfaces and alter the rheology. However, the results showed in this thesis differ from other studies in that no increase in proteins was found in the centrifuged liquor on addition of salt.

It can be concluded that the distribution of water in the dough was not influenced by the levels of salt used in the studies, but fluid can be more easily forced out of the matrix formed in the presence of salt, probably due to changes in the protein network.

**Sodium partition on the dough system**

In a similar way to the measurement of proton mobility, sodium ions can also be measured by NMR. Although sodium NMR is a known technique, it has not been utilised to look at doughs (as far as the current literature would suggest) and the

technique was new to Food Sciences at University of Nottingham. The prototype probes created were found to be quantitative for sodium and as long as temperature and filling heights of the probes were controlled the amount of sodium in the doughs or the centrifuged portions of dough could be followed.

Although the mobility of sodium is governed by many interactions, where starch and protein interactions with the sodium are likely to dominate the mobility of the ions, $^{23}$Na NMR data was found to provide a useful insight into the behaviour of sodium ions in the dough system.

To try and understand how the sodium interacted with the ingredients of the dough, a simple two-state model was evoked. Two alternative models were proposed to understand partition of sodium ions in the dough system.

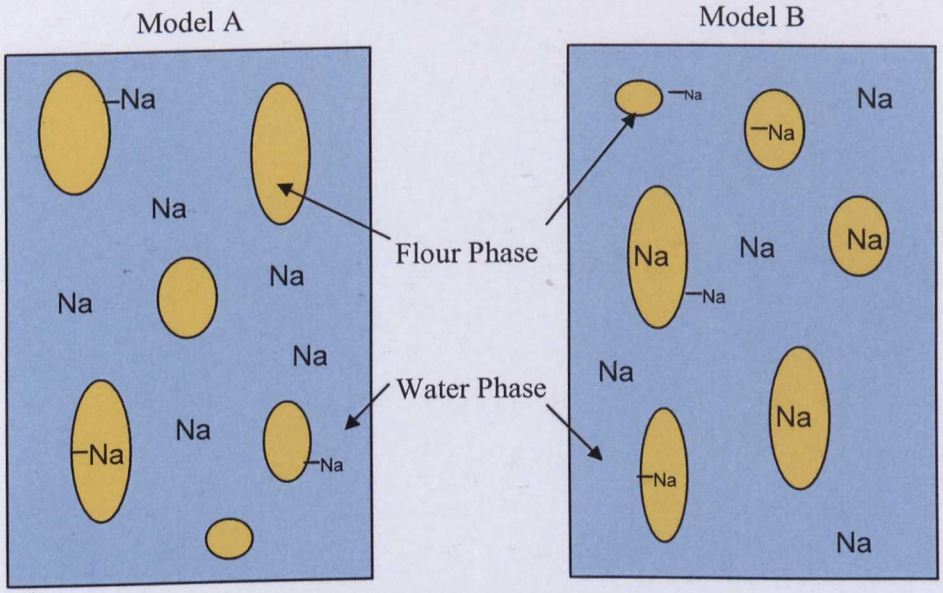


Figure 9-4. Proposed model of salt partition in the dough system, A = the mobile sodium is only in the liquid phase, B= the mobile sodium is equality distributed between the solid flour particulates and the fluid in the dough. –Na represents the restricted motion sodium, (non-detectable) Na represents the free motion sodium (detectable).

Model A assumes that only the sodium ions in the water phase can be detected by the present $^{23}$Na NMR parameters. Flour components act as inert filler in the system and no interactions are occurring between sodium ions and flour. Thus sodium ions are only undergoing free motion.

An alterative model (Model B) is that the sodium ions are equally partitioned in the dough system within the flour components and water phase. In this model, sodium ions associated with flour components and also dissolved in the water phase are in free motion and can be detected from the present $^{23}$Na NMR experimental conditions. Thereby, the concentration of sodium throughout the whole dough would be the same. With this hypothesis, sodium concentration on the whole dough basis can be used to calculate the partition of sodium in the dough system. Figure 9-6 shows the theoretical levels of sodium that would be detected based on the two models of salt dispersion in the dough.

