VALUE ADDED WHEAT CRC

PROJECT REPORT

Project: 2.1.9

Improving Gluten Production: Development of a New Salt-Washing Process

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SUMMARY OF COMMERCIAL IMPLICATIONS

The following potential advantages are offered by the new gluten-washing procedure (listed in order of commercial importance):-

1. **Increased yield of gluten**
2. **Reduced grist rate of flour for gluten washing**
3. **Increased ease of gluten washing**
4. **Increased volume of starch-gluten throughput**
5. **Improved extensibility and elasticity of gluten**
6. **Improved gluten colour and odour**
7. **Reduced lipid and ash contents for gluten and starch**
8. **No extra capital costs to alter equipment: downstream implications**
9. **Low added operating cost**

These expectations are based on two years of laboratory research and limited pilot-scale trials. The potential to achieve these advantages can only be determined by implementing the process at the industrial scale.

The laboratory research involved Food Science Australia, the Value-Added Wheat CRC and the Grains R & D Corporation. The concept of adding ammonium chloride to the gluten wash water came from the advice that 2% sodium chloride (common salt) was used in Canada to reduce the lipid content of gluten. We tried the volatile salt ammonium chloride as a replacement, and found that it was effective at 0.5% (one quarter of the concentration for common salt), bringing other unexpected advantages. This full report describes the research leading to these findings. The following summary indicates the parts of the full report where greater detail is given.

1. Increased yield of gluten

Washing gluten in 0.5% ammonium chloride was found to provide an increase in gluten yield of up to 20% in laboratory experiments (see Section 3.2.1, Tables 3-5). Further yield increases are likely with the use of transglutaminase enzyme (Section 6). These findings from lab experiments could not be checked in the pilot-scale trials, they require full-scale operation for verification.

2. Reduced grist rate of flour for gluten washing

The increases in yield were most marked (23%) for gluten washed with 0.5% ammonium chloride from very poor quality flour (H/J stream, with considerable bran and germ contamination). The reduction of lipid and ash contents in the glutens were also most marked for this flour stream (Section 3.2.1, Table 5). This result suggests that the ammonium chloride-wash process should permit the use of higher-extraction flours.

3. Increased ease of gluten washing

The handling of gluten washing was easier in the presence of 0.5% ammonium chloride. Hand-washing of gluten in the lab is difficult, but the task was facilitated by the addition of salt, even for poor quality flour (Section 3.2.1, Table 5). Handling was also found to be easier with the salt washing during the pilot-scale trials. These advantages are expected to apply for industrial processing.

4. Increased volume of starch-gluten throughput

The combination of easier processing, plus higher yield potential, should permit the rate of processing and thus throughput to be increased industrially.

5. Improved extensibility and elasticity of gluten

Glutens washed with ammonium chloride had considerably greater extensibility than water-washed control glutens, with slightly increased Rmax values for lab and pilot-scale trials (Sections 3.2.2, 3.2.3, 4.3.2, 4.3.3, Figure 17). This advantage is especially significant in helping to meet quality specifications that have traditionally been problematic.

6. Improved gluten colour and odour

Darkening of gluten appears to be associated with lipids. The salt-wash process delivers gluten with low lipid content and much better colour (lighter) and odour (less "cereal smell") (Section 3.2.5, 4.3.4).

7. Reduced lipid and ash contents for gluten and starch

Lipid contents of gluten from ammonium-chloride washing have lipid contents about half that for water-washed control glutens, both for lab-washed glutens (Section 3.2.1, Tables 3-5) and for gluten from pilot-scale trials (Section 4.3.1). Ash contents were also lower. In addition, low lipid levels were found for the starch resulting from the salt-washing (Section 3.2.1). Low-lipid starch should be better suited to processing in general.

8. No extra capital costs to alter equipment: downstream implications

The modifications needed for the new process are modest, requiring only the facility to have ammonium chloride added to the water for dough mixing and for the initial stages of washing. Before any full-scale trials of the process, there needs to be a thorough evaluation of the possible consequences down-stream for the added salt. Initial discussions of this possible problem have not shown up any difficulties. Ammonium chloride should be completely lost as a result of drying of gluten and starch materials. The inclusion of ammonium chloride in the wash water should be an advantage for the fermentation process. If some ammonium chloride carries through into the liquid starch products, care needs to be taken to ensure that this does not interfere with its uses.

9. Low added operating cost

The ongoing operating cost of implementing the new process should be little more than the cost of the ammonium chloride. However, it is critical that any company adopting the process must evaluate any unforeseen effects of the change of process before adopting it as commercial practice.

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1 INTRODUCTION

Wheat gluten, produced commercially by a water washing process of wheat flour dough, is used in a wide range of products, especially for the food industry. Vital wheat gluten is a dried form of the product, which retains its functional properties, characteristic of gluten, namely, the unique cohesive and viscoelastic properties that result when the dried gluten is rehydrated with water to form a water-insoluble proteinaceous mass. The most common usage of gluten has traditionally been, and continues to be, in bakery-goods production, primarily to fortify low-protein flour to increase dough strength, gas retention and texture in the finished products. Gluten is also an important food protein as a water-binding agent or protein enhancer/replacer in other food products, such as breakfast cereals, meats, cheese, snack foods, and texturised meat analogues (reviewed by Day et al., 2004). The unique viscoelastic properties of dough make wheat gluten a highly valued functional plant protein. Nevertheless, gluten is a moderately low-cost ingredient, in comparison to milk protein or soy protein ingredients; therefore it has a potential to be used as a raw material for production of high-value/high-nutrition food products.

Gluten structure and composition is important to its properties – including dough properties – and its use in many foods. Although sold as a protein ingredient, gluten contains more than just protein. As a commodity, dry gluten usually contains approximately 75% protein, up to 8% moisture, and varying amounts of starch, lipid and fibre. The lipid content in gluten can vary between 6 and 10%, depending on the processing conditions and on the lipid content of the flour used. Most of the lipid content of the flour becomes associated with the protein during the washing process. The gluten proteins are largely hydrophobic in nature and the lipids bind to the hydrophobic areas of the protein as they are repelled by the water used in the washing. Lipids are strongly bound to gluten proteins and are removed with much more difficulty after gluten washing than when they are removed from the original flour.

The protein that makes up gluten is a complex mixture of proteins, containing many, probably several hundred, polypeptides, many of them being disulfide cross-linked to one another. The individual proteins are divided into two main classes – monomeric and polymeric. These terms can be confusing in that any protein is a polymer of amino acids. In gluten, the term 'monomeric' refers to individual, discrete polypeptide species, while the term 'polymeric' refers to chains formed from individual monomeric proteins, cross-linked via the disulfide bonds of cystine residues in adjoining chains. The monomeric protein is called 'gliadin' and the polymeric protein is called 'glutenin'.

The development of the rheological properties of gluten depends upon the hydration of the protein and concurrent mixing. Both processes, especially hydration, are needed to promote the unravelling and unfolding of the tightly packed aggregated gluten (glutenin and gliadin) molecules from their anhydrous state and to facilitate the rapid development of a viscoelastic network of gluten throughout. The rheological properties (viscosity, plasticity, extensibility, elasticity, consistency, and strength) of this network are critical for the quality of baked goods, as well as other gluten-containing food products. The development and the properties of the gluten network depend upon a number of cooperative secondary interactions, i.e., electrostatic, van der Waals, hydrophobic, dipole-dipole interactions and hydrogen bonding, in addition to covalent disulfide-bond formation (Wrigley et al., 1998).

Traditionally, sodium chloride has been used and is still used in bread baking, largely to provide taste and enhance the flavour of the bread. It is also well known that sodium chloride influences dough properties (i.e., mixing requirements, optimum water absorption, and ease of processing) and bread quality (loaf volume). Earlier studies showed that sodium chloride decreased the binding of total lipids and of phospholipids in doughs. Accordingly, the lipid content of glutens washed out in salt solutions was lower than in glutens washed out in water. Binding of non-polar lipids is substantially reduced by the presence and level of added

sodium chloride. This observation points to the possibility of lipid binding being altered by the effects of sodium chloride on the gluten proteins. Adding sodium chloride presumably decreases the solubility of gluten proteins and makes them more compact. This, in turn, decreases the binding of non-polar lipids by the hydrophobic regions in the interior of the protein. It has also been speculated that salts exert their effects upon water structure by altering the free energy associated with the transfer of apolar protein residues from a non-polar to aqueous environment rather than by direct binding to hydrophobic sites on the protein. Thus, changes in protein properties can be directly attributed to changes in the inherent hydrophobic properties of the protein rather than to changes in the properties of hydrophobic ligand-protein complexes. Salts induce stronger inter-protein hydrophobic and hydrophilic interactions resulting in increased aggregation (Preston, 1989). The increased inter-protein interactions in the developed doughs would also result in higher Extensograph resistance, and larger Extensograph area. These research evidences suggest that the changes may be related to the effect of sodium chloride on protein-folding structure, thereby influence the binding of lipids by gluten protein.

Our research is focused on investigating the interactions between the gluten proteins and non-protein components, particularly the lipids, to investigate their contributions to wheat gluten quality. The lipid fraction in gluten has also been considered as a significant source of colour, odour and taste problems, which currently limit the use of these proteins as a food ingredient.

The research described in this report was undertaken as part of a three-year project, entitled "Gluten structure and modification for food ingredient use". The project has been mainly funded by the Grains Research and Development Corporation, via the Value-Added Wheat CRC, with the research being carried out in Food Science Australia. The aims of this pat of the work were to:

i) Investigate the use of sodium chloride in gluten washing, and its effect on gluten lipid content, thus upon gluten quality and yield;
ii) Investigate the use of alternative salts, particularly volatile salts, such as ammonium salts, and their effects on gluten lipid content yield and quality;
iii) Pursue the possibility of the process being transferred to the gluten industry.

The use of sodium chloride (at 2%) for gluten washing as a means of reducing the lipid content of gluten was recommended on the personal advice of Prof Walter Bushuk, based on common commercial practice in Canada some decades ago.

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Wheat flours (lab-scale)

Wheat flours for laboratory experiments were supplied by Manildra Group, one from the year 2002 and the others from year 2004 (Table 1). The first two are typical flours used for industrial gluten production at the times specified. The third (H/J S030) represents the "bottom of the mill", a stream containing a high proportion of non-endosperm material and a high ash content. Total protein, total lipid, moisture and ash contents of these flours were determined (Table 1).

A set of commercial vital wheat gluten (produced between October – December, 2003), including one produced with addition of cysteine, was supplied by Manildra Group, and tested for their rheological properties by the conventional / industrial standard method – the extensograph, as well as the newly developed laboratory creep test method.

Table 1. The composition of the flour samples used in laboratory experiments.

Flour sample	Year obtained	Composition			
		Protein (%)	Lipid (%)	Moisture (%)	Ash (%)
Fl S008	2002	10.5	1.5	12.6	0.50
Fl S031	2004	12.6	1.8	13.5	0.68
H/J S030	2004	13.1	2.5	11.8	1.16

2.1.2 Chemicals

All the chemicals used for laboratory experiments were analytical grade purchased from Sigma Aldrich Pty Ltd (Castle Hill, NSW, Australia). Food-grade ammonium chloride (supplied by Manildra) was used for pilot-scale experiments.

2.1.3 Salt solutions

Sodium chloride solutions for laboratory experiments were made in water at concentrations of 0.5%, 1% and 2% (w/v), providing concentrations of 85mM, 171mM and 342mM, respectively. Ammonium chloride solution was also made in water at a concentration of 0.5% (w/v), which is equivalent to an ammonium ion concentration of 100 mM.

2.2 Methods

2.2.1 Gluten washing (lab-scale)

Flour (300 g) was mixed with water (180 ml) using a Hobart dough mixer at setting 1 for 2 min, followed by setting 2 for another 2.5 min, to form a dough. The dough was then rested in water for 30 min. Wet gluten was obtained by hand kneading and washing the dough in water (5 × 5 L) until the wash water was clear. The wet gluten was then freeze dried for 48 – 72 hours. The dried gluten was ground to powder using a coffee grinder. Gluten control (GC), was produced using straight flour; defatted gluten (DFG), was produced using chloroform-defatted flour (DF).

In most cases, when gluten was produced using the salt-washing process, the specified concentration of the salt was used to make the dough as well as for resting and washing (4 × 5 L), except for the last washing, when water (5 L) was used to remove or reduce the salt content of the final wet gluten.

When gluten washing was carried out at 40°C, pre-heated water or salt solution (warmed to 40°C) was used for making the dough. A water bath was used to maintain the temperature at 40°C during resting, and the washing process was carried out in pre-heated water or salt solution.

2.2.2 Protein, lipid, moisture and ash contents

Total nitrogen, total lipid, moisture and ash contents of wheat flour and gluten were analysed according the AACC standard methods 46-30, 30-10, 44-19 and 08-01, respectively. Total protein was calculated using a multiple number of 5.7 × N.

2.2.3 Colour measurement

The colour of wheat flour and gluten samples was measured using a Minolta Colour Analyser. The analyser was calibrated using a white tile (L = 97.51, a = –0.04 and b = +2.01). A ceramic cup (4.0 cm i.d. × 3.5 cm depth) was used to hold flour or gluten powder. Triplicate measurements were taken, and the L, a and b values were recorded for each sample.

2.2.4 Rheological assessment

2.2.4.1 Creep and recovery test

Freeze dried gluten (0.6g) was mixed with water (0.9mL) using a pestle and mortar to make a wet gluten dough weighing 1.5g. The gluten dough was then wrapped up in Cling wrap and allowed to rest for one hour. The creep test was carried out using a Paar Physica Modular Compact Rheometer 300. The rheometer was equipped with 10-mm diameter serrated upper and lower parallel plates that were maintained at 25°C. The gluten dough was placed between the plates, and the upper plate was lowered to a fixed gap of 2.0 mm. A home-made ring surrounded the measuring plates using water-saturated ring-shaped filter paper to minimise drying of the gluten sample during measurements. The creep and recovery tests were carried out by applying a constant shear stress of 200 Pa for 200 sec. The shear stress was then taken off to allow the sample to recover, also for 200 sec.

2.2.4.2 Resistance and extensibility

Dry gluten (40 g) was mixed with starch (260 g), initially by hand with a spatula in a beaker, then in the Farinograph mixing bowl for 2 minutes. Sodium chloride solution (30% w/v, 20g) was mixed with water (160 ml, 30°C), which was added to the gluten / starch mix in the Farinograph mixing bowl. Additional water (at 30°C) was added to give a mean dough viscosity of 400±20 BU at the end of 5 minutes. The amount of water used was recorded for the calculation of water absorption. After mixing, the dough was removed, and cut to three pieces of 150 g each. The dough was then moulded and rested for 45 minutes at 30°C before the test.