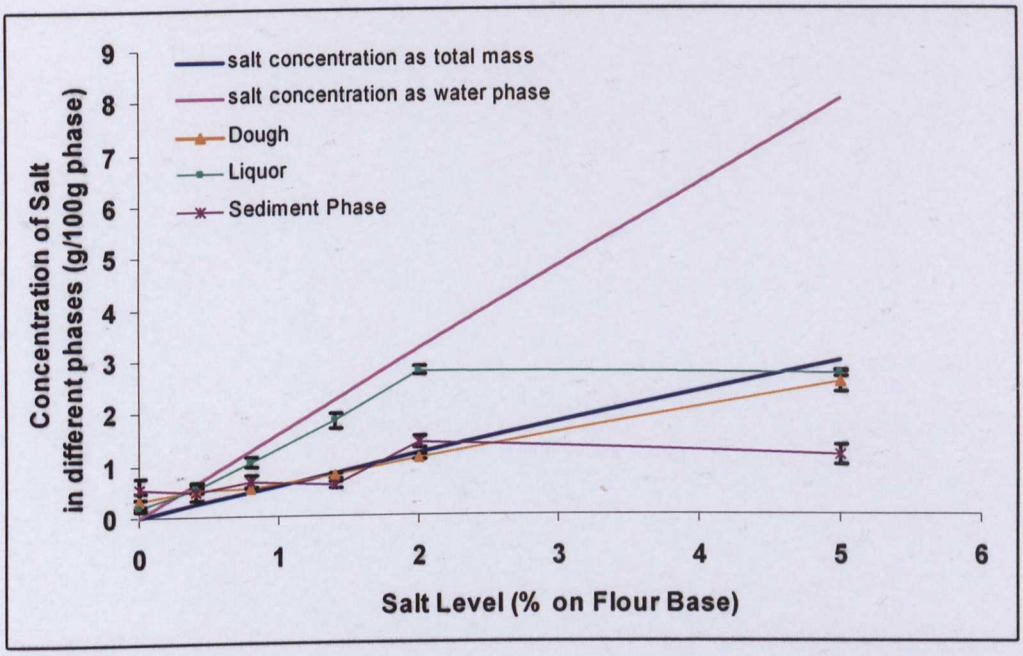


Figure 9-5. The theoretical levels of sodium that would be detected from $^{23}$Na NMR if all the sodium was mobile in the liquid phase (▬); if all sodium was detectable and equally distributed in all phases (▬) and data for the whole doughs, the centrifuged sediment and the supernatant. (Data from hard wheat dough samples)

From the $^{23}$Na NMR data, the level of salt detected in the dough is less than the prediction of either of the models, indicating that a considerable proportion of the sodium is "invisible" to the NMR as an implication of non mobile. The level of sodium detectable in the centrifuges supernatant is lower than the model predicted for all the sodium being partitioned to the fluid phase, and suggests that a major proportion of the salt is fixed to the matrix. The sodium quantities detected in the

fluid phase by NMR similar or to those measured by flame photometry and therefore the NMR method seems to be valid for this type of calculation.

As salt concentration increases to above 1.4%, an equilibrium between the "bound" and "free" sodium ions seems to be established, with about 80% of the sodium being mobile and detectable in the whole dough. Some work with the different components of dough showed that it is the starch that reduces the mobility of the sodium ions more than the gluten. However, in a full formulation, the gluten may interact with the sodium to a different extent.

It was expected that loss of sodium mobility may equate to the number of binding sites in the dough system and that a minimum amount of sodium would have to be present to "fill" these. This did not seem to be the case and the loss of sodium mobility seems to be proportional to the total concentration of the sodium in the formulation. Further work would need to be carried out to establish if this is the case and what impact the different levels of sodium has on the dough systems in terms of its processability and final bread quality.

The studies reported in this thesis provide more evidence on the physicochemical properties that could be involved with salt in bread dough, but there is still no direct proof of how salt influences the stability of dough and thus the dough quality. Further studies are needed to investigate the inter-relationship between salt and the physical properties of bakery products so that compensatory mechanisms can be devised to improve the process tolerance of bread with reduced salt. The fact that this study concentrated on one ingredient (salt) does not imply that other ingredients such as yeast, fat and improver, are any less important and may interact to provide quality products.

**Future work**

Extend the multi-technique study of water distribution to a range of different temperature and resting times to assess the potential factors affecting the dough and bread quality.

- Characterise the soluble components in dough liquor phase to understand the potential role of the aqueous liquid phase.
- Assess the role of salt in whole meal brown bread.
- Measurement of dough quality including stickiness.
- The role of other ingredients such as improvers and yeast in the dough quality in low salt system.

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# APPENDICES

## Appendix A: Supporting information for Chapter 5

### A 1. Table of Samples for Commercial bread dough

| Std Order | RunOrder | CenterPt | Blocks | Improver | Added Water | Starch Damage | Flour | Salt |
|---|---|---|---|---|---|---|---|---|
| 20 | 1 | 1 | 3 | Compound | Lowest | 35 | English | 2 |
| 21 | 2 | 1 | 3 | Basic | Highest | 25 | Canadian | 2 |
| 24 | 3 | 1 | 3 | Compound | Lowest | 35 | Canadian | 1.4 |
| 19 | 4 | 1 | 3 | Basic | Highest | 35 | English | 2 |
| 22 | 5 | 1 | 3 | Compound | Lowest | 25 | Canadian | 2 |
| 23 | 6 | 1 | 3 | Basic | Highest | 35 | Canadian | 1.4 |
| 17 | 7 | 1 | 3 | Basic | Highest | 25 | English | 1.4 |
| 18 | 8 | 1 | 3 | Compound | Lowest | 25 | English | 1.4 |
| 4 | 9 | 1 | 1 | Compound | Lowest | 35 | English | 2 |
| 1 | 10 | 1 | 1 | Basic | Highest | 25 | English | 1.4 |
| 8 | 11 | 1 | 1 | Compound | Lowest | 35 | Canadian | 1.4 |
| 5 | 12 | 1 | 1 | Basic | Highest | 25 | Canadian | 2 |
| 7 | 13 | 1 | 1 | Basic | Highest | 35 | Canadian | 1.4 |
| 6 | 14 | 1 | 1 | Compound | Lowest | 25 | Canadian | 2 |
| 2 | 15 | 1 | 1 | Compound | Lowest | 25 | English | 1.4 |
| 3 | 16 | 1 | 1 | Basic | Highest | 35 | English | 2 |
| 26 | 17 | 1 | 4 | Basic | Lowest | 25 | English | 2 |
| 27 | 18 | 1 | 4 | Compound | Highest | 35 | English | 1.4 |
| 31 | 19 | 1 | 4 | Compound | Highest | 35 | Canadian | 2 |
| 28 | 20 | 1 | 4 | Basic | Lowest | 35 | English | 1.4 |
| 32 | 21 | 1 | 4 | Basic | Lowest | 35 | Canadian | 2 |
| 25 | 22 | 1 | 4 | Compound | Highest | 25 | English | 2 |
| 29 | 23 | 1 | 4 | Compound | Highest | 25 | Canadian | 1.4 |
| 30 | 24 | 1 | 4 | Basic | Lowest | 25 | Canadian | 1.4 |
| 9 | 25 | 1 | 2 | Compound | Highest | 25 | English | 2 |
| 11 | 26 | 1 | 2 | Compound | Highest | 35 | English | 1.4 |
| 10 | 27 | 1 | 2 | Basic | Lowest | 25 | English | 2 |
| 14 | 28 | 1 | 2 | Basic | Lowest | 25 | Canadian | 1.4 |
| 15 | 29 | 1 | 2 | Compound | Highest | 35 | Canadian | 2 |
| 13 | 30 | 1 | 2 | Compound | Highest | 25 | Canadian | 1.4 |
| 16 | 31 | 1 | 2 | Basic | Lowest | 35 | Canadian | 2 |
| 12 | 32 | 1 | 2 | Basic | Lowest | 35 | English | 1.4 |
| 33 | 33 | 1 | 1 | Basic | Highest | 35 | English | 1.4 |
| 34 | 34 | 1 | 1 | Basic | Lowest | 35 | English | 2 |
| 35 | 35 | 1 | 1 | Compound | Lowest | 35 | English | 1.4 |
| 36 | 36 | 1 | 1 | Compound | Highest | 35 | English | 2 |
| 37 | 37 | 1 | 2 | Basic | Lowest | 35 | Canadian | 1.4 |
| 38 | 38 | 1 | 2 | Basic | Highest | 35 | Canadian | 2 |
| 39 | 39 | 1 | 2 | Compound | Lowest | 35 | Canadian | 2 |
| 40 | 40 | 1 | 2 | Compound | Highest | 35 | Canadian | 2 |
| 41 | 41 | 1 | 3 | Basic | Lowest | 25 | Canadian | 2 |
| 42 | 42 | 1 | 3 | Basic | Highest | 25 | Canadian | 1.4 |
| 43 | 43 | 1 | 3 | Compound | Lowest | 25 | Canadian | 1.4 |
| 44 | 44 | 1 | 3 | Compound | Highest | 25 | Canadian | 2 |
| 45 | 45 | 1 | 4 | Basic | Lowest | 25 | English | 1.4 |
| 46 | 46 | 1 | 4 | Basic | Lowest | 25 | English | 2 |
| 47 | 47 | 1 | 4 | Compound | Lowest | 25 | English | 2 |
| 48 | 48 | 1 | 4 | Compound | Highest | 25 | English | 1.4 |