2.2.5 Dynamic water sorption

The measurement of the relationship of food moisture content with temperature and humidity is traditionally a lengthy and labour-intensive process, involving the use of saturated solutions and a series of repetitive weighings. The dynamic vapour system (DVS) simplifies and automates the process by providing both environmental controls and measurement. The DVS measures the uptake and loss of moisture under changing humidity and a range of temperatures. It consists of a microbalance that measures the changing weight of the sample, inside an incubator that maintains a preselected temperature. Computer feedback is used to control the humidity of the air that flows over the sample by a selection of dry or humidified air. The sample was placed on the DVS video pan until heaped. The pan was then tapped to remove excess sample. The initial mass of the sample was between 0.1 g and 0.15 g.

The DVS was set to cycle from 0% to 80% and back to 0% relative humidity (RH) in six RH steps (0, 10, 30, 50, 70 and 80%). The initial sequence at 0% RH is a drying phase. The relative humidity of each step was held until the sample reaches equilibrium, except at 80%, when the sample was held for 5 hours. Runs were conducted at 25°C with an air flow of 500 sccm. The results were then converted to isotherms by taking the final moisture content of each step and plotting it against relative humidity.

3 SALT WASHING RESULTS (LAB-SCALE)

3.1 The effect of sodium chloride washing on gluten properties

Flour S008 was used for this study. Dough forming and the gluten hand-washing process were carried out using sodium chloride solutions at various salt concentrations, from 0.1 to 2% (w/v) (Table 2). A gluten control (GC) was also produced using water only, and gluten with minimum lipid content was prepared using chloroform-defatted flour (DF).

3.1.1 Protein, lipid, ash contents and protein recovery

The chemical compositions (protein, lipid and ash) in freeze-dried gluten samples were analysed according to the standard methods (Table 2), together with the total protein recovery data by the various washing processes. Gluten prepared by water-washing from straight flour S008 contained 6.2% lipid, which accounted for 45% of the lipid in the starting flour. Gluten prepared from chloroform-defatted flour contained less than 1% lipid. When 2% sodium chloride was used to prepare gluten from the same full-fat flour, the amount of lipid in the resulting gluten was clearly reduced, with less than half the amount of lipid compared to the water-washed gluten control. The ability of sodium chloride to reduce the lipid content of gluten was decreased when lower levels of sodium chloride were used. Apparently, at least 2% sodium chloride was needed to achieve worthwhile reduction of the lipid content of the washed gluten.

Table 2. Protein, lipid and ash contents in gluten washed out from flour S008 using sodium chloride at various levels, in comparison with gluten washed out with water from wheat flour S008 (GC) and chloroform-defatted flour S008 (DFG).

Dough forming and gluten washing process	Protein (N×5.7) (% w/w gluten)	Lipid (% w/w gluten)	Ash (% w/w gluten)	Total gluten (% w/w flour)	Protein Recovery (% w/w flour)
Gluten prepared using NaCl from flour S008:					
2% (w/v)	79	2.7	1.1	11.1	83
1% (w/v)	79	3.6	0.7	10.9	83
0.5% (w/v)	80	5.7	0.5	11.7	84
0.1% (w/v)	72	6.3	0.5	10.9	75
Gluten control (GC) – water only	75	6.2	0.3	11.0	79
Gluten from chloroform-defatted flour (DFG) – water only	77	0.8	0.5	10.6	78

The results are in agreement with the personal advice of Prof Walter Bushuk that lipid content is halved by washing with 2% sodium chloride. They also confirm an early report by Pomeranz et al. (1968) in which corn oils were used to study lipid-binding during dough formation and gluten washing. This showed that sodium chloride decreased binding of total lipids and of phospholipids in doughs, and that the lipid content of gluten washed out in salt solutions was lower than in gluten washed out in water. Increasing the level of sodium chloride decreased lipid binding, particularly for non-polar lipids. It has also been suggested that adding sodium chloride decreases the solubility of gluten proteins and makes them more

compact and coherent. This, in turn, decreases binding of non-polar lipids by the hydrophobic regions in the interior of the protein.

The protein recovery results showed that the incorporation of sodium chloride at levels at 0.5% (w/v) and above increased protein recovery in comparison to gluten washed with water. It has been suggested that at low concentrations (0.05 – 0.3M), sodium chloride has a strong salting-out tendency as evidenced by significant decreases in gluten protein extractability (Preston et al., 1981). Amino-acid composition analysis showed that the relative proportion, in washed gluten, of albumin-like proteins increased and that of gliadin-like proteins decreased, as sodium chloride concentration increased (i.e., extractability decreased).

The ash contents in glutens washed out in sodium chloride solutions were higher than those of the gluten washed out in water. Ash contents decreased in relation to decreases in the amounts of salt used, even though a last wash with water had been used following the salt-washing processes. This indicates the likelihood of some residual salt in gluten which could not be completely removed by water once it has been incorporated in the dough-forming and the washing process.

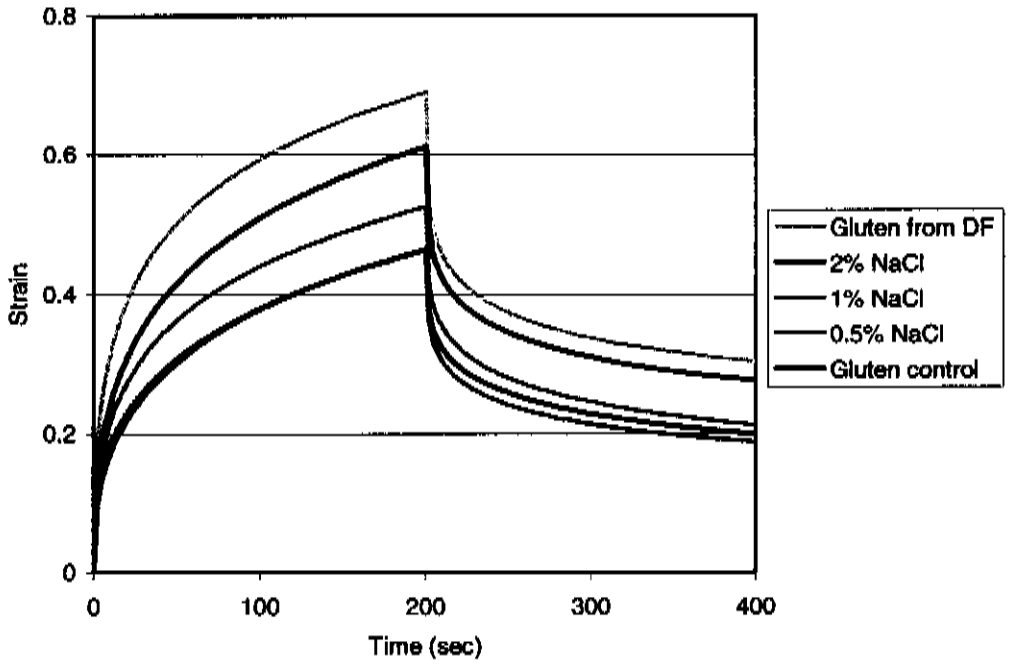
3.1.2 Rheological properties (creep and recovery)

Sodium chloride has long been used as an important ingredient for bread making, largely to enhance flavour. It has also been know that the addition of salt affects the dough rheology by strengthening the gluten, thus enhancing the ease of dough handling and regulating fermentation. The creep and recovery test was used to investigate the rheological properties of the gluten washed out with salt solutions, of the gluten control, which contained highest amount of lipids, and of the gluten water washed from chloroform-defatted flour. The creep and recovery test was carried out by applying constant shear stress of 200 Pa for 200 seconds, and the maximum strain reached by each rehydrated gluten sample was recorded. This is a measure of the extent flow of gluten sample under the applied stress. The shear stress was then taken off after the set time to allow the sample to recover. The recovery was monitored for 200 seconds for all samples (Figure 1).

The gluten produced from chloroform defatted flour 008 had reached highest strain after 200 sec, greater than any gluten washed out with salt solution including the gluten control, which contained the highest amount of lipid. Gluten washed out using 2% NaCl had a slighter lower maximum strain than the gluten from defatted flour (water washed), but higher than the 1% NaCl, which in turn was higher than the 0.5% NaCl and gluten control (Figure 1 (a) and (b)). There were no significant differences between the gluten washed out with 0.5% NaCl and the water-washed gluten control, because a concentration of 0.5% NaCl was not sufficient to have any effect on lipid-binding in gluten. All glutens tested had similar recoveries (Figure 1(c)), once the applied force was taken off during the test, even though they reached different strains within the set time. The results seem to indicate that removing or reducing lipid content in gluten had an effect on the subsequent rheological properties, at least for the flour used here, which is likely to be a poor-quality flour due to the particular year it was harvested i.e. the effects of a drought season. It is possible that lipids may interfere with or diminish non-covalent bonding between or within gluten protein subunits, thereby reducing cohesion within the overall protein network. The use of salt would partially exclude the lipid in gluten depending on the level of its usage, resulting in improved elastic-viscous properties for the resulting gluten.

Figure 1. Creep and recovery of gluten produced using different levels of sodium chloride during the dough formation and washing processes, in comparison with gluten prepared with water only (gluten control and gluten from DF).

(a) Creep and recovery



(b) Maximum strain after 200 sec under a constant stress

(c) Recovery (%) after removal the applied stress

3.1.3 Colour

The colours of the gluten (L, a and b values) were measured using a Minolta Colour Analyser. There were no differences in L (100=white, 0=black) values between the gluten control, the gluten from defatted flour and the gluten washed out with less than 0.5% NaCl (Figure 2a) , but there were increasing of L values from the gluten washed out with 1% and 2% NaCl. However, removal of lipid reduced the b (yellowness (+), blueness (-)) values of water-washed gluten, i.e. the gluten prepared from defatted flour had lower b values than the gluten control (Figure 2b). The b value of the gluten washed out with 2% NaCl was similar to that of gluten from defatted flour, and the b value increased with decreasing levels of NaCl in the washing process. The results showed that the b values, i.e. the yellowness, could be used as a good indication of the relative amount of lipid in gluten. The reduction of yellowness due to the (partial) removal of lipids could improve overall gluten visual quality (lighter / less creamy colour).

3.1.4 Water sorption isotherms

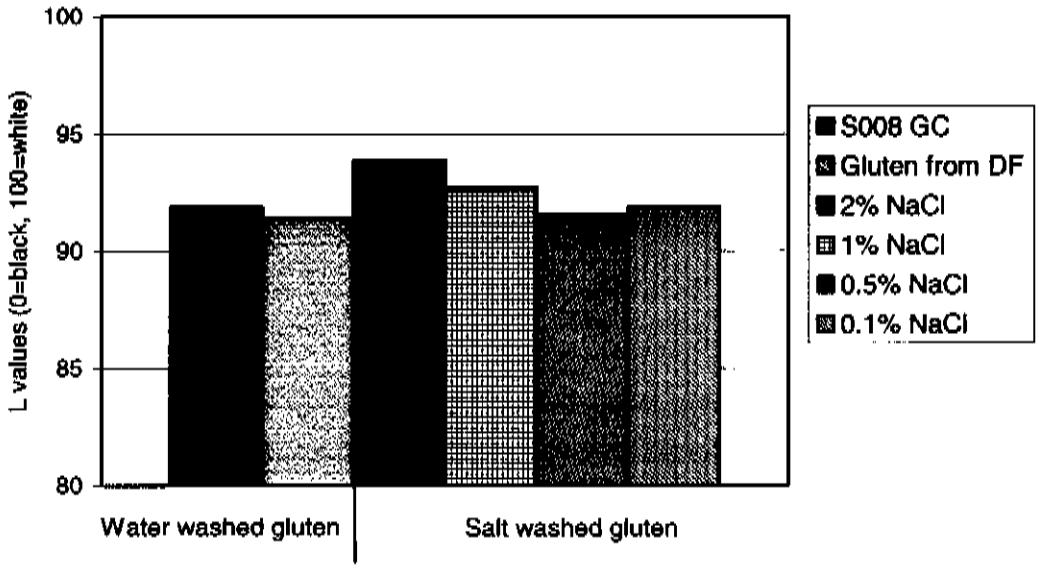
Water is especially important in influencing the structure of protein in its hydrated state, which in turn influences interactions between proteins and lipids. Water sorption isotherms of dried gluten were measured using the Dynamic Vapour Sorption system at six equilibrium steps of relative humidity (RH).

An isotherm is a curve that describes, at a specified moisture content, the equilibrium relation of the amount of water sorbed by the food components, and the vapour pressure or relative humidity. In the low-humidity range, the isotherm is concave in relation to the humidity axis. In the intermediate range, the isotherm has a region of inflection that is basically linear. In the high-humidity range, it is concave in relation to the moisture content axis. The lower portion of the isotherm represents the adsorption of the first layer of water vapour onto the surface of the adsorbing food. The region of inflection represents the adsorption of additional layers. In the initial portion of the isotherm, the water pressure – moisture content relation is governed by the energy of binding between the water molecules and the adsorbing surface. The binding energy depends on the physical structure of the surface, its composition, and the properties of the water. In the intermediate portion, water molecules are deposited on water molecules already present in the first layer and to a small extent on the non-polar sites. The energy is mainly that of condensation of water; the adsorption mainly depends on the water vapour pressure. In the high-moisture range, the vapour pressure is governed mainly by the second layer, which covers the surface. The addition of successive layers is basically the result of a capillary condensation. In this high-moisture range, water sorption increases rapidly but the vapour pressure is affected little.
Figure 3 shows that there are water sorption differences between the gluten control and the gluten from defatted flour. At same target RH between 10-70%, gluten from defatted flour absorbed slightly more water than the gluten control when the sample reached the equilibrium. The figure also shows that at the same moisture level, gluten from the defatted flour had a lower RH than the gluten control, which suggests that it binds water more 'tightly' than the gluten control.

Although the gluten washed out using 2% NaCl, did not appear to absorb significantly more water than the gluten control, at high RH, between 70-80%, the gluten washed out with the salt had a much steeper water sorption curve than either the gluten control or the gluten from defatted flour. It is possible that removal of lipids may also increase the proportion of active sorption sites of protein for water, i.e., locations from where the lipids have been removed become available for water molecules to adsorb, thus facilitating non-covalent hydrogen bonds which are important for gluten viscoelastic properties.

Figure 2. The comparison of the colour (L and b values) of gluten powder measured using a Minolta Colour Analyser, gluten washed out in sodium chloride solutions with gluten washed with water – gluten control (GC) and gluten produced from chloroform defatted flour (DFG).

(a) L values (0 = black, 100 = white)



(b) b values (yellowness (+), blueness (-))

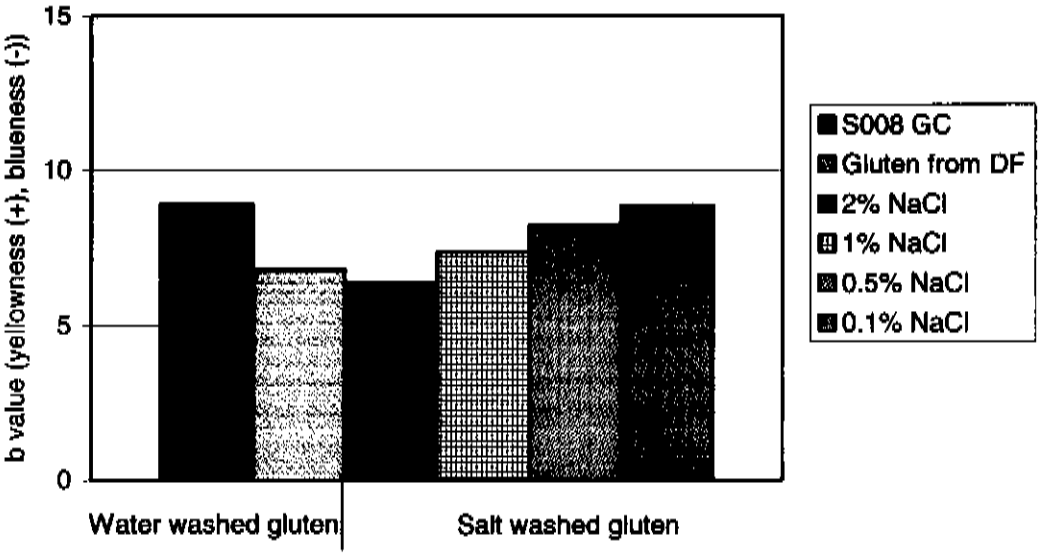
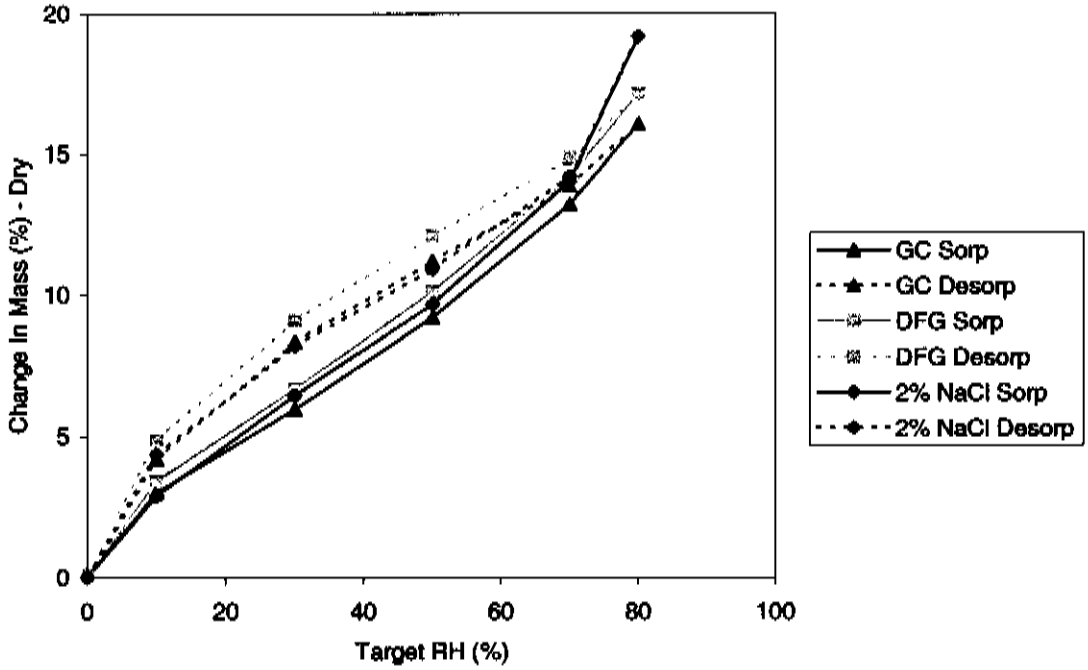


Figure 3. Dynamic vapour system (DVS) isotherm plots of the gluten control, the gluten from defatted flour, and the glutens washed out with 2% NaCl.



3.2 The use of ammonium chloride for gluten washing

The use 2% sodium chloride solution for gluten washing has showed that it could reduce the lipid content in gluten by more than 50%, whilst also improving gluten colour and rheological properties. However, the use of sodium chloride has the disadvantage that a significant level of the salt remains in the washed gluten, despite a final water wash, so that there is an unacceptably high ash content in the resulting gluten (Table 2). Furthermore for industrial production, this level of salt could increase the cost of production, such that its use may counteract the benefits to gluten quality. The use of lower levels such as 1% and 0.5% sodium chloride had much reduced or minimally desirable effects. Sodium chloride, being a non-volatile salt, would have adverse effects on the current gluten production system. An alternative volatile salt would therefore be preferred to sodium chloride, if it shows the same or superior effects compared to sodium chloride. Ammonium chloride, a volatile salt, was therefore chosen as an alternative salt for further exploring the salt-washing process to improve gluten quality.

The NH_4Cl salt-wash process was investigated using three wheat flours (Table 1): -

S008 – flour from Manildra Nowra, year 2002;
S031 – flour from Manildra Nowra, year 2004;
S030 – a mill fraction from reduction rolls H/J, year 2004.

In addition to the analyses already established for accessing gluten quality produced using sodium chloride, the rheological property of gluten was also assessed using the Farinograph and Extensograph methods, as documented by Manildra for Client's specifications. Although the mill fraction H/J is unlikely to be used for gluten production, due to its high germ and bran content, its high lipid content provides an extreme example to help in understanding the effects of lipid on gluten quality.

Gluten was made with three different treatments: -

Gluten control (**GC**) – no treatment, water only for dough making as well as washing;

Salt-washed gluten (**S1G**) – dough was made using 0.5% NH_4Cl (w/v, = 100 mM), and washed in 3 × 5L 0.5% NH_4Cl and 1 × 5L water;

Gluten from defatted flour (**DFG**) – gluten made from chloroform-defatted flour, with water-washing only.

All gluten samples were hand washed, freeze dried and ground to a powder of less than 250 μm particle size. Each gluten sample was prepared using 300 g flour. Four gluten samples were made for each flour and each treatment. All the analyses were carried out on each individual gluten sample except for the Extensograph test where two gluten samples from each treatment, 20 g each, were combined for one test.

3.2.1 Protein, lipid, ash contents and protein recovery

Tables 3, 4 and 5 summarise the protein, lipid and ash contents for all gluten samples prepared using the ammonium-salt and the water-washing processes, and the total protein recoveries from different washing process for flour S008, flour S031 and for the mill fraction H/J S030, respectively.

As expected, gluten controls prepared from the mill fraction H/J contains much higher levels of lipid (12.0% w/w, Table 5) than the gluten controls from wheat flours S008 and S031 (6.6% and 7.7% w/w respectively, Tables 3 and 4), since this mill fraction is from the later reduction rolls of the milling process, thus containing more germ and bran material than any of the early fractions or the final straight-run flour, therefore the flour contains exceptional high levels of lipids (2.5% w/w, Table 1). Flour S031 contains slightly higher amount of lipids than flour S008 (Table 1). This feature is reflected in the respective gluten controls, i.e., gluten controls prepared from flour S031 contained higher amounts of lipid than the gluten controls from flour S008. All gluten prepared from chloroform-defatted flour contained lipid levels less than 1%. This showed that the removal of lipids from flour was effective using chloroform.

The lipid content in the gluten washed out in 0.5% (w/v) ammonium chloride solution was considerably lower than for the gluten controls for all three flours. The percentage of reduction varied from about half of the lipids for flour S008 (Table 3), to about one-third reduction for flour S031 (Table 4) and H/J S030 (Table 5). The performance of 0.5% (w/v) ammonium chloride in reducing lipid content in gluten for flour S008 was similar to that of 1% and 2% (w/v) NaCl. This important finding contrasts with the situation for sodium-chloride washing, for which a salt concentration of 2% was needed to achieve the various advantages to gluten quality. This unexpected finding adds value to the expected advantage of using a volatile salt such as ammonium chloride.

For a further comparison with water washing, laboratory-scale gluten production was increased (600 g flour instead of 300 g) for flour S008 using the 0.5% (w/v) ammonium chloride salt washing process. In addition to studying the gluten produced, starch was collected and freeze-dried. The washing solutions were pooled and part (2 L) of the remaining washing water (after centrifugation to separate starch) was vacuum-evaporated to ~250 ml, then freeze dried. Lipid extraction was then carried out on the gluten, starch and dried materials from the washing water, using 95% ethanol at 70°C. The results showed that the amounts of lipid extracted from the glutens obtained using this salt-washing process and the control, were 5% (w/w) and 10% (w/w), respectively, higher than the total lipid levels determined using the acid hydrolysis method. The resulting lipid extracts were very cloudy

Table 3. Protein and lipid contents in glutens washed out using 0.5% ammonium chloride (S1G) in comparison with gluten washed out with water from wheat flour S008 (GC) and gluten from defatted flour S008 (DFG).

Gluten samples	Protein content (N×5.7) (% w/w gluten)	Lipid content (% w/w gluten)	Total gluten (% w/w flour)	Protein Recovery (% w/w flour)
S008 GC 6[*1]	75.1	6.2	11.0	78.8
S008 GC 7	73.2	7.0	11.3	79.0
S008 GC_{40} 1[*2]	71.2	6.2	11.1	75.3
S080 GC_{40} 2[*2]	70.8	6.8	10.6	71.5
S008 S1G 1	75.4	3.1	10.7	77.1
S008 S1G 2	73.9	3.5	10.8	76.2
S008 S1G 3	72.4	4.0	11.7	80.9
S008 S1G 4	74.3	4.0	11.3	80.0
Mean	74.0	3.7	11.2	78.6
Std dev	1.24	0.44	0.46	2.25
S008 $S1G_{40}$ 1[*2]	68.9	3.8	12.2	79.8
S008 $S1G_{40}$ 2[*2]	74.5	4.0	12.4	88.0
S008 DFG 1	76.1	0.9	10.6	76.8
S008 DFG 2	77.8	0.7	10.6	78.3
Mean	77.0	0.8	10.6	77.5
Std dev	1.20	0.14	0.02	1.05

[*1] Gluten samples pooled from several preparation in year 2003.

[*2] Dough forming and gluten washing was carried at 40°C.

Table 4. The protein, lipid and ash contents in gluten washed out using 0.5% ammonium chloride (S1G) in comparison with gluten washed out with water from wheat flour S031 (GC) and gluten from chloroform defatted flour S031 (DFG).

Gluten samples	Protein content (N×5.7) (% w/w gluten)	Lipid content (% w/w gluten)	Ash content (% w/w gluten)	Total gluten (% w/w flour)	Protein Recovery (% w/w flour)
S031 GC 1	71.6	7.3	1.22	14.5	82.2
S031 GC 2	71.0	7.5	1.14	14.3	80.5
S031 GC 3	72.1	7.9	1.19	14.0	80.2
S031 GC 4	68.9	7.9	1.26	14.5	79.5
Mean	70.9	7.7	1.20	14.3	80.6
Std dev	1.41	0.30	0.05	0.23	1.14
S031 S1G 1	75.9	5.2	0.80	14.2	85.3
S031 S1G 2	74.4	4.8	0.84	14.9	88.2
S031 S1G 3	72.1	5.0	0.80	15.0	85.8
S031 S1G 4	71.8	5.0	0.77	15.3	87.4
Mean	73.6	5.0	0.80	14.9	86.7
Std dev	1.95	0.16	0.03	0.49	1.33
S031 DFG 1	71.8	0.4	1.06	14.0	80.0
S031 DFG 2	72.8	0.9	1.03	14.3	82.6
Mean	72.3	0.7	1.05	14.2	81.3
Std dev	0.71	0.35	0.02	0.19	1.86

Table 5. The protein, lipid and ash contents in gluten washed out using 0.5% ammonium chloride (S1G) in comparison with gluten washed out with water from mill fraction H/J S030 (GC) and gluten from chloroform defatted mill fraction S030 (DFG).

Gluten samples	Protein content (N×5.7) (% w/w gluten)	Lipid content (% w/w gluten)	Ash content (% w/w gluten)	Total gluten (% w/w flour)	Protein Recovery (% w/w flour)
S030 H/J GC 1	71.0	13.0	3.12	12.9	70.1
S030 H/J GC 2	68.7	11.5	2.82	12.0	62.9
S030 H/J GC 3	67.6	12.5	2.93	12.0	61.8
S030 H/J GC 4	68.6	11.1	2.89	12.7	66.3
Mean	69.0	12.0	2.94	12.4	65.3
Std dev	1.44	0.88	0.13	0.48	3.75
S030 H/J S1G 1	74.0	7.9	1.16	15.0	84.5
S030 H/J S1G 2	70.8	8.6	1.19	14.2	76.9
S030 H/J S1G 3	72.3	7.7	1.22	14.5	80.0
S030 H/J S1G 4	74.5	8.5	1.28	14.4	81.9
Mean	72.9	8.2	1.21	14.5	80.8
Std dev	1.69	0.45	0.05	0.31	3.18
S030 H/J DFG 1	76.6	0.6	3.01	13.4	78.5
S030 H/J DFG 2	75.6	1.0	2.43	12.1	69.7
Mean	76.1	0.7	2.72	12.8	74.1
Std dev	0.71	0.28	0.41	0.96	6.25

from the gluten control i.e. difficult to filter, while the lipid extracts from the salt-washed gluten were clear yellow. The amount of lipid extracted from starch was 0.8% (w/w) when the salt-washing process was used, less than the half of the lipids in the starch (1.8% w/w) washed with water. Although lipids were not recovered from the dry material of the salt-wash solution due to the interference of salts during lipid extraction, the dried residues from the washing solution contained a few yellow oily droplets for the salt-wash solution. The evidence from this experiment suggests that not only the amount of lipids in gluten can be halved by using the ammonium chloride washing process, but also the lipid in starch is reduced.

However, in contrast to gluten prepared using sodium chloride, the ash content of the gluten prepared using ammonium chloride was very low, even lower than that of the water-washed glutens (i.e. the gluten control and gluten prepared from defatted flour). These changes in ash content ranged from about one-third reduction for flour S031 to more than two-thirds for mill fraction H/J. Removal of ammonium chloride during drying was expected, since it is a volatile salt, thus offering an advantage over the use of sodium chloride. However, the actual reduction in the ash content in gluten suggests that if the use of ammonium chloride decreases the solubility of gluten proteins and makes them more compact, it not only excludes lipids from gluten, but also other mineral matter largely coming from the germ and bran materials, probably due to the better cohesiveness of protein during the dough formation.

The ammonium-chloride washing caused an increase in the yields of gluten, although this was not evident. This positive result was most evident for the H/J flour (Table 5), and least for the gluten made from flour S008. The gluten yield may thus be expected to be better using the ammonium-chloride washing process, particularly when the flour quality is poor.

In industrial practice, the gluten washing process is carried out with warm water (at about 40°C). However, the majority of the work carried out in the laboratory used washing temperatures at of about 20°C for ease of handling. Accordingly, to mimic industrial practice, some experiments were carried out at 40°C, using flour S008. Ammonium chloride worked equally well in reducing lipid content in gluten at 40°C. Protein recovery was also similar at both temperatures for flour S008 (Table 3).