## A2. Baking Conditions in CCFRA

**Mixing Stage**

- Tweedy 35 Mixer was used with 8kg flour per batch.
- Salt is dissolved in the mix water before adding to the flour.
- Water temperature was adjusted to achieve a final temperature for required mixer work input.
- Speed of mixing was 25Hz for 30% of the time and the remainder to reach about 240 sec needed to reach the energy input.
- First 60 sec at atmospheric pressure then pull vacuum.
- Final dough temperature is 29°C $\pm$ 1 °C.

**Proving Stage**

50 $\pm$ 5 minutes at 43°C at 70%RH to achieve target proof height of 11cm at the dough core temperature of 36°C $\pm$ 2°C.

**Baking Stage**

- Reel oven 244 °C for 30 minutes to achieve 95°C dough core temperature in straps of 3.
- Cool at 21°C for 3 hours, slice at temperature < 30°C, record any stickiness.

# Appendix B: Supporting information for Chapter 8

## B1. A sample calculation of salt partition in hard wheat CBP dough

| **Salt % (on flour base)** | **0** | **2** | **5** |
|---|---|---|---|
| Recipe Salt Added (g in 100g flour) | 0 | 2 | 5 |
| Salt in 100g of dough (g) | 0.000 | 1.203 | 2.953 |
| Recipe water added (g = mL in 100g flour) | 63.2 | 63.2 | 63.2 |
| Concentration of salt inside the dough (g salt /100g dough) | 0.000 | 1.203 | 2.953 |
| Concentration of salt dissolved in the water phase (g salt/ 100g water phase) | 0.000 | 3.210 | 8.026 |
| | | | |
| Dough Liquor recovered (% of total added water) Ratio | 17.114 | 25.561 | 30.225 |
| Dough Liquor trapped in the Solid phase (% of total added water) Ratio | 82.886 | 74.439 | 69.775 |
| Liquor from 100g dough (mL / 100g of dough) | 10.816 | 16.154 | 19.102 |
| Fluid in solid phase (mL/ 100g of dough) | 52.384 | 47.046 | 44.098 |
| | | | |
| **Concentration** | | | |
| Observed Concentration of Salt in Whole Dough Sample (NMR) (g/100ml) | 0.324 | 1.120 | 2.550 |
| Normalized Observed Concentration of Salt in Whole Dough Sample (g/100ml) | 0.000 | 0.797 | 2.227 |
| Observed Concentation of Salt in Dough Liquor Phase (NMR) (g/100ml) | 0.184 | 2.784 | 2.712 |
| Normalized Observed Concentration of Salt in Liquid Sample (g/100ml) | 0.000 | 2.601 | 2.528 |
| Observed Concentration of Salt in Solid Phase (NMR) (g/100ml) | 0.555 | 1.409 | 1.146 |
| Normalized Observed Concentration of Salt in Solid Phase Sample (g/100ml) | 0.000 | 0.854 | 0.591 |
| | | | |
| **From centrifugation, we know the water content distribution between the liquor phase and the solid phase (Mass of Salt)** | | | |