3.2.2 Rheological property (resistance and extensibility)

Gluten was combined with wheat starch, and 2% NaCl solution was added, to make a dough to a viscosity of 400 ± 20 BU in the Farinograph. Gluten made from the three flour samples represented three distinctive patterns (Figure 4). The dough made with the control gluten from flour S008 was 'stiff', i.e. had higher resistance with a poor extensibility. The dough made with the gluten from the mill stream S030 H/J was very soft, i.e. low resistance Rmax with a good extensibility. And the dough made with the gluten from flour S031 had a good extensibility and a reasonable resistance Rmax, i.e. a large area under the graph. The extensibility and the Rmax results of each gluten are summarised in Table 6.

Gluten washed out in 0.5% (w/v) ammonium chloride solution from all three flours exhibited better extensibilities than their respective gluten controls (Figure 5) without reduction of the resistance Rmax. The increase of the extensibility was about 3.5 cm for gluten from S008 and about 2.5 cm for glutens from S031 and S030. In the example of flour S030, the extensibility of gluten was increased from an average of 12.7 cm to 15.2 cm by using the NH_4Cl salt wash process. These increases in extensibility could be crucial to meeting customer's specifications, especially when they are evident for the H/J poor-quality flour. Statistical analyses (ANOVA) showed that the extensibility of the gluten washed out in 0.5% (w/v) ammonium chloride was significantly different to the gluten control for all three

flours (Figure 6). However, these statistical analyses were carried out on a limited number of replications, i.e. three for each extensibility test, due to the labour input required to produce enough gluten for the Extensograph.

Table 6. The extensibility and Rmax of the test gluten (after mixing with wheat starch), made from the three flours.

	Extensibility (cm)			
Flour sample	S008 (20°C)	S008 (40°C)	S031	S030 H/J
Gluten control	3.5 ± 0.2	4.5 ± 0.2	12.7 ± 0.8	11.7 ± 0.3
Salt (NH_4Cl) washed gluten	7.1 ± 0.5	8.0 ± 0.5	15.2 ± 1.3	14.5 ± 0.5
Gluten from defatted flour	9.7 ± 0.3		12.8 ± 0.3	10.5 ± 0.5
	Rmax (BU)			
Flour sample	S008 (20°C)	S008 (40°C)	S031	S030 H/J
Gluten control	730 ± 44	567 ± 40	563 ± 21	220 ± 17
Salt (NH_4Cl) washed gluten	833 ± 25	627 ± 50	623 ± 40	250 ± 10
Gluten from defatted flour	980 ± 35		533 ± 12	297 ± 25

Glutens washed out in 0.5% (w/v) ammonium chloride solution also had slightly increased Rmax, in comparison to the corresponding gluten controls (Figure 7). Although these increases were not statistically significant in all cases, the results demonstrated that the ammonium chloride process would produce gluten with better extensibility without any reduction in dough resistance, i.e. a gluten with overall better rheological property presumably due to the reduction of lipids and overall cohesiveness caused by the use of salt in the process.

The Extensograph tests also showed that there were slight differences between the glutens produced at 20°C (laboratory temperature) and 40°C (industrial practice). Washing at 40°C produced gluten (from flour S008) that was slightly more extensible, but with a lower Rmax., i.e. it forms a softer dough. However, gluten produced using ammonium chloride process still possess equally better extensibility then gluten control at both 20°C and 40°C.

The extensibility results were evaluated for the gluten that was water-washed from defatted flour. By removing lipids completely from flour S008, gluten water-washed from this chloroform-defatted flour had even better extensibility and Rmax than the gluten washed out with ammonium chloride and the gluten control. The extensibility of the gluten from defatted flour S008 was significantly different to salt-washed gluten and the gluten control (Figure 6). In fact, a good linear relationship can be established between the lipid contents of the gluten control, the salt-washed gluten and the gluten from defatted flour, and their corresponding extensibilities (Figure 8). However, removal of lipids completely from flour S031 did not affect the gluten extensibility or Rmax significantly (Figure 9).

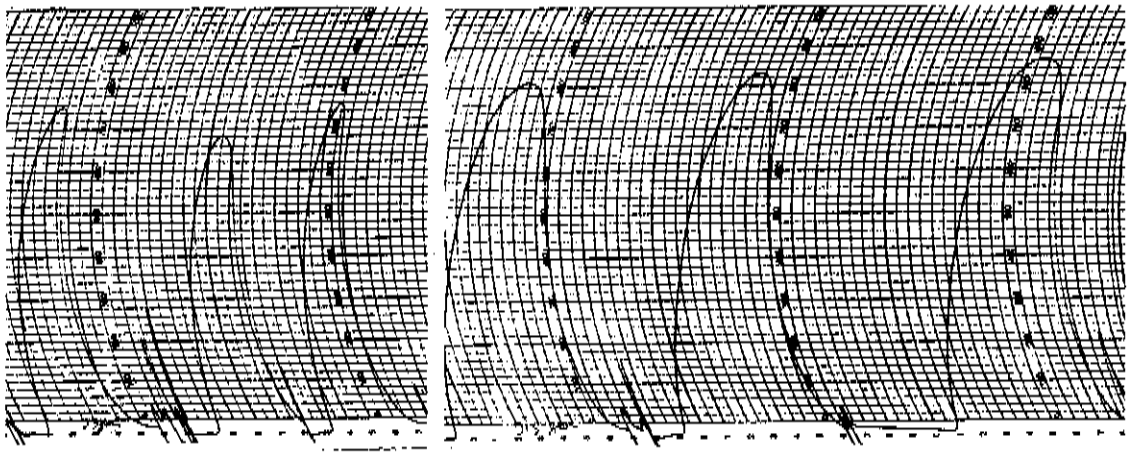
By contrast, for flour H/J S030, the gluten produced from defatted flour had less extensibility, but higher Rmax. The results suggest that although lipids may have some influence on gluten rheology, the main determinant of gluten rheological properties is its protein quality, i.e. different subunits of glutenin and gliadin, the ratios of glutenin and gliadin, etc. Flour S031 produced a much stronger gluten as comparison to flour S008 and H/J S030 as shown by the difference of gluten control from these flours. The lipids in flour S031, although higher in quantity than flour S008, had less influence on gluten rheological properties than for flour S008. This perhaps also demonstrated that the effect of ammonium chloride on gluten rheology was not just by the reduction of lipids, but also by changes in gluten-protein hydrophobicity.

Figure 4. Extensograph diagrams comparing gluten washed out in 0.5% ammonium chloride solution with the gluten washed with water only, from three different flours.

(a) Flour S008

Gluten control

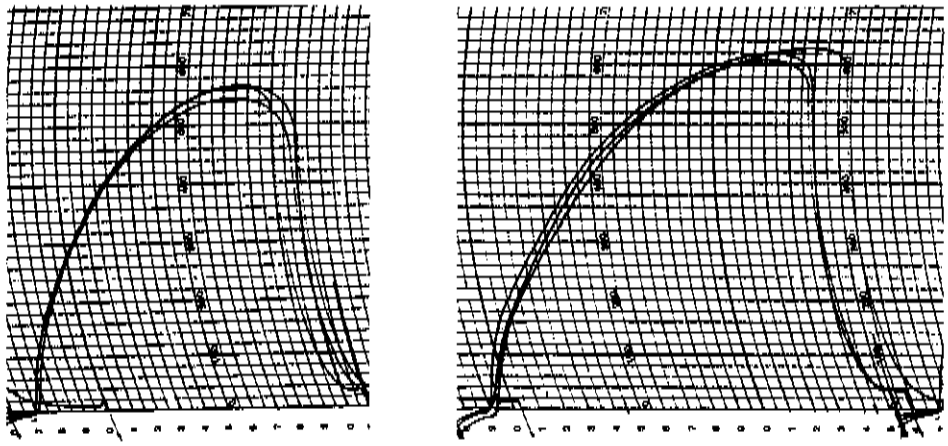
S1G, 0.5% NH_4Cl washed gluten



(b) Flour S031

Gluten control

S1G – 0.5% NH_4Cl washed gluten



(c) Mill stream S030 H/J

Gluten control

S1G – 0.5% NH_4Cl washed gluten

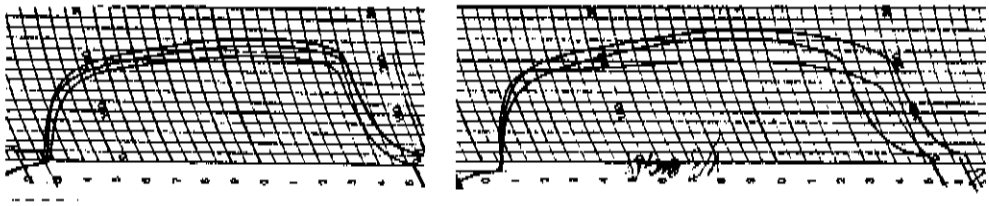


Figure 5. The increase of the extensibility of gluten washed out in 0.5% ammonium chloride in comparison with gluten washed in water, i.e. gluten control.

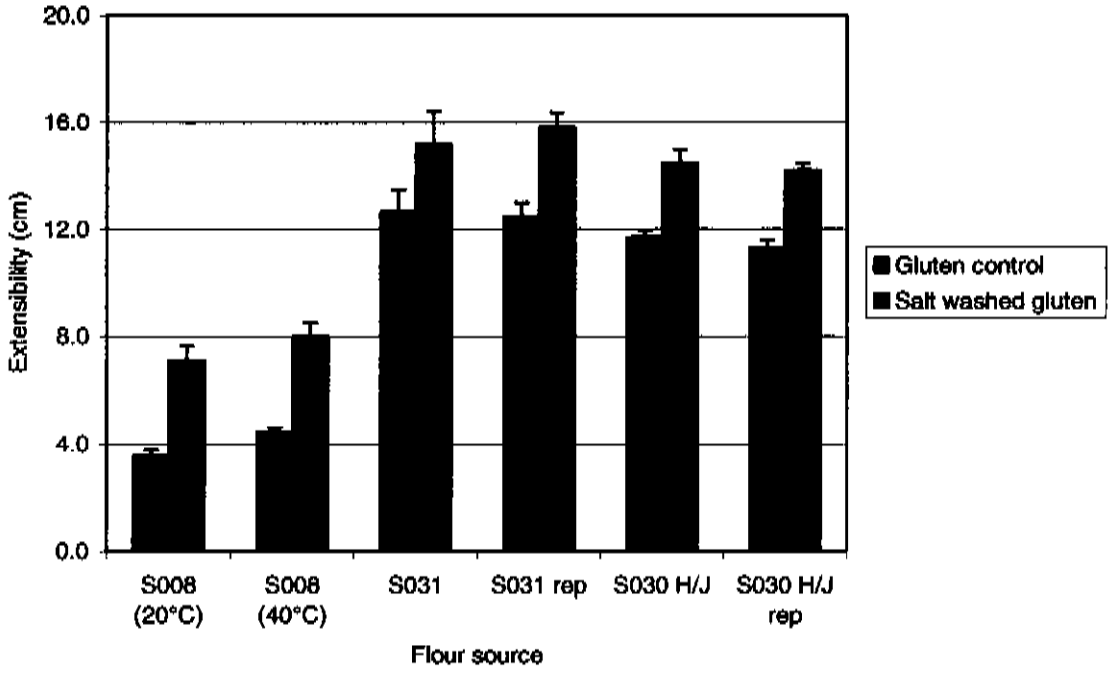


Figure 6. Statistical analysis (ANOVA) of extensibility. Individual bars indicate the statistical significance differences between the treatments, sample bars connecting different samples indicate no significant difference between the treatments.

Flour S008:	GC	GC_{40}	S1G	$S1G_{40}$	DFG
	3.6	4.5	7.1	8.0	9.7
Flour S031:	GC1	GC2	DFG	S1G1	S1G2
	12.5	12.7	12.8	15.2	15.8
Flour H/J S030:	DFG	GC1	GC2	S1G1	S1G2
	10.3	11.3	11.6	14.3	14.6

Figure 7. The comparison of the Rmax of gluten washed out with 0.5% ammonium chloride with the gluten control.

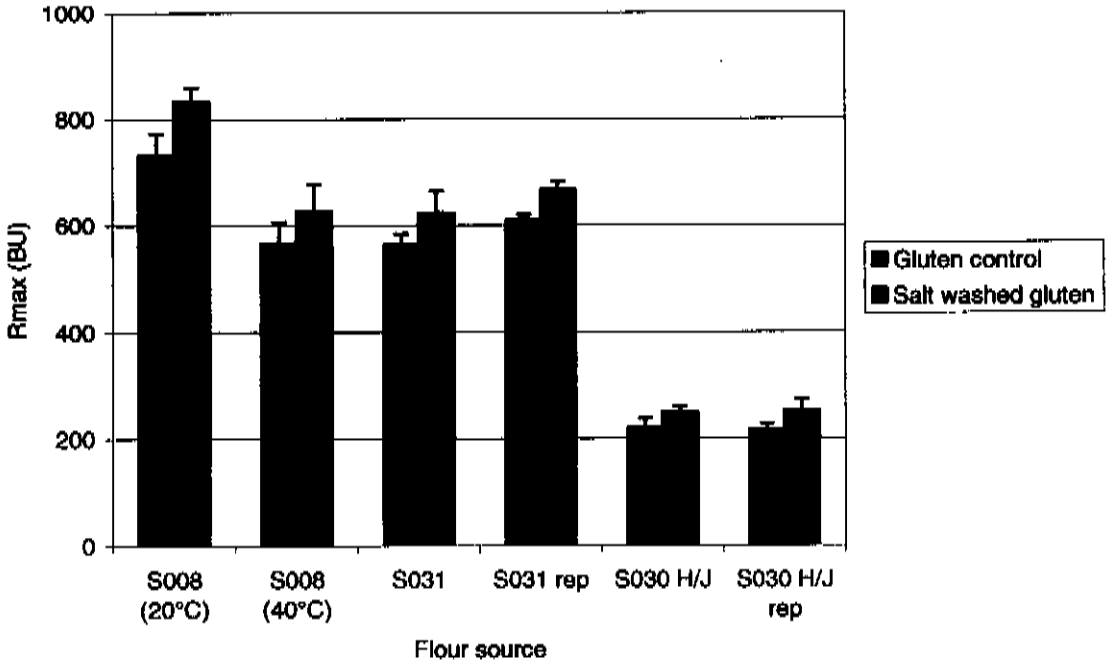


Figure 8. The relationship between the lipid content and the extensibility of gluten control, salt-washed gluten and gluten from defatted flour, from flour S008.

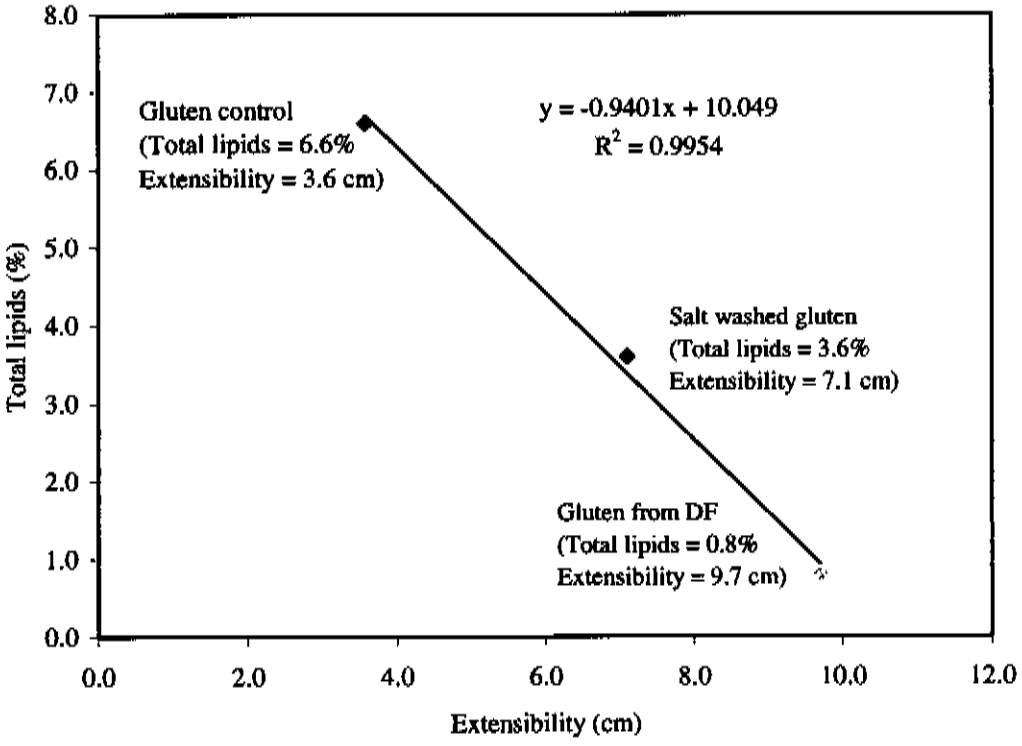
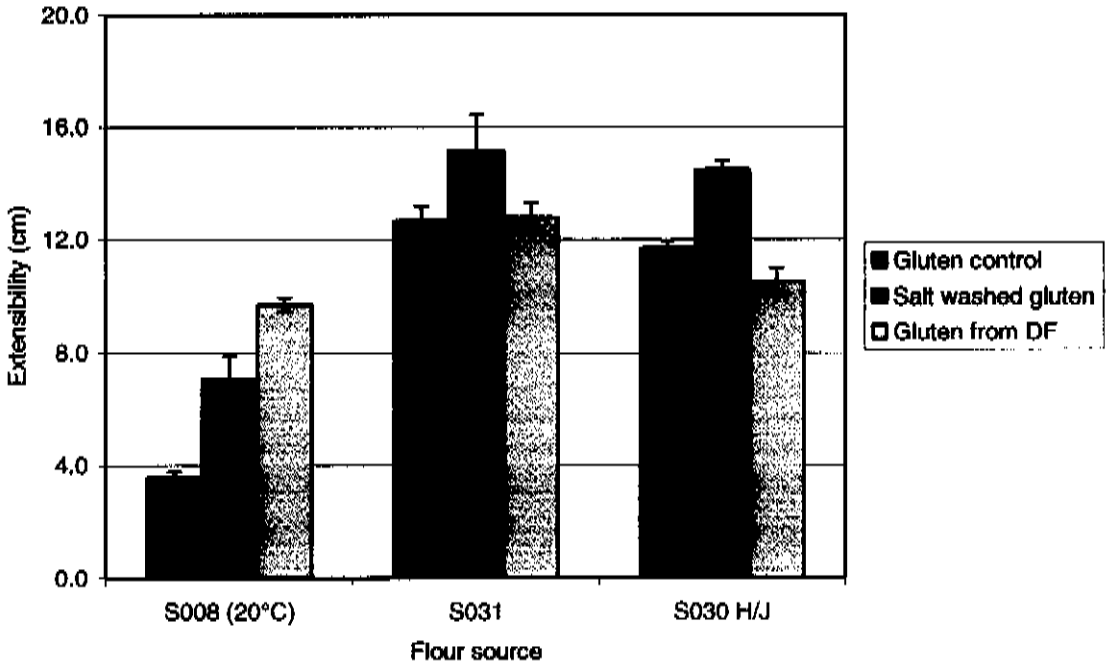


Figure 9. Comparison of the extensibilities of the gluten water-washed, i.e. gluten control, ammonium salt-washed gluten and the gluten water-washed from defatted flour.



3.2.3 Rheological property (creep and recovery)

Similarly to that of the gluten washed out using 2% (w/v) NaCl from flour S008 (Figure 1), the ammonium chloride-washed gluten had also been stretched further, i.e. more extensible, than the gluten control (Figure 10), lying between the gluten from defatted flour and the gluten control. However, unlike the Extensograph results, the creep test results (Figure 11) did not show the any clear differences between gluten washing at different temperatures. The same exercise was also carried out using the ammonium chloride salt-wash process. The creep test results showed that there were no significant differences between the gluten washed at 20°C and 40°C. However, it was noticed that the dough was softer during processing when it was made with warm water, and easier for handling using warm water. This high-lighted the differences between the use of a large-deformation test and the small-deformation creep test to investigate the rheological properties of gluten.

The glutens washed out with ammonium chloride from flour S031 and mill stream H/J (S030) both exhibited further extension under the same rotational stress applied compared to the gluten control (Figures 11 and 12). However, the gluten that was water-washed from the defatted flour showed less extension than the gluten control for flour S031 or no differences for mill fraction S030 H/J. Although this is the opposite to that of flour S008, it is in agreement with the Extensograph results, i.e. the gluten from defatted flour from these two flours showed less or no change in their extensibility (Figure 6). The creep and recovery results showed largely the same trend as the Extensograph results, i.e., all three glutens produced using the ammonium chloride salt-washing process had better extensibility than the gluten washed out with water.

Figure 10. Creep test of the rehydrated gluten (60% water content) produced using flour S008, gluten control (GC7) – water washed, S1G – gluten washed out using 0.5% ammonium chloride, DFG – gluten water washed using chloroform defatted flour.

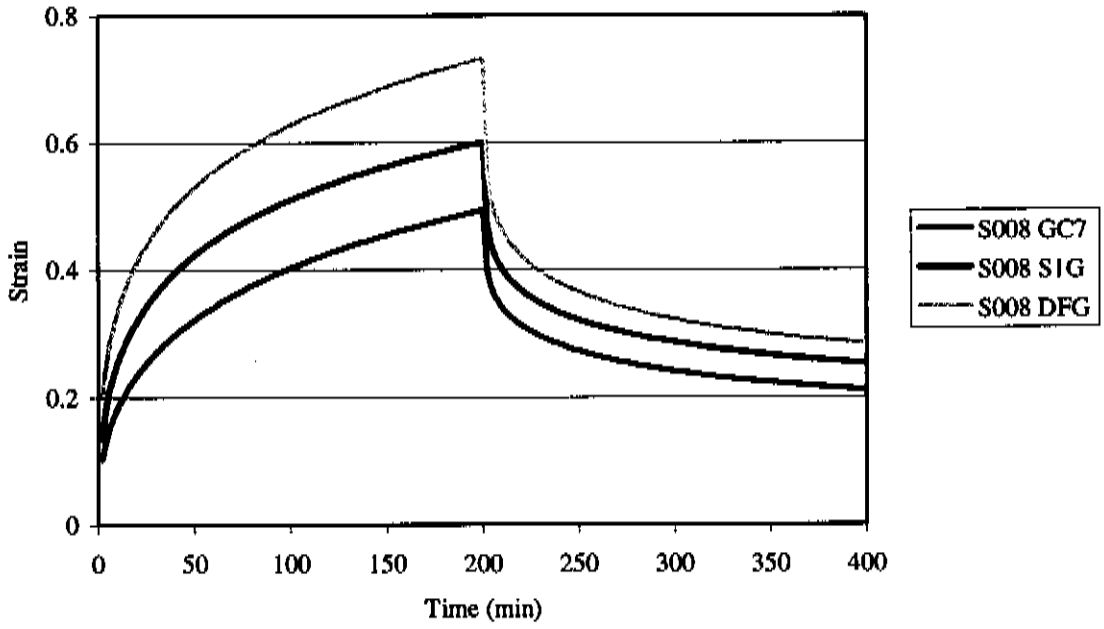


Figure 11. Creep test of the rehydrated gluten (60% w/w water content) produced using flour S008 at different temperatures.

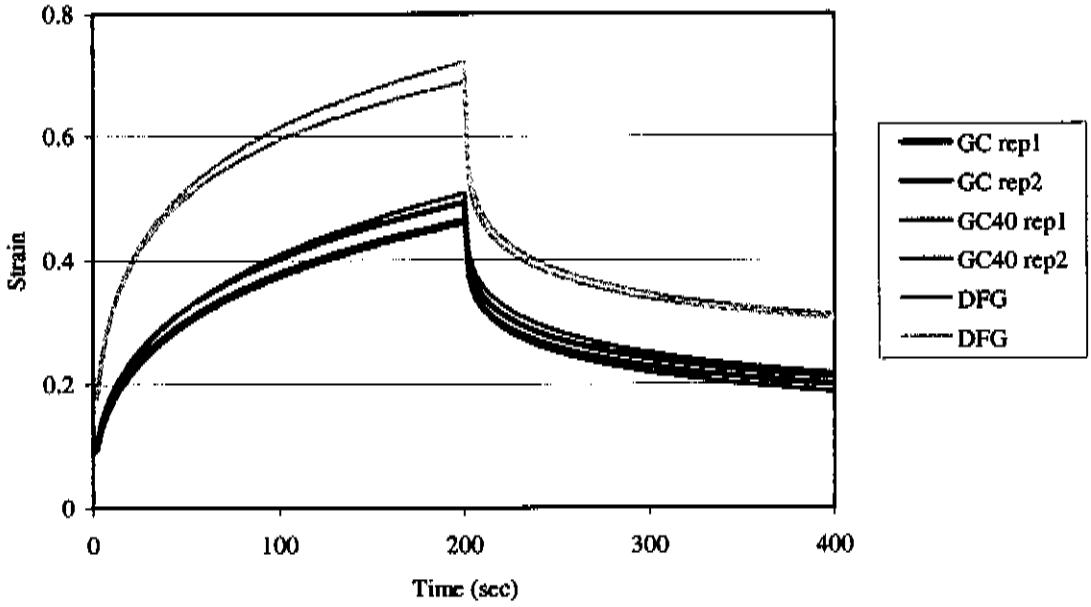


Figure 12. Creep test of the rehydrated gluten (60% w/w water content) produced using flour S031, mean of replicate determination of gluten samples from: gluten control (GC) – water washed, S1G – gluten washed out using 0.5% ammonium chloride, DFG – gluten water washed using chloroform-defatted flour. The error bars show the standard deviation of 8 measurements for GC, S1G and 4 measurements for DFG.

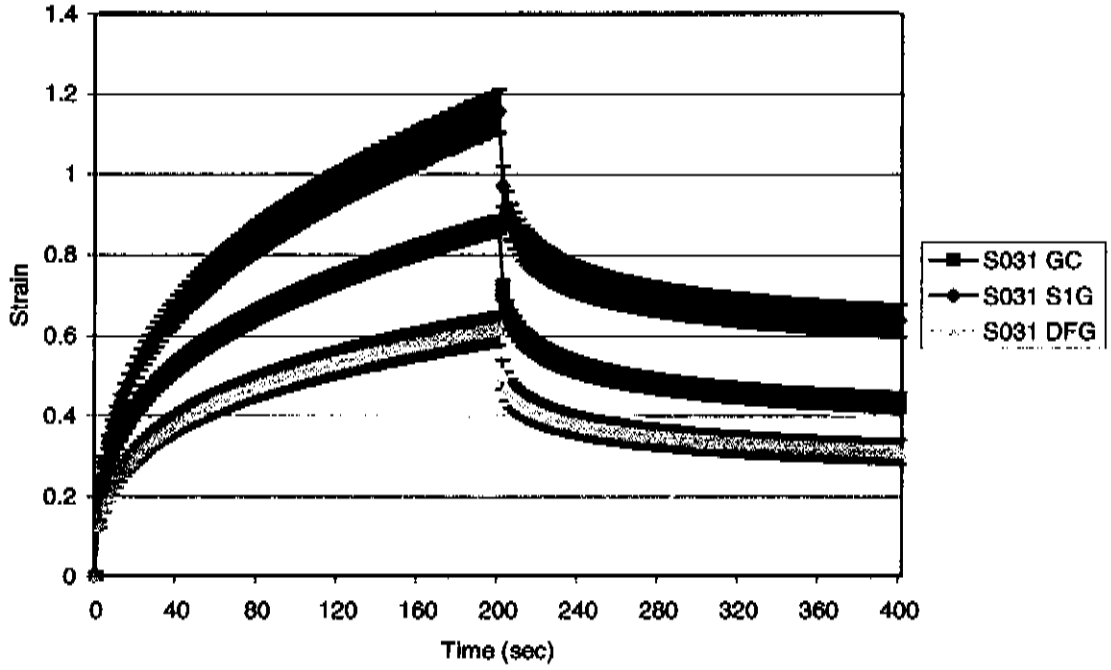
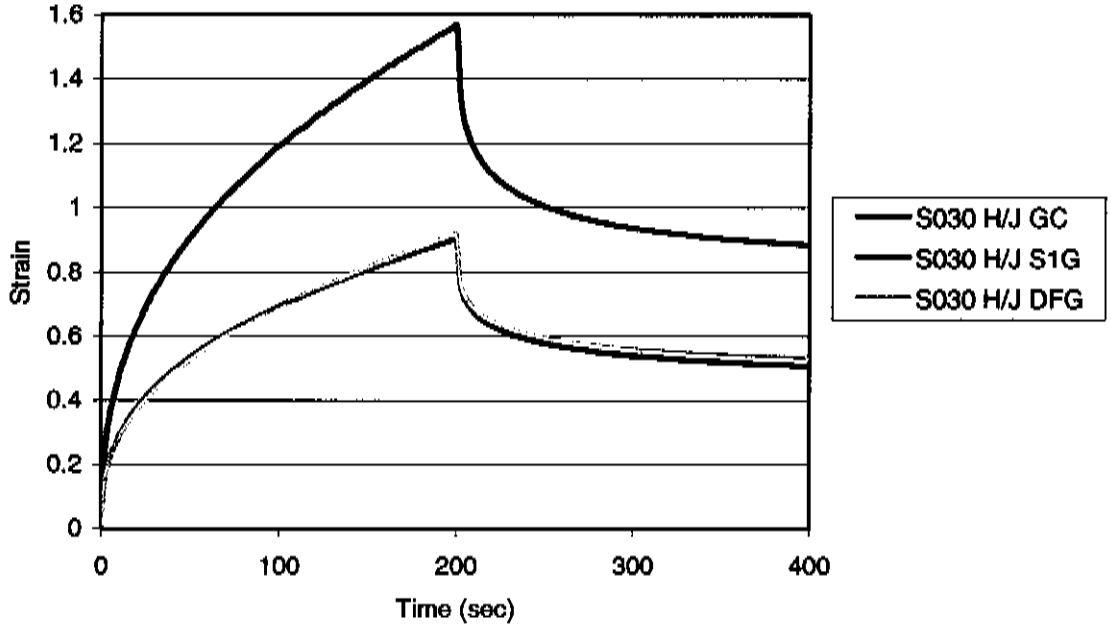


Figure 13. Creep test of the rehydrated gluten (60% water content) produced using mill fraction H/J S030, mean of replicate determination of flour gluten samples from: gluten control (GC) – water washed, S1G – gluten washed out using 0.5% ammonium chloride, DFG – gluten water washed using chloroform-defatted flour.