| NMR observation = visiable amount of salt present in the dough | | | |
| Total amount of salt in the Liquid Phase (g of salt/100g of dough) | 0.020 | 0.450 | 0.518 |
| Normalized | 0.000 | 0.420 | 0.483 |
| Total amount of salt in Solid Phase (g of salt/100g of dough) | 0.291 | 0.663 | 0.505 |
| Normalized | 0.000 | 0.402 | 0.261 |
| Total visible salt in the dough in Liquor + Solid (g of salt in 100g of dough) | 0.311 | 1.113 | 1.023 |
| Normalized | 0.000 | 0.802 | 0.713 |
| | | | |
| **Expectation of Salt Levels** | | | |
| Amount of added Salt visible in dough by NMR (assuming all salt dissolved in water phase) (%) | 0.000 | 34.898 | 31.777 |
| Normalized | 0.000 | 24.816 | 27.745 |
| Amount of added Salt visible in dough by NMR (assuming all salt is equal partition) (%) | 0.000 | 93.155 | 86.355 |
| Normalized | 0.000 | 66.242 | 75.396 |
| | | | |
| Percentage of Total visible Salt in 100g of dough (assuming all salt in water Phase) | | | |
| compare with the recipe amount added to the dough to Whole Dough (NMR) | 0.000 | 34.898 | 31.777 |
| Normalized | 0.000 | 24.816 | 27.745 |
| compare with the recipe amount added to the dough to | 0.000 | 34.658 | 12.753 |

| | | | |
|---|---|---|---|
| Liquor+Solid (NMR) | | | |
| Normalized | 0.000 | 24.981 | 8.882 |
| | | | |
| Percentage of Total visible Salt in 100g of dough (assuming all salt is equal partition in dough) | | | |
| compare with the recipe amount added to the dough to Whole Dough (NMR) | 0.000 | 93.155 | 86.355 |
| Normalized | 0.000 | 66.242 | 75.396 |
| compare with the recipe amount added to the dough to Liquor+Solid (NMR) | 0.000 | 92.513 | 34.655 |
| Normalized | 0.000 | 66.684 | 24.137 |
| | | | |
| **Percentage of Lost Salt** | | | |
| **assuming all salt in the water phase** | | | |
| compare with the recipe amount added to the dough to Whole Dough (NMR) | 0.000 | 65.102 | 68.223 |
| Normalized | 0.000 | 75.184 | 72.255 |
| compare with the recipe amount added to the dough to Liquor+Solid (NMR) | 0.000 | 65.342 | 87.247 |
| Normalized | 0.000 | 75.019 | 91.118 |
| | | | |
| **assuming all salt is equal partition** | | | |
| compare with the recipe amount added to the dough to Whole Dough (NMR) | 0.000 | 6.845 | 13.645 |
| Normalized | 0.000 | 33.758 | 24.604 |
| compare with the recipe amount added to the dough to Liquor+Solid (NMR) | 0.000 | 7.487 | 65.345 |
| Normalized | 0.000 | 33.316 | 75.863 |