3.2.4 Correlation between extensibility and the creep test

To prepare enough gluten materials in laboratory for meaningful extensograph test is a time-consuming and labour-costing task. The creep test developed in this project has showed similar and consistent trend as the extensibility measured by the conventional extensograph test. A set of commercial vital wheat gluten was obtained from Manildra Group and tested for the rheological properties using both the extensograph and the creep test (Table 7), in an attempt to establish any relationship between the two test methods. The establishment of such relationship could provide a comparative guidance from the creep test data to their quality at industrial levels for the laboratory modified gluten.

The results (Figure 14) showed that there is a very good correlation between the extensibility measured using 13.3% gluten in a gluten/starch dough with the maximum stress reached after 200 sec under a constant stress by the creep test using 0.6g 60% (w/v) rehydrated gluten. The correlation also applies to hand washed (with water as well as with 0.5% ammonium chloride) and freeze dried gluten prepared from flour 008 and 031, although there is a shift of the linear line, presumably an indication of different drying condition.

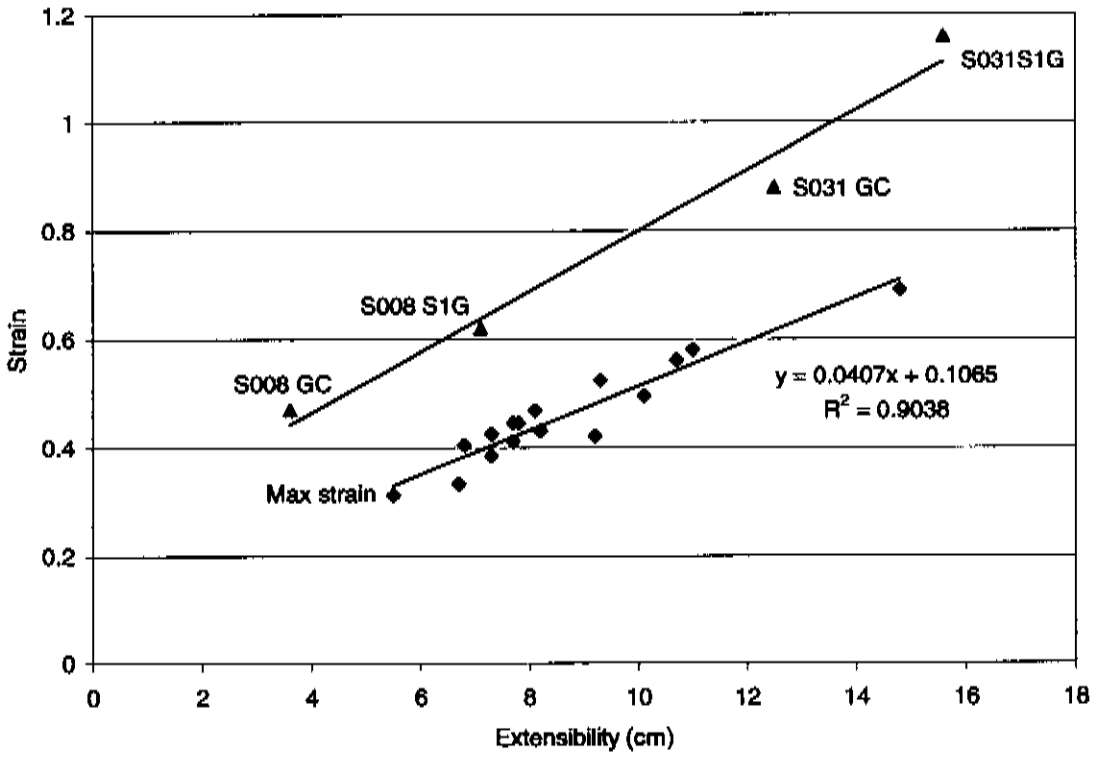
The results showed that creep test of rehydrated gluten can be used to predict its extensibility measured by the traditional extensograph method. The interesting results here were that the increase of the extensibility of the gluten by ammonium chloride process were similar for the flour 008 and 031 – about 3.5 cm increase of the extensibility, and about 0.2 unit increase of the maximum strain by the creep test.

Table 7. Gluten rheological properties – comparison of the extensibility as measured by the conventional entensograph method (13.3% gluten in gluten/starch dough) and the maximum strain reached after 200 sec under the constant stress by the creep test (0.6g 60% rehydrated gluten).

Gluten Samples	Protein content (% w/w)	Extensograph		Creep test	
		Ext (cm)	Rmax (BU)	Max strain (After creep)	Recovery (%)
VWG001	70.4	11.0	747	0.580	54.5
VWG003	70.2	7.3	727	0.425	60.2
VWG009	68.2	5.5	433	0.312	58.7
VWG032	75.2	10.1	787	0.495	55.6
VWG033*	72.9	14.8	328	0.690	56.7
VWG034	79.9	8.1	1000	0.468	56.8
VWG035	74.9	8.2	947	0.431	55.9
VWG036	75.5	6.7	970	0.333	55.6
VWG037	78.4	9.2	1000	0.421	58.0
VWG038	75.0	9.3	813	0.524	54.8
VWG039	77.6	7.3	957	0.384	58.1
VWG040	78.8	7.7	933	0.446	61.2
VWG041	76.6	6.8	940	0.405	61.5
VWG042	76.5	10.7	563	0.561	58.1
VWG043	79.0	7.8	670	0.445	59.1

* Commercial gluten prepared with the addition of cysteine.

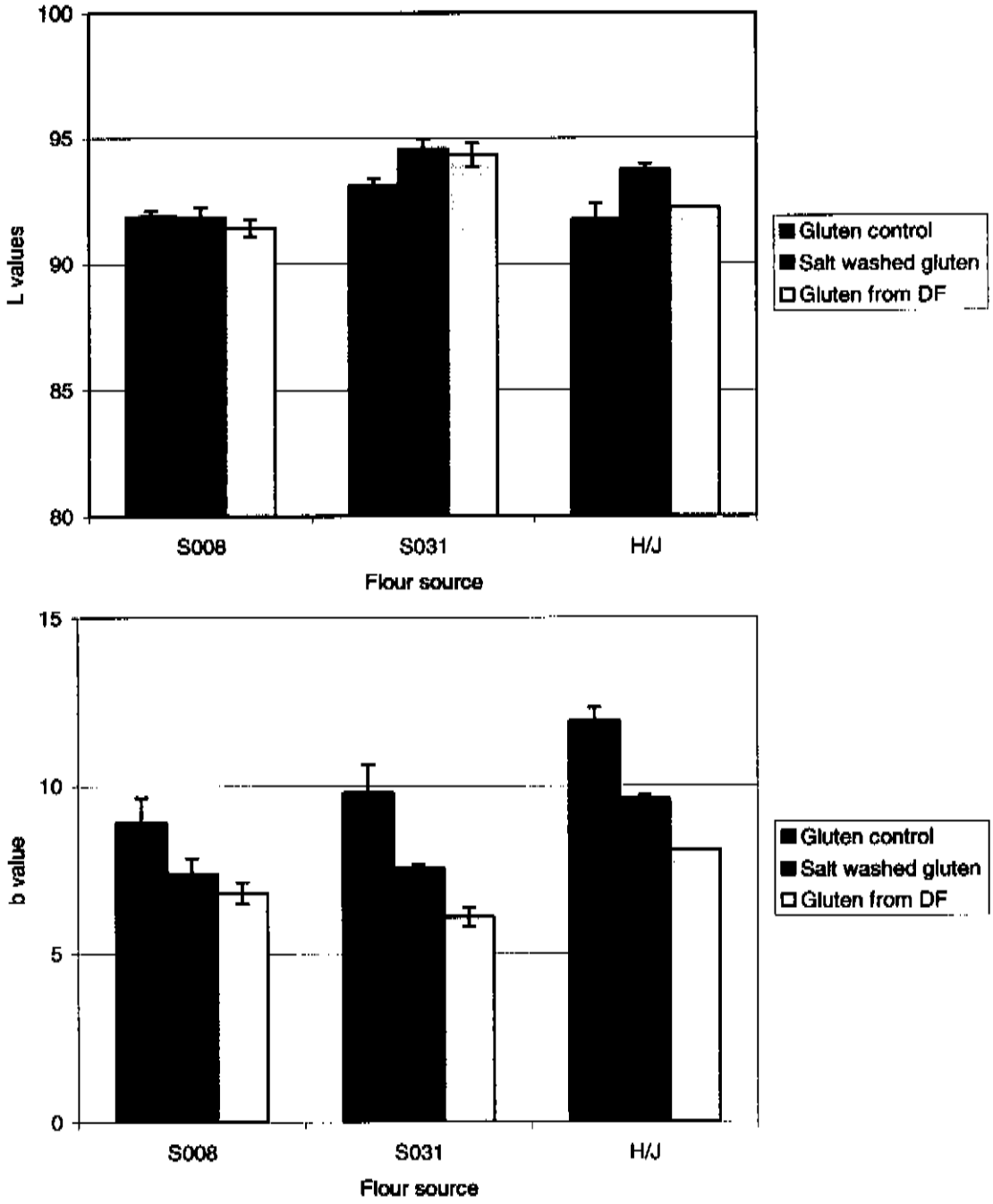
Figure 14. Correlation of the extensibility (cm) measured by the conventional extensograph method (13.3% gluten in gluten / starch dough) and the maximum strain reached after 200 sec under the constant stress by the creep test (0.6g 60% rehydrated gluten).



3.2.5 Colour assessment

The L values of the salt-washed gluten from flour S031 and the mill fraction S030 H/J were clearly higher (lighter) than their respective gluten controls (Figure 15), although there was no significant difference in L values between the gluten control, salt-washed gluten and gluten from defatted flour, prepared from flour S008. It was thus observed that the glutens washed with ammonium salt were generally brighter and whiter than the water-washed gluten controls, especially for gluten from poor-quality flour. The differences in L values were inconsistent between the three flours used, and there was not enough data in the experiment to provide any conclusion. The quality of the flours used varied widely; S008 could have a higher level of polyphenol oxidase, since it came from the 2002 drought-affected harvest. Much of the enzyme-induced darkening may already have take place before the processing of this flour. The increases of the L values of the salt-washed glutens from flour S030 H/J and S031 indicate that the salt-washing process may have some kind of depressing effect on enzyme activities associated with browning during the washing process. The b values (yellowness) of the salt-washed gluten were lower than their respective gluten controls and slightly higher than the gluten from defatted flour. The reduction of the b values was clearly associated with the amounts of lipid in the glutens, further illustrating the advantages of the ammonium chloride-washing process in its ability to reduce the lipid content of the resulting gluten.

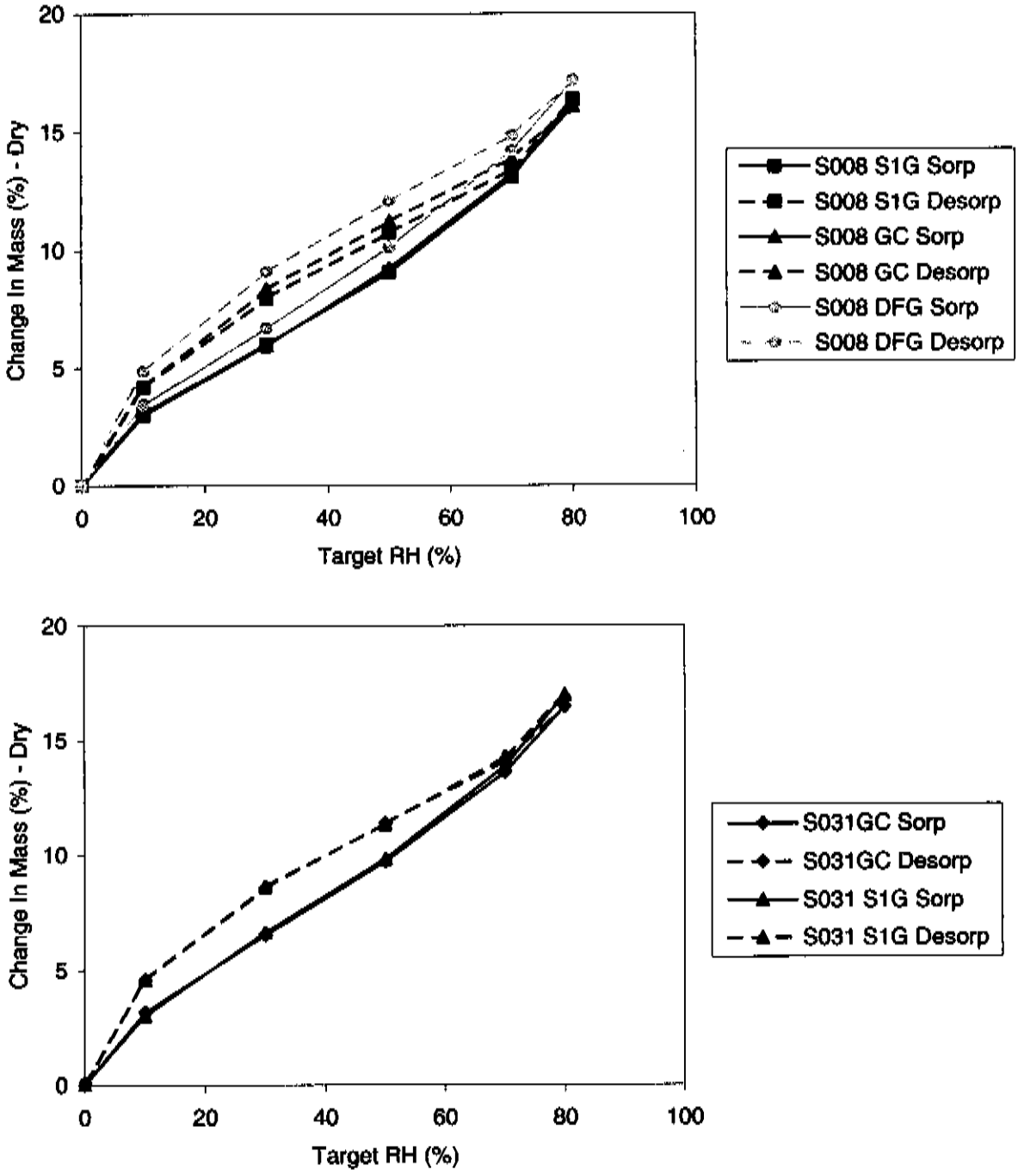
Figure 15. Comparison of colour measured as L and b values of salt-washed gluten, gluten control and gluten produced from chloroform-defatted flour.



3.2.6 Water sorption isotherms

Unlike the gluten washed out in 2% (w/v) sodium chloride solution, the gluten washed out in ammonium chloride solution showed little difference from the gluten control for both flours S008 and S031 with respect to water-sorption isotherms (Figure 16).

Figure 16. Water sorption isotherms of gluten washed with ammonium salt (S1G), gluten control (GC) and gluten from chloroform defatted flour (DFG) from flour S008 and flour S031.



4 USE OF AMMONIUM CHLORIDE FOR GLUTEN WASHING (PILOT-SCALE)

The use 0.5% ammonium chloride was established to provide significant benefits for gluten washing in the laboratory, so the experiments were extended to see if these benefits were also evident when the scale of experimentation was increased to involved multiple-kilogram quantities, using pilot-scale facilities at Manildra's Altona plant. Two pilot-scale trials were carried out separately between 3-5th November and 14-15th December, 2004 using two separate batches of commercial flour.

4.1 Wheat flour and commercial vital wheat gluten

For the first trial carried out on 3-5th November, flour was collected from the rail wagons on arrival at Nowra from Manildra Flour Mill on 26th October. Approximately 25 kg was collected from each of the 14 wagons each containing about 75 tonnes of flour, approximately 1,000 tonnes in total, sufficient for approximately 24 h operation through the starch-gluten plant. Flour, 14 bags × 25 kg, was dry blended at Manildra Altona plant prior to use for the pilot-scale runs. Attempts were also made to collect commercial gluten samples that would correspond to the flour sample as follows:

i) On Tuesday, 26th October, prior to flour unloading, the flour silos were approximately 25% full, sufficient for about 6-8 hours' operation. The train was unloaded between 3.30 and 6.30pm. It was estimated that processing the new flour would commence at about 2200 h on 26th October.

ii) Processing through the starch-gluten plant takes approximately six hours. It was estimated that gluten corresponding to the flour samples taken would first arrive at the silos at about 0400 h on 27th October and would continue through to about 0400 h on 28th October. Packing of dry gluten commenced on 27th October at 1346 h and continued through to 0242 h on 28th October.

iii) A sample of Vital Wheat Gluten was collected from each shipping container for QC evaluation. A 200 g sub-sample of each of these samples was taken for Food Science Australia's evaluation. From the weight-rejected packed product, 4 × 25 kg (50 lb) bags of Vital Wheat Gluten were taken, representing time intervals through production.

For the second trial on 14-15th December, milled flour, approximately 500 kg, was supplied by Manildra from the milling plant at Gunnedah. Total composition of the flour samples were analysed at Food Science Australia.

4.2 The pilot-scale trial procedure

During the first trial, three gluten products were made, one in each day (Table 7), namely, Product 1 – a control gluten involving water washing only, Product 2 – a test gluten with 0.5% (w/v) NH_4Cl for dough mixing, 0.5% (w/v) NH_4Cl for gluten extraction (glomeration), followed by two water washes, the procedure developed based on the laboratory trials and Product 3 – a test gluten with 1% (w/v) NH_4Cl for dough mixing, 0.25% (w/v) NH_4Cl for gluten extraction (glomeration), followed by two water washes, a variate process may suit industrial practice better than the process for the Product 2.

The resulting wet gluten was ring dried under the conditions normally used at Nowra. Hand-washed gluten was also produced, using the same flour for the first trial under the same processing conditions.

During the second trial, two products were made during the second trial – product 1, the control and product 2, 0.5% salt-washed gluten. The dough mixing was adjusted to the speed of 25 kg/h flour and 25 kg/h water.

Table 7. Procedures for pilot-scale gluten washing.

Rate:	Product 1 – Control, water only	Product 2 – 0.5% NH_4Cl for dough mixing 0.5% NH_4Cl for gluten extraction	Product 3 – 1% NH_4Cl for dough mixing 0.25% NH_4Cl for gluten extraction	Total
Dough mixing:				
Flour 15 kg/h	90 kg	90 kg	105 kg	350 kg flour
Water 15 kg/h	80 L	400 L 0.5% NH_4Cl (5g/L)	400 L 1% NH_4Cl (10g/L)	6 kg NH_4Cl
Dough 30 kg/h (3 ×10 kg bucket)	160 kg – 6 hours (14 bucket)	180 kg – 6 hours (18 bucket)	210 kg – 7 hours (21 bucket)	
Hypex gluten extraction:				
Batch:	Dough 6 × 30 kg	Dough 6 × 30 kg	Dough 7 × 30 kg	1 kg NH_4Cl
Dough 20 kg	Water 6 × 20 L	0.5% NH_4Cl (5g/L) 20 L × 6	0.25% NH_4Cl (2.5g/L) 20 L × 7	
Water 20 L				
Washing:				
Water 40 L × 2	6 batch	6 batch	7 batch	
Wet gluten 4.8 kg	* 3 × washing used		* 1st gluten recovery very low	
Dryer:				
1 hr start	Rehydrate VWG			
1 hr flesh at 2 .5 kg /h	2.5 kg dry gluten	2.5 kg dry gluten	2.5 kg dry gluten	
1-2 hr collection	3 kg dry gluten	5 kg dry gluten	5 kg dry gluten	

4.3 Results and discussion

4.3.1 Protein, lipid and ash contents

The total nitrogen, total lipid and ash contents in flour, commercial VWG and gluten samples produced and subsequent lab experiments using the same flour were analysed and listed in Table 8. In both trial, glutens produced by addition of ammonium chloride to dough making and gluten extraction contain lower levels of total lipids and ash in comparison to the gluten prepared using water only. The results were in agreement with previous laboratory experiments (Tables 3-5). This indicates that the process can be scale up and is robust in reducing lipid and ash content in gluten.

The difference between the quality of the gluten Products 2 and 3 with Product 1 was visually noticeable during the trial prior to the drying. Products 2 and 3 were easier, i.e. softer and more cohesive mass, to be handled during the gluten extraction and washing. Due to the batch process nature during the pilot-scale trial, the protein recovery and the total yield cannot be calculated.

Table 8 Protein, lipid and ash contents of the flour, the commercial VWG and gluten samples produced from the pilot-scale trials.

Gluten samples		Protein (N×5.7) (% w/w)	Lipid (% w/w)	Ash (% w/w)
First trial: Flour		12.1	2.0	0.81
Commercial Vital Wheat Gluten				
Customer	Packing data / time			
Manildra Perth	1615	72.8	5.0	0.83
Kyowa Hi Foods	2042	74.4	5.3	0.88
MMC-USA	2310	74.5	5.2	0.89
MMC-USA	0242	73.5	5.2	0.89
Pilot-trial / ring dried				
Control bag 1	03/11/2004 5.45-6.15pm, 1.5kg	72.6	4.9	0.89
Control bag 2	03/11/2004 6.15-6.45pm, 1.5kg	72.5	4.9	0.94
0.5% Salt bag 1	04/11/2004 4.50-5.50pm, 2kg	71.4	3.2	0.79
0.5% Salt bag 2	04/11/2004 3.50-4.50pm, 2kg	71.6	3.8	0.81
1% Salt bag 1	05/11/2004 4.00-5.00pm, 2kg	71.0	3.4	0.73
2% Salt bag 2	05/11/2004 5.00-6.00pm, 2kg	73.5	3.6	0.70
Lab prepared / freeze dried				
Control 5	09/11/04	72.5	6.9	1.11
Control 6	09/11/04	68.9	6.9	1.19
0.5% Salt 5	09/11/04	71.3	3.7	0.86
0.5% Salt 6	09/11/04	71.3	3.2	0.85
1% Salt 5	09/11/04	71.0	4.1	0.87
1% Salt 6	09/11/04	69.0	4.2	0.73
Second trial: Flour		13.3	1.9	0.56
Control bag 2	14/12/04	73.8	5.4	0.74
Control bag 3	14/12/04	73.8	5.4	0.78
0.5% salt bag 2	15/12/04	73.5	4.3	0.62
0.5% salt bag 3	15/12/04	75.0	4.2	0.58

4.3.2 Rheological properties (resistance and extensibility)

The extensibility of gluten was tested using the standard extensograph method. The results (Table 9, Figure 17) showed that Product 1 (the pilot-scale control) was similar to that of the commercial Vital Wheat Gluten from Nowra in the first trial and was a typical commercial gluten in the second trial. This indicate that the pilot-trial process and condition was closed enough to make gluten products resemble to that of commercial products. The extensibility of Product 2 (0.5% NH_4Cl washed) and Product 3 (1.0%) showed an increase of about 50% over the extensibility of the control gluten without any significant reduction of the gluten strength (Rmax).

Table 9. Extensograph results for pilot-scale gluten samples.

Gluten sample	Extensibility (cm) (Mean of three measurements)		Rmax (BU) (Mean of three measurements)	
	Bag 1	Bag 2	Bag 1	Bag 2
First trial				
VWG from Nowra	8.2	7.2	850	920
Product 1 – control	8.3	7.5	>1000	>1000
Product 2 – 0.5% salt	12.0	11.8	800	880
Product 3 – 1.0% salt	10.8	11.8	905	920
Second trial				
Product 1 – control	7.0	6.5	875	865
Product 2 – 0.5% salt	10.5	10.7	>1000	>1000

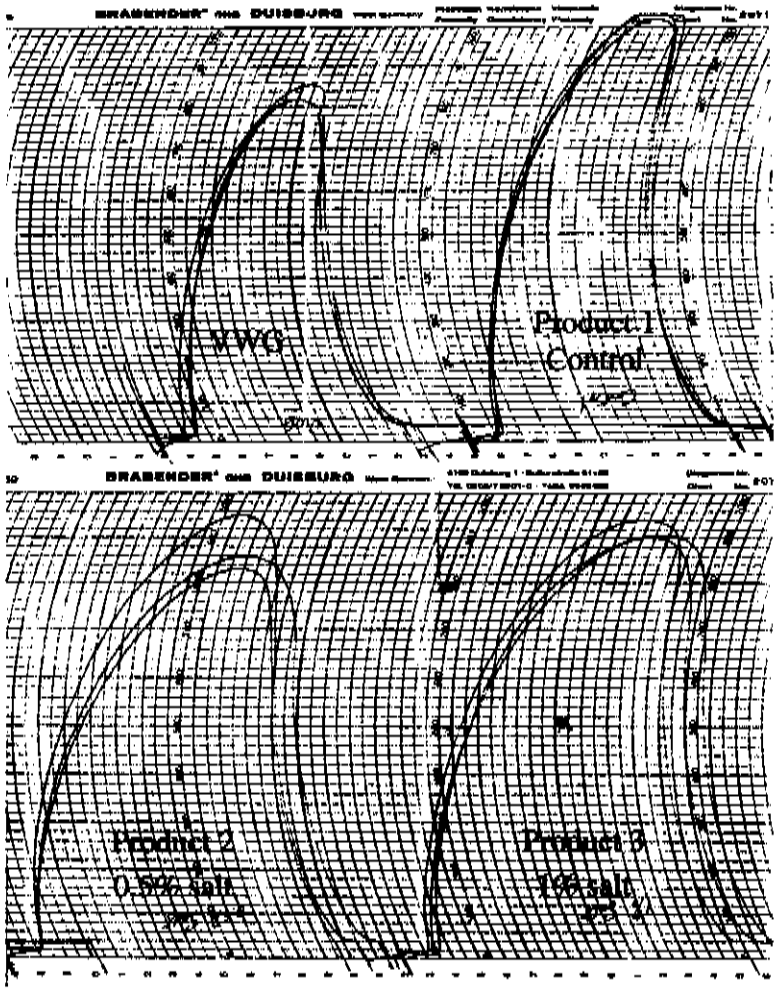
Further experiments were carried out using the Extensograph to investigate the effect of cysteine on gluten, control as well as the 0.5% salt washed gluten from the second trial. Cysteine (2% w/v in water) was added to the NaCl salt solution (170ml) prior to its addition to the starch/gluten mixing in the Farinograph bowl. The amount of cysteine added ranged from 300 to 600ppm (gluten weight base). The results (Figure 18) show that at the level of 300ppm, gluten extensibility increased by about 1.5 cm for both control and the ammonium salt-washed gluten with the reduction of Rmax to about 660 BU. The effect of cysteine on the extensibility of gluten was the same for both gluten control and the salt-washed gluten throughout. Both reached the plateau at about 400 – 450 ppm of cysteine. Further addition of cysteine (to 600 ppm) did not increase the extensibility but reduced the resistance. Gluten extensibility as a result of ammonium salt washing was maintained; addition of cysteine has increased it further. The results suggest that further enhancement of gluten rheological properties can be achieved above the quality already gained through the use of the ammonium salt-washing process.

4.3.3 Rheological properties (creep and recovery)

The creep and recovery curve of Product 1 (control) was similar to that of the commercial gluten (VWG) (Figure 19). The maximum creep (strain) after 200 seconds for Product 2 (0.5% salt) was clearly higher than that for Product 1 and for VWG, i.e., gluten made by using the salt-washing process has better dough properties than the control. The maximum strain for Product 3 (1% salt initially, reduced to 0.25% for gluten extraction) was also higher than Product 1, although it was slightly lower than Product 2. This was also reflected by the Extensograph test, in which the extensibility of Product 3 was also marginally lower than Product 2. The differences between Product 2 and Product 3 are likely to be within the measurement error in both the creep test and the Extensograph.

Figure 17 Extensograph diagrams comparing gluten produced with the 0.5% ammonium chloride process (Product 2) and 1% ammonium chloride process (Product 3) with the gluten washed with water only (Product 1, control) and the commercial vital wheat gluten.

First Trial:



Second trial:

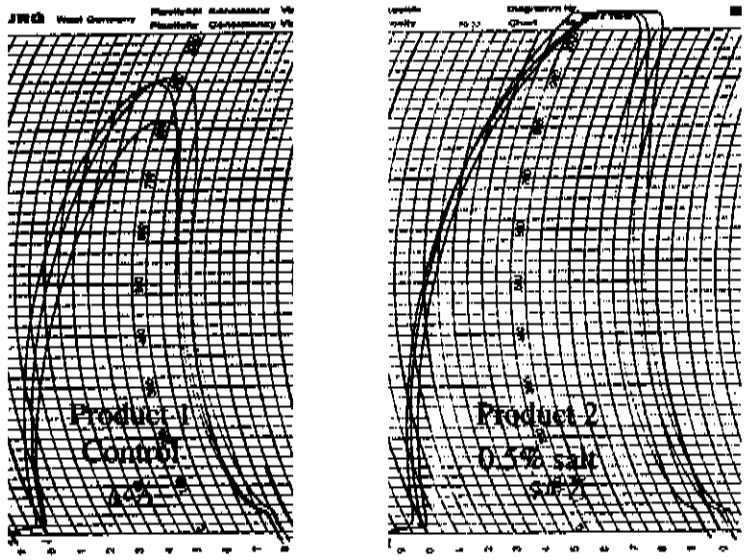


Figure 18. The effect of cysteine on gluten rheological properties. Cysteine (2% w/v in water) was added to the NaCl salt solution prior to the dough mixing in the Fairnograph bowl. The amount of cysteine used was calculated based on weight of gluten (40 g).

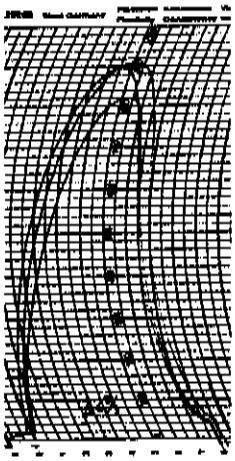
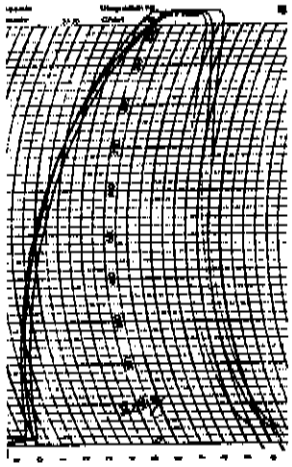
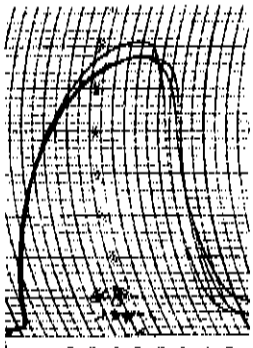
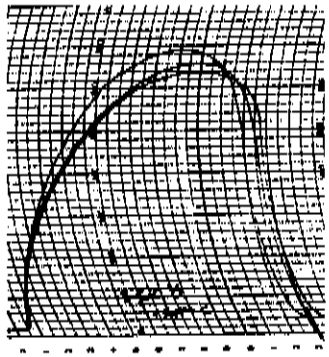
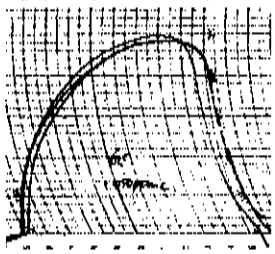
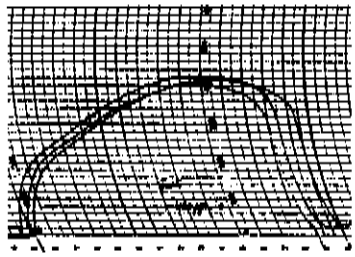
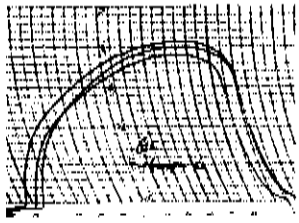
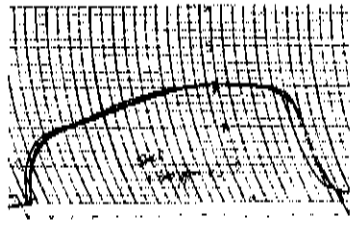
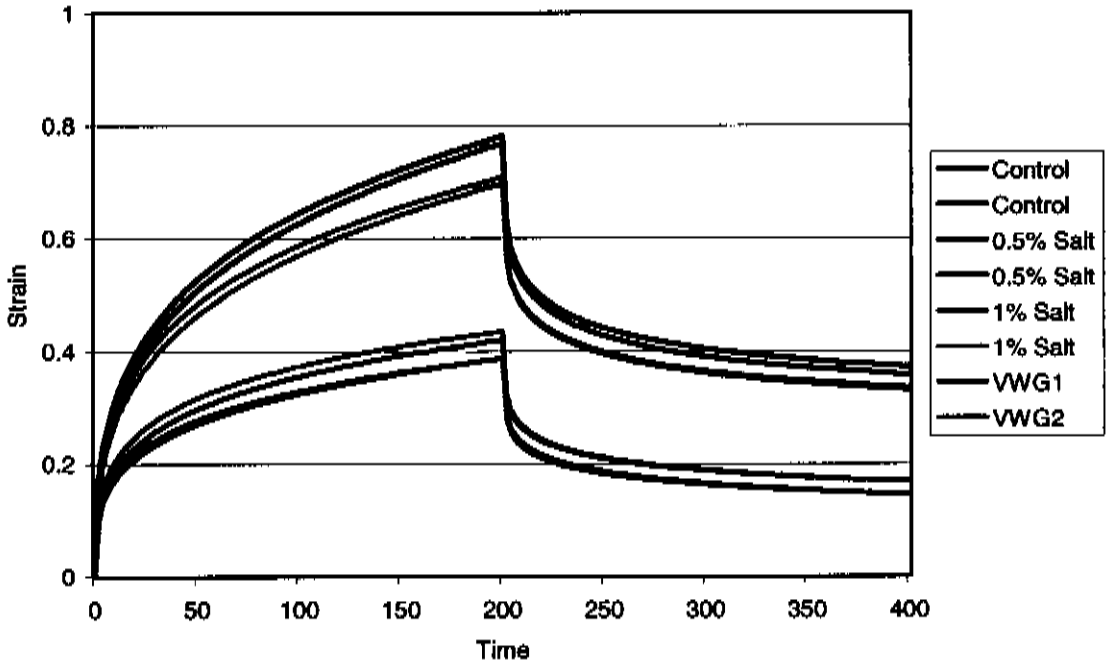
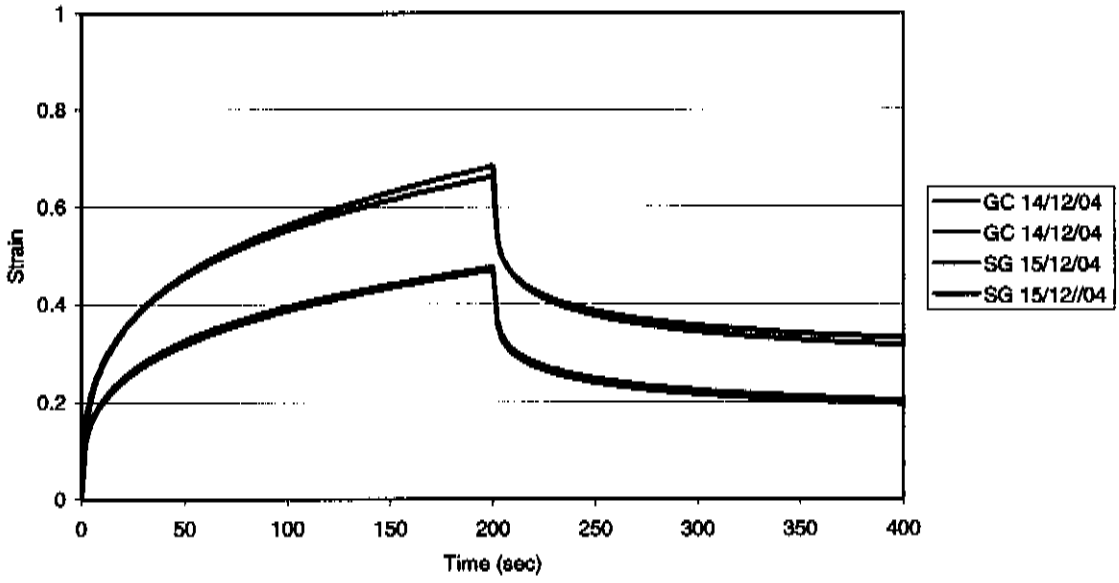
Gluten control	Extensograph reults	0.5% Salt washed gluten	Extensograph reults
	***No cysteine*** Rmax = 875 Ext = 7.0 cm		***No cysteine*** Rmax = >1000 Ext = 10.5 cm
	***300ppm cysteine*** Rmax = 690 Ext = 8.5 cm		***300ppm cysteine*** Rmax = 660 Ext = 12.0 cm
	***450ppm cysteine*** Rmax = 485 Ext = 10.5 cm		***400ppm cysteine*** Rmax = 410 Ext = 14.0 cm
	***600ppm cysteine*** Rmax = 395 Ext = 10.5 cm		***600ppm cysteine*** Rmax = 310 Ext = 14.0 cm

Figure 19. Creep test of the rehydrated gluten (60% w/w water content) produced during the pilot trial in comparison with the commercial VWG.

First trial:



Second trial:



4.3.4 Colour

One issue that arose in the first trial was the colour of the gluten. The product prepared on Day 1 was satisfactory in colour, but the products became successively darker on subsequent days. On Day 3, there was a distinct red tinge about the gluten, and the dough also had a pinkish colour prior to washing. This was showed by their *a* values when measured using a Monitor Colourmeter. Product 3 had the highest *a* value (indicative of redness) followed by Product 2, then Product 1. A later batch was also worse than the early batch in the same day. This was not observed in the subsequent laboratory-prepared gluten samples and had never been seen before in all laboratory trials. This suggests that the redness in Product 3 and later batches of Product 2 could be the equipment used at Altona – either from corrosion in the tanks or plumbing, or from a red dye coming from a hose used to transport hot water to the process. Therefore the second trial was carried out primarily to ascertain that the colour would not be an issue in commercial practice, as well as to further confirm the quality improvement on gluten by the salt-washing process.

The gluten products from the second trial were also tested independently at the QC laboratory at Nowra, Manildra Group. The results showed that there was no increase of *a* values for gluten by using the salt-washing process. Similar to that showed in the laboratory experiments (Figure 15) by using the salt-washing process, gluten (Product 2) had lighter colour in comparison to the water-washed control (Product 1), i.e. higher L value (brighter) and lower *b* value (less yellowier). All the *L-b* results – dried powder, wet gluten and baked, showed that the ammonium salt-washed gluten had scores above the control and normal commercial VWG products.

5 GLUTEN WASHING WITH A WIDER RANGE OF SALT SOLUTIONS

Limited experiments have indicated that a wider range of results are obtainable with other salt solutions, particularly with other ammonium salts. These experiments included the unexpected result that washing with ammonium phosphate produced a gluten that is readily dispersible in water, yet it could be washed satisfactorily by hand. This result is potentially valuable for commercial development because water-dispersible and water-soluble gluten preparations have considerable value as food ingredients and for many non-food applications, e.g., in the pharmaceutical industry (Day et al., 2004).

Further consideration of the underlying principles involved in gluten washing with different salts has led us to consider the likelihood that the behaviour under these different circumstances may be related to the Hofmeister series of anions and actions. This hypothesis offers the possibility that these behaviours might be predicted based on the Hofmeister series, thereby extending the commercial value of this general principle to a range of useful applications.

6 GLUTEN WASHING WITH THE ENZYME TRANSGLUTAMINASE

A further series of laboratory-scale experiments was conducted, using the cross-linking enzyme transglutaminase in an attempt to increase the yield of gluten. This aim was justified in these experiments, but they have not yet been carried through to pilot-scale trials. When gluten was produced with the aid of the enzyme transglutaminase, the enzyme (3 g) was dissolved in water or salt solution (180 ml) prior to mixing with flour (300 g). The dough was then placed in a plastic bag to prevent the loss of the enzyme during resting. The dough was then submerged in water or salt solution for resting (30 min). Gluten washing was then carried out using water or salt in the washing process. The experiment was carried out at both 20 and 40°C. The results indicated that the gluten recovery (yield) obtained from both

temperature treatments was nearly 20% more than the gluten control. The rheological properties as tested in creep test showed the gluten was less extensible then the gluten control. The addition of salt did not have effect on the lipid content of the gluten.

Table 10. Colour measurement for the gluten produced from the second pilot-scale trial.
L value: 0=black, 100=white; *a* value: redness (+), greenness (-); *b* value: yellowness (+), blueness (-).

Dry gluten powder measured at FSA:

Gluten sample	***L* value**	***a* value**	***b* value**	***L* - *b***
Product 1 – control	89.5	0.29	11.6	77.9
Product 2 – 0.5% salt	90.9	0.14	10.5	80.4

Dry gluten powder measured at Nowra:

Gluten sample	***L* value**	***a* value**	***b* value**	***L* - *b***
Product 1 – control	88.09	-0.03	10.22	77.87
Product 2 – 0.5% salt	89.91	-0.25	10.25	79.66

* Normal VWG < 76.00

Wet gluten measured at Nowra:

Gluten sample	***L* value**	***a* value**	***b* value**	***L* - *b***
Product 1 – control	75.49	-0.37	14.47	61.02
Product 2 – 0.5% salt	76.29	-0.22	14.01	62.28

* Normal VWG < 60.00

Baked (5g wet – 6 min sandwich):

Gluten sample	***L* value**	***a* value**	***b* value**	***L* - *b***
Product 1 – control	57.75	9.73	22.37	16.85
Product 2 – 0.5% salt	59.45	11.22	24.18	17.38

* Normal VWG < 16.80

7 CONCLUSIONS

The known use of sodium chloride salt (at 2%) to improve dough properties and gluten extensibility was verified in these studies at the laboratory level. The use of lower concentrations of sodium chloride provided progressively lower levels of improvement, until at 0.5% there was no advantage over washing with water. The experiments with sodium chloride formed a basis for proceeding to evaluate the value of other salts, especially ammonium salts that offer the potential value of volatility. These experiments brought the unexpected advantage that improvements were provided at one quarter of the concentration needed for sodium chloride; the advantages obtained with 2% sodium chloride were achieved with only 0.5% ammonium chloride. The pilot-scale trials have provided results in agreement with the laboratory experiments and it proved that the process is robust and can be scale-up to commercial level. We are not aware that this unexpected result has been obtained previously. It has not been documented, either as a research publication or as a patent, that the use of ammonium chloride for gluten washing has such excellent potential to provide a means of improving gluten quality along the lines desired by industry. These advantages are summarized as follows:-

- Reduced (half) lipid content for both gluten and starch
  - Therefore reductions in associated flavour and aroma problems
- Improved rheological properties
  - Increasing extensibility without reducing the resistance (Rmax)
- Increased gluten whiteness
  - Maybe enzymic activities (PPO) are depressed by salt during washing
- Increased protein yield
- Reduced ash content for gluten and starch
- No adverse effects or increased processing expenses, apart from the small cost of NH_4Cl.

Most of these advantages were also demonstrated in the pilot-scale trials, but in this case (continuous operation versus a batch-wise process), it is difficult in principle to evaluate all these factors, especially such aspects as increased gluten yield.

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